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PROGRAM OVERVIEW

SUN, MARCH 19	MON, MARCH 20	TUES, MARCH 21	WED, MARCH 22
8 am - 7 pm **Registration** *Lobby Level* **Short Courses** **9:00 am - 4:00 pm** Full-Day Course, Day 2 **Design and Analysis of Quantitative Proteomics Experiments** *Grant AB, Lower Level* **9:00 am - 12:00 pm** Morning Short Course **Cross-Linking Mass Spectrometry** *Grant C, Lower Level* **9:00 am - 12:00 pm** Morning Short Course **Glycomics and Glycoproteomics: Basics** *Grant D, Lower Level* **1:00 - 4:00 pm** Afternoon Short Course **Glycomics and Glycoproteomics: Advanced** *Grant D, Lower Level* **1:00 – 4:00 pm** Afternoon Short Course **Stable and Transient Protein-Protein Interactions** *Grant C, Lower Level* **4:30-5:30 pm** Workshop, Open to all **Grantwriting Workshop** *Grant AB, Lower Level*		**6:45 am** Fun Run/Walk Participants go to Conf Reg Desk. Map/info at Reg Desk.	
	8:00 – 8:30 am Early Morning Coffee *Exhibits & Posters, Bivouac Ballroom Lower Level*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits & Posters, Bivouac Ballroom Lower Level*	**8:00 – 8:30 am** Early Morning Coffee *Presidential Foyer, 2nd Level*
	8:30 – 9:20 am **Plenary Lecture** Rommie Amaro *Presidential Ballroom, 2nd Level*	**8:30 – 9:20 am** **Award Presentations + Talks** Alexey Nesvizshkii Peter Nemes, Christine Vogel *Presidential Ballroom, 2nd Level*	**8:30 – 9:20 am** **Tips & Tricks Talks** *Presidential Ballroom, 2nd Level*
	9:20 – 9:50 am Coffee Break *Exhibits & Posters, Bivouac Ballroom Lower Level*	**9:20 – 9:50 am** Coffee Break *Exhibits & Posters, Bivouac Ballroom Lower Level*	**9:20 – 9:50 am** Coffee Break, *Presidential Foyer, 2nd Level*
	9:50 – 11:10 am Parallel Sessions (2) **Cardiovascular** *Presidential Ballroom, 2nd Level* **Neurological Diseases** *Palm Court, Lobby Level*	**9:50 – 11:10 am** Parallel Sessions (2) **Computation and Big Data** *Presidential Ballroom, 2nd Level* **Metabolomics** *Palm Court, Lobby Level*	**9:50 – 11:10 am** Parallel Sessions (2) **Precision Medicine and Metabolic Diseases** *Presidential Ballroom, 2nd Level* **Proteoform Biology** *Palm Court, Lobby Level*
	11:10 am – 12:00 pm Plenary Session **Lightning Talks – Round I** *Presidential Ballroom, 2nd Level*	**11:10 am – 12:00 pm** Plenary Session **Lightning Talks – Round II** *Presidential Ballroom, 2nd Level*	**11:10 am – 12:00 pm** **Plenary Lecture** Leslie Thompson *Presidential Ballroom, 2nd Level*
	12:00 – 1:30 pm **Lunch Seminars** Bruker, *Grant AB, Lower Level* Thermo, *Grant CD, Lower Level*	**12:00 – 1:30 pm** **Lunch Seminar** SCIEX, *Grant AB, Lower Level* Waters, *Grant CD, Lower Level*	
	1:30 - 3:00 pm **Monday Posters** *Exhibits & Posters, Bivouac Ballroom Lower Level*	**1:30 - 3:00 pm** **Tuesday Posters** *Exhibits & Posters, Bivouac Ballroom Lower Level*	
	3:00 – 4:20 pm Parallel Sessions (2) **Cancer Moonshot** *Presidential Ballroom, 2nd Level* **Glycans and Vaccines** *Palm Court, Lobby Level*	**3:00 – 4:20 pm** Parallel Sessions (2) **New Technologies** *Presidential Ballroom, 2nd Level* **Top Down Analysis** *Palm Court, Lobby Level*	
	4:30 – 5:50 pm Parallel Sessions (2) **Metaproteomics and Proteome Diversity** *Presidential Ballroom, 2nd Level* **Disease and Protein-Protein Proximity** *Palm Court, Lobby Level*	**4:30 – 5:50 pm** Parallel Sessions (2) **Single Cell Proteomics** *Presidential Ballroom, 2nd Level* **Cross-Linking/Molecular Painting** *Palm Court, Lobby Level*	
6:00 – 7:00 pm **Opening Session Plenary Lecture** Eric Topol *Presidential Ballroom, 2nd Level*	**5:50 – 6:30 pm** **Mixer** with Exhibitors *Bivouac Ballroom, Lower Level* Munchies & Drinks All are welcome! Grab something to eat before the workshops.	**6:00 – 9:00 pm** **AACC-US HUPO Joint Event + Program** 6-7pm Dinner reception + cash bar in *Presidential Foyer, 2nd Level* 7-9pm Program, *Presidential Ballroom, 2nd Level*	
7:00 – 8:30 pm **Opening Reception** *with Exhibitors, Bivouac Ballroom, Lower Level* Food & Drinks All are welcome!	**6:30 – 8:00 pm** Evening Workshops (3) **Creating Sustainable Value for Your Datasets (BD2K/LINCS)**, *Presidential Ballroom, 2nd Level* **How to Obtain a Faculty Position and Keep It**, *Grant AB, Lower Level* **Implementing Strategies of the HPP**, *Palm Court, Lobby Level*		

GENERAL INFORMATION

VENUE. The conference venue is the US Grant Hotel, a historic property. The meeting is located among three levels: lower, lobby, and 2nd level. Both stairs and elevators connect all three levels.

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

POSTERS AND EXHIBITORS. Posters are located in Bivouac Ballroom, Lower Level, with exhibitors. Monday posters should be mounted between 8-8:30am on Monday, Tuesday posters should be mounted between 8-8:30 am on Tuesday. Exhibit booths will be in place from Sunday evening welcome reception through Tuesday at 3 pm (conclusion of Tuesday Poster Session)

Posters are presented/attended:

- Monday posters on Monday 1:30-3:00 pm
- Tuesday posters on Tuesday 1:30-3:00 pm

TALKS. There are two session rooms on different levels.

- Palm Court, Lobby Level (parallel session only)
- Presidential Ballroom, 2nd Level (plenary and parallel session)

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

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

EVENING WORKSHOPS. All conference attendees are welcome to attend the workshops. There is not a separate registration.
Sunday 4:30-5:30 pm there will be a special Grantwriting workshop in Grant AB, Lower Level.
Monday 6:30-8:00 pm there will be three workshops immediately following an informal mixer with exhibitors. See program schedule for titles and locations.

LUNCH SEMINARS. Free lunch seminars are hosted on Monday and Tuesday on the lower level in Grant AB and CD. All attendees are invited to attend, but are encouraged to RSVP at host company exhibit booths. See program schedule for details.

JOB BOARD. Located in Bivouac Foyer, Lower Level. Use this area to post opportunities or your CV if you are seeking a position.

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

NO PHOTOGRAPHY or RECORDING of posters or talks is permitted.

FUN RUN/WALK. Tuesday morning, meet at 6:45 am in Hotel Lobby. Participants will be directed to outside gathering spot. Main group to depart at 7am. This is at your own pace and preferred distance. Route is out-and-back and turn-around at any point is encouraged. The goal is to get out and move with colleagues and friends, not a race. Please sign up at Registration Desk (even if you indicated your participation online).

CONFERENCE T-SHIRTS. Custom t-shirt is quick-dry jersey and appropriate for exercise or everyday wear. Advance online purchases ($8) will be accommodated first, but there are extra available for purchase onsite ($10). Come to registration for purchase or pick-up of your pre-order.

SPECIAL TUESDAY JOINT EVENT + PROGRAM. Together with AACC (American Association of Clinical Chemistry) Southern California Chapter a dinner reception and program are offered on Tuesday, 6:00-9:00 pm in the Presidential Foyer and Ballroom. See program schedule for additional information. This event + program is free to all conference attendees though RSVP is requested.

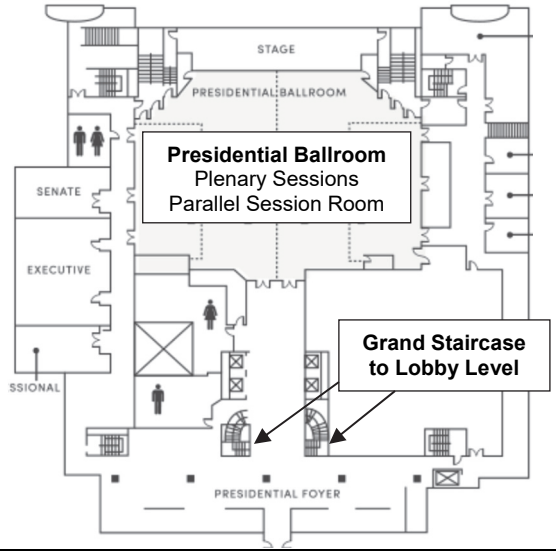
US GRANT HOTEL - THREE LEVELS FOR CONFERENCE

2ND LEVEL

Accessible by grand staircase on lobby level and elevators.

PRESIDENTIAL BALLROOM
Parallel Session Room on Monday-Wednesday
All Plenary Lectures on Monday-Wednesday
Workshop Room on Monday

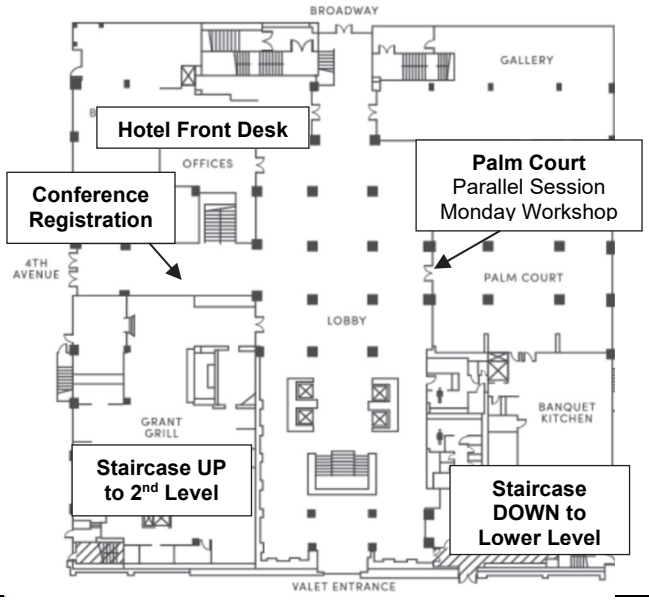
PRESIDENTIAL FOYER
Tuesday Evening Event



LOBBY LEVEL

PALM COURT
Parallel Session Room on Monday-Wednesday
Workshop Room on Monday

REGISTRATION
Registration desk for conference

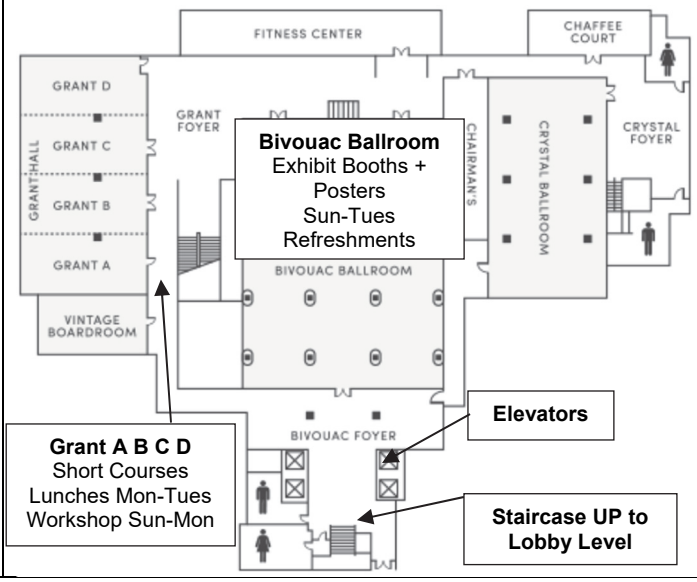


LOWER LEVEL

BIVOUAC BALLROOM
Exhibit Booths + Posters
Sunday Welcome Reception
All Monday-Tuesday Coffee Breaks
Monday Evening Mixer

GRANT A B C D
Short Courses on Saturday- Sunday
Lunch Seminars on Monday-Tuesday
Workshop Room Sunday-Monday

CRYSTAL BALLROOM
Committee Meeting Room



EXHIBITORS

US HUPO is pleased to acknowledge the support of conference exhibitors. Exhibit booths are located in the Bivouac Ballroom, Lower Level along with the technical posters. Sunday welcome reception, Monday-Tuesday coffee breaks, and Monday mixer will be located here with the exhibitors.

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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 program schedule for descriptions.*	**Tuesday, 12:00 – 1:30 pm** *See program schedule for descriptions.*
Bruker, *Grant AB* **Thermo Scientific**, *Grant CD*	**SCIEX**, *Grant AB* **Waters Corporation**, *Grant CD*

AWARDS

US HUPO now has two awards both created to honor the name and contributions of leaders in the field and of the Society.

GILBERT S. OMENN COMPUTATIONAL PROTEOMICS AWARD

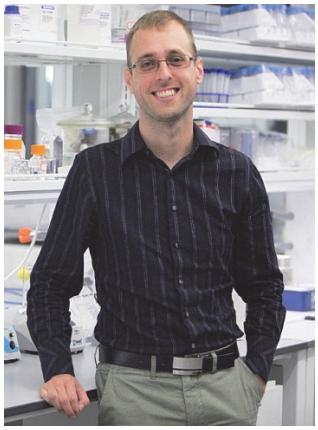
2017 Recipient: **Alexey I. Nesvizshkii**
University of Michigan

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

Dr. Nesvizshskii is recognized for his continuing efforts in providing statistical assessment of proteomics data, for the generation of user-friendly and open source software tools, and for his commitment to educate the proteomics community on the proper use of computational proteomics tools.

ROBERT J. COTTER NEW INVESTIGATOR AWARD

The Robert J. Cotter New Investigator Award will be given to an individual early in his or her career, in recognition of significant achievements in proteomics, broadly defined. This year two recipients were selected to split the award.



2017 Recipient: **Peter Nemes**
George Washington University

Dr. Nemes' laboratory has developed unique single-cell MS strategies and instruments that enabled, for the first time, the discovery proteomic (and metabolomic) and characterization of single embryonic cells in the cleavage stage (16-cell) *Xenopus* embryo. More recently, his lab also advanced MS sensitivity using these instruments to total protein amounts contained by a handful of neurons in the mouse cerebral cortex. Dr. Nemes has deployed these bioanalytical tools to ask basic questions in cell and developmental biology and neuroscience.

2017 Recipient: **Christine Vogel**
New York University

Dr. Vogel's unique contribution to proteomics research lies in the integration of quantitative protein measurements with system-wide information on transcriptome and other changes to derive new insights into principles of gene expression regulation–in steady state and dynamic cellular systems.
The overarching goal of her research is to understand the protein expression response to environmental stress, using the yeast *Saccharomyces cerevisiae* and mammalian cells, integrating proteomics with a diverse set of system-wide data.

Dr. Vogel's lab uses a combination of sequencing and high-resolution mass spectrometry for these system-wide studies, plus computational analysis to provide robust statistical tools that integrate diverse datasets.

SHORT COURSES

9:00 AM – 4:00 PM, SATURDAY AND SUNDAY (Two-day Course)
Design and Analysis of Quantitative Proteomic Experiments: Introduction to Statistical Methods and Practical Examples using Skyline, R and MS Stats
Instructors: Meena Choi (Northeastern University), Brendan MacLean (University Washington) and Olga Vitek (Northeastern University)
Grant AB, Lower Level

This 2-day course will discuss details of statistical experimental design of quantitative mass spectrometry-based proteomic experiments, and the analysis of the acquired data with Skyline and MSstats. It is designed for experimentalists looking to enhance their statistical and data analysis skills, and will contain both lectures and practical hands-on exercises.

First, the course will discuss the fundamental concepts of statistical experimental design, that are key for designing reproducible investigations. Second, the course will introduce the open-source software Skyline. In particular, we will demonstrate the use of Skyline for proteome-wide profiling of samples with label-free shotgun data-dependent spectral acquisition (DDA), and the process of building libraries from DDA spectra to analyze profiling experiments with data-independent acquisition (DIA). The participants will practice these analysis steps using example datasets. Finally, we will discuss the principles of statistical inference, including summarization of protein abundances from multiple spectral features, derivation of confidence intervals for fold changes, and testing proteins for differential abundance. The participants will perform hands-on analyses of the example datasets with open-source software R and MSstats.

The participants should bring their own laptop computers. Instructions regarding downloading and installing the software will be provided prior to the course.

9:00 AM– 12:00 PM, SUNDAY (Morning Half-day Course)
Cross-Linking Mass Spectrometry: Practical Uses in Studying Protein Interactions and Structures
Instructors: Lan Huang (UC Irvine) and Robert Chalkley (UCSF)
Grant C, Lower Level

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.

9:00 AM – 12:00 PM, SUNDAY (Morning Half-day Course)
Glycomics and Glycoproteomics: The Basics (Part I)
Instructor: Parastoo Azadi (Complex Carbohydrate Center, University of Georgia)
Grant D, Lower Level

The basics course will cover different forms of glycosylation and diversity of glycoprotein structures and various chemical (hydrazinolysis and β-eliminations) and enzymatic methods available to release N and O-linked glycans. The pros and cons of each release method and their subsequent yields will be discussed. Participants will learn basic techniques for the isolation, purification and characterization of oligosaccharides by mass spectrometry techniques such as ESI-MS, MALDI-MS and GC-MS. Glycomics protocols including derivatization of glycans and glycomics methods starting from cells, tissues or purified glycoproteins will be covered. The optimization methods for analysis of sialic acid containing oligosaccharides by MS techniques discussed. Various monosaccharide procedures including hydrolysis conditions needed for different residues will be reviewed as well as linkage analysis (methylation) of oligosaccharides.

1:00 – 4:00 PM, SUNDAY (Afternoon Half-day Course)
Glycomics and Glycoproteomics: Advanced (Part II)
Instructor: Parastoo Azadi (Complex Carbohydrate Center, University of Georgia)
Grant D, Lower Level

In the advanced course topics that will be covered include:

- Mapping N and O-linked glycosylation sites (LCMS)
- Glycoproteomics (glycopeptide analysis ETD /HCD fragmentation)
- Glycosylation site occupancy
- Determining the composition of glycans
- Sequencing of oligosaccharides by MS/MS and MSn including branching points and fragmentation pattern
- Glycan quantitation using MS and internal standards
- Optimization methods for analysis of Non-carbohydrate constituents such as sulfates and phosphates on glycans
- Data interpretation and available software and databases
- Glycoprotein biopharmaceuticals and their structural analysis by mass spectrometry

1:00 – 4:00 PM, SUNDAY (Afternoon Half-day Course)
Stable and Transient Protein-Protein Interactions: Discovery, Quantification and Validation
Instructors: Ileana Criste (Princeton University) and Alexey Nesvizshkii (University of Michigan)
Grant C, Lower Level

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:

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

As we will gradually cover fundamental and more advanced topics concerning protein interactions, the course will be appropriate for both beginner- and advanced-level participants. Detailed protocols will be provided, and enough time will be set aside for discussing these topics from either a mass spectrometry or biology perspective.

SUNDAY, MARCH 19

4:30 – 5:30 PM: SPECIAL WORKSHOP
GRANTWRITING, *Grant Hall AB (Lower Level)*
Presented by Peipei Ping (UCLA) and Ileana Cristea (Princenton University)

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

Welcome to the Opening Session

PRECISION PROTEOMICS FOR DISCOVERY AND HEALTH
Session Chair: Peipei Ping (UCLA)

6:00 – 6:10 pm Opening Remarks

6:10 – 7:00 pm **Digital Medicine Revolution**; Eric Topol, *MD, PhD, Director, Scripps Translational Science Institute*

7:00 – 8:30 PM: **OPENING RECEPTION**, *Bivouac Ballroom (Lower Level)*

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

MONDAY, MARCH 20

8:00 – 8:30 AM: **EARLY MORNING COFFEE & PASTRIES**, *Bivouac Ballroom (Lower Level)*

8:30 - 9:20 AM: **PLENARY LECTURE**, *Presidential Ballroom (2nd Level)*

8:30 - 9:20 am **Bringing Molecular Modeling to the Mesoscale**; Rommie E. Amaro; *Director, National Biomedical Computation Resource, Co-Director, Drug Design Data Resource (D3R), University of California, San Diego*

9:20 - 9:50 AM: **COFFEE BREAK**, *Bivouac Ballroom (Lower Level)*
Refresh and visit with the exhibitors.

9:50 – 11:10 AM: PARALLEL SESSION
CARDIOVASCULAR DISEASE, *Presidential Ballroom (2nd Level)*
Session Chair: Merry Lindsey (University of Mississippi)

9:50-10:15 am **New Technologies for Advancing Stem Cell Derived Cardiomyocytes in Drug Discovery, Disease Modeling, and Regenerative Medicine**; Matthew Waas; Chelsea Fujinaka; Theodore Keppel; Ranjuna Weerasekera; Rebekah Gundry; *Medical College of Wisconsin, Milwaukee, WI*

10:15-10:40 am **Top-Down Proteomics of Myofilaments in Heart Failure**; Ying Ge; *Uw-Madison, Madison, WI*

10:40-10:55 am **DIA-MS Reveals Altered Mitochondrial Protein Profile and Activation of mTOR2-signaling during Aneurysm Progression in a Mouse Model of Marfan Syndrome**; Sarah Parker[1]; Aleksander Stotland[1]; Elena Gallo-MacFarlane[2]; Nicole Wilson[2]; Roberta Gottlieb[1]; Hal Dietz[2]; Jennifer VanEyk[1]; *[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]Johns Hopkins University, Baltimore, MD*

10:55-11:10 am **Redox Proteome Dynamics in Mouse Heart During Maladaptive Cardiac Remodeling**; Jie Wang[1, 2]; Quan Cao[1, 2]; Dominic Ng[1, 2]; Peipei Ping[1, 2]; *[1]Department of Physiology, UCLA, Los Angeles, CA; [2]The NIH BD2K Center at UCLA, Los Angeles, CA*

9:50 – 11:10 AM: PARALLEL SESSION
NEUROLOGICAL DISEASES, *Palm Court (Lobby Level)*
Session Chair: Lingjun Li (University of Wisconsin)

9:50-10:15 am **Brain Region and Pathological Stage Specific Proteome-Wide Alterations in Mouse Models of Alzheimer's Disease Like Pathology.**; Jeffrey Savas[1]; Yi-Zhi Wang[1]; Laura DeNardo-Wilke[2]; Salvador Martinez de Bartolome Izquierdo[3]; Daniel McClatchy[3]; Natalie Shanks[4]; Timothy Hark[1]; Kira Cozzolino[1]; Mathieu Lavallee-Adam[3]; Sung Kyu Park[3]; Jeffery Kelly[3]; Edward Koo[4]; Andrew Dillin[5]; Terunaga Nakagawa[6]; Eliezer Masliah[4]; Anirvan Ghosh[4]; John Yates[3]; *[1]Northwestern University, Chicago, Illinois; [2]Stanford University, Stanford, CA; [3]Scripps Research Institute, La Jolla, CA; [4]University of California, San Diego, La Jolla, CA; [5]University of California, Berkeley, Berkeley, CA; [6]Vanderbilt University School of Medicine, Nashville, TN*

10:15-10:40 am **Spatio-Temporal Profile of Synaptic Protein Complexes and Their Role in Brain Disease**; Marcelo Coba; *Zilkha Neurogenetic Institute, Keck School Of Medi, ,*

10:40-10:55 am **Elucidation of Novel Proteoforms of Superoxide Dismutase (SOD1) in Sporadic ALS Patients**; David Muddiman; Philip Loziuk; *North Carolina State University, Raleigh, NC*

10:55-11:10 am **A Multi-Network Approach to Define Pathways Altered in Alzheimer's Disease and Parkinson's Disease**; Lingyan Ping; Duc Duong; Eric Dammer; Marla Gearing; James Lah; Allan Levey; Nicholas Seyfried; *Emory University School of Medicine, Atlanta, GA*

Dr. Savas' talk is sponsored by

Cambridge Isotope Laboratories, Inc.

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

Presentation Order

Mon 1 **A 3-D Proteome Atlas is an Important Component to the Cancer Moonshot**; Kat Tiemann; Carolina Garri; Ruth Alvarez; Sang Bok Lee; Jonathan Katz; Kian Kani; USC, Los Angeles, CA; **See Monday Poster 15**

Mon 2 **A kinetic Proteomics Approach to Identify Targets of Autophagy in Cancer and Recover Chemotherapeutic Effects**; Monique Paré Speirs; Bradley Naylor; John C. Price; Brigham Young University, Provo, UT; **See Monday Poster 11**

Mon 3 **A Novel Method of Quantifying Protein Methylation Utilizing SWATH-MS**; Aaron Robinson; Sarah Parker; Vidya Venkatraman; Ronald Holewinski; Shelly Lu; Jennifer Van Eyk; Cedars Sinai Medical Center, Los Angeles, CA; **See Monday Poster 50**

Mon 4 **CE-MS-based Profiling of BNP Proteoforms from Plasma in One Hour**; Koen Raedschelders; Shenyan Zhang; Jennifer Van Eyk; Cedars-Sinai Medical Center, Los Angeles, CA; **See Monday Poster 62**

Mon 5 **Glycomics and Proteomics of Myelinated versus Non-myelinated Regions of Human Brain Tissue**; Manveen Sethi[1]; Harry Pantazopoulos[2, 3]; Sabina Berretta[2, 3]; Joseph Zaia[1]; [1]Boston University School of Medicine, Boston, MA; [2]Department of Psychiatry, Harvard Medical School, Boston, MA; [3]Translational Neuroscience Laboratory, McLean Hosp, Belmont, MA; **See Monday Poster 41**

Mon 6 **Histone H3 Mutations Drive Aberrant Chromatin-reader Interactions in Diffuse Intrinsic Pontine Glioma**; Dylan M. Marchione; Mariel Coradin; Simone Sidoli; Zuofei Yuan; Benjamin A. Garcia; University of Pennsylvania School of Medicine, Philadelphia, PA; **See Monday Poster 53**

Mon 7 **Investigating the Mechanism of AGE-mediated Cancellation of Calorie Restriction Benefits**; Richard Carson; Bradley Naylor; John Price; Brigham Young University, Provo, UT; **See Monday Poster 59**

Mon 8 **LinkedOmics : A Web-based Platform for Cancer Multi-omics Data Integration and Comparison**; Suhas Vasaikar[1]; Peter Straub[2]; Jing Wang[1]; Bing Zhang[1]; [1]Baylor College of Medicine, Houston, Texas; [2]Vanderbilt University, Nashville, TN; **See Monday Poster 12**

Mon 9 **Mass Spectrometry-based Proteomic and PTM Studies Provide Insight into the Molecular Mechanisms of Restenosis**; Matthew Glover[1]; Qing Yu[1]; Bowen Wang[1]; Xudong Shi[1]; Lian-Wang Guo[1]; K. Craig Kent[1, 2]; Lingjun Li[1]; [1]University of Wisconsin-Madison, Madison, WI; [2]The Ohio State University, Columbus, OH; **See Monday Poster 24**

Mon 10 **Online 2D-NCFC-RP/RPLC System for Efficient and Comprehensive Proteomic Analyses**; Sang-Won Lee; Hangyeore Lee; Jeong Eun So; Korea University, Seoul, Korea, Republic of; **See Monday Poster 48**

Mon 11 **Probing Translational Regulation in Spinal Muscular Atrophy (SMA) using Integrated Proteomics and Transcriptomics Approaches**; Amanda Guise[1]; Shaojun Tang[2]; Ruchi Chauhan[1]; Constantin van Outryve d'Ydewalle[4]; Hendrik Wesseling[1]; Charlotte Sumner[4]; Martin Hemberg[3]; Hanno Steen[1]; Judith S; [1]Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Georgetown University, Washington, DC; [3]Wellcome Trust Sanger Institute, Cambridge, UK; [4]Johns Hopkins School of Medicine, Baltimore, MD; **See Monday Poster 40**

Mon 12 **Proteome-wide Acetylation Dynamics Revealed by Metabolic Labeling and Quantitative Proteomics**; Yekaterina Kori[1]; Simone Sidoli[1]; Zuo-Fei Yuan[1]; Xiaolu Zhao[2]; Benjamin A. Garcia[1]; [1]University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; [2]Wuhan University, Wuhan, China; **See Monday Poster 49**

Mon 13 **Proteomics for Systems Biology: Defining the Cross-talk between Signaling Pathways and chromatin modifications occurring during treatment of acute myeloid leukemia**; Simone Sidoli; Pamela J. Sung; Katarzyna Kulej; Martin Carroll; Benjamin A. Garcia; University of Pennsylvania, Philadelphia, PA; **See Monday Poster 30**

Mon 14 **Proteomics Studies in Synergistic Protection from Retinal Degeneration by Combined Stem Cell Therapies**; Dawn Z Chen[1, 3]; Changqing Zhang[2]; Yang Gao[1]; Bin Lu[2]; Sergey Girman[2]; Benjamin Bakondi[2]; Weston Spivia[1]; Jennifer E. Van Eyk[1]; Shaomei Wang[2, 3]; [1]Advanced Clinical Biosystems Research Institute, Los Angeles, CA; [2]Board of Governors Regenerative Medicine Institute, Los Angeles, CA; [3]David Geffen School of Medicine, UCLA, Los Angeles , CA; **See Monday Poster 46**

Mon 15 **Proteostasis Interactome Remodeling of Amyloidogenic Proteins Governs Improved ER Quality Control Decisions**; Lars Plate[1]; Joseph C. Genereux[2]; R. Luke Wiseman[1]; Jeffery W. Kelly[1]; [1]The Scripps Research Institute, La Jolla, CA; [2]University of California, Riverside, Riverside, CA; **See Monday Poster 58**

Mon 16 **Quantitative Analysis of Newly Synthesized Proteins during Maladaptive Cardiac Remodeling**; Yuanhui Ma[1]; Daniel B. McClatchy[1]; David Liem[2]; Dominic Ng[2]; Peipei Ping[2]; John R. Yates[1]; [1]The Scripps Research Institute, La Jolla, CA; [2]University of California at Los Angeles, Los Angeles, CA; **See Monday Poster 27**

Mon 17 **Quantitative Drug Distribution Mapping in Tissues using IR-MALDESI Mass Spectrometry Imaging**; Mark Bokhart[1]; Elias Rosen[2]; Corbin Thompson[2]; Ken Garrard[1]; Angela Kashuba[2]; David Muddiman[1]; [1]NCSU, Raleigh, NC; [2]UNC Eshelman School of Pharmacy, Chapel Hill, NC; **See Monday Poster 39**

Mon 18 **Quantitative Phosphoproteomic and Proteomic Analysis of Swine Hearts Revealed Novel Insights into Myocardial Stunning**; Xue Wang[1]; Xiaomeng Shen[2]; Rebeccah Young[3]; Jun Li[2]; Shichen Shen[2]; John Canty[3]; Jun Qu[2]; [1]Roswell Park Cancer Institute, Buffalo, NY; [2]SUNY at buffalo, Buffalo, NY; [3]Clinical and Translational Research Center, Buffalo, NY; **See Monday Poster 29**

Mon 19 **Semi-Automated Methods in Skyline and New MS/MS Rules to Define and Assign Xylosylated *N*-Linked Glycans in Populus Trichocarpa**; Elizabeth S. Hecht[1]; Philip Loziuk[1]; Brian Pratt[2]; Brendan Maclean[2]; Mike Maccoss[2]; David Muddiman[1]; [1]North Carolina State University, Raleigh, NC; [2]University of Washington Genome Science, Seattle, WA; **See Monday Poster 35**

Mon 20 **Time-resolved Global and Chromatin Proteomics during Herpes Simplex Virus (HSV-1) Infection**; Katarzyna Kulej[1]; Daphne D. Avgousti[1]; Simone Sidoli[2]; Christin Herrmann[1]; Ashley N. Della Fera[1]; Eui Tae Kim[1]; Benjamin A. Garcia[2]; Matthew D. Weitzman[1]; [1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania, Philadelphia, PA; **See Monday Poster 31**

Mon 21 **Transcriptome and Proteomic Profiling of a Drosophila Seizure Model Reveals Glial Regulation of Key Synaptic Proteins**; Kevin Hope[1]; Daniel Johson[3]; Xiaoyue Jiang[2]; Andreas Huhmer[2]; Daniel Lopez-Ferrer[2]; Lawrence Reiter[1, 4]; [1]Department of Neurology, UTHSC, Memphis, TN; [2]Thermo Fisher Scientific, San Jose, CA; [3]Molecular Bioinformatics Core, UTHSC, Memphis, TN; [4]Department of Anatomy and Neurobiology, UTHSC, Memphis, TN; **See Monday Poster 43**

Mon 22 **Unambiguously and Comprehensively Resolving Cancerous Adenocarcinoma and Stromal Proteomes and their Interactive Signaling without Cell Sorting in Patient-derived Xenograft Models**; Shichen Shen[1]; Jun Li[1]; Ninfa L. Straubinger[1]; Xue Wang[2]; Michelle K. Greene[4]; Christopher J. Scott[4]; Wen Wee Ma[3]; Robert M. Straubinger[1]; Jun Qu[1]; [1]SUNY at Buffalo, Buffalo, NY; [2]Roswell Park Cancer Institute, Buffalo, NY; [3]Mayo Clinic, Rochester, MN; [4]Queen's University, Belfast, Northern Ireland; **See Monday Poster 14**

Mon 23 **Unraveling the Complexity of the Gut-Brain Axis N-Glycoproteome**; Mariana BarbozaGardner[1]; Gege Xu[2]; Melanie Gareau[1]; Helen Raybould[1]; Carlito Lebrilla[2, 3]; [1]Department of Anatomy, Physiology & Cell Biology, UC Davis, CA; [2]Department of Chemistry, UC Davis, CA; [3]Department of Biochemistry, School of Medicine, UC Davis, California; **See Monday Poster 47**

Mon 24 **Urinary Protein Changes in Walker 256 Tumor-bearing Rats**; Jianqiang Wu[1]; Youhe Gao[1, 2]; [1]Peking Union Medical College, Beijing, China; [2]Beijing Normal University, Beijing, China; **See Monday Poster 1**

Mon 25 **Vascular Contributions of Plasma Lipoproteins to Alzheimer's Diseases**; Danni Li; Fangying Huang; U of Minnesota, Minneapolis, MN; **See Monday Poster 42**

MONDAY 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: **BRUKER**, *Grant Hall AB (Lower Level)*

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

Trapped Ion Mobility Mass Spectrometry (TIMS-MS) for Shotgun Proteomics
Scarlet Beck*, Max Planck Institute of Biochemistry, Martinsried (near Munich), Germany*

Proteomic Profiling for Biomarker Discovery and Validation using a Bruker Impact II Q-TOF
Gary H. Kruppa, Ph. D.*, Vice President 'Omics, Bruker Daltonics Inc., Billerica, MA*

12:00 – 1:30 PM: **THERMO SCIENTIFIC**, *Grant Hall CD (Lower Level)*

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

The Essential Roles of System Qualifications, Inter-Sample QC and Intra-Sample QC in 'omic Proteomics
M. Arthur Moseley, Ph.D. *Associate Research Professor, Department of Medicine, Duke University School of Medicine*

1:30 – 3:00 PM: M**ONDAY POSTER SESSION**, *Bivouac Ballroom (Lobby Level)*

3:00 – 4:20 PM: PARALLEL SESSION
CANCER MOONSHOT, *Presidential Ballroom (2nd Level)*
Session Chair: Henry Rodriguez (NIH, NCI)

3:00-3:25 pm **Quantitative Mass Spectrometry Analysis of Immune Checkpoint Protein Expression and N-Glycosylation in Human Melanoma**; Carlos Morales-Betanzos; Hyoungjoo Lee; Paula Gonzalez-Ericsson; Justin Balko; Douglas Johnson; Lisa Zimmerman; Daniel Liebler; *Vanderbilt Univ School of Medicine, Nashville, TN*

3:25-3:50 pm **Using Colon Organoids to Explore Cancer Biology**; Katelyn Ludwig[1]; Xin Liu[1]; Jessica Lukowski[1]; Colin Flinders[2]; Shannon Mumenthaler[2]; Heinz-Joseph Lenz[2]; Amanda Hummon[1]; *[1]University of Notre Dame, Notre Dame, IN; [2]University of Southern California, Los Angeles, CA*

3:50-4:05 pm **Genomic Determinants of Protein Abundance Variation in Colorectal Cancer Cell Lines**; Jyoti Choudhary; *Wellocme Trust Sanger Institute, Cambridge, United Kingdom*

4:05-4:20 pm **Antigen Presentation Profiling Reveals T-cell Recognition of Lymphoma Immunoglobulin Neoantigens**; Niclas Olsson[1]; Michael Khodadoust[1]; Lisa Wagar[1]; Ole Audun Werner Haabeth[1]; Binbin Chen[1]; Keith Rawson[1]; Chih Long Liu[1]; David Steiner[1]; Samhita Rao[1]; Lichao Zhang[1]; Henning Stehr[1]; Aaron Newman[1]; Debra K Czerwinski[1]; Victoria Carlton[2]; Martin Moorhead[2]; Malek Faham[2]; Holbrook Kohrt[1]; Michael R Green[3]; Mark M Davis[1]; Ron Levy[1]; Ash A. Alizadeh[1]; Joshua Elias[1]; *[1]Stanford University, Stanford, CA; [2]Adaptive Biotechnologies, Seattle, Washington; [3]University of Nebraska Medical Center, Omaha, NE*

3:00 – 4:20 PM: PARALLEL SESSION
GLYCANS AND VACCINES, *Palm Court (Lobby Level)*
Session Chair: Susan Old (NIH, NIAID)

3:00-3:25 pm **Germline-targeting Vaccine Design for HIV: a Strategy to Overcome the Sequence Diversity and Heavy Glycosylation of the Envelope Trimer**; William Schief, *The Scripps Research Institute, La Jolla, CA*

3:25-3:50 pm **A Focus on Analytics: Glycosylation Profiling to Assess HIV-1 Env Quality.**; Heather Desaire; *University of Kansas, Lawrence, KS*

3:50-4:05 pm **Systematic Quantification of Human Cell Surface Glycoprotein Dynamics**; Haopeng Xiao; Ronghu Wu; *Georgia Tech, Atlanta, GA*

4:05-4:20 pm **High Resolution CESI-MS Analysis of Formalin-Fixed Paraffin-Embedded, Released N-glycans**; Bryan Fonslow[1]; Boglarka Donczo[2]; Marton Szigeti[2]; Andras Guttman[1, 2]; *[1]SCIEX, San Diego, CA; [2]University of Debrecen, Debrecen, Hungary*

4:30 – 5:50 PM: PARALLEL SESSION
METAPROTEOMICS AND PROTEOME DIVERSITY, *Presidential Ballroom (2nd Level)*
Session Chair: Nuno Bandeira (UCSD)

4:30-4:55 pm **Characterizing Temporal and Inter-Individual Functional Differences in Pre-Term Human Infant Gut Microbiome Development by a Metaproteomics Approach**; Robert Hettich[1]; Weili Xiong[1]; Alfredo Blakeley-Ruiz[1]; Christopher Brown[2]; Matthew Olm[2]; Matthew Rogers[3]; MIchael Morowitz[3]; Jillian Banfield[2]; *[1]ORNL, Oak Ridge, TN; [2]University of California, Berkeley, CA; [3]University of Pittsburgh, Pittsburgh, PA*

4:55-5:20 pm **New Quantitative Methods to Study the Gut Microbiota**; X, Zhang[1]; C.K. Chiang[1]; L. Li[1]; W. Chen[2]; A.E. Starr[1]; K. Cheng[1]; Z. Ning[1]; J. Mayne[1]; S. A. Deeke[1]; R. Tian[2]; D. Mack[3]; A. Stintzi[1]; Daniel Figeys[1]; *[1]Ottawa Institute of Systems Biology, U. of Ottawa, Ottawa, Canada; [2]SUSTC, China, Shenzhen, China; [3]Department of Pediatrics, University of Ottawa, Ottawa, Canada*

5:20-5:35 pm **Optimization of the Number of Proteins and Biological Replicates in Large-scale Proteomic Studies**; Ting Huang[1]; Tiannan Guo[2]; Ruedi Aebersold[2]; Olga Vitek[1]; *[1]Northeastern University, BOSTON, MA; [2]ETH Zurich, Zurich, Switzerland*

5:35-5:50 pm **Integrated Proteogenomics Analyses to Study Tumor Heterogeneity in Human Lung Adenocarcinoma Using Sequential Biopsies and Rapid/Warm Autopsies**; Nitin Roper[1]; Xu Zhang[1]; Tapan K. Maity[1]; Shaojian Gao[1]; Abhilash Venugopalan[1]; Paul Rudnick[2]; Romi Biswas[1]; Constance M. Cultraro[1]; David Fenyo[3]; David Kleiner[4]; Stephen Hewitt[4]; Udayan Guha[1]; *[1]Thoracic and GI Oncology Branch, CCR , NCI, NIH, Bethesda, MD; [2]Spectragen Informatics LLC, Bainbridge Island, WA; [3]NYU School of Medicine, New York, NY; [4]Pathology, CCR, NCI, NIH, Bethesda, MD*

4:30 – 5:50 PM: PARALLEL SESSION
DISEASE AND PROTEIN-PROTEIN PROXIMITY, *Palm Court (Lobby Level)*
Session Chair: Ying Ge (University of Wisconsin)

4:30-4:55 pm Brian Raught; *University of Toronto, Toronto, ON, Canada*

4:55-5:20 pm **Integrating Proteomics and Virology to Define Cellular Immune Signaling Mechanisms During Herpesvirus Infection**; Ileana Cristea; *Princeton University, Princeton, NJ*

5:20-5:35 pm **A Post-Translational Modification Code for CFTR Maturation is Disturbed in Cystic Fibrosis**; Sandra Pankow; Casimir Bamberger; John R. Yates; *The Scripps Research Institute, La Jolla, CA*

5:35-5:50 pm **Monitoring Signal-Specific Changes to *in vivo* Ribosomal Structure and Activity**; Bradley Naylor[1]; Andrew Mathis[2]; Richard Carson[1]; Nathan Keyes[1]; Ryne Peters[1]; John Price[1]; *[1]Brigham Young University, Provo, UT; [2]UT Southwestern, Dallas, TX*

5:50 – 6:30 PM: **INFORMAL MIXER**, *Bivouac Ballroom (Lower Level)*

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

EVENING WORKSHOPS

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

6:30 – 8:00 PM: PARALLEL EVENING WORKSHOP
HOW TO OBTAIN AN ACADEMIC FACULTY POSITION AND KEEP IT!, *Grant Hall AB (Lower Level)*
Presented by Ben Garcia (University of Pennsylvania and Laurie Parker (University of Minnesota)

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
FOUNTAIN OF YOUTH: CREATING SUSTAINABLE VALUE FOR YOUR DATASETS (BD2K / LINCS)
Presidential Ballroom (2nd Level)

Two NIH programs, Big Data to Knowledge (BD2K) and Library of Network-Based Cellular Signatures (LINCS), together with the BD2K-LINCS Data Coordination and Integration Center (DCIC), present benefits and challenges of large proteomic data sets, current efforts to generate a comprehensive library of signatures representative of chemical and genetic perturbations in cellular systems, as well as analysis tools and platforms for integration across different data types. Paving the road to a shared vision of powerful biological knowledge gains from large proteomic data sets queried individually or combined with omic integration studies, now and into the future.

6:30 – 8:00 PM: PARALLEL EVENING WORKSHOP
IMPLEMENTING THE STRATEGIES OF THE HUPO HUMAN PROTEOME PROJECT (HPP), *Palm Court (Lobby Level)*
Organized by Maggie Lam (UCLA)

An Overview of the Strategies and Plans for the HUPO Human Proteome Project; Gil Omenn (University of Michigan), HPP Chair
Designing Cutting-edge Organ-specific Biological Studies using Popular Proteins and SRM Assays; Maggie Lam (UCLA) and discussant Jennifer Van Eyk (Cedars-Sinai Medical Center)
Proteogenomic Analyses of Cancer Genes and Proteins: a Collaborative cHPP and B/D HPP Study, with a Focus on the CPTAC Studies of Ovarian Cancers; Hui Zhang and discussant Alexey Nesvizhskii (University of Michigan)

TUESDAY, MARCH 21

6:45 AM: Fun Run/Walk Participants go to Conference Registration to be directed to outside meeting point. Main group departs at 7:00 am. More information and map available to pick up at Conference Registration (anytime).

8:00 – 8:30 AM: **EARLY MORNING COFFEE & PASTRIES**, *Bivouac Ballroom (Lower Level)*

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

8:30 – 8:55 am **Gilbert S. Omenn Computational Proteomics Award: Alexey Nesvizshkii**, *University of Michigan*
Award presentation followed by 20 minute talk.

8:55 – 9:08 am **Robert J. Cotter New Investigator Award: Peter Nemes**, *George Washington Universrity*
Award presentation followed by 10 minute talk.

Peter Nemes will also give an additional talk on Tuesday afternoon in the Single Cell Proteomics session (4:30-5:50 pm), *Presidential Ballroom*.

9:08 – 9:20 am **Robert J. Cotter New Investigator Award: Christine Vogel**, *New York Universrity*
Award presentation followed by 10 minute talk.

Christine Vogel will also give an additional talk on Tuesday afternoon in the New Technologies session (3:00-4:20 pm), *Presidential Ballroom*.

9:20 - 9:50 AM: **COFFEE BREAK**, *Bivouac Ballroom (Lower Level)*
Coffee and pastries with the exhibitors.

9:50 – 11:10 AM: PARALLEL SESSION
COMPUTATION AND BIG DATA, *Presidential Ballroom (2nd Level)*
Session Chair: Peipei Ping (UCLA)

9:50-10:15 am **Characterization of the Cardiac Proteome with Big Data Proteomics**; Maggie PY Lam; *University of California at Los Angeles, Los Angeles, CA*

10:15-10:40 am **Big Data to Knowledge: Integrated Bioinformatics towards Systems Biology and Precision Medicine**; Cathy H Wu; *University of Delaware, Newark, DE*

10:40-10:55 am **Proteogenomic Classifications and Outcome in Squamous Cell Carcinoma of the Lung**; Robbert Slebos; Paul Stewart; Eric Welsh; Guolin Zhang; Bin Fang; Sean Yoder; Katherine Fellows; Y Ann Chen; Jamie Teer; Steven Eschrich; John Koomen; Eric Haura; *Moffitt Cancer Center, Tampa, FL*

10:55-11:10 am **Missing Protein Node Prediction and Protein Quantitation in Bipartite Network Representations of Complex Proteomes**; Casimir Bamberger; Salvador Martinez de Bartolome Izquierdo; Miranda Montgomery; Sandra Pankow; John Yates III; *The Scripps Research Institute, La Jolla, CA*

9:50 – 11:10 AM: PARALLEL SESSION
METABOLOMICS, *Palm Court (Lobby Level)*
Session Chair: Alan Saghatelian (Salk Institute)

9:50-10:15 am **Using Metabolomics to Identifying Non-Genetic Determinants of Disease**; Caroline Johnson; *Yale School of Public Health, New Haven, CT*

10:15-10:40 am **New Technologies for Large Scale and Targeted Analyses of Small Molecules**; David Wishart; *University of Alberta, Edmonton, Canada*

10:40-10:55 am **Establishing a Novel NanoLC-MS/MS Platform for Detecting and Quantifying DNA Modification**; Ranran Wu; Kevin Janssen; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA*

10:55-11:10 am **Dilu: High Resolution Metabolomics Quantification and Identification Platform**; Yu Gao; *The Scripps Research Institute, La Jolla, California*

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

Presentation Order

Tues 1 **Linear B Cell Epitope Prediction by Using High Throughput Peptide Microarrays**; Robayet Chowdhury[1, 2]; Taylor Brown[1, 2]; Neal Woodbury[1, 2]; [1]Innovations in Medicine,The Biodesign Institute, Tempe, Arizona (AZ); [2]Arizona State University, Tempe, AZ; **See Tuesday Poster 12**

Tues 2 **A Spin Column-Free Permethylation Procedure for Glycan Analysis**; Yueming Hu[1, 2]; Chad R. Borges[1, 2]; [1]Arizona State University, Tempe, AZ; [2]The Biodesign Institute, Arizona State University, Tempe, Az; **See Tuesday Poster 4**

Tues 3 **Comparative Proteomic Analysis of the Influence of Gender and Acid Stimulation on Normal Human Saliva Using LC/MS/MS**; Xiaoping Xiao[1]; Yaoran Liu[2]; Wei Sun[1]; Qian Li[2]; [1]Chinese Academy of Medical Sciences, Beijing, China; [2]Peking Union Medical College hospital, Beijing, China; **See Tuesday Poster 8**

Tues 4 **Development of Protein Biomarkers for Effects of Radiation Exposure using Quantitative Mass Spectrometry**; Kate Liu; Elizabeth Singer; Whitaker Cohn; Julian Whitelegge; William McBride; Joseph Loo; UCLA, Los Angeles, California; **See Tuesday Poster 3**

Tues 5 **Early Detection in Urinary Proteome for the Effective Early Treatment of Bleomycin-Induced Pulmonary Fibrosis in a Rat Model**; Jianqiang Wu[1]; Xundou Li[1]; Youhe Gao[1, 2]; [1]Peking Union Medical College, Beijing, China; [2]Beijing Normal University, Beijing, China; **See Tuesday Poster 9**

Tues 6 **Elucidating the Biological Implications of Aluminum Binding to Osteocalcin**; Stephanie Thibert[1, 2]; Olgica Trenchevska[1]; Mario Kratz[3]; Ian de Boer[4]; Mian Yang[1]; Richard Hervig[5]; Peter Williams[2]; Joshua Jeffs[1, 2]; Chad Borges[1, 2</SU; [1]Biodesign Institute, Arizona State University, Tempe, AZ; [2]School of Molecular Sciences, ASU, Tempe, AZ; [3]Fred Hutchinson Cancer Research Center, Seattle, WA; [4]University of Washington, Seattle, WA; **See Tuesday Poster 49**

Tues 7 **Evidence of Human Antagonistic Auto-antibodies as a Mechanism of Insulin Resistance**; Andrew Lipchik; Anil Narasimha; Michael Snyder; Stanford University, Stanford, CA; **See Tuesday Poster 46**

Tues 8 **Exosomal EphA2 Transmits Chemoresistance and Predicts Pancreatic Cancer Patient Responses to therapy**; Jia Fan1; Qian Wei2; Eugene J. Koay[3]; Yang Liu[1, 2]; Zhen Zhao[4]; Tony Y. Hu[1, 2]; [1]Arizona State University, Tempe, AZ; [2]Houston Methodist Research Institute, Houston, TX; [3]University of Texas M.D. Anderson Cancer Center, Houston, TX; [4]National Institutes of Health, Bethesda, MD; **See Tuesday Poster 2**

Tues 9 **GiaPronto: A One-Click Graph Visualization Software for Proteomics Datasets**; Amber K. Weiner[1, 2]; Simone Sidoli[1]; Sharon J. Diskin[2]; Benjamin A. Garcia[1]; [1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia, PA; **See Tuesday Poster 15**

Tues 10 **Histone H2A Proteolysis During Mouse Embryonic Stem Cell Differentiation**; Mariel Coradin[1]; Simone Sidoli[1, 3]; Kelly Karch[1, 2]; Benjamin A. Garcia[1, 2]; [1]Department of Biochemsitry and Molecular Biophysic, Philadelphia, PA; [2]Epigenetics Program, Philadelphia, PA; [3]University of Pennsylvania School of Medicine, Philadelphia, PA; **See Tuesday Poster 6**

Tues 11 **Identification of FMS-like Tyrosine Kinase 3 (FLT3) Substrates Using KALIP**; Minervo Perez; University of Minnesota, Minneapolis, MN; **See Tuesday Poster 63**

Tues 12 **Influence of the Gut Microbiota on Histone Modifications in Intestinal Epithelial Cells**; Peder J. Lund; Sarah A. Smith; Johayra Simithy; Zuo-Fei Yuan; Kevin Janssen; Gary D. Wu; Benjamin A. Garcia; University of Pennsylvania, Philadelphia, PA; **See Tuesday Poster 5**

Tues 13 **Integrative Proteogenomic Characterization of Colorectal Cancer Cell Lines and Primary Tumors**; Jing Wang[1]; Dmitri Mouradov[2]; Xiaojing Wang[1]; Robert Jorissen[2]; Matthew Chambers[8]; Lisa Zimmerman[8]; Suhas Vasaikar[1]; Christopher Love[2]; Shan Li[2]; Kym; [1]Baylor College of Medicine, Houston, TX; [2]The Walter and Eliza Hall Institute of Medial Rese, Parkville, Australia; [3]The University of Melbourne, Parkville, Australia; [4]University of Oxford, Oxford, United King; **See Tuesday Poster 52**

Tues 14 **MetaProt: A Cloud-based Platform to Analyze, Annotate, and Integrate Metabolomics Datasets with Proteomics Information**; Howard Choi[1]; Vincent Kyi[1]; Brian Bleakley[1]; Ding Wang[1]; Henning Hermjakob[2]; Peipei Ping[1]; [1]NIH BD2K Center at UCLA, Los Angeles, CA; [2]EMBL-EBI, Hinxton, UK; **See Tuesday Poster 19**

Tues 15 **Phosphoproteomics Reveals Potential Crosstalk Between mTORC2 and MAP Kinases**; Samuel Entwisle[1]; Camila Martinez-Calejman[2]; David Guertin[2]; Judit Villen[1]; [1]University of Washington, Seattle, WA; [2]University of Massachusetts Medical Center, Worcester, MA; **See Tuesday Poster 1**

Tues 16 **proBAMsuite, a Bioinformatics Framework for Genome-Based Representation and Analysis of Proteomics Data**; Xiaojing Wang; Baylor College of Medicine, HOUSTON, TX; **See Tuesday Poster 57**

TUESDAY, MARCH 21

Tues 17 **Profiling Biochemical Individuality: Human Personal Omics Profiling (hPOP)**; Sara Ahadi[1]; Hannes Rost[1]; Christie Hunter[2]; Liang Liang[1]; Shannon Rego[1]; Orit Dagan-Rosenfeld[1]; Denis Salins[1]; Mike Snyder[1]; [1]Stanford University, Stanford, CA; [2]SCIEX, Redwood City, CA; **See Tuesday Poster 45**

Tues 18 **RNA Mononucleoside Modification Detection, Quantitation, and Multiplexing by nanoLC-MS/MS**; Kevin A. Janssen; Ranran Wu; Benjamin A. Garcia; University of Pennsylvania School of Medicine, Philadelphia, PA; **See Tuesday Poster 18**

Tues 19 **Selection and Validation of Endogenous Retention Time Standards and Quality Control Peptides for Plasma Proteomics Study**; Shenyan Zhang; Vidya Venkatraman; Qin Fu; Ronald Holewinski; Mitra Mastali; Jennifer Van Eyk; Cedars Sinai Medical Center, Los Angeles, CA; **See Tuesday Poster 47**

Tues 20 **TargetSeeker-MS: A Bayesian Inference Approach for Drug Target Discovery using Protein Fractionation Coupled to Mass Spectrometry**; Mathieu Lavallée-Adam[1, 2]; Jolene Diedrich[1, 3]; Alexander Pelletier[1]; William Low[3]; Antonio Pinto[3]; Salvador Martínez-Bartolomé[1]; Michael Petrascheck[1]; James Moresco[1; 1]The Scripps Research Institute, Lajolla, CA; [2]University of Ottawa, Ottawa, Canada; [3]Salk Institute for Biological Studies, La Jolla, CA; **See Tuesday Poster 62**

Tues 21 **The Hybrid Search: A Mass Spectral Library Search Method for Discovery of Modifications in Proteomics**; Meghan C. Burke; Yuri A. Mirokhin; Dmitrii V. Tchekhovskoi; Sanford P. Markey; Stephen E. Stein; Mass Spectrometry Data Center, NIST, Gaithersburg, MD; **See Tuesday Poster 16**

Tues 22 **Understanding Mechanism of Action of Drug Resistance Reversal Potential of Usnic Acid Using Proteomic Profiling**; Sneha Sinha; csir-cimap, Lucknow, India; **See Tuesday Poster 39**

Tues 23 **Validation of Tumor Proteogenomic Annotations**; Anindya Bhattacharya[1, 2]; Vineet Bafna[1, 2]; [1]UC San Diego, La Jolla, CA; [2]UC San Diego, La Jolla, CA; **See Tuesday Poster 53**

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: **SCIEX**, *Grant Hall AB (Lower Level)*

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

Enabling Precision Medicine with Industrialized Quantitative Proteomics
Randy Arnold & Christie Hunter, *SCIEX*

12:00 – 1:30 PM: **WATERS CORPORATION**, *Grant Hall CD (Lower Level)*

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

Quantitative Bottom up Proteomics by Data Independent Analysis using Quadrupole Scanning and Ion mobility
LeRoy B. Martin, III, Ph.D, *Sr. Manager, Biological Mass Spectrometry, Waters Corporation*

1:30 – 3:00 PM: **TUESDAY POSTER SESSION**, *Bivouac Ballroom (Lobby Level)*

3:00 – 4:20 PM: PARALLEL SESSION
NEW TECHNOLOGIES, *Presidential Ballroom (2nd Level)*
Session Chair: Michael Maccoss (University of Washington)

3:00-3:25 pm **Multi-Omic Mass Spectrometry Profiling Technology to Assign Protein Function** ; Joshua Coon; *University of Wisconsin-Madison, Madison, WI*

3:25-3:50 pm **New Technologies to Interrogate the Dynamics and Function of the Phosphoproteome**; Judit Villen, *University of Washington*

3:50-4:05 pm **The Multiple Dimensions of Translation Regulation During the Eukaryotic Stress Response**; Justin Rendleman[1]; Zhe Cheng[1]; Scott Kuersten[2]; Guoshou Teo[1]; Hyungwon Choi[3]; Christine Vogel[1]; *[1]New York University, New York, NY; [2]Illumina, San Diego, CA; [3]National University, Singapore, Singapore*

4:05-4:20 pm **Advancing Top-Down Proteomics Through Instrumentation and Data Acquisition Method Development**; Lissa C. Anderson[1]; Chad R. Weisbrod[1]; Nathan K. Kaiser[1]; Greg T. Blakney[1]; Christopher L. Hendrickson[1, 2]; Alan G. Marshall[1, 2]; *[1]NHMFL, Tallahassee, FL; [2]Dept of Chemistry & Biochemistry, FSU, Tallahassee, FL*

3:00 – 4:20 PM: PARALLEL SESSION
TOP-DOWN ANALYSIS OF PROTEIN COMPLEXES, *Palm Court (Lobby Level)*
Session Chair: Michael Greig (Pfizer)

3:00-3:25 pm **Democratizing Top-Down in Both Denatured and Native Modes: Has the time now come?**; Neil Kelleher; *Northwestern University, Evanston, IL*

3:25-3:50 pm **Flexible Software for Evolving Instrumentation in Top Down Proteomics**; Samuel Payne; *Pacific Northwest National Laboratory, Richland, WA*

3:50-4:05 pm **Proteoform Suite Software: A New Tool for Rapidly Identifying and Quantifying Proteoforms and Constructing Proteoform Families**; Michael Shortreed; Anthony Cesnik; Leah Schaffer; Brian Frey; Rachel Knoener; Zachary Rolfs; Yunxiang Dai; Katherine Buxton; Mark Scalf; Lloyd Smith; *University of Wisconsin, Madison, WI*

4:05-4:20 pm **Measuring Intact Protein Turnover on Drosophila Melanogaster Head Using Tunable Intact Protein Mass Increases Method (TIPMI)**; Jeniffer V. Quijada[1, 2]; Jeffrey N. Agar[1, 2]; *[1]Northeastern University, Boston, MA; [2]Barnett Inst., Northeastern University, Boston, MA*

4:30 – 5:50 PM: PARALLEL SESSION
SINGLE CELL PROTEOMICS, *Presidential Ballroom (2nd Level)*
Session Chair: Jun Qu (University at Buffalo)

4:30-4:55 pm **Proteomics of Single EarlySstage Blastomeres from *Xenopus laevis***; Liangliang Sun[2]; Kyle Dubiak[1]; Elizabeth Peuchen[1]; Zhenbin Zhang[1]; Paul Huber[1]; Norman Dovichi[1]; *[1]University of Notre Dame, Notre Dame, IN; [2]Michigan State University, East Lansing, MI*

4:55-5:20 pm **Massively Multiplexed Cellular Analysis in Human Health and Disease**; Sean Bendall; *Stanford University, Stanford, CA*

5:20-5:35 pm ***In situ* Microsampling Single-cell Capillary Electrophoresis Mass Spectrometry Uncovers Proteomic Cell Heterogeneity in the Live Frog (*Xenopus laevis*) Embryo**; Peter Nemes; Camille Lombard-Banek; Aparna Baxi; Sally Moody; *George Washington University, Washington, DC*

5:35-5:50 pm **A Twist to Increase Biomarker Specificity: Exploiting CESI Mass Spectrometry and Protein Modifications**; Jennifer Van Eyk, *Cedars-Sinai Medical Center, Los Angeles, CA*

Dr. Van Eyk's talk is sponsored by

4:30 – 5:50 PM: PARALLEL SESSION
CROSS-LINKING / MOLECULAR PAINTING, *Presidential Ballroom (2nd Level)*
Session Chair: Lan Huang (UC Irvine)

4:30-4:55 pm **Advancements in Protein Cross-Linking Biochemistry, Software, and Data Visualization Uncover Protein Complex Architecture.**; Trisha Davis[1]; Michael Riffle[1]; Alex Zelter[1]; Daniel Jaschob[1]; Michael Hoopmann[2]; Richard Johnson[1]; Robert Moritz[2]; Michael Maccoss[1]; *[1]University of Washington, Seattle, WA; [2]Institute for Systems Biology, Seattle, WA*

4:55-5:20 pm **In Cell Protein Footprinting for the Structural Analysis of Proteins in their Native Environment**; Lisa M. Jones; *University of Maryland, Baltimore, MD*

5:20-5:35 pm **Dual Cleavabale Crosslinking Technology (DUCCT): A New Strategy for High Confidence Identification of Crosslinked Peptides**; Saiful Chowdhury[1]; Jayanta Chakrabarty[1]; Gerhard Munske[2]; Aishwarya Naik[1]; *[1]Univ of Texas at Arlington, Arlington, TX; [2]WSU, Pullman, WA*

5:35-5:50 pm **Assessing Availability of Primary Amines in Proteins *in vivo* in Order to Determine Structure and Interaction of Proteins**; Casimir Bamberger; Sandra Pankow; John R. Yates; *The Scripps Research Institute, La Jolla, CA*

6:00 – 9:00 PM: **JOINT AACC-US HUPO PROGRAM**
Presidential Foyer &Ballroom (2nd Level)
Kimia Sobhani and Jennifer Van Eyk, presiding

All US HUPO conference attendees and AACC (American Association of Clinical Chemistry) SoCal members are invited to attend this special joint program. The program kicks off with a reception from 6:00 - 7:00 pm (Presidential Ballroom Foyer) followed by a series of talks.

There is no charge for this event, but registration (RSVP) is requested. Find online RSVP link at www.ushupo.org.

7:00 - 7:45 pm **Applications of Next-Generation Sequencing for Virus Surveillance and Discovery**, Michael Berg, PhD (Abbott Laboratories)

8:00-8:12 pm **Machine Learning to Extract Morphometric Biomarkers from Tissue Architecture**, Beatrice Knudsen, MD, PhD (Cedars-Sinai Medical Center)

8:13-8:25 pm **From Proteomics to Bedside: Translating Discoveries in the Next Generation**; Joshua LaBaer, MD, PhD (Arizone State University, Biodesign Institute)

8:26-8:38 pm **High Sensitivity and Multiplex MRM Assays for Quantitation of Hundreds of Proteins in Clinical Specimens**; Christoph Borchers, PhD (University of Victoria - Genome BC Proteomics Centre)

8:39-8:51 pm **Molecular Glycopathology by Capillary Electrophoresis: Analysis of the N-Glycome of Formalin Fixed Paraffin Embedded Mouse Tissue Samples**; Andras Guttman PhD, D.Sc. (University of Debrecen)

This special joint program is sponsored by

8:00 – 8:30 AM: **EARLY MORNING COFFEE & PASTRIES**, *Presidential Ballroom Foyer (2nd Level)*

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

TIPS & TRICKS (TECHNOLOGY FOCUS) LIGHTNING SESSION
Presidential Ballroom (2nd Level)
High-energy, three-minute presentations selected from poster presentations.
Focus is on work-arounds, hacks, and new technology. Posters will be on display in foyer during the coffee break.

Presentation Order

Wed 1 ***Ex Vivo* Protein Oxidation as a Metric of Blood Plasma/Serum Integrity**; Chad R. Borges; Joshua Jeffs; Shadi Ferdosi; Arizona State University, Tempe, AZ; **See TP Poster 48**

Wed 2 **Time-Dependent Metabolomics in Systems Biology Context for Mechanism of Action Studies**; Akos Vertes[1]; Andrew Korte[1]; Hang Li[1]; Peter Nemes[1]; Lida Parvin[1]; Sylwia Stopka[1]; Sunil Hwang[1]; Ziad Sahab[1]; Deborah Bunin[2]; Merrill Knapp[2</SU; [1]George Washington University, Washington, DC; [2]SRI International, Menlo Park, CA; [3]GE Global Research, Niskayuna, NY; [4]Protea Biosciences Inc., Morgantown, WV; **See TP Poster 28**

Wed 3 **A Promising Alternative to MS2-DIA: IonStar Enables Large-scale, Accurate and Extensive Quantification with Low Missing Data and False Positives**; Jun Qu; Xiaomeng Shen; Shichen Shen; SUNY-Buffalo, Buffalo, NY; **See TP Poster 34**

Wed 4 **A Single UHPLC System for both High Flow and Nano Flow LC-MS/MS: Application in Discovery and Targeted Proteomics**; Linfeng Wu[1]; Alex Zhu[2]; Paul Goodley[1]; Pat Perkins[1]; [1]Agilent Technologies, Santa Clara, CALIFORNIA; [2]Agilent Technologies, Wilmington, DE; **See TP Poster 27**

Wed 5 **Decoding Site-specific Alteration of Sialo-glycoproteome in EGFR-subtype of Non-small Cell Lung Cancer**; Yi-Ju Chen; Yu-Hsien Lin; Ta-Chi Yen; Kay-Hooi Khoo; Yu-Ju Chen; Academia Sinica, Taipei, Taiwan, Province of China; **See MP Poster 56**

Wed 6 **Global Identification of Functional Phosphorylation Sites in *Saccharomyces cerevisiae***; Ian Smith; University Of Washington, Seattle, WA; **See TP Poster 24**

Wed 7 **In-depth Quantitation of Changes in Protein Expression Levels in Complex Samples on a Q-TOF Instrument Using Data-Independent Acquisition (DIA)**; Stephanie Kaspar-Schoenefeld[1]; Thomas Kosinski[1]; Pierre-Olivier Schmit[2]; Na Parra[3]; [1]Bruker Daltonik GmbH, Bremen, Germany; [2]Bruker Daltonique S.A., Wissembourg, France; [3]Bruker Daltonics, Billerica, USA; **See TP Poster 35**

Wed 8 **PCT-HD for Tissue Biopsy Samples: Comparison to a Standard Method.**; Vera Gross[1]; Peter Hains[2]; Keith Ashman[3]; Valentina Valova[2]; Alexander Lazarev[1]; [1]Pressure BioSciences, Inc., South Easton, MA; [2]Children's Medical Research Institute, Westmead NSW, Australia; [3]Sciex, Framingham, Massachusetts; **See TP Poster 32**

Wed 9 **Quantification of Circulating *M. tuberculosis* Antigen Peptides Allows Rapid Diagnosis of Active Disease and Treatment Monitoring**; Chang Liu; Jia Fan; Christopher Lyon; Ye Hu; Arizona State University, Tempe, AZ; **See TP Poster 33**

Wed 10 **Quantitative Bottom Up Proteomics Using a Novel Scanning Quadrupole Data Independent Acquisition (DIA) Method**; Jim Langridge[1]; Chris Hughes[1]; Lee Gethings[1]; Roy Martin[2]; Keith Richardson[1]; Johannes Vissers[1]; [1]Waters Corporation, Wilmslow, UK; [2]Waters, Beverly, MA; **See TP Poster 31**

Wed 11 **Quantitative Phosphoproteomic Analysis Reveals System-wide Signaling Networks in Chronic Lymphocytic Leukemia (CLL) B cells**; Hsin-Yi Wu[1]; Jung-Lin Wu[1]; Shang-Ju Wu[2]; Kuo-I Lin[1]; Yu-Ju Chen[1]; [1]Academia Sinica, Taipei, Taiwan; [2]National Taiwan University Hospital, Taipei, Taiwan; **See MP Poster 57**

Wed 12 **Two-dimensional Reversed Phase-Reversed Phase Liquid Chromatography for Top-down Proteomics**; Zhe Wang; Hongyan Ma; Toni Woodard; Si Wu; University of Oklahoma, Norman, OK; **See MP Poster 60**

Wed 13 **Ultrasensitive Microanalytical CE-nanoESI-MS for Bottom-up Proteomic Characterization of Mouse Hippocampal Neurons**; Sam Choi; Eric Corcoran; Marta Zamarbide; M. Chiara Manzini; Peter Nemes; The George Washington University, Washington DC, District of Columbia; **See TP Poster 37**

9:20 - 9:50 AM: **COFFEE BREAK**, *Presidential Ballroom Foyer (2nd Level)*
Tips & Tricks posters will be featured in the foyer.

9:50 – 11:10 AM: PARALLEL SESSION
PRECISION MEDICINE AND METABOLIC DISEASES, *Presidential Ballroom (2nd Level)*
Session Chair: Pothur Srinivas (NIH, NHLBI)

9:50-10:15 am **Managing Health and Disease Using Big Data;** Michael Snyder, *Stanford University*

10:15-10:40 am **Precision Tuning of Therapeutics Targeting PPARs for Treatment of Diabetic Bone**; Patrick Griffin; *Scripps Research Institute, Jupiter, FL*

10:40-10:55 am **Systemic or Non-Systemic? Co-regulation Analysis of the Urinary Proteome to Identify Plasma-derived Proteins in Urine**; Tue Bjerg Bennike[1]; Saima Ahmed[3]; Hanno Steen[2]; *[1]Harvard Medical School, Boston, MA; [2]Boston Children's Hospital, Boston, MA; [3]Boston Children's Hospital - Harvard Univeristy, Boston MA, MA*

10:55-11:10 am **A Precision Proteomics Pipeline for Remote Blood Monitoring: Integrating Volumetric Absorptive Microsampling with Targeted and Data-Independent Acquisition Mass Spectrometry**; Irene Van Den Broek[1]; Qin Fu[1]; Stuart Kushon[2]; Kim Chansky[2]; Michael Kowalski[3]; Kevin Millis[4]; Andrew Percy[4]; Tasha Agreste[4]; A.Lenore Ackerman[1]; Jennifer Anger[1]; Ron Holewinski[1]; Vidya Venkatraman[1]; Jennifer Van Eyk[1]; *[1]Cedars-Sinai Medical Center, Los Angeles, CA; [2]Neoteryx, Torrance, CA; [3]Beckman Coulter Life Sciences, Indianapolis, IN; [4]Cambridge Isotope Laboratories, Tewksbury, MA*

9:50 – 11:10 AM: PARALLEL SESSION
PROTEOFORM BIOLOGY, *Palm Court (Lobby Level)*
Session Chair: James Wohlschlegel (UCLA)

9:50-10:15 am **Metabolic Labeling in Middle-Down Proteomics Allows for Comprehensive Interpretation of the Dynamic Histone Code**; Simone Sidoli[1]; Kelly Karch[1]; Chrystian Ruminowicz[2]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Private developer, Białystok , Poland*

10:15-10:40 am **LINCS PCCSE: A Mineable Resource of Epigenetic and Phosphoproteomic Cellular Drug Responses**; Jacob Jaffe; PCCSE Team; *Broad Institute of Harvard and MIT, Cambridge, MA*

10:40-10:55 am **Quantitative Proteomic Approach Reveals Landscape of Regulatory Elements for Lysine 2-Hydroxyisobutyrylation Pathway**; He Huang; Mathew Perez-Neut; Kyle Delaney; Okwang Kwon; Yingming Zhao; *University of Chicago, Chicago, Illinois*

10:55-11:10 am **Immediate-Early Histone Proteoform Dynamics in Response to Epigenetic Inhibitors**; Tao Wang; Matthew V. Holt; Nicolas L. Young; *Baylor College of Medicine, Houston, TX*

11:10 AM – 12:00 PM: **PLENARY LECTURE + CLOSING SESSION**, *Presidential Ballroom (2nd Level)*
Jennifer Van Eyk, presiding

11:10 -11:55 am **'OMICS' and Neurodegenerative Disease – Approaches and Insights Gained**; Leslie Thompson, *University of California, Irvine*

11:55 am-12:00 pm Closing Remarks

MONDAY 9:50 – 11:10 AM
CARDIOVASCULAR DISEASE *Presidential, 2nd Level*

Mon Talk 09:50-10:15 am: New Technologies for Advancing Stem Cell Derived Cardiomyocytes in Drug Discovery, Disease Modeling, and Regenerative Medicine

Matthew Waas; Chelsea Fujinaka; Theodore Keppel; Ranjuna Weerasekera; Rebekah Gundry
Medical College of Wisconsin, Milwaukee, WI

Using modern differentiation approaches, human pluripotent stem cells (hPSCs) are a virtually unlimited source of in vitro derived cardiomyocytes (hPSC-CM) that can be used for developmental studies, disease modeling, drug testing, and ultimately cell therapy. However, we are currently unable to produce homogeneous cultures of cells of defined sub-types and maturation stages. Thus, novel non-transgene based methods for tracking and isolating well-characterized subtype and stage-specific cells is required to promote the use of these cells for research and clinical applications. Using the cell surface capture technology, a chemoproteomic approach that enables capture and identification of extracellular protein domains, we have identified >1900 cell surface proteins on hPSC-CM. Using hierarchical clustering and qualitative comparisons of cell surfaceomes of >50 human cell types, we have identified >40 cell surface proteins that are potentially informative for cell type, subtype, and maturation stage identity within the context of cardiomyocyte differentiation. Using parallel reaction monitoring (PRM), we have begun to quantify cell surface proteins over the first 100 days of differentiation and have found that the cell surface proteome is quite dynamic during early differentiation, supporting the identification of maturation-stage specific markers. We have also used PRM to probe primary human tissue to gain insight into tissue distribution of new markers *in vivo*. Technologies under development include novel antibodies that recognize a new pan-cardiomyocyte marker, novel candidates for chamber specificity, and proteins restricted to specific stages of maturation. Altogether, by combining cell type, subtype, and maturation stage specific markers, these data support the development of cell surface marker "barcodes" that will enable the identification and isolation of functionally defined cells most useful for research and therapy.

Mon Talk 10:15-10:40 am: Top-Down Proteomics of Myofilaments in Heart Failure

Ying Ge
Uw-Madison, Madison, WI

Heart failure (HF) is the leading cause of morbidity and mortality in developed countries. The underlying mechanisms of HF are very complex and remain poorly understood. Myofilament proteins, responsible for cardiac contraction and relaxation, play critical roles in signal reception and transduction in HF. Post-translational modifications (PTMs) of myofilament proteins afford a mechanism for the beat-to-beat regulation of cardiac function. Herein, we aim to establish a top-down mass spectrometry (MS)-based disease proteomics platform to examine myofilament proteins extracted from both normal and diseased tissues to establish a relationship between altered modifications of myofilaments and cardiac dysfunction. Top-down MS directly analyzes intact proteins providing a “bird’s eye view” to observe all types of modifications including PTMs (phosphorylation, acetylation, etc.) and sequence variants (mutants, alternatively spliced isoforms, amino acid polymorphisms) simultaneously in one spectrum. We have shown that top-down MS has unique advantages in unraveling the molecular complexity, quantifying multiple modified protein forms, complete mapping of modifications with full sequence coverage, discovering unexpected modifications, and identifying and quantifying positional isomers and determining the order of multiple modifications. Recently, we have developed a novel liquid chromatography (LC)/MS-based top-down proteomics strategy to comprehensively assess the modifications of myofilament and associated proteins extracted from a minimal amount of myocardial tissue (~500 µg tissue/experiment) with high reproducibility and throughput. The entire procedure that includes tissue homogenization, myofilament extraction, and on-line LC/MS takes less than 3 hours. Enabled by this novel top-down proteomics technology, we discovered a concerted reduction in the phosphorylation of myofilament and Z-disc proteins in acutely-infarcted swine myocardium and significantly augmented ratio of α-skeletal actin vs. α-cardiac actin in human dilated cardiomyopathy. We have shown the promise of top-down proteomics in conjunction with functional studies using both animal models of HF and clinical human heart samples for understanding HF.

Mon Talk 10:40-10:55 am: DIA-MS Reveals Altered Mitochondrial Protein Profile and Activation of mTOR2-signaling During Aneurysm Progression in a Mouse Model of Marfan Syndrome

Sarah Parker[1]; Aleksander Stotland[1]; Elena Gallo-MacFarlane[2]; Nicole Wilson[2]; Roberta Gottlieb[1]; Hal Dietz[2]; Jennifer VanEyk[1]
[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]Johns Hopkins University, Baltimore, MD

Aortic aneurysm describes abnormal growth and remodeling of the aorta and predisposes to fatal vascular dissection and rupture. Marfan syndrome (MFS) is a hereditary disorder caused by deficiency of extracellular fibrillin-1, with multiple characteristic phenotypes including highly penetrant aneurysm. The discovery that deficiency of fibrillin-1 lead to excessive activation of transforming growth factor β (TGFβ) signaling has led to major advances in the understanding of AA in MFS, though many details regarding the molecular mechanisms remain unclear. In the current study we described the molecular changes correlating with aneurysm development in a mouse model of MFS. Data Independent Acquisition MS label-free quantitative proteomic analysis was performed on ascending aorta segments from young (10-weeks; N=3 WT, N=3 MFS) and aged (12 months, N=3 WT, N=4 MFS) mice. We identified 382 proteins with altered abundance (e.g., Log2FC > |0.6| and < 1% FDR) in the MFS mice relative to WT, with 73 and 249 specific to young and aged mice, respectively, and 60 consistently altered at either age. Pathway analysis predicted TGFβ as a dominantly activated upstream regulator at both ages. Interestingly, in comparison to their WT counterparts, aged MFS mice showed markedly lower mitochondrial protein expression and a reversal in predicted activation status of the mTOR2 component RICTOR, with predicted inhibition in young MFS mice and strong activation in the aged MFS mice. Immunoblotting revealed increased phosphorylation of RICTOR and activation of its downstream signaling target AKT in aged MFS aorta. Furthermore, we demonstrate that TGFβ promotes both RICTOR and AKT phosphorylation in cultured aortic vascular smooth muscle cells. These data suggest that activation of RICTOR/mTOR2 and/or AKT may be previously unrecognized determinants of aneurysm progression in MFS and may represent therapeutic targets for the most catastrophic phenotypic consequence of this and mechanistically-related disorders of vessel wall homeostasis.

Mon Talk 10:55-11:10 am: Redox Proteome Dynamics in Mouse Heart During Maladaptive Cardiac Remodeling

Jie Wang[1, 2]; Quan Cao[1, 2]; Dominic Ng[1, 2]; Peipei Ping[1, 2]
[1]Department of Physiology, UCLA, Los Angeles, CA; [2]The NIH BD2K Center at UCLA, Los Angeles, CA

Increased reactive oxygen species (ROS) production during maladaptive cardiac remodeling has been well documented by a number of experimental and clinical studies. As a consequence of ROS accumulation, reversible cysteine oxidation emerges to regulate protein function in cardiac signaling pathways. However, a systematic evaluation of reversible cysteine oxidation in ROS mediated cardiac remodeling is missing.

We conducted an exploratory study to identify reversibly oxidized proteins or signaling pathways involved in cardiac remodeling. We induced cardiac remodeling in C57BL/6J mice using 15mg/kg/day isoproterenol (ISO) at three time points (day0, day1, and day8) and developed a biotin switch-based mass spectrometry method to detect the dynamics of the reversible oxidized proteome in vivo. Reversibly oxidized thiols in extracts of control and ISO-treated samples were blocked by adding N-ethylmaleimide, then reduced and labeled with biotin maleimide for enrichment of oxidized cysteines and mass spectrometry analysis. Compared to conventional methods such as oxi-ICAT and cys-TMT, this method provides a more economical alternative that allows for analysis of large sample sizes.

For the first time in the ISO induced model of cardiac maladaptive remodeling, we systematically evaluated the transient status of reversible oxidation, enabling future identification of association between redox dynamics and disease pathology. Our preliminary study identified 483 proteins in total and approximately 250 proteins at each time point. Compared to the control sample (day0), oxidized proteins

within both ISO-treated samples (day1 and day8) exhibited increased upregulation. Specifically, the redox protein SERCA2 showed increased reversible oxidation after ISO treatment, which validates this novel method. Gene ontology analysis through DAVID on proteins clustered according to their oxidation changes during cardiac remodeling reveals ECM family proteins to be strongly upregulated in day1. As these proteins are tightly associated with the pathogenesis of fibrosis, their reversible oxidation can potentially ameliorate fibrosis, conferring beneficial effects to the remodeling heart.

MONDAY 9:50 – 11:10 AM
NEUROLOGICAL DISEASES *Palm Court, Lobby Level*

Mon Talk 09:50-10:15 am: Brain Region and Pathological Stage Specific Proteome-Wide Alterations in Mouse Models of Alzheimer's Disease Like Pathology.

Jeffrey Savas[1]; Yi-Zhi Wang[1]; Laura DeNardo-Wilke[2]; Salvador Martinez de Bartolome Izquierdo[3]; Daniel McClatchy[3]; Natalie Shanks[4]; Timothy Hark[1]; Kira Cozzolino[1]; Mathieu Lavallee-Adam[3]; Sung Kyu Park[3]; Jeffery Kelly[3]; Edward Koo[4]; Andrew Dillin[5]; Terunaga Nakagawa[6]; Eliezer Masliah[4]; Anirvan Ghosh[4]; John Yates[3]
[1]Northwestern University, Chicago, Illinois; [2]Stanford University, Stanford, CA; [3]Scripps Research Institute, La Jolla, CA; [4]University of California, San Diego, La Jolla, CA; [5]University of California, Berkeley, Berkeley, CA; [6]Vanderbilt University School of Medicine, Nashville, TN

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that impairs memory and reduces cognition. The incidence of AD is increasing and is now the leading cause of dementia in adults. Pathological hallmarks of AD include the presence of amyloid plaques, neurofibrillary tangles, astrogliosis, and changes in vasculature in the brain, which are currently believed to cause neurodegeneration and premature death. Amyloid beta peptides impair multiple cellular pathways in the brain and play a causative role in Alzheimer's disease pathology, but how the brain proteome is remodeled during this process is unknown. To identify new protein networks associated with AD-like pathology, we performed global quantitative proteomic analysis in three complementary mouse models at pre- and post-symptomatic ages. To correlate protein expression levels with AD-like pathology, we determined the level of amyloid beta peptides by ELISA, quantified the plaque load with two amyloid stains in each brain, and analyzed the samples by mass spectrometry. Our results reveal changes in the abundance of hundreds of proteins never before associated with AD and the identification of protein modules, both of which correlate with disease progression in affected brain regions. Hippocampal modules associated with age-dependent changes revealed an impairment of distinct protein networks involved with mitochondrion, myelin sheath, cell—cell adherens junctions, and excitatory synaptic function. Reduced levels of post-synaptic receptor complex subunits were confirmed in the context of immunopurifed complexes, human AD brain tissue, and were functionally verified to potentially rescue AD like pathology in vivo. Our results show that at the molecular level AD brain pathology is highly complex and represents feedback and feed forward responses in multiple cell types.

Mon Talk 10:15-10:40 am: Spatio-Temporal Profile of Synaptic Protein Complexes and Their Role in Brain Disease

Marcelo Coba
Zilkha Neurogenetic Institute, Keck School Of Medi, ,

The postsynaptic density (PSD) is a morphological and functional specialization of the postsynaptic membrane. This specialized signaling machinery is composed of 1,500~2,000 proteins arranged in protein interactions that can be modulated by post-translation modifications (PTMs) such as phosphorylation. Although components of the PSD have been individually studied, it is less clear how they associate in protein-protein interaction networks. Disruption of this signaling machinery might be implicated in a variety of brain disorders. However it is not known how the core-scaffold machinery associates this collection of proteins through development and how proteins coding for genes involved in psychiatric and other brain disorders are distributed through spatio-temporal protein complexes. Here, using immunopurification, proteomics, bioinformatics and mouse genetics, we isolated 4300 in-vivo protein interactions across 48 protein complexes and determined their protein domain composition, their correlation to gene expression levels and their developmental integration to the PSD. We defined major clusters for enrichment of schizophrenia (SCZ), autism spectrum disorders (ASD), developmental delay (DD) and intellectual disability (ID) risk factors at embryonic day 14, postnatal day 7, 14 and the adult PSD. These protein complexes contained a discrete number of protein domains defining molecular functions.

I will discuss how mutations in highly-connected nodes alter protein-protein interactions that modulate the assembly of macromolecular complexes enriched in disease risk candidates, and their role in synaptic plasticity and disease. These results are integrated into a software platform enabling the prioritization of brain disease risk factors and their placement within synaptic protein interaction networks.

Mon Talk 10:40-10:55 am: Elucidation of Novel Proteoforms of Superoxide Dismutase (SOD1) in Sporadic ALS Patients

David Muddiman; Philip Loziuk
North Carolina State University, Raleigh, NC

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease which currently impacts more than 12,000 people in the U.S. Currently, treatment is limited and diagnosis is performed by exclusion of other diseases, with no biochemical test available. Only about 5-10% of cases are familial or inherited with the remaining 90-95% being sporadic cases, occurring at random with no clearly associated risk factors. About 20% of familial cases result from mutations in the gene that encodes the enzyme copper-zinc superoxide dismutase 1 (SOD1). It has been demonstrated that SNPs and modifications of SOD1 can cause dissociation of the native dimer, formation of trimers which are toxic to motor neurons. To date, an in-depth proteomic profile of SOD1 in humans diagnosed with sporadic ALS has remained elusive. Purified SOD1 from human erythrocytes of 10 control and 7 patients diagnosed with sporadic ALS. Abundant proteoforms of SOD1 could be identified and quantified by Intact MS measurements. In comparison, bottom-up data reduced the <g class="gr_ gr_15 gr-alert gr_spell gr_run_anim ContextualSpelling" id="15" data-gr-id="15">proteoform</g> complexity and demonstrated the ability achieve greater depth, particularly with low abundant modifications. These data revealed 30 highly confident sites in SOD1 modified by 10 distinct modifications, <g class="gr_ gr_24 gr-alert gr_gramm gr_run_anim Grammar multiReplace" id="24" data-gr-id="24">many</g> related to ROS which haven't been observed previously as well as SNPs. By label-free quantification, many of these differ between control and ALS groups. In addition, <g class="gr_ gr_26 gr-alert gr_gramm gr_run_anim Grammar multiReplace" id="26" data-gr-id="26">a SIL</g> peptide was produced by chemical synthesis and utilized for PC-IDMS to identify incorporation of a non-protein amino acid. Finally, we <g class="gr_ gr_16 gr-alert gr_spell gr_run_anim ContextualSpelling ins-del multiReplace" id="16" data-gr-id="16">present</g> synthesis of a new $^{13}C_3{}^{15}N_2$ SIL non-protein amino acid which we demonstrate by CE/LC-MS. These findings present the potential for a biochemical method of diagnosing sporadic ALS.

Mon Talk 10:55-11:10 am: A Multi-Network Approach to Define Pathways Altered in Alzheimer's Disease and Parkinson's Disease

Lingyan Ping; Duc Duong; Eric Dammer; Marla Gearing; James Lah; Allan Levey; Nicholas Seyfried
Emory University School of Medicine, Atlanta, GA

Neurodegenerative diseases are progressive and debilitating disorders that commonly reveal the co-occurrence of multiple protein aggregates in brain. For example, greater than 50% of Alzheimer's Disease (AD) cases have Lewy bodies comprised of aggregated α-synuclein, whereas co-morbid AD pathologies, including amyloid beta (Aβ) plaques and tau pathology, are often found in Parkinson's Disease (PD) brains at death. Despite these common pathologies, shared cellular pathways that link AD and PD are poorly understood. To fill this gap in knowledge, we used a systems biology approach to define protein co-expression networks from postmortem human brain tissue of individuals with AD, PD and co-morbid AD/PD pathologies. Employing liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) on an Orbitrap Fusion mass spectrometer we comparatively analyzed the total brain proteome of cortical samples representing four groups (n=10 cases each): i) pathology free controls, ii) AD, iii) PD and iv) AD/PD cases with co-morbid pathologies. Approximately 8,000

proteins were identified and quantified across all 40 individual cases using isobaric tandem-mass tags (TMT). Weighted co-expression network analysis (WGCNA) was used to define protein modules linked to cell-type that were specific to AD and those shared with PD, the latter representing common mechanisms associated with neurodegeneration. A subset of modules also overlapped with RNA co-expression networks generated from previously published AD microarray data. These included modules associated with neurons and astroglial cell types, showing altered expression in AD. Overlap of RNA and protein networks was otherwise modest, with many modules specific to the proteome, including those linked to microtubule function and RNA/DNA binding. These results highlight the utility of integrating proteomics and transcriptomics to define unique and common mechanisms associated with AD and PD.

MONDAY 3:00 – 4:20 PM
CANCER MOONSHOT *Presidential, 2nd Level*

Mon Talk 3:00-3:25 pm: Quantitative Mass Spectrometry Analysis of Immune Checkpoint Protein Expression and N-glycosylation in Human Melanoma

Carlos Morales-Betanzos; Hyoungjoo Lee; Paula Gonzalez-Ericsson; Justin Balko; Douglas Johnson; Lisa Zimmerman; Daniel Liebler
Vanderbilt Univ School of Medicine, Nashville, TN

Immune checkpoint proteins have become targets of a new generation of immuno-therapeutics. Although immune checkpoint inhibitors directed against programmed cell death-1 (PD-1) and programmed cell death 1 ligand 1 (PD-L1) produce dramatic responses in several cancers, most patients do not benefit. Available biomarkers, such as immunohistochemical (IHC) analysis of PD-L1 do not reliably predict individual responses. We developed an addressable fractionation, stable isotope dilution parallel reaction monitoring (PRM) platform to quantify PD-1, PD-L1, and programmed cell death 1 ligand 2 (PD-L2) at fmol/microgram protein levels in formalin fixed, paraffin-embedded sections from 22 human melanomas. PD-L1 was quantified in all samples at 0.02-1.5 fmol/microgram and the PRM data were largely concordant with total PD-L1-positive cell content, as analyzed by IHC using the E1L3N antibody. PD-1 was quantifiable in 10 of the 22 specimens at 0.03 to 0.15 fmol/microgram. PD-L1 was present in excess over PD-1 in 20 samples at ratios up to 56-fold. PD-L2 levels ranged from 0.03 to 1.9 fmol/microgram and the PD-L2/PD-L1 ratio in half the samples exceeded 0.5, which suggests that PD-L2, a higher affinity PD-1 ligand, may contribute significantly to T-cell downregulation. We also detected N-glycosylation in the melanomas at PD-L1 position 192 and identified 5 mannose and N-acetylglucosamine glycan forms by MS/MS. Extent of PD-L1 glycan modification varied by approximately 10-fold, based on MS/MS signal intensity normalized to PD-L1 amount. Interestingly, the melanoma with the highest PD-L1 protein abundance and most abundant glycan modification yielded a very low PD-L1 IHC estimate, thus suggesting that N-glycosylation may affect IHC measurement. Additional PRM analyses quantified immune checkpoint/co-regulator proteins LAG3, IDO1, TIM-3, VISTA and CD40, which all displayed distinct expression independent of PD-1, PD-L1 and PD-L2. Targeted MS can provide a next-generation analysis platform to advance cancer immuno-therapeutic research and diagnostics. (Supported by NIH grant U24CA159988)

Mon Talk 3:25-3:50 pm: Using Colon Organoids to Explore Cancer Biology

Katelyn Ludwig[1]; Xin Liu[1]; Jessica Lukowski[1]; Colin Flinders[2]; Shannon Mumenthaler[2]; Heinz-Joseph Lenz[2]; Amanda Hummon[1]
[1]University of Notre Dame, Notre Dame, IN; [2]University of Southern California, Los Angeles, CA

Organoids are valuable in vitro models that are derived directly from primary cells. They offer unique insights into the biology of a specific patient. For cancer patients, the organoid system can provide data regarding the proteins that are aberrantly expressed in a patient's tumor or provide an indication how a patient will respond to a new therapy. Organoids can also be implanted into mouse models as xenografts so that they can propagate for longer periods of time. We have investigated the biology of human-derived colon organoids in two ways. First, we have tested patient-derived organoids with different therapeutics and used Matrix Assisted Laser Desorption/Ionization (MALDI) imaging mass spectrometry to determine whether the drugs would penetrate the organoid. With MALDI imaging, we can also establish whether the drug was metabolized and if the metabolites co-localize with the parent drug. In a second project, we are examining the proteomic composition of organoids obtained from colon tumors that derive from the right and left sides of the colon and have been xenografted into mice. The colon is a large organ, with different physiological and molecular characteristics for the ascending and transverse colon (right side) versus the descending colon (left side). While the genomic and transcriptomic differences between these two sides have been explored, the proteomic composition has never been explored. Using iTRAQ quantitative labeling, we are investigating the proteomic differences for right and left-derived human colon organoids. This proteomic study will elucidate the protein expression patterns that differ between the two sides of the colon.

Mon Talk 3:50-4:05 pm: Genomic Determinants of Protein Abundance Variation in Colorectal Cancer Cell Lines

Jyoti Choudhary
Wellocme Trust Sanger Institute, Cambridge, United Kingdom

Understanding the extent to which genomic alterations compromise the integrity of the proteome is fundamental in identifying the mechanisms that shape cancer heterogeneity. We have used quantitative mass spectrometry to characterize the proteomes and phosphoproteomes of 50 colorectal cancer cell lines that serve as a model of variable tumour clonal expansions. We have used the variation in abundance between 12k proteins and 27k phosphosites to classify the cell lines into subgroups that show distinct yet overlapping organisation with other genomic classifiers. Tightly organised protein co-variation networks deconvoluted the complexity of the molecular landscapes, revealed the proteome-wide interdependencies of biological processes and identified collateral effects of mutations on protein complexes. Proteomic analysis of human iPS cells engineered with gene knockouts confirms the transmission of co-variation as a characteristic of protein interactions and exposes that stoichiometry is tightly regulated at the protein level. Proteomics, genomics and gene expression models all appeared to be largely mutually exclusive in predicting drug responses for 265 compounds further highlighting the utility of proteomics. Overall, we provide a deep integrative view of the molecular structure underlying the variation of colorectal cancer cells.

Mon Talk 4:05-4:20 pm: Antigen Presentation Profiling Reveals T-cell Recognition of Lymphoma Immunoglobulin Neoantigens

Niclas Olsson[1]; Michael Khodadoust[1]; Lisa Wagar[1]; Ole Audun Werner Haabeth[1]; Binbin Chen[1]; Keith Rawson[1]; Chih Long Liu[1]; David Steiner[1]; Samhita Rao[1]; Lichao Zhang[1]; Henning Stehr[1]; Aaron Newman[1]; Debra K Czerwinski[1]; Victoria Carlton[2]; Martin Moorhead[2]; Malek Faham[2]; Holbrook Kohrt[1]; Michael R Green[3]; Mark M Davis[1]; Ron Levy[1]; Ash A. Alizadeh[1]; Joshua Elias[1]
[1]Stanford University, Stanford, CA; [2]Adaptive Biotechnologies, Seattle, Washington; [3]University of Nebraska Medical Center, Omaha, NE

Adaptive immune responses are important determinants of clinical outcomes in patients with diverse cancer types, and have great potential for emerging cancer therapies. A central unmet need is an accurate and efficient way to select high-priority targets for effective immunotherapies and vaccination strategies. Towards the management of mantle cell lymphoma (MCL), one emerging hypothesis is that clinically relevant immunological responses can be generated against somatic mutations encoded in their tumor genomes. Alterations in the tumor proteome – particularly the presentation of novel antigenic peptides (neoantigens) that distinguish malignant from normal cells by major histocompatibility complexes (MHC), can serve as potent substrates for specific anti-tumor immune responses.

We sought to identify lymphoma neoantigens by taking an integrated genomic and proteomic strategy that interrogates antigen peptides presented by MHC-class I and class II. Peptides bound to MHC were purified via immunoprecipitation followed by identification using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Mass spectra were searched against patient-specific proteome databases generated by whole exome sequencing and targeted immunoglobulin gene sequencing. This approach was applied to systematically characterize 36,500 immunopeptides from 17 patients' tumor specimens. Interestingly, 52 neoantigenic peptides were derived from

the lymphoma immunoglobulin (Ig) heavy or light chain variable regions. Although we identified MHC presentation of private germline polymorphic alleles, no mutated peptides were recovered from non-Ig somatically mutated genes. Furthermore, somatic mutations within the immunoglobulin variable region were almost exclusively presented by MHC-II. T-cells specific for immunoglobulin-derived neoantigens were found in two patients. Following ex vivo activation and expansion, the T-cells were remarkably able to mediate killing of autologous lymphoma cells.

These results demonstrate that an integrative approach combining MHC isolation, peptide identification and exome sequencing is an effective platform to uncover tumor neoantigens. Application of this strategy to human lymphoma implicates immunoglobulin neoantigens as targets for lymphoma immunotherapy.

MONDAY 3:00 – 4:20 PM
GLYCANS AND VACCINES *Palm Court, Lobby Level*

Mon Talk 3:25-3:50 pm: A Focus on Analytics: Glycosylation Profiling to Assess HIV-1 Env Quality.

Heather Desaire
University of Kansas, Lawrence, KS

Our overarching goal is to provide analytical support for those producing the recombinant protein, HIV-1 Env, for use in vaccine trials. Our studies are primarily focused on profiling Env glycan content using glycopeptide analysis methods, because the glycans on this protein are components of multiple broadly neutralizing antibody epitopes, while shielding other sites that might otherwise be immunogenic. Env glycosylation is now known to be influenced by a variety of factors, including the genotype of the protein variant, the cell line used for its expression, and the details of the construct design. We used a mass spectrometry-based approach to map the complete glycosylation profiles at every site in several Env trimers, and we compared this profile to that of truncated Envs, monomeric gp120's and oligomeric gp140's. This comparison has allowed us to infer the construct design variables that most impact Env's glycosylation profile.

Our results indicate that glycosylation analysis is a very useful tool for Env quality assessment, and we show that some immunogens destined for clinical trials contain much better glycosylation profiles than others. We found that a conserved "native-like" glycosylation profile exists among compact Env trimers, but monomeric gp120s or uncleaved gp140s can yield highly divergent glycosylation profiles. While the glycosylation of gp120 can vary substantially, based on the expression and purification conditions, our analyses are useful in identifying conditions that produce near-native glycosylation profiles in gp120, prior to full scale manufacturing for clinical trials. Additionally, we determined that a reasonably good approximation of native Env glycosylation can be achieved using CHO cells. In summary, this presentation provides a case-study showing how both analytical glycobiology and mass spectrometry contribute to the development of next-generation vaccine candidates.

Mon Talk 3:50-4:05 pm: Systematic Quantification of Human Cell Surface Glycoprotein Dynamics

Haopeng Xiao; Ronghu Wu
Georgia Tech, Atlanta, GA

Protein glycosylation is one of the most common protein modifications, and it regulates nearly every extracellular event. Surface glycoproteins are dynamic for cells to adapt the ever-changing extracellular environment. These glycoproteins contain wealthy information of cellular development and disease statuses, and have significant biomedical implications. Systematic investigation of surface glycoproteins will result in a better understanding of surface protein functions, cellular activities and the molecular mechanisms of disease. However, it is extraordinarily challenging to specifically and globally analyze surface glycoproteins. Recently we have designed the first method to systematically analyze surface glycoprotein dynamics and measure their half-lives by integrating pulse-chase labeling, selective enrichment of surface glycoproteins, and multiplexed proteomics. The current results clearly demonstrated that surface glycoproteins with catalytic activities were much more stable than those with binding and receptor activities. Glycans can effectively protect extracellular enzymes from being degraded by many proteases outside of the cells. Glycosylation sites located outside of any domain had a notably longer median half-life than those within domains, which strongly suggests that glycans within domains regulate protein interactions with other molecules while those outside of domains majorly play a role in protecting protein from degradation. This method can have extensive applications in biological and biomedical research fields.

Mon Talk 4:05-4:20 pm: High resolution CESI-MS Analysis of Formalin-Fixed Paraffin-Embedded, Released N-Glycans

Bryan Fonslow[1]; Boglarka Donczo[2]; Marton Szigeti[2]; Andras Guttman[1, 2]
[1]SCIEX, San Diego, CA; [2]University of Debrecen, Debrecen, Hungary

Since introduction as a biological tissue sample fixative by F. Blum in 1893, formalin fixation has almost exclusively been the method for tissue preservation. Fixation using formalin makes clinically relevant samples stable that can be stored at ambient condition for decades in depositories. Combined with paraffin embedment, formalin-fixed tissues (FFPE specimens) are amenable to long-term storage and can be universally used in histopathological laboratories for development of several clinico-molecular assays for oncological prognosis and treatment decisions. Glycosylation plays an important role in cellular interactions, protein folding, and stability. Recently capillary electrophoresis has been introduced as a method to probe the N-glycosylation pattern of these precious samples.

For characterization, N-linked glycosylation is routinely analyzed by capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) after endoglycosidase-based glycan release and fluorophore labeling. CE separation provides structural resolution and migration time-based identification of glycans, but coupling with MS detection offers additional structural information. The integration of CE and ESI into a single dynamic process (CESI) provides the capability of performing CE separation and MS ionization with ultra-low flow rate, resulting in reduced ion suppression and improved sensitivity. CESI-MS has been optimized and evaluated for released, APTS-labeled N-glycan analysis.

The CESI-MS method was evaluated using real samples with additional sample preparation. Prior to CESI-MS experiments, the released glycan samples were first characterized with CE-LIF analyses. Glycan sequencing was performed with a combination of glycolytic enzymes to determine the carbohydrate content of each glycan. Analysis of FFPE mouse lung and brain glycans were then performed by CESI-MS. Similar numbers of glycan peaks were extracted from the MS data for each sample. Accurate molecular masses were calculated from m/z's and charge states to find potential carbohydrate compositions for each APTS-glycan electrophoretic peak. FFPE glycans from different mouse tissues were analyzed and compared.

MONDAY 4:30 – 5:50 PM
GLYCANS AND VACCINES *Presidential, 2nd Level*

Mon Talk 4:30-4:55 pm: Characterizing Temporal and Inter-Individual Functional Differences in Pre-Term Human Infant Gut Microbiome Development by a Metaproteomics Approach

Robert Hettich[1]; Weili Xiong[1]; Alfredo Blakeley-Ruiz[1]; Christopher Brown[2]; Matthew Olm[2]; Matthew Rogers[3]; MIchael Morowitz[3]; Jillian Banfield[2]
[1]ORNL, Oak Ridge, TN; [2]University of California, Berkeley, CA; [3]University of Pittsburgh, Pittsburgh, PA

Details about microbial species population structure and functional dynamics during microbiome establishment are poorly understood in the human infant gut. The objective of this work is to exploit a high performance LC-MS/MS based metaproteomic approach to explore host and microbiome temporal functional shifts during microbial colonization of the pre-term infant gut. Fecal metaproteomes of nine pre-term infants were measured at discrete time-points over several months. Approximately 10,000 human and microbial protein groups were identified in each infant. In early time points, human proteins were more abundant than microbial proteins, and comprised pathways involving epithelial barrier establishment and immune response, whereas vitamin and short-chain fatty acid production pathways were active in the microbiome. Intriguingly, comparison of genomic and proteomic patterns identified some microbial species that were both

active and in low abundance. Most functional core metabolic processes (glycolysis, TCA, oxidative phosphorylation, fatty acid biosynthesis, nucleotide metabolism) were conserved in the microbiome; however, temporal and inter-individual variations were identified. For example, lipid metabolism (specifically glycerol degradation) was dominant early in one infant, and progressed to predominantly amino acid metabolism over time, in stark contrast to other pre-term infants. These differences are likely related to microbiome or environmental factors that distinguished the infants. In response to bacterial colonization, the human hosts expressed tight junction, actin cytoskeleton, and mucin proteins that play pivotal roles in the integrity and barrier properties of mucosal epithelial layers. The time-course metaproteomics measurements revealed core metabolic pathways in both human and microbial proteins, revealing the establishment of the mutualistic relationship between the microbiome and human host early in infancy. In total, these results reveal functional stability and inter-individual signatures of the preterm infant gut microbiome.

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Mon Talk 4:55-5:20 pm: New Quantitative Methods to Study the Gut Microbiota

X. Zhang[1]; C.K. Chiang[1]; L. Li[1]; W. Chen[2]; A.E. Starr[1]; K. Cheng[1]; Z. Ning[1]; J. Mayne[1]; S. A. Deeke[1]; R. Tian[2]; D. Mack[3]; A. Stintzi[1]; Daniel Figeys[1]

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Host–microbiota interactions have been associated to a growing list of diseases, including inflammatory bowel diseases (IBD), obesity, diabetes, cardiovascular diseases. We are particularly interested in studying the molecular changes that occurs in host and microbiota during gut dysbiosis and to develop screening techniques for microbiome.

Our understanding of the microbiome has been primarily driven by Next-generation sequencing (NGS). Particularly, large-scale cohort studies through either 16S rRNA gene or shotgun metagenomic sequencing have provided fine taxonomic characterizations of the human microbiome. While NGS-based techniques have been widely applied, the application of metaproteomics, which measures the expressed proteins in the microbiome, is far less common. In the past, metaproteomics was limited by the insufficient sensitivity and resolution of mass spectrometers to measure low abundant proteins from complex microbial communities such as intestinal microbiota. In addition, the lack of easy-to-use metaproteomic computational platforms that can handle the huge reference protein database also limited the application of metaproteomics.

Here we will report on i) the development of a metaproteomics protein identification platform called MetaPro-IQ (universal workflow for gut MetaProteome Identification and Quantification), ii) the application of metaproteomics to study human-microbiome interactions in pediatric IBD and iii) the development of a rapid assay for individual's microbiome (called RapidAIM), an ex vivo assays to study the effects of drugs on the human gut microbiome. These metaproteomics technologies make it possible to measure changes in individual microbiome during dysbiosis and to screen compounds against individual microbiomes.

Mon Talk 5:20-5:35 pm: Optimization of the Number of Proteins and Biological Replicates in Large-scale Proteomic Studies

Ting Huang[1]; Tiannan Guo[2]; Ruedi Aebersold[2]; Olga Vitek[1]

[1]Northeastern University, Boston, MA; [2]ETH Zurich, Zurich, Switzerland

Due to technical limitations in measurement methods and the high dynamic range of protein expression, current protein quantification methods suffer the trade-off between quantitative accuracy, proteome coverage, sample throughput and sample consumption. This trade-off might be manageable for studies that aim at measuring proteins from samples for which the sample amount is not limiting as is the case for cancer cell lines that can be expanded perpetually. However, the tradeoffs become substantial in studies which explore quantitative proteomic profiles of a large number (hundreds) of (clinical) bio-samples and aim at extracting biologically significant information from statistical inference, as is frequently the case for biomarker or classification studies. For such studies it is presently unknown which factor(s) should be favored. Yet, the optimal choice of factors is of great importance for the design of large scale proteomic studies.

In this study we systematically analyzed the effects of the number of proteins quantified and the number of biological replicates on detecting predictive proteins to stratify biological and clinical phenotypes, and on the prediction power of those detected predictive proteins. Six datasets generated by different quantification methods, including DDA-MS, SRM-MS and SWATH-MS, were used for the analysis. We first re-sampled different numbers of proteins and biological replicates from three datasets with phenotype information. Then, we simulated datasets with different numbers of proteins and biological replicates based on all the six datasets. A random forest classification model was built on the training set and evaluated on another independent validation set. The considered evaluation metrics included reproducibility and sensitivity of identifying predictive proteins, and prediction power of resulting classification models.

The results showed that model performance increased with the number of biological replicates in the training set. On the other hand, model performance eventually decreased when more proteins were kept in the quantification dataset.

Mon Talk 5:35-5:50 pm: Integrated Proteogenomics Analyses to Study Tumor Heterogeneity in Human Lung Adenocarcinoma Using Sequential Bbiopsies and Rapid/Warm Autopsies

Nitin Roper[1]; Xu Zhang[1]; Tapan K. Maity[1]; Shaojian Gao[1]; Abhilash Venugopalan[1]; Paul Rudnick[2]; Romi Biswas[1]; Constance M. Cultraro[1]; David Fenyo[3]; David Kleiner[4]; Stephen Hewitt[4]; Udayan Guha[1]

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Tumor heterogeneity modulates treatment response to targeted therapy. Both intra-tumor and inter-tumor heterogeneity is well characterized in various cancers, including lung cancer, the commonest cause of cancer death in both men and women. Tumor heterogeneity studies have been conducted mostly for early stage lung cancer. Furthermore, these studies have focused primarily on next gen sequencing (NGS). Here, we report an integrated analysis at the level of somatic variants, copy number, transcript, protein expression, and the phosphoproteome to demonstrate the extent of tumor heterogeneity and its potential impact on tumor biology. We have recently reported a patient with unprecedented heterogeneity with <1% of somatic SNVs common between the lung and lymph node metastatic sites (Biswas et.al., CSHL Molecular Case Studies, Nov 2016) using whole genome (WGS) and targeted iontorrent sequencing. Here, we report how mass spectrometry-based quantitative proteomics complements the prior genomics analysis to reveal the extent of heterogeneity. In addition, 33 tumor regions from metastatic sites including lung, liver and kidney, obtained by rapid/warm autopsy from 4 patients with metastatic lung adenocarcinoma were analyzed using whole exome sequencing (WES), RNA-seq, CNV-seq, and quantitative mass spectrometry-based proteomics. We used the "super-SILAC" and TMT labeling strategies for quantitative proteomics using a Thermo Orbitrap Elite mass spectrometer. Patient-specific databases were built incorporating all somatic variants identified by NGS to interrogate the mass spectrometry data and an extensive validation pipeline was built for confirmation of variant peptides. RNA-seq, CNV-seq and proteomics analyses complemented the clonal evolution analyses performed using WES. There were notable discrepancies in the clustering of specific metastatic sites based on transcript or protein expression and their placement in the phylogenetic tree constructed from WES-based clonal evolution analyses. Interestingly, we discovered increased overall mutation burden, intratumor heterogeneity, and subclonal mutations in one of our patients with no known drivers.

MONDAY 4:30 – 5:50 PM
DISEASE AND PROTEIN-PROTEIN PROXIMITY
Palm Court, Lobby Level

Mon Talk 4:55-5:20 pm: Integrating Proteomics and Virology to Define Cellular Immune Signaling Mechanisms During Herpesvirus Infection

Ileana Cristea
Princeton University, Princeton, NJ

The mammalian immune system relies on its remarkable ability to distinguish self from non-self in order to trigger defense responses against pathogens. In the case of viral infections, the viral nucleic acids provide pathogen signatures that are recognized by the infected cells. However, as viruses are obligate host parasites, their DNA or RNA genomes are derived directly from cellular nucleotide pools. Thus, limited features distinguish viral and cellular nucleic acids. This is particularly the case for viral DNA, as chemical or structural moieties that differentiate viral DNA from cellular DNA have not been yet established. As a solution to the conceptual challenge of distinguishing two similar molecules (viral and cellular DNA), host recognition of viral DNA has been thought to occur only in cellular compartments normally devoid of host DNA. Indeed, specialized cellular proteins that sense viral DNA have been identified in the cytoplasm and endosomes. A prominent cytoplasmic DNA sensor is the cyclic dinucleotide GMP-AMP synthase (cGAS). However, the majority of known pathogenic human DNA viruses, including herpesviruses, deposit their DNA and replicate in the nucleus. In recent years, we and others have established that, contrary to the prior notion, viral DNA can be distinguished from host DNA within nuclei of infected cells. The host interferon inducible protein IFI16 was identified as a nuclear sensor of viral DNA. However, the mechanisms involved in the detection of nuclear viral DNA and the differences between cytoplasmic- and nuclear-derived antiviral responses remain poorly understood. Here, we decipher the distinct functions of two viral DNA sensors, IFI16 and cGAS, during active immune signaling upon infection with two herpesviruses, herpes simplex virus 1 (HSV-1) and human cytomegalovirus (HCMV). Our quantitative mass spectrometry-based proteomics, live-cell imaging, optogenetics, and CRISPR-based cellular assays underscore the value of integrative approaches to uncover complex cellular responses against pathogens.

Mon Talk 5:20-5:35 pm: A Post-Translational Modification Code for CFTR Maturation is Disturbed in Cystic Fibrosis

Sandra Pankow; Casimir Bamberger; John R. Yates
The Scripps Research Institute, La Jolla, CA

The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is critical for maintaining the salt balance in polarized epithelial tissues and deletion of CFTR phenylalanine 508 (ΔF508 CFTR) is the prevalent cause for Cystic Fibrosis (CF), one of the most common inherited and lethal childhood diseases. Here, we describe the discovery of a post-translational modification (PTM) code that determines CFTR maturation and degradation. By carrying out the first quantitative analysis of the global PTM landscape of wt and ΔF508 CFTR in vivo, we identified over 40 CFTR PTMs that cluster in several modification hotspots at which methylation, ubiquitination and phosphorylation occur in very close proximity to each other and are mutually exclusive, – forming a PTM code. This code is severely disturbed in ΔF508 CFTR, contributing to the disease phenotype. In particular, ΔF508 CFTR shows significantly reduced phosphorylation in the first nucleotide binding domain (NBD1), which is crucial for maturation and transport of wt CFTR to the plasma membrane. NBD1 phosphorylation is also essential for rescue of ΔF508 CFTR function at permissive temperature, as simultaneous inhibition of the mediating kinase prevented the rescue. Monitoring ΔF508 CFTR PTM changes under various conditions such as culture at permissive temperature or treatment with the recently FDA approved CF drug VX-809 revealed that restoring a wt CFTR-like PTM pattern is essential for rescue of ΔF508 CFTR function, and that the quantitative PTM code represents a molecular signature that is predictive of the efficacy of treatments in restoring ΔF508 CFTR activity.

Mon Talk 5:35-5:50 pm: Monitoring Signal-Specific Changes to *in vivo* Ribosomal Structure and Activity

Bradley Naylor[1]; Andrew Mathis[2]; Richard Carson[1]; Nathan Keyes[1]; Ryne Peters[1]; John Price[1]
[1]Brigham Young University, Provo, UT; [2]UT Southwestern, Dallas, Tx

Control of protein homeostasis is fundamental to the health and longevity of all organisms. Because the rate of protein synthesis by ribosomes is a central control point in this process, regulation and maintenance of ribosome function could have amplified importance in the overall regulatory circuit. Indeed, ribosomal defects are commonly associated with loss of protein homeostasis, aging and disease, whereas improved protein homeostasis, implying optimal ribosomal function, is associated with disease resistance and increased lifespan. To maintain a high quality ribosome population within the cell, dysfunctional ribosomes are targeted for autophagic degradation. It is not known if complete degradation is the only mechanism for eukaryotic ribosome maintenance or if they might also be repaired by replacement of defective components.

We used stable-isotope feeding and protein mass-spectrometry to measure the kinetics of turnover of ribosomal RNA (rRNA) and 71 ribosomal proteins (r-proteins) in mice as well as the synthesis rates of ~1000 proteins within the cell. In general, peripheral r-proteins and those with more direct roles in peptide-bond formation are replaced multiple times during the lifespan of the assembled structure, presumably by exchange with a free cytoplasmic pool, whereas the majority of r-proteins are stably incorporated for the lifetime of the ribosome. Dietary signals impact both the rates of new ribosome assembly and component protein exchange. The results indicate that changes in ribosome activity are accompanied by variations in in vivo maintenance mechanisms. Signal-specific modulation of ribosomal repair and degradation could provide a mechanistic link in the frequently observed associations between diminished rates of protein synthesis, increased autophagy, and greater longevity.

TUESDAY 9:50 – 11:10 am
COMPUTATION AND BIG DATA *Presidential, 2nd Level*

Tues Talk 09:50-10:15 am: Characterization of the Cardiac Proteome with Big Data Proteomics

Maggie PY Lam
University of California at Los Angeles, Los Angeles, CA

Characterization of the Cardiac Proteome with Big Data Proteomics Introduction. Advances in proteomics technologies have created new opportunities to understand complex diseases from a systems perspective. It is increasingly recognized that protein functions are the complex outputs orchestrated by multiple molecular parameters including the abundance of protein species, their modifications, localization, interactions, and others. The continual development of analytical and data science methods to characterize these proteome parameters is imperative in advancing our understanding of disease mechanisms and aiding in the discovery of protein biomarkers. In this study, we developed technological and computational platforms to elucidate differential protein isoform expression and turnover kinetics under cardiac hypertrophy.

Methods. Using metabolic labeling, high-resolution mass <g class="gr_ gr_22 gr-alert gr_gramm gr_disable_anim_appear Punctuation only-ins replaceWithoutSep" id="22" data-gr-id="22">spectrometry</g> and mathematical modeling, we created a technological platform to measure the individual protein expressions and turnover dynamics in a mouse model of cardiac hypertrophy. In parallel, we developed a data science approach that combined transcriptomics and proteomics technologies to elucidate protein changes in the model with isoform resolution.

Results and Discussion. Application of this platform led to findings including (i) the recognition that cardiac mitochondrial proteins have diverse half-lives spanning multiple orders of magnitude, suggesting a finer and independent level of regulation in parallel to mitophagy; (ii) the discovery of differential protein turnover dynamics in metabolic pathways and the identification of novel markers of cardiac hypertrophy; and (iii) the elucidation of differential alternative isoform expressions in the model of cardiac hypertrophy using a data science method.

Conclusion. Taken together, our results demonstrate that regulations of protein functions in the cardiovascular system are more complex than previously thought, where individual proteins and pathways may alter synchronously in turnover/degradation in response to stress, without necessarily changing in abundance.

Tues Talk 10:15-10:40 am: Big Data to Knowledge: Integrated Bioinformatics Towards Systems Biology and Precision Medicine

Cathy H Wu
University of Delaware, Newark, DE

With the advent of next-generation sequencing, proteomics and other high-throughput technologies, systems integration is becoming the driving force for the 21st century biology and medicine. To fully realize the value of such genome-scale data requires advanced bioinformatics for integration, mining, comparative analysis, and functional interpretation at different molecular levels from gene, mRNA and miRNA to protein and post-translational modification in systems biology context. We have developed a bioinformatics research infrastructure linking data mining and text mining with ontologies and biological network analysis for knowledge discovery and disease understanding from panomics data. Our natural language processing (NLP) framework is being extended for full-scale literature mining and generalizable relation extraction in a semantic network to connect gene/protein, mutation, miRNA to drug, disease and phenotype in personalized medicine context. This talk will highlight collaborative projects with the analysis of proteomic, phosphoproteomic and transcriptomic data from large-scale national initiatives, including CPTAC (Cancer Proteomic Tumor Analysis Consortium) and LINCS (Library of Integrated Network-based Cellular Signatures), for disease knowledge network discovery.

Tues Talk 10:40-10:55 am: Proteogenomic Classifications and Outcome in Squamous Cell Carcinoma of the Lung

Robbert Slebos; Paul Stewart; Eric Welsh; Guolin Zhang; Bin Fang; Sean Yoder; Katherine Fellows; Y Ann Chen; Jamie Teer; Steven Eschrich; John Koomen; Eric Haura
Moffitt Cancer Center, Tampa, FL

Integration of proteomic analyses with genomics and gene expression profiling allows a more detailed description of the biological processes that can potentially be used for better classification and help to guide patient selection for targeted therapies. We analyzed 116 surgically resected squamous cell lung carcinoma samples for copy-number alterations, gene expression profiling, targeted exome-sequencing and global proteomic profiling. Copy number and DNA mutation status were assessed using standard technologies, while gene expression profiling was carried out by RNA-sequencing. Proteomic analysis was performed using TMT labeling, 12- fraction bRPLC separation and LC-MS/MS analysis with a Thermo Q-Exactive mass spectrometer. Database searches were performed using three search engines against RefSeq version 78, and summarized using IDPicker 3. We detected more than 8,000 protein groups (FDR <5%). Tumors were classified into 4 gene expression-based classes: Classical, Basal, Primitive and Secretory. At the protein level, the Classical subtype was associated with xenobiotic and energy metabolism; the Basal subtype with defense responses and extracellular matrix changes; the Primitive subtype with nucleic acid metabolism; and the Secretory subtype with p38 signaling. Proteomics-based classification identified two sub-categories within the Classical subtype, characterized by inflammatory and stress response signaling. Within this group, patients with high expression of inflammation-associated proteins had better cancer-specific survival than those with low expression (p=0.04, Log-rank test). Targeted exome sequencing revealed frequent mutations in TP53, CDKN2A, NFE2L2, and in other genes. Proteomic expression of genes located in amplified chromosomal regions was used to identify driver genes in squamous cell lung carcinoma. Our results provide new biological insights from the addition of protein measurements to genomic datasets that have the potential to improve classification. The data suggest that proteins involved in immune responses are important for the biological behavior and outcome of the Classical subtype in squamous cell lung carcinoma.

Tues Talk 10:55-11:10 am: Missing Protein Node Prediction and Protein Quantitation in Bipartite Network Representations of Complex Proteomes

Casimir Bamberger; Salvador Martinez de Bartolome Izquierdo; Miranda Montgomery; Sandra Pankow; John Yates III
The Scripps Research Institute, La Jolla, CA

In bottom–up proteomics proteins in samples are digested into peptides. We analyzed the protein-peptide relationships via bipartite networks in order to quantify the varying proteoforms present in samples. A detailed analysis of these networks showcases the power of using bottom-up proteomics: The direct presentation of all peptide to protein relationships accurately determines the weights of the protein nodes based on the quantitative measurements of the peptide nodes. An initial set of experiments were run using different Drosophila species (D. melanogaster and D. virilis) in order to test the accuracy. The distribution of weights in peptide nodes showed the species specificity of the peptide nodes, and therefore we were able to identify significantly regulated protein variants and predict the presence of additional species-specific orthologues that were not included in the initial analysis. The networking algorithm was also tested on Cystic Fibrosis cells CFBE41o- compared to HBE41o-, and identified serine/threonine protein kinase variants PKN1 and PKN3 were less whereas PKN2 was more abundant in the Cystic Fibrosis cells. The bipartite network analysis of peptide-protein relationships allows for a quantitation at protein level and prediction of additional proteoform nodes in a given sample.

TUESDAY 9:50 – 11:10 am
METABOLOMICS *Palm Court, Lobby Level*

Tues Talk 09:50-10:15 am: Using Metabolomics to Identifying Non-Genetic Determinants of Disease

Caroline Johnson
Yale School of Public Health, New Haven, CT

Since the completion of the human genome project in 2003, it is now understood that only 10-30% of human diseases can be explained by genetics alone. Thus environmental exposures are the predominant cause of human disease, however most of these exposures are unknown. Colon cancer is one such disease in which exposures have a major role in its development, and play out on different genetic backgrounds, but their mechanisms are not fully understood. Metabolomics is an all-encompassing tool that can reveal novel biomarkers and mechanisms of an exposure in relation to disease. Using a combination of four different metabolomics platforms we identified metabolites that were produced by bacterial biofilms (conglomerates of bacteria encased in a polymeric matrix) in patients with colon cancer. Interestingly, both biofilms and metabolites resided predominantly on the right-side of the colon. This is an area of the colon which has worse prognosis for the patient upon cancer presentation. Orthogonal biological experiments revealed a possible pro-carcinogenic mechanism for biofilms in colon cancer and indicated that bacteria use host metabolites to build biofilms. Furthermore, we identified additional influences that modulate metabolism in right-sided colon cancer. Thus using metabolomics our work has revealed potential biomarkers of colon cancer and mechanisms of disease progression.

Tues Talk 10:15-10:40 am: New Technologies for Large Scale and Targeted Analyses of Small Molecules

David Wishart
University of Alberta, Edmonton, Canada

Metabolomics is a technology-driven science. For the past decade it has benefited tremendously from rapid advances in both molecular separation and mass spectrometry technologies. These developments have not only improved the speed, sensitivity, resolution of LC-MS and GC-MS-based metabolomics, they have led to the discovery of many new metabolites and metabolic pathways. More recently, an increasing desire for automated, quantitative, low-cost metabolomics has led to an important shift in the field. In particular metabolomics is now being pushed to become much simpler and far more accessible to a larger community of users. This shift is being driven not only by user demand, but by important technology and software developments. In this presentation I will begin by describing a number of software tools that we, and others, are developing that can help make metabolomics more

of a turnkey operation. These include new programs for MS spectral prediction and metabolite matching, new software for automated spectral deconvolution and new algorithms for novel metabolite prediction. I will also describe the growing trend towards metabolomics "kits" which not only exploit these software developments but which also enable larger scale, cheaper, automated and more targeted analysis of small molecules. Finally, I will describe the development of hand-held, low-cost metabolomic devices, which are being created to permit rapid, inexpensive and targeted analysis of smaller numbers of metabolites. The appearance of targeted metabolomics kits and the development of portable metabolomics devices (so-called "tricorders") is making metabolomics far more accessible and much more routine than ever before. They are also enabling metabolomics tests to be more easily migrated to routine clinical applications.

Tues Talk 10:40-10:55 am: Establishing a Novel NanoLC-MS/MS Platform for Detecting and Quantifying DNA Modification

Ranran Wu; Kevin Janssen; Benjamin A. Garcia
University of Pennsylvania, Philadelphia, PA

Epigenetics is the study of heritable changes in gene expression that does not involve changes in DNA sequence. DNA methylation is one of the major contributors to epigenetic mechanisms. The most studied DNA methylation is 5-methylcytosine (5mC), as it is found to be more prevalent in eukaryotes. Thanks to the discovery of the ten eleven translocation (TET) enzymes, 5mC can be readily oxidized to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC). Besides cytosine methylation and oxidation, N^6-methyladenine has recently been identified in eukaryotes as well. In this study, we established a robust analytical method using nanoLC-MS/MS technique to detect and quantify global DNA modifications. We first optimized the nanoLC-MS/MS parameters using commercially purchased DNA nucleosides. Choosing the right stationary phase is crucial as the DNA nucleosides are of high hydrophilicities. Genomic DNA was extracted from cultured cells and further digested to single DNA nucleosides. These DNA nucleosides are being detected and fragmented using the optimized nanoLC-MS/MS platform. A novel approach of stable ^{13}C isotope labeling is incorporated into the optimized nanoLC-MS/MS platform. This approach is currently under development to achieve the ultimate goal of an accurate detection and multiplexed quantification for the already-identified as well as rare DNA modifications *in vivo*

Tues Talk 10:55-11:10 am: Dilu: High Resolution Metabolomics Quantification and Identification Platform

Yu Gao
The Scripps Research Institute, La Jolla, California

Metabolite profiling requires data preprocessing approaches to correlate specific metabolites to their biological origin. Within all these, quantification is probably one of the most important process. When a data set is generated through chromatography, peak alignment and peak detection will play a huge role in data quantification and directly affect the outcome of metabolite identification. In the past ten years, XCMS has been the standard and most used program for data quantification. It was developed by the Siuzdak laboratory at Scripps in 2006 using R and very little change has been made since its initial release. Recent years, the development of new mass spectroscopy instrumentations such as Orbitrap brought the metabolomic data acquisition to a whole new level with +/- 1ppm mass drift and high resolution MS2 capabilities. Current software programs such as XCMS cannot meet the full needs for current research. Here we present the whole new metabolomic data quantification engine "Dilu" that utilize the high resolution mass data to perform nonlinear retention time alignment, matched filtration, peak detection and peak matching. In collaboration with the Saghatelian lab, we tested both XCMS and Dilu on lipidomic data. Dilu outperforms XCMS on high resolution mass data by a large margin in terms of time consumption, data accuracy and data integrity.

TUESDAY 3:00 – 4:20 pm
NEW TECHNOLOGIES *Presidential, 2nd Level*

Tues Talk 3:00-3:25 pm: Multi-Omic Mass Spectrometry Profiling Technology to Assign Protein Function

Joshua Coon
University of Wisconsin-Madison, Madison, WI

Mitochondrial dysfunction is associated with many human diseases, including neurodegeneration and cancer, that are often caused by changes in proteins or pathways that are not well-characterized. We mapped the proteomes, lipidomes and metabolomes of 174 yeast strains, each of which has a single gene deleted, using mass spectrometry. 144 of these genes have human homologs, 60 of which are associated with disease and 39 of which are uncharacterized. We present a multi-omic data analysis and visualization tool that we use to find covariance networks that can predict molecular functions, correlations between the perturbation profiles of related gene deletions, gene-specific perturbations that reflect protein functions, and a global respiration deficiency response. Using this multi-omic approach, we link seven proteins including Hfd1p and its human homolog ALDH3A1 to mitochondrial coenzyme Q (CoQ) biosynthesis, an essential pathway that is disrupted in many human diseases.

Tues Talk 3:50-4:05 pm: The Multiple Dimensions of Translation Regulation During the Eukaryotic Stress Response

Justin Rendleman[1]; Zhe Cheng[1]; Scott Kuersten[2]; Guoshou Teo[1]; Hyungwon Choi[3]; Christine Vogel[1]
[1]New York University, New York, NY; [2]Illumina, San Diego, CA; [3]National University, Singapore, Singapore

The correlation between protein and RNA concentration, and its relationship to translation have received enormous attention thanks to recent development of high-resolution techniques that allow for detailed mapping of genome-wide events. However, integrative analysis, that monitor changes transcriptional and post-transcriptional dynamics in one system, in particular under perturbed conditions, are still rare.

In a unique, multi-dimensional study, we investigated the response of mammalian cancer cells to oxidative and protein misfolding stress over time. We collected high-resolution datasets on transcript and protein concentration changes using RNA-seq and mass spectrometry based proteomics, monitoring four time points over 8 hours for ~9,000 proteins. Importantly, we complemented these concentration data with information on the binding of both ribosomal and non-ribosomal proteins to the mRNAs, affecting RNA stability, translation and localization. The study represents the first time that all four parameters — RNA, protein, ribosomal and non-ribosomal footprints — were monitored within one system.

We expanded our time series tool PECA (Protein Expression Control Analysis) to provide a consistent statistical framework to intregrate and analyze the datasets. PECA includes time series information and allows us to deconvolute the contributions of transcription, translation, and degradation to concentration changes. We observed different relationships between transcription, translation, and degradation for different sets of genes. We also noticed unique and unexpected patterns: some genes increased in their mRNA level without increased translation, and the binding of non-ribosomal proteins suggested that the genes' RNA was stored and degraded. Further, we observed local footprints in the 5'UTR which were consistent with regulatory elements known as upstream Open Reading Frames.

The presentation will discuss these and other examples of translation regulatory trends and events that we identified in our data.

Tues Talk 4:05-4:20 pm: Advancing Top-Down Proteomics Through Instrumentation and Data Acquisition Method Development

Lissa C. Anderson[1]; Chad R. Weisbrod[1]; Nathan K. Kaiser[1]; Greg T. Blakney[1]; Christopher L. Hendrickson[1, 2]; Alan G. Marshall[1, 2]
[1]NHMFL, Tallahassee, FL; [2]Dept of Chemistry & Biochemistry, FSU, Tallahassee, FL

The 21T FT ICR-MS at NHMFL was constructed to achieve extraordinary performance with respect to top-down analysis. This is achieved by the increased field strength and the culmination of several technologies included during its construction. Here we focus on the

inclusion of front-end electron transfer dissociation (FETD) coupled with an external multipole storage device (MSD), which allows for analysis of larger cumulative ion targets than ever before and lessens the need transient summing. We demonstrate that the linear operational range in terms of cumulative ion target (<5.0E4-3E7 total charges) permits acquisition of spectra which illicit very high sequence coverage, in-spectrum dynamic range, and mass measurement accuracy despite the large aggregate ion populations.

We recently demonstrated high-throughput intact proteome analysis of human colorectal cancer cells by LC-21T FT ICR-MS/MS. Over 1800 proteoforms were identified (1% FDR) from 8 injections, the highest number per injection of any similar study to date. ETD outperformed CID with regard to sequence coverage, which is key to comprehensive proteoform characterization. However, as protein size increases, total ion current is distributed among more fragment ion channels, requiring compensation through increased spectral averages or ion target. Even with molecular-weight-based prefractionation, proteins spanning 5-30 kDa were observed in a single LC-MS experiment.

This evidence provided motivation to scale ion targets based on precursor MW, since we already introduce "fills" of ETD reaction products to the MSD until the desired target is achieved. This approach yielded unprecedented sequence coverage for proteins ranging from 2.8 kDa to 29 kDa without intensive spectral averaging (e.g., ~50% sequence coverage for carbonic anhydrase with 4 averaged acquisitions). An empirical relationship was established between ion target and protein MW, then incorporated within the instrument control software. Real-time scaling of ETD fragment ion fills based on observed MW improved our ability to perform on-line top-down analysis.

TUESDAY 3:00 – 4:20 pm
TOP DOWN ANALYSIS OF PROTEIN COMPLEXES
Palm Court, Lobby Level

Tues Talk 3:00-3:25 pm: Democratizing Top-Down in Both Denatured and Native Modes: Has the time now come?

Neil Kelleher
Northwestern University, Evanston, IL

While top down mass spectrometry has become synonymous with the direct analysis of intact proteins via mass spectrometry, the term more generally denotes an approach to analytical measurement that recognizes the value of retaining as much information as possible about a system prior to analysis. By avoiding proteolytic digestion, proteoform-specific identifications can be made. Notably, the top down philosophy is equally applicable to the level of protein-protein and protein-ligand interactions. This talk will describe an approach that enables the direct analysis of protein interactions in an untargeted fashion via novel separation strategies coupled to multistage native mass spectrometry (MS2 and MS3). Providing information from intact complex mass (MS1) to subunits and their backbone fragment ions, native top-down MS even enables identification and characterization of unknown protein interactions. We posit that these advances form the basis for a platform for the untargeted characterization of protein complexes and protein-ligand interactions from a variety of biological systems. By preserving non-covalent interaction throughout sample preparation and ionization, top down mass spectrometry can operate at the next level of hierarchy in the biology of proteins underlying human wellness and disease.

Tues Talk 3:50-4:05 pm: Proteoform Suite Software: A New Tool for Rapidly Identifying and Quantifying Proteoforms and Constructing Proteoform Families

Michael Shortreed; Anthony Cesnik; Leah Schaffer; Brian Frey; Rachel Knoener; Zachary Rolfs; Yunxiang Dai; Katherine Buxton; Mark Scalf; 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. This approach is highly effective at identifying large numbers of proteins present in complex samples, but the digestion of proteins into peptides renders it impossible to identify the proteoforms from which they were derived. We recently reported a powerful new strategy for the identification of proteoforms and the elucidation of proteoform families (groups of related proteoforms) from experimental determinations of the accurate proteoform mass and number of lysine residues. Accurate proteoform masses are determined by standard MS analysis of undigested protein mixtures and the lysine counts are determined using NeuCode isotopic tagging. We have now created a software package, called Proteoform Suite, for the rapid identification of proteoforms and construction of proteoform families. We have also implemented both relative quantification of proteoforms using isotopic tagging and gene ontology (GO) analysis complete with calculation of enrichment and statistical confidence testing. The capabilities of the program will be demonstrated in proteoform analysis for several different systems including E. coli, yeast, mouse, and human cell lines. We will also describe how the use of other software, which we developed for the comprehensive identification of post-translational modifications in bottom-up data, greatly enhances the creation of sample-specific proteoform databases, thereby increasing the number and accuracy of proteoform identifications.

Tues Talk 4:05-4:20 pm: Measuring Intact Protein Turnover on Drosophila Melanogaster Heads using Tunable Intact Protein Mass Increases Method (TIPMI)

Jeniffer V. Quijada[1, 2]; Jeffrey N. Agar[1, 2]
[1]Northeastern University, Boston, MA; [2]Barnett Inst., Northeastern University, Boston, MA

Quantitative proteomics provides a comparison between two static conditions for protein abundances differences. However, proteins are constantly in a dynamic state between synthesis and degradation in a living organism. Protein synthesis and degradation are controlled by a canonical pathway for a regulation of the protein abundance. Abnormal function of protein degradation process has been associated with various neurodegenerative diseases, like Alzheimer, Parkinson, as a result of the accumulation of prions, and proteins aggregation. The measurement of the protein turnover from healthy and diseased cells may help in the understanding of the molecular mechanism of those neurodegenerative diseases. Previous studies have been done by a bottom-up approach using peptide analysis. Here we present an application of tunable intact protein mass increases TIPMI 1 methodology for a top-down protein turnover measurement applied to Drosophila Melanogaster heads.

TIPMI uses stable isotope metabolic precursor for labeling proteins. In this study, 2H-water was used to label Drosophila Melanogaster (insect). Flies were cultivated in natural abundance (light) and 15% 2H-water (heavy) feed for many generations. Flies were transferred to newly labeled food using two labeling approaches, first light labeled Drosophila was transferred to heavy feed. Second, heavy labeled fly was transferred to light feed. Harvest was done at different time points 0,2,4,8,16, and 32 days. Ten flies heads were lysed, proteins were extracted and precipitated with methanol:chloroform:water, and analyzed by LC-FTICR MS.

The LC-MS chromatogram was summed into 15-seconds interval and MaxEnt deconvoluted. Individual spectrum or 2D pseudo-gel could be used to identify peak pairs by inspection and their areas Light-Heavy peaks were calculated using custom MatLab and LabView routines. TIPMI method allowed us to characterize around 15 intact proteins in Drosophila head using compartment modeling analysis for fitting.2

This is the first application for a measure of intact protein turnover by LC-MS using TIPMI.

TUESDAY 4:30 – 5:50 pm
SINGLE CELL PROTEOMICS *Presidential, 2nd Level*

Tues Talk 4:30-4:55 pm: Proteomics of Single Early-Stage Blastomeres from *Xenopus laevis*

Liangliang Sun[2]; Kyle Dubiak[1]; Elizabeth Peuchen[1]; Zhenbin Zhang[1]; Paul Huber[1]; Norman Dovichi[1]
[1]University of Notre Dame, Notre Dame, IN; [2]Michigan State University, East Lansing, MI

We propose that single cells (blastomeres) isolated from early stage invertebrate, amphibian, or fish embryos are ideal model systems for the development of technologies for single cell analysis. These embryos develop outside the mother and are simple to manipulate. For these embryos, although cell cleavage is not exactly symmetric, the

content per blastomere decreases roughly by half with each cell division, creating a geometric progression in cellular content. This progression forms a ladder of single-cell targets for the development of successively higher sensitivity instruments. We performed bottom-up proteomics on single blastomeres isolated by microdissection from 2-, 4-, 8-, 16-, 32- and 50-cell *Xenopus laevis* (African clawed frog) embryos. Over 1,400 protein groups were identified in single-run reversed-phase liquid chromatography-electrospray ionization-tandem mass spectrometry from single balstomeres isolated from a 16-cell embryo. When the mass of yolk-free proteins in single blastomeres decreased from ~0.8 µg (16-cell embryo) to ~0.2 µg (50-cell embryo), the number of protein group identifications declined from 1,466 to 644. Around 800 protein groups were quantified across four blastomeres isolated from a 16-cell embryo. By comparing the protein expression among different blastomeres, we observed that the blastomere-to-blastomere heterogeneity in 8-, 16-, 32- and 50-cell embryos increases with development stage, presumably due to cellular differentiation.

Tues Talk 4:55-5:20 pm: Massively Multiplexed Cellular Analysis in Human Health and Disease

Sean Bendall
Stanford University, Stanford, CA

Single cell analysis, starting with the earliest low parameter fluorescent cytometry and microscopy experiments, helped define the major cell subsets of human cellular systems as we understand them today (i.e. T-cells, B-cells, macrophages). As single cell analysis parameters have increased, so has the identification of rare immune subsets and stem cells. This progression has been stymied by the limit of light-based parameters measurable, realistically capped at 12-15 due to boundaries in instrumentation and spectral overlap considerations in fluorophore-based tagging methods.

Now, a novel combination of elemental mass spectrometry with single cell analysis (mass cytometry - CyTOF) and imaging (multiplexed ionbeam imaging – MIBI, Nature Med. 2014) offers routine examination of 30-50 parameters without fluorescent agents or interference from spectral overlap with heavy metal isotopes as reporters. Using these new platforms we have been able to reach new levels of hematopoietic immune organization (Science, 2011, 2015) combined with novel single-cell visualization and analysis methods (SPADE, viSNE, Wanderlust, Phenograph, SCAFFOLD - Nature Biotech 2011, 2013, Cell 2014, 2015, 2017) we detailed hematopoietic immune function and dysfunction, identifying new cell populations, regulatory relationships and clinically predictive features underlying disease.

Together, these collective works expose unappreciated layers of human cellular organization, and provide an opportunity to reevaluate diseases and pharmacological therapeutics as specific perturbations to this inherent order.

Tues Talk 5:20-5:35 pm: *In situ* Microsampling Single-cell Capillary Electrophoresis Mass Spectrometry Uncovers Proteomic Cell Heterogeneity in the Live Frog (*Xenopus laevis*) Embryo

Peter Nemes; Camille Lombard-Banek; Aparna Baxi; Sally Moody
George Washington University, Washington, DC

A critical step during normal embryonic development is differentiation of stem cells into all the different types of tissues of the body. Unbiased and label-free detection by high-resolution mass spectrometry (HRMS) has recently uncovered gross proteomic changes between developmental stages of the South African clawed frog (*Xenopus laevis*), a powerful model of vertebrate embryonic development. Traditional proteomic HRMS enhances detection sensitivity by pooling together large populations of cells, albeit at the risk of averaging out cell-characteristic processes such as those responsible for acquiring cell-specific tissue fates. We recently custom-built a high-sensitivity capillary electrophoresis electrospray ionization (CE-ESI) platform and dissected single identified cells from the 16-cell *X. laevis* embryo to uncover proteomic cell heterogeneity during early embryonic development (Nemes *et al*. Angew. Chem. Int. Ed. 2016). Here we report the development of an in situ single-cell CE-ESI-MS approach that enables, for the first time, the discovery characterization of protein expression directly in single cells in the live *X. laevis* embryo. The approach utilizes a high-resolution stereomicroscope and a precision-controlled microcapillary to aspirate a 1–10 nL from identified single cells in the developing embryo. Culturing under normal laboratory conditions and sampling with miniscule damage to the cell was found compatible with embryonic development. Using this technology, we asked how the proteome changes in dorsal-animal cells that give rise to the nervous system. Proteins from the collected cell material were trypsin-digested in 1–4 µL solution. CE-MS analysis of 10 ng of the resulting peptides identified ~300–500 proteins from each cell in the 16-to-128-cell embryo. Label-free quantification and multivariate data analysis uncovered reproducible proteomic changes between cells in the neural-fated cell clones. Capillary microsampling single-cell CE-ESI-MS opens new potentials to study how differential gene expression orchestrates cell fates during early vertebrate development.

TUESDAY 4:30 – 5:50 pm
CROSS-LINKING / MOLECULAR PAINTING
Palm Court Lobby Level

Tues Talk 4:30-4:55 pm: Advancements in Protein Cross-Linking Biochemistry, Software, and Data Visualization Uncover Protein Complex Architecture.

Trisha Davis[1]; Michael Riffle[1]; Alex Zelter[1]; Daniel Jaschob[1]; Michael Hoopmann[2]; Richard Johnson[1]; Robert Moritz[2]; Michael Maccoss[1]
[1]University of Washington, Seattle, WA; [2]Institute for Systems Biology, Seattle, WA

Chemical cross-linking of proteins by fixed-length linkers and subsequent identification of cross-linked peptides by mass spectrometry has the potential to quickly and cheaply provide high-resolution knowledge of protein complex architecture. However, limitations in instrumentation, biochemical methods and computer software have historically resulted in low yields of identified cross-linked peptides—thus limiting the utility of this approach in practice. Here we describe advancements in three areas that have made cross-linking a practical reality for the determination of protein complex architecture. First the optimization of chemical cross-linking reaction conditions to ensure the presence of as many biologically meaningful cross-links as possible (and as few confounding cross-links as possible). Second the development of the Kojak and Percolator software pipeline to confidently identify large numbers of cross-linked peptide sequences from unlabeled tandem mass spectrometry data. And third, the development of ProxI to visualize, compare, perform quality control (QC), and disseminate experimental cross-linking results and underlying proteomics data. ProxI has greatly aided in the upstream optimization of biochemical cross-linking conditions and search software by providing tools to rapidly gauge the comparative impact of different conditions in a structural context. Finally, real-world examples will be given for biomedical research that both drove and were enabled by this work.

Tues Talk 4:55-5:20 pm: In Cell Protein Footprinting for the Structural Analysis of Proteins in their Native Environment

Lisa M. Jones
University of Maryland, Baltimore, MD

Protein footprinting coupled with mass spectrometry has emerged in recent years as a valuable tool to study protein structure. These methods have been successfully used to identify protein-protein and protein-ligand interactions in varying protein systems. To date, these footprinting methods have been performed on relatively pure proteins *in vitro*. The development of an in cell footprinting method would provide a powerful platform to study proteins in their native cellular environment. We have extended the use of the hydroxyl radical based footprinting method fast photochemical oxidation of proteins (FPOP) for in cell structural analysis of proteins. FPOP generates hydroxyl radicals via excimer laser photolysis of hydrogen peroxide. These radicals then oxidatively modify solvent accessible sites in proteins. Oxidative labeling is advantageous for in cell labeling owing to its irreversible nature, which allows for purification of the proteins of interest from the cell membrane prior to MS analysis. We show that in cell FPOP (IC-FPOP) can successfully oxidatively modify over a thousand proteins within the cell. These proteins are found in varying cellular compartments including the cytoplasm, mitochondria, and nucleus. We also show that IC-FPOP successfully probes solvent accessibility within the cell similar to *in vitro* FPOP. This demonstrates the efficacy of using IC-FPOP to study protein structure within the cell.

Tues Talk 5:20-5:35 pm: Dual Cleavabale Crosslinking Technology (DUCCT): A New Strategy for High Confidence Identification of Crosslinked Peptides

Saiful Chowdhury[1]; Jayanta Chakrabarty[1]; Gerhard Munske[2]; Aishwarya Naik[1]

[1]Univ of Texas at Arlington, Arlington, TX; [2]WSU, Pullman, WA

Chemical crosslinking and mass spectrometry is now emerging as a confident technique for covalent labeling of nearby proteins. This produces a stable link so weak and transient interactions can be identified during pulldown or antibody-based experiments. Recently, mass spectrometry-cleavable crosslinkers are coming out as one of the most prominent method for crosslinking proteins. In the mass spectrometry-cleavable methods, crosslinked peptides can be cleaved into two signature peptides so their sequence can be identified by tandem mass spectrometry. Unfortunately, confident identification of crosslinked products by cleavable crosslinkers is also challenging. Further MS3rd sequencing of the cleaved peptides is required, which is sometimes very ambiguous due to the fragmentation of the crosslinked residues attached to peptides. In order to provide high confidence in crosslinked-peptide identifications, we introduced a novel crosslinker which we called DUCCT (Dual Cleavable Crosslinking Technology). This crosslinker contains two lysine reactive groups and two mass spectrometry-cleavable bonds. One cleavable bond is selective for CID-MS cleavage where as other one selectively cleaves by ETD tandem mass spectrometry. This produces two signature mass spectra of the same crosslinked peptides after CID and ETD tandem mass spectrometry. Crosslinked peptides can be identified with high confidence from these two signature mass spectra of the same crosslinked peptides. If needed, further confidence on the crosslinked-peptide identification can be possible by MS3rd sequencing of the cleaved peptides. Crosslinker was synthesized using peptide synthesis chemistry and its molecular mass was verified. DUCCT was tested in peptide and protein level using neurotensin peptide as well as several standard proteins. In addition, its labeling efficiency was tested in macrophage immune cell upon exposures to microbial ligand lipopolysaccharide. Moreover, to study crosslinking in a large-scale sample, a stand -alone software was also developed. We believe this crosslinking approach will provide high confidence in identifying protein-protein interactions globally.

Tues Talk 5:35-5:50 pm: Assessing Availability of Primary Amines in Proteins *in vivo* in Order to Determine Structure and Interaction of Proteins

Casimir Bamberger; Sandra Pankow; John R. Yates

The Scripps Research Institute, La Jolla, CA

The ε-amino group of Lysine residues in proteins is highly polar in solution. Here, Lysine methylation is chemically initiated in order to determine the degree of accessibility of Lysine residues. Depending on its accessibility on the surface of proteins, it can be partially chemically modified by small molecules or, – if engaged in protein-protein interactions –, chemical methylation fails. Here, we append the classical protocol for fixing cells with formaldehyde such that Lysine residues are di-methylated *in vivo*.

First results are presented that assess labeling efficiency as well as dynamic range of sensitivity. Specifically, changes in surface accessibility of the Cystic Transmembrane Conductance Receptor (CFTR) upon deletion of F508, – which is responsible for most cases of the disease Cystic Fibrosis –, demonstrates the performance of the protocol on a single protein level. In a second example, human embryonic kidney (HEK) cells were mildly heat shocked and a change in relative Lysine accessibility compared to controls. In this case change in protein surface accessibility is determined across a proteome based on a relative change in surface accessibility of lysine residues measured in individual peptides.

TUESDAY 7:00 – 9:00 pm
AACC-US HUPO JOINT PROGRAM
Presidential Ballroom, 2nd Level

Tues Talk 8:39-8:51 pm: Molecular Glycopathology by Capillary Electrophoresis: Analysis of the N-glycome of Formalin Fixed Paraffin Embedded Mouse Tissue Samples

Andras Guttman

HLBS, San Diego, Hungary

Capillary electrophoresis with laser induced fluorescence (CE-LIF) detection was used to analyze endoglycosidase released and fluorophore labeled N-glycans from formalin-fixed paraffin-embedded (FFPE) mouse tissue samples of lung, brain, heart, spleen, liver, kidney and intestine. The FFPE samples were first deparaffinized followed by solubilization and glycoprotein retrieval. PNGase F mediated release of the N-linked oligosaccharides was followed by labeling with aminopyrene trisulfonate (APTS). After CE-LIF glycoprofiling of the FFPE mouse tissues, the N-glycan pool of the lung specimen was subject to further investigation by exoglycosidase array based carbohydrate sequencing. Structural assignment of the oligosaccharides was accomplished by the help of the GUcal software and the associated database, based on the mobility shifts after treatments with the corresponding exoglycosidase reaction mixtures. Sixteen major N-linked carbohydrate structures were sequenced from the mouse lung FFPE tissue glycome and identified, as high mannose (3) neutral biantennary (3) and sialylated bianennary (10) oligosaccharides. Two of these latter ones also possessed alpha linked galactose residues.

WEDNESDAY 9:50 – 11:10 am
PRECISION MEDICINE & METABOLIC DISEASES
Presidential Ballroom, 2nd Level

Wed Talk 9:50-10:15 am: Managing Health and Disease Using Big Data

Michael Snyder, Brian Piening, Wenyu Zhou, Kevin Contrepois, Hannes Roest, Dalia Perelman, Gucci Gu, Xiao Li, Jessilyn Dunn, Denis Salins, Shannon Rego, Sophia Miryam Schussler-Fiorenza Rose, Shana Leopold, Jessica Sibal, Tejas Mishra, Liang Liang, Varsha Rao, Nastaran Heidari, Reza Sailani, Lihua Jiang, Colleen Craig, Candice Allistar, Erica Weinstock, Justin Sonnenburg, George Weinstock, Tracy MacLaughlin

Department of Genetics, Stanford University

Understanding health and disease requires a detailed analysis of both our DNA and the molecular events that determine human physiology. We performed an integrated Personal Omics Profiling (iPOP) of 100 healthy and prediabetic participants over three years including periods of viral infection as well as during controlled weight gain and loss. Our iPOP integrates multiomics information from the host (genomics, epigenomics, transcriptomics, proteomics and metabolomics) and from the gut microbiome as well as wearable information. Longitudinal multiomics profiling reveals extensive dynamic biomolecular changes occur during times of perturbation, and the different perturbations have distinct effects on different biological pathways. Wearable data also adds unique early detection information. Overall, our results demonstrate a global and system-wide level of biochemical and cellular changes occur during environment exposures and omics profiling can be used to manage health.

Wed Talk 10:15-10:40 am: Precision Tuning of Therapeutics Targeting PPARs for Treatment of Diabetic Bone

Patrick Griffin

Scripps Research Institute, Jupiter, FL

The nuclear receptor PPARG (NR1C3) regulates osteoblast and osteoclast differentiation, and is the molecular target of thiazolidinediones (TZDs), insulin sensitizers that enhance glucose utilization and adipocyte differentiation. However, clinical use of TZDs has been limited by side effects including a higher risk of fractures and bone loss. Recently we demonstrated that the post-translational modifications at S112 and S273, which influence PPARG pro-adipocytic and insulin sensitizing activities, also determine PPARG osteoblastic (pS112) and osteoclastic (p273) activities. Thus, we sought to develop selective modulators that would repress the PPARG controlled adipogenic program which enhancing the PPARG controlled anti-diabetic program. These efforts led to the development of

SR10171, an inverse agonist that blocks pS273 but not pS112. Treatment of either hyperglycemic or normoglycemic animals with SR10171 increased trabecular and cortical bone while normalizing metabolic parameters. Interestingly, SR10171 treatment modulated osteocyte, osteoblast, and osteoclast activities, and decreased marrow adiposity. These data demonstrate that regulation of bone mass and energy metabolism share similar mechanisms suggesting that one pharmacologic agent could be developed to treat both diabetes and metabolic bone disease.

Wed Talk 10:40-10:55 am: Systemic or Non-Systemic? Co-regulation Analysis of the Urinary Proteome to Identify Plasma-Derived Proteins in Urine

Tue Bjerg Bennike[1]; Saima Ahmed[3]; Hanno Steen[2]
[1]Harvard Medical School, Boston, MA; [2]Boston Children's Hospital, Boston, MA; [3]Boston Children's Hospital - Harvard Univeristy, Boston MA

The urine proteome is thought to reflect systemic changes throughout the body, and thus holds great promise for better understanding of disease etiologies and discovering disease-specific biomarkers. Compared to serum or plasma as blood-based body fluids, urine has the advantage of being easily, non-invasively and plentifully available. Furthermore, it is analytically less challenging, more stable, and significantly less biohazardous.

The majority of the urinary proteins are shed from kidney and/or genitourinary organs into the urine. However, ~1/3 of the urinary proteins derive from blood by being glomerularly filtered in the kidney. Thus, urine serves as a proximal body fluid as well as a systemic body fluid.

Despite these two distinct major origins of urinary proteomes, surprisingly little is known about the exact origin of many urinary proteins. To overcome this limitation, we adopted our previously developed co-regulation proteomics approach (EMBO J. 2014;33:385) to identify the origin of various urinary proteins. As a proof of concept, we analyzed the proteome of 34 urine samples. Of the >2,200 identified proteins 1,130 proteins met our strict quantification criteria. In the dataset, we next separated proteins of plasma origin and kidney/genitourinary tract proteins based on how similar their respective abundance profiles were to the abundance profiles of serum albumin or uromodulin. The list of protein whose abundance profiles resembled the serum albumin abundance profile was highly enriched in bona fide plasma proteins ($p = 0.0017$). Interestingly, the total abundances of the plasma derived proteins in urine did NOT correlate with the abundance in plasma. This co-regulation analysis also identified numerous 'functional hubs', i.e. sets of proteins that share abundance profile, which provided insights beyond the binary case-control analysis. Identifying the functional hubs will enable future urinary biomarker studies as e.g. disease-specific differential abundances in previously identified hubs supports the meaningfulness of the observed differences.

Wed Talk 10:55-11:10 am: A Precision Proteomics Pipeline for Remote Blood Monitoring: Integrating Volumetric Absorptive Microsampling with Targeted and Data-Independent Acquisition Mass Spectrometry

Irene Van Den Broek[1]; Qin Fu[1]; Stuart Kushon[2]; Kim Chansky[2]; Michael Kowalski[3]; Kevin Millis[4]; Andrew Percy[4]; Tasha Agreste[4]; A.Lenore Ackerman[1]; Jennifer Anger[1]; Ron Holewinski[1]; Vidya Venkatraman[1]; Jennifer Van Eyk[1]
[1]Cedars-Sinai Medical Center, Los Angeles, CA; [2]Neoteryx, Torrance, CA; [3]Beckman Coulter Life Sciences, Indianapolis, IN; [4]Cambridge Isotope Laboratories, Tewksbury, MA

Volumetric absorptive microsampling (VAMS™) allows accurate sampling of a fixed small volume of blood (10 µL) from a minimally invasive finger prick, while overcoming hematocrit and sample heterogeneity issues associated with dried blood spots. Application of VAMS for quantification of biomarkers would enable remote, population-scale sample access and accelerate advancements in precision medicine.

We have developed a precision proteomics pipeline that integrates the first application of VAMS for proteins into an automated sample preparation platform, enabling high-throughput mass spectrometric proteotyping, using both targeted (SRM) and discovery (DIA) proteomics workflows.

Validation of the precision proteomics pipeline demonstrated excellent extraction recovery (100.3% - 111.8% for 3 replicates at 3 hematocrit levels) and within- and between day reproducibility (average 5.9% CV for 5 replicates on 5 days). Broader applicability to a larger number of proteins was demonstrated for both a targeted (average 11.0 %CV for 180 peptides from 73 proteins, n=6 on 3 days) and discovery (average 15.7% CV for 1661 peptides from 423 proteins, n=3 on 1 day) proteomics workflow. Moreover, the correlation between peptide abundance in dried blood and corresponding plasma ($R=0.9360$ for the targeted and $R=0.8383$ for the discovery workflow) indicate general robustness of sample storage and automated extraction and digestion, as well as the suitability of dried blood as a surrogate for traditional plasma biomarker studies.

The precision proteomics pipeline, using SRM and DIA, is now being applied to a clinical cohort of plasma and dried blood from 40 patients with lower urinary tract pathology. All samples, plasma and blood, are spiked with 15N-labeled apolipoprotein A-I as well as 112 stable-isotope labeled peptides from 44 candidate biomarker proteins.

We believe that the precision proteomics pipeline for remote blood monitoring will open doors for personalized, longitudinal, and population-based proteomics research.

WEDNESDAY 9:50 – 11:10 am
PROTEOFORM BIOLOGY *Palm Court, Lobby Level*

Wed Talk 9:50-10:15 am: Metabolic Labeling in Middle-Down Proteomics Allows for Comprehensive Interpretation of the Dynamic Histone Code

Simone Sidoli[1]; Kelly Karch[1]; Chrystian Ruminowicz[2]; Benjamin A. Garcia[1]
[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Private developer, Białystok , Poland

Advances in the performance of liquid chromatography (LC) and mass spectrometry (MS) have allowed for more efficient separation and accurate identification of longer (~50-60 aa) peptide sequences, namely middle-down proteomics. Middle-down proteomics is currently the most widely adopted method to characterize combinatorial histone post-translational modifications (PTMs). Histone PTMs have a synergistic role in modulating gene expression and other chromatin functions, and thus determination of co-existing PTM frequencies rather than single PTM abundances is essential. Here, we demonstrate that the middle-down strategy can be combined with metabolic labeling using SILAC for histone proteins and PTM quantification. First, we mixed equal amounts of HeLa cells grown in light and heavy media (lysine (13C6, 15N2) and arginine (13C6, 15N4)), purified histone H3 and verified that unlabeled and labeled histone tails were selected for MS/MS fragmentation without biases and provided comparable results in histone PTM quantification. Then, we applied SILAC quantification and middle-down proteomics into an epithelial to mesenchymal transition (EMT) model system to monitor dynamic changes in histone PTM abundances. The analysis showed good reproducibility with bottom-up data and highlighted H3K27me3 as upregulated mark in mesenchymal cells. Finally, we characterized the dynamics of histone H3 methylations in EMT using Methionine-(methyl-13C,D3) labeling, the precursor of the methyl donor S-adenosylmethionine (SAM). Results confirmed that H3K27me3 was the fastest and most abundant catalyzed methylation in EMT cells, and we determined which combinatorial PTMs are associated with such mark during cell phenotypic transition. Our in-house developed software isoScale to process middle-down data has been here upgraded to process peptide results containing any kind of PTM, including heavy labeled methylations, and isotopic labeling of amino acid residues. Collectively, we demonstrate that middle-down is now a technique that permits characterization of dynamics of protein or PTM synthesis as an additional layer of information to quantification of PTM combinatorial patterns.

Wed Talk 10:15-10:40 am: LINCS PCCSE: A Mineable Resource of Epigenetic and Phosphoproteomic Cellular Drug Responses

Jacob Jaffe; PCCSE Team
Broad Institute of Harvard and MIT, Cambridge, MA

The Library of Integrated Network-based Cellular Signatures (LINCS) Proteomics Characterization Center for Signaling and Epigenetics (PCCSE) collects molecular profiles of the cellular responses to drug, genetic, and environmental perturbations. We are focused on understanding how the phosphosignaling and epigenetic states of cells change as a result of compound treatment, and especially on how these two distinct signaling mechanisms interact in specific cell types and more generically across cell types. To do this, we have developed two targeted proteomic signature assays that focus on quantifying changes in selected phosphopeptides (reduced-representation phosphoproteomics, or P100) and histone modifications (global chromatin profiling, or GCP) We have embarked on an ambitious effort to systematically collect profiles from 100s of drug and genetic perturbations across multiple models of disease using our signature assays, and expect to profile over 10,000 samples over the course of the project. Our initial data release (https://bit.ly/PCCSEData) includes profiles from 90 distinct compounds profiled in 6 distinct cell types, one of which is a model for neurobiology. Both assays demonstrate extremely good replicate recall and a surprising amount of signal from the compounds tested.

We introduce the concept of "connectivity" to universally integrate the data across assays and provide an intuitive means of query to identify similar molecular phenotypes. Connectivity can be used to help establish the mechanism of action of a perturbation or reveal associations among seemingly unrelated conditions. We use connectivity to demonstrate differences in cell type-specific phosphosignaling responses as opposed to more general responses that transcend cell types in epigenetics. We also highlight the unique responses of a neurobiology cell model that behaves very distinctly from more typical oncology cell models in response to signaling modulatory compounds.

Wed Talk 10:40-10:55 am: Quantitative Proteomic Approach Reveals Landscape of Regulatory Elements for Lysine 2-Hydroxyisobutyrylation Pathway

He Huang; Mathew Perez-Neut; Kyle Delaney; Okwang Kwon; Yingming Zhao
University of Chicago, Chicago, Illinois

Protein post-translational modifications (PTMs) underlie a core mechanism of biological regulation conserved in all forms of life. The chemical structure and position of PTMs modulate protein structure and function dictating diverse biological outputs, such as driving signal transduction pathways and/or histone-mediated epigenetic programs. We previously discovered lysine 2-hydroxyisobutyrylation (K_{hib}) and demonstrated that K_{hib} is associated with active gene expression; however, the key elements regulating K_{hib} remain unknown, hindering further efforts to study K_{hib} in physiological contexts and diseases like cancer. Therefore, the purpose of this study was to systematically identify the key K_{hib} regulatory pathways in cells. Using HPLC-MS/MS and stable isotopic labeling of amino acids on cultured Hek293t cells, we identified 6548 K_{hib} sites on 1725 substrate proteins. Bioinformatics analysis revealed that the substrate proteins are involved in transcription, translation, protein degradation, and metabolism. Using a synthetic histone pull down array, we identified over 300 proteins that can bind to histone K_{hib} marks. Additionally, we identified Tip60, a MYST family acetyltransferase, can cataylze the addition 2-hydroyisobutyryl moiety to lysine *in vitro* and *in vivo*. In conclusion, we found that K_{hib} is a widespread, enzymatically regulated PTM recognized by a host of binding partners. Drugs targeting enzymatic regulators and PTM-specific binding partners have previously shown efficacy for the treatment of cancer. Our study provides a solid foundation for further analysis of the functions of K_{hib} in physiology and potentially opens new avenues for the treatment of diseases like cancer.

Wed Talk 10:55-11:10 am: Immediate-Early Histone Proteoform Dynamics in Response to Epigenetic Inhibitors

Tao Wang; Matthew V. Holt; Nicolas L. Young
Baylor College of Medicine, Houston, TX

Epigenetic inhibitors are exceptionally effective for a subset of cancers and in overcoming resistance to other therapies, despite unclear underlying mechanisms. Nearly all previous studies of epigenetic inhibitors have focused on long time frames and isolated post-translational modifications.

We show here that there are dramatic changes in histone proteoform abundances in response to epigenetic inhibitors that are unexpectedly quick, dramatic and proteoform specific. These new insights will be useful in understanding mechanisms of action and effective time coordination of multiple treatments.

We have developed a high-throughput online liquid chromatography-top-down proteomic method that is: sensitive, high throughput, quantitative, proteoform specific, and capable of capturing fast dynamics in 15 minute increments over many hours with multiple biological replicates and robust statistics.

We have established the immediate-early proteoform-level response to several epigenetic inhibitors in multiple human cell lines. For example, different cell lines have variable responses to A196 (an inhibitor of H4K20 methyltransferase SUV420). A 19% decrease in H4K20me2 was observed in SUM159 cells, compared to 8% decrease in MCF7 cells. However, in both cell lines the binary combination of H4K16acK20me2 decreases dramatically within 15 minutes of treatment, suggesting proteoform specific activity. Interestingly, only H4K20me2 and not H4K20me3 is decreased by A196 in this time frame, despite SUV420 being accepted as the enzyme that writes both H4K20me2 and K20me3, suggesting different mechanisms. Surprisingly, the level of H4K5ac is ~5x increased with or without K20me2. H4K16ac does not change abundance while the H4K16acK20me2 proteoform rapidly decreases. Reciprocally, H4K16acK20me2 dramatically increases within 15 minutes of histone deacetylase inhibition (HDACi). Remarkably, histone deacetylase and SUV420 inhibition in combination reveals even deeper interplay between these marks dependent on the order of application. The increased acetylation expected upon HDACi treatment is even greater with pretreatment with A196. While pretreating with HDACi obliterates A196 activity altogether.

MONDAY POSTER LIST

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Lightning Talks

Posters featured in the Monday *Lightning Session - Round I* and Wednesday *Technology and Tips & Tricks* are noted.

Biomarkers and Targeted MS Assays, Mon Poster 01-10

Mon Poster 01 **Urinary Protein Changes in Walker 256 Tumor-Bearing Rats;** Jianqiang Wu[1]; Youhe Gao[1, 2]; *[1]Peking Union Medical College, Beijing, China; [2]Beijing Normal University, Beijing, China* Monday Lightning Session

Mon Poster 02 **Glycan "Nodes" as Cancer Markers: Clinical Performance in Muscle Invasive Bladder Cancer;** Shadi Ferdosi[1]; Erik Castle[2]; Thai Ho[2]; Melissa Stanton[2]; Garrick Wallstrom[3]; Chad R. Borges[1]; *[1]Biodesign Institute, Arizona State University, Tempe, AZ; [2]Mayo Clinic, Phoenix, AZ; [3]Department of Biomedical Informatics, Arizona State University, Tempe, AZ*

Mon Poster 03 **Limited Tryptic Digestion Isotope Dilution Mass Spectrometry (LTD-IDMS) for Hemagglutinin Stability Assessment in Influenza Vaccines;** Keith R Morgenstern[1]; Tracie L Williams[1]; Yingxia Wen[2]; Giuseppe Palladino[2]; Yuhong Xie[2]; Ethan C Settembre[2]; John R Barr[1]; *[1]Centers for Disease Control and Prevention, Atlanta, GA; [2]Seqirus, Cambridge, MA*

Mon Poster 04 **A Standard Assessment of Extracellular Vesicle Extraction Methods by Targeted Mass Spectrometry;** Tingting Wang[1, 2]; Kyle Anderson[1, 2]; Illarion Turko[1, 2]; *[1]Institute for Bioscience & Biotechnology Research, Rockville, MD; [2]National Institute of Standards and Technology, Rockville, MD*

Mon Poster 05 **Development of a Multiplexed Assay for Oral Cancer Candidate Biomarkers Using Peptide Immunoaffinity Enrichment and Targeted Mass Spectrometry;** Jau-Song Yu[1]; Yung-Chin Hsiao[1]; Lang-Ming Chi[2]; Kun-Yi Chien[1]; Wei-Fan Chiang[3]; Yi-Ting Chen[1]; Kai-Ping Chang[2]; Yu-Sun Chang[1]; *[1]Chang Gung University, Taoyuan, Taiwan; [2]Chang Gung Memorial Hospital, Taoyuan, Taiwan; [3]Chi-Mei Medical Center, Liouying, Taiwan*

Mon Poster 06 **Multiplexed Kinase Biosensor Technology and SWATH-MS for Monitoring Chronic Myelogenous Leukemia (CML) Signaling;** Tzu-Yi Yang; Laurie Parker; *University of Minnesota, Minneapolis, MN*

Mon Poster 07 **High Throughput and Highly Multiplexed 73 Proteins MRM Assay with Accuracy: Combination of Automated Sample Preparation and Scheduled MRM Analysis;** Qin Fu; Mitra Mastali; Irene van den Broek; Vidya Venkatraman Venkatraman; Jennifer E Van Eyk; *Cedars Sinai Medical Center, Los Angeles, CA*

Mon Poster 08 **A Robust and Multiplexed ImmunoMRM Assay for Relative Quantitation of Key Phosphopeptides in the DNA Damage Response (DDR) Pathway;** Si Mou[1]; Lei Xiong[1]; Jeffery Whiteaker[2]; Lei Zhao[2]; Yihan Li[1]; Amanda Paulovich[2]; Hua-Fen Liu[1]; Christie Hunter[1]; *[1]SCIEX, Redwood City, California; [2]Fred Hutchinson Cancer Research Center, Seattle, WA*

Mon Poster 09 **Improving Depth of Coverage of SWATH® Acquisition Using SWATH Xtend to Build Better Libraries;** Christie Hunter[1]; Sara Ahadi[3]; Jemma Wu[2]; Mark Molloy[2]; *[1]SCIEX, Redwood City, California; [2]APAF, Sydney, Australia; [3]Stanford University, Stanford, CA*

Mon Poster 10 **TargetedMSQC: an R Package for Quality Control of Chromatographic Peaks in Targeted Proteomics;** Shadi Toghi Eshghi; Qingling Li; Paul Auger; Kristin Wildsmith; Veronica Anania; W. Rodney Mathews; *Genentech, Inc., South San Francisco, CA*

Cancer Proteomics, Mon Poster 11-23

Mon Poster 11 **A Kinetic Proteomics Approach to Identify Targets of Autophagy in Cancer and Recover Chemotherapeutic Effects;** Monique Paré Speirs; Bradley Naylor; John C. Price; *Brigham Young University, Provo, UT* Monday Lightning Session

Mon Poster 12 **LinkedOmics : A Web-Based Platform for Cancer Multi-omics Data Integration and Comparison;** Suhas Vasaikar[1]; Peter Straub[2]; Jing Wang[1]; Bing Zhang[1]; *[1]Baylor College of Medicine, Houston, Texas; [2]Vanderbilt University, Nashville, TN* Monday Lightning Session

Mon Poster 13 **Time-Resolved Quantitative Analysis of Cellular Signaling and Epigenetic Mechanisms During Epithelial-Mesenchymal Transition;** Congcong Lu[2, 3]; Simone Sidoli[3]; Zuofei Yuan[3]; Benjamin A. Garcia[1, 2]; *[1]Epigenetics Program, Philadelphia, PA; [2]Department of Biochemistry and Molecular Biophysic, Philadelphia, PA; [3]University of Pennsylvania, Philadelphia, PA*

Mon Poster 14 **Unambiguously and Comprehensively Resolving Cancerous Adenocarcinoma and Stromal Proteomes and Their Interactive Signaling Without Cell Sorting in Patient-Derived Xenograft Models;** Shichen Shen[1]; Jun Li[1]; Ninfa L. Straubinger[1]; Xue Wang[2]; Michelle K. Greene[4]; Christopher J. Scott[4]; Wen Wee Ma[3]; Robert M. Straubinger[1]; Jun Qu[1]; *[1]SUNY at Buffalo, Buffalo, NY; [2]Roswell Park Cancer Institute, Buffalo, NY; [3]Mayo Clinic, Rochester, MN; [4]Queen's University, Belfast, Northern Ireland* Monday Lightning Session

MONDAY POSTER LIST

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Mon Poster 15 **A 3-D Proteome Atlas is an Important Component to the Cancer Moonshot;** Kat Tiemann; Carolina Garri; Ruth Alvarez; Sang Bok Lee; Jonathan Katz; Kian Kani; *USC, Los Angeles, CA* Monday Lightning Session

Mon Poster 16 **Analysis of Blood Plasma Suggests Tumor Re-Seeding may be Facilitated by Wound Creation During Radiotherapy Treatment of Breast Cancer Patients;** Catherine Going; Marta Vilalta; Marjan Rafat; Melissa Jenkins; Jie Jane Chen; Kathleen Horst; Edward Graves; Sharon Pitteri; *Stanford University, Palo Alto, CA*

Mon Poster 17 **Quantitative Glycoproteomic Profiling of Prostate Cancer Tissue Reveals Alterations in Glycosylation Patterns in Men with Recurrent Disease;** Sarah M. Totten; Cheylene Tanimoto; Abel Bermudez; Amy Hembree; James D. Brooks; Sharon J. Pitteri; *Stanford University School of Medicine, Palo Alto, CA*

Mon Poster 18 **Global Quantitative Phosphoproteomics to Identify Mechanisms of Resistance to the Third Generation EGFR TKIs in Human Lung Adenocarcinoma;** Xu Zhang[1]; Tapan K. Maity[1]; Karen E. Ross[2]; Stephanie C. Pitts[1]; Cathy Wu[3]; Udayan Guha[1]; *[1]Thoracic and GI Oncology Branch, CCR, NCI, NIH, Bethesda, MD; [2]Georgetown University, Washington D.C., D.C.; [3]University of Delaware, Newark, DE*

Mon Poster 19 **Mapping the Surfaceome of Plasma Cells Reveals Novel Immunotherapy Targets for Multiple Myeloma;** Nathan Schuld[1]; Ensaf Al-Hujaily[2]; Parameswaran Hari[3]; Jeffrey Medin[1, 2]; Rebekah Gundry[1, 4]; *[1]Department of Biochemistry, Milwaukee, WI; [2]Department of Pediatrics, Milwaukee, WI; [3]Dept. of Medicine/Hematology Oncology, Milwaukee, WI; [4]Medical College of Wisconsin, Milwaukee, WI*

Mon Poster 20 **Proteomic and lipidomic Analyzes Reveal Significant Lipid Metabolism Alteration in Colon Cancer Cells;** Lili Niu[1]; Tanxi Cai[2]; Ying Zhang[1]; Fuquan Yang[2]; *[1]Huazhong University of Science and Technology, Wuhan, China; [2]Institute of Biophysics, CAS, Beijing, China*

Mon Poster 21 **Method Development and Evaluation of the Protein Phosphatase 2 Phosphoproteome Using the Chip iFunnel QTOF Platform;** Paulos Chumala[1]; Vadiraja Bhat[2]; Chelsea Cunningham[1]; Brooke Thompson[1]; Frederick Vizeacoumar[1]; Franco Vizeacoumar[1]; George Katselis[1]; *[1]College of Medicine, University of Saskatchewan, Saskatoon, Canada; [2]Agilent Technologies, Wilmington, DE*

Mon Poster 22 **Multi-Omics Analysis for the Validation of Differential Transcripts in PAX8 Overexpressed MOSE Cells;** Melissa R. Pergande[1]; Laura M. Rodgers[1]; Vadiraja Bhat[2]; Carol Haney-Ball[2]; Joanna E. Burdette[1]; Stephanie M. Cologna[1]; *[1]Un of Illinois at Chicago, Chicago, IL; [2]Agilent Technologies Inc, Santa Clara, CA*

Mon Poster 23 **P-MartCancer-Interactive Online Software to Enable Biomaker Discovery from Shotgun Cancer Proteomic Datasets;** Bobbie-Jo Webb-Robertson; Lisa Bramer; Jeffrey Jensen; Markus Kobold; Kelly Stratton; Amanda White; *Pacific Northwest National Laboratory, Richland, WA*

Cardiovascular Disease, Mon Poster 24-29

Mon Poster 24 **Mass Spectrometry-based Proteomic and PTM Studies Provide Insight into the Molecular Mechanisms of Restenosis;** Matthew Glover[1]; Qing Yu[1]; Bowen Wang[1]; Xudong Shi[1]; Lian-Wang Guo[1]; K. Craig Kent[1, 2]; Lingjun Li[1]; *[1]University of Wisconsin-Madison, Madison, WI; [2]The Ohio State University, Columbus, OH* Monday Lightning Session

Mon Poster 25 **Integrated Omics Analysis of Protein Isoform Switch Under Cardiac Hypertrophy;** Maggie PY Lam[1]; Yi Xing[2]; Peipei Ping[1]; *[1]NIH BD2K Center of Excellence, UCLA, Los Angeles, CA; [2]Dept. of MIMG, UCLA, Los Angeles, CA*

Mon Poster 26 **DDA vs DIA: Convergence of Increased Breadth, Reproducibility and Precision.;** Ronald Holewinski[1]; Vidya Venkatraman[1]; Sarah Parker[1]; Georgia Saylor[2]; Chunhong Mao[3]; Grace Athas[4]; David Herrington[2]; Jennifer Van Eyk[1]; *[1]Cedars-Sinai Medical Center, Los Angeles, CA; [2]Wake Forest Baptist Medical Center, Winston-Salem, NC; [3]Virginia Tech University, Blacksburg, VA; [4]Louisiana State University School of Medicine, New Orleans, LA*

Mon Poster 27 **Quantitative Analysis of Newly Synthesized Proteins During Maladaptive Cardiac Remodeling;** Yuanhui Ma[1]; Daniel B. McClatchy[1]; David Liem[2]; Dominic Ng[2]; Peipei Ping[2]; John R. Yates[1]; *[1]The Scripps Research Institute, La Jolla, CA; [2]University of California at Los Angeles, Los Angeles, CA* Monday Lightning Session

Mon Poster 28 **Temporal Quantitative Phosphoproteomics of ADP Stimulation Reveals Novel Central Nodes in Platelet Activation and Inhibition;** Florian Beck[1]; Joerg Geiger[2]; Stepan Gambaryan[4]; Fiorella A. Solari[1]; Margherita Dell'Aica[1]; Stefan Loroch[1]; Nadine Mattheij[3]; Igor Mindukshev[4]; Oliver Poetz[5]; Kerstin Jurk[6]; Julia M Burkhart[1]; Christian Fufezan[7]; Johan W.M. Heemskerk[3]; Ulrich Walter[6]; Rene P. Zahedi[1]; Albert Sickmann[1]; *[1]ISAS, Dortmund, Germany; [2]Interdisciplinary Bank of Biomaterials and Data, Wuerzburg, Germany; [3]Department of Biochemistry, CARIM, Maastricht Univ, Maastricht, Netherlands; [4]Sechenov Institute of Evolutionary Physiology, St. Petersburg, Russian Federation; [5]NMI Natural and Medical Sciences Institute, Tuebingen, Germany; [6]Center for Thrombosis and Hemostasis, Mainz, Germany; [7]Institute for Biology and Biotechnology of Plants, Muenster, Germany*

MONDAY POSTER LIST

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Mon Poster 29 **Quantitative Phosphoproteomic and Proteomic Analysis of Swine Hearts Revealed Novel Insights into Myocardial Stunning;** Xue Wang[1]; Xiaomeng Shen[2]; Rebeccah Young[3]; Jun Li[2]; Shichen Shen[2]; John Canty[3]; Jun Qu[2]; *[1]Roswell Park Cancer Institute, Buffalo, NY; [2]SUNY at buffalo, Buffalo, NY; [3]Clinical and Translational Research Center, Buffalo, NY* Monday Lightning Session

Chromatin Dynamics, Mon Poster 30-32

Mon Poster 30 **Proteomics for Systems Biology: Defining the Cross-Talk Between Signaling Pathways and Chromatin Modifications Occurring During Treatment of Acute Myeloid Leukemia;** Simone Sidoli; Pamela J. Sung; Katarzyna Kulej; Martin Carroll; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA* Monday Lightning Session

Mon Poster 31 **Time-resolved Global and Chromatin Proteomics during Herpes Simplex Virus (HSV-1) Infection;** Katarzyna Kulej[1]; Daphne D. Avgousti[1]; Simone Sidoli[2]; Christin Herrmann[1]; Ashley N. Della Fera[1]; Eui Tae Kim[1]; Benjamin A. Garcia[2]; Matthew D. Weitzman[1]; *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania, Philadelphia, PA* Monday Lightning Session

Mon Poster 32 **Identification of Actin Arginine Methylation Site in Chromatin Remodeling Complexes Relevant to Transcriptional Regulation and Human Vascular Disease;** Prabodh Kapoor[1]; Zhenan Liu[2]; Vinesh Vinayachandran[3]; Rohit Reja[3]; Chia-Fang Lee[4]; Ashutosh Kumar[5]; Jiyuan Chen[6]; Jing Xiao[1]; Jianjun Shen[1]; Bongsoo Park[3]; Michelle Gadush[4]; Maria Person[4]; Kathleen Trybus[7]; Kam Zhang[5]; Franklin Pugh[3]; Kristine Kamm[2]; Dianna Milewicz[6]; Xuetong Shen[1]; *[1]University of Texas M.D. Anderson Cancer Center, Smithville, TX; [2]University of Texas Southwestern Medical Center, Dallas, TX; [3]Pennsylvania State University, University Park, PA; [4]University of Texas at Austin, Austin, TX; [5]RIKEN Center for Life Science Technologies, Yokohama, Japan; [6]UT Health Science Center at Houston, Houston, TX; [7]University of Vermont, Burlington, VT*

Disease and Protein-Protein Interactions and Proximity, Mon Poster 33

Mon Poster 33 **Discovery of a Novel Interaction Between Apolipoprotein E and Interferon-γ (IFNγ) that can be Modulated by Oxidation of IFNγ;** Erandi Kapuruge; Chad Borges; *Arizona State University, Tempe, Arizona*

Glycans and Vaccines, Mon Poster 34-36

Mon Poster 34 **Combinining SEC Separations with Isotope Dilution Mass Spectrometry for Improving the Quality of Influenza Vaccines.;** Jonathan Bundy; Wanda Santana; Yulanda Williamson; Tracie Williams; John Barr; *Centers for Disease Control, Atlanta, Georgia*

Mon Poster 35 **Semi-Automated Methods in Skyline and New MS/MS Rules to Define and Assign Xylosylated *N*-Linked Glycans in Populus Trichocarpa;** Elizabeth S. Hecht[1]; Philip Loziuk[1]; Brian Pratt[2]; Brendan Maclean[2]; Mike Maccoss[2]; David Muddiman[1]; *[1]North Carolina State University, Raleigh, NC; [2]University of Washington Genome Science, Seattle, WA* Monday Lightning Session

Mon Poster 36 **PEAKS AB: A Software Platform for LC-MS/MS Based Therapeutic Protein Characterization;** Baozhen Shan; Lin He; *Bioinformatics Solutions Inc, Waterloo, Canada*

Imaging, Mon Poster 37-39

Mon Poster 37 **Advances in Multi-modal Mass Spectrometry Imaging for Biomedical Applications;** Roy Martin[1]; James Langridge[2]; Emmanuelle Claude[2]; Philippa Hart[2]; Towers Mark[2]; Emrys Jones[2]; *[1]Waters, Beverly, MA; [2]Waters Corporation, Wilmslow, UK*

Mon Poster 38 **Relative and Absolute Quantification of Metabolites in Hen Ovarian Tissues using IR-MALDESI Mass Spectrometry Imaging;** Milad Nazari; Mark Bokhart; David Muddiman; *North Carolina State University, Raleigh, NC*

Mon Poster 39 **Quantitative Drug Distribution Mapping in Tissues Using IR-MALDESI Mass Spectrometry Imaging;** Mark Bokhart[1]; Elias Rosen[2]; Corbin Thompson[2]; Ken Garrard[1]; Angela Kashuba[2]; David Muddiman[1]; *[1]NCSU, Raleigh, NC; [2]UNC Eshelman School of Pharmacy, Chapel Hill, NC* Monday Lightning Session

Neurological Diseases and Neuroproteomics, Mon Poster 40-47

Mon Poster 40 **Probing Translational Regulation in Spinal Muscular Atrophy (SMA) Using Integrated Proteomics and Transcriptomics Approaches;** Amanda Guise[1]; Shaojun Tang[2]; Ruchi Chauhan[1]; Constantin van Outryve d'Ydewalle[4]; Hendrik Wesseling[1]; Charlotte Sumner[4]; Martin Hemberg[3]; Hanno Steen[1]; Judith Steen[1]; *[1]Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Georgetown University, Washington, DC; [3]Wellcome Trust Sanger Institute, Cambridge, UK; [4]Johns Hopkins School of Medicine, Baltimore, MD* Monday Lightning Session

MONDAY POSTER LIST

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Mon Poster 41 **Glycomics and Proteomics of Myelinated Versus Non-Myelinated Regions of Human Brain Tissue;** Manveen Sethi[1]; Harry Pantazopoulos[2, 3]; Sabina Berretta[2, 3]; Joseph Zaia[1]; *[1]Boston University School of Medicine, Boston, MA; [2]Department of Psychiatry, Harvard Medical School, Boston, MA; [3]Translational Neuroscience Laboratory, McLean Hosp, Belmont, MA* Monday Lightning Session

Mon Poster 42 **Vascular Contributions of Plasma Lipoproteins to Alzheimer's Diseases;** Danni Li; Fangying Huang; *U of Minnesota, Minneapolis, MN* Monday Lightning Session

Mon Poster 43 **Transcriptome and Proteomic Profiling of a Drosophila Seizure Model Reveals Glial Regulation of Key Synaptic Proteins;** Kevin Hope[1]; Daniel Johson[3]; Xiaoyue Jiang[2]; Andreas Huhmer[2]; Daniel Lopez-Ferrer[2]; Lawrence Reiter[1, 4]; *[1]Department of Neurology, UTHSC, Memphis, TN; [2]Thermo Fisher Scientific, San Jose, CA; [3]Molecular Bioinformatics Core, UTHSC, Memphis, TN; [4]Department of Anatomy and Neurobiology, UTHSC, Memphis, TN* Monday Lightning Session

Mon Poster 44 **Protein Signatures Differentiate Cancer Comorbidity in ALS Patients;** Young Ah Goo[1]; Kevin Murnan[2]; N. Polat[3]; Alissa Schunter[1]; H. Fen[2]; D.B. Gursel[2]; H. Idrisoglu[3]; Paul Thomas[1]; Pembe H. Ozdinler[2]; *[1]Proteomics Center of Excellence, Northwestern Univ, Chicago, IL; [2]Department of Neurology, Northwestern University, Chicago, IL; [3]Istanbul University Medical School, Istanbu, Turkey*

Mon Poster 45 **Protein Expression and Phosphorylation Changes During Neuronal Differentiation Reveal Novel Maturity Markers;** Matthew Berberich[1]; Steven Rodriguez[2]; Kartik Subramanian[1]; Robert Everley[1]; Peter Sorger[1]; *[1]Harvard Medical School, Boston, MA; [2]Massachusetts General Hospital, Boston, MA*

Mon Poster 46 **Proteomics Studies in Synergistic Protection from Retinal Degeneration by Combined Stem Cell Therapies;** Dawn Z Chen[1, 3]; Changqing Zhang[2]; Yang Gao[1]; Bin Lu[2]; Sergey Girman[2]; Benjamin Bakondi[2]; Weston Spivia[1]; Jennifer E. Van Eyk[1]; Shaomei Wang[2, 3]; *[1]Advanced Clinical Biosystems Research Institute, Los Angeles, CA; [2]Board of Governors Regenerative Medicine Institute, Los Angeles, CA; [3]David Geffen School of Medicine, UCLA, Los Angeles , CA* Monday Lightning Session

Mon Poster 47 **Unraveling the Complexity of the Gut-Brain Axis N-Glycoproteome;** Mariana BarbozaGardner[1]; Gege Xu[2]; Melanie Gareau[1]; Helen Raybould[1]; Carlito Lebrilla[2, 3]; *[1]Department of Anatomy, Physiology & Cell Biology, UC Davis, CA; [2]Department of Chemistry, UC Davis, CA; [3]Department of Biochemistry, School of Medicine, UC Davis, California* Monday Lightning Session

New Technologies, Mon Poster 48

Mon Poster 48 **Online 2D-NCFC-RP/RPLC System for Efficient and Comprehensive Proteomic Analyses;** Sang-Won Lee; Hangyeore Lee; Jeong Eun So; *Korea University, Seoul, Korea* Monday Lightning Session

Post-Translational Modifications, Mon Poster 49-57

Mon Poster 49 **Proteome-Wide Acetylation Dynamics Revealed by Metabolic Labeling and Quantitative Proteomics;** Yekaterina Kori[1]; Simone Sidoli[1]; Zuo-Fei Yuan[1]; Xiaolu Zhao[2]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; [2]Wuhan University, Wuhan, China* Monday Lightning Session

Mon Poster 50 **A Novel Method of Quantifying Protein Methylation Utilizing SWATH-MS;** Aaron Robinson; Sarah Parker; Vidya Venkatraman; Ronald Holewinski; Shelly Lu; Jennifer Van Eyk; *Cedars Sinai Medical Center, Los Angeles, CA* Monday Lightning Session

Mon Poster 51 **Post-Translational Crosstalk Networks Associated with EMT-Mediated Resistance to EGFR Inhibitors;** Guolin Zhang; Paul A Stewart; Bin Fang; Eric A Welsh; John M Koomen; Steven Eschrich; Eric B Haura; *H.Lee Moffitt Cancer Center & Research Institute, Tampa, FL*

Mon Poster 52 **Phosphorylation Knowledge Networks Integrated with LINCS Phosphoproteomic and Drug-Target Data to Interpret Cancer Cell Responses to Kinase Inhibitors ;** Karen E Ross[1]; Jake Jaffee[2]; Stephan Schurer[3]; Cathy H Wu[4]; *[1]Georgetown University Medical Center, Washington, DC; [2]Broad Institute of Harvard and MIT, Cambridge, MA; [3]University of Miami, Coral Gables, FL; [4]University of Delaware, Newark, DE*

Mon Poster 53 **Histone H3 Mutations Drive Aberrant Chromatin-Reader Interactions in Diffuse Intrinsic Pontine Glioma;** Dylan M. Marchione; Mariel Coradin; Simone Sidoli; Zuofei Yuan; Benjamin A. Garcia; *University of Pennsylvania School of Medicine, Philadelphia, PA* Monday Lightning Session

Mon Poster 54 **Using Protein Modifications in Serum to Diagnose Patients with Rheumatoid Arthritis;** Stephen Ames; *Brigham Young University, Idaho Falls, ID*

Mon Poster 55 **Reprogramming the SNO-Proteome in the Brain of the Shank3-KO Model of Autism Spectrum Disorder;** Haitham Amal[1]; Vadiraja Bhat[2]; John S Wishnok[1]; Guoping Feng[3]; Steven R Tannenbaum[1, 3]; *[1]Massachusetts Institute of Technology, Cambridge, MA; [2]Agilent Technologies, Wilmington, DE; [3]McGovern Institute for Brain Research, Cambridge, MA*

MONDAY POSTER LIST

Monday posters should be set up 8:00-8:30 am Monday morning and removed at 6:30 pm Monday evening.

Mon Poster 56 **Decoding Site-Specific Alteration of Sialo-glycoproteome in EGFR-Subtype of Non-Small Cell Lung Cancer;** Yi-Ju Chen; Yu-Hsien Lin; Ta-Chi Yen; Kay-Hooi Khoo; Yu-Ju Chen; *Academia Sinica, Taipei, Taiwan* **Wednesday Tips & Tricks**

Mon Poster 57 **Quantitative Phosphoproteomic Analysis Reveals System-wide Signaling Networksin Chronic Lymphocytic Leukemia (CLL) B cells;** Hsin-Yi Wu[1]; Jung-Lin Wu[1]; Shang-Ju Wu[2]; Kuo-I Lin[1]; Yu-Ju Chen[1]; *[1]Academia Sinica, Taipei, Taiwan; [2]National Taiwan University Hospital, Taipei, Taiwan* **Wednesday Tips & Tricks**

Protein Quality Control, Mon Poster 58

Mon Poster 58 **Proteostasis Interactome Remodeling of Amyloidogenic Proteins Governs Improved ER Quality Control Decisions;** Lars Plate[1]; Joseph C. Genereux[2]; R. Luke Wiseman[1]; Jeffery W. Kelly[1]; *[1]The Scripps Research Institute, La Jolla, CA; [2]University of California, Riverside, Riverside, CA* Monday Lightning Session

Proteomics in Ageing, Mon Poster 59

Mon Poster 59 **Investigating the Mechanism of AGE-Mediated Cancellation of Calorie Restriction Benefits;** Richard Carson; Bradley Naylor; John Price; *Brigham Young University, Provo, UT* Monday Lightning Session

Top-Down Proteomics, Mon Poster 60-63

Mon Poster 60 **Two-dimensional Reversed Phase-Reversed Phase Liquid Chromatography for Top-Down Proteomics;** Zhe Wang; Hongyan Ma; Toni Woodard; Si Wu; *University of Oklahoma, Norman, OK* Wednesday Tips & Tricks

Mon Poster 61 **Profiling of Intact Proteins in the CSF of Alzheimer's Disease Patients: Revealing Specific Proteoform Biomarker Candidates;** Gary Kruppa[1]; Jerome Vialaret[2]; Sylvan Lehmann[2]; Audrey Gabelle[2, 3]; Pierre-Olivier Schmit[4]; Christophe Hirtz[2]; *[1]Bruker Daltonics Inc., Billerica, MA; [2]Laboratoire de Biochimie...CHU Montpellier, Montpellier, France; [3]Cent. Mémoire Ressources Recherche,CHU Montpellier, Montpellier, France; [4]Bruker France, Wissembourg, France*

Mon Poster 62 **CE-MS-Based Profiling of BNP Proteoforms from Plasma in One Hour;** Koen Raedschelders; Shenyan Zhang; Jennifer Van Eyk; *Cedars-Sinai Medical Center, Los Angeles, CA* Monday Lightning Session

Mon Poster 63 **Development of an All-Recombinant Intact Protein Standard for LC MS Application Development and System Suitability Testing**; Rosa Viner[2]; Kay Opperman[1]; Daniel Lopez Ferrer[2]; Helene Cardasis[2]; Viktorija Vitkovske[3]; Shane Bechler[2]; Lukas Taujenis[3]; Vikrant Gohil[3]; John Rogers[1]; Egle Capkauske[3]; Kestutis Bargaila[3]; Agne Alminaite[3]; Juozas Siurkus[3]; *[1]Thermo Fisher Scientific, Rockford, IL; [2]Thermo Fisher Scientific, San Jose, Ca; [3]Thermo Fisher Scientific, Vilnius, Lithuania*

MONDAY POSTER ABSTRACTS

Monday posters should be set up 8:00-8:30 am Monday morning and removed at 6:30 pm Monday evening.

Mon Poster 01: Urinary Protein Changes in Walker 256 Tumor-Bearing Rats

Jianqiang Wu[1]; Youhe Gao[1, 2]
[1]Peking Union Medical College, Beijing, China; [2]Beijing Normal University, Beijing, China

Early diagnosis of cancer can significantly improve survival rates for cancer patients. Cancer biomarkers are measurable changes associated with the cancer. Without homeostatic control, urine reflects early changes in the body with a prospect in cancer early diagnosis. In this study, the Walker 256 tumor rat model was established by subcutaneous injection of 2*106 Walker 256 tumor cells into the right armpit. To identify urinary proteome changes during the entire development of cancer, urine samples of walker 256 tumor-bearing rats were collected at five time points corresponding to before cancer cell implant, before tumor mass palpable, tumor mass appearance, tumor rapid growth and cachexia respectively.

The urinary protein patterns on SDS-PAGE changes significantly as tumors progress. Urinary proteins were identified using an Orbitrap-Lumos mass spectrometry by label-free quantitation. Interesting, seven differential urine proteins before tumor mass even palpable could be identified with a fold change >2 and p value <0.05. And these early changes in urine could be also identified at tumor mass appearance, tumor rapid growth and cachexia. Twenty-four differential proteins were annotated before as biomarkers of cancer diseases and nine proteins as biomarkers of breast cancer. Additionally, it was found that those differential proteins were involved in several pathways related to cancer, including IL-6 and IL-12 signaling, production of nitric oxide and ROS and apoptosis. Finally, 30 dynamically changed urinary proteins were selected as more reliable cancer biomarkers, and they were validated by targeted proteomics. Our study suggested that urine is a sensitive biomarker source for early detection of cancer. And systemic changes reflected in urine proteome during cancer progression can improve the understanding of pathophysiological changes of cancer.

Mon Poster 02: Glycan "Nodes" as Cancer Markers: Clinical Performance in Muscle Invasive Bladder Cancer

Shadi Ferdosi[1]; Erik Castle[2]; Thai Ho[2]; Melissa Stanton[2]; Garrick Wallstrom[3]; Chad R. Borges[1]
[1]Biodesign Institute, Arizona State University, Tempe, AZ; [2]Mayo Clinic, Phoenix, AZ; [3]Department of Biomedical Informatics, Arizona State University, Tempe, AZ

Bladder cancer is one of the top ten causes of cancer deaths annually. The 5-year survival for invasive bladder cancer is low, but early identification of invasive tumors can lead to proper selection of patients for curative treatment with combined chemotherapy and surgery. Distinguishing between low-grade non-invasive tumors and high-grade muscle-invasive tumors is critical as treatment is drastically different between the two. To address this unmet need we have designed a novel peripheral blood test based on malignancy-associated glycans that is cost-effective, compatible with standard blood collection tubes, and relies only upon conventional clinical reference lab equipment (GC/MS). Our approach, which we refer to as "glycan node analysis", constitutes the first example of molecularly "bottom-up" glycomics. It is based on a global glycan methylation analysis procedure that is applied to whole blood plasma/serum. The approach quantifies in a single assay over two dozen monosaccharide-and-linkage-specific, pre-defined types of glycan polymer chain links—"glycan nodes"—many of which represent direct 1:1 molecular surrogates for specific glycosyltransferase activities. 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. Pilot data based on 6-sialylation of glycans in blood plasma from 15 muscle-invasive vs. 15 non muscle-invasive bladder cancer patients indicated significant potential utility in distinguishing these cohorts—with a clinical sensitivity of 57% at a specificity of 97%. Validation data have been collected and will be presented from an expanded study of 259 patients from across the spectrum of bladder cancer—ranging from cancer-free to non-invasive bladder cancer to muscle-invasive and metastatic bladder cancer. The ability of multivariate-based glycan node models to define a biosignature of muscle invasive bladder cancer will also be presented.

Mon Poster 03: Limited Tryptic Digestion Isotope Dilution Mass Spectrometry (LTD-IDMS) for Hemagglutinin Stability Assessment in Influenza Vaccines

Keith R Morgenstern[1]; Tracie L Williams[1]; Yingxia Wen[2]; Giuseppe Palladino[2]; Yuhong Xie[2]; Ethan C Settembre[2]; John R Barr[1]
[1]Centers for Disease Control and Prevention, Atlanta, GA; [2]Seqirus, Cambridge, MA

Influenza vaccine potency assays should be able to quantify immunologically active hemagglutinin (HA). The current technique, single-radial immunodiffusion (SRID), does so through measurement of an immunoprecipitin ring that forms when vaccine is added to a strain-specific antibody:agarose gel solution, but requires a lengthy antibody and reference antigen development period. The limited tryptic digestion (LTD)-isotope dilution mass spectrometry (IDMS) method offers a purely biophysical, alternative means to assess vaccine potency. This method, developed with Seqirus, relies on understanding the irreversible conformational change HA undergoes during low pH triggered fusion in the host cell endosome as HA mediates fusion between the cell and viral membranes to enable influenza infection. This conformational change also exposes HA1 region-peptides making them susceptible to protease cleavage. The post-fusion conformation has been recognized in *in vivo* experiments to be significantly less immunologically active than the pre-fusion form. One function of SRID is to differentiate the more active pre-fusion HA from the less active post-fusion HA conformations. Through performing LTD of the sample after transient pH treatment, one can measure HA1 peptide fragments specific for the non-pre-fusion conformation. IDMS results have therefore offered a way to monitor HA stability. This technique, while still in early development stages, offers a potential new method for measuring influenza vaccine potency without the need for strain-specific antibodies.

Mon Poster 04: A Standard Assessment of Extracellular Vesicle Extraction Methods by Targeted Mass Spectrometry

Tingting Wang[1, 2]; Kyle Anderson[1, 2]; Illarion Turko[1, 2]
[1]Institute for Bioscience & Biotechnology Research, Rockville, MD; [2]National Institute of Standards and Technology, Rockville, MD

Extracellular vesicles (EV) are a group of heterogeneous membrane vesicles generated and released by cells to the extracellular environment. They are widely present in body fluids and contain proteins, lipids, and nucleic acids that are involved in cell communication and various pathological processes. The unique yet unclear properties and functions of EV have stimulated studies of their isolation and characterization. However, due to the diversity of the EVs source and the variety of isolation and characterization techniques, no standard practice exists to provide a normative assessment to the current EV analysis methods. The demand for standardization has motivated the evaluation of EV analysis methods in the field.

In this work, we evaluated different EV isolation protocols, which include both conventional (high-speed centrifugation) and commercial (precipitation) EV extraction methods by analyzing the representative EV proteins in human serum and cerebrospinal fluid using targeted mass spectrometry coupled with liquid chromatography. 15N-Labeled EV internal standards were expressed in E.coli and purified with whole gel elution. The relative concentrations of the extracted EV proteins were normalized by the total protein, and the yield and purity were compared with both methods.

Mon Poster 05: Development of a multiplexed assay for oral cancer candidate biomarkers using peptide immunoaffinity enrichment and targeted mass spectrometry

Jau-Song Yu[1]; Yung-Chin Hsiao[1]; Lang-Ming Chi[2]; Kun-Yi Chien[1]; Wei-Fan Chiang[3]; Yi-Ting Chen[1]; Kai-Ping Chang[2]; Yu-Sun Chang[1]
[1]Chang Gung University, Taoyuan, Taiwan; [2]Chang Gung Memorial Hospital, Taoyuan, Taiwan; [3]Chi-Mei Medical Center, Liouying, Taiwan

Oral cancer is one of the most common cancers worldwide, and there are currently no biomarkers approved for aiding its management.

Although many potential oral cancer biomarkers have been discovered, very few have been verified in body fluid specimens in parallel to evaluate their clinical utility. The lack of appropriate multiplexed assays for chosen targets represents one of the bottlenecks to achieving this goal. In the present study, we develop a peptide immunoaffinity enrichment-coupled multiple reaction monitoring-mass spectrometry (SISCAPA-MRM) assay for verifying multiple reported oral cancer biomarkers in saliva. We successfully produced 363 clones of mouse anti-peptide monoclonal antibodies (mAbs) against 36 of 49 selected targets, and characterized useful mAbs against 24 targets in terms of their binding affinity for peptide antigens and immuno-capture ability. Comparative analyses revealed that an equilibrium dissociation constant (KD) cut-off value < 2.82 x 10^{-9} M could identify most clones with an immuno-capture recovery rate > 5%. Using these mAbs, we assembled a 24-plex SISCAPA - MRM assay and optimized assay conditions in a 25-ug saliva matrix background. This multiplexed assay showed reasonable precision (median coefficient of variation, 7.16 to 32.09%), with lower limits of quantitation (LLOQ) of <10, 10–50, and >50 ng/ml for 14, 7 and 3 targets, respectively. When applied to a model saliva sample pooled from oral cancer patients, this assay could detect 19 targets at higher salivary levels than their LLOQs. Finally, we demonstrated the utility of this assay for quantification of multiple targets in individual saliva samples (20 healthy donors and 21 oral cancer patients), showing that levels of six targets were significantly altered in cancer compared with the control group. We propose that this assay could be used in future studies to compare the clinical utility of multiple oral cancer biomarker candidates in a large cohort of saliva samples.

Mon Poster 06: Multiplexed kinase biosensor technology and SWATH-MS for monitoring chronic myelogenous leukemia (CML) signaling

Tzu-Yi Yang; Laurie Parker
University of Minnesota, Minneapolis, MN

The development of tyrosine kinase inhibitors (TKIs) have revolutionized strategies for treating 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. In treating CML, it has been shown 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.

The goal of this project is to develop a technique for measuring patients' response to TKI treatments by investigating kinase profiles in cancer cells. Artificial peptide biosensors that are specific substrates of Abl, Syk, JAK2, and Src family kinases were used to probe kinase activity in model cell lines and CML patients' mononuclear cells. The biosensors are comprised of peptide sequences that serve as surrogate kinase substrates and a cell penetrating sequence that drives the biosensors into cells. Phosphorylation levels of the peptide biosensors were examined using multiple reaction monitoring and/or SWATH-MS. Additionally, we examine the protein expression profile in patient samples.

Our data suggest that multiplexed biosensors can be used for monitoring activity of kinases in primary samples. We are able to detect Bcr-Abl activity and inhibition by imatinib in the human CML cell line K562. MRM enabled reproducible detection of the peptide biosensor at fmol levels. While specific culture conditions, reproducibility and precision need to be further optimized, our data suggested that SWATH-MS has the potential of providing a comprehensive way of studying the machineries involved in various biological processes. Future directions will be to profile proteome signatures for CML patients pre- and post- treated with imatinib using SWATH-MS.

Mon Poster 07: High throughput and highly multiplexed 73 proteins MRM assay with accuracy: Combination of automated sample preparation and scheduled MRM analysis

Qin Fu; Mitra Mastali; Irene van den Broek; Vidya Venkatraman Venkatraman; Jennifer E Van Eyk
Cedars Sinai Medical Center, Los Angeles, CA

Liquid chromatography (LC) coupled with selected reaction monitoring mass spectrometry (SRM-MS) allows precise quantitation of proteotypic peptides (as surrogates for the corresponding protein) in 10s to 1000s of plasma samples. However, throughput, reproducibility, time, and cost remain longstanding barriers to the necessary large-scale MS based protein quantification. We developed a highly multiplexed MRM analysis in a single scheduled LC MS/MS run targeting 73 plasma proteins with important biological functions and pathways including: acute phase responses (35 proteins); cholesterol and fat metabolism (32 proteins), coagulation systems (13 proteins); atherosclerosis signaling (16 proteins), complement systems (13 proteins), productions of nitric oxide and reactive oxygen species (16 proteins), Intrinsic Prothrombin Activation Pathway (6 proteins), hematopoiesis from pluripotent stem cells (3 proteins) and other biological functional pathways. Sample preparation for proteomic analysis of complex biological samples by mass spectrometry is a tedious and time-consuming process with many steps where technical variations can be introduced and propagated. We optimized an automated trypsin digestion workflow that yields uniformly-processed 96 samples in less than 5 hours. 90% of the transitions had total CVs below 20%, as measured from six replicate automated sample preparations on three independent days. Our reproducible quantitation of hundreds of peptides from numerous proteins was seen across replicates and days, demonstrating the broad applicability of this approach. In addition, with only minor adjustment in the automated sample preparation protocol, the highly multiplexed MRM method was applicable to 10 µL dried blood from volumetric absorptive microsamplers (Mitra®) with similar performance characteristics as plasma. We then evaluate 73 proteins expressions with the fast, highly accurate, and completely hands-free MS protein sample preparation workflow in a chronic kidney disease plasma cohort. The results of 73 proteins expression profile in controls and diseased plasma (with two different time points) will be presented.

Mon Poster 08: A Robust and Multiplexed ImmunoMRM Assay for Relative Quantitation of Key Phosphopeptides in the DNA Damage Response (DDR) Pathway

Si Mou[1]; Lei Xiong[1]; Jeffery Whiteaker[2]; Lei Zhao[2]; Yihan Li[1]; Amanda Paulovich[2]; Hua-Fen Liu[1]; Christie Hunter[1]
[1]SCIEX, Redwood City, California; [2]Fred Hutchinson Cancer Research Center, Seattle, WA

Robust quantitative tools for studying DDR pathway are in high demand due to the importance of this pathway in cancer research. Current methods using antibody detection such as ELISA or Western Blots are sensitive and high-throughput but suffer from potential interferences or lack of multiplexing capability. ImmunoMRM assays rely on antibodies for enrichment but use mass spectrometry detection for high peptide specificity. Coupled with the ease of multiplexing and internal standard creation, ImmunoMRM provides a great strategy for quantitative protein/peptide assays. However lack of commercially available reagents make these assays inaccessible to the majority of researchers. Here, we describe the key steps involved in developing a full ImmunoMRM kit for quantifying key phosphopeptides in the DDR pathway (47 peptides in a single multiplexed assay) with the goal of making this assay more broadly available to researchers.

The total workflow from sample preparation to LC-MS/MS analysis and data processing has been extensively optimized and integrated with the explicit goal of removing key user pain points. First, a full reagent kit for ImmunoMRM sample preparation has been developed; each individual component from digestion reagents to capture antibodies has been extensively optimized for good performance and stability in kit format, including storage stability and ease of automation. Studies were performed to simplify and validate performance of each protocol step including antibody-bead conjugation, digestion, immuno-capture efficiency and recovery. In addition, the LC-MRM conditions have been adapted for microflow LC and the data processing pipeline has been established to further

simplify the full workflow. Robustness of the assay was verified by analyzing multiple biological samples across multiple laboratories. This validated, multiplexed ImmunoMRM assay for the DDR pathway has been demonstrated to be highly robust and easily adaptable into multiple research labs for targeted quantitation of key phosphopeptides from important proteins in the DDR pathway.

Mon Poster 09: Improving Depth of Coverage of SWATH® Acquisition using SWATH Xtend to Build Better Libraries

Christie Hunter[1]; Sara Ahadi[3]; Jemma Wu[2]; Mark Molloy[2]
[1]SCIEX, Redwood City, California; [2]APAF, Sydney, Australia; [3]Stanford University, Stanford, CA

Great improvements have been made to the depth of quantitative coverage obtained in data independent acquisition experiments over the last few years through the improvements in both hardware and acquisition strategies. The variable Q1 window acquisition strategy has provided the biggest improvements in results, allowing small Q1 windows for specificity but maintaining broad m/z coverage, for an overall data quality improvement. To fully interrogate the dataset however, deep libraries are needed which require extensive sample fractionation and multiple LC-MS/MS acquisitions. Database search algorithms are used to process the data to combine the peptides identifications into a single ion library, however they typically do not handle retention time alignment. Here, we demonstrate how SWATH Xtend1 can be used to combine multiple fractionation experiments into a single ion library for improved SWATH coverage.

Protein fraction from peripheral blood mononuclear cells (PBMCs) was digested and fractionated using multiple strategies (SCX, high pH, 1D LC) then processed by LC-MS/MS on TripleTOF® 6600 System. The individual fractionation experiments were processed individually using ProteinPilot™ Software. 1D SWATH acquisition and IDA experiments were also performed on the same sample and were used as the retention time (RT) context (SEED library). Using the cloud implementation of SWATH Xtend in the OneOmics™ Project, each of the fractionated libraries was added to the SEED library and RT alignment was performed to convert the added library to the original RT context. The resultant library was compared to a library were all fractions were processed in one large database search (no RT alignment). We observed almost double the number of proteins and peptides that were reproducibly quantified from a SWATH datafile using SWATH Xtend. This new application provides an easy way to build larger libraries for deeper SWATH quantitative experiments.

1 Wu et al,. (2016) Mol Cell Prot 15(7) 2501-14.

Mon Poster 10: TargetedMSQC: an R package for quality control of chromatographic peaks in targeted proteomics

Shadi Toghi Eshghi; Qingling Li; Paul Auger; Kristin Wildsmith; Veronica Anania; W. Rodney Mathews
Genentech, Inc., South San Francisco, CA

Recent advancements in the field of targeted proteomics and mass spectrometry have significantly improved assay sensitivity and multiplexing capacity. The high-throughput nature of targeted proteomics experiments results in an increase in the rate of data production which requires development of novel analytical tools to keep up with data processing demand. Currently, development and validation of targeted mass spectrometry assays require manual inspection of chromatographic peaks from large datasets to ensure quality, a process that is time consuming, prone to inter- and intra-operator variability and limits the efficiency of data interpretation from targeted proteomics analyses. To address this challenge, we are developing TargetedMSQC, an R package that facilitates quality control and verification of chromatographic peaks from targeted proteomics datasets. This tool calculates metrics to quantify several quality aspects of a chromatographic peak, e.g. symmetry, jaggedness, and co-elution and shape similarity of monitored transitions in a peak group, as well as the consistency of transitions' ratios between endogenous analytes and isotopically labeled internal standards. The algorithm takes advantage of machine learning to identify peaks with interference or poor chromatography. Using TargetedMSQC to analyze targeted proteomics data significantly reduces the time spent on manual inspection of peaks and improves both speed and accuracy of interference detection. In addition, the scoring system is designed to explicitly state the underlying cause for peak quality failure, thus simplifying the troubleshooting process. Furthermore, automated and quantitative assessment of peak quality offers a more objective and systematic framework for high throughput analysis of targeted mass spectrometry assay datasets. In summary, this open source tool improves data interrogation efficiency and facilitates automated peak inspection for targeted proteomics data analysis and is a step towards more robust and faster assay development and implementation.

Mon Poster 11: A kinetic proteomics approach to identify targets of autophagy in cancer and recover chemotherapeutic effects

Monique Paré Speirs; Bradley Naylor; John C. Price
Brigham Young University, Provo, UT

The majority of metastatic cancers develop chemoresistance. These chemoresistant cancers often use autophagy, an alternative protein degradation system, to modify cellular metabolism and overcome the chemotherapy. It is well known that increased autophagy in cancer facilitates rapid protein turnover and cell fitness. However, the contributions of autophagy to chemoresistance are unresolved. Pharmacological regulation of autophagy is a potential strategy to counteract chemoresistance mechanisms and sensitize tumors to traditional treatments. Current clinical trials are attempting to control autophagy flux using nonspecific inhibitors such as chloroquine (CQ) with limited knowledge of the mechanisms and side effects of these drugs. We used stable isotopic labeling and mass spectrometry to measure time dependent changes in autophagy substrate turnover *in vitro* and in an HCT-116 xenograft mouse model. Recent studies and our preliminary *in vitro* data suggest that the autophagy substrate selection process is an important component of chemoresistance. Our *in vivo* data suggest that specific protein classes are significantly more sensitive to CQ-induced autophagy inhibition compared to other cytoplasmic components in HCT-116 xenograft tumor cells. Understanding which proteins are selectively degraded in the tumor and neighboring tissues will help us delineate cancer pro-survival mechanisms and consider ways to exploit autophagy as a tool for expanding the capacity of chemotherapeutics.

Mon Poster 12: LinkedOmics : A web-based platform for cancer multi-omics data integration and comparison

Suhas Vasaikar[1]; Peter Straub[2]; Jing Wang[1]; Bing Zhang[1]
[1]Baylor College of Medicine, Houston, Texas; [2]Vanderbilt University, Nashville, TN

Background

With the development of high-throughput technologies, massive and complex omics such as genomic, epigenomic, transcriptomic and proteomic data are increasingly being generated, which provide a comprehensive view for characterizing cancer mechanism. However, these heterogeneous data are not easily or directly available to the biologists and high dimensionality of the large-scale data increases difficulty level for extracting biological insights. Thus, we developed a platform LinkedOmics (http://aws2.zhang-lab.org/linkedomics/) that used the association analysis to help cancer biologists and clinicians effectively explore the relationships between the more than 100,000 attributes from cancer multi-omics data and yield novel biological and clinical insights.

Results

Currenlty, LinkedOmics includes three modules LinkFinder, LinkInterpreter and LinkCompare, as well as 1,620,049 attributes from genomic, epigenomic, transcriptomic, proteomic, and clinical data for the breast, colorectal and ovarian tumors of The Cancer Genome Atlas (TCGA) and the Clinical Proteomics Tumor Analysis Consortium (CPTAC) portal and multi-omics data of other 32 cancer types from TCGA portal. LinkFinder allows users to search for attributes that are associated with a query attribute, which can perform eQTL/pQTL analysis for copy number alteration data, mutation signature identification for mutation data, biomarker identification for clinical data, regulated target gene identification for microRNA data and other

analyses. The analysis results can also be plotted by scatter plot, box plot or Kaplan-Meier plot. To explore biological insights from the association results, LinkInterpreter performs the enrichment analysis based on the gene ontology, pathway, network module, phenotype, disease and drug and chromosomal location data. The LinkCompare uses visualization function and meta-analysis to compare and integrate association results generated by the LinkFinder module, which can easily perform multi-omics analysis in one cancer type or pan-cancer analysis.

Conclusion

LinkedOmics provides a unique platform for cancer biologists and clinicians to access, analyze and compare high-dimensional cancer multi-omics data.

Mon Poster 13: Time-resolved quantitative analysis of cellular signaling and epigenetic mechanisms during epithelial-mesenchymal transition

Congcong Lu[2, 3]; Simone Sidoli[3]; Zuofei Yuan[3]; Benjamin A. Garcia[1, 2]

[1]Epigenetics Program, Philadelphia, PA; [2]Department of Biochemistry and Molecular Biophysic, Philadelphia, PA; [3]University of Pennsylvania, Philadelphia, PA

As an important step in cancer progression, epithelial-mesenchymal transition (EMT) goes along with dramatic changes in cellular morphology, the loss and remodeling of cell-cell and cell-matrix adhesions, and the gain of migratory and invasive capabilities. There are several signaling pathways that may induce EMT and phosphorylation of proteins is considered to play a predominant role in those pathways. However, how transcriptional regulatory signaling, epigenetic proteome level networks combine to regulate this process are still not well understood. Here, we use TGF-β to induce EMT in mouse mammary epithelial cells. A systems-level characterization of phosphoproteome and proteome dynamics during EMT was studied by stable isotope labeling with amino acids in cell culture (SILAC)-based quantitative analysis. In total, we detected 3492 proteins, and fifteen percent were up-regulated significantly at least 2-fold by TGF-β after 2 days. 7272 phosphorylation sites on 2303 proteins were identified, where seventeen percent protein had multiple phosphorylation sites (more than 4). We analyzed phosphoproteomic and proteomic changes across six time points after TGF-β stimulation (0 min, 5 min, 30 min, 60 min, 1 day and 2 days) and clustered trends in abundance using fuzzy c-means with at least four values and ANOVA test smaller than 0.05. GO analysis showed that nuclear proteins were more enriched in the short-term (5 min) increase of phosphorylation sites, while mitochondrial proteins in short-term increase of protein. And cytoskeletal proteins were enriched in both long-term (2 days) increase of phosphoproteome and proteome. In addition, we investigated histone modification changes by label free quantification. A significant increase of histone trimethylation (H3K27me3 and H4K20me3) was found during EMT. Our data provide a detailed time resolved resource of proteome, epi-proteome and phosphoproteome dynamics during EMT, which improve our understanding of dynamic cell signaling networks and how they contribute to tumor progression.

Mon Poster 14: Unambiguously and comprehensively resolving cancerous adenocarcinoma and stromal proteomes and their interactive signaling without cell sorting in patient-derived xenograft models

Shichen Shen[1]; Jun Li[1]; Ninfa L. Straubinger[1]; Xue Wang[2]; Michelle K. Greene[4]; Christopher J. Scott[4]; Wen Wee Ma[3]; Robert M. Straubinger[1]; Jun Qu[1]

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Patient-derived xenograft (PDX) models recapitulate faithfully the characteristics of diseases such as pancreatic adenocarcinoma (PDAC), because the tumor cells reside in their stromal microenvironment, and represent an excellent tool for preclinical development of diagnostic/treatment biomarkers and therapeutic targets. However, analyzing the PDX proteome is complex owing to the difficulty in determining the origin of protein changes (i.e., cancer vs stromal cells). Here we describe a novel proteomics approach to quantify unambiguously, in situ, individual cancer and stromal proteomes. SCID mice bearing PDAC PDX tumor #18269 were treated with standard-of-care drugs gemcitabine (Gem) and nab-paclitaxel (nPTX), alone or combined. Tumors were procured 24h or 192h post-treatment (n=4/group, 48 in total). An in-house proteomics pipeline, IonStar, was employed to allow quantification with excellent accuracy/precision, and extremely low missing data and false-biomarker-discovery rate. Because PDAC cells are human and stromal cells are murine, we developed a suite of bioinformatics methods providing unambiguous, species-specific protein quantification based on origin-unique domains, e.g. selective shared-peptide removal and data quality control based on multivariate mean-variation modeling. Despite the high sequence homology between the two species, we achieved the quantification of 4,516 species-unique proteins under stringent cutoffs, with excellent quantitative precision (~5% intra-group CV%) and extremely low-missing values (<0.2%) among the 48 biological replicates. Distinct and complementary signaling pathways implicated in treatment response were attributable to cancer cells vs. stroma. A panel of biomarker candidates was suggested with high confidence for early detection of treatment effects (@24h) and/or tumor recurrence (@192h),, e.g. CLIC4, IMon PosterAD1, FAM3C, MSMO1, AKAP1, and TMEM30A. Key findings were validated with immunoassays. Overall, this study provides a generic method to investigate a mixed-origin system such as PDX, which permits unambiguous, in situ study of target cells vs. environment interaction without cell sorting and thus is highly valuable for pre-clinical studies.

Mon Poster 15: A 3-D Proteome Atlas is an important component to the Cancer Moonshot

Kat Tiemann; Carolina Garri; Ruth Alvarez; Sang Bok Lee; Jonathan Katz; Kian Kani

USC, Los Angeles, CA

In our effort to address the Cancer Moonshot's Blue Ribbon Panel recommendation to develop methods of overcoming resistance to therapy, we constructed a 3-D proteomic atlas which helped reveal the significance of cellular localization of a key proto-oncogene and cancer biomarker, Anterior Gradient 2 (AGR2).

AGR2 has been shown to be involved in cell transformation (proto-oncogene), drug resistance, and metastasis. AGR2 has also been characterized as a biomarker because its expression is elevated in virtually all adenocarcinomas. Surprisingly however, some cancer patients with increased expression of AGR2 have a favorable prognosis. To resolve this apparent paradox, we investigated the significance of AGR2 localization.

AGR2 has structural motifs that are conserved in a class of Endoplasmic Reticulum (ER) proteins known as protein disulfide isomerases, and its biology has therefore been studied in the context of chaperonins. However, AGR2 also has biological activity as a secreted protein (sAGR2) and has been detected in high abundance in plasma and urine of metastatic cancer patients. Although there is a clear correlation between sAGR2 levels and the occurrence of metastatic disease, the phenotypes associated with AGR2 biology have not been studied in a context that can discriminate between the role of the ER versus secreted protein.

In order to identify the effects of cellular localization on AGR2 expression and patient prognosis, we utilized a combination of interactome mapping technologies (BioID and AP-MS/MS) to create a 3-D proteome atlas. The AGR2 interactome revealed novel pathways that are differentially regulated based on the localization of AGR2. As a result we were able to demonstrate that the secreted versus ER resident protein have opposing effects on response to chemotherapy and cancer metastasis *in vivo*.

This talk will summarize our findings and provide a framework for the importance of a 3-D proteome atlas as part of the Cancer Moonshot.

Monday posters should be set up 8:00-8:30 am Monday morning and removed at 6:30 pm Monday evening.

Mon Poster 16: Analysis of blood plasma suggests tumor re-seeding may be facilitated by wound creation during radiotherapy treatment of breast cancer patients

Catherine Going; Marta Vilalta; Marjan Rafat; Melissa Jenkins; Jie Jane Chen; Kathleen Horst; Edward Graves; Sharon Pitteri
Stanford University, Palo Alto, CA

Radiotherapy (RT) is an essential component of breast cancer treatment and is often combined with chemotherapy. It has been observed that RT of a primary tumor recruits circulating tumor cells (CTCs) back to the primary tumor, leading to cancer recurrence after treatment, but very little is known about the molecular mechanism of this tumor re-seeding. In this study, we performed the first quantitative analysis of changes induced by RT in the levels of low-abundance proteins in the blood plasma of breast cancer patients with the aim of discovering pathways implicated in CTC migration and cancer recurrence after RT.

Blood plasma was collected from breast cancer patients prior to, during, and after RT. Samples were depleted of abundant plasma proteins, labelled with either light (prior to RT) or heavy (after RT) C12/C13 acrylamide, and fractionated by RP-HPLC before trypsin digestion and analysis by LC-MS on a Thermo LTQ-Orbitrap Elite mass spectrometer. Peptide fragmentation was performed using CID, and the ratio of heavy-to-light (H/L) labeled peptides was used to determine whether a protein increased (H/L > 1.5) or decreased (H/L < 0.67) in abundance after RT.

In preliminary analysis, approximately 700 proteins were identified in each sample, with quantitation obtained for around 50% of these proteins. During treatment with RT, an increase in GPX3, PRDX2, and PRDX6 was observed due to catabolism of hydrogen peroxide created during radiation. Also observed were several signatures of wound healing, such as increases in immunoglobulins, FCN3, and MBL2, and decreases in structural proteins such as ZYX, CCN3, ACTC1, FLNA, FGB, and COL1A1, facilitating tissue migration during wound healing. Creating a wound at the tumor site with RT over the course of several weeks of treatment may create the right environment for CTCs to migrate back to the original tumor site and re-seed the tumor.

Mon Poster 17: Quantitative Glycoproteomic Profiling of Prostate Cancer Tissue Reveals Alterations in Glycosylation Patterns in Men with Recurrent Disease

Sarah M. Totten; Cheylene Tanimoto; Abel Bermudez; Amy Hembree; James D. Brooks; Sharon J. Pitteri
Stanford University School of Medicine, Palo Alto, CA

The primary goal of this study was to identify and quantify aberrancies in N-glycosylation patterns and N-linked glycoprotein levels expressed in high grade prostate cancer tissue from men with and without recurrence following radical prostatectomy using multi-lectin affinity chromatography and tandem mass spectrometry techniques. Glycosylation is a common and highly complex posttranslational modification, and plays a role in regulating cellular functions. Aberrant glycosylation has been observed in serum and tissue samples with various types of cancer, demonstrating that alterations in glycosylation can be disruptive to cellular activity. Furthermore, glycan characterization can lead to more effective biomarkers with increased clinical utility. In this study, multi-lectin affinity chromatography and advanced tandem mass spectrometry were used to identify thousands of glycoproteins in fresh-frozen prostate tissue. Proteins were extracted from cancer tissue and matched normal tissue from ten donors, five of which experienced recurrence after surgery. Isotopic labels were incorporated during alkylation for quantitation. Extracted proteins were separated on a multi-lectin affinity column packed in-house with lectins selected to specifically capture proteins with sialylated (SNA/MAL), core-fucosylated (AAL), and highly branched complex-type (PHA-E/L) glycan moieties. Each lectin fraction was eluted separately to retain four discrete fractions containing specific glycoforms. LC-MS/MS was used to analyze the tryptic digest from each fraction for protein identification and quantitation. Glycosylation patterns across thousands of prostate tissue proteins and their glycoforms were systematically screened. Preliminary results reveal the identification of 6,202 unique proteins, and quantitation of 2,894 proteins, most of which contained sialylated glycoforms. In all patients (regardless of treatment outcome), several hundred proteins, including tumor-associated calcium transducer 2 (TACD2) and lipoma-preferred partner (LPP), had elevated total protein levels, while other proteins demonstrated upregulation only among certain glycoforms found in specific M-LAC fractions. Other glycoproteins were up-regulated in cancer only in recurrent patients, including CPNE1, TXND5, and CASC4.

Mon Poster 18: Global quantitative phosphoproteomics to identify mechanisms of resistance to the third generation EGFR TKIs in human lung adenocarcinoma

Xu Zhang[1]; Tapan K. Maity[1]; Karen E. Ross[2]; Stephanie C. Pitts[1]; Cathy Wu[3]; Udayan Guha[1]
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Lung adenocarcinoma patients with activating EGFR mutations initially exhibit dramatic response to the first or second generation EGFR TKIs. However, they eventually develop resistance. The most common mechanism is a secondary T790M mutation. Rociletinib and osimertinib are two novel, irreversible, mutant-selective third generation EGFR TKIs that specifically target the mutant forms of EGFR, including T790M. We sought to identify novel mechanisms of resistance to the third generation EGFR TKIs using SILAC-based quantitative mass spectrometry to quantitate the proteome and phosphoproteome.

H1975, the parental lung adenocarcinoma cell line, expresses the most common acquired resistance mutation, T790M. Isogenic resistant cells to both rociletinib (COR1, COR10) and osimertinib (AZR3, AZR4) have been developed from H1975 using a stepwise increase in TKI treatment over several months. SILAC mass spectrometry was performed after basic reverse phase fractionation and TiO2 enrichment of tryptic peptides of mixed lysates to elucidate the differences between the TKI-sensitive and -resistant cells with and without drug treatment. Data analyses was performed using MaxQuant, Proteome Discoverer and PEAKs software algorithms.

Overall, we identified 5700 proteins, and 10298 phosphosites, including 8248 pS sites, 1560 pT sites and 490 pY sites. 4018 class I phosphosites were quantified and the ratios of phosphorylation changes were normalized based on the protein ratio in the same experimental condition. Surprisingly, there was a greater degree of inhibition of phosphorylation in the resistant cells. Experimentally validated kinases for the differentially phosphorylated sites between sensitive and resistant cells were identified using iPTMnet v3.1. Protein kinase A and AKT1 were significantly more likely to be associated with hyperphosphorylated sites ($p < 0.05$); conversely, the cyclin-dependent kinases CDK2 ($p < 0.05$) and CDK1 ($p < 0.1$) were more likely to be associated with hypophosphorylated sites, suggesting drug-resistance may be associated with increased protein kinase A or AKT activity and decreased CDK activity.

Mon Poster 19: Mapping the Surfaceome of Plasma Cells Reveals Novel Immunotherapy Targets for Multiple Myeloma

Nathan Schuld[1]; Ensaf Al-Hujaily[2]; Parameswaran Hari[3]; Jeffrey Medin[1, 2]; Rebekah Gundry[1, 4]
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Multiple myeloma (MM) is a cancer of the plasma cell whereby diseased plasma cells can form masses in the bone marrow and produce aberrant antibodies. MM disease progression involves several genetic mutations and results in the production of several MM clonal populations. This heterogeneity, in addition to the complex tumor microenvironment that contains closely related hematopoietic derivatives (T-cells, B-cells, etc.), poses challenges to the treatment of MM. Although immunotherapy is a promising approach, robust and specific antigens that mark MM cells for destruction by immune system cells are not yet defined. To address this need, the current study uses mass spectrometry to identify and quantify extracellular epitopes of cell surface proteins on MM and closely related cell types

to reveal candidates that may be informative for the MM phenotype and targeted by immunotherapy or antibody-drug conjugates. In the first round of discovery, the Cell Surface Capture Technology (CSC-Technology) was used to identify extracellular domains of >400 cell surface N-glycoproteins present on four MM cell lines (U226, R5, RPMI 8226, MM.1R) and two B-lymphoblast (BLCL, RPMI 1788) cell lines (n=3). Subsequently, bioinformatic analyses exploited a large repository of CSC-Technology data generated from >60 human cell types to determine which proteins are commonly observed among many cell types vs. restricted to a few (i.e. blood cancer) cell types. Also, hierarchical clustering was used to reveal combinations of markers that may be informative for distinguishing among closely related cell types. Altogether, these analyses revealed >30 candidates, of which three are uniquely identified in the MM cell types examined. Current efforts use parallel reaction monitoring to quantify these candidates among a larger cohort of diseased and non-diseased blood cell types, including primary cells, to further refine the candidate list to proteins that may be informative for the MM phenotype and targeted by immunotherapy.

Mon Poster 20: Proteomic and lipidomic analyzes reveal significant lipid metabolism alteration in colon cancer cells

Lili Niu[1]; Tanxi Cai[2]; Ying Zhang[1]; Fuquan Yang[2]

[1]Huazhong University of Science and Technology, Wuhan, China; [2]Institute of Biophysics, CAS, Beijing, China

Metabolic reprogramming is now firmly established as a hallmark of cancer. Alterations in lipid profiling and lipid metabolism pathways encountered in tumors, which were commonly disregarded in the past, are also well recognized and more frequently described now. In the present study, integrative lipidomic and proteomic analyzes were employed to systematically assess the lipid metabolism in colon cancer cells (HT-29 and HCT-116), as well as in FHC, which served as normal cells. Our lipidomic profiling has revealed significant lipid changes at the class and molecular species levels in colon cancer cells. Firstly, elevated both phospholipids and triacylglycerols in the total content were observed in cancerous cells. Meanwhile, 18 phospholipid species were found to be strongly associated with colon cancer progression based on a partial least-squares discriminant analysis model. The resultant was further validated using cancerous tissues and uninfected tissues obtained from the same clinical patients. 13 out 18 species were detected and quantified in tissues and displayed accordant changes. Furthermore, a significant up-regulation of ceramides with different acyl chain lengths (C16-, C22-, C24-, C26- ceramides) was also observed in cancer cells. In consistence, the proteomic data indicates several lipid metabolic pathways, such as fatty acid biosynthesis, glycerophospholipid metabolism, sphingolipid metabolism, as well as lipid-induced apoptosis, were found to be dynamically changed. Our results provide a valuable inventory of lipids and proteins produced during tumor development and may contribute to unravel new potential lipid-related targets for drug development or new treatments.

Mon Poster 21: Method Development and Evaluation of the Protein Phosphatase 2 Phosphoproteome Using the Chip iFunnel QTOF Platform

Paulos Chumala[1]; Vadiraja Bhat[2]; Chelsea Cunningham[1]; Brooke Thompson[1]; Frederick Vizeacoumar[1]; Franco Vizeacoumar[1]; George Katselis[1]

[1]College of Medicine, University of Saskatchewan, Saskatoon, Canada; [2]Agilent Technologies, Wilmington, DE

Protein Phosphatase 2 is a complex playing an important role in control of cell cycle, apoptosis, and cell-fate determination. PP2A is a trimeric enzyme consisting of catalytic, scaffolding, and regulatory subunits. Regulatory subunits provide specificity to substrate interactions and allow the complex to regulate the number of different pathways. Initially reported as tumor suppressor, recent studies have found that PP2A individual subunits have context specific oncogenic or tumor suppressor function. Recent evidences have shown that targeting specific subunits of that complex has the potential to overcome tumor heterogeneity. To gain mechanistic insight in PP2A substrates, we are developing MS-based phosphoproteomics methodologies to identify and characterize substrates of the individual holoenzyme. Methodologies are developed using the Chip iFunnel QTOF platform. HCT116 colorectal cells are grown in light and heavy SILAC media, cells mixed, lysed, and proteins are digested in-solution. Enrichment of phosphorylated peptides is done using a) TiO2 beads and b) high-select Fe-NTA and analyzed by LC-MS/MS. Data are analyzed by MassHunter Bioconfirm software and protein identification is done on SpectrumMill searching against SwissProt Human database. A gene ontology analysis is performed to characterize cellular and functional processes of proteins using Mon PosterP and Pathways Architect Software. There are two isoforms of the catalytic subunit, two isoforms of the scaffolding subunit, and twelve isoforms of the regulatory subunits. We knockdown (using shRNA) as well as knockout (using CRISPR) individual subunits and generate profiles of candidate proteins that are functionally modified. We currently use PPP2R5D as model gene to develop different methodologies and 2,110 unique human proteins (360 proteins containing at least one phosphopeptide) have been identified. Work is under way to enhance phosphopeptide presence. To quantify and compare phosphoproteins between different genes, we are growing cells in SILAC media and proteins will be evaluated using the phosphoproteomic method developed during qualitative analysis.

Mon Poster 22: Multi-omics analysis for the validation of differential transcripts in PAX8 overexpressed MOSE cells

Melissa R. Pergande[1]; Laura M. Rodgers[1]; Vadiraja Bhat[2]; Carol Haney-Ball[2]; Joanna E. Burdette[1]; Stephanie M. Cologna[1]

[1]Un of Illinois at Chicago, Chicago, IL; [2]Agilent Technologies Inc, Santa Clara, CA

Introduction: Multi-omics approaches can be useful in understanding disease pathogenesis including gene pathway/network relationships and regulation. Mouse ovarian surface epithelial (MOSE) cells engineered to overexpress paired box 8 (PAX8) can be used as a model to understand essential proteins in ovarian cancer. In this study, we obtained candidate transcripts markers via RNA sequencing technology, which were significantly changed upon overexpression of PAX8. Additionally, a quantitative proteomics study was performed to validate transcriptional changes, identify new biomarkers, and to reveal mechanistic insight and pharmacological targets for ovarian cancer.

Methods: RNA sequencing was performed on isolated mRNA from MOSE cells. Quantitative proteomics was performed using isobaric tags (iTRAQ 8-plex) where samples were fractionated and analyzed on an iFunnel QTOF interfaced to a chipcube system. Differential proteome changes were subsequently compared to a list of candidate transcripts predicted to be either up or downregulated.

Results: RNA-sequencing revealed 4259 differentially expressed transcripts ($p < 0.05$) while our proteomics study resulted in a total of 3110 identified proteins and 228 differentially expressed proteins (≥ 2 fold, $p \leq 0.01$). Analysis of differentially expressed transcripts and proteins revealed 123 overlapping genes. Among them, Vimentin, Hexokinase 2 and Cadherin 2 were increased and Collagen alpha-1, Annexin A2 and Heat shock 70 protein were decreased in response to PAX8 overexpression.

Conclusions: We have performed a complementary transcriptomic and proteomic analysis in order to evaluate the differential proteome of PAX8 over expressing cells that give rise to ovarian cancers. Further, we validated differential transcripts predicted to be either up or downregulated which may represent multiple pathways and biological networks associated with the disease progression in ovarian cancer. Our future work will be focused on a dynamic proteomic profiling of both the secreted and intracellular proteins via SILAC in order to validate additional differential transcripts.

MONDAY POSTER ABSTRACTS

Monday posters should be set up 8:00-8:30 am Monday morning and removed at 6:30 pm Monday evening.

Mon Poster 23: P-MartCancer-Interactive Online Software to Enable Biomaker Discovery from Shotgun Cancer Proteomic Datasets

Bobbie-Jo Webb-Robertson; Lisa Bramer; Jeffrey Jensen; Markus Kobold; Kelly Stratton; Amanda White

Pacific Northwest National Laboratory, Richland, WA

For the field of clinical proteomics to successfully identify new prognostics or diagnostic markers of disease or response to therapies requires both high quality data and statistical analysis of the data. Obtaining robust cancer biomarker candidates from global shotgun mass spectrometry (MS)-based proteomics experiments remains a challenge. In particular, the processing of these large and complex datasets in a manner that accounts for appropriate quality control processing and data issues, such as missing values, to assure the most robust statistical results is essential. P-Mart is a new interactive web-based software environment that enables biomedical and biological scientists to perform in-depth analyses of global proteomics data without requiring knowledge of pre-processing complex matrices of peptide abundances or direct interaction with the data. P-Mart offers a series of statistical modules associated with quality assessment, peptide and protein statistics, protein quantification and exploratory data analyses. Currently, P-Mart offers access to multiple cancer proteomic datasets generated through the Clinical Proteomics Tumor Analysis Consortium (CPTAC) at the peptide, gene and protein levels. Analyses are performed in P-Mart via customized workflows and interactive visualizations. P-Mart is deployed using Azure technologies (http://pmart.labworks.org/). The web-service is also available via Docker Hub and the statistical functions can be utilized directly from a package available on GitHub. P-Mart is funded through the National Cancer Institute's Informatics Technology for Cancer Research (ITCR) program under grant U01-CA184738-01.

Mon Poster 24: Mass Spectrometry-based Proteomic and PTM Studies Provide Insight into the Molecular Mechanisms of Restenosis

Matthew Glover[1]; Qing Yu[1]; Bowen Wang[1]; Xudong Shi[1]; Lian-Wang Guo[1]; K. Craig Kent[1, 2]; Lingjun Li[1]

[1]University of Wisconsin-Madison, Madison, WI; [2]The Ohio State University, Columbus, OH

Atherosclerosis and associated diseases are the leading cause of death in the United States. However, a significant percentage of surgical interventions to open occluded arteries eventually fail due to renarrowing of the vessel, known as restenosis. In order to further understand the molecular mechanisms of restenosis and ultimately identify novel therapeutic targets, we are developing mass spectrometry (MS)-based strategies to perform large-scale quantitative proteomic and post-translational modification (PTM) studies of both *in vivo* and *in vitro* models of restenosis. By combining filter-aided sample preparation (FASP), dimethylated leucine (DiLeu) isobaric labelling, and high-resolution MS methods, we have obtained relative quantitation for >1000 proteins comparing uninjured versus injured carotid arteries at 3, 7, and 14 days post injury in a rat model. From this data, we identified >100 proteins that are significantly up- or down-regulated post-injury. Gene ontology (GO) analysis of differentially regulated proteins with DAVID bioinformatics tools shows enrichment of a variety of GO terms associated with extracellular matrix remodeling, structure of actin cytoskeleton, and smooth muscle cell dedifferentiation. To complement the total proteome studies in the rat model, we are developing an MS-based workflow for the large-scale examination of phosphorylation and glycosylation in primary human endothelial cells (ECs) and smooth muscle cells (SMCs) activated with TNF-α, a pro-restenotic cytokine. We are interested in comparing ECs versus SMCs as drugs currently used to prevent restenosis by inhibiting SMC proliferation also prevent the formation of the protective EC layer, which leads to complications such as thrombosis. This strategy combines DiLeu labeling and electron-transfer and higher-energy collision dissociation (EThcD) to perform glycopeptide and phosphopeptide quantitation. The goal of these studies is to understand the role of PTM crosstalk in order to assist with the development of therapies that inhibit restenosis after vascular interventions without causing thrombosis.

Mon Poster 25: Integrated Omics Analysis of Protein Isoform Switch under Cardiac Hypertrophy

Maggie PY Lam[1]; Yi Xing[2]; Peipei Ping[1]

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Introduction. Isoform switch is increasingly recognized to be associated with complex diseases such as cancer and heart disease. Alternative splicing is a major mechanism that governs the expression of isoform in the proteome. However, despite advances in proteomics technologies, isoforms from alternative splicing events remain underexplored due to a number of analytical and computational challenges. In our current study, we devised an integrated omics workflow to identify protein alternative isoforms under cardiac hypertrophy.

Methods. We utilized a combined transcriptomics and proteomics workflow (ProteoSeq) to derive alternative isoforms at the protein level. Using a publicly available ENCODE dataset on C57BL/6 mouse heart, we inferred alternative isoforms using a Bowtie-Tophat-rMATS pipeline. An In-house proteomics dataset on an isoproterenol-stimulated cardiac hypertrophy model was searched against protein sequences generated from the inferred isoforms to identify alternatively spliced proteins.

Results. Using our integrated omics approach, we identified 816 distinct splice junction peptides in the mammalian heart. These include the identification of ~50 proteins expressing 2 or more splice isoforms. Our analysis has provided, for the first time, protein-level evidence of many of the alternative splicing events under cardiac hypertrophy. Quantification efforts have also revealed isoform switches of alternative isoforms under disease conditions (e.g., pyruvate kinase, alpha-enolase).

Conclusion. We obtained protein-level evidence of isoform switches under cardiac hypertrophy. Integrated omics approach has the potential to further expand our knowledge of alternative splicing events in heart diseases and others.

Mon Poster 26: DDA vs DIA: Convergence of increased breadth, reproducibility and precision.

Ronald Holewinski[1]; Vidya Venkatraman[1]; Sarah Parker[1]; Georgia Saylor[2]; Chunhong Mao[3]; Grace Athas[4]; David Herrington[2]; Jennifer Van Eyk[1]

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Liquid chromatography-mass spectrometry (LC/MS) based proteomics has become the method of choice of analyzing large scale changes in protein expression in biological systems. Currently, there are two main methods of data acquisition when performing LC-MS based proteomics experiments: 1) data dependent acquisition (DDA), where the most abundant ions/peptides are selected for fragmentation and sequence but is biased by the stochastic nature of precursor selection and low sampling efficiency due to the limited speed of mass spectrometers and 2) data independent acquisition (DIA), in which all ions/peptides are fragmented and potentially sequenced against a spectral library. These challenges result in missing individual peptide identification across LC-MS/MS runs within a larger dataset. In recent years, DIA has gained considerable interest due to the ability to theoretically fragment every ion in a biological sample and potentially identify considerably more proteins than DDA methods. In this study we acquired data for the same 99 abdominal aorta samples in DDA mode using an Orbitrap Elite and DIA mode using a TripleTOF 6600. We were able to identify 1495 and 1833 proteins by DDA and DIA, respectively, with 1257 of the proteins overlapping. While DIA provided only a modest increase in the number of proteins identified (~18% increase), it provided a large advantage in data completeness with 1334 of the proteins having less than 50% missingness (72%), while the DDA data only provided 725 proteins with less than 50% missingness (48%). Therefore, while DIA provided only a modest gain in the number of proteins identified compared to DDA, the gain in data completeness was substantial

(84% increase for DIA). Compared with traditional DDA workflows, these new DIA techniques achieve deeper proteome coverage, fewer missing values, and lower quantification variance to enable flexible and robust proteome characterization.

Mon Poster 27: Quantitative Analysis of Newly Synthesized Proteins during Maladaptive Cardiac Remodeling

Yuanhui Ma[1]; Daniel B. McClatchy[1]; David Liem[2]; Dominic Ng[2]; Peipei Ping[2]; John R. Yates[1]

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Maladaptive cardiac remodeling (MCR) is a common stage of many heart diseases with increasing incidence and prevalence. We recently developed a new quantitative proteomic technique PALM (Pulse Azidohomoalanine Labeling in Mammals) to quantitate newly-synthesized proteins (NSPs). PALM relies on azidohomoalanine (AHA) which is a non-canonical amino acid that is accepted by the endogenous methionine tRNA and inserted into proteins in vivo. Here we applied PALM strategy with a Heavy isotope labeled AHA (HAHA)to the isoproterenol (ISO) induced MCR mouse model to quantify NSPs during an early phase of ISO induced MCR and a late phase to enhance our insights into MCR. ISO or saline was delivered to mice for 14days. To determine the acute and prolonged effect of ISO, the mice were fed a diet of either AHA or HAHA for four days at different time points. The hearts were dissected and homogenized. AHA and H-AHA tissues were mixed 1:1(wt/wt).Three biological replicates including a label swap were analyzed. There were 417 and 362 proteins quantified in all three replicates at the 4 and 14 day, respectively. Both of these quantified datasets had a similar protein distribution and good correlation between biological replicates. 93 and 75 proteins were significantly different between the ISO and control hearts in the 4 and 14day, respectively. In the 4day analysis, these proteins were significantly enriched mitochondrial and oxidative phosphorylation pathways and in the 14day analysis, the proteins were similarly enriched in mitochondria but in the TCA cycle, suggests there are distinct acute and chronic cardiac responses to ISO. There were 20 NSP that were statistically significant at both time points indicating these are strongly associated with ISO treated hearts. Six proteins within that have never been reported in MCR before. Overall, our study could increase the understanding of MCR and provide potential novel therapeutic targets.

Mon Poster 28: Temporal quantitative phosphoproteomics of ADP stimulation reveals novel central nodes in platelet activation and inhibition

Florian Beck[1]; Joerg Geiger[2]; Stepan Gambaryan[4]; Fiorella A. Solari[1]; Margherita Dell'Aica[1]; Stefan Loroch[1]; Nadine Mattheij[3]; Igor Mindukshev[4]; Oliver Poetz[5]; Kerstin Jurk[6]; Julia M Burkhart[1]; Christian Fufezan[7]; Johan W.M. Heemskerk[3]; Ulrich Walter[6]; Rene P. Zahedi[1]; Albert Sickmann[1]

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ADP enhances platelet activation by virtually any other stimulant to complete aggregation. It binds specifically to the G-protein coupled membrane receptors P2Y1 and P2Y12, stimulating intracellular signaling cascades leading to integrin αIIbβ3 activation, a process that is antagonized by endothelial prostacyclin. P2Y12 inhibitors are among the most successful anti-platelet drugs, however, show remarkable variability in efficacy. We reasoned whether a more detailed molecular understanding of ADP-induced protein phosphorylation could identify (i) critical hubs in platelet signaling towards aggregation, and (ii) novel molecular targets for anti-platelet treatment strategies. We thus applied quantitative temporal phosphoproteomics to study ADP-mediated signaling at unprecedented molecular resolution. Furthermore, to mimic the antagonistic efficacy of endothelial-derived prostacyclin, we determined how Iloprost reverses ADP-mediated signaling events. We provide temporal profiles of 4,797 phosphopeptides, 608 of which showed significant regulation. Regulated proteins are implicated in well-known activating functions such as degranulation and cytoskeletal re-organization, but also in less well-understood pathways, involving ubiquitin ligases and GTPase exchange factors/GTPase-activating proteins (GEF/GAP). Our data demonstrate that ADP-triggered phosphorylation occurs predominantly within the first 10 seconds, with many short rather than sustained changes. For a set of phosphorylation sites (e.g. PDE3ASer312, CALDAG-GEFISer587, ENSASer109) we demonstrate an inverse regulation by ADP and Iloprost, suggesting that these are central modulators of platelet homeostasis. This study demonstrates an extensive spectrum of human platelet protein phosphorylation in response to ADP and Iloprost which inversely overlap and represent major activating and inhibitory pathways.

Mon Poster 29: Quantitative Phosphoproteomic and Proteomic Analysis of Swine Hearts Revealed Novel Insights into Myocardial Stunning

Xue Wang[1]; Xiaomeng Shen[2]; Rebeccah Young[3]; Jun Li[2]; Shichen Shen[2]; John Canty[3]; Jun Qu[2]

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As a part of coronary artery disease, myocardial stunning is the prolonged depression of cardiac contractility after reperfusion, commonly occurring after the salvage of reversible ischemia and leading to delayed recovery of contractile function of myocardium. Postischemic myocardial stunning often contributes to the morbidity associated with acute coronary syndrome, and the exact molecular mechanism underlying this common disorder is poorly understood. Herein, we employed comprehensive, quantitative phosphoproteomics and accurate, reproducible ion-current based proteomics(IonStar) to globally analyze the altered phosphosites and protein abundance on swine myocardium (n=10, 5 LAD stunned region vs. 5 remote normal region) with myocardial stunning. We totally quantified 4699 phosphorylation sites in 1847 phosphorylated proteins, and 2099 proteins using in-house proteomics pipeline with extremely-low missing value(<0.2%). To our knowledge, this represents the most in-depth analysis of swine myocardial proteome and phosphoproteome. Consistent with previous report, proteins (e.g CNN1,MYL9,ARPC3,PALLD,ACTN1,CSRP1) conferring contractile function were significantly decreased during myocardial stunning. Moreover, we discovered the reduced extracellular cellular matrix function indicated by the decreased collagen (COL14A1,COL1A2) in stunned myocardium. Molecular evidence revealed by proteomics suggested apoptosis might be involved in the stunning, corresponding to our TUNNEL staining data. Furthermore, the systematic analysis of phosphorylation network based on phosphoproteomic data demonstrated the abnormal phosphorylations of functional molecules were regulated by classic upstream kinases (e.g CKII,ATM,CDK,MAPK,PKA,INSR) and signaling transductions such as insulin signaling, EGFR signaling, mTOR signaling, Wnt signaling, which are likely implicated in the pathogenesis of stunning. In conclusion, this study sheds new lights into the molecular mechanisms of postischemic myocardial stunning enhancing our understanding of this disorder and will provide scientific basics for therapeutics. Additionally, based on RNA-seq and LC/MS sequencing, this work established the first-ever confirmed swine heart protein database with complete annotation, an important resource for future study of this important model.

Mon Poster 30: Proteomics for systems biology: defining the cross-talk between signaling pathways and chromatin modifications occurring during treatment of acute myeloid leukemia

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We present an integrative analysis of phosphoproteomics and histone post-translational modifications (PTMs) of acute myeloid leukemia (AML) human cell lines responding to the FLT3 inhibitor Quizartinib (AC220). Fms like tyrosine kinase 3 (FLT3) mutated with an internal tandem duplication (ITD) is one of the most common oncogenes in AML. Recent data demonstrate that leukemic cells in most patients

responded to therapy through differentiation and not with cell death as expected. This raises the question whether FLT3-ITD inhibits myeloid differentiation via epigenetic mechanisms involving histone PTMs.

We incubated FLT3 mutant MV4-11 and MOLM14 cell lines with Quizartinib to model differentiation during early response to treatment. We collected 6 time points up to 16 hours and performed proteomics, phosphoproteomics and histone profiling. Data was analyzed using unsupervised clustering analysis, and filtered features (proteins, phosphorylations, histone PTMs) were assessed for abundance trends significantly correlating with the phenotypic trend, i.e. differentiation.

Data revealed that almost every epigenetic enzyme known to be mutated in subgroups of AML patients had regulated phosphorylations or protein levels, providing first evidence of why mutations on such proteins might prevent effective treatment. By combining our results with experimentally verified interaction data (databases) we could draw a clear pathway of regulated phosphorylation events connecting FLT3 with histone readers and writers. The PRC2 complex (catalyzing histone H3 methylation on lysine 27) had most significant steadily enriched phosphorylations upon treatment, known to inhibit its activity. Two of its components, EZH2 and Suz12, were downregulated also at the protein level. The histone PTM H3K27me was downregulated accordingly and its antithetical PTM H3K27ac had opposite trend. Together, we prove that a holistic clustering analysis of multi–omics data assisted the identification of a regulated interplay of epigenetic factors around H3K27me in differentiating AML cells. Inhibitors targeting PRC2 might facilitate AML cell differentiation and thus proliferation arrest.

Mon Poster 31: Time-resolved Global and Chromatin Proteomics during Herpes Simplex Virus (HSV-1) Infection

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Herpes simplex virus (HSV-1) lytic infection results in global changes to the host cell proteome and the proteins associated with host chromatin. We present a system level characterization of proteome dynamics during infection by performing a multi-dimensional analysis during HSV-1 lytic infection of human foreskin fibroblast (HFF) cells. Our study includes characterization of the host and viral proteomes, phosphoproteomes, chromatin bound proteomes and post-translational modifications (PTMs) on cellular histones during infection. Our goal was to track the alterations of signaling pathways upon viral infection and correlate them with modulation of host chromatin organization and proteome expression. We analyzed proteomes across six time points of virus infection (from 0 to 15 hours post-infection) and clustered trends in abundance using fuzzy c-means. Globally, we accurately quantified more than 4,000 proteins, 200 differently modified histone peptides and 9,000 phosphorylation sites on cellular proteins. In addition, we identified 67 viral proteins and quantified 571 phosphorylation events on viral proteins. Interestingly, we observed an enrichment of threonine phosphorylation (~34% of total phosphosites) on viral proteins as compared to the expected 80:20:1 ratio of serine:threonine:tyrosine phosphorylation frequencies on cellular proteins. We investigated chromatin bound proteins by proteomic analysis of the high-salt chromatin fraction and identified 510 proteins significantly different in abundance during infection. We found 53 histone marks significantly regulated during virus infection, including a steady increase of histone H3 acetylation (H3K9ac and H3K14ac). Our data provide a resource of unprecedented depth for human and viral proteome dynamics during infection. Collectively, our results indicate that the proteome composition of the chromatin of HFF cells is highly affected during HSV-1 infection, and that phosphorylation events are abundant on viral proteins. We propose that our epi-proteomics approach will prove to be important in the characterization of other model infectious systems that involve changes to chromatin composition.

Mon Poster 32: Identification of Actin Arginine Methylation Site in Chromatin Remodeling Complexes Relevant to Transcriptional Regulation and Human Vascular Disease

Prabodh Kapoor[1]; Zhenan Liu[2]; Vinesh Vinayachandran[3]; Rohit Reja[3]; Chia-Fang Lee[4]; Ashutosh Kumar[5]; Jiyuan Chen[6]; Jing Xiao[1]; Jianjun Shen[1]; Bongsoo Park[3]; Michelle Gadush[4]; Maria Person[4]; Kathleen Trybus[7]; Kam Zhang[5]; Franklin Pugh[3]; Kristine Kamm[2]; Dianna Milewicz[6]; Xuetong Shen[1]

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Nuclear actin has distinct functions from the cytoskeletal roles of bulk actin. The function of nuclear actin is studied by purifying the Ino80, NuA4 and SWR1 chromatin remodeling complexes from yeast and separating the proteins by SDS-PAGE. LC-MS/MS on actin using an Orbitrap Elite characterized multiple post-translational modifications. In particular, we identified arginine mono-methylation on the R256 residue. The R256me1 mark is evolutionarily conserved, and an antibody raised against it is active for nuclear actins from yeast, mouse and humans (as R258me1). A genome-wide survey of the actin R256me1 mark in yeast using ChIP-exo sequencing shows a high degree of correlation with active transcription. From a human disease perspective, R258 mutations in human smooth muscle alpha-actin cause thoracic aortic aneurysms and dissections (TAAD). Western blot analysis on R258C patient cells show reduced methylation. Molecular dynamics simulations suggest that arginine methylation results in a significant conformational change of actin. In conclusion, the R258me1 modification provides a distinct mark for identifying nuclear actin. The ability of actin to influence transcription and affect vascular disease proceeds by a mechanism involving methylation of a conserved arginine residue.

Mon Poster 33: Discovery of a novel interaction between Apolipoprotein E and Interferon-γ (IFNγ) that can be modulated by oxidation of IFNγ

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Arizona State University, Tempe, Arizona

Apolipoprotein E (ApoE) is a 34 kD, partially O-glycosylated protein present in many lipoprotein particles. ApoE is mainly synthesized in the liver, but is also synthesized by many other tissues including macrophages. Other than its role in lipid transport, ApoE plays significant roles in atherosclerosis, neurodegenerative diseases and autoimmune diseases. Crosstalk between several cytokines and ApoE highlights the involvement of ApoE in regulating inflammation in these conditions. In atherosclerosis, the pro-inflammatory cytokine, IFNγ has been shown to promote and modify atherogenesis in the absence of ApoE expression. In addition, IFNγ has been shown to inhibit the production of ApoE in macrophages via a yet-undefined posttranslational mechanism. Recently, using ELISA experiments, we have found evidence of a physical binding interaction between recombinant IFNγ and ApoE. Interestingly, this interaction was partially abrogated upon oxidation of methionine residues within IFNγ. Experiments are underway to determine the dissociation constant (K_D) for the observed interaction and to assess its behavior in lipid-rich environments. Findings from this study may help identify how IFNγ exerts its regulatory effect on ApoE production and facilitate a better understanding of the molecular mechanisms underlying the roles of ApoE and IFNγ in the pathogenesis of atherosclerosis.

Mon Poster 34: Combinining SEC separations with Isotope Dilution Mass Spectrometry for Improving the Quality of Influenza Vaccines.

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The regulated protein in influenza vaccine is hemagglutinin (HA). Currently, vaccine potency is determined by the single radial immunodiffusion assay (SRID), that was previously correlated with the production of neutralizing antibodies to HA *in vivo*. This assay requires the production of both reference antigens and polyclonal

antibodies raised against HA, which takes several months. In case of an influenza pandemic, it is desirable to develop an alternative assay that can operate independently or an adjunct of SRID.

Size Exclusion Chromatography Isotope Dilution Mass Spectrometry (SEC-IDMS) is a recently developed alternative potency assay for influenza vaccine, which combines the ability of SEC to separate macromolecular species based on their size and shape with the gold standard precision and accuracy of IDMS for protein quantification. Samples of vaccine bulk material are injected onto a SEC column. Two fractions are collected, the first corresponding to larger oligomers and aggregates of HA and other viral proteins and the second in the region where trimeric HA species should elute. The fractions were then subjected to a conventional IDMS protocol for HA quantification. Values obtained from different vaccine lots and samples that have been subjected to stress (such as extremes of pH) can then be compared by the absolute amount of HA detected from both fractions and/or the ratio between HA content present in the trimeric and the oligomeric/aggregate fractions.

We hypothesized that the proportion of observed trimeric HA to oligomeric/aggregated HA might be correlated with vaccine potency. As an example, for a given stress/ unstressed vaccine bulk sample pair, the trimeric to oligomeric HA fraction ratios (T:O ratio) observed was 2.61and 0.62, respectively. The great majority of unstressed samples had a T:O ratio greater than 2, and all stressed samples examined had a T:O ratio much less than 2.0, demonstrating the viability of this technique.

Mon Poster 35: Semi-Automated Methods in Skyline and New MS/MS Rules to Define and Assign Xylosylated *N*-Linked Glycans in Populus Trichocarpa

Elizabeth S. Hecht[1]; Philip Loziuk[1]; Brian Pratt[2]; Brendan Maclean[2]; Mike Maccoss[2]; David Muddiman[1]

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Defining the glycosylation patterns of enzymes is critical to better understanding the enzymatic activity of biological pathways. Here, the in-line glycosite and glycomics Filter Aided *N*-linked Glycan Separation (FANGS) coupled to the Individuality when Normalizing with Glycan Hydrazide Tags (INLIGHT) strategy was applied for the first time in Populus trichocarpa, a model system for the production of lignin. The specificity of the in-line protocol to identify glycosites by deamidation was significantly different than in mammalian plasma and tissue. Over 500 glycosites were identified and belonged to the N/X/S/T motif, and 13/25 enzymes involved in the monolignol pathway were found to be glycosylated (occupancy levels 10-90 %). The glycome of Populus trichocarpa was also defined for the first time by developing an untargeted, semi-automated method, integrating Hardklor to extract monoisotopic mass, an in-house program, and Skyline analysis. Identifications were assigned on the basis of exact mass, INLIGHT tagged pairs, retention time, spectral accuracy, and MS/MS. The program had a 3.6% error rate, and identified 27 new glycans, with three glycans observed rarely in a similar plant species, and a single glycan validated by MS/MS as a novel species. Quantification in Skyline was compared to the XCalibur Quan browser, and the small molecule workflow analysis had significantly less variation in the area-under-the curve quantitation (Levene's test, $p < 0.05$). Plants have unique elements compared to the mammalian glycome (1,3 fucosylation, xylose saccharide incorporation) and glycomics studies have been conducted on few plant systems. Through this work, and follow-up studies using the well-characterized horseradish peroxidase glycoprotein standard, we discovered the production of a new xylose-rearranged ion during CID and HCD, and validated its identity using MS^3. This information is critical to the assignment of tandem mass spectra across all xylosylated species.

Mon Poster 36: PEAKS AB: A software platform for LC-MS/MS based therapeutic protein characterization

Baozhen Shan; Lin He

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Studies of therapeutic proteins, especially monoclonal antibodies (mAbs), require the complete and accurate protein sequences and the comprehensive characterization before the proceeding of downstream applications. Especially, as a special post-translational modification (PTM), N-linked glycosylation plays important roles in biological functions of mAbs. The qualitative and quantitative analysis of glycosylation, after retrieving the protein sequences, is thus demanded by researchers. Therefore, we propose PEAKS AB to facilitate mAb protein de novo sequencing and the analysis N-linked glycosylation.

We used a standardized procedure to prepare the mAb sample for LC-MS/MS analysis. The heavy and light chains were separated, reduced, and alkylated. Five enzymes were used to digest each of the separated chains. The digested peptides were extracted and subjected to LC-MS/MS analysis on a Thermo-Fisher Scientific Q-Exactive mass spectrometer.

The protein sequences of both the heavy and light chains were assembled automatically and the confidence of each amino acid was calculated according to the MS/MS data. To evaluate the performance of PEAKS AB for protein sequencing, the assembled sequences were compared to the true ones and the comparison showed 100% accuracy.

The analysis of N-linked glycosylation using PEAKS AB includes three parts: 1) glycan form identification, 2) glycosylated peptide identification, and 3) glycosylation quantification. PEAKS AB searched in the integrated N-linked glycan database for best matching glycan form for each tandem mass spectrum that was possibly generated from an intact glycosylated peptide. The in silico digested peptides from the assembled protein sequence was used with the best matching glycan to validate the correctness of the glycosylated peptides. The extracted ion count (XIC) for each glycosylated peptide was then calculated from LC-MS data with the consideration of isotopic peaks. These quantity information of each N-linked glycan form was directly shown in PEAKS AB and was ready to be reported for follow-up analyses.

Mon Poster 37: Advances in Multi-modal Mass Spectrometry imaging for Biomedical Applications

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Desorption electrospray ionisation (DESI) is an established technique in the field of mass spectrometry, and has over recent years been used for tissue imaging (MSI). DESI results for lipids and small molecules can be of high quality compared to the more established matrix assisted laser desorption ionisation (MALDI) imaging technique. An additional advantage of DESI is that it can be performed without further sample preparation, such as matrix deposition. Using optimized gas and solvent flow rates, the DESI technique can be non-destructive and doesn't destroy the tissue surface.

Multi modal imaging is a concept that combines the strengths of different imaging modalities to provide a more complete picture of the biological question. This can involve the use of morphological imaging such as H&E staining, functional imaging like Positron Emission Tomography (PET) or the use of molecular imaging like Mass Spectrometry Imaging (MSI). There can be significant benefit of combining or fusing these technologies.

Within the scope of MSI, different ionisation techniques have been demonstrated to have complementary strengths. Here we will describe technology improvements made to both matrix assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI), and demonstrate the benefits of combining the two approaches with examples in the pre-clinical and clinical application space.

Monday posters should be set up 8:00-8:30 am Monday morning and removed at 6:30 pm Monday evening.

Mon Poster 38: Relative and Absolute Quantification of Metabolites in Hen Ovarian Tissues using IR-MALDESI Mass Spectrometry Imaging

Milad Nazari; Mark Bokhart; David Muddiman
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Mass spectrometry imaging (MSI) is a rapidly evolving mode of analysis for examining the spatial distribution and relative abundance of metabolites in biological tissue sections. MS-based metabolomics studies require positive and negative polarities to obtain comprehensive metabolite data since different metabolites exhibit significantly different ionization efficiencies based on their structure. Infrared Matrix-Assisted Laser Desorption Electrospray Ionization (IR-MALDESI) is an ambient ionization method that has been employed for MSI analysis of metabolites. In IR-MALDESI analyses, a thin layer of ice is used as the energy-absorbing matrix, and a mid-IR laser pulse (2940 nm) is used to desorb tissue material at each rastered position. The desorbed materials partition into the orthogonal electrospray droplets where ions are generated by an ESI-like process and subsequently measured by the MS.

A polarity switching IR-MALDESI MSI method was developed to obtain comprehensive metabolite coverage. Using this method, the differences in the metabolomes of healthy and cancerous hen ovarian tissue sections were examined, and differences in spatial distribution and relative abundance of more than 700 lipids and metabolites between the tissues were differentiated. Glutathione was one of the metabolites that exhibited an order of magnitude increase in the cancerous tissue, and therefore was chosen for quantitative analysis based on its high degree of upregulation in the cancerous tissue and its well-known role in the detoxification of cells. A quantitative MSI method was developed to quantify endogenous glutathione in healthy and cancerous tissue sections. To help reduce variability in ablation and ionization events, specimen slides were uniformly coated with a normalization compound using an automated pneumatic sprayer. The ratio of analyte abundance to normalization compound abundance reduces variability across changing tissue microenvironments. A calibration curve consisting of SIL-glutathione was spotted directly on the tissue allowing direct correlation of SIL-glutathione abundance to native glutathione for absolute quantification in tissue.

Mon Poster 39: Quantitative drug distribution mapping in tissues using IR-MALDESI mass spectrometry imaging

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Mass spectrometry imaging (MSI) is an emerging technology for the visualization of endogenous and exogenous analytes in biological tissues. Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) is an ionization technique well-suited for the analysis of fresh-frozen tissues. A mid-IR laser is used for the complete ablation of a voxel of tissue from a surface. The resulting ablation plume interacts with an orthogonal electrospray plume where analytes partition into electrospray droplets and are ionized in an ESI-like manner. The patterned acquisition of this spatially resolved analysis can then be reconstructed into ion maps depicting the distribution of analytes in the tissue.

A MSI method for the absolute quantification of small molecule pharmaceuticals was developed and applied to antiretroviral compounds in tissues. Variability in MSI data was reduced using a microscope slide uniformly coated with a structural analogue normalization compound prior to mounting a 10 µm thick tissue section. Voxel to voxel differences in ablation dynamics, ionization efficiency and MS detection are minimized by taking the ion abundance ratios of analyte to normalization compound on a per-voxel basis. The incorporation of a MSI calibration curve allows the ion abundance of an analyte to be related to an absolute concentration. The quantification procedure has been developed into the MSiQuantification tool within the open-source software MSiReader, improving throughput of quantitative MSI experiments. Additionally, the spatial resolution of IR-MALDESI analysis was reduced through improved laser focusing optics. An iris and beam expander were used to spatially filter the laser beam waist in addition to an aspherical focusing lens to improve the IR laser ablation diameter to less than 100 µm. This reduced ablation diameter improves the quality of drug and metabolite distribution images by allowing smaller tissue features to be visualized.

Mon Poster 40: Probing translational regulation in spinal muscular atrophy (SMA) using integrated proteomics and transcriptomics approaches

Amanda Guise[1]; Shaojun Tang[2]; Ruchi Chauhan[1]; Constantin van Outryve d'Ydewalle[4]; Hendrik Wesseling[1]; Charlotte Sumner[4]; Martin Hemberg[3]; Hanno Steen[1]; Judith Steen[1]
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Spinal muscular atrophy (SMA) is a leading genetic cause of infant mortality and is caused by loss-of-function of the survival of motor neuron (SMN) protein critical for snRNP assembly and splicing. Recent clinical trials show the promise of antisense oligonucleotides to restore functional SMN, and complementary therapeutics targeting non-SMN proteins will improve current treatments. To investigate non-SMN proteins involved in SMA from a global perspective, we undertook an integrated proteomic and transcriptomic analysis of murine and human spinal cord tissue using quantitative mass spectrometry analysis coupled with next-generation RNA-sequencing to investigate changes in protein synthesis rates and translational machinery in SMA. Specifically, we hypothesize that global disruption of translation in SMA contributes to neurodevelopmental defects, neuronal dysfunction, and neurodegeneration. To examine proteomic and transcriptomic changes in SMA mice throughout development and neurodegeneration we isolated protein and RNA from SMNΔ7 and control spinal cords over a developmental timecourse. Protein samples were enzymatically digested, labeled at the peptide-level with isobaric tags, pooled, fractionated, and analyzed by mass spectrometry on a hybrid quadrupole-orbitrap instrument. In-depth proteome analysis identified ~10,000 proteins, with hundreds of proteins exhibiting condition-dependent abundance changes. Clustering and covariate analyses uncovered protein group co-regulation, suggesting altered regulation of translation machinery in SMA. We assessed differential expression in the transcriptome and proteome using an f-divergence cutoff index (fCI) algorithm developed in our lab. To further investigate protein synthesis rates, we probed our proteo-transcriptomic dataset using the PECA algorithm (Choi Lab). To investigate the presence of SMA-specific spliceoforms and non-canonical translation products we searched MS data against a custom database built from RNAseq alignments. We present a comprehensive analysis of time-associated global proteome and transcriptome changes in mouse and human tissues to identify proteins and pathway targets for therapeutic intervention. Together these results give insight into molecular mechanisms underlying SMA and neurodegenerative disease.

Mon Poster 41: Glycomics and proteomics of myelinated versus non-myelinated regions of human brain tissue

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Chondroitin sulfate proteoglycans (CSPGs) and hyaluronan aggregates compose a significant portion of the brain extracellular matrix. CSPGs are involved in crucial brain processes such as brain connectivity, axon guidance, and myelination. Furthermore, CSPG abnormalities have been reported in subjects with schizophrenia (SZ), in which thalamo-cortical disconnectivity has been repeatedly reported in brain imaging studies. We propose that CSPG expression in the thalamus thus may play an essential role in the disruption of thalamo-cortical connectivity. Preliminary immunohistochemistry results on the mediodorsal nucleus (MD), a large thalamic nucleus, of SZ subjects showed altered organization of myelinated fiber bundles and glycomics studies on SZ and control MD tissues showed a significant increase in unsulfated CS disaccharide in SZ. In this study,

our aim was to test the hypothesis that CSPGs, myelin, and related extracellular matrix proteins are altered in subjects with SZ. To date, no studies have compared the CS abundance and proteoglycan expression in myelinated (MY) versus non-myelinated (NM) regions.

To test this, preliminary glycomics and proteomics analysis was performed on MY fiber bundles and NM regions of the thalamus sections from healthy controls. The CS disaccharides were released using chondroitinase ABC enzyme followed by trypsin digestion peptides from MY and NM spots of brain sections by manual on-slide digestion and analyzed using liquid chromatography-mass spectrometry analysis. Preliminary glycomics results showed an significant increase in unsulfated CS disaccharide abundance in MY compared to NM regions suggesting differential CS patterns in myelin bundles. However, proteomics data showed similar coverage of core protein form PGs. Although, a comparatively higher coverage was seen for myelin related proteins for MY compared to NM regions. We plan follow-up studies using samples from SZ patients and control subjects in order to compare MY and NM regions of the thalamus. This information will contribute to the understanding of the molecular and neuropathological underpinnings of thalamo-cortical disconnectivity in SZ.

Mon Poster 42: Vascular Contributions of Plasma Lipoproteins to Alzheimer's Diseases

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Scientific evidence continues to identify vascular contributions to Alzheimer's Disease (AD). Plasma lipoproteins (VLDL, IDL, LDL, and HDL) have long been implicated in cardiovascular and cerebrovascular health and disease because of its role in maintaining the integrity of vascular systems. To this end, we apply a MS-based shotgun proteomics coupled with spectral counts to conduct relative quantitative analyses of abundance of proteins in plasma lipoprotein fractions. We demonstrated that the combined workflow of fractionating plasma lipoproteins (i.e., sequential ultracentrifugation) and subsequently the MS-based proteomic analysis was reproducible both within a day and between days. Next, we applied the MS-based proteomics to biochemically characterize plasma lipoproteins fractionated from plasma of two randomly selected adults P1 and P2. We identified 52, 46, 50, and 37 proteins in the VLDL, IDL, LDL, and HDL fractionated from the P1's plasma, respectively, and identified 49, 48, 53, and 65 proteins in the VLDL, IDL, LDL, and HDL fractionated from the P2's plasma, respectively. Heat map analysis indicated that many proteins were uniquely present in only one or two, but not all four, plasma lipoprotein fractions. Furthermore, we performed pathway and network analyses using Ingenuity Pathway Analysis (IPA) software (QIAGEN, Redwood City, CA) and identified pathways and networks represented by the proteins in the VLDL, IDL, LDL, and HDL fractions of P1 and P2. Interestingly, we found that certain proteins in the plasma HDL of P2 formed an interactive network for immune cell trafficking were not represented by the plasma HDL proteins of P1. Furthermore, our pathway analyses suggest that fractionated plasma lipoproteins harbor proteins, networks, and pathways (e.g., LXR/RXR activation, IL12 signaling, production of nitric oxide and reactive oxygen species, clathrin-mediated endocytosis, IL1-mediated inhibition of RXR function, and IGF-1 signaling) that have been implicated in AD.

Mon Poster 43: Transcriptome and Proteomic Profiling of a Drosophila Seizure Model Reveals Glial Regulation of Key Synaptic Proteins

Kevin Hope[1]; Daniel Johson[3]; Xiaoyue Jiang[2]; Andreas Huhmer[2]; Daniel Lopez-Ferrer[2]; Lawrence Reiter[1, 4]

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We have developed a model of Duplication 15q syndrome which is associated with autism and epilepsy. Flies expressing elevated levels of the UBE3A homologue, Dube3a, in glial cells, but not neurons, show a significant increase in seizure susceptibility. In order to identify the transcripts and proteins in the brain affected by glial specific elevation of Dube3a, we performed RNAseq and quantitative proteomic analysis of animals with elevated levels of Dube3a in glia or neurons versus control. We found more significantly differentiated whole brain transcripts in flies expressing Dube3a in glia using the GAL4 driver Repo-GAL4 than in brains expressing only in neurons with elav-GAL4 (1222 vs 497). Pathway analysis revealed a significant decrease for transcripts encoding 26 synapse associated proteins in the Repo>Dube3a animals, despite the fact that these changes occur in neurons and Dube3a was expressed in glial cells. Further immunohistochemistry of the pre-synaptic marker bruchpilot (brp), confirmed that this protein changes in neurons as predicted. In order to reveal the molecular mechanism of seizure in our model, we correlated transcriptional changes from RNAseq to protein changes using quantitative proteomics. The proteomic analysis identified over 3200 protein groups corresponding to more than 23000 peptides in a single LC-MS run. And it allowed to confirm that 8/26 of the synaptic proteins predicted to be less abundant in the repo>Dube3a samples were found to be significantly down-regulated. One of these proteins, the Na+/K+ exchanger ATPalpha, may be the key connection between glia and neurons in this model since we have previously shown that ATPalpha is a ubiquitination target of Dube3a in flies. Overall, the direct correlation between transcriptional and protein level changes in our disease model points out that we can use quantitative proteomics to validate our RNAseq data and also identify new direct protein targets of Dube3a in the brain.

Mon Poster 44: Protein signatures differentiate cancer comorbidity in ALS patients

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Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons in the brain and the spinal cord. The progressive degeneration of the motor neuron circuitry in ALS eventually leads to paralysis in patients. With voluntary muscle movement progressively degraded, people lose the ability to speak, eat, and move, eventually leading to death usually within 5 years of diagnosis. ALS patients often suffer from comorbidities - diabetes, heart disease, dementia or cancer – which further complicates diagnosis, treatment and proper care.

In an effort to investigate potential biomarkers that distinguish ALS patients with breast or prostate cancer, serum samples isolated from a cohort of 33 ALS patients with prostate cancer, breast cancer, or with frontotemporal dementia (ALS/FTD) were investigated, along with seven age and sex-matched healthy controls. 12 highly abundant serum proteins were depleted from the samples and subjected to LC-MS/MS analysis on a hybrid linear ion trap – Orbitrap mass spectrometer. For label-free protein quantification, the MaxLFQ algorithm was used as part of the MaxQuant environment. Statistical and bioinformatics analyses were performed using the Perseus software.

Our study revealed the presence of a distinct set of proteins in the serum of ALS patients with prostate cancer as well as breast cancer, suggesting unique biological underlying factors contribute to disease pathology. A better understanding of the pathological features characterizing ALS, coexisting with other disorders, will shed light into the common and unique cellular events shared among these disorders and will help develop effective diagnostic tools and will potentially identify novel treatment strategies.

Mon Poster 45: Protein expression and phosphorylation changes during neuronal differentiation reveal novel maturity markers

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The differentiation of neuronal stems cells into fully functional neurons is an intricate process that involves the precise regulation and coordination of diverse cellular and molecular pathways. Use of in

vitro model systems to investigate this process has led to a better understanding of a wide variety of neurological diseases. The ReNcell VM immortalized cell line (Millipore) has been used as an effective model to investigate the process of neuron differentiation and produces both the tangles and plaques Alzheimer's disease (AD) phenotypes in AD mutants. Upon removal of growth factors from the culture media, these cells have been demonstrated to differentiate from neuronal stem cells to fully functional neurons. To better understand this process, we isolated proteins from cultured ReNcells at 10 different time points over a period of two weeks, in biological duplicate, and performed multiplexed quantitative mass spectrometry-based proteomics analysis on them. In addition, measuring the relative abundance changes of phosphopeptides adds another layer of information about the role of signal transduction during neuronal differentiation. Herein we describe the application of system-wide proteomics and phosphoproteomics to better understand the expression and signaling changes that occur during the process of neuronal differentiation. The findings of this study provide mechanistic insight into why Statins and GSK3 inhibitors promote neuronal differentiation and serve as a resource for identifying other potential differentiation promoting compounds. These data comprise nearly 4,500 protein and 3,000 phosphorylation maturity markers including REST, a protein shown to play a role in the maturity reversal of Alzheimer's neurons. We believe the in-depth quantification of protein and phosphorylation changes will serve as a resource for neuroscientists and data scientists alike.

Mon Poster 46: Proteomics Studies in Synergistic Protection from Retinal Degeneration by Combined Stem Cell Therapies

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The advance of stem cell biology offers real promise to save vision. Synergistic protection from retinal degeneration has been revealed through a combined treatment of a sub-retinal injection of neural progenitor cells (NPC) (NPC^{SRI}) with intravenous injection of Mesenchymal stem cells (MSC) (MSC^{IV}) into the Royal College of Surgeons (RCS) rats with inherited retinal degeneration. However, the mechanism of this adjuvant therapy in rescuing vision is largely unknown. A strategic proteomics workflow particularly designed for the discovery and validation of proteins playing important roles in vision preservation has been established. Label-free nanoLC-MS/MS was performed on grouped RCS rat retina tissue (NPC^{SRI+IV}, NPC^{SRI} only, untreated) collected at postnatal day (P) 35 and blood samples drawn at days 3 and 7 after the MSC^{IV}. This study found nearly 2000 non-redundant proteins in the retina and 388 in plasma, including growth factors, cytokines, kinases, and extracellular matrix proteins. Compounds known to be locally involved in neurogenesis, visual perception, and inflammatory response proteins were identified. Compared with NPC^{SRI} alone, further biostatistical analysis indicated that proteins responsible for improving the retina microenvironment, such as vision cell regenerations (quality of photoreceptor, outgrowth of neuritis, engraftment, migration, endocytosis, organization of cytoskeleton, metabolites transport) were significantly upregulated and retinal degeneration proteins (photoreceptor degeneration, apoptosis of neuron) were considerably down-regulated in retina. The enhanced efficacy was further indicated by the proteins involved in immune response regulation and complement cascades in the blood system. The changes of specific proteins were verified by multiple reaction monitoring (MRM), immunohistochemistry, and western blotting analysis. This first proteomics analysis lays the foundation for uncovering the mechanism of neural-protection and better understanding in how to enhance the efficacy of stem cell-based therapies for retinal degenerative diseases in regenerative medicine.

Mon Poster 47: Unraveling the Complexity of the Gut-Brain Axis N-Glycoproteome

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The Gut-Brain axis is a bidirectional communication system that integrates neural, hormonal and immunological routes to properly maintain gastrointestinal homeostasis, digestion, the immune system, mood, behavior and cognition. Protein N-glycosylation can influence the nervous system in a variety of ways by affecting functions of glycoproteins involved in brain development and normal physiology. Moreover, gut-microbes can communicate with the brain and modulate brain development, function as well as behavior through mechanisms that are yet unclear. However, interactions of microbes with host-cell surface N-glycans of intestinal epithelial cells and neural cells are poorly understood mainly due to the lack of structural knowledge of host-glycans and glycoconjugates. In this work, we developed a glycoproteomic approach to a) obtain in-vivo detailed profiles of host cell-surface N-glycans along the gastrointestinal tract and in different functional brain areas in mice; b) identify corresponding host cell-surface N-glycoproteins; and c) to determine the site-specific glycoprotein occupancy. The surface N-glycome of gastrointestinal tract and brain areas including cortex, hypothalamus, hippocampus, cerebellum and brain stem were obtained from C57bl/6J mouse by nano liquid-chromatography-time of flight mass spectrometric analysis (nanoLC-CHIP-TOF/Q-TOF-MS). LC-Orbitrap-MS analysis of tryptic peptides derived from mouse pre-cortex, hypothalamus, and hippocampus cell surface proteins allowed for the identification of more than 3100 proteins, 1600 of which were plasma membrane glycoproteins. Enrichment and analysis of N-glycopeptides derived from select forebrain areas rendered the identification of more than 500 glycopeptides and permitted the assignment of more than 150 cell-surface glycoprotein site–specific occupancy. The results obtained reveled the complexity of host cell surface glycans and glycoproteins in the gut-brain axis, that can serve as targets for interactions with gut microbes and participate in the microbiota-gut-brain axis cross talk.

Mon Poster 48: Online 2D-NCFC-RP/RPLC system for efficient and comprehensive proteomic analyses

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Proteomics aims to achieve complete profiling of the protein content and protein modifications in cells, tissues and biofluids, and to quantitatively determine changes in their abundances. This information serves to elucidate cellular processes and signaling pathways, and to identify candidate protein biomarkers and/or therapeutic targets. Analyses must therefore be both comprehensive and efficient. Here, we present a novel online two dimensional reverse phase/reverse phase liquid chromatography separation platform, based on a newly developed online non-contiguous fractionating and concatenating device (NCFC fractionator). In bottom-up proteomics analyses of a complex proteome, this system provided significantly improved exploitation of the separation space of the two RPs, considerably increasing the numbers of peptides identified compared to a contiguous 2D-RP/RPLC method. The fully automated online 2D-NCFC-RP/RPLC system bypassed a number of laborintensive manual processes required with the previously described offline 2D-NCFC RP/RPLC method, and it thus offers minimal sample loss in a context of highly reproducible 2D-RP/RPLC experiments.

Mon Poster 49: Proteome-wide acetylation dynamics revealed by metabolic labeling and quantitative proteomics

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Protein acetylation is known to play a critical role in biological processes such as protein stability, function, and localization. Histone acetylation in particular is known to modulate gene expression, and

thus the dynamics and turnover of this modification have been extensively studied. In the current study we performed a comprehensive characterization of acetylation rates on non-histone proteins using mass spectrometry. Protein acetylation rates were determined by incorporating an isotopically labeled donor (13C-glucose or D3-acetate), which is metabolically converted into heavy acetyl-CoA and subsequently incorporated into proteins. These compounds were introduced into HeLa cell cultures and samples were collected at eight time points. After identification using nanoLC-MS/MS on an Orbitrap Fusion, quantification of antibody enriched acetylated peptides was performed using an in-house tool to extract isotopic patterns. Results showed that out of 1821 acetylated peptides detected by glucose labeling, 900 peptides had a significantly increasing acetylation trend. Predictably, as the acetate pathway is less utilized by HeLa cells during growth (glucose:acetate = 9:1), only 126 out of 2120 peptides from the acetate labeling experiment had a significantly increasing acetylation trend. Acetyl sites were then grouped based on their turnover speed; faster rates were enriched on proteins associated with chromatin and RNA metabolism, while slower rates were more typical on lipid metabolism related proteins. No sequence motif was found to be significantly enriched for any turnover group, although a depletion of histidine residues was typical of all acetylated peptides detected. Finally, we developed a script to automatically map whether detected acetylations were within known protein domains. With this approach we characterized an acetylation with fast turnover within the activation loop of the histone acetyltransferase p300, which could explain self-acetylation as an important feedback mechanism to regulate acetyltransferases. In summary, our work is the most comprehensive map of protein acetylation dynamics to date.

Mon Poster 50: A Novel Method of Quantifying Protein Methylation Utilizing SWATH-MS

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Protein methylation of arginine and lysine is thought to be an important post-translational modification in disease but very little of its specific effect is known. Global protein methylation analysis has been limited until the recent development of enrichment techniques utilizing peptide immunoprecipitations to methylated lysine and arginine and subsequent mass spectrometry. However, enrichment techniques require large amounts of antibody and sample in order to maximize coverage. We hypothesize that protein methylation can be studied without the need to enrich each sample via application of a data independent acquisition approach, creating a hyper-methylated peptide library to which each experimental sample is compared. We anticipate this would increase coverage of the methylome and provide an opportunity to study global protein methylation as well as obtaining total protein quantification from the same sample.

We have developed an informatics approach of distinguishing a methylated peptide from its un-methylated form, giving us the ability to quantify methylated peptides in a complex protein lysate. To create a hyper-methylated library, we utilized a mouse model of nonalchoholic steatohepatitis (NASH) where methylation is constitutively elevated via genetic knockout of Glycine N-Methyltransferase (GNMT). The mouse has an excess of S-Adenosyl Methionine (SAMe), the primary methyl donor. Furthermore, DNA/histone methylation is known to be increased in this model, but protein methylation has yet to be studied at a global level.

GNMT knockout and wild-type livers were digested with trypsin and the peptides were respectively pooled and run in DDA to create a hyper-methylated library. A total of 48 unique peptides containing a lysine or arginine methyl site were found. The individual liver samples (n=5) were then run by DIA and compared to the library. We found 34 of the methylated peptides in our library and saw a significant increase in methylation in the GNMT knockout mice.

Mon Poster 51: Post-translational crosstalk networks associated with EMT-mediated resistance to EGFR inhibitors

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Epithelial-mesenchymal transition (EMT) mediated resistance to kinase inhibitors is a major hurdle in lung cancer treatment, which leaves physicians and patients without an effective therapeutic strategy. Post-translational modifications (PTM) play critical roles in regulating EMT. We hypothesized that multiple PTMs and their cross-talk mechanisms may provide insights into the specificity and combinatorial logic to EMT signal processing and identify new therapeutic strategies.

SILAC SEPTM experiments compared the proteome, global phosphoproteome, ubiquitinome and acetylome ("tri-PTMomes") of paired HCC4006 parental/HCC4006ER resistant cells with EMT mediated resistance to EGFR kinase inhibitors (i.e. erlotinib). Strategies included modified peptide immunoprecipitation, bRPLC peptide fractionation, and IMAC enrichment prior to LC-MS/MS and quantification with MaxQuant. Then, a bioinformatics pipeline integrating heavy/light ratios of recombined normalized intensities, network analysis, proteome annotation, and literature mining was applied to characterize crosstalk among tri-PTMomes to pinpoint new therapeutic targets. Relative quantification of expression proteome included 6,641 proteins (FDR<5%). Of 1,654 differentially expressed proteins between sensitive parental and resistant cells, 17 classic EMT markers (11 decreased and 6 increased) were observed. We identified basal tri-PTMomes including 2,418 unique pSTY sites on 967 proteins, 784 unique UbK-sites on 687 proteins and 713 unique AcK-sites on 402 proteins. Interestingly, EMT led to changes of pSTY-sites (141 increase, 191 decrease), UbK-sites (29 increase, 32 decrease) and AcK-sites (14 increase, 46 decrease). Network analysis revealed global crosstalk among activated writers and erasers of phosphorylation (90 kinases, 42 phosphatases), ubiquitination (18 Deubiquitinating enzymes, 54 E1-E2-E3-Ligases) and acetylation (12 acetyltransferases, 4 deacetylases), and their substrates. Integrated analysis identified 28 proteins associated with HCC4006ER resistance, which include the EMT proteins: HMGA1, PML, PTPN12, MET and FAK.

Collectively, these findings begin to describe complex interplay in PTM modulation. Combinatorial targeting of key proteins or networks associated with PTM crosstalk represents a potential strategy for overcoming EMT-induced resistance.

Mon Poster 52: Phosphorylation Knowledge Networks Integrated with LINCS Phosphoproteomic and Drug-Target Data to Interpret Cancer Cell Responses to Kinase Inhibitors

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Targeted kinase inhibitors have shown great promise as anti-cancer drugs, but their use is complicated by development of resistance and off-target effects. As part of its effort to catalog molecular responses to a wide range of perturbing agents, the NIH LINCS Program (http://www.lincsproject.org/) has collected valuable large-scale data on kinase inhibitors, including their effects on kinase activity and on phosphosite abundance in cancer cells. The next step toward understanding how kinase inhibitors affect cancer cells is to interpret this data in the context of known kinase-substrate relationships and signaling pathways. Therefore, we have developed a workflow for integration of LINCS phosphoproteomic and drug-target data with literature-based phosphorylation knowledge networks using the therapeutic cyclin-dependent kinase (CDK) inhibitor, dinaciclib, as a test case.

We constructed a network of known kinase-site relationships using iPTMnet (http://proteininformationresource.org/iPTMnet), a resource that combines phosphorylation information from literature mining, databases and ontologies. This network was then overlaid with LINCS phosphoproteomic (P100) and kinase inhibition (KINOMEscan) data

from dinaciclib-treated breast cancer cells. For each kinase in the network, we analyzed the relationship between its degree of inhibition by dinaciclib and changes in the phosphorylation level of its substrates. For known targets of dinaciclib, such as CDK2, substrate phosphorylation was lower, as expected; interestingly, substrates of some dinaciclib-insensitive kinases, such as AURKB, were also less phosphorylated, indicating indirect effects potentially caused by changes in cell cycle distribution.

Currently, we are expanding the network to include additional sources of kinase-site relationships as well as LINCS gene expression data (L1000) on the substrates. We plan to automate the analysis workflow and apply it to all of the kinase inhibitor data available from LINCS. This effort will facilitate comparisons among kinase inhibitors, cells types, and experimental conditions, thereby furthering our understanding of the impact of kinase inhibitors on signaling pathways in cancer cells.

Mon Poster 53: Histone H3 mutations drive aberrant chromatin-reader interactions in diffuse intrinsic pontine glioma

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Despite representing only 10% of pediatric brain cancers, diffuse intrinsic pontine glioma (DIPG) is the leading cause of brain cancer death in children. It was recently determined that nearly 80% of DIPGs harbor a mutation in one of the sixteen genes encoding histone H3. Interestingly, the mutation always causes a K-to-M substitution at position 27 (K27M). Subsequently, our group helped show that the mutant histone proteins function via a dominant negative mechanism, stably associating with a subunit of the polycomb repressive complex (PRC2) and thereby reducing H3K27 di- and trimethylation genome-wide (K27me2 and K27me3, respectively). Preliminary data using mass spectrometry in a HEK 293T cell model has demonstrated that histone H3.3 K27M expression also has dramatic effects on patterns of H3K36 dimethylation (K36me2). Associated with active transcription, K36me2 was present on approximately 40% of detected histone H3 peptides in both control and H3.3 K27M mutant cells. However, in control cells it almost always coexisted with some form of K27 methylation. Strikingly, in K27M cells this association was compromised, leading to a tenfold increase in the abundance of K36me2 associated with unmodified K27. Analysis of three H3.3 K27M+ patient-derived DIPG cell lines revealed the same pattern of epigenetic alterations, and this was not observed in three glioma lines which lacked histone mutations. Therefore, this epigenetic rearrangement appears to be a specific, disease-relevant consequence of mutant histone expression. We hypothesize that the mutation-induced inhibition of PRC2 induces a chromatin environment that is more permissive of K36me2-driven transcription. Here we present a stable isotope labeling with amino acids in cell culture (SILAC)-based proteomics strategy to identify chromatin readers that uniquely interact with H3K36me2 when the neighboring K27 is unmethylated. Preliminary data and functional validation efforts are described.

Mon Poster 54: Using Protein Modifications in Serum to Diagnose Patients with Rheumatoid Arthritis

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Current diagnosis of Rheumatoid Arthritis (RA) is accomplished by identifying a low-abundance protein found in human serum. To identify RA in a faster, more cost-effective manner, we are using heat denaturation curves (HDC) of patients with RA and age matched controls. Only heavily abundant proteins can alter the shape of HDC in any appreciable manner. Thus, if these abundant proteins have been modified in patients with RA, diagnosing RA may be accomplished through HDC. We have measured differences in the HDC of the afore-mentioned patients using differential scanning calorimetry (DSC), and the data shows that the samples can be separated into three distinct groups. The proposed goal is to identify the protein modifications responsible for the differences in the HDC of these groups. If the differences correlate positively to patients with RA and patients without, the use of DSC could be used to diagnose patients with RA at a faster and more economical rate. In addition to lowering the cost and difficulty of RA diagnosis, we will work towards discovering the mechanisms of RA, enabling the continued development of suitable RA treatments.

Mon Poster 55: Reprogramming the SNO-Proteome in the brain of the Shank3-KO model of Autism Spectrum Disorder

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It is well established that Nitric Oxide (NO) and S-nitrosylation of neuronal proteins play a key role in several neuropathologies. Autism-Spectrum-Disorders (ASDs) are neurodevelopmental disorders based on three-behavioral characteristics: impaired social interaction, lack in communication and repetitive/restricted behavior. One high confidence ASD mutated gene is Shank3, which codes for a post-synaptic scaffolding protein and plays a critical role in neuronal development. Recently, several groups have developed mass-spectrometric approaches for analysis of the SNO-proteome in different tissues, including the brain that has revealed hundreds of proteins associated with a pathological state. This has allowed analysis of the role of SNO on a systems level. To test our hypothesis that NO is involved in ASD, and to unravel its effect on synaptic transmission and signaling pathways, we used a Shank3-KO mouse model.

Using SNOTRAP we have tested Adults and 6-weeks mouse age groups in, cortex and striatum.

In the adults, we found a large increase of SNO-proteins in the KO model compared to the WT in both cortex and striatum, while in 6-weeks mice, we found a large increase in the cortex with a smaller increase in the striatum. Gene Ontology analysis showed that the SNO-proteome in the KO-Adults-Cortex identified SNO-proteins that are critical and important to neuron projection, post-synaptic density, synaptic vesicle fusion, nervous system development, synapse, myelin sheath, glutamate biosynthesis and other neuronal related processes. None of the above appeared in the WT-Adults-Cortex. Specific proteins were identified in the KO-6 weeks-Cortex that are related to the glutamatergic synapse, neuron projection, myelin sheath, regulation of signal transduction, and nervous system development, none of which appeared in the WT-6 weeks-Cortex.

In conclusion, our findings could lead to a comprehensive analysis of the role of S-nitrosylation in one form of ASD and shed light on unclear mechanisms that lead to the neurodevelopmental disorder.

Mon Poster 56: Decoding site-specific alteration of Sialo-glycoproteome in EGFR-subtype of non-small cell lung cancer

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Altered sialylation of cell surface glycoproteins has been correlated with cancer development. Due to extreme heterogeneity, variable branching and extension of glycans, decoding the complex site-specific glycan structure on glycoproteins still presents great analytical challenges in mass spectrometry-based glycoproteomics. A highly specific enrichment and quantitation approach for intact glycopeptides is critical for comprehensive sialo-glycoproteomics profiling. In non-small cell lung cancer (NSCLC), sialylation has been reported to regulate EGFR activity and enhance its sensitivity to tyrosine kinase inhibitors. Here, we report a new glycopeptide enrichment material, ZIC-cHILIC, and incorporating TMT6plex labeling approach to decode the altered sialo-proteome in different EGFR subtypes of NSCLC cells. The intact glycopeptides were enriched by our home-made ZIC-cHILIC stage-tip from NSCLC membrane fraction, separated into 5 fractions by stepwise elution and analyzed by Orbitrap Fusion MS with product-dependent stepped HCD, which targets 3 diagnostic oxonium ions (m/z 204.08 HexNAc, 138.06 Hex fragment, and 366.11 HexNAcHex) to induce the glycopeptide fragmentation. ZIC-cHILIC stage-tip afforded good enrichment specificity. By presence of sialic acid-specific diagnostic

oxonium ions in MS/MS spectra, our results showed as high as 88% specificity for enrichment of sialo-glycopeptides. On the sialo-glycoproteomic scale, 2346 unique intact sialo-glycopeptides corresponding to 747 proteins were quantified by TMT6plex labeling. The comparison of the 4 NSCLC cell lines with different EGFR subtypes revealed 29 unique glycopeptides site-specifically present in wild-type NSCLC, such as integrin a3/b1 with fucosylated monosialo-biantennary glycan on N86/N363. In addition, 19 and 24 glycopeptides were uniquely identified in NSCLC cells with exon-19 deletion and L858R/T790M mutation, respectively. The proteome-scale profiling also revealed high glyco-site identification coverage of individual glycoprotein. Without immunoprecipitation, 11 of 13 N-glycosylation sites on EGFR were identified and altered site-specific sialylated glycoforms were also quantified. On the model study of NSCLC, our results may allow better understanding how the glycosylation correlates with EGFR subtypes.

Mon Poster 57: Quantitative Phosphoproteomic Analysis Reveals System-wide Signaling Networks in Chronic Lymphocytic Leukemia (CLL) B cells

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Targeting various kinases in B cell receptor (BCR) signaling have demonstrated promising efficiency for treating chronic lymphocytic leukemia (CLL). However, the efficacy varies since these kinases contribute to other signaling pathways and the clinical effects stem from simultaneous inhibition of multiple signaling responses. To gain better understanding in the response of targeted therapy for individual patient, identification of abnormal protein kinase activity and phosphorylation signaling network from individual patient may provide improved or new therapeutic opportunity. Here, personalized phosphoproteome signatures of the primary B cells purified from the blood of 6 CLL patients and 3 healthy controls delineated the differential phosphorylation events in CLL. Among 4438 phosphopeptides, 289 phosphopeptides (193 proteins) were found to be significantly changed ($p < 0.05$). Pathway analysis reveals that 21 pathways were differentially enriched in the CLL patients ($p<0.05$). BCR signaling were the top pathway along with many BCR related signaling pathways, such as ERK/MAPK signaling and PI3K signaling. Interestingly, the Systemic Lupus Erythematosus Signaling is ranked the top pathway which has been well documented with close association with autoimmune diseases and lymphoproliferative disorders, but the detailed mechanism remained to be studied so far. The phosphoproteomic analysis mapped many molecules in BCR signaling such as CD22, CD19, and NFAT along with their site-specific alteration in CLL compared to healthy control. Linking these differentially altered phosphoproteins, involved in cell proliferation and apoptosis, to BCR signaling through protein-protein interaction compiles a system-wide signaling network in CLL lymphomagenesis. We have validated two proteins to confirm their higher expression level of specific phosphorylation sites in CLL patients compared to healthy controls. These two proteins are under investigation to elucidate their potential functional roles in CLL. Altered phosphoproteome of malignant B cells may provide novel insights into the pathogenesis of B-CLL and new directions of strategies for controlling B cell malignancies.

Mon Poster 58: Proteostasis Interactome Remodeling of Amyloidogenic Proteins Governs Improved ER Quality Control Decisions

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The folding and trafficking of secreted proteins versus their degradation in the endoplasmic reticulum (ER) is controlled by the combined activity of chaperones, co-chaperones, folding and quality control factors, which together comprise the ER proteostasis network. Imbalances in ER proteostasis are associated with etiologically diverse protein misfolding diseases. For instance, systemic amyloid diseases arise when a destabilized protein escapes the ER quality control mechanism and traffics to the extracellular space where it misfolds and aggregates into proteotoxic species that damage post-mitotic tissue. Activation of the Unfolded Protein Response (UPR) stress-responsive signaling pathway adjusts the ER proteostasis capacity through upregulation of chaperones and quality control factors that counteract the protein folding stress. ER proteostasis remodeling through stress-independent activation of UPR-associated transcription factors has emerged as a promising strategy to enhance the ER quality control stringency and prevent the secretion of destabilized variants of structurally diverse proteins, whose extracellular misfolding is associated with amyloid diseases. Here, I will describe mechanistic insights into how remodeling of the ER proteostasis network can influence quality control decisions for destabilized proteins and selectively reduce their secretion. Using a multiplexed interactomics approach consisting of cross-linking affinity purification, coupled to tandem mass tag (TMT)-based quantitative proteomics, we elucidate interaction changes between destabilized amyloidogenic client proteins and ER proteostasis network components. Biochemical follow-up studies establish the role of prioritized components and sub-pathways in mediating the attenuated secretion of destabilized, amyloidogenic proteins upon UPR-dependent ER proteostasis remodeling, uncovering emergent properties of proteostasis networks accessed through activation of stress-responsive signaling pathways. These results highlight the utility of multiplexed quantitative interactomics to define how altered client protein engagement of proteostasis pathways can influence the quality control decisions. The identification of key steps in the protein quality control mechanisms can be exploited to develop new strategies to correct protein misfolding diseases.

Mon Poster 59: Investigating the Mechanism of AGE-mediated Cancellation of Calorie Restriction Benefits

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Calorie restriction (CR) is the gold standard method for increasing lifespan and overall health in laboratory animals, and provides one of the best non-genetic models for studying aging processes. However, the mechanism of lifespan extension is the subject of intense debate and remains unknown. The addition of advanced glycation end products (AGEs), which are 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. This mechanism is also unknown, although the most likely cause is pro-inflammatory remodeling of the proteome. Our overall objective is to determine the mechanism by which dietary AGE-modified proteins cancel the benefits of CR. We have applied kinetic proteomics and quantitative mass spectrometry to a mouse model in order to explore the correlation between calorie restriction, dietary AGEs, and resulting perturbations in the proteome. Our lab uses a heavy water (D_2O) metabolic labeling method to measure the synthesis and degradation rates of thousands of proteins simultaneously in vivo, allowing direct investigation of proteome maintenance within the cell. Additionally, we have paired our proteomic data with mRNA sequencing, allowing us to discern between transcriptional and post-transcriptional effects of AGE-mediated signaling. Our preliminary data indicate widespread changes in protein homeostasis, resulting at least in part from dietary mediated remodeling of the transcriptome. Understanding how AGE-related signaling decreases cellular fitness and lifespan at the level of protein regulation is expected to lead to the eventual identification of novel therapeutic targets for age-related diseases such as diabetes, cardiovascular disease, and dementia.

Mon Poster 60: Two-dimensional Reversed Phase-Reversed Phase Liquid Chromatography for Top-down Proteomics

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The single dimension reversed-phase liquid chromatography (RPLC) is the most prevalent separation tool to study complex intact protein samples in top-down proteomics. However, due to extreme complexities and large dynamic ranges, 1D-RPLC only may not provide sufficient intact proteome coverage. In addition, confident intact protein characterization often requires good quality fragmentation peaks through averaging several MS/MS scans, and it is a rate-limitation step for 1D RPLC analysis. To address these

challenges, the orthogonal separation techniques (i.e., 2D LC) are often combined to improve proteome coverage and increase dynamic range of detection.

In our study, we evaluated a "salt-free" high-pH RPLC approach as the first dimension separation. We chose a commercially available polyethoxysilane-based packing material to achieve maximize efficiency and occupancy of the silanol groups of the fused silica material to avoid mixed mode retention of proteins when dealing with high pH buffers. The intact Escherichia coli lysate were used to evaluate the proposed separation approach. Through bottom-up analysis of the fractions, we noticed a significant difference between the distributions of proteins identified in each fraction was observed. A total of 3713 unique proteins were identified in low pH mode, and 2080 proteins were identified in high pH mode with an overlap of 1008 proteins. Our preliminary results demonstrated that RPLC methods with mobile phases of different pH's have good orthogonality. Encouraged by the results, we perform both 1D-RPLC and 2D RPLC-RPLC separation of the intact E. coli proteins. Compared to the 147 proteins and 244 proteoforms identified using 1D RPLC-MS top-down study, 244 proteins and 1515 proteoforms were identified using 2D RPLC-RPLC-MS top-down approach.

Therefore, we have developed a 2D pH-RPLC-MS/MS platform for top-down proteomics study and successfully applied it to analyze complex sample.

Mon Poster 61: Profiling of Intact Proteins in the CSF of Alzheimer's Disease Patients: Revealing Specific Proteoform Biomarker Candidates

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Thanks to proteomics investigations, the idea that protein biomarkers like tau, amyloid peptides, ApoE, cystatin, and neurogranin are represented in body fluids as single species has evolved, as most proteins are present in different proteoforms and subjected to numerous processing events (proteolytic processing or alternative splicing) and post-translational modifications (PTM). Measuring the intact mass of proteins by MS in biofluids has the advantage of providing information on the presence and relative amount of the distribution of different proteoforms. This approach is known as proteomform profiling.

In this study we have used a state-of-the-art benchtop UHR-Q-TOF (Impact II, Bruker Daltonics) in a proteoform profiling approach with the objective of detecting and identifying proteoform biomarkers in human cerebrospinal fluids (CSF). CSF samples from 30 patients already diagnosed with Alzheimer's disease (AD), multiple sclerosis (MS) and other non-degenerative neurological disorders (OND) were included in the study.

Statistical analyses were performed on more than 5000 mass spectral features reproducibly detected in patient samples. Theses MS results have been correlated with clinical data, to derive a first list of more than 70 differentially expressed features. Using additional LC-MS/MS runs, the identified features were fragmented, sequenced and *de-novo* identified and validated. For the first time, results confirmed that several isoforms of APP, clusterin or transthyretin were differentially expressed in the CSF of AD patients, when compared to the CSF of the other groups.

Moreover, we have been able to detect and characterize several isoforms of chromogranin/secretogranin, a synaptic biomarker family which have been recently described as possible biomarkers of neurodegeneration. Among all chromogranin/secretogranin isoforms detected, some show differential expression in the three clinical groups assayed.

Mon Poster 62: CE-MS-based Profiling of BNP Proteoforms from Plasma in One Hour

Koen Raedschelders; Shenyan Zhang; Jennifer Van Eyk

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B-type Natriuretic Peptide (BNP) is a biologically active 32 amino acid peptide secreted by ventricular cells into the circulation. Its concentration is routinely used in the diagnosis of heart failure. Multiple plasma enzymes are responsible for the cleavage of BNP into at least 7 known proteoforms. We coupled capillary electrophoresis to high-resolution mass spectrometry with a view to profiling BNP proteolysis in plasma. Our method relies on pulsing exogenous BNP into a known quantity of plasma where proteolysis is allowed to occur. Using a neutral coated CESI capillary, we can sequentially sample this reaction vial directly using electrokinetic injection to monitor the dynamic generation and breakdown of up to five BNP proteoforms. In an abbreviated iteration of our method using multisegment injection, we are able to produce a multi-point BNP proteolysis profile from one patient within an hour, including all sample preparation steps.

Mon Poster 63: Development of an All-recombinant Intact Protein Standard for LC MS Application Development and System Suitability Testing

Rosa Viner[2]; Kay Opperman[1]; Daniel Lopez Ferrer[2]; Helene Cardasis[2]; Viktorija Vitkovske[3]; Shane Bechler[2]; Lukas Taujenis[3]; Vikrant Gohil[3]; John Rogers[1]; Egle Capkauske[3]; Kestutis Bargaila[3]; Agne Alminaite[3]; Juozas Siurkus[3]

[1]Thermo Fisher Scientific, Rockford, IL; [2]Thermo Fisher Scientific, San Jose, Ca; [3]Thermo Fisher Scientific, Vilnius, Lithuania

Introduction

In recent years, interest in intact protein analysis by HPLC, LC-MS, and MSMS has increased significantly. This can be attributed to both improvements to LC and MS hardware, instrument control software, data processing software, as well as conceptual shifts in how we can best address and answer biological questions given these emerging commercially available capabilities. Here, we describe the development of a multi-purpose intact protein standard for LC, LC-MS, and LC-MSMS quality control and application development for the Top-down proteomics field.

Methods

Protein standards were expressed in *E. coli* or purchased from Peprotech. Multiple proteins were screened and six were selected that 1) evenly covered a MW range of 9kD – 68kD, 2) presented clean, modification and adduct-free ESI spectra, and 3) whose ESI charge state distributions covered a wide m/z range from 500-2000. Mixing ratios were adjusted such that all six proteins could be detected simultaneously in a single infusion MS experiment (R&D standard). Quality and stability of selected proteins was verified by SDS-PAGE, UV HPLC, and infusion or LC-MS using an Orbitrap mass spectrometer at both high and low resolution settings.

Data

To extend utility of the standard to the broader intact protein analysis community, we utilized recombinant proteins that met the above requirements as well as satisfy FDA regulations. As such, new non-animal, recombinant protein candidates were synthesized, affinity captured, and purified.<ins cite="mailto:Viner,%20Rosa" datetime="2016-01-17T15:54"> </ins>Affinity tags were removed and proteins were dialyzed into MS-compatible buffer. Selected candidates were fully characterized by UV HPLC and LC/MS/MS, and accelerated stability tests were performed on those which met purity, MW, and m/z distribution requirements. Data from this recombinant high quality intact protein standard will be presented.

TUESDAY POSTER LIST

Tuesday posters should be set up 8:00-8:30 am Tuesday morning and removed at 3:00 pm Tuesday afternoon.

Lightning Talks

Posters featured in the Tuesday *Lightning Session - Round II* and Wednesday *Technology and Tips & Tricks* are noted.

Biochemical Pathway Elucidation, Tues Poster 01

Tues Poster 01 **Phosphoproteomics Reveals Potential Crosstalk Between mTORC2 and MAP kKnases;** Samuel Entwisle[1]; Camila Martinez-Calejman[2]; David Guertin[2]; Judit Villen[1]; *[1]University of Washington, Seattle, WA; [2]University of Massachusetts Medical Center, Worcester, MA* **Tuesday Lightning Session**

Biomarkers and Targeted MS Assays, Tues Poster 02-04

Tues Poster 02 **Exosomal EphA2 Transmits Chemoresistance and Predicts Pancreatic Cancer Patient Responses to Therapy;** Jia Fan[1]; Qian Wei[2]; Eugene J. Koay[3]; Yang Liu[1, 2]; Zhen Zhao[4]; Tony Y. Hu[1, 2]; *[1]Arizona State University, Tempe, AZ; [2]Houston Methodist Research Institute, Houston, TX; [3]University of Texas M.D. Anderson Cancer Center, Houston, TX; [4]National Institutes of Health, Bethesda, MD* Tuesday Lightning Session

Tues Poster 03 **Development of Protein Biomarkers for Effects of Radiation Exposure using Quantitative Mass Spectrometry;** Kate Liu; Elizabeth Singer; Whitaker Cohn; Julian Whitelegge; William McBride; Joseph Loo; *UCLA, Los Angeles, CA* Tuesday Lightning Session

Tues Poster 04 **A Spin Column-Free Permethylation Procedure for Glycan Analysis;** Yueming Hu[1, 2]; Chad R. Borges[1, 2]; *[1]Arizona State University, Tempe, AZ; [2]The Biodesign Institute, Arizona State University, Tempe, Az* Tuesday Lightning Session

Chromatin Dynamics, Tues Poster 05-06

Tues Poster 05 **Influence of the Gut Microbiota on Histone Modifications in Intestinal Epithelial Cells;** Peder J. Lund; Sarah A. Smith; Johayra Simithy; Zuo-Fei Yuan; Kevin Janssen; Gary D. Wu; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA* Tuesday Lightning Session

Tues Poster 06 **Histone H2A Proteolysis During Mouse Embryonic Stem Cell Differentiation;** Mariel Coradin[1]; Simone Sidoli[1, 3]; Kelly Karch[1, 2]; Benjamin A. Garcia[1, 2]; *[1]Department of Biochemsitry and Molecular Biophysic, Philadelphia, PA; [2]Epigenetics Program, Philadelphia, PA; [3]University of Pennsylvania School of Medicine, Philadelphia, PA* Tuesday Lightning Session

Clinical Proteomics, Tues Poster 08-10

Tues Poster 08 **Comparative Proteomic Analysis of the Influence of Gender and Acid Stimulation on Normal Human Saliva Using LC/MS/MS;** Xiaoping Xiao[1]; Yaoran Liu[2]; Wei Sun[1]; Qian Li[2]; *[1]Chinese Academy of Medical Sciences, Beijing, China; [2]Peking Union Medical College hospital, Beijing, China* Tuesday Lightning Session

Tues Poster 09 **Early Detection in Urinary Proteome for the Effective Early Treatment of Bleomycin-Induced Pulmonary Fibrosis in a Rat Model;** Jianqiang Wu[1]; Xundou Li[1]; Youhe Gao[1, 2]; *[1]Peking Union Medical College, Beijing, China; [2]Beijing Normal University, Beijing, China* Tuesday Lightning Session

Tues Poster 10 **A Standardized Method to Produce a Digested Yeast Protein Extract Reference Material for Mass Spectrometry;** Candice Johnson; Ashley Beasley Green; Karen Phinney; *National Institute of Standards and Technology, Gaithersburg, MD*

Clinical Proteomics, Tues Poster 11-16

Tues Poster 11 **Quantitative Interrogation of Large Mass Spectrometry Datasets Using a Flexible Hierarchical Organization Scheme;** Phillip Seitzer[1]; Seth Just[1]; Susan Ludwigsen[1]; Caleb Emmons[1]; Brian Searle[2]; *[1]Proteome Software, Portland, Oregon; [2]University of Washington, Seattle, WA*

Tues Poster 12 **Linear B Cell Epitope Prediction by Using High Throughput Peptide Microarrays;** Robayet Chowdhury[1, 2]; Taylor Brown[1, 2]; Neal Woodbury[1, 2]; *[1]Innovations in Medicine,The Biodesign Institute, Tempe, Arizona (AZ); [2]Arizona State University, Tempe, AZ* Tuesday Lightning Session

Tues Poster 13 **Methods for Estimating False Discovery Rates in High-Resolution Peptide Spectral Library Searches;** Zheng Zhang; Yuri Mirokhin; Dmitrii Tchekhovskoi; Sanford Markey; Stephen Stein; *NIST, Gaithersburg, Maryland*

Tues Poster 14 **A Multi-Level Quality Control Workflow for MS-Based Proteomics Using a Complex Biological Standard;** Hossein Fazelinia; Lynn Spruce; Hua Ding; Steven Seeholzer; *Children's Hospital of Philadelphia, Philadelphia, PA*

TUESDAY POSTER LIST

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Tues Poster 15 **GiaPronto: A One-Click Graph Visualization Software for Proteomics Datasets;** Amber K. Weiner[1, 2]; Simone Sidoli[1]; Sharon J. Diskin[2]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia, PA* Tuesday Lightning Session

Tues Poster 16 **The Hybrid Search: A Mass Spectral Library Search Method for Discovery of Modifications in Proteomics;** Meghan C. Burke; Yuri A. Mirokhin; Dmitrii V. Tchekhovskoi; Sanford P. Markey; Stephen E. Stein; *Mass Spectrometry Data Center, NIST, Gaithersburg, MD* Tuesday Lightning Session

High Resolution Mass Spectrometry, Tues Poster 17

Tues Poster 17 **Applying DDA Methods for Global Profiling: Evaluating DDA Acquisition Parameters for Exhaustive Data Mining and Reproducibility;** Scott M. Peterman[1]; David Sarracino[1]; Shen Luan[1]; Amol Prakash[2]; Bradley J. Hart[3]; *[1]Thermo Fisher Scientific, Cambridge, MA; [2]Optys Tech Corporation, SHREWSBURY, MA; [3]Thermo Scientific, Trabuco Canyon, CA*

High Resolution Mass Spectrometry, Tues Poster 18-22

Tues Poster 18 **RNA Mononucleoside Modification Detection, Quantitation, and Multiplexing by NanoLC-MS/MS;** Kevin A. Janssen; Ranran Wu; Benjamin A. Garcia; *University of Pennsylvania School of Medicine, Philadelphia, PA* Tuesday Lightning Session

Tues Poster 19 **MetaProt: A Cloud-based Platform to Analyze, Annotate, and Integrate Metabolomics Datasets with Proteomics Information;** Howard Choi[1]; Vincent Kyi[1]; Brian Bleakley[1]; Ding Wang[1]; Henning Hermjakob[2]; Peipei Ping[1]; *[1]NIH BD2K Center at UCLA, Los Angeles, CA; [2]EMBL-EBI, Hinxton, UK* Tuesday Lightning Session

Tues Poster 20 **Bladder Cancer Metabolomics Using the UPLC/MS-Based AbsoluteIDQ p180 Kit;** Sri Ramya Donepudi[1]; Vasanta Putluri[1]; Feng Jin[1]; Suman Maity[1]; Vadiraj Bhat[2]; Arun Sreekumar[1]; Nagireddy Putluri[1]; *[1]Baylor College of Medicine, Houston, TX; [2]Agilent Technologies, Santa Clara, CA*

Tues Poster 21 **Extending an Integrated Reference Tandem Mass Spectral Library for Comprehensive Identification of Metabolites and Bioactive Peptides;** Xiaoyu Yang; Pedatsur Neta; Stephen Stein; *NIST, Gaithersburg, MD*

Tues Poster 22 **Racial Disparity in Bladder Cancer and Identification of Altered Metabolism in African American Compared to European Bladder Cancer;** Venkatrao Vantaku[1]; Tiffany Dorsey[2]; Vasanta Putluri[1]; Sri Ramya Donepudi[1]; Suman Maity[1]; Wei Tang[2]; Feng Jin[1]; Danthasinghe Waduge Badrajee Piyarathna[1]; Kimal Rajapakshe[1]; Shyam Kavuri[1]; Vadiraja Bhat[3]; Seth Lerner[1]; Yair Lotan[4]; Wei Liu[5]; Cristian Coarfa[1]; Arun Sreekumar[1]; Stefan Ambs[2]; Nagireddy Putluri[1]; *[1]Baylor College of Medicine, Houston, texas; [2]NIH/ACI, Bethesda, MD; [3]Agilent Technologies, Santa Clara, CA; [4]UTSouthwestern, Dallas, TX; [5]Agios Pharmaceuticals, Boston, MA*

Metaproteomics, Tues Poster 23

Tues Poster 23 **Mucosal Microbiome and Vaccine Response in HIV-Exposed Uninfected African Infants;** Suereta Fortuin; *Cape Town University., Cape Town, South Africa*

New Technologies, Tues Poster 24-37

Tues Poster 24 **Global Identification of Functional Phosphorylation Sites in *Saccharomyces cerevisiae*;** Ian Smith; *University Of Washington, Seattle, WA* Wednesday Tips & Tricks

Tues Poster 25 **Investigations of Kinase Signaling in Cancer Metabolism with Cell-Active, KinaseSspecific Biosensors;** Laura Marholz; *University of Minnesota, Minneapolis, MN*

Tues Poster 26 **Parallel Accumulation - Serial Fragmentation (PASEF) on a Novel Trapped Ion Mobility Spectrometry (TIMS) – QTOF instrument;** Scarlet Beck[1]; Heiner Koch[1]; Florian Meier[1]; Markus Lubeck[2]; Stephanie Kaspar-Schoenefeld[2]; Niels Goedecke[2]; Oliver Raether[2]; Juergen Cox[1]; Matthias Mann[1]; *[1]Max Planck Institute of Biochemistry, Martinsried/Munich, Germany; [2]Bruker Daltonik GmbH, Bremen, Germany*

Tues Poster 27 **A Single UHPLC System for Both High Flow and Nano Flow LC-MS/MS: Application in Discovery and Targeted Proteomics;** Linfeng Wu[1]; Alex Zhu[2]; Paul Goodley[1]; Pat Perkins[1]; *[1]Agilent Technologies, Santa Clara, CA; [2]Agilent Technologies, Wilmington, DE* Wednesday Tips & Tricks

Tues Poster 28 **Time-Dependent Metabolomics in Systems Biology Context for Mechanism of Action Studies;** Akos Vertes[1]; Andrew Korte[1]; Hang Li[1]; Peter Nemes[1]; Lida Parvin[1]; Sylwia Stopka[1]; Sunil Hwang[1]; Ziad Sahab[1]; Deborah Bunin[2]; Merrill Knapp[2]; Andrew Poggio[2]; Carolyn Talcott[2]; Brian Davis[3]; Christine Morton[3]; Christopher Sevinsky[3]; Maria Zavodsky[3]; Nicholas Morris[4]; Matthew Powell[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* Wednesday Tips & Tricks

TUESDAY POSTER LIST

Tuesday posters should be set up 8:00-8:30 am Tuesday morning and removed at 3:00 pm Tuesday afternoon.

Tues Poster 29 **Reducing Your Coomassie Footprint: An Enviromentally Friendly Protein Stain with Sensitivity Similar to SYPRO Ruby and Deep Purple Gel Stains;** Gary Smejkal; Donald Mousseau; *Focus Proteomics, Hudson, NH*

Tues Poster 30 **Quantification of Protein Stability in Mammalian Tissues;** Dan B. Mcclatchy; Nam-Kyung Yu; John R. Yates; *The Scripps Research Institute, La Jolla, CA*

Tues Poster 31 **Quantitative Bottom Up Proteomics Using a Novel Scanning Quadrupole Data Independent Acquisition (DIA) Method;** Jim Langridge[1]; Chris Hughes[1]; Lee Gethings[1]; Roy Martin[2]; Keith Richardson[1]; Johannes Vissers[1]; *[1]Waters Corporation, Wilmslow, UK; [2]Waters, Beverly, MA* Wednesday Tips & Tricks

Tues Poster 32 **PCT-HD for Tissue Biopsy Samples: Comparison to a Standard Method.;** Vera Gross[1]; Peter Hains[2]; Keith Ashman[3]; Valentina Valova[2]; Alexander Lazarev[1]; *[1]Pressure BioSciences, Inc., South Easton, MA; [2]Children's Medical Research Institute, Westmead NSW, Australia; [3]Sciex, Framingham, MA* Wednesday Tips & Tricks

Tues Poster 33 **Quantification of Circulating *M. Tuberculosis* Antigen Peptides Allows Rapid Diagnosis of Active Disease and tTeatment Monitoring;** Chang Liu; Jia Fan; Christopher Lyon; Ye Hu; *Arizona State University, Tempe, AZ* Wednesday Tips & Tricks

Tues Poster 34 **A Promising Alternative to MS2-DIA: IonStar Enables Large-Scale, Accurate and Extensive Quantification with Low Missing Data and Ffalse Positives;** Jun Qu; Xiaomeng Shen; Shichen Shen; *SUNY-Buffalo, Buffalo, NY* Wednesday Tips & Tricks

Tues Poster 35 **In-Depth Quantitation of Changes in Protein Expression Levels in Complex Samples on a Q-TOF Instrument Using Data-Independent Acquisition (DIA);** Stephanie Kaspar-Schoenefeld[1]; Thomas Kosinski[1]; Pierre-Olivier Schmit[2]; Na Parra[3]; *[1]Bruker Daltonik GmbH, Bremen, Germany; [2]Bruker Daltonique S.A., Wissembourg, France; [3]Bruker Daltonics, Billerica, MA* Wednesday Tips & Tricks

Tues Poster 36 **Developing Novel Biosensors for Serine/Threonine Kinases Involved in Cancer;** Joel Zembles; *University Of Minnesota, Minneapolis, Minnesota*

Tues Poster 37 **Ultrasensitive Microanalytical CE-nanoESI-MS for Bottom-up Proteomic Characterization of Mouse Hippocampal Neurons;** Sam Choi; Eric Corcoran; Marta Zamarbide; M. Chiara Manzini; Peter Nemes; *The George Washington University, Washington DC* Wednesday Tips & Tricks

Pathogen Proteomics, Tues Poster 38-39

Tues Poster 38 **Comprehensive Proteomics Analysis to Identify Differentially Expressed Proteins in Cells with ASC Speck Formation;** I-Che Chung; Chih-Ching Wu; Yu-Sun Chang; *Chang Gung University, Taoyuan, Taiwan*

Tues Poster 39 **Understanding Mechanism of Action of Drug Resistance Reversal Potential of Usnic Acid Using Proteomic Profiling;** Sneha Sinha; *csir-cimap, Lucknow, India* Tuesday Lightning Session

Post-Translational Modifications, Tues Poster 40-43

Tues Poster 40 **Global Proteomic Analysis of Lysine Acetylation During Zebrafish Embryogenesis;** Sunjoo Kim; Oh Kwang Kwon; Sangkyu Lee; *BK21 Plus KNU Multi-Omics /Drug Research Team, daegu, South of Korea*

Tues Poster 41 **Turnover Profiles of Histone Post-Translational Modifications in a Myogenic Model Using SILAC Labeling, Enzyme Networks and Trend Clustering Analysis;** Natarajan Bhanu; *University of Pennsylvania, Philadelphia, PA*

Tues Poster 42 **Glycation of Human Serum Albumin Increases Ex Vivo in Poorly Handled Samples;** Joshua W. Jeffs[1, 2]; Chad R. Borges, PhD[1, 2]; Douglas S. Rehder[2]; *[1]Arizona State University, Tempe, AZ; [2]The Biodesign Institute, Arizona State University, Tempe, AZ*

Tues Poster 43 **High Throughput and Accurate Quantitation of Phosphoproteomics for Biological Signaling;** Xiaoyue Jiang[1]; Ryan Bomgarden[2]; Rosa Viner[1]; Andreas Huhmer[1]; *[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Rockford, IL*

Precision Medicine, Tues Poster 44-46

Tues Poster 44 **Fast Second Dimension Microflow LC for High-Throughput Deep Proteome Coverage;** Randy Arnold[1]; Nick Morrice[2]; Joerg Dojahn[3]; Christie Hunter[1]; *[1]SCIEX, Redwood City, CA; [2]SCIEX, Warrington, UK; [3]SCIEX, Darmstadt, Germany*

Tues Poster 45 **Profiling Biochemical Individuality: Human Personal Omics Profiling (hPOP);** Sara Ahadi[1]; Hannes Rost[1]; Christie Hunter[2]; Liang Liang[1]; Shannon Rego[1]; Orit Dagan-Rosenfeld[1]; Denis Salins[1]; Mike Snyder[1]; *[1]Stanford University, Stanford, CA; [2]Sciex, Redwood City, CA* Tuesday Lightning Session

TUESDAY POSTER LIST

Tuesday posters should be set up 8:00-8:30 am Tuesday morning and removed at 3:00 pm Tuesday afternoon.

Tues Poster 46 **Evidence of Human Antagonistic Auto-Antibodies as a Mechanism of Insulin Resistance;** Andrew Lipchik; Anil Narasimha; Michael Snyder; *Stanford University, Stanford, CA* Tuesday Lightning Session

Protein Quality Control, Tues Poster 47-48

Tues Poster 47 **Selection and Validation of Endogenous Retention Time Standards and Quality Control Peptides for Plasma Proteomics Study;** Shenyan Zhang; Vidya Venkatraman; Qin Fu; Ronald Holewinski; Mitra Mastali; Jennifer Van Eyk; *Cedars Sinai Medical Center, Los Angeles, CA* Tuesday Lightning Session

Tues Poster 48 ***Ex Vivo* Protein Oxidation as a Metric of Blood Plasma/Serum Integrity;** Chad R. Borges; Joshua Jeffs; Shadi Ferdosi; *Arizona State University, Tempe, AZ* Wednesday Tips & Tricks

Proteoform Biology, Tues Poster 49-50

Tues Poster 49 **Elucidating the Biological Implications of Aluminum Binding to Osteocalcin;** Stephanie Thibert[1, 2]; Olgica Trenchevska[1]; Mario Kratz[3]; Ian de Boer[4]; Mian Yang[1]; Richard Hervig[5]; Peter Williams[2]; Joshua Jeffs[1, 2]; Chad Borges[1, 2]; *[1]Biodesign Institute, Arizona State University, Tempe, AZ; [2]School of Molecular Sciences, ASU, Tempe, AZ; [3]Fred Hutchinson Cancer Research Center, Seattle, WA; [4]University of Washington, Seattle, WA; [5]School of Earth & Space Exploration, ASU, Tempe, AZ* Tuesday Lightning Session

Tues Poster 50 **Novel Algorithm for Quantifying Proteoforms of Tropomyosin;** Amol Prakash[1]; Irina Tchernyshyov[2]; Irene van den Broek[2]; Vidya Venkatraman[2]; Scott Peterman[3]; Jennifer Van Eyk[2]; *[1]Optys Tech Corporation, Shrewsbury, MA; [2]Cedars Sinai Medical Center, Los Angeles, CA; [3]Thermo Scientific BRIMS, Cambridge, MA*

Proteogenomics, Tues Poster 51-57

Tues Poster 51 **Temporal Analysis of Proteome and Transcriptome in Differentiating Human Pancreatic Endocrine Cells identifies Developmentally Regulated Protein Networks.;** Ertugrul Cansizoglu[1]; Quinn P. Peterson[2]; Shaojun Tang[3]; Judith Steen[1]; Hanno Steen[1]; *[1]Harvard Medical School / BCH, Boston, MA; [2]Harvard Department of Stem Cell and Regenerative B, Cambridge, MA; [3]Georgetown University Medical Center, Washington, DC*

Tues Poster 52 **Integrative Proteogenomic Characterization of ColorectalCcancer Cell Lines and Primary Tumors;** Jing Wang[1]; Dmitri Mouradov[2]; Xiaojing Wang[1]; Robert Jorissen[2]; Matthew Chambers[8]; Lisa Zimmerman[8]; Suhas Vasaikar[1]; Christopher Love[2]; Shan Li[2]; Kym Lowes[2]; Helene Jousset[2]; Janet Weinstock[2]; Christopher Yau[4]; John Mariadason[5, 6]; Zhiao Shi[1]; Yuguan Ban[7]; Xi Chen[7]; Robert Coffey[8, 9]; Robbert Slebos[10]; Antony Burgess[2, 3]; Daniel Liebler[8]; Bing Zhang[1]; Oliver Sieber[2, 3]; *[1]Baylor College of Medicine, Houston, TX; [2]The Walter and Eliza Hall Institute of Medial Rese, Parkville, Australia; [3]The University of Melbourne, Parkville, Australia; [4]University of Oxford, Oxford, United Kingdom; [5]Olivia Newton-John Cancer Research Institute, Heidelberg, Australia; [6]La Trobe University School of Cancer Medicine, Melbourne, Australia; [7]University of Miami Miller School of Medicine, Miami, FL; [8]Vanderbilt University School of Medicine, Nashville, TN; [9]Veterans Affairs Medical Center, Nashville, TN; [10]Moffitt Cancer Center, Tampa, FL* Tuesday Lightning Session

Tues Poster 53 **Validation of Tumor Proteogenomic Annotations;** Anindya Bhattacharya[1, 2]; Vineet Bafna[1, 2]; *[1]UC San Diego, La Jolla, CA; [2]UC San Diego, La Jolla, CA* Tuesday Lightning Session

Tues Poster 54 **Proteogenomics: *in Silico* Analysis to Investigate the Low Discovery Rate of Variant Peptides in Shotgun Proteomics;** Tung-Shing Lih[1, 2]; Wai-Kok Choong[1, 2]; ChiTing Lai[2, 3]; Chia-Li Han[4]; Yu-Ju Chen[2]; Ting-Yi Sung[1]; *[1]Institute of Information Science, Academia Sinica, Taipei, Taiwan; [2]Institute of Chemistry, Academia Sinica, Taipei, Taiwan; [3]GSB Degree Program, National Taiwan University, Taipei, Taiwan; [4]School of Pharmacy, Taipei Medical University, Taipei, Taiwan*

Tues Poster 55 **Expression Profiling of miRNA, mRNA and Protein Cargo of Myeloid Derived Suppressor Cells and Their Exosomes;** Catherine Fenselau[1]; Lucia Geis-Asteggiante[1]; Ashton Belew[1]; Nathan Edwards[2]; Suzanne Ostrand-Rosenberg[3]; Najib El-Sayed[1]; *[1]University of Maryland, College Park, MD; [2]Georgetown University Medical Center, Washington, DC; [3]University of Maryland Baltimore County, Baltimore, MD*

Tues Poster 56 **Proteogenomic Characterization of Drug Resistance in the K562 CML Cell line;** Vinh Nguyen; Laurie Parker; *University of Minnesota, Minneapolis, MN*

Tues Poster 57 **proBAMsuite, a Bioinformatics Framework for Genome-Based Representation and Analysis of Proteomics Data;** Xiaojing Wang; *Baylor College of Medicine, Houston, TX* Tuesday Lightning Session

Proteomics in Ageing, Tues Poster 58-61

Tues Poster 58 **Analysis of the Effects of Dietary Signals on Protein Homeostasis;** Bradley Naylor; Richard Carson; Monique Speirs; John Price; *Brigham Young University, Provo, UT*

TUESDAY POSTER LIST

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Tues Poster 59 **Characterization of Cell-Surface, Exosomal, and Secreted Proteins of Senescent Human Fibroblasts by DIA/SWATH;** Nathan Basisty; Judy Campisi; Bradford Gibson; Birgit Schilling; *Buck Institute for Research on Aging, Novato, CA*

Tues Poster 60 **Proteomic and Metabolic Characterization of Oxidative Stress Responses Using a Stem-Cell Derived Multi-Tissue Disease Model of Age-Related Macular Degeneration;** Jesse G. Meyer[1]; Thelma Y. Garcia[1]; Birgit Schilling[1]; Arvind Ramanathan[1]; Deepak Lamba[1, 2]; Bradford Gibson[1, 3]; *[1]Buck Institute for Research on Aging, Novato, CA; [2]University of Washington, Seattle, WA; [3]Amgen, Thousand Oaks, CA*

Tues Poster 61 **Monitoring Riboprotein Turnover Kinetics in Vivo Through Stable Heavy Isotope Labeling.;** Nathan Keyes; Richard Carson; Ryne Peters; Bradley Naylor; John Price; *Brigham Young University, Provo, Utah*

Proteomics in Drug Development, Tues Poster 62-63

Tues Poster 62 **TargetSeeker-MS: A Bayesian Inference Approach for Drug Target Discovery Using Protein Fractionation Coupled to Mass Spectrometry;** Mathieu Lavallée-Adam[1, 2]; Jolene Diedrich[1, 3]; Alexander Pelletier[1]; William Low[3]; Antonio Pinto[3]; Salvador Martínez-Bartolomé[1]; Michael Petrascheck[1]; James Moresco[1, 3]; John R. Yates[1]; *[1]The Scripps Research Institute, Lajolla, CA; [2]University of Ottawa, Ottawa, Canada; [3]Salk Institute for Biological Studies, La Jolla, CA* Tuesday Lightning Session

Tues Poster 63 **Identification of FMS-Like Tyrosine Kinase 3 (FLT3) Substrates Using KALIP;** Minervo Perez; *University of Minnesota, Minneapolis, MN* Tuesday Lightning Session

TUESDAY POSTER ABSTRACTS

Tuesday posters should be set up 8:00-8:30 am Tuesday morning and removed at 3:00 pm Tuesday afternoon.

Tues Poster 01: Phosphoproteomics reveals potential crosstalk between mTORC2 and MAP kinases

Samuel Entwisle[1]; Camila Martinez-Calejman[2]; David Guertin[2]; Judit Villen[1]

[1]University of Washington, Seattle, WA; [2]University of Massachusetts Medical Center, Worcester, MA

The Mechanistic Target of Rapamycin complex 2 (mTORC2) is becoming appreciated as an important regulator of cell growth and metabolism, yet remains poorly understood. It was recently shown that mTORC2 regulates lipid metabolism in mouse brown adipose tissue (BAT). Specifically, knockout of mTORC2 in mouse BAT leads to increased lipid oxidation, decreased lipid synthesis, and resistance to diet-induced obesity. mTORC2 phosphorylates and activates Akt in response to insulin, however Akt can also act independently of mTORC2. In general, the downstream effectors of mTORC2 in the context of insulin signaling are poorly understood. To address this knowledge gap, we used mass spectrometry to measure the proteome and phosphoproteome for a 15 minute insulin treatment of brown adipocytes possessing an inducible mTORC2 knockout (mTORC2-KO). In an approach recently developed in our lab, we collected data-dependent acquisition (DDA) data for discovery-driven analysis, and used it to rapidly generate targeted, parallel reaction monitoring (PRM) phosphoproteomic assays. We found very few proteome changes in response to insulin. However, comparison of the control with mTORC2-KO revealed that enzymes related to fatty acid metabolism were decreased in the mTORC2-KO. The phosphoproteomic insulin responses of the control and mTORC2-KO were remarkably similar, but with some notable differences. We present preliminary evidence suggesting that mTORC2 participates in insulin-dependent cross-talk with the MAP kinase Erk. Specifically, we report a potentially novel regulatory mechanism whereby phosphorylation of the tyrosine, but not the threonine residue, in the activation loop is dependent on mTORC2. These, along with other candidate mTORC2 effectors, were also measured using PRM, which allows for more precise quantification, consistent detection, and enhanced phosphorylation site localization compared with DDA. The overall goal of this work is to discover phosphorylation events downstream of mTORC2 that could ultimately be targeted therapeutically to improve BAT function.

Tues Poster 02: Exosomal EphA2 transmits chemoresistance and predicts pancreatic cancer patient responses to therapy

Jia Fan[1]; Qian Wei[2]; Eugene J. Koay[3]; Yang Liu[1, 2]; Zhen Zhao[4]; Tony Y. Hu[1, 2]

[1]Arizona State University, Tempe, AZ; [2]Houston Methodist Research Institute, Houston, TX; [3]University of Texas M.D. Anderson Cancer Center, Houston, TX; [4]National Institutes of Health, Bethesda, MD

Exosomes are secreted by most cells, including tumor cells, and exhibit paracrine and endocrine effects to alter cell behavior. However, the ability of cancer-derived exosomes to regulate drug resistance, which primarily arises from clonal cell changes, is less well understood. We therefore analyzed whether exosomes and specific exosomal proteins could transfer chemoresistance between heterogeneous pancreatic cancer (PC) cell lines. Exosomes isolated from three PC cell lines with variable gembicitabine (GEM) sensitivity (PANC-1, MIA PaCa-2, and BxPC-3) were tested for their capacity to transmit chemoresistance and then analyzed by comparative proteomics to identify candidate resistance factors. Our results revealed that exosomes of chemoresistant PANC-1-derived exosomes were internalized by and increased GEM resistance of chemosensitive PC cells. PANC-1 exosomes were subsequently found to overexpress Ephrin type-A receptor 2 (EphA2), which is associated with therapy resistance in other tumor types, and chemoresistance transmission was inhibited by shRNA-mediated EphA2 knockdown in PANC-1 exosome donor cells, while direct treatment with recombinant EphA2 did not promote chemoresistance. Notably, circulating exosomal EphA2 levels were dramatically increased in mice bearing PANC-1 tumors, and in human PC patients who subsequently revealed poor responses to various chemotherapy and/or chemoradiation regimens. Based on these results, we conclude that exosomal EphA2 can transmit therapy resistance between heterogeneous PC cells and may potentially serve as a non-invasive biomarker to predict treatment response in patients with pancreatic cancer. However, it remains to be seen if additional exosomal factors regulate resistance to other cancer therapeutic agents in pancreatic cancer or other cancer types.

Tues Poster 03: Development of Protein Biomarkers for Effects of Radiation Exposure using Quantitative Mass Spectrometry

Kate Liu; Elizabeth Singer; Whitaker Cohn; Julian Whitelegge; William McBride; Joseph Loo

UCLA, Los Angeles, California

With increasing concern about nuclear/radiological terrorism, there is a national interest in developing biodosimetry methods that can guide triage and treatment decisions following an incident. Proper diagnosis and prognosis have been challenging since radiation victims may present delayed symptoms and there is considerable person-to-person variability in the response. To address these issues, we aim to develop protein biomarkers that can provide an individual's radiation dosimetry and to predict organ-specific response in the form of a blood test taken days after radiation exposure.

One of the protein biomarker panels that we are evaluating arises from the known role of the transcription factor Nrf2 in the cellular response to radiation. We focus on eight proteins that are most prominently modulated by Nrf2 and the anti-oxidant response element (ARE) promotor that it targets. Using targeted mass spectrometry, we are measuring the response of these proteins in the bone marrow of mice (n=8) at 8-hour and 1, 2, 4 days after exposure to 6 Gy total body irradiation (TBI). This quantitative study also includes comparisons of male versus female and C57Bl6 versus C3H strains.

Preliminary data of C3H mice presented considerable difference between male and female protein expression profiles. For most of the detectable Nrf2-modulated proteins, female mice showed more prominent upregulation after irradiation than male mice. There is few literature so far that addresses gender differences in radiation response. This experiment may yield new insight on gender differences in radiation susceptibility.

Compared to traditional biodosimetry markers that only measure absorbed dose, Nrf2-regulated proteins as part of antioxidant response have prognostic potential in predicting individual patient outcome, which is more valuable for personalized treatment.

Tues Poster 04: A spin column-free permethylation procedure for glycan analysis

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Glycan permethylation was introduced as a tool to facilitate the study of glycans in 1903. Since that time, permethylation procedures have been continually modified to improve permethylation efficiency and qualitative applicability. Typically, however, either laborious preparation steps or cumbersome and uneconomical spin columns have been needed to obtain decent permethylation yields on small glycan samples. Here we describe a spin column-free (SCF) glycan permethylation procedure that is applicable to both O- and N-linked glycans and can be employed upstream to intact glycan analysis by MALDI-MS, ESI-MS, or glycan linkage analysis by GC-MS. The SCF procedure involves neutralization of NaOH beads by acidified phosphate buffer, which eliminates the risk of glycan oxidative degradation and avoids the use of spin columns. Optimization of the new permethylation procedure provided high permethylation efficiency for both hexose (> 98%) and HexNAc (> 99%) residues—yields which were comparable to (or better than) those of some widely used spin column-based procedures. A light vs. heavy labelling approach was employed to compare a popular spin-column based approach to the SCF approach. Recovery of intact N-glycans was significantly better with the SCF procedure, but overall yield of O-glycans was similar or slightly diminished. When the SCF procedure was employed upstream to hydrolysis, reduction and acetylation for glycan linkage analysis of pooled glycans from unfractionated blood plasma, analytical reproducibility was on par with that from previous spin column-based "glycan node" analysis results that we have reported. When applied to blood plasma samples from Stage III-IV breast

cancer patients (n=20) and age-matched controls (n=20), the SCF procedure facilitated identification of three glycan nodes with significantly different distributions between the cases and controls (ROC c-statistics > 0.75; p < 0.01). In summary, the SCF permethylation procedure expedites and economizes both intact glycan analysis and linkage analysis of glycans from whole biospecimens.

Tues Poster 05: Influence of the gut microbiota on histone modifications in intestinal epithelial cells

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The gut microbiota exerts a heavy influence on the physiology of its host organism, especially through the production of small molecule metabolites like butyrate. Since butyrate is known to inhibit histone deacetylases (HDACs), the microbiota is expected to have an impact on the global epigenetic landscape of intestinal epithelial cells in the gut. To study the effect of the microbiota on host cell epigenetics, we employed mass spectrometry to comprehensively and quantitatively catalog hundreds of unique combinations of post-translational modifications on the histones of intestinal epithelial cells from germ-free and conventionally raised mice. Consistent with butyrate altering global patterns of histone acetylation, germ-free mice harbored reduced levels of acetylation compared to conventional mice, primarily on histone H4. While the ability of butyrate to negatively regulate HDACs has long been appreciated, more recent studies have focused on the potential for butyrate to positively regulate histone acetyltransferases (HATs) by serving as a source of acetyl-CoA through beta oxidation. Thus, to directly assess whether butyrate can be converted into acetyl-CoA for subsequent use by HATs, we treated an intestinal epithelial cell line with 13C-labeled butyrate and then used mass spectrometry to measure isotope incorporation into various acyl-CoA species and post-translationally modified histone peptides. Incubation with 13C-labeled butyrate clearly shifted the isotopic distributions of acetyl-CoA and numerous acetylated peptides from histones H2A, H3, and H4, providing strong support that butyrate can serve as a carbon source for histone acetylation. Overall, our work suggests that the flow of carbon from the diet to the epigenome through the microbiota via butyrate metabolism may be a significant epigenetic mechanism in addition to butyrate acting as an HDAC inhibitor. Adding to a growing body of evidence, this study highlights the molecular level intersection of metabolic and epigenetic networks, which may hold mechanistic significance in dysbiosis and inflammatory conditions.

Tues Poster 06: Histone H2A proteolysis during mouse embryonic stem cell differentiation

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Lineage commitment of embryonic stem cells (ESCs) is driven by changes in gene expression regulated by chromatin rearrangement. For instance, our and others data have demonstrated that during differentiation the levels of histone H3 and H4 acetylation substantially decrease. Moreover, a few studies have also showed that histone H3 and H2B tails can be proteolytically cleaved as irreversible post-translational modification (PTM), which functional role is still poorly understood. We present a systematic analysis of PTMs on histone H2A during mouse ESCs differentiation using both bottom-up and top-down mass spectrometry. By using our histone MS platform we identified the combinatorial PTMs H2AK5acK9ac to be significantly regulated during ESC differentiation. Using affinity pull-down approach with synthetic modified peptide baits, we identified a group of proteins that bind this specific H2A acetyl combination. These proteins are potential candidates to play a critical role in maintaining the pluripotent stem cell state. Using top-down proteomics, we identified extensive proteolysis on histone H2A (clipped H2A, cH2A. cH2A sites that were most abundant during ESC differentiation at L23 and G44, while other degradation products were identified as well. Parallel in vitro studies indicated that clipping of H2A at G44 is performed by the protease Cathepsin L. Lastly, we generated the histone dimer protein complex H2A/H2B using intact and G44 cH2A, and performed hydrogen/deuterium exchange (H/DX) to evaluate nucleosome stability. Preliminary experiments have demonstrated that the cleaved form destabilizes the dimer H2A/H2B complex as compared to the intact H2A form. In conclusion, our results suggests that histone H2A is extensively regulated with PTMs during mouse ESC differentiation, and that protease processing potentially promotes nucleosome eviction, probably to facilitate gene transcription mediated through acetylated H2A. As the mouse histone nucleosome is highly similar to human, we are confident we will be able to translate our studies into human chromatin.

Tues Poster 07: Aberrant Protein S-Nitrosylation ;A New Perspective In Hypertensive And Diabetic Hypertensive Disorder

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Background: Diabetes and hypertension frequently coexist, leading to additive increases in the risk of life-threatening cardiovascular events. Large scale proteomic studies implicate the role of aberrant protein expression in these groups' specifically post translational modifications. Protein S-nitrosylation conveys a large part of the ubiquitous effect on cellular signal transduction, accumulating evidence indicates important roles in normal physiology. Dysregulated S-nitrosylation has been implicated as a cause or consequence of a broad range of diseases, including asthma, cystic fibrosis, Parkinson disease, heart failure, and stroke. The purpose of study is to identify molecular changes and potential alterations in expression of specific aberrant s-nitrosylation in hypertensive and diabetic hypertensive patients. We aimed to identify such blood biomarkers and potential drug targets which can provide insight into the underlying molecular mechanisms, associated with its pathology.

Methods: Proteome mapping of hypertensive, diabetic hypertensive serum samples was conducted to get the expression of aberrant nitrosylated proteins. Serum samples (n=15 from each group) by using sodium dodecyl sulphate polyacrylamide gel electrophoresis coupled with immunoblot by using anti S-nitrosylated antibody followed by imaging and statistical analysis by Quantity-One software (BioRad).

Results: We have identified in total fifteen nitrosylated protein components with altered expression among the studied groups. The 177.8KDa, 119 KDa, 74.02KDa, 61.5KDa, 52.3KDa protein, and 24.93KDa proteins are showing hyper-nitrosylation in diabetic hypertensive serum samples. However, the 119 KDa, 74.02KDa and 61.5KDa protein components showed hyper-nitrosylation in hypertensive serum samples as compared to normal controls while rest of the proteins component were found hypo-nitrosylated.

Conclusion: The characterization of aberrantly expressed nitrosylated proteins globally and their association with disease associated pathways probably are playing modulatory roles in the pathophysiology of the disease, following post-translational modifications.

Tues Poster 08: Comparative proteomic analysis of the influence of gender and acid stimulation on normal human saliva using LC/MS/MS

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Human saliva is an important source for disease biomarker discovery. In this work, we investigate the influence of gender and acid stimulation on the normal human salivary proteome. Un-stimulated and acid-stimulated saliva samples from 5 males and 5 females were labeled with 4-plex iTRAQ and analyzed by 2-DLC MS/MS. A total of 1,770 proteins were identified, and 82 proteins in un-stimulated saliva were found to be gender-specific. These proteins were mainly associated with immune function, metabolism and inflammation. However, no gender-specific proteins were found in acid-stimulated saliva. In addition, 182 and 307 differential proteins were found to be acid stimulation-specific in male samples and female samples,

respectively. Of these, 137 proteins were common to both genders and mainly participated in the process of cellular movement, immune function and inflammatory response. By comparing the influence of gender and acid stimulation on the salivary proteome, it was found that acid stimulation caused more significant alteration and played a more important role in the human salivary proteome than gender. The above results may be helpful for salivary proteome research in the future.

Tues Poster 09: Early detection in urinary proteome for the effective early treatment of bleomycin-induced pulmonary fibrosis in a rat model

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Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal fibrosis lung disease. With limited effective treatment in the late stage, IPF has a very poor prognosis with an average survival less than 3 years. Molecular biomarkers are highly desired in IPF especially in its early phase. Without homeostatic control, urine is a better biomarker source than blood for detection of small and early pathological changes.

In this study, urine samples were collected at 4 time points from bleomycin-induced pulmonary fibrosis rats. By labeled proteome quantitation, 30 differential proteins were identified as diagnostic biomarkers for disease monitoring. Many differential proteins were reported to be associated with the pathogenesis of IPF. Then urine samples at slight fibrosis were used for early diagnostic biomarkers identification, 11 differential proteins were identified. Four of them had been reported being associated with fibrosis process in previous studies. Prednisone treatment was administered at different phases. It was found that prompt prednisone treatment after early diagnosis effectively inhibited lung fibrosis, whereas same treatment at late phase had very limited effects. Compared with differential proteins during lung fibrosis, change trends of 5 differential proteins after prednisone treatment were reversed, and they could serve as therapeutic monitoring biomarkers.

Urinary proteomics have been underutilized in respiratory diseases for decades. This is the first urinary proteomics application in lung fibrosis. Our results showed that urine proteins have the potential for early diagnosis, monitoring disease progression and reflecting treatment efficacy in IPF and probably other lung diseases. These findings may lead to an increased understanding of pathogenesis of pulmonary fibrosis.

Tues Poster 10: A Standardized Method to Produce a Digested Yeast Protein Extract Reference Material for Mass Spectrometry

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There is currently a well-established need for high quality measurements in mass spectrometry based clinical proteomic analysis. This need has emerged due to increased applications of mass spectrometry to the identification and quantification of proteins in clinical specimens. Currently, there are few reference materials or quality control materials available that can be used to evaluate the performance of the various steps in proteomics workflows. NIST previously developed RM 8323 Yeast Protein Extract as a proteomics quality control material. Customer feedback received after RM 8323 was issued indicated that a digested yeast extract material would also be of interest to the clinical proteomics community. RM 8313 Digested Yeast Protein Extract is being developed as a quality control material for mass spectrometry-based workflows and for validation of new methods and measurement approaches used to identify tryptic digested peptides in complex protein samples. Preparation of a yeast based complex tryptic peptide material required optimization of factors such as yeast cell growth and protein expression, protein extraction, protein digestion, and finally the LC-MS analysis of the material produced. Performing a large-scale protein digestion also requires optimization. In the production of RM 8313, trypsin is the most essential and costly reagent in the process, and lower grade (less efficient than sequencing grade) trypsins were compared to an industry standard enzyme for mass spectrometry. Lastly, the raw data files from the LC-MS^2 analysis will be subjected to NIST MSQC performance metrics to evaluate both sample preparation methods and instrument performance. These results will be used to guide production of this digested yeast material in preparation for scale-up, aliquoting and stability checks. By producing RM 8313 we intend to provide a digested yeast protein material which is consistent and economical to support measurements used to identify tryptic peptides in complex protein mixtures in mass spectrometry based workflows.

Tues Poster 11: Quantitative interrogation of large mass spectrometry datasets using a flexible hierarchical organization scheme

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Proteomics and metabolomics experiments often result in the generation of a large number of mass spectrometry samples. In general, the set of samples associated with an experiment may contain technical and biological replicates, blank or quality control samples, and often includes several different experiment-level variables assessed simultaneously (for example, time point, tissue type, and treatment or control). In order to identify meaningful quantitative trends in these datasets, it is essential to organize samples appropriately and use this organization to inform quantitative value computation. To this end, we have developed a flexible hierarchical organization scheme and roll-up based on assigning any number of attributes to samples, and organizing these attributes at, below, or above the level of technical replicate, biological replicate, and comparison level. In our scheme, the comparison level is always at or above the biological replicate level and the biological replicate level is always at or above the technical replicate level. Attributes organized below the technical replicate level are treated as fractionations, while attributes at or above the comparison level are treated as independent experimental variables. Depending on the question being asked, multiple organization schemes may be appropriate for a given dataset. Our system allows for efficient re-computation upon re-organization, facilitating exploration of different attribute hierarchies.

To demonstrate the efficacy of our solution, we have re-analyzed several previously published datasets. Our sample organization scheme works equally well on untargeted proteomics and metabolomics datasets, where precursor intensity was used as our preferred quantitation method. By integrating our hierarchical organization approach into an analysis framework with statistical testing (including but not limited to ANOVA and t-test) and Principal Component Analysis, we were able to identify analytes that were significantly differentiated between experimental conditions and formulate biological hypotheses useful for further investigation.

Tues Poster 12: Linear B cell epitope prediction by using high throughput peptide microarrays

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Elucidation of Antigen-Antibody (Ag-Ab) interactions is critical to understanding the humoral immune response against pathogenic invasion. The sites at which antibodies bind to antigens are called B cell epitopes. dentification of B cell epitopes in the target antigens is pivotal to designing effective epitope driven vaccines, immunoassays and antibody production. Expensive and resource intensive experimental mapping of B cell epitopes make in-silico methods more effective and practical. In the last three decades, several prediction methods have been developed to predict linear (continuous) and conformational (discontinuous) B cell epitopes. The majority of B cell epitopes are conformational but the number of structurally characterized Ag-Ab complexes is limited which inherently limits the

prediction methods. In addition, even linear B cell epitope prediction is limited by factors such as small and redundant datasets of experimentally characterized epitopes. High throughput peptide microarrays generate large datasets which can be used to develop an improved prediction algorithm. We are utilizing 130 K random sequence peptide microarrays where antibodies from diluted human serum bind to peptides which represent potential linear B cell epitopes on a silicon based solid surface. As this technology provides a distinctive signature related to antibody profiles circulating in blood a healthy and diseased states, it is referred to as an immunosignature assay. The large datasets of Ab-peptide binding produced by the immunosignaure assays should allow us to develop algorithms (e.g. machine learning) which will be able to predict linear B cell epitopes. This would help us to improve immunodiagnostic tests, rationalize synthetic vaccine design and minimize the cross reactivity of antibodies to proteins in terms of protein purification.

Tues Poster 13: Methods for estimating false discovery rates in high-resolution peptide spectral library searches

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Spectral library searching (SLS) is an attractive alternative to sequence database searching (SDS) for peptide identification due to its speed, sensitivity and ability to include any selected mass spectra. However, SLS does not have a tested and accepted process for calculating false discovery rates (FDR) as does SDS. The present study reports the development and testing of FDR procedures for SLS based on decoy libraries. Two types of decoy libraries were found suitable for high resolution peptide SLS. The first constructs spectra by reversing all sequences except the C-terminal residue. The second randomly replaces all non-C-terminal residues while maintaining the library's amino-acid distribution, followed by shifting the m/z of fragment ions accordingly. Determination of FDR is performed in a manner equivalent to SDS, concatenating a library with its decoy prior to a search. The SLS target-decoy approach yields thresholds for 1% FDR equivalent to those of using decoy-free PeptideProphet algorithm, but quite different from precursor m/z shifting methods published previously for low resolution SLS. For a human label-free hcd spectral library (containing 1,127,970 spectra), using CPTAC label-free data (containing 605,113 spectra): at 1% FDR, MSPepSearch identified 330,946 IDs (PeptideProphet algorithm), 328,512 IDs (Reversed-sequence library) and 334,940 IDs (Random-sequence library). We tested a CPTAC iTRAQ4-labeled data containing 994,133 spectra searched using a human iTRAQ4-labeled hcd spectral library containing 1,201,632 spectra. The 1% FDR identifications were 303,141 IDs (PeptideProphet algorithm), 307,497 IDs (Reverse-sequence library) and 307,588 IDs (Random-sequence library). The numbers of IDs varied in a 1.4 – 1.9% range. MSPepSearch led to up to 20% more identifications at 1% FDR when compared with other widely-used SDS engines coupled to post-processing approaches such as Percolator. The application of decoy methods for spectral libraries provides a measure of confidence for high resolution SLS, allowing SLS and SDS results to be compared and integrated.

Tues Poster 14: A multi-level quality control workflow for MS-based proteomics using a complex biological standard

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Mass spectrometry (MS)-based proteomics has continued to advance over the past few years, and a multitude of new technologies and different approaches have been developed. Despite the great success of modern MS in biological research, the reproducibility of MS results and the meaningful comparison of analysis results obtained by different laboratories and platforms remain cumbersome. This is partly because of absence of universal and well-established quality control (QC) and quality analysis (QA) methodology in MS-based proteomics. Sample preparation, chromatography, and MS instrumentation are main sources of variability in proteomics experiments that can eventually affect identification and quantification of peptides. Here, we present some of our efforts to establish a systematic QC/QA methodology using open source packages such as PTXQC, iMonDB, and etc. to identify the source of the variation within the proteomics data and thereby recognize early the occurrence of batch effects arising from the various workflow phases and make corrections to the workflow accordingly. Early efforts in developing this standard focused on sample stability and aliquot-to-aliquot reproducibility. The throughput penalty of these QC runs is far offset by the confidence gained in final experimental data quality, especially for long-term projects involving many samples run over several months.

Tues Poster 15: GiaPronto: A one-click graph visualization software for proteomics datasets

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The high sensitivity, accuracy and versatility of mass spectrometry (MS) in characterizing both protein samples and protein post-translational modifications (PTMs) led to the development of proteomics as a widely adopted discipline, especially if combined with online separation using liquid chromatography (LC). Despite the vast versatility of proteomics applications, quality control of LC-MS runs, data distribution, significance changes between datasets and protein functional analysis are often represented with similar plots. We implemented a freely available software to aid in analyzing and visualizing proteomic data. Once the input table contains the appropriate headers and protein/peptide quantitative values, it automatically performs normalization, statistics and outputs several graphs for data visualization. Moreover, the output includes several tables formatted for further data analysis. The program was developed in Shiny and was optimized to use as input the output table from MaxQuant [Cox et al 2008]. However, any input table is accepted. The program currently analyzes label-free, SILAC and Isobaric Tag datasets. Once statistical tests have been applied, our program outputs several plots and figure legends that allow the user to determine the quality of their data, regulated or abundant proteins, and global overviews of Gene Ontology or motif enrichments. These graphs include canonical box plots, principal component analysis (PCA), and scatterplots for correlation, but also more creative representations that adopt the absolute intensity of the protein in the mixture as an additional dimension. These last graphs are potentially very useful for selection of biomarkers, as they highlight the most detectable proteins in an LC-MS run. These plots are publication quality and can be customized by the user. Overall, this program not only provides a simplified tool for data representation, but also suggests a standardized data processing and quality control guideline for proteomics data, which is still largely left to the individual initiative of proteomics laboratories.

Tues Poster 16: The Hybrid Search: A Mass Spectral Library Search Method for Discovery of Modifications in Proteomics

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We introduce a mass spectral library based method to identify high resolution tandem mass spectra of peptides that contain unanticipated modifications. The 'hybrid' mass spectral library search method is based on the hypothesis that if two precursor ions differ by a single modification, which does not significantly alter the fragmentation mechanism, each product ion that does not contain the modification will have the same m/z; however, product ions that contain the modification will differ by the mass difference, or DeltaMass, normalized for charge. This allows each peak in a query spectrum to match either directly to a peak in the library spectrum or to a library peak whose m/z has been shifted by the normalized accurate DeltaMass. This method was evaluated using recurrent unidentified spectra from the Clinical Proteomic Tumor Analysis Consortium (CPTAC). The unidentified spectra were clustered into consensus spectra using in-house tools, generating a total of 1,428,188 spectra, which were searched against the publicly available human and mouse iTRAQ libraries.

Results from the hybrid mass spectral library search evaluation demonstrate the ability to identify unanticipated modifications including experimental artifacts, such as desulfurization of cysteine, amino acid substitutions and amino acid insertions or deletions. To date, 74 amino acid substitutions that may be due to single nucleotide polymorphisms (SNP) have been identified as well as 32 unique amino acid substitutions that cannot be explained by a SNP. The ability of the hybrid mass spectral library search method to consider the matching product ions before and after shifting is novel compared to current methods available. The peptide identifications that have been recovered illustrate how the spectral library search method serves as a powerful tool for the identification of unidentified high quality spectra due to various causes including an incomplete protein database or search settings that exclude the correct identification.

Tues Poster 17: Applying DDA methods for global profiling: evaluating DDA acquisition parameters for exhaustive data mining and reproducibility

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Global protein profiling methods are designed to determine the relative/absolute expression levels for the sample-specific proteome. Experimental methods generally incorporate bottom-up sample preparation performing qualitative/quantitative analysis on peptides which effectively become the protein surrogate marker. Thus, instrumental data acquisition methods are taxed with sampling the sample-specific peptides with not only the greatest depth and breadth, but maintaining high reproducibility across all samples. Data independent analysis (DIA) has primarily been associated with global profiling, but there are severe limitations when coupled with UHPLC separations. Chromatographic peak widths are reduced from ca. 30 seconds to 10 requiring larger and fewer precursor isolation windows enabling sufficient data points to be acquired per peak width for reproducible quantitation. Our approach is to enhance data dependent acquisition (DDA) methods by increasing the precursor isolation window for each tandem mass spectral event. Thus, high resolution/accurate mass (HRAM) measurement can be maximized for full scan MS acquisition (used for quantitation) and the resulting set of product ion spectra can be processed for exhaustive spectral matching. This presentation will demonstrate the advantages of using wide-window DDA methods as compared to DIA for reproducible peptide detection for a set of peripheral blood mononuclear cell (PBMC) digest across 10 technical replicates. In addition, a matrix of instrument settings was used to collect comparative data to maximize the optimized DDA method. The settings compared were the precursor isolation window, AGC, maximum ion fill times, and dynamic exclusion settings. In addition, the effects of wider DDA windows were evaluated for data processing routines compared between sequence and spectral matching for protein and peptide identification rates. The final results were also compared to DIA on the same sample.

Tues Poster 18: RNA Mononucleoside Modification Detection, Quantitation, and Multiplexing by nanoLC-MS/MS

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Over 140 modifications to RNA nucleosides have been identified, but many of these modifications have not been functionally characterized in detail, and new modifications are discovered annually. While modifications like m6A and m5C have been studied in a variety of contexts, the full profile of modifications has not been the focus of much research. Here, we present a method that allows for detection of abundant and low level modifications by separating nucleosides on a porous graphite carbon column nanoLC system and analyzing eluents by data independent acquisition (DIA) MS/MS. This system enables the quantitative study of many RNA nucleosides simultaneously in any RNA model system, and the application of DIA provides both improved sensitivity and an advancement in the ability to detect new modifications: even if a peak has low abundance at the MS1 level, a mass change corresponding to a ribose loss in the MS2 can often be identified. Further, stable isotope labeling in cell culture can allow for multiplexing of multiple samples to obtain relative quantitation of their respective RNA PTM levels. This is achieved by treating four different samples with 12C, $^{13}C_1$, $^{13}C_3$, or $^{13}C_6$ glucose for three to nine days in order to label the ribose moiety of the mononucleosides. This process selectively labels the ribose ring but not the base of a nucleoside, which means that all nucleosides will have uniform mass shifts and each sample can easily be differentiated. This powerful technology has the potential to rapidly advance knowledge on poorly studied RNA modifications in various cellular contexts.

Tues Poster 19: MetaProt: A Cloud-based Platform to Analyze, Annotate, and Integrate Metabolomics Datasets with Proteomics Information

Howard Choi[1]; Vincent Kyi[1]; Brian Bleakley[1]; Ding Wang[1]; Henning Hermjakob[2]; Peipei Ping[1]

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Introduction: The rapid development of analytical technologies has drastically increased the amount of data generated by both proteomics and metabolomics experiments. Both proteins and metabolites are essential building blocks of cellular processes that collaboratively formulate biological pathways. However, many large datasets of either proteins or metabolites are often fragmented and rarely integrated. We have addressed this data science challenge via developing an integrated platform for collective analyses of metabolite data with protein information.

Approach: We have formulated our strategy based on the considerations where metabolome meets proteome at the following intersections: <g class="gr_ gr_41 gr-alert gr_spell gr_run_anim ContextualSpelling" id="41" data-gr-id="41">their</g> shared biological and cellular pathways, chemical and biochemical reactions, post-translational modifications, metabolites as <g class="gr_ gr_40 gr-alert gr_gramm gr_run_anim Grammar only-ins doubleReplace replaceWithoutSep" id="40" data-gr-id="40">ligand</g> for protein-based receptors, and <g class="gr_ gr_39 gr-alert gr_spell gr_run_anim ContextualSpelling" id="39" data-gr-id="39">metabolites-based</g> transcriptional regulation. Importantly, both metabolome and proteome are characterized by mass spectrometry. MetaProt consists of a Wiki user interface and a distributed system architecture; it fetches information via application programming interfaces (APIs), retrieving data from multiple resources including biological pathways from Reactome/KEGG PATHWAY, chemical reactions from UniProt/HMDB, PTMs from dbPTM/PhosphoSitePlus, and ligand and drug information from KEGG LIGAND/DrugBank. Accordingly, the collected information from multiple resources <g class="gr_ gr_43 gr-alert gr_gramm gr_run_anim Grammar multiReplace" id="43" data-gr-id="43">are</g> used to construct a network of direct and indirect interactions between proteins and metabolites. In addition, biological and chemical functional annotations for proteins and metabolites are retrieved from COPaKB/Gene Wiki and MetaboLights/ChEBI. MetaProt will be hosted on Amazon Web Services (AWS), enabling users to perform these analyses on the cloud and generate integrated reports, including a table view of protein-metabolite interactions, and functional annotations as well as graphical views of networks and pathways.

Summary: We are establishing a cloud-based platform, MetaProt, for quantifying, triaging and analyzing omics datasets, finding existing as well as novel connections between proteins and metabolites, annotating molecular functions, and providing biomedical insights.

Tues Poster 20: Bladder Cancer Metabolomics Using the UPLC/MS-based AbsoluteIDQ p180 Kit

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Biocrates is a targeted quantitative metabolomics kit p180. This kit runs on a number of mass spectrometry platform and measures 188 metabolites, covering a biologically relevant panel of amino acids, sugars, acylcarnitines and phospholipids, using flow injection

electrospray ionization tandem mass spectrometry (ESI-MS/MS) and LC-MS/MS analysis. We analyzed bladder cancer tissues samples from African American (n = 35) and European American (n = 33) using the AbsoluteIDQ p180 Kit (Biocrates Life Sciences), which included quantitative analysis of 40 acylcarnitines, 21 amino acids, 19 biogenic amines, 15 sphingolipids, 90 glycerophospholipids, and sum of hexoses. We found that individual lipid metabolites can differentiate AA BCa from EA BCa with relevant significance. The Ratio between PC and lysoPC differentiates AA BCa from EA BCa with greater significance.Along the same lines, phospholipase A1 (PLA1A), Lecithin retinol acyltransferase (LRAT), two key enzymes required for conversion of (PC) into LPC were significantly elevated in AA BCa tissues. Consistent with this finding, global levels of PC were significantly lower in AA BCa tumors than EA BCa tissues. In Summary, these data show that the biogenetic amines and lipid metabolism is altered in AA BCa tissues, resulting in accumulation of key metabolites that could result in oncogenic transformation and/or disease progression

Tues Poster 21: Extending an Integrated Reference Tandem Mass Spectral Library for Comprehensive Identification of Metabolites and Bioactive Peptides

Xiaoyu Yang; Pedatsur Neta; Stephen Stein

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Tandem mass spectrometry is becoming a routine technique for compound identification in metabolomics and proteomics. However, accurately identifying compounds from millions of mass spectra is highly challenging for data analysis. Tandem mass spectral library searching has been proven to be a fast and reliable data analysis technique assisting metabolite and peptide identification. We have been extending an integrated tandem mass spectral library with a wide range of metabolites and bioactive peptides and MS^2 spectra of various precursor types, including isotopic precursors, ions produced in-source, and MS^n spectra, for more accurate identification. Mass spectra of each authentic compound and its in-source fragments were acquired with an Orbitrap Elite instrument (LTQ, FT, and HCD). A new top-down hierarchical clustering algorithm was developed for clustering MS^2, MS^3 and MS^4 spectra to generate consensus spectra based on the fragmentations. Software were developed for identifying fragments in high resolution spectra. For peptides, association rules were also used to investigate the relationship between the product ions and the amino acids and their neighbors. Using these methods, we examined > 8,000 metabolites and bioactive peptides, collecting > 400,000 new spectra. For protonated and deprotonated peptides, we identified > 100 fragment ions from modifications and from neutral losses (e.g. loss of NH_2COCH_2SH from carbamidomethylated cysteine). Some metabolites were identified with not only $[M+H]^+$ and $[M-H]^-$, but also $[M+Na]^+$, $[M+2H]^{2+}$, $[M-2H]^{2-}$, and other ions. Ions produced in-source by loss of H_2O or NH_3 or larger fragments (e.g. $[M+H-C_5H_7NO_3]+$ from oxidized glutathione) were also identified. Isomeric metabolites such as N-methylphenylalanine and α-methylphenylalanine were distinguished by their MS^3 spectra. The current library contains a total of > 15,000 compounds with > 600,000 spectra from different types of mass spectrometers. This library has been applied for identifying metabolites and ions produced in-source in human urine and *E. Coli* metabolomic studies.

Tues Poster 22: Racial Disparity in Bladder Cancer and Identification of Altered Metabolism in African American Compared to European Bladder Cancer

Venkatrao Vantaku[1]; Tiffany Dorsey[2]; Vasanta Putluri[1]; Sri Ramya Donepudi[1]; Suman Maity[1]; Wei Tang[2]; Feng Jin[1]; Danthasinghe Waduge Badrajee Piyarathna[1]; Kimal Rajapakshe[1]; Shyam Kavuri[1]; Vadiraja Bhat[3]; Seth Lerner[1]; Yair Lotan[4]; Wei Liu[5]; Cristian Coarfa[1]; Arun Sreekumar[1]; Stefan Ambs[2]; Nagireddy Putluri[1]

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Bladder cancer (BCa) incidence and mortality rates vary substantially among racial and ethnic groups. Most notably, European-Americans (EA) have a higher incidence of the disease, while African-Americans (AA) experience higher mortality rates and poorer survival. To date, a metabolomic analyses aimed at understanding of bladder cancer health disparity has not been reported. Our results using AA and EA BCa tissues have confirmed elevated expression of select enzymes involved in the metabolism of mitochondrial metabolites and lipids, specifically in AA BCa. For example, glutaminase 1 (GLS1), isocitrate dehydrogenase 2 (IDH2), and iron-containing alcohol dehydrogenase 1 (ADHFE1), three enzymes involved in D-2HG synthesis, were significantly elevated at mRNA and protein levels in AA BCa compared with EA BCa tissues. N-Acetyltransferase 8-Like Protein (NAT8L), which is required for synthesis of NAA, was higher in AA BCa, but ASPA, which is needed for NAA breakdown, was significantly lower in AA BCa tissues. Taken together, these data strongly support the notion that mitochondrial and lipid metabolism is uniquely wired in AA BCa tissues, resulting in accumulation of key metabolites that could contribute to oncogenic transformation and/or disease progression

Tues Poster 23: Mucosal microbiome and vaccine response in HIV-exposed uninfected African infants

Suereta Fortuin

Cape Town University., Cape Town, South Africa

Infants born to HIV-infected mothers have altered cellular immunity despite being HIV-uninfected themselves. Recently we explored the relationship between gut microbiome, as determined by 16S rRNA sequencing, and BCG vaccine responses in these infants. A continuing weakness of metagenomics approaches lies in the fact that predicted Open reading frames (ORFs) might not be expressed under in vivo conditions, incomplete DNA sequence data can result in partial ORFs which will be excluded during annotation, plus bioinformatics prediction of likely cellular localization of the encoded proteins (e.g. cell surface or secreted) may be inaccurate. 16S rRNA data yields information on bacterial diversity of species within the microbiome but does not describe metabolic function. To describe this we aim to use metaproteomic approaches annotate the gut microbiome of these HIV-exposed uninfected infants. We will use a MS-based approach to directly identify and quantify the expressed proteins of the infant gut microbiome, including experimental and bioinformatics segregation of the microbiome. Proteomic material from eight infant stools samples, between 4 to 8 days after birth, were analyzed using ultra-high-performance liquid chromatography (UHPLC) coupled to a quadrople mass filter Orbitrap analyzer (QExactive). MS raw data was analyzed using an unbaised approach using an in-house developed bioinformatics pipeline in conjunction with the Uniref100 (universal database) to identify proteins from organisms represented in these infant stool samples.

To date our results provide insight functional community in these HIV-exposed uninfected infant gut microbiota.

Tues Poster 24: Global Identification of Functional Phosphorylation Sites in *Saccharomyces cerevisiae*

Ian Smith

University Of Washington, Seattle, WA

Over 20,000 phosphorylation sites have been identified in Saccharomyces cerevisiae due to modern advances in mass spectrometry, however only roughly 5% of these phosphorylation events have been functionally annotated. Current attempts to identify functional phosphorylation sites globally have been limited to bioinformatic inference using sequence conservation and protein structural information as a prioritization criteria. But these methods have limited accuracy and experimental validation is preferable. To address this major bottleneck, we developed a high-throughput validation approach for identifying functional phosphorylation sites utilizing a pulsed stable isotope labeling by amino acids in cell culture (pulsed-SILAC) strategy with a heavy lysine isotope label to experimentally identify functional phosphorylation sites proteome-wide. The pulsed-SILAC approach coupled with LC-MS/MS was used to directly quantify and compare protein turnover between the phosphorylation modified protein and its unmodified protein counterpart to predict functional phosphorylation sites. Of the 2,715 phosphorylation events with turnover ratios in three biological replicates, we determined that 11% of the phosphorylation sites were functional based on a significant deviation in turnover from its unmodified protein counterpart. 78% of these experimentally identified

functional phosphorylation sites demonstrated a slower turnover compared to its unmodified counterpart, suggesting that most functional phosphorylation sites in this study are stabilizing the modified protein. The destabilizing phosphorylation sites demonstrated enrichment in PEST-like sequence motifs, which have been implicated in promoting protein degradation. An orthogonal size-exclusion mass spectrometry experiment demonstrated that many of the significantly slower turnover phosphorylation events from the pulsed-SILAC experiment are mediating complex formation and stabilizing the modified protein. Overall, our results indicate that a pulsed-SILAC mass spectrometry approach can successfully annotate functional protein phosphorylation events globally.

Tues Poster 25: Investigations of kinase signaling in cancer metabolism with cell-active, kinase-specific biosensors

Laura Marholz

University of Minnesota, Minneapolis, MN

Alterations in the metabolism of cancerous cells have been well established and include the upregulated intake of nutrients and the enhanced reliance on aerobic glycolysis (the so-called "Warburg effect"). These changes may help cancer cells proliferate and survive stressful conditions, and may even help explain the development of drug resistance to commonly prescribed cancer treatments. The purpose of this research is to investigate the mechanisms of these metabolic changes by studying the signaling activity of a panel of serine/threonine kinases (LKB1, AMPK, mTOR, and Akt) that are all intimately involved in regulating aspects of metabolism. Their signaling will be measured both in a drug-sensitive and drug-resistant cancer model. Previous work in our lab has focused on the discovery of cell-active, peptidic biosensors of specific tyrosine kinases to measure the activity of these proteins in living cells. These were rationally designed through a bioinformatics approach which transforms an input library of verified substrate sequences to an output set of specific candidate biosensor sequences based on a statistical analysis of amino acid preferences at positions neighboring the phosphosite. This project uses this workflow to design specific biosensors for the panel of selected metabolically-relevant kinases, compatible for high-throughput experiments and live-cell applications. These biosensors are chemical tools which will be able to quantitatively measure the signaling activity of these kinases in drug-sensitive chronic myelogenous leukemia (CML) cancer cell lines as well as those cultured specifically for drug resistances to imatinib, nilotinib, and dasatinib, three commonly prescribed kinase inhibitors in the clinic. This drug-resistant model system is being probed with re-sensitization strategies based on information gained from the metabolic profiling. Knowledge of differential metabolic pathway regulation will provide novel therapeutic targets, which could be broadly applicable as many cancers are known to acquire drug resistance to many currently prescribed first-line clinical treatments.

Tues Poster 26: Parallel accumulation - serial fragmentation (PASEF) on a novel trapped ion mobility spectrometry (TIMS) – QTOF instrument

Scarlet Beck[1]; Heiner Koch[1]; Florian Meier[1]; Markus Lubeck[2]; Stephanie Kaspar-Schoenefeld[2]; Niels Goedecke[2]; Oliver Raether[2]; Juergen Cox[1]; Matthias Mann[1]

[1]Max Planck Institute of Biochemistry, Martinsried/Munich, Germany; [2]Bruker Daltonik GmbH, Bremen, Germany

We recently described the potential of the parallel accumulation - serial fragmentation (PASEF) method on a TIMS-QTOF instrument for shotgun proteomics (Meier et al., JPR 2015, PMID: 26538118). Briefly, peptide ions are stored in an RF tunnel according to their cross sections. When they are released, the quadrupole selects them for fragmentation, which multiplies the number of measured MS/MS spectra compared to traditional approaches. Here we describe further development of the instrumental hardware, firmware and software, with a view to fully integrate the powerful PASEF-TIMS capabilities in an online shotgun proteomics workflow.

In PASEF mode multiple precursors per TIMS scan were selected by sub-millisecond switching of the quadrupole isolation window. Raw data were then analyzed with DataAnalysis (Bruker Daltonics) and MaxQuant (Cox group), which was adapted for PASEF-TIMS-QTOF data. In the TIMS device (~10cm long) ions are accumulated and released for a user-defined length of time. We tested different accumulation and release times 25ms, 50ms, 75ms and 100ms corresponding to median ion mobility resolutions of 22 (25ms) up to 78 (100ms). The PASEF scan mode increased the sequencing speed up to 5-fold and sensitivity by targeting low abundant precursor ions several times. Analyzing as little as 200ng of a human cancer cell line (HeLa) protein digest with the PASEF method on a prototype TIMS-QTOF mass spectrometer (Bruker Daltonics) in a 90min gradient led to the acquisition of more than 500,000 MS/MS spectra.

Our results demonstrate the potential of PASEF-TIMS-MS for deep shotgun proteomic analysis with low sample amounts. Further developments will focus on improving current instrument capabilities to allow a routine analysis of low abundant samples.

Tues Poster 27: A Single UHPLC System for both High Flow and Nano Flow LC-MS/MS: Application in Discovery and Targeted Proteomics

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Liquid Chromatography Mass Spectrometry (LC-MS) technologies have emerged as popular methods for proteome biomarker identification and quantification analysis. Traditional studies by high-performance liquid chromatography (HPLC) are performed using columns with internal diameter (i.d.) of 1.0–4.6 mm and have typical flow rates of 0.2–1.0 mL/min. An alternative to conventional LC is Nano-LC, which use smaller columns of 10–100 µm i.d. and flow rates of nanoliters per minute. The prior gives high reproducibility and great robustness which is suitable for large scale routine analysis, and the latter gives high sensitivity and allows small sample consumption which is widely used for protein biomarker discovery.

Here we demonstrated a LC-MS/MS based proteome identification and quantification workflow using a single Ultra High Performance Liquid Chromatography (UHPLC) system. Fast switch between nano and conventional flows was achieved by using a new designed Nanodapter which is cost effective and has much less solvent consumption than traditional split system. By testing various protein digest samples, i.e. BSA digest, yeast lysate and human cell extract spiked with protein standards, we showed this system provides high sensitivity performance for sample limited discovery application as well as excellent reproducibility and robustness for targeted routine analysis.

Tues Poster 28: Time-Dependent Metabolomics in Systems Biology Context for Mechanism of Action Studies

Akos Vertes[1]; Andrew Korte[1]; Hang Li[1]; Peter Nemes[1]; Lida Parvin[1]; Sylwia Stopka[1]; Sunil Hwang[1]; Ziad Sahab[1]; Deborah Bunin[2]; Merrill Knapp[2]; Andrew Poggio[2]; Carolyn Talcott[2]; Brian Davis[3]; Christine Morton[3]; Christopher Sevinsky[3]; Maria Zavodsky[3]; Nicholas Morris[4]; Matthew Powell[4]

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Rapid identification of the mechanism of action (MoA) for toxins or drug candidates requires high-throughput omics technologies. Multiplexed approaches have successfully accelerated protein and transcript analyses on a systemic scale. However, the development of high-throughput quantitative metabolomics has been delayed by the rapid degradation or turnover of metabolites during sample preparation, and by the lack of non-targeted analysis protocols for these molecular classes. Here we describe a high-throughput methodology for the identification of metabolic changes in HepG2/C3A hepatocytes exposed to bendamustine, a nitrogen mustard, and other challenge agents, using laser ablation electrospray ionization mass spectrometry (LAESI-MS), in combination with shot-gun proteomics and microarray-based transcriptomics. Quantitative in situ analysis of metabolites by LAESI-MS was enhanced by ion mobility separation (IMS) and stable isotope labeling. Direct analysis of cells at ten timepoints between 30 s and 48 h during exposure to bendamustine by LAESI-IMS-MS resulted in

the detection of over a thousand spectral features, and the identification and quantitation of 122 metabolites. Copy numbers of the identified metabolites ranged from 3□106 to 1011 molecules per cell. Quantitative levels of >67,000 transcripts, and >5,000 proteins, were also followed as a function of time. Statistically significant changes in biomolecule levels were evaluated using knowledge-based methods, including gene set enrichment analysis, and Ingenuity Pathway Analysis. Beyond the successful reconstruction of the canonical MoA known for bendamustine, i.e., DNA damage repair and p53 stabilization, we observed the activation of other pathways, including S-phase arrest at 24 h and G2/M arrest at 48 h via senescence. The discovered MoA was then validated by conventional phenotypic and intracellular signaling assays, and immunocytochemistry. In addition to the greater detail our time-dependent omics approach affords to the identification of MoA, our data-driven inference methods also apply to the discovery of unknown MoAs.

Tues Poster 29: Reducing Your Coomassie Footprint: An enviromentally friendly protein stain with sensitivity similar to SYPRO Ruby and Deep Purple gel stains

Gary Smejkal; Donald Mousseau
Focus Proteomics, Hudson, NH

KUMASI is a stabilized Coomassie colloid for staining proteins in polyacrylamide gels with sensitivity similar to SYPRO Ruby and Deep Purple gel stains. The new stain detected over 1000 protein spots in two-dimensional gels of Escherichia coli K-12 cell lysates, comparable to staining with SYPRO Ruby or Deep Purple fluorescent gels stains, and detected 28% more protein spots than competing Coomassie formulations.

Tues Poster 30: Quantification of Protein Stability in Mammalian Tissues

Dan B. Mcclatchy; Nam-Kyung Yu; John R. Yates
The Scripps Research Institute, La Jolla, CA

We developed QUAD (Quantification of Azidohomoalanine Degradation) analysis to quantify protein stability from rodent tissues by mass spectrometry. Azidohomoalanine (AHA) is modified methionine that is accepted by the endogenous methionine tRNA and inserted into proteins in vivo. Biotin-alkynes can be covalently added to AHA with click chemistry and then AHA containing proteins can be enriched with avidin beads. We have previously reported that AHA can safely be added to a rodent diet to identify and quantify newly synthesized proteins from tissues. In this study, we placed mice on an AHA diet for 4 days, then the mice were returned to a normal diet for 3, 7, and 14 days. Using biotin-alkynes with heavy stable isotopes, AHA proteins were quantified to generate hundreds of protein stability trajectories. Our data demonstrates that protein stability varies greatly within a tissue proteome but global patterns can distinguish different tissues. Examining identical proteins in multiple tissues, we conclude that the cellular environment affects protein stability. In brain tissue, different subcellular compartments possessed unique patterns of protein stability. Furthermore, different protein functions were significantly enriched in the stable and unstable protein datasets. Finally, we further investigated the TRIC chaperone complex with additional MS and non-MS experiments since it surprisingly contained both stable and unstable protein subunits. This is the first study to investigate the TRIC complex from brain tissue and our data suggests that the structure of the TRIC complex in brain tissue differs from previous reports in other biological samples. In conclusion, QUAD is a simple and robust method for quantifying global protein stability in mammalian tissues.

Tues Poster 31: Quantitative Bottom Up Proteomics Using a Novel Scanning Quadrupole Data Independent Acquisition (DIA) Method

Jim Langridge[1]; Chris Hughes[1]; Lee Gethings[1]; Roy Martin[2]; Keith Richardson[1]; Johannes Vissers[1]
[1]Waters Corporation, Wilmslow, UK; [2]Waters, Beverly, MA

A novel LC-MS DIA mode of operation is described for bottom up proteomics analysis, providing both qualitative and quantitative information on the digested peptides. The approach has been developed for a Q-Tof mass spectrometer with the acquired data applied to both discovery and targeted proteomics experiments. The data was acquired with the quadrupole continuously scanned, isolating specific m/z regions prior to the oa-TOF. Data was acquired up to a frequency of 400 spectra/s. Alternate MS scans comprised precursor and CID product ions. The resulting 2D data, m/z (ToF) vs. m/z (quadrupole) were processed with both PLGS, Progenesis (Waters) and MASCOT (Matrix Science, UK) for untargeted discovery. For specific targeted proteomics analysis transition extraction lists in Skyline (University of Washington, US)

Quantitative proof-of-principle data were acquired by serially diluting a four-protein digest mixture into a tryptic digest of an E.coli cell lysate, and the analysis of a tryptic digest from a Human cell line (HeLa) extract. Optimization was performed with regard to proteins identification and quantitation versus protein load and quadrupole transmission width. Analysis of the data indicates that the scanning quad DIA enables over an order of magnitude more specificity than a static quad operated with the same resolution and a quadrupole transmission window of approximately 25Da provided optimum protein identifications

Aliquots of control, diabetic and obese human plasma samples were digested with trypsin overnight. 2DMS data were collected and differentially expressed peptides and hence proteins across the three conditions were quantified. Results show that peptides from apolipoproteins exhibited the most significant changes in good agreement with expected changes in relation to disease and/or phenotype.

Tues Poster 32: PCT-HD for Tissue Biopsy Samples: Comparison to a Standard Method.

Vera Gross[1]; Peter Hains[2]; Keith Ashman[3]; Valentina Valova[2]; Alexander Lazarev[1]
[1]Pressure BioSciences, Inc., South Easton, MA; [2]Children's Medical Research Institute, Westmead NSW, Australia; [3]Sciex, Framingham, Massachusetts

We present the results of an investigation comparing traditional tissue homogenization followed by overnight digestion, with PCT-HD, a significantly faster method involving tissue homogenization with PCT microPestles under pressure, followed by pressure-accelerated digestion. High hydrostatic pressure and, specifically, pressure cycling, have previously been shown to increase protease activity and accelerate protein digestion, compared to reactions carried out at ambient pressure.

Traditional tissue preparation and digestion methods often involve manual sample homogenization, long digest times, and multi-step procedures that inevitably lead to loss of analytes and therefore require large amounts of starting material. Quantitative proteomics of small samples such as biopsies, especially when carried out on a large scale, require scaled-down methods that reduce sample loss while maximizing digest efficiency. The PCT-HD method allows the entire procedure, from tissue to peptides, to be carried out in a single 150ul MicroTube, minimizing sample loss and maximizing peptide yield.

Here we show that tissue samples prepared using the ~3 hour PCT-HD protocol, yield comparable or superior results to samples processed using a traditional method that requires ~20 hours from start to finish. The PCT-HD protocol resulted in significantly greater sequence coverage and more protein and peptide IDs, while producing slightly higher rates of miscleavage and slightly lower rates of semi-tryptic cleavage. In addition, rates of protein modifications such as oxidation, deamidation, and carbamylation were examined. Carbamylation rates were significantly lower, deamidation was essentially unaffected, and there was a slight increase in methionine oxidation in samples processed under pressure. In summary, the PCT-HD method minimizes manual sample handling and reduces total preparation time from ~20 hours to ~3 hours, while maximizing quantitative recovery of proteotypic peptides from biopsy-size tissue samples.

Tuesday posters should be set up 8:00-8:30 am Tuesday morning and removed at 3:00 pm Tuesday afternoon.

Tues Poster 33: Quantification of circulating *M. tuberculosis* antigen peptides allows rapid diagnosis of active disease and treatment monitoring

Chang Liu; Jia Fan; Christopher Lyon; Ye Hu
Arizona State University, Tempe, AZ

Tuberculosis (TB) is a major global health threat, resulting in an urgent unmet need for a rapid, non-sputum-based quantitative test to detect active *Mycobacterium tuberculosis* (*Mtb*) infections in clinically diverse populations and quickly assess *Mtb* treatment responses for emerging drug-resistant strains. We have identified *Mtb*-specific peptide fragments and developed a method to rapidly quantify their serum concentrations, using antibody-labeled and energy-focusing porous discoidal silicon nanoparticles (NanoDisks) and high-throughput mass spectrometry (MS) to enhance sensitivity and specificity. NanoDisk-MS diagnosed active *Mtb* cases with high-sensitivity and -specificity in a case-control study with cohorts reflecting the complexity of clinical practice. Similar robust sensitivities were obtained for culture-positive pulmonary TB (PTB, 91.3%) and extrapulmonary TB (EPTB, 92.3%) cases, while sensitivities obtained for culture-negative PTB (82.4%) and EPTB (75.0%) cases in HIV-positive patients significantly outperformed those reported for other available assays. NanoDisk-MS also exhibited high specificity (87.1% - 100%) in both healthy and high-risk groups. Absolute quantification of serum *Mtb* antigen concentration was informative in assessing responses to anti-mycobacterial treatment. A NanoDisk-MS assay approach could thus significantly improve the diagnosis and management of active TB cases, and perhaps other infectious diseases.

Tues Poster 34: A promising alternative to MS2-DIA: IonStar enables large-scale, accurate and extensive quantification with low missing data and false positives

Jun Qu; Xiaomeng Shen; Shichen Shen
SUNY-Buffalo, Buffalo, NY

In-depth and reproducible protein measurement especially with many biological/individual replicates is critical for pharmaceutical/clinical proteomics, but remains highly challenging owing to suboptimal accuracy, reproducibility and false-positive-biomarker-discovery, and high levels of missing data. MS2-based Data-Independent-Acquisition(MS2-DIA) approaches were developed to lower missing values but often with limited depth. To address these challenges, we developed an "IonStar" workflow consisting of experimental components and a data processing pipeline to take full advantage of high-resolution MS1 ion-current-based quantification, which achieves accurate, precise and in-depth protein quantification with low-missing-values in large cohorts. A surfactant-aided-extraction/precipitation/on-pellet-digestion procedure provides highly efficient(>80%recovery) and reproducible(<15%CV) sample preparation across large biological cohorts, and a unique nano-LC setup affords comprehensive, consistent and robust separation across >100 samples with high loading capacity; the MS1 quantitative features are acquired in a data-independent manner under high-resolution(FWHM=120k), achieving excellent S/N and selectivity. The data processing pipeline includes accurate chromatographic alignment, a sensitive DICE-type feature generation to enhance matching among many runs and to achieve sensitive quantification with low-missing-data, accurate ID/feature mapping, normalization, low-quality-data removal and aggregation. This pipeline was thoroughly compared to popular methods including spectral counting, Proteome-Discoverer, OpenMS and Maxquant, using a 5-group, 20-replicates benchmark sampleset containing ~6200 true-/false-positives. IonStar showed by far the most in-depth analysis, best accuracy, lowest intra-group-variation(~5%CV vs.11-18% by others), missing data(<0.2% vs.18-44%) and false-positive-biomarker-discovery-rate(<4% vs.7-31%), with quantified protein abundances spanning ~5.8 orders of magnitudes(vs.3.2-4.4). We demonstrate an application of IonStar in large-scale proteomics investigation for temporal cellular response to novel chemotherapeutics combinations, and quantified >6000 unique proteins without any missing data in 48 samples under stringent cutoffs, with <3% false-positive-biomarker-discovery-rate. To our knowledge, this was the first time a label-free protein measurement achieves such depth and reproducibility in large cohort. This study demonstrated IonStar may serve as a promising alternative to MS2-DIA owing to its superior proteome coverage and quantitative performances.

Tues Poster 35: In-depth quantitation of changes in protein expression levels in complex samples on a Q-TOF instrument using Data-Independent Acquisition (DIA)

Stephanie Kaspar-Schoenefeld[1]; Thomas Kosinski[1]; Pierre-Olivier Schmit[2]; Na Parra[3]
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Understanding the dynamic of the proteome is a challenge that raises the interest of many biologists, as the proteome variations can be associated with all important biological processes. Untargeted label-free quantitative proteomics approaches have been widely adopted and used to detect differences in protein expression. Measuring peptide and protein ratios with a high level of accuracy requires being able to reproducibly detect, identify and quantify compounds over different batches of samples. DIA workflows have gained in popularity over the last years as they overcome the issue of stochastic selection of peptide precursors encountered in typical data-dependent approaches (DDA). The success of DIA approaches strongly relies on key instrumental capabilities, as it implies the repeated acquisition of full-scan MS/MS (where resolution, sensitivity, accuracy and dynamic range shall be ideally preserved) at a very fast rate.

We present here the excellent capabilities of Bruker's impact II Q-TOF for DIA-based approaches by challenging the instrument with a complex sample consisting of a mixture of three proteomes (HeLa, *E.coli*, and yeast) in different ratios according to Navarro *et al.* (2016). Data have been acquired in DIA mode and processed with the Spectronaut™ software suite. We have analyzed two sample sets differing in their theoretical regulation ratios, ranging from small fold changes (1:2 and 1:4) to large fold changes (1:10 and 10:1), with the latter being the more challenging one. Results show extremely good reproducibility with the median coefficient of variation for the human background proteome among replicate runs of 5% and 7%. For both experiments we found human proteins and peptides to be centered at a log2 ratio of 0, which nicely corresponds to the theoretical ratio of 1:1, across the complete dynamic range of 4 orders of magnitude further supporting the extremely good capabilities of the used Q-TOF instrument for presented DIA approach.

Tues Poster 36: Developing Novel Biosensors for Serine/Threonine Kinases Involved in Cancer

Joel Zembles
University Of Minnesota, Minneapolis, Minnesota

A panel of nine serine/threonine kinases were chosen based on their relevance in the misregulation of metabolic processes relating to cancer. Two kinases on the panel are mitogen-activated protein kinases (MAPK), MAPK3 also named Erk1 and MAK14 or p38 alpha. Using the KINATEST-ID Pipeline as a guide, positional matrix scoring of substrates for these kinases was done.1 Referencing online databases, substrates were found for each kinase, and verified.2 Criteria for verification involved finding evidence in the literature that a substrate with a mutation at the modification site replacing the serine or threonine with a hydrophobic residue that is unable to be phosphorylated such as alanine and observing a difference in activity in an autoradiograph. MAK3 had 33 verified substrates out of the potential 280 substrates found, while MAK14 had 15 verified out of the potential 154 substrates found. Continuing the steps in the pipeline, binding partners of the panel kinases were determined by the Human Protein Reference Database.3 These binding partners were cross-referenced against the verified substrate list so they must have interactions with the kinases but are not substrates that can be phosphorylated. The collected data will be used in the positional matrix scoring of the first step in the KINATEST-ID Pipeline to assist in the development of peptide biosensors for the kinase panel.

References:

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Tues Poster 37: Ultrasensitive Microanalytical CE-nanoESI-MS for Bottom-up Proteomic Characterization of Mouse Hippocampal Neurons

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The George Washington University, Washington DC, District of Columbia

Unbiased proteomic characterization of neurons provides information on molecular processes involved in normal development of the nervous system. However, sensitive detection technology is required to address cell-to-cell heterogeneity, specifically those capable of measuring trace amounts of proteins in single cells or small populations of neurons. High-resolution mass spectrometry (HRMS) excels at identifying proteins from large populations, usually millions of cells. Here, we report a HRMS-based microanalytical platform that enables ultrasensitive characterization of proteins from small populations of neurons in the mouse cerebral cortex. Our platform integrates a custom-built capillary electrophoresis (CE) nanoelectrospray ionization (nanoESI) interface for HRMS to achieve trace-level sensitivity. First, we constructed micro-loading stage capable of injecting 1 nL of peptides into a CE capillary where peptides were electrophoretically separated by applying 21 kV across the 90-cm fused silica capillary. The peptides were then ionized in a custom-built CE-nanoESI source that features a tapered-tip metal emitter to generate stable cone-jet regime. Using peptide standards, the CE-nanoESI-HRMS platform was characterized with an ~260-zmol (156,000 copies) lower limit of detection and high separation power (~330,000 theoretical plates). The instrument was able to detect ~15 amol (~1 pg) of bovine serum albumin and cytochrome c in a bottom-up approach, raising sufficient sensitivity to measure proteins in small neuron populations. We utilized this platform to analyze ~500 pg of protein digest from cultured hippocampal neurons isolated from pup mouse (embryonic day 16). The platform was able identify over 1,000 different peptides belonging to 361 nonredundant protein groups (<1 % FDR). The label-free quantitation intensities calculated for these proteins suggested a 4-log-order dynamic concentration range. Identified proteins were enriched in many genes that are classical neuron markers. Ultrasensitive characterization of proteins by CE-nanoESI-HRMS raises new potentials to investigate how differential gene expression establishes neuron heterogeneity for normal brain function.

Tues Poster 38: Comprehensive proteomics analysis to identify differentially expressed proteins in cells with ASC speck formation

I-Che Chung; Chih-Ching Wu; Yu-Sun Chang
Chang Gung University, Taoyuan, Taiwan

Upon stimulation, pattern recognition receptors NLRP3, AIM2 and RIG-I will be recruited to ASC, a key platform protein for inflammasome activation, resulting in formation of a protein complex or speck-like particles triggering caspase 1 activation. Activated caspase 1 cleaves pro-IL-1β into a mature form IL-1β, which is then secreted from cell. As noted that the activated inflammasome complexes, or the speck-like particles were detected in some but not all treated cells. To reveal the difference between the speck $^{(+)}$and speck $^{(-)}$cells, we used a well-characterized ASC-mCherry-expressing THP-1-derived macrophage cell line, which can be easily induced by microbial toxins nigericin to activate NLRP3 inflammasomes (speck $^{(+)}$) cells. Approximately 25-30% cells treated with nigericin formed specks. We then isolated the speck $^{(+)}$cells by flow cytometry. Further purification of speck $^{(+)}$population into nigericin-treated speck $^{(-)}$and nigericin-treated speck $^{(+)}$groups improved the purity of cells to about 85% for the subsequent proteomic analysis (iTRAQ LC-MS/MS). A total of 995 proteins were enriched (overexpressed) in speck $^{(+)}$THP-1-ASC-mCherry cells after nigericin stimulation from duplicated experiments. GeneGo process network of these enriched proteins are mainly participated in two biological pathways: oxidative phosphorylation and ubiquinone metabolism, suggesting that mitochondria is participated in NLRP3 inflammasome activation. The electron transport chain (ETC) in the mitochondria inner membrane is the major site of oxidative phsophorylation and is involved in the generation of ATues Poster, and is also the main source of cellular ROS. We demonstrated that mitochondrial dysfunctions (increased mitochondrial ROS production and decreased intracellular ATues Poster production) are detected in speck $^{(+)}$THP-1-ASC-mCherry cells after nigericin stimulation. Together, these results provide a new direction for studying the molecular mechanisms involved in NLRP3-ASC speck formation and inflammasome activation.

Tues Poster 39: Understanding mechanism of action of drug resistance reversal potential of Usnic Acid using proteomic profiling

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Antibiotic resistance is an overwhelming problem worldwide to combat the infectious diseases. Staphylococcus aureus is an uncompromised human pathogen which causes wide range of infections such as sepsis, toxic shock syndrome, endocarditis or osteomyelitis. Controlling this pathogen is highly complicated due to emergence of MRSA, VRSA and MDR strains. Over past few years, researchers around the globe are putting serious efforts for searching novel antibiotics against multi drug resistant (MDR) microbes. In recent years, the herbal medicine has attained popularity due to its advantageous features. Moreover, many natural compounds such as berberine, totarol, curcumin, khusimol, gallic acid etc has been reported recently against quit a few MDR strains.

Usnic acid is a natural dibenzofuran compound extracted from several species of Lichen. It has been used in medicines, beauty products, perfumes, cosmetics and it also has antiviral, antimicrobial, anti-inflammatory, anti-growth and so on. Though recent studies showed its anti-bacterial against gram positive organism such as S. aureus but its mechanism of action is still unclear. In current study, we used state-of-the-art proteomic technology such as two dimentional electrophoresis coupled with Matrix assisted laser desorption and ionization- time of flight (MALDI-TOF/TOF) mass spectrometry to understand the proteome alteration in Staphylococcus aureus (MRSA2071). Our proteomics analysis has highlighted alteration of 25 protein spots (18 protein spots were down regulated and 7 protein spots were up regulated) with respect to untreated control. The altered proteins were involved membrane biosynthesis, metabolic processes and electron transport chain pathways which are essential for the survival of the bacteria. To the best of our knowledge, this is the first report describing the effect of Usnic acid on S. aureus and resistance reversal ability and we are doing validation experiments to get insight into the mechanism of action.

Tues Poster 40: Global proteomic analysis of lysine acetylation during zebrafish embryogenesis

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Lysine acetylation is an important post-translational modification. Since the development of mass spectrometry (MS)-based proteomics technology, important roles of lysine acetylation beyond histones have focused on chromatin remodeling during the cell cycle and regulation of nuclear transport, metabolism, and translation. Zebrafish (*Danio rerio*) is a widely used vertebrate model in genetics and biologic studies. Although studies in several mammalian species have been performed, the mechanism of lysine acetylation in *D. rerio* embryos is incompletely understood. Here, we investigated the global acetylome in *D. rerio* embryos by using an MS-based proteomics approach. We identified 351 acetylated peptides and 377 non-redundant acetylation sites on 189 lysine-acetylated proteins in 5-day post-fertilization (hpf) embryos of *D. rerio*. Among lysine-acetylated peptides, 40.2% indicated 3 motifs: (ac)KxxxK, (ac)KxxxxK, and Lx(ac)K. Of 190 acetylated proteins, 81 (42.6%) were mainly distributed in the cytoplasm. Gene ontology enrichment and Kyoto Encyclopedia of Genes and Genomes pathway analyses showed that lysine

acetylation in *D. rerio* was enriched in metabolic pathways. Additionally, 17 of 30 acetylated ribosomal proteins were evolutionarily conserved between zebrafish and humans. Our results indicate that acetyllysine might have regulatory effects on ribosomal proteins involved in protein biosynthesis.

Tues Poster 41: Turnover profiles of histone post-translational modifications in a myogenic model using SILAC labeling, enzyme networks and trend clustering analysis

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Distinctive epigenetic and chromatin states direct cell type-specific gene expression both during proliferation and post-replicative differentiation during development. In human skeletal myogenesis model, we examined the replacement of 'old' histones by 'new' histones in the chromatin of myoblasts and myotubes.

Light human myoblast and myotubes were expanded and differentiated respectively in heavy medium containing [$^{13}C_6$] L-arginine and were harvested on days 1, 2, 3, and 7, in triplicate. The histones extracted from proliferating and differentiated cells were derivatized with propionic anhydride and the relative abundance was analyzed using nano liquid chromatography-tandem mass spectrometry on an LTQ-Orbitrap Elite (Thermo Scientific).

Differentiating myotube cells had an overall slower turnover of all histones compared to proliferating myoblasts. Among the hundreds of histone post-translational modifications (PTMs), turnovers correlated between myoblast and myotube samples, overall slower in myotubes. Notably, acetylations were faster than methylations. Hybrid marks (i.e. peptides carrying acetylation and methylations on different residues) and higher-order methylations (i.e. trimethylations) had lower turnover rates. Interestingly, we observed modifications with a very tight turnover profile across days and cell states, in some cases belonging to different histone proteins. By performing k-means clustering of the trends of all peptides, we determined classes of difference variances, including tightly related PTMs or PTMs with peculiar behavior. Next, we superimposed literature-based knowledge about enzymes catalyzing the PTMs; interestingly, PTMs with fast turnover seemed to be catalyzed by a large family of enzymes. Moreover, PTMs with very similar turnover were often those which are catalyzed by the same enzyme. Finally, by performing a comparative analysis between PTM turnover and their known function, we observed a clear slowing down of turnover for PTMs related to heterochromatin and gene repression, and speeding up for active marks. This confirmed that histones in open chromatin are replaced much faster than histones in condensed chromatin.

Tues Poster 42: Glycation of Human Serum Albumin Increases Ex Vivo in Poorly Handled Samples

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Blood plasma proteins and their posttranslational modifications (PTMs) are frequently unstable outside of the body (*ex vivo*). This can be due to residual enzymatic processes or non-specific chemical reactions. Chemical reactions in blood are commonly not at equilibrium *in vivo*—prompting certain natural shifts to occur over time *ex vivo.* Actual equilibrium constants for redox reactions involving molecular oxygen, however, can shift wildly in plasma samples *ex vivo* where the dissolved oxygen concentration goes from near zero (since oxygen is carried on hemoglobin within red blood cells in circulation) to 0.25 mM. We have previously shown that albumin S-cysteinylation (an oxidative modification) and apolipoprotein A-1 methionine oxidation increase spontaneously *ex vivo* when blood plasma/serum samples are exposed to temperatures above their freezing point of -30 ºC. Prompted by the fact that oxidative processes can enhance protein glycation, we have now evaluated changes in albumin glycation at -20 ºC and at room temperature, as well as in samples subjected to freeze-thaw cycles and different surface area-to-volume ratios. A simple dilute-and-shoot method utilizing trap-and-elute liquid chromatography coupled with ESI mass spectrometry (LC-ESI-MS) was used to analyze the relative abundance of the glycated form of albumin. Significant increases in the relative abundance of glycated albumin were found to occur within hours at room temperature, and within days at -20 °C. It was also shown that samples stored at higher surface area-to-volume ratios experienced more rapid *ex vivo* glycation. Because the reaction between glucose and albumin is non-specific and non-enzymatic, these results are likely to be relevant to glycation of most proteins in archived samples.

Tues Poster 43: High throughput and accurate quantitation of phosphoproteomics for biological signaling

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The most accurate quantitation on protein complex mixtures can be accomplished by employing TMT SPS MS3 method on Tribrid mass spectrometers. However, this method is not optimal for phosphopeptide quantitation due to special characteristics in phosphopeptide fragmentation patterns. In this study, we developed methods to address this limitation and provide high phosphopeptide identifications and accurate quantitation.

Digested HeLa cells were labeled with TMT10plex™ reagents and mixed at ratios of 16:8:4:2:1:1:2:4:8:16. Yeast digest was labeled with the last 5 channels mixed equimolar (0:0:0:0:0:1:1:1:1:1) and spiked into TMT-labeled HeLa digest sample as interference. This resulted in Hela digests with first five channels that were free of interference and the last five channels interfered by yeast proteome. The mixed samples were further enriched for phosphopeptides and analyzed on Orbitrap Fusion and Lumos MS.

Ratio distortion was observed for phosphopeptide analysis on HeLa and yeast mixture, when using MS2 workflow. This was due to interfering ions co-isolated with precursor ions. The use of SPS MS3 improved quantitation accuracy. However there was 50% of loss in the number of phosphopeptides identified. The loss was due to the strong presence of the neutral loss peak specific to phosphopeptide, which limited the identifications from CID MS2 spectra. We thus developed and optimized two new MS3 instrument methods to reduce the loss of phosphopeptide identifications. This approach minimized loss to less than 30%, while maintaining quantitation accuracy benefits given by SPS MS3.

The new MS3 methods were applied to large scale phosphoproteome characterization in A549 cell line upon insulin and IGF-1 treatments. Overall, 3,378 protein groups and 12,465 phosphopeptides were identified of which 10,436 were quantifiable. The accurate and reproducible measurement enabled mapping regulated phosphorylation sites to numerous signaling pathways including mTOR signaling and AMPK signaling pathways.

Tues Poster 44: Fast Second Dimension microflow LC for High-Throughput Deep Proteome Coverage

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The complexity of a proteomics digest requires extensive fractionation to deeply interrogate the proteome. The key goal is to spread the peptides out across fractions such that when each is analyzed by LC-MS/MS, the mass spectrometer has time to maximize collection of high quality peptide MS/MS spectra. While nanoLC can provide sensitivity advantages for limited samples, the use of microLC for the second dimension enables more robust and faster separations.

A human cell lysate digest was fractionated using high pH reverse phase chromatography using a Shimadzu Nexera system. A Durashell RP column (250 x 4.6mm, 5um, Agela Technologies) operating at 1 mL/min was used, running a gradient of 2 – 90% acetonitrile in 2mM ammonium hydroxide. 15 fractions were collected, one every every 2 mins.

Each fraction was then separated using low pH reverse phase gradient on the NanoLC™ 425 system operating in microflow mode. A Triart C18 150 x 0.3mm column (YMC) was used at 5 µL/min flow rate

with a 45 min gradient from 2-40% acetonitrile in 0.1% formic acid for a total run time of 1 hour /fraction.

The eluent was analyzed using the TripleTOF® 6600 system equipped with a DuoSpray™ Source and a 25 µm ID hybrid electrode. Data dependent acquisition (IDA) was performed with 30 MS/MS per cycle, each with 50 msec accumulation. All IDA data was processed using using ProteinPilot™ Software 5.0, both individually and all in one large search.

Using high pH fractionation in the first dimension, good even peptide separation was obtained with ~9000 peptides identified in each fraction. Only about 25% of peptides were found in multiple fractions, providing 106849 distinct peptides in total. Using just 15 hours of instrument time, the high quality separation combined with the high speed acquisition of the TripleTOF 6600 system provided 7164 proteins identifications.

Tues Poster 45: Profiling Biochemical Individuality: Human Personal Omics Profiling (hPOP)

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<title>RESOURCES AND ENVIRONMENT</title> Recent advances in high throughput technologies allow profiling of thousands of analytes within a single experiment. These measurements could potentially be used to diagnose disease early, monitor treatment progression and stratify patient groups to ensure each individual obtains the treatment best suited to their needs. This personalized approach to medicine would include continuous monitoring of thousands of parameters over a whole lifetime. However, in order to be able to interpret such data, we need to have a better understanding of the underlying natural variation of biological molecules in large crowds. Only if we know the natural ranges of individual analytes, the expected responses to perturbations and the long-term trends in their levels, can we draw meaningful conclusions from comprehensive personalized profiling.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become one of the most powerful techniques in profiling human proteome. Data independent acquisition with “Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra” (SWATH) provides data-independent fragmentation of all precursor ions entering the mass spectrometer in isolated window. Continuous stepping of isolation window covers the whole m/z range of interest. This allows repeated analyses of each window during the elution of a single chromatographic peak and therefore a deeper fragment ion map of the sample.

Human blood plasma is the prime source of protein biomarkers and contains protein biomarkers that indicate biological changes associated with disease. However large dynamic range of protein concentrations plasma make plasma proteome technically challenging and time-consumable to study. We performed several optimizations on current SWATH mass spectroscopy method and we can now quantify more than 490 proteins in human plasma sample with false discovery rate of 1% in only 60 minutes. SWATH-MS provides a stage for high-through put proteomics that can be used for our large cohort study with exquisite quantitative accuracy and reproducibility.

Tues Poster 46: Evidence of Human Antagonistic Auto-antibodies as a Mechanism of Insulin Resistance

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Type 2 diabetes mellitus (T2D) is a metabolic disease that is strongly tied to obesity and often preceded by insulin resistance and chronic inflammation in adipose, muscle and liver tissues. Recently, cells of the adaptive immune system, B and T lymphocytes, have been demonstrated to be important regulators of insulin sensitivity. B cells have been shown to drive obesity-associated insulin resistance through numerous mechanisms including activation of T cells, secretion of inflammatory cytokines and production of pathogenic autoreactive immunoglobulin G antibodies (pIgGs) in visceral adipose tissue. Previously, pIgGs, distinct from those characteristic of type 1 diabetes, have been shown to predominately target a variety of intercellular self-antigens from multiple tissues during the onset of insulin resistance. However, the link between pathogenic antibodies and onset of insulin resistance remains poorly understood. Here we examine the cellular mechanism linking pIgGs to insulin resistance using proteomic and metabolic techniques. An ultra-high density overlapping peptide array containing 2.7 million features covering all human proteins was used to profile autoreactive IgG epitopes associated with insulin sensitivity. Differential epitope reactivity analysis of insulin resistant and sensitive individuals revealed enrichment of autoreactivity toward surface proteins involved in insulin signaling. The ability of the IgGs to cause reduced mitochondrial respiration, a hallmark of insulin resistance, was used to access their pathogenicity. The cellular oxygen consumption rate was measured in the presence of purified IgG and observed to be significantly decreased with IgGs from insulin resistant individuals suggesting a pathogenic function. Together these results demonstrate a direct link between pIgGs and insulin resistance as well as offers a potential novel antibody-based diagnostic for determining insulin resistance.

Tues Poster 47: Selection and validation of endogenous retention time standards and quality control peptides for plasma proteomics study

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Plasma is one of the most common proteomics samples in clinical or cohort study. With the rapid development of data-in-dependent acquisition(DIA) mass spectrometry, nowadays we are able to identify and quantify hundreds of proteins in each patient, which becomes a great data resource to support population or disease study. Monitoring sample preparation quality and instrument status are very important to the data quality of large cohort study. However, ion library-dependent data analysis can only extract those peptides in the library and cannot tell us if there is abnormal digestion or modifications which may indicates sample preparation issue. Retention calibration by synthetic RT standards like iRT can fail in ion library-independent data analysis workflow such as DIA-Umpire during low signal extraction. In our study, we selected 286 peptides from 70 plasma MS data sets of healthy and patients as endogenous retention time standards and validated it in all kinds of plasma samples with different sample preparation including depletion or fractionation and various LC-MS acquisition method, such as micro and nano flow LC separation or variable DIA windows settings. Combined with thorough DDA search, those assembled peptide spectra show the good coverage of non-tryptic or semi-tryptic peptides that are usually not in existing ion library. Among those 286 peptides, we chose 85 peptides of 39 proteins with stable peak intensity and good retention time reproducibility as endogenous QC peptides for plasma study.

Tues Poster 48: *Ex Vivo* Protein Oxidation as a Metric of Blood Plasma/Serum Integrity

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Every year, improprieties in pre-analytical handling and storage of blood plasma/serum (P/S) specimens generate false leads in biomedical research. Considering the entire life of a research specimen, temporary exposure to the thawed state (including -20°C) is not uncommon—yet no other pre-analytical variable is more difficult to control and track. As demonstrated by data that will be presented, even the best SOPs in the most respected hands cannot guarantee that specimens are handled ideally. Surprisingly, no gold standard marker of P/S integrity has been established. To address these problems, we developed a simple, inexpensive, rapid test requiring 10 µL of P/S that provides an assessment of the oxidative damage that P/S proteins have incurred due to exposure to the thawed state. The test is based on the fact that the relative abundance of S-cysteinylated (oxidized) albumin (S-Cys-Alb) will always increase over time (but to a maximum value) when P/S is handled/stored above its melting point of -30°C. Thus by measuring S-Cys-Alb before and after an intentional incubation period that causes S-Cys-Alb to hit its

maximum value, the *difference* between these values, ΔS-Cys-Alb, is readily interpreted as *inversely* proportional to the degree of *ex vivo* oxidation that occurred *prior* to the first measurement of S-Cys-Alb. Thus, for example, a ΔS-Cys-Alb value of zero would indicate a badly mistreated sample. When applied to a set of stage I lung adenocarcinoma cases and age/gender/smoking matched controls that were collected under the same SOP as part of an NCI-sponsored research program, the ΔS-Cys-Alb assay revealed a major biospecimen integrity discrepancy between the cases and controls (ROC c-statistic for ΔS-Cys-Alb of 0.96). The degree of oxidation observed in the damaged samples is not uncommon amongst archived P/S biospecimens; the impact of such protein oxidation on P/S biomarker discovery, however, remains to be investigated.

Tues Poster 49: Elucidating the Biological Implications of Aluminum Binding to Osteocalcin

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Osteocalcin (Oc), the most abundant non-collagen protein in bone, is a 6-kDa protein in which 0-3 mid-region Glu residues may be γ-carboxylated (converted to Gla residues) in a vitamin K-dependent manner. Γ-carboxylation of Oc imparts its ability to bind calcium within the hydroxyapatite bone mineral and thereby influence bone mineralization. In 2015 we showed that the vitamin K-dependency of γ-carboxylation results in highly variable inter-individual levels of Oc γ-carboxylation that depend directly on vitamin K nutritional status. Recently, while employing our electrospray ionization (ESI)-based mass spectrometric immunoassay (MSIA) to characterize the relative abundance of circulating osteocalcin PTMs in clinical samples, we noticed that the fully γ-carboxylated protein (3-Gla-Oc) was always partially bound to aluminum (Al^{3+})—at anywhere from 5-50% relative abundance. Control experiments with synthetic 3-Gla-Oc showed that neither LC solvents or the LC-ESI-MS system was the source of the Al^{3+}, but that contamination of immunocapture buffers with trace concentrations of Al^{3+} contributed low relative percent abundances of aluminum-bound 3-Gla-Oc. An ESI-MS approach to determine the K_d of the 3-Gla osteocalcin-aluminum interaction at pH 7.4 revealed a minimum binding affinity of 20 nM. 2-Gla-Oc did not bind Al^{3+} as avidly and 0-Gla-Oc did not bind Al^{3+} at all. Bone is the major repository for aluminum within the human body—facilitating its half-life of about 7 years. The fact that Oc binds Al^{3+} in a γ-carboxylation (vitamin K) dependent manner implies that vitamin K nutritional status may impact long term aluminum excretion rates. Intriguingly, ApoE allele genetics impact vitamin K uptake by bone—creating a trifecta of factors (genetic, environmental, and lifestyle) that may be relevant to Alzheimer's disease. We are working to determine the fraction of circulating Oc that is actually bound to Al^{3+} *in vivo* and whether or not γ-carboxylation of Oc impacts overall Al excretion rates.

Tues Poster 50: Novel Algorithm for Quantifying Proteoforms of Tropomyosin

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Quantitative measurement of proteins is an important aspect in the discovery of new diagnostic or prognostic biomarkers. Mammalian genes can undergo multiple mutations, rearrangements and splicing events, thereby translating into protein sequence isoforms (alleles). Out of the approximately 20,000 human proteins annotated in Uniprot database, more than half have known multiple sequence isoforms, and almost 13% have four or more annotated isoforms. Knowing the relative or absolute amounts of these protein isoforms can advance biotechnology and clinical domains, as well as be a major step towards personalized medicine. Very few technologies, e.g., mass spectrometry, have the capability to resolve the individual isoforms at the protein or peptide level. Previous LCMS studies to quantify protein isoforms include the use of peptides that are unique to each protein isoform, as a way to measure each isoform. Unfortunately, of the proteins in Uniprot database that have four or more isoforms, only 17% have one unique peptide per isoform, and only 0.2% have three of more unique peptides per isoform. We have, therefore, developed an algorithm that quantifies protein isoforms using quantitative measurement of peptides that belong to one or more isoform and share sequence homology. From the proteins in Uniprot database that have four or more isoforms, over 40% exhibit this characteristic. We have tested this algorithm, still in its preliminary stages of development, on a simulated setup involving a synthetically developed mixture of four isoforms of the protein Tropomyosin (an important cardiac diseases biomarker), where in an unsupervised manner, our algorithm was able to calculate back the mixture ratio within 21% error. Thus, our approach provides an important alternative tool to scientists to enrich identification of potential isoforms regardless of availability or detectability of unique peptides and could be employed for LCMS data interpretation for both biomarker and pre-clinical studies.

Tues Poster 51: Temporal Analysis of Proteome and Transcriptome in Differentiating Human Pancreatic Endocrine Cells identifies Developmentally Regulated Protein Networks.

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Diabetes is among the most prominent global health concerns. The underlying factors that cause the disease are complex and show correlation with unhealthy diet and obesity. Diabetes can be associated with either loss of pancreatic islet cells responsible for production of the essential hormone insulin for glucose metabolism regulation or loss of sensitivity to insulin in the bloodstream by the target cells. In either case, a continuous exogenous insulin supplement is required for survival and no permanent cure currently exists for diabetes.

While the differentiation of stem cells into pancreatic beta cells offers tremendous potential as a cure for diabetes, many challenges exist toward developing these cells into an effective therapy. To better understand the maturation process of endocrine cells, we performed a global, quantitative, and temporal analysis of human stem cells as they differentiate into pancreatic islet cell types using proteomics and transcriptomics. Human pancreatic cells from postmortem tissue were used to compare and characterize the maturity of the hESC-derived islet cells. This temporal developmental profiling approach identified key candidate factors unique to specific differentiation states, stage-specific changes in protein complements, as well as specific markers of pancreatic beta cell progenitors. We have identified number of regulatory molecules that take part in islet cell formation progression specific to different stages of differentiation.

Tues Poster 52: Integrative proteogenomic characterization of colorectal cancer cell lines and primary tumors

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Human tumor-derived cell lines are widely used to study cancer biology and therapy, but controversy remains whether cell lines provide appropriate representation of primary tumors. Here, we report an integrative proteogenomic analysis of human colorectal cancer

(CRC) cell lines, primary tumors and corresponding normal tissues. We found a significant, systematic difference between cell line and tumor proteomes, with major differences arising from tumor stroma. Nevertheless, cell lines mirrored the proteomic differences between tumors and normal tissues, in particular for intrinsic molecular programs, indicating that cell lines provide informative models for cancer cells *in vivo*. The intersection of cell line and tumor data identified tumor cell-specific proteome alterations driven by genomic aberrations. Our integration of cell line proteogenomic data with drug sensitivity measurements highlights the potential of proteomic data for predicting therapeutic responses. We identified representative cell lines for all proteomic subtypes of primary tumors with evidence for subtype-specific drug responses.

Tues Poster 53: Validation of Tumor Proteogenomic Annotations

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In Proteogenomics pipelines, tandem mass spectra are searched against theoretical spectra of peptide sequences derived from genomic and transcriptomic data, to identify mutations, splice variants, and proteins disrupted by structural variation. Proteogenomics is a specific focus of cancer proteome analysis (including the CPTAC projects), as cancer is marked by somatic genome lesions. While many somatic mutations have been identified using cancer proteogenomic pipelines, the increased sensitivity comes at the expense of false-discoveries. Standard p-value and FDR controls are not effective as the decoy databases do not account for all mutations. Here we ask if “a proteogenomically discovered mutation has a simpler explanation as a reference (modified) peptide”. We describe a new method, Proteogenomics-Event-Validation (PEV) to address this. PEV takes as input a list of mutated peptide spectrum matches (PSMs). For each PSM, it enumerates reference (modified) isobaric peptides that match the spectrum. The reference peptides are prioritized and scored using a Bayesian framework to determine if the original identification was better than the reference based alternative.

We applied PEV to spectra from 95 CPTAC colorectal samples previously searched against a database constructed from 88 TCGA RNA-seq data. PEV analysis on the PSMs from the 1035 mutated peptides suggested 543 peptides with at least one reference (modified) alternative. PEV used MS-GF+ to rescore the mutated and alternative peptides. In a vast majority of the cases, the mutated peptide remained the best explanation suggesting robustness of proteogenomics discovery. For example, the T->S mutation in peptide K.VQLPTE(T->S)LQELLDLHR.D appears correct because the best alternative explanation required Oxidation of Proline. However, in a small number of cases, the alternative explanation dominated. For example, an “N->D” mutation was better explained by Deamidation of Asn. PEV promises to be a useful resource for validating proteogenomic discoveries.

Tues Poster 54: Proteogenomics: *in silico* analysis to investigate the low discovery rate of variant peptides in shotgun proteomics

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In current proteogenomics studies, the number of identified variant peptides in shotgun proteomics is often much less than the number of variants discovered via genomics analysis. Therefore, we conducted *in silico* analysis on several human protein sequence datasets with different scales of sample complexity (i.e. whole human proteome from UniProt to a targeted protein sequence) and then compared to mass spectrometry (MS)- based datasets (e.g. experimentally observed peptides collected from PeptideAtlas) to investigate the possible reasons of relatively low discovery of variant peptides in shotgun proteomics. In the analysis, we considered peptide length of six ranges: 7-15, 7-20, …, 7-40 amino acids (aa). We first examined the difference between theoretical and experiment protein coverages of wild-type human proteins which were computed using fully tryptic *in silico* peptides and experimentally observed peptides, respectively. The theoretical protein coverage was more closely representing the experimental protein coverage by using peptides with <g class="gr_ gr_19 gr-alert gr_gramm gr_run_anim Grammar only-ins doubleReplace replaceWithoutSep" id="19" data-gr-id="19">length</g> of 7-20 aa. Thus, we tried different proteases (e.g. trypsin, chymotrypsin, Arg-C, Asp-N, Glu-C, Lys-C and Lys-N) and species (e.g. *Escherichia coli*). Our results suggested most of <g class="gr_ gr_20 gr-alert gr_gramm gr_run_anim Grammar only-ins replaceWithoutSep" id="20" data-gr-id="20">experimentally</g> observed tryptic peptides having <g class="gr_ gr_21 gr-alert gr_gramm gr_run_anim Grammar only-ins doubleReplace replaceWithoutSep" id="21" data-gr-id="21">length</g> of 7-20aa that was probably affected by the MS. In addition, peptides with length 7-20aa were probably more suitable for discovering variant proteins, regardless of protease and species. We then examined <g class="gr_ gr_23 gr-alert gr_gramm gr_run_anim Grammar multiReplace" id="23" data-gr-id="23">the amount of</g> variants that we could observe ideally using multiple proteases in parallel. With different overlapped peptides being generated, parallel use of multiple proteases could improve the variant coverage. Finally, we validated our findings from *in silico* variant peptides by using their counterpart wild-type peptides to check the experimental coverage in PeptideAtlas. Overall, our analysis indicated that probably only at most ~40% of the variants could be experimentally detected with the consideration of similar peptide properties shared between wild-type and variant peptides.

Tues Poster 55: Expression profiling of miRNA, mRNA and protein cargo of myeloid derived suppressor cells and their exosomes

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Myeloid-derived suppressor cells (MDSCs) are known to accumulate in the tumor microenvironment where they suppress adaptive and innate immunity. MDSCs release exosomes (~30 nm in diameter), which have been shown to mediate their suppressive activity. Inflammatory conditions increase the abundance and suppressive activity of MDSCs, facilitating tumor progression. The aim of this study was to identify and quantify the protein, mRNA and miRNA contents of murine MDSCs and MDSC-derived exosomes collected under conventional and heightened inflammation. The protein and RNA cargoes of replicate samples from matched MDSCs and released exosomes were determined by shotgun proteomics and next generation sequencing, respectively. A total of 1249 proteins were identified in exosomes, from which 28% (353) had “nucleic acid binding” functions based on gene ontology (GO). In the case of MDSCs, 1423 proteins were identified from both inflammatory conditions. More than half (58%) of the identified proteins were shared between MDSC and their exosomes, and 371 proteins were found in greater abundance in exosomes. Enriched GO categories of these 371 proteins included the cellular locations “extracellular region”, “extracellular space” and “cell surface”; and the molecular functions “antigen binding”, “signal transducer” and “peptidase activity”. Both miRNAs and mRNAs were shown to be present in exosomes shed under both inflammation conditions. A total of 1453 miRNAs and 40433 mRNA transcript isoforms confidently identified in MDSC and exosomes were used for quantitative comparisons. This study provided, for the first time, experimental identification of ~624 predicted miRNAs by Ensembl. Functional analyses of miRNAs and mRNAs found to be in greater abundance in exosomes showed enriched GO biological processes related to regulation of apoptosis, angiogenesis and T cell function. The integration of these protein and RNA analyses provides complementary and reinforcing information about signaling pathways in receiver cells that may be affected by the differential exosome cargo profiles.

Tues Poster 56: Proteogenomic Characterization of Drug Resistance in the K562 CML Cell line

Vinh Nguyen; Laurie Parker

Tuesday posters should be set up 8:00-8:30 am Tuesday morning and removed at 3:00 pm Tuesday afternoon.

University of Minnesota, Minneapolis, MN

Tyrosine kinase inhibitors (TKIs) are the major treatment for chronic myelogenous leukemia (CML). CML results from a mutation that forms the Bcr-Abl protein, a kinase with constitutive activity. The Bcr-Abl inhibitor imatinib led to a transformational improvement in outcomes for CML patients, However, resistance has emerged as a clinical problem. Studies show that second line drugs do little to improve the long term outlook of patients—the majority fail follow-up treatment within a year. Drug target mutation is a significant mechanism of first line response failure, however second line failure has been determined to be mostly mutation-independent. While studies have examined individual mechanisms for CML resistance to TKIs, few larger-scale evaluations of transcriptomic or proteomic profiles of TKI resistant cells have been performed. Given that some CML patients exhibit suboptimal response to imatinib, and many are unlikely to achieve remission on next-line inhibitors, understanding mechanisms that lead to TKI resistance could be instrumental in guiding therapy for these patients.

In this work, we sought to identify markers and/or therapeutic targets arising from global effects of TKI exposure on CML cells by performing transcriptomic and data-independent acquisition proteomic analyses of CML cell line K562 and three TKI-resistant derivatives: K562-IR (imatinib resistant), K562-NR (nilotinib resistant), and K562-DR (dasatinib resistant). Integrated analysis of transcriptomic (RNAseq, Illumina HiSeq) and proteomic (SWATH, SCIEX 5600+) experiments was performed along with confirmation by immunoanalysis using capillary electrophoresis (Wes, ProteinSimple). The data describe common features of resistance to these inhibitors, which share common targets, including downregulation of Myc targets, apparent engagement of hypoxia-related signaling, evidence for alteration of ubiquitin-mediated protein degradation, and metabolic reprogramming to apparently rely more heavily on glycolysis for survival. Overall, this suggests that ubiquitin-mediated proteolysis mechanisms and stress-related changes in metabolism may be valuable targets for avoiding and/or overcoming target mutation-independent resistance in CML.

Tues Poster 57: proBAMsuite, a bioinformatics framework for genome-based representation and analysis of proteomics data

Xiaojing Wang
Baylor College of Medicine, HOUSTON, TX

As an essential complement to genomics, proteomics holds the promise of surveying the proteomes of biological and clinical samples both qualitatively and quantitatively. Mass spectrometry based shotgun proteomics technology has advanced rapidly during the past decade. An emerging demand is to effectively integrate them with genomic and transcriptomic data, particularly in disease related samples. However, a standard data format for sharing and exchanging such information has not been established. Although a genome-based representation of proteomics data may lead to novel data analysis and interpretation opportunities that go beyond visualization, these opportunities have barely been explored.

To facilitate genome-based representation and analysis of proteomics data, we developed a new bioinformatics framework, proBAMsuite, in which a central component is the protein BAM (proBAM) file format for organizing peptide spectrum matches (PSMs) within the context of the genome. proBAMsuite also includes two R packages, proBAMr and proBAMtools, for generating and analyzing proBAM files, respectively. Applying proBAMsuite to three recently published proteomics datasets, we demonstrated its utility in facilitating efficient genome-based sharing, interpretation, and integration of proteomics data. First, the interpretation of proteomics data is significantly enhanced with the rich genomic annotation information. Second, PSMs can be easily reannotated using user-specified gene annotation schemes and assembled into both protein and gene identifications. Third, using the genome as a common reference, proBAMsuitefacilitates seamless proteomics and proteogenomics data integration. Finally, proBAM files can be readily visualized in genome browsers and thus bring proteomics data analysis to a general audience beyond the proteomics community. Results from this study establish proBAMsuite as a useful bioinformatics framework for proteomics and proteogenomics research.

Tues Poster 58: Analysis of the Effects of Dietary Signals on Protein Homeostasis

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

Dietary changes such as calorie restriction (CR) have been shown to significantly alter protein homeostasis, the rate at which new protein is created and old protein is degraded. This change in protein homeostasis is hypothesized to be responsible for the significant increase in longevity and other beneficial effects experienced by organisms fed a CR diet. We have observed that diet composition is a significant modifier of the change in protein homeostasis. We are exploring this change through global proteomic kinetic and quantitation measurements to calculate changes in protein synthesis and degradation. We then compare changes in relative synthesis and degradation for altered diets. This work will aid in understanding dietary regulatory signals, and how such changes regulate the proteome on a global level.

Tues Poster 59: Characterization of Cell-Surface, Exosomal, and Secreted Proteins of Senescent Human Fibroblasts by DIA/SWATH

Nathan Basisty; Judy Campisi; Bradford Gibson; Birgit Schilling
Buck Institute for Research on Aging, Novato, CA

Introduction: Senescent cells are irreversibly growth arrested cells that secrete myriad of cytokines, chemokines, growth factors, and proteases collectively termed the senescence-associated secretory phenotype (SASP). The persistence of senescent cells and chronic SASP contribute to aging and numerous diseases by promoting chronic inflammation, tumorigenesis, and defective stem cell renewal. Selectively killing senescent cells is a promising strategy to combat these effects which has shown great benefits in mouse models, however, senescence biomarkers will be required to take this approach in humans. In this study we perform an unbiased characterization of surface proteins, secreted proteins, and exosome-associated proteins in senescent human fibroblasts.

Methods: To generate senescent (SEN) and control (CTL) cells, respectively, IMR90 primary human fibroblasts were x-ray irradiated or mock irradiated and cultured for 10 days. Growth medium was then replaced with serum-free medium and collected after 24 hours to obtain secreted protein fractions (10x SEN & 10x CTL). Exosomes were depleted from these fractions by high-speed centrifugation and pooled into separate exosome fractions (5x SEN & 5x CTL). To obtain cell surface proteins, we utilized a variant of the Cell Surface Capture method in which surface lysine residues are biotinylated with sulfo-NHS-SS-biotin followed by lysis, digestion, and affinity enrichment of biotinylated peptides.

Results: In total we identified >1000 total secreted proteins with >500 significantly changed ($q<0.05$) for SEN/CTL. These were almost exclusively increased in senescent cell fractions and consisted largely of cytokines, growth factors, proteases, cytoskeletal and adhesion proteins, clotting factors, and metabolic proteins. We identified over 100 surface proteins differentially expressed in SEN and CTL groups ($q<0.05$) including increased expression of proteins in integrin signaling, Rho GTues Posterase signaling, and cell death signaling and decreased expression of proteins in translation, mRNA processing, and TGF beta signaling. Finally, over 30 exosomal proteins were significantly increased in SEN/CTL cells.

Tues Poster 60: Proteomic and metabolic characterization of oxidative stress responses using a stem-cell derived multi-tissue disease model of Age-related Macular Degeneration

Jesse G. Meyer[1]; Thelma Y. Garcia[1]; Birgit Schilling[1]; Arvind Ramanathan[1]; Deepak Lamba[1, 2]; Bradford Gibson[1, 3]
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Model organisms are an invaluable starting point for understanding human biology, but often treatments successful in model organisms fail in human trials. To facilitate more efficient translational research,

we hope to bypass use of model organisms in favor of patient-derived organ models grown *in vitro* from induced pluripotent stem cells (iPSCs). AMD affects two key cell types in the eye, the retinal pigment epithelium (RPE) and photoreceptor cells. As a first step toward an *in vitro* model of age-related macular degeneration (AMD), we characterized the proteomic response to oxidative stress in human stem cell-derived RPE using data-independent acquisition (DIA). Using Spectronaut and the pan-human library (Aebersold group), we identify and quantify 5,000 human proteins from unfractionated digestions in each 4 hour acquisition. We found a large number of protein-level changes due to paraquat (PQ) or 4-HNE stress after 1 or 3 weeks of treatment, and interestingly, most changes are oxidant specific. Paraquat-specific protein-level changes suggest altered metabolism in the TCA cycle and fatty acid beta-oxidation. To verify this, we are using targeted metabolite profiling starting with various acyl carnitines. Next, since photoreceptor death in AMD is linked to dysfunction and/or death of the RPE layer, we have generated a multi-tissue model of the retina *in vitro* using stem cell derived RPE and retinal neurons containing photoreceptors. This system allows us to study to response of photoreceptors co-cultured with stressed RPE. Preliminary experiments revealed subtle but significant protein-level changes in photoreceptors that were cultured with PQ-stressed RPE. Functional analysis of these protein-level changes suggests photoreceptor re-entry into the cell cycle, a known step toward photoreceptor death in several heritable eye diseases. In conclusion, stem-cell derived tissue models provide an opportunity to model human disease, and understanding of proteomic and secretomic changes will provide insights into pathogenesis and potential biomarkers.

Tues Poster 61: Monitoring Riboprotein Turnover Kinetics in vivo through Stable Heavy Isotope Labeling.

Nathan Keyes; Richard Carson; Ryne Peters; Bradley Naylor; John Price

Brigham Young University, Provo, Utah

The ribosome plays a critical role in cellular function. Poor ribosomal function is associated with loss of protein homeostasis, aging and disease. Understanding the mechanism behind how the ribosome is maintained may open doors to future therapies treating aging and disease associated with loss of protein homeostasis. The ribosome is a massive protein, and degrading the ribosome anytime a subunit breaks would not be an efficient use of energy. We have observed that riboproteins are exchanged at different rates than the overall ribosome, implying some form of ribosomal maintenance. We are investigating whether this exchange rate is driven by gene expression of the riboproteins or by post translational modifications.

We are performing a two pronged study into ribosomal homeostasis. First, we are using stable isotope feeding and mass-spectrometry to measure riboprotein turnover data in mice. Second, we are scanning the same samples for post translational modifications (PTMs) on the Orbitrap mass spectrometer on nine different dietary cohorts.

Tues Poster 62: TargetSeeker-MS: A Bayesian Inference Approach for Drug Target Discovery using Protein Fractionation Coupled to Mass Spectrometry

Mathieu Lavallée-Adam[1, 2]; Jolene Diedrich[1, 3]; Alexander Pelletier[1]; William Low[3]; Antonio Pinto[3]; Salvador Martínez-Bartolomé[1]; Michael Petrascheck[1]; James Moresco[1, 3]; John R. Yates[1]

[1]The Scripps Research Institute, Lajolla, CA; [2]University of Ottawa, Ottawa, Canada; [3]Salk Institute for Biological Studies, La Jolla, CA

Determining the affinity of a drug to its putative targets and off-targets is critical to the understanding of its mechanism and to assess its clinical usefulness. Stability-based protein separation techniques, such as thermal shift assay, when coupled to mass spectrometry (MS), have shown great potential to identify the targets and off-targets of a drug on a proteome scale. These approaches support the hypothesis that proteins that are bound by a given drug will have a change in stability. Nevertheless, the computational analyses associated with these methods have remained rudimentary and tightly tied to the protocol under which the datasets were produced.

We propose a novel flexible Bayesian inference approach named TargetSeeker-MS to identify drug targets and off-targets in datasets produced using stability-based protein fractionation techniques coupled to MS. The algorithm quantifies proteins in each fraction and evaluates the significance of the difference in the abundance distribution across the fractions for each protein between the untreated and drug treated samples, thereby assessing the confidence that the protein is bound by the compound.

We analyzed with TargetSeeker-MS C. elegans protein lysate samples untreated and treated with benomyl, a fungicide. TargetSeeker-MS identified several proteins with fractionation profiles that were significantly altered by benomyl, including aldehyde dehydrogenase, a known target. We demonstrate that TargetSeeker-MS is flexible and that its drug target identifications are reproducible in C. elegans samples that were processed using two different stability-based protein separation techniques (thermal shift assay and an acetic acid-based separation). We also validate a novel benomyl target by measuring in vitro its altered enzymatic activity upon drug treatment.

TargetSeeker-MS, which is available on the web, allows the rapid confident identification of the targets and off-targets of a drug on a proteome scale, thereby providing a better understanding of its mechanisms and ease the evaluation of its clinical viability.

Tues Poster 63: Identification of FMS-like tyrosine kinase 3 (FLT3) substrates using KALIP

Minervo Perez

University of Minnesota, Minneapolis, MN

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

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