US HUPO
from genes to function

9th Annual Conference

Translational Proteomics:
Biology, Technology and Clinical Advances

March 10 - March 13, 2013
Baltimore, MD

Join us next year in Seattle
April 6 - 9, 2014

www.ushupo.org

Society Sponsors
MCP molecular and cellular proteomics announces...

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American Society for Biochemistry and Molecular Biology
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## CONFERENCE ORGANIZERS

Daniel Chan, Chair, *Johns Hopkins University*

Nathan Edwards, *Georgetown University*

Akhilesh Pandey, *Johns Hopkins University*

Karin Rodland, *Pacific Northwest National Laboratory*

Hui Zhang, *Johns Hopkins University*

## US HUPO 2012 – 2013 BOARD OF DIRECTORS

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Yingming Zhao, *University of Chicago*
## Program Overview

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<th>SUN, MARCH 10</th>
<th>MON, MARCH 11</th>
<th>TUES, MARCH 12</th>
<th>WED, MARCH 13</th>
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<tr>
<td><strong>8 am - 7 pm</strong>&lt;br&gt;Registration&lt;br&gt;Key Ballroom Foyer</td>
<td><strong>8:00 – 8:30 am</strong>&lt;br&gt;Early Coffee&lt;br&gt;Key Ballroom 7-12</td>
<td><strong>8:00 – 8:30 am</strong>&lt;br&gt;Early Coffee&lt;br&gt;Key Ballroom 7-12</td>
<td><strong>8:00 – 8:30 am</strong>&lt;br&gt;Early Coffee&lt;br&gt;South Key Foyer</td>
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<tr>
<td><strong>Full-day Short Courses</strong>&lt;br&gt;9:00 am - 4:00 pm&lt;br&gt;Essentials of Proteome ID &amp; Quantification&lt;br&gt;Carroll A, Level 3</td>
<td><strong>8:30 – 9:20 am</strong>&lt;br&gt;Plenary Lecture&lt;br&gt;Key Ballroom 3,4,6&lt;br&gt;John Yates</td>
<td><strong>8:30 – 9:20 am</strong>&lt;br&gt;Plenary Lecture&lt;br&gt;Key Ballroom 3,4,6&lt;br&gt;Alexander Makarov</td>
<td><strong>8:30 am - 12:00 pm</strong>&lt;br&gt;US HUPO - EDRN Joint Symposium&lt;br&gt;Key Ballroom 3,4,6</td>
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<tr>
<td><strong>Half-day Short Courses</strong>&lt;br&gt;9:00 am - 12:00 pm&lt;br&gt;Statistical Methods&lt;br&gt;Carroll B, Level 3</td>
<td><strong>9:20 – 10:30 am</strong>&lt;br&gt;Parallel Sessions (3)&lt;br&gt;Clinical Proteomics&lt;br&gt;Key Ballroom 3,4,6</td>
<td><strong>9:20 – 10:30 am</strong>&lt;br&gt;Parallel Sessions (3)&lt;br&gt;Clinical Glycoproteomics and Glycomics&lt;br&gt;Key Ballroom 3,4,6</td>
<td><strong>8:30 – 9:30 am</strong>&lt;br&gt;Cancer Biomarker: Discovery&lt;br&gt;Key Ballroom 3,4,6</td>
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<tr>
<td><strong>9:00 am - 12:00 pm&lt;br&gt;Sample Prep for Proteomics Experiments&lt;br&gt;Tubman A, Level 3</strong></td>
<td><strong>10:50 am – 12:00 pm</strong>&lt;br&gt;Parallel Sessions (3)&lt;br&gt;Cardiovascular Proteomics&lt;br&gt;Key Ballroom 3,4,6&lt;br&gt;Proteogenomics&lt;br&gt;Key Ballroom 5</td>
<td><strong>10:50 am – 12:00 pm</strong>&lt;br&gt;Parallel Sessions (3)&lt;br&gt;Systems Proteomics&lt;br&gt;Key Ballroom 3,4,6</td>
<td><strong>9:20 – 10:30 am</strong>&lt;br&gt;Cancer Biomarker: Translation&lt;br&gt;Key Ballroom 3,4,6</td>
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<td><strong>9:00 am - 12:00 pm&lt;br&gt;Technical Improvements in MS&lt;br&gt;Key Ballroom 5</strong></td>
<td><strong>12:00 – 1:30 pm</strong>&lt;br&gt;Lunch Seminars&lt;br&gt;Agilent, Tubman AB&lt;br&gt;Thermo, Paca</td>
<td><strong>12:00 – 1:30 pm</strong>&lt;br&gt;Vendor Seminars&lt;br&gt;Shimadzu, Tubman AB&lt;br&gt;Waters, Paca</td>
<td><strong>10:30 am – 12:00 pm</strong>&lt;br&gt;Coffee Break&lt;br&gt;Key Ballroom 7-12</td>
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<tr>
<td><strong>1:00 - 4:00 pm&lt;br&gt;Glycoproteomics &amp; Glycomics&lt;br&gt;Tubman A, Level 3</strong></td>
<td><strong>1:30 - 2:00 pm</strong>&lt;br&gt;MCP Lectureship&lt;br&gt;Key Ballroom 3,4,6&lt;br&gt;Catherine E. Costello</td>
<td><strong>1:30 – 3:00 pm</strong>&lt;br&gt;Interactive Parallel Sessions (2)&lt;br&gt;Industry Panel: Academic Collaboration&lt;br&gt;Key Ballroom 3,4,6</td>
<td><strong>1:30 – 3:00 pm</strong>&lt;br&gt;FDA Panel&lt;br&gt;Key Ballroom 5</td>
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<tr>
<td><strong>1:00 - 4:00 pm&lt;br&gt;Stable and Transient Protein-Protein Interactions&lt;br&gt;Tubman B, Level 3</strong></td>
<td><strong>2:00 – 3:30 pm</strong>&lt;br&gt;Parallel Sessions (3)&lt;br&gt;Advances in Technology&lt;br&gt;Key Ballroom 3,4,6&lt;br&gt;Cell Regulation&lt;br&gt;Key Ballroom 5</td>
<td><strong>3:00 – 3:30 pm</strong>&lt;br&gt;Robert J. Cotter New Investigator Award Lecture: Rebekah Gundry&lt;br&gt;Key Ballroom 3,4,6</td>
<td><strong>3:00 – 3:30 pm</strong>&lt;br&gt;Evening Workshop&lt;br&gt;Integration of Proteomics&lt;br&gt;Key Ballroom 3,4,6</td>
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<td><strong>6:00 – 7:00 pm&lt;br&gt;Opening Session&lt;br&gt;Key Ballroom 3,4,6</strong>&lt;br&gt;Plenary Lecture: Denis Hochstrasser</td>
<td><strong>3:30 – 5:00 pm</strong>&lt;br&gt;Poster Session&lt;br&gt;Key Ballroom 7-12</td>
<td><strong>3:30 – 4:00 pm</strong>&lt;br&gt;US HUPO Business Meeting&lt;br&gt;Key Ballroom 3,4,6</td>
<td><strong>6:00 – 7:00 pm</strong>&lt;br&gt;Opening Reception&lt;br&gt;Key Ballroom 7-12&lt;br&gt;Food &amp; Drinks&lt;br&gt;All are welcome!</td>
</tr>
<tr>
<td><strong>7:00 – 8:30 pm&lt;br&gt;Opening Reception&lt;br&gt;Key Ballroom 7-12</strong></td>
<td><strong>5:00 – 5:30 pm</strong>&lt;br&gt;Happy Half-Hour&lt;br&gt;Key Ballroom 7-12</td>
<td><strong>4:00 - 5:30 pm</strong>&lt;br&gt;Poster Session&lt;br&gt;Key Ballroom 7-12</td>
<td><strong>5:30 – 6:00 pm</strong>&lt;br&gt;Happy Half-Hour&lt;br&gt;Key Ballroom 7-12</td>
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<td><strong>5:30 - 6:30 pm&lt;br&gt;NCI: Proteomic Research Highlights, Key Ballroom 5</strong></td>
<td><strong>5:30 - 6:30 pm&lt;br&gt;NHLBI Proteomics&lt;br&gt;Key Ballroom 1-2</strong></td>
<td><strong>5:30 – 6:00 pm&lt;br&gt;Evening Workshop&lt;br&gt;Integration of Proteomics&lt;br&gt;Key Ballroom 3,4,6</strong></td>
<td><strong>6:00 – 7:00 pm&lt;br&gt;Evening Workshop&lt;br&gt;Integration of Proteomics&lt;br&gt;Key Ballroom 3,4,6</strong></td>
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GENERAL INFORMATION AND MAP

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

POSTERS. Posters are located in Key Ballroom 7-12. All posters should be mounted Monday morning by 10:00 am. Posters must be removed by 6:00 pm Tuesday evening.

All posters present on Monday and Tuesday.

MONDAY
- Odd-numbered boards are attended 3:30 - 4:15 pm
- Even-numbered boards are attended 4:15 - 5:00 pm

TUESDAY
- Odd-numbered boards are attended 4:00 - 4:45 pm
- Even-numbered boards are attended 4:45 - 5:30 pm

TALKS. All Plenary and Concurrent Sessions are located in sections of the Key Ballroom accessible via the South and West Foyers. See schedule for details.

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.

EVENING WORKSHOPS. Evening workshops are scheduled on Monday and Tuesday immediately following the Happy Half-Hour Mixer. All conference attendees are welcome to attend the workshops. There is not a separate registration.

LUNCH SEMINARS. Concurrent lunch seminars take place on Monday and Tuesday, 12:00 – 1:30 pm in the Paca and Tubman Rooms on the Third Level (one floor up from posters and sessions). Go to pages 9 and 14 for more information on seminar talks. RSVP is encouraged (at company exhibit booth.)


STUDENTS / POST-DOC 'BREAKFAST WITH EXPERTS'. Students and post-docs conference attendees are invited to attend a special breakfast with invited speakers and US HUPO Board members on Tuesday, March 12, 7:15 - 8:15 am (Paca Room, Third Level).

INTERNET ACCESS. Free wireless internet access is provided in the Poster-Exhibits Room (Key Ballroom 7-12) and in the hotel bar (main level).

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

NO PHOTOGRAPHY or RECORDING of posters or talks is permitted.

RESTAURANTS & BALTIMORE CITY INFORMATION. Restaurant list and maps are available at Registration and at Concierge Desk (main lobby.)

BALTIMORE HILTON HOTEL

Level Two - Exhibits & Posters, Session Rooms, Registration

Key Ballroom 1-2
Parallel Sessions

Key Ballroom 5
Parallel Sessions

Key Ballroom 3,4,6
Plenary Session
Parallel Sessions

To Key Ballroom 5

Express Elevators UP to Level 3
Carroll, Paca and Tubman
Lunch Seminars, Short Courses

Escalators DOWN to Lobby

Exhibits & Posters
Coffee Breaks
Receptions

Washrooms
EXHIBITORS
US HUPO is pleased to acknowledge the support of conference exhibitors. Exhibit booths are located in Key Ballroom 7-12 with the posters. Attendees are invited and encouraged to visit exhibit booths Sunday through Tuesday.

<table>
<thead>
<tr>
<th>Exhibit Booth Schedule</th>
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<tr>
<td>7:00 – 8:30 pm</td>
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<td>10:30 – 10:50 am</td>
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<tr>
<td>3:30 – 5:40 pm</td>
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<tr>
<td>4:00 - 6:00 pm</td>
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Exhibitors may be present at other times during the day. The exhibit and poster space will be open all-day Monday and Tuesday.

<table>
<thead>
<tr>
<th>VENDOR LUNCH SEMINARS</th>
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<tr>
<td>The following companies offer lunch seminars to conference participants on Monday and Tuesday. Seating is limited. Please reserve a seat at company exhibit booths.</td>
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<table>
<thead>
<tr>
<th>Monday, 12:00 – 1:30 pm</th>
<th>Tuesday, 12:00 – 1:30 pm</th>
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<tr>
<td>See page 9 for more information.</td>
<td>See page 14 for more information.</td>
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<tr>
<td>Agilent Technologies, Tubman AB, Level 3</td>
<td>Shimadzu, Tubman, Level 3</td>
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<tr>
<td>Thermo Scientific, Paca, Level 3</td>
<td>Waters Corporation, Paca, Level 3</td>
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AWARDS

Robert J. Cotter New Investigator Award
In honor of Robert J. Cotter’s contributions to US HUPO an award in his name will be made annually. This award will recognize outstanding proteomics scientists in the early years of their faculty careers.

2013 Recipient: Rebekah Gundry, Medical College of Wisconsin
Award lecture: Tuesday, March 12, 3:00 - 3:30 pm, Key Ballroom 3,4,6.

Student and Post-doc Travel Stipends
US HUPO supports graduate students and post-doctoral fellows with travel stipends of $500 each. We are pleased to announce the 2013 recipients.

Jagat Adhikari, Brown University
Kristin Boggio, University of Massachusetts
Zheng Cao, Van Andel Institute
Benjamin Diner, Princeton University
Jia Fan, The Methodist Research Institute
Jan Heng, Samuel Lunenfeld Research Institute
Aparna Kumar, Carnegie Mellon University
Jean-Philippe Lambert, Samuel Lunenfeld Institute
Liu Liu, Peking Union Medical College
Brianne Petritis, Arizona State University
Lee Sam, University of Michigan
Steven Hunter Walker, North Carolina State University
Tzu-Yi Yang, Purdue University
Sung-Hwan Yoon, University of Washington
Yiying Zhu, Brown University

Poster Awards
A poster award will be given to the best graduate student poster and the top post-doc poster. Posters will be judged and scored during the Monday poster session. The winners will be announced at the US HUPO Business Meeting on Tuesday, March 12, 3:30 - 4:00 pm in Key Ballroom 3,4,6.
Newcomers to the field of proteomics will benefit from this introduction to the algorithms employed in identifying tandem mass spectra and quantifying protein changes. Identification topics will include database search algorithms, error rate estimation, and protein assembly. Additional discussion will include sequence database selection, data repositories, and other on-line tools.

Both global and targeted quantitative proteomic workflows rely on statistical considerations during both experimental planning and interpretation of the results. The course will introduce to the experimentalist how to approach all the elements (experimental design, mass spectrometry based measurements, computation / statistics, and biological interpretation) with the objective of getting the best outcome for preliminary investigations, and then refinements for follow-up. The course will contain both lectures and analysis of example experimental datasets.

Details of sample preparation; what is needed to DIY. Topics will include methods for solubilization, protein level separation, proteolysis, peptide separation, membrane prep, micro-nan sample prep, and liquid chromatography tips that are practical and state-of-the-art. Examples will be reviewed in depth, with discussion to include reagents, volumes, column packings, etc.

This course is designed for both mass spectrometrists and biologists who want to understand more about the fundamental and practical aspects of systematic PTM analysis using mass spectrometry. Topics include: an introduction to PTMs; sample preparation for PTM analysis; HPLC/MS/MS analysis and manual verification of PTM peptides; full-spectrum PTM analysis using non-restrictive sequence alignment algorithms; proteomics analysis of phosphorylation, ubiquination, glycosylation, and lysine acetylation as case examples.

This course will cover the structural and functional analysis of glycoproteins including O-glycans, N-glycans, and their corresponding glycosylation sites and enzymatic regulation. The analytical methods include molecular and cellular biology and high throughput technologies such as mass spectrometry and protein microarrays. Examples of applying these glycoproteomics and glycomics analysis to uncover biological and clinical significance of protein glycosylation will also be introduced. This course is designed to provide materials and resources to connect researchers in biology, high throughput facilities, and clinical investigations to the current glycoproteomics and glycomics technologies.

Affinity purification of protein complexes is one of the most common technologies coupled with mass spectrometric proteomic analyses. There are many technical considerations required to produce useful results both with respect to the biochemistry and data processing.

**Welcome to the Opening Session**

<table>
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<tr>
<th>Time</th>
<th>Description</th>
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<td>6:00 - 6:15 pm</td>
<td>Opening Remarks</td>
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<tr>
<td>6:15 - 7:00 pm</td>
<td>Omics, Mass Spectrometry &amp; The Medical Avatar; Denis Hochstrasser; <em>University Hospital of Geneva; Geneva, Switzerland</em></td>
</tr>
<tr>
<td>7:00 - 8:30 pm</td>
<td>OPENING RECEPTION Key Ballroom 7-12</td>
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Join the exhibitors for hors d'oeuvres and drinks following the opening lecture.
MONDAY MORNING, MARCH 11

8:00 - 8:30 AM: EARLY COFFEE, Key Ballroom 7-12

8:30 - 9:20 AM: PLENARY LECTURE, Key Ballroom 3,4,6
Karim Rodland, presiding

8:30 - 9:20 am
Unbiased Identification of Synaptic Receptor Ligands; John R. Yates, III; Jeffrey Savas; Anirvan Ghosh; Joris de Wit; The Scripps Research Institute, La Jolla, CA; University of California San Diego, La Jolla, CA; CNS Discovery, F. Hoffman-La Roche, Basel, Switzerland; VIB Center for the Biology of Disease, K.U. Leuven, Leuven, Belgium

9:20 - 10:30 AM: PARALLEL SESSION
CLINICAL PROTEOMICS, Key Ballroom 3,4,6
Hui Zhang, presiding

9:20 - 9:30 am Opening Remarks; Hui Zhang, Johns Hopkins University

9:30 - 10:00 am
Mass Spectrometric Strategies for Protein Biomarker Discovery and Validation; Ruedi Aebersold; ETH Zurich and University of Zurich

10:00 - 10:15 am
Development of Multiplexed MSIA (Mass Spectrometric Immunoassay)-SRM Assays for Proteins Associated with Alzheimer’s Disease and Application to Clinical Samples; Mary F Lopez; Bryan Krastins; David Sarracino; Maryann S Vogelsang; Scott M. Peterman; Amol Prakash; Gouri Vadali; Bruno Darbouret; Johan Gobom; Erik Portelius; Josef Pannee; Eric Niederkofler; Urban Kiernan; Dobrin Nedelkov; Kai Blennow; Thermo Fisher Scientific Scientific, Cambridge, MA; Thermo Fisher Scientific Scientific, Grimes, IA; Sahlgrenska Academy at University of Gothenberg, Gothenberg, Sweden

10:15 - 10:30 am
Discovery and Validation of Multiple Colon Cancer Serological Biomarkers Using In-depth Proteome Analysis and Multiple Reaction Monitoring Assays; Wona Joo; Hsin Yao Tang; Tony Chang-Wong; David W. Speicher; The Wistar Institute, Philadelphia, PA

9:20 - 10:30 AM: PARALLEL SESSION
IDENTIFICATION OF PROTEIN MODIFICATIONS AND ISOFORMS, Key Ballroom 5
John S. Garavelli, presiding

9:20 - 9:30 am Opening Remarks; John S. Garavelli, Delaware Biotechnology Institute, University of Delaware

9:30 - 10:00 am
Bioinformatics of Phosphopeptide Identification, Phosphosite Localization, and iTRAQ Quantitation in Phosphoproteomics using LC-MS/MS; Karl Clauser; Philipp Mertins; Jana Qiao; DR Mani; Michael Gillette; Steven A. Carr; Broad Institute of MIT and Harvard, Cambridge, MA

10:00 - 10:15 am
Mining Human Proteomic Data for PTM Discovery; Min-Sik Kim; Sneha Pinto; FNU Samarjeet; Jyoti Sharma; Akhilesh Pandey; Johns Hopkins University School of Medicine, Baltimore, MD; Institute of Bioinformatics, Bangalore, India

10:15 - 10:30 am
LuciPHOr: Powerful Phosphorylation Site Localization Algorithm with False Localization Rate Estimation Using Target-decoy Approach; Damian Fermin; Anne-Claude Gingras; Alexey Nesvizhskii; Hyungwon Choi; University of Michigan, Ann Arbor, MI; Samuel Lunenfeld Research Institute, Toronto, ON; National University of Singapore, Singapore, Singapore

9:20 - 10:30 AM: PARALLEL SESSION
TECHNICAL IMPROVEMENTS IN MASS SPECTROMETRY, Key Ballroom 1-2
Dan Liebler, presiding

9:20 - 9:30 am Opening Remarks; Dan Liebler, Vanderbilt University

9:30 - 10:00 am
UV Photodissociation for Bottom-up and Top-down Proteomics; Jared B. Shaw, Michelle Robinson and Jennifer S. Brodbelt; The University of Texas at Austin, Austin, TX

10:00 - 10:15 am
Protein Identification Using Surface Acoustic Wave Nebulization (SAWN); Sung Hwan Yoon; Young Ah Goo; Michael Wilson; Yue Huang; John Edgar; Scott Heron; David Goodlett; University of Washington, Seattle, WA

10:15 - 10:30 am
Antibody-free, Targeted Mass Spectrometry Quantification of Low-Abundance Candidate Cancer Biomarkers in Human Serum and Urine; Tujin Shi; Thomas Fillmore; Rui Zhao; Yuqian Gao; Carrie Nicora; Athena Schepmoes; Jacob Kagan; Karin Rodland; Tao Liu; Keqi Tang; Richard Smith; David Camp; Alvin Liu; Wei-Jun Qian; Pacific Northwest National Laboratory, Richland, WA; National Cancer Institute, NIH, Rockville, MD; University of Washington, Seattle, WA
10:50 AM - 12:00 PM: PARALLEL SESSION  
CARDIOVASCULAR PROTEOMICS, Key Ballroom 3,4,6  
Peipei Ping, presiding

10:50 - 11:00 am  Opening Remarks; Peipei Ping, UCLA
11:00 - 11:30 am  The Role of Disease-modifying Post-translational Modifications in Heart Failure: Complexity in Designing New Therapies; Jennifer Van Eyk; Johns Hopkins University
11:30 - 11:45 am  Cardiomyopathy of Chagas Disease: Cysteiny1-S-Nitrosylation of Key Host Proteins as Candidate Biomarkers; John Wiktorowicz1,2; Susan Stafford1,2; Kizhake Soman1,2; Hyunsu Ju1,2; Paola Zago1,5; Nisha Garg1,5; Allan Brasier1,2; 1NIAD Clinical Proteomics Center, Galveston, TX; 2University of Texas Medical Branch, Galveston, TX; 3Instituto de Patologia Experimental, Salta, Argentina; 4Department of Immunology and Pathology, Galveston, TX; 5Universidad Nacional de Salta, Salta, Argentina
11:45 am - 12:00 pm  Characterization of the Cardiac Myosin Binding Protein-C Phosphoproteome in Healthy and Failing Human Hearts; Viola Kooij; Ronald Holewinski; Anne Murphy; Jennifer Van Eyk; Johns Hopkins University, Baltimore, MD

10:50 AM - 12:00 PM: PARALLEL SESSION  
PROTEOGENOMICS: FUSING THE GENOME AND PROTEOME, Key Ballroom 5  
Reid Townsend, presiding

10:50 - 11:00 am  Opening Remarks; Reid Townsend, Washington University
11:00 - 11:30 am  Protein and Transcript Discordance in a Prostate Cancer Model; Lee Sam; Damian Fermin; Anastasia Yocum; Arul Chinnaian; Alexey Nesvizhskii; University of Michigan, Ann Arbor, MI
11:30 - 11:45 am  A Multi-Omics Investigation of a Human Hematopoietic Cell Type; Derese Getnet1; Min-Sik Kim1; Chris Mitchell1; Praveen Kumar1; Srinivas Srinanth1; Sneha Pinto1; Mio Iwasaki1; Tai-Chung Huang1; Patrick Shaw1; Xinyan Wu1; Jun Zhong1; Rajgothama Chaerkady1; Babylakshmi Muthusamy1; Rajasekhar Nurjogi1; Nandini Sahasrabuddhe1; Rajesh Raju2; Caitlyn Bowman1; Ludmila Donilova1; Jeovon Cutler1; Dhanashree Kelkar1; T. S. Keshava Prasad1; Charles G Drake1; Luigi Marchionni1; Peter Murakami1; Alan Scott1; Leeming Shi1; Jean Thierry-Mieg5; Danielle Thierry-Mieg5; Rafael Irizarry1; Charles Wang1; Leslie Cope1; Yasushi Ishihama1; H. C. Harsha Gowda1; Akhilesh Pandey1; 1Johns Hopkins University, Baltimore, MD; 2Institute of Bioinformatics, Bengaluru, India; 3Kyoto University, Kyoto, Japan; 4Food and Drug Administration, Jefferson, AR; 5National Center for Biotechnology Information, Bethesda, MD; 6Beckman Research Institute of City of Hope, Duarte, CA
11:45 am - 12:00 pm  An Integrative Approach for Genomic and Proteomic Data Analysis; Li Chen1; Bai Zhang1; Yuan Tian1; Caitlin Choi1; Shisheng Sun1; Jianyong Zhou1; Hui Zhang1; Daniel Chan1; Jianhua Xuan1; Yue Wang1; Zhen Zhang1; 1Johns Hopkins School of Medicine, Baltimore, MD; 2Virginia Tech, Arlington, VA

10:50 AM - 12:00 PM: PARALLEL SESSION  
Controversies and Challenges in Clinical Proteomics, Key Ballroom 1-2  
Zhen Zhang, presiding

10:50 - 11:00 am  Opening Remarks; Zhen Zhang, Johns Hopkins University
11:00 - 11:30 am  Reproducibility and Forensic Bioinformatics in High-Throughput Biology; Keith Baggerly; UT MD Anderson Cancer Center
11:30 - 11:45 am  Quality Control in Building a High-resolution MS/MS Library of Peptides from Tryptic Digestion of Human Serum Albumin; Xiaoyu Yang; Pedatsur Neta; Lisa Kilpatrick; Yuri Mirokhin; Yuxue Liang; Stephen Stein; NIST, Gaithersburg, MD
11:45 am - 12:00 pm  An Integrative Approach for Genomic and Proteomic Data Analysis; Li Chen1; Bai Zhang1; Yuan Tian1; Caitlin Choi1; Shisheng Sun1; Jianyong Zhou1; Hui Zhang1; Daniel Chan1; Jianhua Xuan1; Yue Wang1; Zhen Zhang1; 1Johns Hopkins School of Medicine, Baltimore, MD; 2Virginia Tech, Arlington, VA
MONDAY AFTERNOON, MARCH 11

12:00 – 1:30 PM: **VENDOR LUNCH SEMINARS** or **LUNCH-ON-YOUR OWN**
Attendees are encouraged to RSVP for lunch seminars at company exhibit booths.
Lunch seminars are located on the Level Three

**AGILENT TECHNOLOGIES**, Tubman AB, Level Three

**Molecular Dissection of Complex Systems Using Label-Free Intensity-based Mass Spectrometry**
Michael E. Wright, Ph.D.; Molecular Physiology & Biophysics, Carver College of Medicine, Iowa City, IA

**Defining the Next Experiment: Pathway-directed Analysis from Protein Discovery Data**
Christine Miller; Senior Application Scientist, Agilent Technologies, Inc., Santa Clara, CA

**THERMO SCIENTIFIC**, Paca, Level Three

**Latest Advances in Functional Proteomics Workshop**
In the past few years, proteomics has evolved from Discovery Proteomics, cataloguing of proteins, to Functional Proteomics, with an emphasis on developing assays to hypothesis-driven questions. In this workshop, industry experts will present different mass spectrometry-based strategies for functional proteomics - from traditional approaches for validation to using HR/AM mass spectrometry for verification - while at the same time leveraging many of the technologies that biologists have been using for years.

Speakers:
- Harsha Gunawardena, Ph.D., UNC School of Medicine
- Hui Zhang, Ph.D., Johns Hopkins University

1:30 - 2:00 PM: **PLENARY SESSION**
**MCP LECTURESHIP**, Key Ballroom 3,4,6

**1:30 - 2:00 pm**
**The Present and Future Frontiers of Proteomics and Glycomics**; Catherine E. Costello; Boston University, Boston, MA

2:00 - 3:30 PM: **PARALLEL SESSION**
**EARLY CAREER SESSION I: FOCUS ON ADVANCES IN TECHNOLOGY**, Key Ballroom 3,4,6

2:00 - 2:10 pm
Opening Remarks; Catherine Fenselau, University of Maryland

2:10 - 2:20 pm
**High Throughput Stable-Isotope Labeled Derivatization Strategy and Data Analysis for the Relative Quantification of N-linked Glycans in Complex Biological Samples**; S. Hunter Walker; Amber D. Taylor; David C. Muddiman; North Carolina State University, Raleigh, NC

2:20 - 2:30 pm
**The Importance of Being Proportional: Shifting the Intensity-based Label Free Relative Quantification Paradigm**; Susan Van Riper; Ebbing De Jong; LeeAnn Higgins; John Carlis; Tim Griffin; University of Minnesota, Minneapolis, MN

2:30 - 2:40 pm
**Use of Data Dependent and Independent Mass Spectrometry Acquisition for the Systematic Study of the Human Acetylome Components**; Jean-Philippe Lambert; Sarah Picaud; Brett Larsen; Beatriz Gonzalez Badillo; Tony Pawson; Stefan Knapp; Panagis Filippakopoulos; Anne-Claude Gingras; Samuel Lunenfeld Research Institute, Toronto, Canada; Structural Genomics Consortium, Oxford, UK; University of Toronto, Toronto, Canada

2:40 - 2:50 pm
**Improved Protein Extraction and Identification from Archival Formalin-fixed Paraffin-embedded Human Aortas Using High Pressure and Heat**; Zongming Fu; Kun Yan; Avraham Rosenberg; Grace Grace; Richard S. Vander Heide; Allen Everett; David Herrington; Jennifer Van Eyk; John Hopkins University School of Medicine, Baltimore, MD; Louisiana State University School of Medicine, New Orleans, LA; Wake Forest University School of Medicine, Winston-Salem, NC

2:50 - 3:00 pm
**Automating Proteomic Sample Preparation with the Perfinity Workstation to Improve Mass Spectrometric Assay Reproducibility and Throughput**; Christine A Jelinek; Rachel Lieberman; Brian J Feild; Kevin Meyer; Robert Cotter; Jennifer Van Eyk; Johns Hopkins School of Medicine, Baltimore, MD; Perfinity Biosciences, West Lafayette, IN; Shimadzu Scientific Instruments, Columbia, MD; Johns Hopkins University, Baltimore, MD
MONDAY AFTERNOON, MARCH 11

3:00 - 3:10 pm Direct Profiling and Imaging of N-linked Glycans from Formalin-fixed Paraffin-embedded Tissues by Matrix-assisted Laser Desorption/Ionization Mass Spectrometry; Shadi Toghi Eshghi; Shuang Yang; Xiangchun Wang; Xingde Li; Hui Zhang; Johns Hopkins University, Baltimore, MD

3:10 - 3:20 pm Targeted Quantification of Membrane-associated Proteins Using Multiple Reaction Monitoring Mass Spectrometry and Full-length Isotope-labeled Protein Standards; Meiyao Wang1, 2; Hua-Jun He3; Gun-Young Heo3; Irina Pikuleva4; Lili Wang3; Illario Turko1, 2; 1Institute for Bioscience and Biotechnology Research, Rockville, MD; 2National Institute of Standards and Technology, Gaithersburg, MD; 3Case Western Reserve University, Cleveland, OH

2:00 - 3:30 PM: PARALLEL SESSION
EARLY CAREER SESSION II: FOCUS ON CELL REGULATION, Key Ballroom 5
Natalie Ahn, presiding

2:00 - 2:10 pm Opening Remarks; Natalie Ahn, University of Colorado at Boulder

2:10 - 2:20 pm Identification of Potential Early Diagnostic Biomarkers for Plasmodium falciparum and P. vivax Infections through Serum Proteomics; Sanjeeva Srivastava; Sandipan Ray; Indian Institute of Technology Bombay, Mumbai, India

2:20 - 2:30 pm SILAC-Based Proteomics Platform for the Large Scale Analysis of Protein-Ligand Binding Interactions; Jagat Adhikari; Duc Tran; Michael C. Fitzgerald1, 2; 1Duke University Medical Center, Durham, NC; 2Duke University, Durham, NC

2:30 - 2:40 pm Mass Spectrometry Characterization of Acrolein Protein Targets in the Liver: Focus on Site-Specific Pneumonectomy; Yiving Zhu; Carthene Bazemore-Walker; Brown University, Providence, RI

2:40 - 2:50 pm Macrophage Plasticity Illustrated by Ligand-induced Transient and Permanent Proteome and Transcriptome Changes Studied Using Discovery-mode and Targeted Proteomics; Eunkyung An; Nathan Manes; Aleksandra Nita-Lazar; NIAID, NIH, Bethesda, MD

2:50 - 3:00 pm The Interactome of Human Histone Deacetylases: Insights into the Stability and Cellular Functions of Chromatin Remodeling Complexes; Todd Greco1; Preeti Joshi1; Amanda Guise1; Yang Luo1; Fang Yu1; Alexey Nesvizhskii2; Ileana Cristea1; 1Princeton University, Princeton, NJ; 2University of Michigan, Ann Arbor, MI

3:00 - 3:10 pm Identification of Potential NSCLC Exosomal Biomarkers from Mutation-specific Lung Cancer Cell Lines; David Clark1; Zhongping Liao2; Austin Yang3; Li Mao1; 1UMB, School of Dentistry, Baltimore, MD; 2UMB, Geenebaum Cancer Center, Baltimore, MD

3:10 - 3:20 pm Identifying Targets of Pleiotropic Drugs by Flourorous Ligand Affinity Chromatography Combined with Activity-affinity Correlation Proteomics; Alex Kentis1; Jason J. Marneau1, 2; Alejandro Gutierrez1, 2; Casie Reed1; Li Pan1, 2; Jon C. Aster1, 2; A. Thomas Look1, 2; James E. Bradner1, 2; Hanno Steen1, 2; 1Harvard Medical School, Boston, MA; 2Dana Farber Cancer Institute, Boston, MA; 3Harvard Medical School, Boston, MA

3:20 - 3:30 pm Increased Throughput for 2D LC in the Analysis of Human Placental Samples; Martha Stapels1; Keith Fadgen1; J. Will Thompson2; Arthur Moseley2; James Langridge1; 1Waters Corporation, Milford, MA; 2Duke University, Durham, NC

2:00 - 3:30 PM: PARALLEL SESSION
EARLY CAREER SESSION II: FOCUS ON BIOMARKERS AND BIOINFORMATICS, Key Ballroom 1-2
Gil Omenn, presiding

2:00 - 2:10 pm Opening Remarks; Gil Omenn, University of Michigan

2:10 - 2:20 pm A Priori Identification of Bevacizumab Response in Recurrent Glioblastoma; Maxime Heroux; Maria Chesnik; Mona Al Gizawy; Elizabeth Cochran; Scott Rand; Jennifer Connelly; Wade Mueller; Mark Malkin; Kathleen Schmainda; Shama P. Mirza; Medical College of Wisconsin, Milwaukee, WI

2:20 - 2:30 pm Discovering Biomarkers to Predict Threatened Preterm Labour using SWATH and iTRAQ; Jan Heng1; Lorne Taylor1; Brett Larson2; Hon Nian Chua3; Monika Tucholska2; Soke May Pung2; Mary Lee2; Stephen Tate2; Craig Pennell3; Tony Pawson1; Stephen Lye1; 1Samuel Lunenfeld Research Institute, Toronto, ON, Canada; 2Institute for Infocomm Research, A*STAR, Singapore, Singapore; 3AB SCIEX, Concord, ON, Canada; 4University of Western Australia, Crawley, WA, Australia
MONDAY AFTERNOON, MARCH 11

2:30 - 2:40 pm
A Multiple Reaction Monitoring (MRM) Method to Detect BCR-Abl Kinase Activity in CML Using a Peptide Biosensor; Tzu-Yi Yang; Laurie L. Parker; Purdue University, West Lafayette, IN

2:40 - 2:50 pm
Discovery of Functional Determinants in Plasmodium Falciparum Red Blood Cell Invasion Using Long-lived Merozoites and a Systems Biology Approach; Krishan Kumar; Prakash Srinivasan; Michael J. Nold; J. David Haynes; Kathleen Moch; Karine Reiter; Dan Sturdevant; Steve F. Porcella; Scott Geromanos; David L. Narum; 1LMIV, NIAID, NIH, rockville, MD; 2LNVR, NIAID, NIH, rockville, MD; 3Waters Corporation, Milford, MA; 4Walter Reed Army Institute of Research, Silver Spring, MD; 5NIAID, NIH, Hamilton, Montana

2:50 - 3:00 pm
Proteomic Definition of the Balance Between Host Innate Immunity and Virus-mediated Immunosuppression; Benjamin Diner; Tuo Li; John Fuesler; Ileana Cristea; Princeton University, Princeton, NJ

3:00 - 3:10 pm
Identification of miR-145 Targets in Pancreatic Cancer Through an Integrated Omics Analysis; Tai-Chung Huang; Santosh Renuse; Praveen Kumar; Sneha Pinto; Yi Yang; Raghothama Chaerkady; Brian Godsey; Joshua Mendell; Curt Civin; Luigi Marchionni; Akhilesh Pandey; 1Johns Hopkins University School of Medicine, Baltimore, MD; 2Institute of Bioinformatics, Bangalore, India; 3University of Maryland, Baltimore, MD; 4University of Texas Southwestern, Dallas, TX

3:10 - 3:20 pm
Comparative Proteomic Analysis of Human Embryonic Stem Cells (ESCs) and human induced Pluripotent Stem Cells (iPSCs) of Different Origin; Natalia Pripuzova; Melkamu Getie-Kebtie; Christopher Grunseich; Colin Sweeney; Harry Malech; Michail Alterman; 1FDA, Center for Biologics Evaluation and Research, Rockville, MD; 2Neurogenetics Branch, NINDS, NIH, Bethesda, MD; 3Laboratory of Host Defenses, NIAID, NIH, Bethesda, MD

3:20 - 3:30 pm
Building a Reliable Proteomics Database Requires Stringent Protein Identification Criteria; John Tra; Vidya Vidya Venkatraman; Jennifer Van Eyk; Johns Hopkins University, Baltimore, MD

3:30 - 5:00 PM: POSTER SESSION, Key Ballroom 7 - 12
Exhibits-Posters
Odd-numbered posters present 3:30 - 4:15 pm. Even-numbered posters present 4:15 - 5:00 pm.

5:00 - 5:30 PM: HAPPY HALF-HOUR, Key Ballroom 7-12
Exhibits-Posters Room

5:30 - 6:50 PM: PARALLEL EVENING WORKSHOP, Key Ballroom 5
NATIONAL CANCER INSTITUTE: HIGHLIGHTS FROM PROTEOMIC RESEARCH PROGRAMS
Jacob Kagan and Mehdi Mesri, presiding

5:30 - 5:35 pm
The Early Detection Network: Proteomics Based Biomarkers; Sudhir Srivastava, NCI

5:35 - 5:40 pm
Clinical Proteomic Tumor Analysis Consortium; Henry Rodriguez, NCI

5:40 - 6:00 pm
The Pathway from Biomarkers Discovery to FDA Approval; Daniel Chan, Johns Hopkins University, Baltimore, MD

6:00 - 6:20 pm
Mass Spectrometry-based Tumor Tissue Analysis; Daniel C. Liebler; Vanderbilt University Medical Center, Nashville, TN

6:20 - 6:40 pm
Targeted SRM Quantification at the PNBL EDRN BRL: Toward Clinical Utility; David G. Camp II; Pacific Northwest National Laboratory, Richland, WA

5:30 - 6:30 PM: PARALLEL EVENING WORKSHOP, Key Ballroom 1-2
NHLBI PROTEOMICS
TUESDAY MORNING, MARCH 12

8:00 - 8:30 AM: EARLY COFFEE, Key Ballroom 7-12

8:30 - 9:20 AM: PLENARY LECTURE, Key Ballroom 3,4,6
Catherine Fenselau, presiding

8:30 - 9:20 am
Frontiers of Orbitrap Mass Spectrometry; Alexander Makarov; Thermo Fisher Scientific

9:20 - 10:30 AM: PARALLEL SESSION
CLINICAL GLYCOPROTEOMICS AND GLYCOMICS, Key Ballroom 3,4,6
Jerry Hart, presiding

9:20 - 9:30 am
Opening Remarks; Jerry Hart, Johns Hopkins University

9:30 - 10:00 am
**Approaching Quantitative High Throughput Glycomics**; Michael Tiemeyer\(^1\); Mindy Porterfield\(^1\); Rene Ranzinger\(^1\); Matthew Eavenson\(^2\); Will York\(^1\); Kazuhiro Aoki\(^1\); \(^1\)Complex Carbohydrate Research Center, Athens, GA; \(^2\)University of Georgia, Athens, GA

10:00 - 10:15 am
Comparative Glycomics Analysis of Influenza Hemagglutinin (H5N1) Produced in Vaccine and Vaccine Research Relevant Cell Substrates; Yanming An; John Cipollo; FDA Center for Biologics Evaluation and Research, Bethesda, MD

10:00 - 10:15 am
Comparative Glycomics Analysis of Influenza Hemagglutinin (H5N1) Produced in Vaccine and Vaccine Research Relevant Cell Substrates; Yanming An; John Cipollo; FDA Center for Biologics Evaluation and Research, Bethesda, MD

10:15 - 10:30 am
**Solid Phase Labeling of Sialylated Glycans for Quantitative Analysis using Mass Spectrometry**; Punit Shah; Shuang Yang; Shisheng Sun; Paul Aiyetan; Kevin Yarema; Hui Zhang; Johns Hopkins University, Baltimore, MD

10:30 - 10:50 AM: COFFEE BREAK, Key Ballroom 7-12
Coffee and pastries with the exhibitors.

9:20 - 10:30 AM: PARALLEL SESSION
ASSESSMENT OF QUALITY, CONSISTENCY, AND REPRODUCIBILITY, Key Ballroom 5
Steve Stein, presiding

9:20 - 9:30 am
Opening Remarks; Steve Stein, NIST

9:30 - 10:00 am
**Assessment of Factors Affecting the Reproducibility of Quantitative Proteomics Using LC-MS/MS**; Jian-Ying Zhou; Yuan Tian; Shisheng Sun; Caitlin Choi; Lijun Chen; Bai Zhang; Lily Chen; Zhen Zhang; Daniel Chan; Hui Zhang; Johns Hopkins School of Medicine, Baltimore, MD

10:00 - 10:15 am
External Calibration: Why Should I?; Andrew Hoofnagle; University of Washington, Seattle, WA

9:20 - 10:30 AM: PARALLEL SESSION
MICROARRAY FOR PROTEIN AND LECTIN, Key Ballroom 1-2
Mike Snyder, presiding

9:20 - 9:30 am
Opening Remarks; Mike Snyder, Stanford University

9:30 - 10:00 am
**DNA Methylation Presents Distinct Binding Sites for Human Transcription Factors**; Heng Zhu; Johns Hopkins University, Baltimore, MD

10:00 - 10:15 am
**Nanopore Assay for Low Abundance Blood Biomarker Discovery and Quantification from Human Bodily Fluids**; Tony Hu; The Methodist Hospital Research Institute, Houston, TX

10:15 - 10:30 am
**Measurement of the Glycosylation Changes of Serum Proteins by Lectin Immunosorbant Assay**; Danni Li\(^1\); Hang-Ching Chiu\(^2\); Hui Zhang\(^2\); Daniel Chan\(^2\); \(^1\)University of Minnesota, Minneapolis, MN; \(^2\)Johns Hopkins University, Baltimore, MD

10:30 - 10:50 AM: COFFEE BREAK, Key Ballroom 7-12
Coffee and pastries with the exhibitors.
### TUESDAY MORNING, MARCH 12

#### 10:50 AM - 12:00 PM: PARALLEL SESSION

**SYSTEMS PROTEOMICS, Key Ballroom 3,4,6**

Alexey Nesvizhskii, presiding

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<th>Time</th>
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<tr>
<td>10:50 - 11:00 am</td>
<td>Opening Remarks; Alexey Nesvizhskii, <em>University of Michigan</em></td>
<td>Key Ballroom 3,4,6</td>
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<tr>
<td>11:00 - 11:30 am</td>
<td><strong>Interactome Networks and Human Disease</strong>; Marc Vidal; Dana Farber Cancer Institute</td>
<td>Key Ballroom 3,4,6</td>
<td>Marc Vidal, Dana Farber Cancer Institute</td>
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<td>11:30 - 11:45 am</td>
<td><strong>Signaling Interactomes in Cancer; Opportunities for New Targets and Molecular Markers</strong>; Eric Haura, Jiannong Li, Guolin Zhang, Matthew Smith, Richard Hall, John Koomen, Keiryn Bennett, Giulio Superti Furga, H. Lee Moffitt Cancer Center, Tampa, FL, Center for Molecular Medicine, Vienna, Austria</td>
<td>Key Ballroom 3,4,6</td>
<td>Eric Haura, Jiannong Li, Guolin Zhang, Matthew Smith, Richard Hall, John Koomen, Keiryn Bennett, Giulio Superti Furga, H. Lee Moffitt Cancer Center, Tampa, FL, Center for Molecular Medicine, Vienna, Austria</td>
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<tr>
<td>11:45 am - 12:00 pm</td>
<td><strong>Application of Generalized Protein Parsimony and Spectral Counting to Functional Enrichment Analysis and Protein Isoform Detection</strong>; Nathan Edwards, Georgetown University Medical Center, Washington, DC</td>
<td>Key Ballroom 3,4,6</td>
<td>Nathan Edwards, Georgetown University Medical Center, Washington, DC</td>
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#### 10:50 AM - 12:00 PM: PARALLEL SESSION

**TARGETED QUANTITATION: PROTEOMICS/INFORMATICS, Key Ballroom 5**

Robert Moritz, presiding

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<td>10:50 - 11:00 am</td>
<td>Opening Remarks; Robert Moritz, <em>Institute for Systems Biology</em></td>
<td>Key Ballroom 5</td>
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<td>11:00 - 11:30 am</td>
<td><strong>Shifting the Quantitative Proteomics Paradigm from Hypothesis Generating to Hypothesis Testing</strong>; Michael MacCoss, University of Washington</td>
<td>Key Ballroom 5</td>
<td>Michael MacCoss, University of Washington</td>
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<td>11:30 - 11:45 am</td>
<td><strong>Quantification of PACIFIC Data with Progenesis Software</strong>; Robert Moulder, Santosh Bhosale, Jussi Salmi, Michael Wilson, John Chapman, Young Ah Goo, David Goodlett, University of Turku, Turku, Finland, University of Washington, Seattle, WA, University of Maryland, Baltimore, MD</td>
<td>Key Ballroom 5</td>
<td>Robert Moulder, Santosh Bhosale, Jussi Salmi, Michael Wilson, John Chapman, Young Ah Goo, David Goodlett, University of Turku, Turku, Finland, University of Washington, Seattle, WA, University of Maryland, Baltimore, MD</td>
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#### 10:50 AM - 12:00 PM: PARALLEL SESSION

**CHARACTERIZATION OF PTMs, Key Ballroom 1-2**

Michael Pierce, presiding

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<td>10:50 - 11:00 am</td>
<td>Opening Remarks; Michael Pierce, <em>University of Georgia</em></td>
<td>Key Ballroom 1-2</td>
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<td>11:00 - 11:30 am</td>
<td><strong>Targeting Protein Post-Translational Modifications for Non-invasive Diagnosis of Oral Cancer</strong>; Tim Griffin, University of Minnesota, Minneapolis, MN</td>
<td>Key Ballroom 1-2</td>
<td>Tim Griffin, University of Minnesota, Minneapolis, MN</td>
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<td>11:30 - 11:45 am</td>
<td><strong>Global Analysis of Bacteria-host Interaction by AMPylation Using Human Nucleic Acid Programmable Protein Arrays</strong>; Xiaobo Yu, Andrew R Woolery, Phi Luong, Yi Heng Hao, Markus Grammel, Nathan Westcott, Jin Park, Jie Wang, Xiaofang Bian, Gokhan Demirkiran, Howard C. Hang, Kim Orth, Joshua LaBaer, Arizona State University, Tempe, AZ, University of Texas Southwestern Medical Center, Dallas, TX, The Rockefeller University, New York, NY</td>
<td>Key Ballroom 1-2</td>
<td>Xiaobo Yu, Andrew R Woolery, Phi Luong, Yi Heng Hao, Markus Grammel, Nathan Westcott, Jin Park, Jie Wang, Xiaofang Bian, Gokhan Demirkiran, Howard C. Hang, Kim Orth, Joshua LaBaer, Arizona State University, Tempe, AZ, University of Texas Southwestern Medical Center, Dallas, TX, The Rockefeller University, New York, NY</td>
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<tr>
<td>11:45 am - 12:00 pm</td>
<td><strong>Temporal Phosphorylation Dynamics Analysis of TLR Stimulation: Role of MARCKS in TLR Signaling</strong>; Virginie Sjoelund, Iain Fraser, Aleksandra Nita-Lazar, NIAID, NIH, Bethesda, MD</td>
<td>Key Ballroom 1-2</td>
<td>Virginie Sjoelund, Iain Fraser, Aleksandra Nita-Lazar, NIAID, NIH, Bethesda, MD</td>
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TUESDAY AFTERNOON, MARCH 12

12:00 – 1:30 PM: VENDOR LUNCH SEMINARS or LUNCH-ON-YOUR OWN
Attendees are encouraged to RSVP for lunch seminars at company exhibit booths.
Lunch seminars are located on the Level Three

SHIMADZU, Tubman AB, Level Three
Prepare Your Lab For the Future-
Affinity Mass Spectrometry, One Minute Protein Digests and Cloud-based Informatics
Fred Regnier, J.H. Law Distinguished Professor, Purdue University
Christine Jelinek, Johns Hopkins NHLBI
Gautam Saxena, President & CEO, Integrated Analysis Inc.
This workshop will provide your organization with the facts and critical lessons you need to know for the successful adoption and integration of affinity mass spectrometry, ultra-fast protein digestions, ultra-fast mass spectrometry and powerful new cloud-based informatics in your laboratory. Please join us for this interactive workshop where you will discover new Perfinity integrated Digestion Platform (iDP) technologies that enable automated, reproducible protein digestions on a sub-minute timescale, greatly improving the applicability of peptide based SRM assays. Cloud-based informatics solutions that provide a unique opportunity to improve not only laboratory data storage, but also enable on-cloud workflow execution and project sharing will also be presented. These tools will save your laboratory time, money and resources and will greatly improve data sharing with collaborators.

WATERS CORPORATION, Paca, Level Three
A Systems Biology Approach for the Discovery of Drug and/or Vaccine Targets in Plasmodium falciparum Using Irradiated Long-lived Merozoites
David L. Narum, PhD; Head, Process Development Unit, Laboratory of Malaria Immunology and Vaccinology, NIAID/NIH

1:30 - 3:00 PM: PARALLEL INTERACTIVE SESSION
INDUSTRY PANEL: COLLABORATION WITH ACADEMICS FOR MOVING PROTEOMICS FORWARD, Key 3,4,6
William Clarke, moderator
Panelists: Aaron Hudson (AB SCIEX), Scott Kuzdzal (Shimadzu), Mary Lopez (Thermo Scientific), Christine Miller (Agilent Technologies), Barry Schweitzer (Life Technologies)

1:30 - 3:00 PM: PARALLEL INTERACTIVE SESSION
FDA PANEL: THE PATH LEADING TO REGULATORY APPROVAL OF PROTEOMICS DIAGNOSTICS AND TECHNOLOGIES, Key Ballroom 5
Maria Chan, moderator
Speakers: Julia Lathrop and Elizabeth Stafford (FDA)
Panelists: Reena Philip, Yun-Fu Hu, Abraham Tzou, Meijuan Li (FDA)

3:00 - 3:30 PM: ROBERT J. COTTER NEW INVESTIGATOR AWARD, Key 3,4,6
Recipient: Rebekah Gundry, Medical College of Wisconsin
Cell Surface Chemoproteomics for Capturing States of Human Cardiac Differentiation from Pluripotent Stem Cells

3:30 - 4:00 PM: US HUPO BUSINESS MEETING, Key Ballroom 3,4,6
All attendees are welcome!
Natalie Ahn, presiding

4:00 - 5:30 PM: POSTER SESSION, Key Ballroom 7-12
Exhibits-Posters
Odd-numbered posters present 4:00 - 4:45 pm. Even-numbered posters present 4:45 - 5:30 pm

5:30 - 6:00 PM: HAPPY HALF-HOUR, Key Ballroom 7-12
Exhibits-Posters
TUESDAY EVENING, MARCH 12 - WEDNESDAY MORNING, MARCH 13

6:00 - 7:00 PM: EVENING WORKSHOP, Key Ballroom 3,4,6
INTEGRATION OF PROTEOMICS - BIOLOGY, TECHNOLOGY, AND CLINICAL ADVANCES
Karin Rodland, presiding

6:00 – 6:15 pm Biology Drivers; Karin Rodland; Pacific Northwest National Laboratory, Richland, WA
6:15 – 6:30 pm Technology Developments; Alexander Makarov; Thermo Fisher Scientific
6:30 – 6:45 pm Transitioning Technology to the Clinic; Jennifer Van Eyk, Johns Hopkins University, Baltimore, MD
6:45 - 7:00 pm Discussion

WEDNESDAY MORNING, MARCH 13

8:00 - 9:20 AM: EARLY COFFEE, Key Foyer

US HUPO - EDRN JOINT SYMPOSIUM

8:20 - 9:20 AM: PLENARY LECTURE, Key Ballroom 3,4,6
Sudhir Srivastava, presiding

8:20 - 8:30 am Opening Remarks; Sudhir Srivastava, NCI, EDRN
8:30 - 9:20 am Cancer Genomes and Their Implications for Research and Patients; Bert Vogelstein; Johns Hopkins University, Baltimore, MD

9:20 - 10:30 AM: CANCER BIOMARKER: DISCOVERY, Key Ballroom 3, 4, 6
Sam Hanash, presiding

9:20 - 9:30 am Opening Remarks; Sam Hanash, M D Anderson Cancer Center
9:30 - 10:00 am Implementing“-Omics” Approaches for Precision Medicine; Arul Chinnaian, University of Michigan; Ann Arbor, MI
10:00 - 10:15 am Proteomic Analysis of Liver Tissue Interstitial Fluid and Its Potential in Biomarker Discovery for HBV Associated HCC; Zhilei Liu; Wei Sun; Longqin Sun; Ying Jiang; Fuchu He; Beijing Proteome Research Center, Beijing, China
10:15 - 10:30 am Serum Fucosylated Forms of DPP-4 and tPA Improve the Detection of Prostate Cancer (Pca); Qing Kay Li; Joyce Chiu; Hui Zhang; Daniel Chan; The Johns Hopkins Medical Institutions, Baltimore, MD

10:30 - 10:50 AM: COFFEE BREAK, Key Foyer

10:50 AM - 12:00 PM: CANCER BIOMARKER: TRANSLATION, Key Ballroom 3, 4, 6
Daniel Chan, presiding

10:50 - 11:00 am Opening Remarks; Daniel Chan, Johns Hopkins University
11:00 - 11:30 am Markers for Early Cancer Detection and Personalized Treatment; David Sidransky, Johns Hopkins University, Baltimore, MD
11:30 - 11:45 am Specific Glycoforms of MUC5AC and Endorepellin Accurately Distinguish Mucinous from Non-Mucinous Pancreatic Cysts; Zheng Cao; Kevin Maupin; Bryan Curnulite; Brian Fallon; Christa Feasley; Elizabeth Brought; Richard Kwon; Christopher West; John Cunningham; Randall Brand; Paola Castelli; Stefano Crippa; Peter Allen; Diane Simeone; Brian Haab; Van Andel Institute, Grand Rapids, MI; University of Michigan, Ann Arbor, MI; University of Arizona Health Sciences Center, Phoenix, AZ; University of Pittsburgh Medical Center, Pittsburgh, PA; Ospedale Sacro Cuore Don Calabria, Negrar (VR), Italy; Memorial Sloan-Kettering Cancer Center, New York, NY
11:45 am - 12:00 pm Transfer of Selected Reaction Monitoring (SRM) Assays Between Laboratories; Robert Harlan; Jing Chen; Yansheng Liu; Amol Prakash; Mary F Lopez; Daniel Chan; Ruedi Aebersold; Hui Zhang; Johns Hopkins University, Baltimore, MD; ThermoFisher Scientific, Cambridge, MA; ETH Zurich, Zurich, Switzerland; ThermoFisher, Cambridge, MA
they cannot differentiate between intra- and extra-cellular protein-protein interactions. Nearly every study aimed to identify synaptic ligand/receptors has utilized candidate approaches through invertebrate genetics or cell biology. Candidate binding assays depend on gels, which has limited their identification to the most abundant interactors and resulted in their slow and incremental identification. Consequently, many “orphan receptors” without a known ligand-binding partner remain. We've developed an unbiased, robust, and sensitive method based on mass spectrometry to identify trans-synaptic ligand/receptor interactions using extracellular domain Fc fusion (ectodomain) baits and brain protein extracts as prey. We show that our approach is broadly applicable by first focusing on several well known synaptic adhesion protein families and then on the newly appreciated leucine repeat protein family. Sequential application of our method, or network walking, by sequential bait/prey swapping can elucidate neuronal synaptic networks.

### Sunday 6:00 - 7:00 pm
**Plenary Lecture / Opening Session, Key Ballroom 3,4,6**

Sun 6:15 - 7:00 pm: Omics, Mass Spectrometry & The Medical Avatar

Denis Hochstrasser; Didia Coelho Graça; Annarita Farina; Alex Scherl; Pierre Lesueur; University Hospital of Geneva, Geneva, Switzerland

Diseases have two main origins, the genes and the environment. The environment could be divided in two, microbes and toxics. Therefore, in an analytical perspective, we should analyze in laboratory medicine, gene and gene products, microbes and microbial actions, and toxins and their effects at all levels.

Omic technologies offer today very powerful tools to decipher genomes, transcriptomes, partial proteomes and metabolomes. Deep sequencing, deep mass spectrometry and deep flow cytometry provide a) the full genome or transcriptome sequence in hours at low cost, b) thousands of proteins and metabolites analysis at the atomole level, and c) few cells characterization, all in small body fluid samples.

For example, concerning microbes, proteomic MALDI-TOF analysis of bacterial colonies speed up and refine the antibiotic treatment of patients. Concerning genes and toxics, ETD MS-MS of hemoglobin unravel sequence and post-translational modifications, and chain concentration differences in hours or minutes useful in hemoglobin disorders characterization. Proteomic analysis of biliary or pancreatic cyst fluids display hundreds of interesting proteins and potential cancer biomarkers.

Consequently to these tremendous developments and others, clinical medicine faces an unprecedented problem of a data and knowledge tsunami. How should we extract the patient relevant information out of this omics tsunami? How should we display, in a clever and organized manner, patient genomic, transcriptomic, proteomic and metabolomic information? It is already known today that, even in the best hospitals, more than 3% of the patient data, at the time of discharge, have not been seen and interpreted by any member of the medical staff. How could we therefore cope with all the omics data and accumulating knowledge?

The most likely solution is the development of a medical avatar that would "intelligently" highlight to the patient and his or her medical team the relevant medical, gene and environmental information organized by problems.

### Monday 8:30 - 9:20 am
**Plenary Lecture, Key Ballroom 3,4,6**

**MOA 8:30 - 9:20 am: Unbiased Identification of Synaptic Receptor Ligands**

John R. Yates, III; Jeffrey Savas; Anirvan Ghosh; Joris de Wit; ThermoFisher Scientific, Cambridge, MA; "University of California San Diego, La Jolla, CA; "CNS Discovery, F. Hoffman-La Roche, Basel, Switzerland; "VIB Center for the Biology of Disease, K.U. Leuven, Leuven, Belgium

The identification of trans-synaptic ligand receptor interactions has proven critically important for our understanding of synapse development, stability, maturation, function, neuronal circuit formation, and plays a critical role in neurological diseases such as autism and schizophrenia. Binding of specific ligands to target receptors represent the trigger point for many critical biological processes including -cell adhesion, survival and death signaling, and synapse formation. Further, while many intracellular protein/protein interactions have questionable or little functional consequences, ligand / receptors interactions represent key mechanisms for cells to communicate to the extracellular space and each other. While much effort has gone in to the development of methods for the identification of intracellular protein/protein interactions, much less progress has been made developing methods for the identification ligand / receptor interactions. While antibodies and epitope tags provide powerful molecular handles for the isolation of binary protein-protein interactions, protein complexes and even membrane bound receptor complexes, they are not particularly effective for finding ligand / receptor pairs since...
promotion and clearance roles of Apo E might be due to the different binding capacities of Apo E isoforms (Apo E2,3,4) for Aβ. Disturbances of copper homeostasis may also contribute to the neurodegeneration associated with AD and the level of the copper enzyme ceruloplasmin (CP) is increased in the CSF of AD affected individuals. In order to further investigate the relationship of these markers to AD, we developed multiplexed, Mass spectrometric immunocassay (MSIA)-SRM assays that allow quantification of CP, APOE monitoring and isoform-specific peptides and several Aβ peptides. The MSIA technology provides rapid enrichment for low abundance analytes from fluids such as plasma, serum and cerebrospinal fluid (CSF). We used these assays to interrogate a small cohort of clinical plasma samples from patients with AD and matched controls. The results demonstrated (for the first time) quantitative MS detection of Aβ and other peptides in human plasma.

Mon 10:15 - 10:30 am: Discovery and Validation of Multiple Colorectal Cancer Serological Biomarkers Using In-depth Proteome Analysis and Multiple Reaction Monitoring Assays

Wona Joo; Hsin Yao Tang; Tony Chang-Wong; David W. Speicher; The Wistar Institute, Philadelphia, PA

Proteomics discovery of novel cancer plasma biomarkers is impeded by the vast complexity of plasma, variability of human specimens, and difficulty distinguishing cancer specific proteins from less specific plasma protein changes associated with the acute-phase inflammatory reaction. We addressed these hurdles by utilizing a xenograft mouse model developed with an in-depth 4-D protein profiling method to identify human proteins in the mouse plasma. Proteins confidently identified as human were definitely shed by the tumor into the blood and were therefore candidate serological cancer biomarkers. Parallel proteome analysis of cell culture conditioned medium (secretome) further enhanced sequence coverage of most proteins shed by the same human cell lines in the xenograft model. More than 800 human proteins were identified in a recently analyzed dataset from several low passage number colon carcinoma cell lines (WC013, WC007, WC020, and WC008), and 83 of these proteins were categorized as high priority candidate biomarkers. These candidates included novel candidate biomarkers as well as several proteins previously reported to be elevated in either CRC tumors or the blood of CRC patients. Most identified candidates were involved in either the TGF/FAS signaling transduction pathway or transcription factors associated with TP53 or Myc in pathway analysis. A group of 45 colon cancer candidate biomarkers in the 15-65 kDa region of 1D SDS gels was used for follow-up verification and validation in patient plasma samples. After developing multiplexed MRM assays, these candidate biomarkers were quantitated in pooled 25 control and 25 colorectal cancer patient plasma samples. A subset of 22 candidates was selected for subsequent analysis in individual patient specimens. Stable-isotope labeled peptides were used in the final MRM assay to enable absolute quantitation in individual patients and control subjects. Results suggest that several of these biomarkers are superior to CEA and CA-19-9.

Monday 9:20 - 10:30 am

Identification of Protein Modifications and Isomors

Key Ballroom 5

MOC 9:30 - 10:00 am: Bioinformatics of Phosphopeptide Identification, Phosphosite Localization, and iTRAQ Quantitation in Phosphoproteomics using LC-MS/MS

Karl Clauser; Philipp Mertins; Jana Qiao; DR Mani; Michael Gillette; Steven A. Carr; Broad Institute of MIT and Harvard, Cambridge, MA

We are engaged in a large-scale phosphoproteomic project using human breast cancer tumor tissue obtained from ~100 patients that include luminal A, luminal B, Her-2 enriched and basal-like subtypes. The project is being done under the auspices of the Clinical Proteomic Tumor Analysis Consortium (CPTAC) of the National Cancer Institute (NCI), and the tumor samples were genomically characterized in The Cancer Genome Atlas (TCGA) initiative of the NCI. After cryofracturing frozen tumor tissue, protein was extracted and digested into peptides with trypsin.

Groups of 3 patient samples and a common control were prepared for multiplexed quantitation, after iTRAQ labeling of peptides. High pH reversed phase peptide fractionation and IMAC enrichment of phosphopeptides were followed by LC-MS/MS on high resolution Thermo QExactive mass spectrometer. This presentation will focus on bioinformatic methods for mass spectral data analysis to address issues in phosphopeptide identification and phosphosite localization when combining multiple observations of peptides containing the same phosphosite(s) to produce quantitative results at the phosphosite level. Particular attention will be devoted to the consequences of possible ambiguity in phosphosite localization. This inherent feature of phosphoproteomic datasets emerges when MS/MS fragmentation of a peptide is incomplete for individual peptides that have more phosphorylatable Ser, Thr, or Tyr residues than phosphates present.

Mon 10:00 - 10:15 am: Mining Human Proteomic Data for PTM Discovery

Min-Sik Kim; Sneha Pinto; FNU Samarjeet; Jyoti Sharma; Akhilesh Pandey; Johns Hopkins University School of Medicine, Baltimore, MD; Institute of Bioinformatics, Bangalore, India

Mass spectrometry-based proteomics has become a state-of-the-art technology. Many proteomics studies are often carried out using disease-related cell lines and tissues. To be complementary to community-based collaboration for drawing of a baseline of human proteome, our laboratory along with collaboration with other institutions have systematically analyzed multiple human samples including 6 fetus tissues and 18 adult tissues as well as 6 purified adult hematopoietic cells for last two years. As a result, a draft of human proteome has been announced in the international HUPO congress. >24 millions of high resolution tandem mass spectra by HCD from thirty human samples were acquired on Orbitrap mass spectrometers, but only ~1/3 of total tandem spectra were finally matched to peptide sequences at 1% false discovery rates.

Although known in the field, this indicates a lot of good fragmentation information remains to be unassigned. Here, we report a systematic study towards an overview of extent of PTMs throughout the human body. >16 millions of high quality unmatched tandem spectra were used to search a series of PTMs including phosphorylation at serine, threonine and tyrosine residues, hydroxylation at proline residue, ubiquitination at lysine residues, and O-GlcNAcylation at serine and threonine residues and so on. We were able to identify thousands of new PTMs that were otherwise undiscovered. Further we have generated a script to extract tandem mass spectra which contains signature ions for a specific modification. For example, 204,087 m/z value, the oxonium ion of GlcNAc moiety can be used to enrich tandem mass spectra potentially derived from modified peptides with O-GlcNAc using the in-house script. We were able to achieve high enrichment (>80%) of modified peptides in the search result when compared to blind modification searches as described above. This new method can be implemented to the current proteomics workflow as a routine protocol.

Mon 10:15 - 10:30 am: LuciferPHOr: Powerful Phosphorylation Site Localization Algorithm with False Localization Rate Estimation Using Target-Decoy Approach

Damian Fermin; Anne-Claude Gingras; Alexey Nesvizhskii; Hyungwon Choi; University of Michigan, Ann Arbor, MI; Samuel Lunenfeld Research Institute, Toronto, ON; National University of Singapore, Singapore, Singapore

Localization of phosphorylation sites on ambiguous candidate peptide sequences based on MS/MS spectra is a major bottleneck for automated large-scale phospho-proteomics analysis. Pervasive noise in the spectral data, intense neutral loss peaks, and co-existence of serine/threonine and tyrosine are limiting factors for objectively scoring site patterns across thousands of peptides, and create a major challenge to estimating the overall false localization rate (FLR). To date, a few computational methods have been proposed for assessing phosphorylation site localization, including Mascot Delta score (ModScore) and PhosphoRS, but none of the existing methods is capable of evaluating the FLR. Here we propose LuciPHOr, a model-based method for phosphorylation site localization with rigorous FLR estimation. It takes as input the database search results reported from any search engine,
processed by the Trans-Proteomic Pipeline, and re-examines all permutations on the peptide for localization. The specificity of site permutations is scored based on spectral features such as peak intensity and mass accuracy, and this score is reported along with the local and global FLR. We assessed the performance of LuciPHOr using a dataset comprised of 180 synthetic peptides with known phosphorylation sites in terms of the receiver-operating characteristic. LuciPHOr demonstrated higher sensitivity and specificity than Ascore and MScore and offered accurate FLR estimates for both low (CID) and high (HCD) mass accuracy data. We will discuss the utility of local FLR in the context of phosphoproteomic analysis, and suggest a reporting guideline of site localization analysis at the level of individual candidate sites. LuciPHOr is freely available at http://www.nesvilab.org, and it is currently built for use in linux environment with straightforward installation steps.

Mon 9:30 - 10:00 am: UV Photodissociation for Bottom-Up and Top-Down Proteomics
Jared B. Shaw; Michelle Robinson; Jennifer Brodbelt; The University of Texas at Austin, Austin, TX

193 nm ultraviolet photodissociation (UVPD) yields extensive fragmentation for peptides and proteins without loss of site specific information about labile PTMs such as phosphorylation. Moreover, UVPD allows fast activation and rapid switching between positive and negative ionization modes for more extensive coverage of both basic and acidic peptides. The ability to efficiently and extensively dissociate intact gas phase protein ions remains a significant challenge of top down mass spectrometry. Traditional dissociation methods, such as CID and IRMPD, tend to preferentially cleave at the weakest bonds (e.g. N-terminal to proline, PTMs, etc.) limiting sequence coverage and the ability to localize PTMs. These limitations impede the confident characterization of protein isoforms arising from post translational modifications (PTMs), single nucleotide polymorphisms (SNPs), and alternative splicing. In this work, we present the implementation of 193 nm ultraviolet photodissociation (UVPD) in an Orbitrap Elite mass spectrometer and compare the performance of UVPD to ETD, HCD and CID for both bottom-up and top-down applications. Highly efficient dissociation is achieved using a single 5 ns pulse, with broad coverage obtained primarily through the production of a, x, y and z ions with fewer b, c and v ions.

Mon 10:00 - 10:15 am: Protein Identification Using Surface Acoustic Wave Nebulization (SAWN)
Sung Hwan Yoon; Young Ah Goo; Michael Wilson; Yue Huang; John Edgar; Scott Heron; David Goodlett; University of Washington, Seattle, WA

Electrospray ionization (ESI) has been one of the most popular and standard ionization methods for the proteomics research. We have recently introduced an alternative ionization method, known as surface acoustic wave nebulization (SAWN), which provides much softer ionization compare to the ESI (Huang et al. JASMS 2012). This new technology uses surface acoustic waves (SAW) for nebulization directly from a solution deposited on the flat surface of a piezoelectric Lithium Niobate wafer. The LiNbO3 SAWN chip is mounted on a platform placed in front of the mass spectrometer and ions in the generated plume are drawn into the mass spectrometer by the pressure gradient that exists a priori with an atmospheric pressure ion interface. The SAWN generated ions come directly from a planar surface simplifying the sample preparation process and circumventing any clogging or fracturing capillaries required for ESI. Also, as a soft ionization method, SAWN can limit uncontrolled fragmentation of the precursor ions (Yoon et al. Anal Chem 2012). We have previously demonstrated the utility of SAWN to lipids, peptide and protein analysis. In this study we examine the use of SAWN for proteomic applications by analyzing trypsin digested proteins. Tryptic peptides from β-casein, insulin, and cytochrome C were analyzed by direct infusion MS/MS and SAWN-MS/MS. Peptide precursor ions with the same masses were fragmented and the fragment ions were compared. SAWN-MS/MS reproduced the same MS2 results comparing with direct infusion MS/MS. The total experimental time by SAWN was the half of direct infusion. These preliminary results show promise for using SAWN for proteomic applications in comparison to direct infusion MS2 approach.

Mon 10:15 - 10:30 am: Antibody-free, Targeted Mass Spectrometry Quantification of Low-abundance Candidate Cancer Biomarkers in Human Serum and Urine
Tujin Shi; Thomas Fillmore; Rui Zhao; Yuqian Gao; Carrie Nicora; Athena Schepmoe; Jacob Kagan; Karin Rodland; Tao Liu; Kegi Tang; Richard Smith; David Camp; Alvin Liu; Wei-Jun Qian; Pacific Northwest National Laboratory, Richland, WA; National Cancer Institute, NIH, Rockville, MD; University of Washington, Seattle, WA

Selected reaction monitoring (SRM) has been regarded as a promising high throughput targeted protein quantification technology; however, one of major limitations for current SRM technology is the lack of sufficient sensitivity for detecting low-abundance proteins present at sub-ng/mL in human plasma/serum. To address these challenges, we have recently developed an antibody-free strategy that involves high-pressure, high-resolution separations coupled with intelligent selection and multiplexing (PRISM) for highly sensitive SRM-based targeted protein quantification. The strategy capitalizes on high resolution high pH reversed-phase LC separations for analyte enrichment, intelligent selection of target fractions via on-line SRM monitoring of internal standards, and fraction multiplexing prior to LC-SRM quantification. We demonstrate the use of the multiplexing (PRISM) for highly sensitive SRM-based targeted protein quantification. The strategy capitalizes on high resolution high pH reversed-phase LC separations for analyte enrichment, intelligent selection of target fractions via on-line SRM monitoring of internal standards, and fraction multiplexing prior to LC-SRM quantification. We demonstrate the use of fraction multiplexing. Applications to clinical serum samples illustrated the detection of several endogenous ng/mL-level proteins, including prostate-specific antigen (PSA), and an excellent correlation between the results obtained from the PRISM-SRM assay and those from clinical immunoassay for PSA. This approach was also applied for the verification of a number of prostate cancer candidate biomarkers in human urine, including anterior-granluciferin 2 (AGR2) and proPSA, where the data displayed a preliminary discrimination between prostate cancer and non-cancer subjects. Our results demonstrate that PRISM-SRM is an effective method for quantification of low-abundance endogenous proteins in highly complex biofluid samples. We anticipate broad applications for targeted quantification of low-abundance proteins in systems biology and candidate biomarker verification studies.
the heart’s response to CRT. However, it is not so simple. The “existing PTM-status” of cell may dictate the effectiveness of these signaling pathways, thus, ultimately, define the efficacy of CRT for each individual patient. Recent investigations using new Cys based MS-tag methods, have shown that competition of selective Cys residues for different oxidative PTMs can influence the protein’s phosphorylation response and even dominant the protein functional activity. In fact, the kinase/phosphatase axis with CRT appears to be tuned by their Cys-oxidative state. Thus, further efficacy of the ongoing clinical trials focused on augment heart failure by manipulating kinase pathways may be required by controlling the cardiac redox environment or determining the individual’s redox-status prior to dictating a particular therapy.

Mon 11:30 - 11:45 am: Cardiomyopathy of Chagas Disease: Cysteinyl-S-Nitrosylation of Key Host Proteins as Candidate Biomarkers
John Wiktorowicz 1, 2; Szusan Stafford 1, 2; Kizhake Somani 1, 2; Hynus Ju 1, 2; Paola Zago 3, 5; Nisha Garg 4; Allan Brasier 1, 7; NIAID Clinical Proteomics Center, Galveston, TX; 1University of Texas Medical Branch, Galveston, TX; 2 Instituto de Patologia Experimental, Salta, Argentina; 3Department of Immunology and Pathology, Galveston, TX; 4Universidad Nacional de Salta, Salta, Argentina

Chagas disease, transmitted by injection of Trypanosoma cruzi through reduviidae insect bites (kissing bugs), is designated as the most important emerging disease in developed countries and a “neglected emergency” by the CDC and NIH, with approximately 16-18 million cases in Latin America and 120 million (~25% of the population) more at risk of infection. In 30-40% of the infected individuals, the disease may progress to irreversible cardiomyopathy after many years, with infected individuals serving as carriers and exhibiting considerable morbidity and high risk of mortality. Therapies in use exhibit high toxicity in adults and are largely ineffective in limiting the disease progression. Accordingly, it is crucial that biomarkers are identified that permit a risk assessment of asymptomatic individuals of developing chagas cardiomyopathy. Additionally, studies for this purpose may inform our understanding of the causative pathological processes and allow the identification of new therapies that arrest or prevent the progression of clinical disease, as well as suggest tools to assess the efficacy of new therapies. Preliminary studies of acute and chronic chagas animals suggest that pathological processes leading to chagas cardiomyopathy would cause characteristic changes in the concentration/nitrosation of proteins in the circulating blood cells and generate a detectable disease-specific molecular phenotype. We present here our results comparing 25 seropositive/asymptomatic patient samples with 28 seropositive/symptomatic samples, in which we investigated protein abundance and cysteinyl-S-nitrosylated (SNO) changes in isolated PBMCs using our novel, quantitative SNOFlo technology. Statistical analyses—including t-tests, multivariate adaptive regression splines, and Bayesian stochastic search—were used to select a reduced set of proteins that create an accurate risk model. Statistical analyses—including t-statistics, multivariate adaptive regression splines, and Bayesian stochastic search—were used to select a reduced set of proteins that create an accurate risk model. Each individual’s disease status was predicted with 89% accuracy. Preliminary studies of acute and chronic chagas animals suggest that pathological processes leading to chagas cardiomyopathy would cause characteristic changes in the concentration/nitrosation of proteins in the circulating blood cells and generate a detectable disease-specific molecular phenotype. We present here our results comparing 25 seropositive/asymptomatic patient samples with 28 seropositive/symptomatic samples, in which we investigated protein abundance and cysteinyl-S-nitrosylated (SNO) changes in isolated PBMCs using our novel, quantitative SNOFlo technology. Statistical analyses—including t-tests, multivariate adaptive regression splines, and Bayesian stochastic search—were used to select a reduced set of proteins that create an accurate risk model. Each individual’s disease status was predicted with 89% accuracy.

Mon 11:30 - 11:45 am: Protein and Transcript Discordance in a Prostate Cancer Model
Lee Sam; Damian Ferrini; Anastasia Yocum; Arul Chinnaiyan; Alexey Nesvizhskii; University of Michigan, Ann Arbor; MI
Prostate cancer is the most common non-skin cancer seen in men, with a 1 in 6 lifetime risk of the disease in the United States. mRNA transcript abundance levels are often used in research as an easily derived metric of biological activity in cells, a proxy for protein abundance and activity. However, studies in human cancers using low-throughput methods have found significant discordance in relative mRNA and protein abundance. This relationship is complicated - affected by numerous external factors such as RNA structure, degradation through the microRNA pathway, and numerous post-translational modifications of protein products. In prostate cancer, this discordance is observed in a number of functionally important molecular networks such as those associated with NF-kB and insulin signaling. The most recent research looking at the relationship between transcript and protein abundances has leveraged advances in next-generation sequencing and higher throughput proteomics methods to produce a more global view of the transcriptome and proteome. In this study, we use these methods with the VCaP and RWPE human prostate cell lines to study this relationship. We developed a novel method to perform an “apples-to-apples” comparison of transcript and protein abundances using a common reference database derived from RefSeq, containing 34,728 transcripts and matching protein sequences, which we used to align RNA-seq reads and quantify peptides and proteins from MS/MS data. Using this methodology, we derived two datasets of mRNA and protein abundance levels: an inclusive 5% FDR dataset of totaling 10,162 genes and a highly accurate 1% FDR dataset composed of 6,620 genes. Our analysis focused on the relationship between mRNA and protein in both VCaP and RWPE as well as the differences in this relationship specific to our VCaP cancer model.

Mon 11:45 am - 12:00 pm: Characterization of the Cardiac Myosin Binding Protein-C Phosphoproteome in Healthy and Failing Human Hearts
Viola Kooij; Ronald Holewinski; Anne Murphy; Jennifer Van Eyk; Johns Hopkins University, Baltimore, MD
Introduction: Cardiac myosin binding protein-C (cMyBP-C) becomes dephosphorylated in the failing heart and reduced phosphorylation-dependent regulation of cMyBP-C has been implicated in contractile dysfunction. To date, several phosphorylation sites have been identified for human cMyBP-C; however, a comprehensive characterization of the cMyBP-C phosphoproteome is lacking. This study aimed to characterize the cMyBP-C phosphoproteome using two different proteomic-based methods in explanted control and end-stage failing hearts. Methods: The first approach used to characterize the cMyBP-C phosphoproteome employed a strong-cation exchange chromatography (SCX)-based fractionation method (10 pooled samples, technical replicates = 4) and the second employed a sodium dodecylsulfate polyacrylamide gel electrophoresis method (n = 10; technical replicates = 2). Each subsequently underwent titanium dioxide (TiO2) affinity chromatography to enrich for the tryptic phosphopeptides, which were analyzed using an LTQ-Orbitrap mass spectrometer. Moreover, recombinant C0-C2 fragment of mouse cMyBP-C incubated with PKA, PKC, CamKII and CK2 was analyzed to identify the kinases involved with phosphorylation of cMyBP-C. Results: Seventeen phosphorylation sites on cMyBP-C were identified, with the majority localized in the N-terminal domains C0-C2. The three most abundant phosphorylated sites, Ser284, Ser286 and Thr290, are located in the regulatory M-domain of cMyBP-C. Ser284 showed a significant reduction in phosphorylation in HF, while Ser286 and Thr290 showed a trend towards a reduced phosphorylation. Conclusion: This study demonstrates that cMyBP-C is more extensively phosphorylated than previously known, with 10 novel sites identified. Most sites were primarily located within the N-terminal side of the protein. The three most highly phosphorylated sites on cMyBP-C were Ser284, Ser286 and Thr290 and these three sites showed decreased phosphorylation in the failing heart, which implicate their importance for fine-tuning contractility. To date, the functional importance of Ser286 and Thr290 is unknown. In addition, 16 sites were identified after in vitro kinase incubation. Monday 10:50 am - 12:00 pm: Proteogenomics: Fusing the Genome and Proteome
We believe that such multi-Omics profiling studies can inform us about methylation patterns with mRNA, miRNAs and proteins. We transcribe and translate of genes/proteins by correlating the transcription and translation of genes/proteins with coordinated changes are consolidated by obtaining data regarding protein expression and phosphorylation of naïve CD4+ T cells along with their methylation profiling, RNA sequencing, miRNA sequencing, proteomic and phosphoproteomic profiling of naïve CD4+ T cells from a single individual. We have generated one of the most extensive proteomic catalogs of any primary cell type in humans by obtaining data regarding protein expression and phosphorylation of naïve CD4+ T cells. This study establishes expression of >7,000 proteins in naïve CD4+ve CD4+ T cells along with their various alternative forms including splice variants and post-translationally modified versions. We also obtained evidence for novel genes, unannotated exons, novel splice sites, fused genes, amino acid changes resulting from SNPs and novel N-termini. Finally, we obtained novel, and surprising, insights into amino acid changes resulting from SNPs and novel N-termini. Advances in the field of genomics, epigenetics, transcriptomics and proteomics have provided researchers the technical capability to carry out unbiased genomewide studies. These strategies are often used in isolation while investigating biological problems. It is now apparent that a unified approach that allows us to study genome, epigenome, transcriptome and proteome would provide better insights than any single Omics study. However, the challenges and opportunities of such a unified approach is the lack of appropriate computational tools to deal with such data. In an effort to determine the feasibility of embarking on a multi-Omics approach, we carried out whole genome sequencing, genome wide methylation profiling, RNA sequencing, miRNA sequencing, proteomic and phosphoproteomic profiling of naïve CD4+ T cells from a single individual. We have generated one of the most extensive proteomic catalogs of any primary cell type in humans by obtaining data regarding protein expression and phosphorylation of naïve CD4+ T cells. This study establishes expression of >7,000 proteins in naïve CD4+ve CD4+ T cells along with their various alternative forms including splice variants and post-translationally modified versions. We also obtained evidence for novel genes, unannotated exons, novel splice sites, fused genes, amino acid changes resulting from SNPs and novel N-termini. Finally, we obtained novel, and surprising, insights into amino acid changes resulting from SNPs and novel N-termini.

**Monday 10:50 am - 12:00 pm**

**Controversies and Challenges in Clinical Proteomics**

**Key Ballroom 1-2**

**Mon 11:00 - 11:30 am: Reproducibility and Forensic Bioinformatics in High-Throughput Biology**

Keith Baggerly; UT MD Anderson Cancer Center, Houston, TX

Modern high-throughput biological assays let us ask detailed questions about how diseases operate, and promise to let us personalize care. Careful data processing is essential, because our intuition about what the answers “should” look like is very poor when we have to juggle thousands of things at once. Unfortunately, documentation of precisely what was done is often lacking. When such documentation is absent, we must apply “forensic bioinformatics” to infer from the raw data and reported results what the methods must have been. The issues are basic, but the implications are far from trivial.

We examine several related papers where signatures of drug sensitivity derived from cell lines were used to predict patient response. Patients in clinical trials were allocated to treatment arms on the basis of these results. However, we show in several case studies that the results incorporated several simple errors that may have put patients at risk. One theme that emerges is that the most common errors are simple (e.g., row or column offsets); conversely, it is our experience that the most simple errors are common. We briefly discuss steps we are taking to avoid such errors in our own investigations, and discuss reproducible research efforts more broadly.

**Mon 11:45 am - 12:00 pm: An Integrative Approach for Genomic and Proteomic Data Analysis**

Li Chen; Bai Zhang; Yuan Tian; Caitlin Choi; Shisheng Sun; Jianying Zhou; Hui Zhang; Daniel Chan; Jianhua Xuan; Yue Wang; Zhen Zhang; Johns Hopkins School of Medicine, Baltimore, MD; Virginia Tech, Arlington, VA

Advances in high-throughput genomic and proteomic technologies have made it possible for large-scale simultaneous quantification of mRNA and protein expressions from the same samples. Integrated analysis of such data allows us to identify and characterize molecular activities and interactions in cells at multiple levels that are involved in tumorigenesis and related biological/pathological processes. Potential biomarkers identified through such analyses could further improve our ability to diagnose, treat, and prevent cancer. In this study, we propose an integrative approach to analyze genomic and proteomic data. The proposed approach utilizes a hierarchical structure to first identify differentially expressed genes and proteins between two phenotypes from mRNA expression data and LC-MS/MS proteomic data, independently and respectively. Then the genes/proteins with coordinated changes are consolidated by mapping their unique gene/protein IDs and examining evidence from both mRNA and protein expressions. A differential dependency network signature is further estimated between two
phenotypes, by incorporating other data sources and prior knowledge to enhance statistical power and biological interpretability. Finally, a network-based classifier for these two phenotypes is trained using the network signature and the performance is evaluated using independent data sets. In a case study, we applied our proposed approach in a breast cancer (basal vs. luminal) study. The results showed that the proposed integrative approach could identify reliable gene/protein network signature that achieved good prediction performance with high reproducibility across different data sets. More importantly, the identified networks were biologically meaningful and highly associated with breast cancer basal and luminal phenotypes.

**Monday 1:30 - 2:00 pm**
**MCP Lectureship, Key Ballroom 3,4,6**

Mon 1:30 - 2:00 pm: The Present and Future Frontiers of Proteomics and Glycomics
Catherine E. Costello; Boston University, Boston, MA

In recent years, Genomics and Proteomics generated significant scientific accomplishments and gained public notice. Both still must expand to meet the broad needs of medicine, public health, agriculture and the environment. The Human Genome Project and Human Proteome Project focus attention and resources, attract commercial interest and accelerate technological progress. Glycomics can benefit from the experience of Genomics and Proteomics, but poses further challenges: structural complexity, absence of a template, dynamic changes, limited availability of databases/software and the community’s minimal appreciation of glycan modifications. Nevertheless, these stiff challenges present irresistible opportunities for development of novel approaches and co-operation in testing/evaluation of methods, and applications to biology and medicine.

What have we learned? (1) Avoid assembly/dissemination of data that is insufficiently robust; (2) Develop rapid, sensitive, reliable, high throughput, cost-effective analytical methods for comprehensive characterization of complex mixtures and demand honest representation of results – what was fully determined, what was presumed, what remains uncertain. (3) Establish and maintain close collaborative interactions between clinicians and/or basic science investigators throughout any study, including final presentation of results and conclusions in scientific and public media. (4) Require long-term, practical plans for maintenance/sharing of raw data, databases, and tools.

Dynamic post-translational modifications of proteins, e.g., glycosylation, phosphorylation, acetylation, and their specific position(s), site occupancy, co-occurrence and kinetics, affect the properties of proteins and whole cells, their interactions, transport, activity, and lifetimes. MS approaches that drive novel, emerging capabilities are essential for investigation of normal development, cardiovascular, neurodegenerative, infectious and parasite-borne diseases and cancer. A representative selection of applications will be discussed, with emphasis on glycomics. We are examining every step of the process, including handling/separation of samples, ionization/dissociation methods, manual/automated data interpretation. Examples chosen from our laboratory, colleagues and scientific societies will illustrate promising approaches and remaining challenges. Our research is supported by NIH.

**Monday 2:00 - 3:30 pm**
**Early Career I: Focus on Advances in Technology**
**Key Ballroom 3,4,6**

Mon 2:00 - 3:30 pm: High Throughput Stable-Isotope Labeled Derivatization Strategy and Data Analysis for the Relative Quantification of N-linked Glycans in Complex Biological Samples
S. Hunter Walker; Amber D. Taylor; David C. Muddiman; North Carolina State University, Raleigh, NC

The ubiquity and importance of glycosylation in systems biology is becoming more evident as the fields of glycomics and glyco-biology expand. Due to the increasing interest, it is important to continually develop new strategies to profile and quantify glycans in numerous sample types including cell cultures, tissues, and biological fluid. Herein, a high throughput strategy is presented for global N-linked glycan relative quantification via stable-isotope labeled hydrazide derivatization. This hydrophobic derivatization strategy allows for additional advantages such as an increase in electrospray ionization efficiency due to the hydrophobic effect and the capability to separate N-linked glycans by reversed-phase liquid chromatography. These advantages allow us to relatively quantify >70 unique glycan compositions between two different samples in a LCMS experiment, and this strategy can be applied to any glycomic sample in which the glycans have a reducing terminus. Furthermore, the derivatization strategy adds <4 hr to native glycan sample preparation time with no additional clean-up steps, making this an efficient strategy that can be implemented in nearly all glycomics laboratories. The analysis of glycan stable-isotope labeled relative quantification data is complicated by the lack of robust and accessible glycan bioinformatic platforms. Thus, we have developed a processing protocol using the instrument manufacturer software to extract peak areas using a database of N-linked glycan compositions and exact masses. Additionally, further data analysis is presented taking into account sample preparation variability and isotopic distribution overlap. These analysis strategies combined with the <4 hr derivatization make this a strategy that is immediately beneficial to the glycomics community, and using this strategy, comparisons between the entire glycan profiles of two different samples (e.g. healthy and diseased) can be made in a single LCMS experiment.

Mon 2:20 - 2:30 pm: The Importance of Being Proportional: Shifting the Intensity-based Label Free Relative Quantification Paradigm
Susan Van Riper; Ebbing De Jong; LeeAnn Higgins; John Carlis; Tim Griffin; University of Minnesota, Minneapolis, MN

Intensity-based label free relative quantification (LFRQ) via liquid chromatography (LC) coupled with mass spectrometry (MS) is well suited for proteomic and peptidomic clinical translational studies. Under the current LFRQ paradigm, researchers commonly assert that an analyte, e.g., a peptide, is differentially abundant if its fold-change between samples (relative abundance as measured by its surrogate ion intensity ratio across LC-MS runs) satisfies some criterion. Unfortunately, LC-MS workflows suffer from poor reproducibility and repeatability which interfere with accurately reporting ion intensity. This leads to excessive false positives, which may be discarded via hypothesis-driven experiments, and false negatives, which will never be looked at by researchers. Consequently, possible insights are missed and incorrect conclusions drawn. Thus, we posit that the current relative abundance paradigm is ill-suited to discover differentially abundant analytes, i.e., biomarkers. Here, we propose an alternative which measures compositional proportionality rather than simple relative abundance: the proportionality paradigm. We highlight the proportionality paradigm’s importance by:

1) using a simple example to demonstrate that, when compared to the current paradigm, it reduces false positives, and more importantly false negatives; and
2) using reduction in complex mixture variance (as measured by CV and PEV) to demonstrate its dominance over the current paradigm when embodied in a new method: proximity-based intensity normalization (PIN); and
3) applying it to the CPTAC Study 6 biomarker data set (where standard human protein mixture (Sigma UPS1) was spiked at different levels in constant levels of yeast reference sample) to demonstrate its efficacy in discovering biomarkers.

Our results indicate that, in addition to proteomics and peptidomics, the proportionality paradigm and PIN should be widely applicable to many ‘omics fields using LFRQ via LC-MS, e.g., lipidomics, glycomics, and metabolomics. We expect it will deliver improved reproducibility and repeatability, thereby increasing discovery of statistically significant biological variation in clinical translational studies.
Mon 2:30 - 2:40 pm: Use of Data Dependent and Independent Mass Spectrometry Acquisition for the Systematic Study of the Human Acetylome Components
Jean-Philippe Landry; Sarah Pincock; Brett Larsen; Beatrix Gonzalez Badillo; Tony Pawson; Stefan Knapp; Panagis Filippakopoulos; Anne-Claude Gingras; \textsuperscript{1} Samuel Lunenfeld Research Institute, Toronto, Canada; \textsuperscript{2} Structural Genomics Consortium, Oxford, UK; \textsuperscript{3} University of Toronto, Toronto, Canada

Ly思索 acetylation is one of the key post-translational modifications regulating chromatin structure and function. Recent proteomics studies have detected lysine acetylation on a wide array of proteins beyond chromatin-containing histones, implying a broader role in cellular functions. However, how specificity is acquired within the acetylation system remains largely unknown. Multiple components of the human acetylome are misregulated in diverse cancers or directly involved in numerous diseases such as schizophrenia, and thus constitute attractive targets for therapeutic modulation. In order to characterize the acetylome machinery in an unbiased manner, we embarked on the systematic characterization of all human acetyltransferases, deacetylases and bromodomain-containing proteins (the only known acetylation mark “reader”). Using an array of mass spectrometry techniques, we are exploring the protein complexes surrounding acetylome components, and identifying novel substrates for the acetylation machinery. Firstly, protein complexes encompassing all acetylating enzymes and acetylated proteins have been defined by an affinity purification approach optimized for chromatin-associated proteins. This revealed intricate interaction networks involving acetylome components, which are being validated by reciprocal purifications and targeted in vitro binding assays, and that implicate specific acetylome components in a wide array of biological pathways. Additionally, to gain further biological insight into these novel interaction networks, we are defining their response to chemical and genetic perturbations, with an emphasis on bromodomain inhibitors, including the recently described inhibitor JQ1. JQ1 displaces BET-family bromodomain containing proteins from their acetyllysine targets (in that case, histones), resulting in strong anticancer properties: here we are using JQ1 as a tool compound to analyze network rewiring. Finally, we are elucidating the specificity of human bromodomains for mono- and poly-acetylated substrates by combining recombinant bromodomain pull-downs with data independent mass spectrometry acquisition (SWATH). This unbiased workflow enabled the systematic quantitation of acetylated substrates and the definition of specificity in the acetylation system.

Mon 2:40 - 2:50 pm: Improved Protein Extraction and Identification from Archival Formalin-fixed Paraffin-embedded Human Aortas Using High Pressure and Heat
Zongming Fu; Kun Yan; Avraham Rosenberg; Grace Grace; Richard S. Vander Heide; Allen Everett; David Herrington; Jennifer Van Eyk; Allen Everett; Brett Larsen; Louisiamp; C; 2; 3; Wake Forest University, Winston-Salem, NC

Formaldehyde-fixed (FF), paraffin-embedded (FFPE) aorta arterial repositories are a valuable resource for studying vascular diseases including atherosclerosis; however, such repositories have not yet been exploited in the area of proteomics. This is due, in part, to the fact that aortic tissues including the aorta with have a significant connective tissue component poses particular difficulties in obtaining high which limits yield of protein extraction. This problem is exacerbated by the covalent cross-linking and protein degradation that occurs with formalin fixation and long-term storage, further compromising the ability to extract and identify proteins from those samples. Here we describe an improved method for the protein extraction from FF and FFPE aortas using high temperature (95°C) and high hydrostatic pressure (40,000 psi). Combined heat and elevated pressure increased protein extraction yield from human FF or FFPE compared to matched aortic tissues with heat alone (average 1.52 fold) or at room temperature (average 8.29 fold), resulting in a greater number of proteins identified with more amino acid sequence coverage. For FF tissue, the length of storage did affect the quality of protein based on mass spectrometry analysis.
sections are followed by antigen retrieval and denaturing of the proteins. Peptide-N-Glycosidase F (PNGase F) is sprayed over the tissue sections to release the N-linked glycans from the proteins, while preserving their spatial distribution. Samples are sprayed-coated with matrix and analyzed by MALDI-MS-MS2-MSn using Shimadzu Axima Resonance in positive mode. In preliminary results, a minimum of 15 N-linked glycans were detected from FFPE LnCap cell sections. Of these glycans, seven were fucosylated on the core, one was siaiylated and two had bisecting N-Acetylgalcosamine (GlcNAc) structures. Using the developed N-linked glycan imaging technique, we were able to analyze 20% of the glycans that were previously identified in the same cell lysate by solid-phase glycan extraction (SPGE) and mass spectrometry. By co-registering the mass spectrometry images with histology and lectin histochemical images, this technique is capable of identifying disease-associated glycans directly from FFPE tissues.

**Mon 3:10 - 3:20 pm: Targeted Quantification of Membrane-associated Proteins Using Multiple Reaction Monitoring Mass Spectrometry and Full-length Isotope-labeled Protein Standards**

Meiying Wang,‡ 1, 2; Hua-Jun He,‡ 1; Gun-Young Heo,‡ 3; Irina Pikuleva,‡ 3; Lili Wang,‡ Iliran Turko,‡ 2, 3; †Institute for Bioscience and Biotechnology Research, Rockville, MD; ‡National Institute of Standards and Technology, Gaithersburg, MD; 3Case Western Reserve University, Cleveland, OH

Liquid chromatography (LC) coupled mass spectrometry has emerged as a versatile platform for quantitative proteomics due to its high specificity and sensitivity. Successful targeted quantitation of membrane proteins and cellular functions has not always been reported, but analyses of membrane-associated proteins are still very limited due to low abundance and aqueous insolubility of the target molecules. Given the important cellular functions and their potential as drug targets, we report our quantitative targeted proteomics study for the analysis of membrane-associated proteins using LC coupled multiple reaction monitoring mass spectrometry (MRM MS). By using our optimized sample preparation protocol combined with full-length isotope-labeled protein standards, we successfully performed quantitative analysis for multiple target proteins, including the Alzheimer’s disease risk factor apolipoprotein E4 (apoE4) isoform from human frontal cortex, the clinical cell surface marker cluster of differentiation 4 (CD4) from human T lymphocytes and cholesterol-metabolizing cytochrome P450s with their redox partners from human retinal. Our study addressed the following issues: 1) quantification of membrane-associated proteins; 2) quantitative differentiation of specific protein isoforms; and 3) quantitative analysis of protein cleavage and fragment accumulation associated with human disease. We demonstrate that our quantitative workflow based on LC MRM MS can be used for absolute quantification of membrane-associated proteins as well as for the exploration of basic mechanisms with human diseases. We believe this method can be widely applied for studies of other membrane-associated protein.

**Mon 3:20 - 3:30 pm: SILAC-Based Proteomics Platform for the Large Scale Analysis of Protein-Ligand Binding Interactions**

Jagat Adhikari,‡ 1; Duc Tran;‡ 2; Michael C. Fitzgerald,‡ 1; Duke University Medical Center, Durham, NC; 2Duke University, Durham, NC

Thermodynamic measurements of protein folding and stability can provide important biological information about ligand binding and can help to understand the systems level thermodynamics of protein-ligand binding interactions. An advantage of mass spectrometry-based methods over other approaches is that they are amenable to the study of unpurified proteins in complex biological mixtures. This allows for the detection and quantitation of protein ligand binding interactions at the systems level. Here, we describe a quantitative mass spectrometry-based proteomics method for the large-scale thermodynamic analysis of protein-ligand binding interactions. The methodology utilizes the Stability of Proteins from Rates of Oxidation (SPROX) technique in combination with a stable isotope labeling with amino acids in cell culture (SILAC) approach to compare the equilibrium unfolding/folding properties of proteins in the absence and in the presence of target ligands. The methodology, which is general with respect to ligand, enables measurement of the ligand-induced stability changes associated with protein-ligand binding interactions. The capabilities of the methodology are demonstrated and benchmarked here in two protein-ligand binding studies including one in which the endogenous proteins in a yeast cell lysate are analyzed for binding to cyclosporine A (an immunosuppressant with well-characterized protein targets) and one in which the endogenous proteins in a yeast cell lysate to adenosine triphosphate (ATP, a ubiquitous enzyme co-factor with less well-characterized protein targets). In the cyclosporine A binding study, the already well-characterized tight-binding interaction between cyclosporine A and cyclophilin A was successfully detected and quantified using the methodology. A number of known and some unknown protein targets of ATP were also identified and quantified in the ATP-binding experiment described here. The approach shows promise for future studies of protein-ligand interactions at the systems level (e.g., in cellular processes and disease states).

**Monday 2:00 - 3:30 pm**

**Early Career I: Focus on Cell Regulation**

**Key Ballroom 5**

**Early Career I: Focus on Cell Regulation**

**Key Ballroom 5**

**Mon 2:10 - 2:20 pm: Identification of Potential Early Diagnostic Biomarkers for Plasmodium falciparum and P. vivax Infections through Serum Proteomics**

Sanjeeva Srivastava; Sandipan Ray; Indian Institute of Technology Bombay, Mumbai, India

Malaria is by far the world’s most significant tropical infectious disease and over the last few decades large-scale malaria epidemics have happened in almost all continents. Early detection of malaria effectively reduces the possibilities of complications of secondary infections, fatalities, and gratuitous expenses due to improper and delayed diagnosis and treatments. Although existing malaria diagnosis approaches including microscopic examination, PCR-based molecular diagnostic methods and rapid diagnostic tests are adequately robust and sensitive for detection of the parasite in symptomatic patients, sensitivity level found to be reduced massively in case of detection of asymptomatic malaria with very low parasitemia. Furthermore, existing techniques can not provide any prognostic information regarding the infection. In this study by using discovery proteomics approach we have identified potential serum markers, which can discriminate malaria patients from healthy subjects and other non-malarial infections like dengue or leptospirosis. Malaria patients with diverse range of parasitemia; mainly low and moderate parasitemic were used for the validation of the target proteins using ELISA and western blotting. Capability of our analytical approach for the detection of very low-level of parasitemia (<500 parasites/μL blood) testifies its diagnostic potential. Multivariate statistical analysis by Correspondence Analysis, PLSDA, Decision Trees, Naïve Bayes and support vector machines employing a panel of identified proteins consists of six candidates; serum amyloid A, hemopexin, apolipoprotein E, haptoglobin, retinol-binding protein and apolipoprotein A-I provided over 90% prediction accuracy for malaria. ROC curve analysis was carried out to evaluate the individual performance of classifier proteins. Our findings may contribute in early diagnosis of malaria patients with very low parasitemia and asymptomatic cases, hypozoite detection on the basis of host serum proteome responses and discrimination of malaria from other clinically related non-malarial febrile illness.
alcohol. Total protein carbonylation is elevated in mouse hepatocytes due to acrolein exposure. However, the specific proteins that are modified by acrolein, and importantly, the amino acid sites changed by the aldehyde are not known. Thus, in this study, we determined the identity of the proteins selectively adducted by acrolein with a focus on characterizing carbonylated amino acid residues. Rat liver microsomes were resuspended in buffer and the non-denatured mix was treated with acrolein. Microsomal proteins were not denatured in order to determine acrolein accessible sites in their native conformation. Carbonylated proteins were labeled with biotin hydrazide and then digested with trypsin. Next, biotinylated peptides were isolated from the mixture with avidin affinity chromatography and analyzed via LC-MS/MS. Eighty-seven different proteins and 125 unique modification sites were discerned. Targeted proteins include 9 cytochrome P450 isotypes which play important role in metabolism and 12 ribosomal proteins which are critical for protein synthesis. Our results validate this peptide-centric methodology and should facilitate an understanding of the role that protein carbonylation plays in acrolein’s toxicity within the liver.

Mon 2:40 - 2:50 pm: Macrophage Plasticity Illustrated by Ligand-induced Transient and Permanent Proteome and Transcriptome Changes Studied Using Discovery-mode and Targeted Proteomics
Eunkyung An; Nathan Manes; Aleksandra Nita-Lazar; NIAID, NIH, Bethesda, MD

Cells of monocyte-macrophage lineage display enormous functional and morphological plasticity. We examined the changes in the proteome and transcriptome in the RAW 264.7 macrophages stimulated with receptor activator of nuclear factor-κB ligand (RANKL), the cytokine inducing macrophage differentiation into osteoclasts.

Osteoclasts specialize in bone resorption and many skeletal diseases are due to excess osteoclastic activity.

Differences in the osteoclast and osteoclast precursor morphology and the need for tight control of osteoclast migration suggest differential protein expression. To determine rewired molecular signaling in osteoclasts, we compared proteome and transcriptome of osteoclast precursors, multinucleate osteoclasts, and TRAP+ mononucleate cells, using 3-plex SILAC coupled with LC-MS/MS strategy for proteomics and bead array for genomics. ProLuCID and Census were used for protein identification and quantification. Ingenuity Pathway Analysis was used for network evaluation.

2575 proteins were identified and more than 70% of these were quantified. We found that proteome- and genome- profiles of TRAP+ mono cells were closer to the precursors than to the osteoclasts suggesting it is critical to enrich osteoclasts from TRAP+ mono cells after the treatment in order to obtain accurate information about osteoclasts. Our findings suggest that mitochondria (and specific mitochondrial proteins) are highly involved in osteoclast formation and differentiation and we can focus on finding therapeutic targets in mitochondria to control osteoclast activity.

Sphingosine-1-phosphate (S1P) induces macrophage chemotaxis towards or away from the bones depending on S1P concentration gradient. We used the macrophage discovery-mode proteomic data as well as existing peptide libraries to design proteotypic peptides for targeted proteomics of proteins from the S1P signaling network. Single Reaction Monitoring (SRM) with heavy labeled proteotypic peptides was performed for absolute quantitation and modeling of protein networks involved in S1P induced macrophage migration.

This research was supported by the Intramural Research Program of the NIH, NIAID.
Identification of biological targets of small molecules and drugs remains a significant challenge. Recent developments in quantitative high-resolution mass spectrometry have led to significant improvements in the sensitivity and specificity of drug target discovery. However, identification of targets of small molecules with low binding affinities or those binding to diverse proteins in cells remains particularly difficult. In this study we overcame three major challenges associated with drug target discovery: i) maximizing the activity and native fold of proteins while effectively lysing cells, ii) minimizing proteins non-specifically bound to the affinity chromatography material, and iii) identifying the relevant specifically interacting protein. The solutions to these challenges include the use of novel poloxamer-based protein extraction, fluorous ligand affinity chromatography, and SILAC-based affinity-activity correlation proteomics. We applied this novel integrated workflow to find the targets of the phenothiazine perphenazine, which was recently described as exhibiting anti-leukemic effects. This study unambiguously identified the protein phosphatase 2A (PP2A) Aα subunit as the intracellular target of perphenazine.

Mon 3:20 - 3:30 pm: Increased Throughput for 2D LC in the Analysis of Human Placental Samples
Martha Stapels; Keith Fadgen; J. Will Thompson; Arthur Moseley; James Langridge; Waters Corporation, Milford, MA; Duke University, Durham, NC

Most proteomic samples generate peptides with similar distributions of hydrophobicities and mass (1). The complexity of proteomic samples requires orthogonal methods of separation to identify and quantify all peptides in a sample. Data-independent analysis yields reproducible fragmentation and peak area information for all detectable peptides (2). The use of ion mobility during this analysis inserts an orthogonal separation in the gas phase between chromatographic and mass spectral analyses. In this study, 2D chromatography is combined with ion mobility to resolve peptides in multiple dimensions in a high-throughput manner.

Proteins were extracted from human placenta samples into two solvents, fractionating using TRizol reagent with conical microtubes. Proteins were then reduced, alkylated, and digested in-solution with trypsin. Samples were injected in triplicate onto a nanoflow liquid chromatography system and analyzed with a data-independent analysis platform. The 2D chromatography is based on the combination of the first dimension (2D chromatography) and second dimension (ion mobility) to resolve peptides. We observed that the use of ion mobility during this analysis allows for the reproducible fragmentation and peak area information for all detectable peptides. The combination of these two orthogonal methods allows for the increased throughput and sensitivity in the analysis of human placental samples.

A comparison was made between a traditional 2D method and a faster technique that utilized simultaneous gradients in both dimensions. The faster technique took 68% of the time of the traditional 3-fraction method and the percent savings in time will increase as the number of desired fractions increases. Use of the faster method allowed for a 70% increase in the number of ions detected per minute, a 54% increase in the number of peptides identified per minute, and a 46% increase in the number of proteins identified per minute. Incorporation of ion mobility into the analysis yielded an increase in peak capacity of at least another order of magnitude.

References:
differentially expressed (ALBU, HNRNPC, HNRNPD, UBE2N, TPM1 and TPM3). For each 8-plex iTRAQ, 7 samples randomly selected from each group were labelled (25ug) using channels 113 to 119; reference pool (25 ug) was labelled with channel 121; 5 ug of each 8-plex run were injected into the TripleTOF 5600 (6 runs in total). Six out of 354 quantified proteins were differentially expressed in at least 21 samples (ALBU, HNRNPC, HXK3, MIF, ST1A2 and UBQ). This study compares the discovery and differentially expressed profiles using SWATH and iTRAQ. The small overlap of differentially expressed proteins highlights the need to choose a reliable discovery method and validate biomarkers. Nevertheless, these proteins involved in cytoskeleton dynamic, metabolic processes, cellular signaling and mRNA regulation in leukocytes may provide further insights into the pathophysiology of TPTL.

Mon 2:30 - 2:40 pm: A Multiple Reaction Monitoring (MRM) Method to Detect Bcr-Abl Kinase Activity in CML Using a Peptide Biosensor

Tzu-Yi Yang; Laurie L. Parker; Purdue University, West Lafayette, IN

Abl kinase plays important roles in regulating blood cell proliferation; the mutated oncogenic Bcr-Abl protein drives the initiation of chronic myeloid leukemia (CML). Studies have shown that clinical outcomes and kinase inhibitor drug response in CML patients highly correlate to both Bcr-Abl kinase activity and the kinase’s interplay with downstream signaling proteins. Conventional techniques for measuring kinase activities and substrate phosphorylation levels such as 32P-radioactive assays and Western blots are well developed, but usually require large amount of cellular samples or use radiolabeled reagents. Our group has developed a novel technique for detecting kinase activities and protein phosphorylation: we exploit a cell-penetrating-peptide conjugated Abl substrate peptide as a biosensor for probing Abl kinase activities. The biosensor was incubated with CML cells and the permeabilizing biosensor was isolated with cell lysates. Cell lysates were analyzed on a triple quadrupole mass spectrometer using the multiple reaction monitoring (MRM) technique. Ion intensities of phosphorylated and non-phosphorylated biosensor ‘signatures’ were quantified by integrating the extracted ion chromatograms (XIC) and measuring area under the curve. We are able to detect Bcr-Abl activity and inhibition by imatinib in the human CML cell line K562. MRM enabled reproducible, selective detection of the peptide biosensor at low levels from aliquots of cell lysate equivalent to ~15,000 cells. Our data demonstrate the sensitivity of the biosensor and the translational potential of the biosensor technique.


Krishan Kumar; Prakash Srinivasan; Michael J. Nold; Prakash Srinivasan; Michael J. Nold; 1LMIV, NIAID, NIH, Rockville, MD; 2LNVR, NIAID, NIH, Rockville, Maryland; 3Waters Corporation, Milford, MA; 4Walter Reed Army Institute of Research, Silver Spring, MD; 5NIAID, NIH, Hamilton, Montana

Malaria caused by Plasmodium falciparum causes several hundred million cases of clinical disease each year & nearly 1 million deaths. Passive transfer of hyper-immune human IgG from endemic areas protects children against malaria. Investigational recombiant protein vaccines developed and evaluated over the last decade targeting blood stage parasites or specifically merozoite proteins identified from endemic sera have shown limited or no success in clinical trials. Thus new discovery efforts are important. To this end, we are using a systems biology approach to evaluate the differences between a long-lived merozoite phenotype due to selection pressure on γ-irradiated blood stage parasite line as compared to its parent line. Cell-chieve purified long-lived merozoites retain their capacity to invade RBCs at greater than 3 to 5 times that of the parent line. Comparative analyses of the transcriptomes of immature schizonts (4–8 nuclei) and purified merozoites identified a 2-fold change in a significant number of immature schizonts and only 4 significant changes in purified merozoites. An analysis of parallel comparative analyses of immature schizont proteome identified 2 fold changes in protein abundance using a label free data-independent alternate scanning LC/MS proteomics approach (LC/MSE). Using this same approach with or without ion-mobility, a thorough comparative analysis of the merozoite proteome has identified a higher level of protein abundance in the long lived merozoites, including known invasion ligands. In addition, the use of these invasive merozoites has enabled the protein identification of putative parasite proteins likely involved in cell cycle control and cell signaling. Finally, a more extensive evaluation of the peptidome using TransOomics® has shown that individual peptide signatures may be identified within the different lines for some of the more than 1300 merozoite proteins quantitatively identified. Currently, these unique data sets are being collated in order to provide a more complete understanding of invasive long-lived merozoites.

Mon 2:50 - 3:00 pm: Proteomic Definition of the Balance between Host Innate Immunity and Virus-mediated Immunosuppression

Benjamin Diner; Tuo Li; John Fuesler; Ileana Cristea; Princeton University, Princeton, NJ

The innate immune system utilizes specialized receptors to sense pathogens and elicit antiviral responses. The interferon-inducible HIN200 proteins IFI16 and AIM2 have emerged as sensors of cytoplasmatic viral DNA. We recently demonstrated that IFI16 also functions in the nucleus to detect and respond to herpesviruses. It is still unclear how IFI16 and AIM2 propagate immune signaling and what co-factors are involved. Furthermore, little is known about the other two HIN200 proteins, IFIX and MND2. To gain mechanistic insights into HIN200-mediated innate immunity, we employed a multidisciplinary approach integrating proteomics, molecular virology, and bioinformatics. We built cell systems to study all four HIN200 proteins, and used affinity purification-mass spectrometry to construct functional protein interaction networks. HIN200 proteins were also isolated from interferon-treated cells to identify interactions involved in activating innate immunity. Identified proteins were filtered using SAINT and NSAF/PAX values to assign confidence and fold-enrichment. High confidence and enriched proteins were validated using reciprocal isolations and microscopy, and interactomes were constructed using Cytoscape and functional clustering. This is the first evidence that HIN200 proteins exist within shared protein complexes, while still maintaining unique interactions with proteins regulating immunity, cell cycle, and DNA damage. These results defined the interactions and mechanisms utilized by hosts to elicit immune responses against viruses. However, viruses have also evolved strategies to counteract these host defense mechanisms. Understanding these strategies can provide therapeutic targets for restoring the immune response in infected cells. To define these mechanisms, we characterized HIN200 protein levels and interactions during infection with herpes simplex virus-1 (HSV-1). We found that IFI16 was degraded early in infection by the HSV-1 E3 ubiquitin ligase ICP0. Using wild type or ICP0-deficient viruses we demonstrated ICP0 requirement for suppression of IFI16-mediated innate immunity, and viral infection with HSV-1. Using wild type or ICP0-deficient viruses we demonstrated ICP0 requirement for suppression of IFI16-dependent innate immune responses. Together, our results provide insights into the molecular details underlying both HIN200-mediated innate immunity and virus-mediated immunosuppression.

Mon 3:00 - 3:10 pm: Identification of miR-145 Targets in Pancreatic Cancer Through an Integrated Omics Analysis

Tai-Chung Huang; Santosh Renuse; Praveen Kumar; Sneha Pinto; Yi Yang; Raghothama Chaerkady; Brian Godsey; Joshua Mendell; Curt Clevin; Luigi Marchionni; Akhiles Pandey; Johns Hopkins University School of Medicine, Baltimore, MD; Institute of Bioinformatics, Bangalore, India; 1University of Maryland, Baltimore, MD; University of Texas Southwestern, Dallas, TX

MicroRNAs (miRNAs) are short non-coding RNA regulating gene expression and protein synthesis. For characterizing functions of miRNAs and evaluating their global impacts, an integrated multi-omics analysis is especially informative. We took this approach to study miR-145, a tumor suppressor miRNA markedly reduced in
pancreatic cancer among many others. To investigate how miR-145 counteracts development of pancreatic cancer, in this study, we employed quantitative proteomics (SILAC), gene expression microarray and miRNA microarray to identify potential targets of miR-145 and its regulated network. In our transcriptionomics analysis, miR-145 suppressed expression of genes related to different aspects of cancer development. In addition to its known targets such as FSCN1, YES1 and PODXL, miR-145 also downregulated ITGA11 and MAGEA4. Interestingly, miR-145 also upregulated other miRNAs such as miR-124 and miR-133b, both of which were implicated in tumor suppression. Revealed through SILAC, miR-145 downregulated several oncogenes including multidrug resistance-associated protein 1 (ABCC1), serine palmitoyltransferase 1 (SPTLC1), histone acetyltransferase p300 (EP300) and protein SET (SET) among others. To further the understanding of miR-145-mediated repression of protein synthesis, we compared different types of binding between miR-145 seed sequence and its targets. Globally, “8mer” binding in the 3’-untranslated region of transcripts tended to have lowest protein abundance.

Since miR-145 did not cause all proteome changes directly, we sought to find other contributors by integrating multi-omics along with bioinformatics prediction from TargetScan. Focusing on target genes with concordant miRNA and protein changes, collectively, miRNAs upregulated by miR-145 have an overall stronger binding with their target genes than those downregulated by miR-145.

In summary, to better characterize regulation of gene expression and protein synthesis by miR-145 in the context of pancreatic cancer, we integrated multi-omics analysis that not only helped identify novel targets of miR-145 but also provides a broader insight into the involved network.

Mon 3:10 - 3:20 pm: Comparative Proteomic Analysis of Human Embryonic Stem Cells (ESCs) and Human Induced Pluripotent Stem Cells (iPSCs) of Different Origin
Natalia Pripuzova1; Melkamu Gete-Kebtite1; Christopher Grunseicht1; Colin Sweeney2; Harry Mameh3; Michail Alterman4.
1FDA, Center for Biologics Evaluation and Research, Rockville, MD; 2Laboratory of Host Defenses, NIAID, NIH, Bethesda, MD; 3Neurogenetics Branch, NINDS, NIH, Bethesda, MD

The generation of human induced pluripotent stem cells (hiPSCs) from adult somatic cells holds important scientific and clinical implications. While many studies have characterized and compared gene expression profiles of hiPSCs and human embryonic stem cells (hESCs), only few have dealt with their proteomes. iPSCs have been found to be highly similar to ESCs, but their expression profiles differ from ESCs: iPSCs may not completely silence the expression pattern of somatic cells, however, ESC signature genes are expressed at higher levels in iPSCs after extended culture. To build the protein marker database for characterization of quality and functionality of iPSCs we performed LC/ESI/MS/MS-based proteome characterization of hiPSCs generated from two sources: CD34+ cells circulating in peripheral blood and fibroblasts of healthy donors. Both iPSC lines were established using exciscable polyclonistic lentivirus vector “STEMMCA-loxP” (Millipore). Two iPSC lines were compared with the H9 ESC line, at the early (P.36, P.28, P.34) and late (P.43, P.54) passaging levels, as well as with original donors’ primary cells (FBMC and fibroblasts). Label-free absolute quantification of protein was performed based on ”universal signal response factor” using normalization against an internal reference standard. A total of up to 2,500 proteins were confidently quantified in different cell lines. The level of expression of 10 known pluripotency markers was validated by Western blots. Quantitative comparison between 5 cell lines (H9, two iPSC and two primary cell lines) revealed about 230 proteins differentially expressed in these pluripotent cell lines compared to primary cells. Using classification by PANTHER and functional networks mapping of the over- and under-expressed proteins by Ingenuity Pathway Analysis revealed that differentially expressed proteins in both iPSCs and ESCs showed very similar profiles. Differentially expressed proteins with a significant fold change will be further verified by Western blot and other methods.

Mon 3:20 - 3:30 pm: Building a Reliable Proteomics Database Requires Stringent Protein Identification Criteria
John Tria; Vidya Vidya Venkatraman; Jennifer Van Eyk; Johns Hopkins University, Baltimore, MD

Building reference proteomics database relies on advanced proteomic techniques but obtaining good protein identity necessitates advanced bioinformatics to help optimize true protein identification.

The Lack of uniform protein selection criteria and the wide range of sensitivity in mass spectrometers have remained limiting factors in building reliable databases. The aim of our study was to build a human disease plasma proteome database containing confident protein identities. The database was built using six human disease cohorts: a normal patient, two Lung disease, and three cardiovascular disease cohorts.

Each cohort underwent sample fractionation, tryptic digestion and analysis by LTQ Orbitrap Mass Spectrometer. The raw data were then searched using OMSAA and X!tandem against the non redundant human protein sequence database UNIPROT. We then applied protein selection criteria of 1% FDR and 90% minimum protein probability and manually validated the one peptide hits using peptide spectra. We retained 961 protein names from 9606 unique peptides. We then compared our list to the HUPO’s canonical protein list of 2011. We found 480 proteins also present in the HUPO’s list and 518 proteins only observed in our study. We hope to add the new proteins to the HUPO’s plasma proteome database.

Tuesday 8:30 - 9:20 am
Plenary Lecture, Key Ballroom 3,4,6

Tues 8:30 - 9:20 am: Frontiers of Orbitrap Mass Spectrometry
Alexander Makarov; Thermo Scientific, Bremen, Germany

The Orbitrap mass analyzer is the first high-performance mass analyzer which employs trapping of ions in electrostatic fields. Originating from an ideal (and therefore never realized) Kingdon trap, this analyzer can provide high performance analytical characteristics only when it is highly integrated with the ion injection process. The advent of pulsed injection from an external ion storage device allowed the Orbitrap analyzer to enter mainstream mass spectrometry, initially as a part of a hybrid instrument. Since its introduction the utility of the Orbitrap has been extended by coupling with additional capabilities such as higher-energy dissociation (HCD), ETD, FAIMS and MALDI ionization.

Two major families of instruments, LTQ Orbitrap and Exactive, have entered recently a new phase in their short but eventful history. For the former, a new construction of the Orbitrap mass analyzer with a higher field strength and new methods of data processing, drastically increase spectral acquisition rate, improve sensitivity and performance for proteins in the Orbitrap Elite instrument. For the latter, the addition of a quadrupole mass filter enables a variety of new methods of analysis in the Q Exactive instrument, such as parallel filling and detection, combination of quantitative and qualitative analysis, and spectra multiplexing.

It is shown that quantitative analysis in Orbitrap mass spectrometry is now highly feasible due to a unique combination of high space charge capacity of the C-trap, improved control over the number of stored ions, intelligent filling and high sensitivity with built-in high mass accuracy, resolving power and dynamic range of analyzer.

The performance of new Orbitrap-based instruments is described in comparison to their predecessors as well as to time-of-flight and high-field FT-ICR instruments. The talk also describes both current and emerging applications, such as analysis of intact proteins and protein complexes under native conditions, e.g. antibody-antigen conjugates.
Therapeutics production continues to increase in the drug market share.

Sialylated glycans are typically found at the terminal residue of glycans. Sialic acids play a crucial role in cell surface interactions, protect cells from membrane proteolysis, help in cell adhesion, and determine the half-life of glycoprotein in the blood. The degree of sialylation has been demonstrated to be a consequence of diseases.

Glycans are typically analyzed with Matrix Assisted Laser Desorption Ionization (MALDI) mass spectrometry. However, identification and accurate quantification of sialylated glycans using MALDI is challenging. The labile nature of the sialic acid during matrix ionization leads to loss of the sialic acid. Sialylated glycans have negative charges decreasing the ionization efficiency compared to neutral glycans, also makes analysis of glycans challenging during positive ion mode MALDI.

Robust, automated annotation and quantification of mass spectral data is essential to achieve qHTG but is currently unavailable. We have developed tools and databases to facilitate high throughput approaches for spectral interpretation and quantification of N-linked, O-linked glycans (GSL) glycan structures that allow us to achieve analytic throughput addressing six biological samples per week. This volume of analyses supports the acquisition of datasets with sufficient statistical power to achieve biological samples per week. This volume of analyses supports the acquisition of datasets with sufficient statistical power to achieve biological significance and reproducibility of quantification, we identified a set of analytical factors in the process including sample preparation, data acquisition, and data analysis. In this study, we assessed the impact of LC-MS/MS conditions in the reproducibility of quantitative proteomics for the large throughput analysis of tissue specimens. The studies evaluated the effects of various LC-MS conditions on proteomic quantification results. These conditions included LC gradient, MS setting, peptide loading amount, as well as some disastrous conditions, such as sample contamination, sample storage, column clogging, and instrument power outage. By comparing the number of protein identification contaminants, sample storage, column clogging, and instrument power outage. By comparing the number of protein identification and reproducibility of quantification, we identified a set of experimental conditions that significantly affected the reliability of proteomic quantification by LC-MS/MS. These data were useful to...
assess the data quality and identify the data sets affected by the disastrous conditions during data acquisition and help to achieve high quality proteomic quantification data for a large number of specimens.

Tues 10:15 - 10:30 am: External Calibration: Why Should I?
Andrew Hoofnagle; University of Washington, Seattle, WA

Background: For large retrospective and prospective clinical research studies and for patient diagnosis, prognosis, and management, meaningful longitudinal protein measurements are essential to success. The calibration of the mass spectrometric response into a peptide or protein concentration provides a foundation for reproducible results over time. With traceable reference materials, calibration also has the potential to greatly improve accuracy of the measurements and to further reduce inter-laboratory variability.

Methods: To evaluate the potential benefit of calibration in several assays of proteins in human serum or plasma, our laboratory has experimented with different approaches to calibration: (1) multiplying the response ratio by the concentration of the internal standard, (2) preparing external calibration curves from purified protein spiked into an appropriate matrix, and (3) using native human serum samples as calibrators. In collaboration with many other laboratories, we have also evaluated the effects of calibration on inter-laboratory comparisons.

Results: We have focused on (1) apolipoproteins A-I and B-100, highly abundant plasma proteins important in evaluating cardiovascular disease risk, (2) IGF-1, a low-abundance marker of growth hormone dysregulation or doping, and (3) thromboglobulin, an important low-abundance tumor marker used to monitor therapy in differentiated thyroid carcinoma. We have determined that calibration greatly improves day-to-day reproducibility of measurements within a single laboratory. Additionally, we have shown that human serum provides the best matrix for calibration materials in quantitative protein assays by mass spectrometry and that native human serum sample calibrators improve inter-laboratory agreement.

Conclusions: There has been a rapid increase in the number of clinically relevant protein assays that are being performed using mass spectrometry, which will fundamentally change our ability to diagnose and care for our patients. Choosing the correct methods of calibration will be important in ensuring that the measurements we perform are useful in research and clinical settings.

Tuesday 9:20 - 10:30 am
Microarray for Protein and Lectin, Key Ballroom 1-2

Tues 9:30 - 10:00 am: DNA Methylation Presents Distinct Binding Sites for Human Transcription Factors
Heng Zhu; Johns Hopkins University, Baltimore, MD
DNA methylation, especially CpG methylation at promoter regions, has been generally considered as a potent epigenetic modification that prohibits transcription factor (TF) recruitment, resulting in transcription suppression. Here, we used a protein microarray-based approach to systematically survey the entire human TF family and found numerous purified TFs with methylated CpG (mCpG)-dependent DNA-binding activities. Interestingly, some TFs exhibit specific binding activity to methylated and unmethylated DNA motifs of distinct sequences. To elucidate the underlying mechanism, we focused on Kruppel-like factor 4 (KLF4), and decoupled its mCpG- and CpG-binding activities via site-directed mutagenesis. Furthermore, KLF4 binds specific methylated or unmethylated motifs in human embryonic stem cells in vivo. Our study suggests that mCpG-dependent TF binding activity is a widespread phenomenon and provides a new framework to understand the role and mechanism of TFs in epigenetic regulation of gene transcription.

Tues 10:00 - 10:15 am: Nanopore Assay for Low Abundance Blood Biomarker Discovery and Quantification from Human Bodily Fluids
Tony Hu; The Methodist Hospital Research Institute, Houston, TX

Endogenous serum peptides carried important information of disease are considered to be great potential biomarkers for clinical diagnosis. However, due to the complexity of serum proteins and the interference of high abundant and low abundant proteins, the detection of the serum peptides and low molecular weight (LMW) proteins, especially for a target peptide biomarker is still a challenge. Herein we report a high-throughput, label-free and low-cost nanopore-based assay that is employed to selectively enrich and quantify a low abundant peptide, hepcidin, from human serum and urine. The development of effective assays for quantifying hepcidin levels in biological fluids remains a challenge due to the inherent problems related to concentrating and identifying peptides from biological fluids and the lack of appropriate monoclonal antibodies. To address this challenge, we describe a novel approach based on nanoporous silica (NPS) thin films with nanotextures (pore size, surface and structure) specifically and precisely engineered for hepcidin enrichment. Hep-25 and its isofoms can easily be enriched in the optimized nanopores, and then analyzed by benchtop MALDI-TOF MS. Our method requires only microliter sample volumes and eliminates time-consuming sample pretreatment, while maintaining a high degree of precision, accuracy, and sensitivity. To clinically validate our technique, hepcidin-25 levels were quantified in both serum and urine from 119 healthy volunteers and 19 patients suffering from non-specific inflammation. The levels were determined to be gender, menopausal, and inflammation status dependent. We have therefore adapted and validated this nanotechnology based approach to accurately, reliably, and inexpensively measure pathophysiological hepcidin concentrations in complex human biological fluids.

Tues 10:15 - 10:30 am: Measurement of the Glycosylation Changes of Serum Proteins by Lectin Immunosorbant Assay
Danni Li; Hang-Ching Chiu; Hui Zhang; Daniel Chan; University of Minnesota, Minneapolis, MN; Johns Hopkins University, Baltimore, MD
Lectin immunosorbant assays (LISAs) have been widely used for the analysis of protein glycosylation. Since the great majority of serum proteins are glycosylated, the analysis of serum samples by LISAs could suffer from high sample-dependent background noises, mainly due to non-specific binding of serum proteins. This could reduce the analytical sensitivity of LISAs, resulting in poor sample-to-sample comparison. In this study, first, we established dose-response curves through differential removals of the protein of interest. Then, we determined experimentally that in LISA, the slope of the dose-response curve qualitatively measures the glycosylation changes of serum proteins. We used tissue inhibitor of metallopeptidase 1 (TIMP-1) UEA LISA as the model. Serum samples depleted of endogenous TIMP-1 and supplemented with recombinant TIMP-1 were used to demonstrate that the slopes were not affected by serum matrix. Serum samples supplemented with differentially fucosylated recombinant TIMP-1 were used to demonstrate that the slopes measured the degree of TIMP-1 fucosylation. In conclusion, we developed a LISA based method to measure the glycosylation changes of serum proteins. This method improves upon LISAs and is indifferent to serum interferences. Using 28 serum samples from cancer and non-cancer patients, the TIMP-1 UEA LISA assay showed slopes approaching zero, indicating that serum TIMP-1 had little or no UEA α 1,2-fucosylation.

Tuesday 10:50 am - 12:00 pm
Systems Proteomics, Key Ballroom 3,4,6

Tues 11:00 - 11:30 am: Interactome Networks and Human Disease
Marc Vidal; Dana-Farber Cancer Institute, Boston, MA; Harvard Medical School, Boston, MA
For over half a century it has been conjectured that macromolecules form complex networks of functionally interacting components, and that the molecular mechanisms underlying most biological processes correspond to particular steady states adopted by such cellular networks. However, until a decade ago, systems-level theoretical conjectures remained largely...
unappreciated, mainly because of lack of supporting experimental data.

To generate the information necessary to eventually address how complex cellular networks relate to biology, we initiated, at the scale of the whole proteome, an integrated approach for modeling protein-protein interaction or “interactome” networks. Our main questions are: How are interactome networks organized at the scale of the whole cell? How can we uncover local and global features underlying this organization, and how are interactome networks modified in human disease, such as cancer?

Tues 11:30 - 11:45 am: Signaling Interactomes in Cancer:
Opportunities for New Targets and Molecular Markers
Eric Haura; Jianlong Li; Guolin Zhang; Matthew Smith; Richard Hall; John Koomen; Keiryn Bennett; Giulio Superti Furga; H. Lee Moffitt Cancer Center, Tampa, FL; Center for Molecular Medicine, Vienna, Austria

Mutations in the epidermal growth factor receptor (EGFR) or translocation of echinoderm microtubule associated protein like 4 – anaplastic lymphoma kinase (EML4-ALK) define two unique subsets of lung cancer characterized by sensitivity to tyrosine kinase inhibitors (TKI). We hypothesized that elucidating the interactomes of these oncogene addicted lung cancers may identify vulnerabilities and a more systems-level perspective the molecular etiology. We characterized signaling interactomes using tandem affinity purification (TAP) and liquid chromatography-mass spectrometry (LC-MS/MS) along side anti-phosphotyrosine (pTy) immunocomplexes coupled with LC-MS/MS to map tyrosine phosphorylation. Baited proteins expressed in drug sensitive cell lines are purified using TAP and proteins in complex identified using LC-MS/MS. Parallel studies identify and quantify pTy peptides that are subsequently added to the interactome backbone discovered from TAP-LC-MS/MS. In lung cancer cells with mutated EGFR, we characterized a physical EGFR network consisting of 265 proteins. A focused shRNA screen based on this interactome defines a core set of 14 proteins critical to survival of multiple EGFR addicted lung cancer cells. Overlaying drug binding data identified compounds targeting key nodes that have either singular or combination effects on killing lung cancer cells. Similarly, we characterized a EML4-ALK driven network consists of nearly 480 proteins. Ongoing studies will examine this network for vulnerabilities using a focused shRNA library screen. To translate these interactome-based mass spectrometry studies to clinic, we developed in situ assays that identify and quantify interactions using proximity ligation assays (PLA). PLA can detect and quantify binary interactions between two proteins on a per cell basis using an immunohistochemistry approach. Pilot studies suggest that EGFR or ALK complexes can distinguish tumor types. These systems-level approaches have the potential to enable ‘network medicine’ by identifying novel vulnerabilities in lung cancers and identifying cancer subsets based on interactomes.

Tues 11:45 am - 12:00 pm: Application of Generalized Protein Parsimony and Spectral Counting to Functional Enrichment Analysis and Protein Isoform Detection
Nathan Edwards; Georgetown University Medical Center, Washington, DC

Functional enrichment analysis is used extensively in systems biology analyses of transcriptomics data, linking phenotypically distinct experimental samples via differentially expressed genes to knowledgebases that categorize genes by function, cellular location, domain, or canonical pathway. We address the challenges in applying functional enrichment analysis to proteomic data by ensuring a minimal protein set is considered, and by using spectral counting to detect differential abundant proteins and protein isoforms.

We propose a stringent criteria for inferring proteins from bottom-up peptide-fragmentation spectra - requiring that each inferred protein be supported by at least two unshared peptides, and that the proportion of peptide identifications omitted be consistent with FDR-based peptide identification filtering strategies. We also show that hypergeometric based statistical models and Fisher exact tests can be readily applied to spectral counts to determine differentially abundant proteins, and when coupled with stringent protein inference to ensure an appropriate statistical background, can be used to infer functional enrichment of differential proteins using the DAVID tool. Furthermore, we show that we can test functional protein sets directly, finding evidence for differentially abundant proteins sets based on spectral counts - avoiding the perils of counting proteins. Lastly, we show that spectral counts can be used for the detection of alternative splicing events, even when the underlying set of observed peptides do not provide evidence of distinguishing amino-acid sequence.

Tues 11:00 - 11:30 am: Shifting the Quantitative Proteomics Paradigm from Hypothesis Generating to Hypothesis Testing
Michael MacCoss; University of Washington, Seattle, WA

Proteomics technology has improved dramatically over the last decade. The technology developments have largely been directed around instrument hardware, where instruments have been developed that scan faster, are more sensitive, and have greater mass measurement accuracy. However, the basic workflow has remained largely unchanged – mass spectrometers are directed toward the acquisition of tandem mass spectra on the most abundant molecular species eluting from a chromatography column. More recently, efforts have been focused on the acquisition of mass spectrometry data on target peptides of interest. With improvements in instrument hardware and instrument control software, the practical experimental difference between a targeted and discovery proteomics is beginning to become blurred. These analyses are a significant change from the traditional proteomics workflow and have required the development of novel computational strategies to analyze, visualize, and interpret these data. We will present work illustrating our efforts in the development of targeted proteomics and provide a vision for challenges that still need to be overcome before these analyses become routine and replace more traditional discovery proteomics methodology.

Tues 11:30 - 11:45 am: Quantification of PACIFIC Data with Progenesis Software
Robert Moulder; Santosh Bhosale; Jussi Salmi; Michael Wilson; John Chapman; Young Ah Goo; David Goodlett; University of Turku, Turku, Finland; 1University of Washington, Seattle, WA; 2University of Maryland, Baltimore, MD

The Precursor Acquisition Independent From Ion Count (PACIFIC) methodology increases the detectable dynamic range and percentage of total proteome coverage of complex proteomes such as P. aeruginosa and serum compared to traditional means of data-dependent acquisition (Panchaud, 2009). PACIFIC is a data-independent acquisition strategy that acquires tandem mass spectra from a series of pre-defined 2.5 u channels regardless of the presence of a precursor ion. These 2.5 u channels are systematically stepped by 1.5 u to cover a specified m/z range.

Due to the overall processing time and need for liquid chromatography compatibility, PACIFIC requires separated batching of m/z channels and multiple sample injections to ensure each chromatographic peak is repetitively sampled.

In view of the fact that precursor ion scans are not necessary for PACIFIC analysis, previous quantitative strategies for PACIFIC have relied on spectral counting (Hengel 2011; Goo 2012) and isobaric labels (Panchaud 2011; Acosta-Martin 2011). However, inclusion of accurate mass precursor ion scans in the data acquisition strategy can provide the opportunity to record replicate precursor ion intensities and perform area under the curve (AUC) quantification.

Here, we demonstrate the utility of Progenesis software (Nonlinear Systems) for the quantitative analysis of PACIFIC data from LC-MS/MS measurements of serum and yeast samples.

Samples were analysed in replicate using an Orbitrap-Velos with an online EASY-nLC (Thermo Scientific). High mass accuracy and resolution precursor ion scans were amended to the PACIFIC scheme. The Progenesis derived quantitative results were
compared with spectral counts, documented concentrations for serum, and established cell copy numbers for yeast. As well as providing consistency and coverage, these data indicated the advantages of how composite protein and peptide spectral matches from multiple data files and feature identification in Progenesis can enhance quantification by calculation of peptide AUC’s from PACIFIC measurements.

**Tues 11:45 am - 12:00 pm: Public Spectrum Libraries Evaluation for Building SRM Assay**

Shadab Ahmad; Amol Prakash; Barbara Frewen; Scott M. Peterman; Bruno Domon; Sebastien Gailhun; Gregory Byram; Bryan Krasins; David Sarracino; Maryann S. Vogelsang; Gouri Vadali; Jennifer Sutton; Mary F Lopez; Thermo Scientific, BRIMS, Cambridge, MA; Luxembourg Clinical Proteomics Center, Strassen, Luxembourg

Single Reaction Monitoring (SRM) has emerged as influential and reliable technique in targeted shotgun proteomics that can verify and/or quantify targeted proteins with high precision. The most challenging part of the technique is selecting good transitions. Ideally, one should have purified and/or recombinant proteins for optimisation of transitions, but this is a time consuming and labour intensive process.

Alternatively, discovery data from global spectral repositories can be utilised for fast selection of transitions. The best strategy to use these libraries and their strengths and weaknesses are still not clear. Herein, we study the most popular global spectral repositories, PeptideAtlas, The GPM, and NIST, and utilises their diverse peptide rankings criteria to build transitions for ten commercially available recombinant proteins. Other transitions were also built utilising publicly available tools for building SRM assay (PASSEL, SRMAtlas and GPM-ERM). We compared these library-based transitions with those we optimised empirically for seven of the ten proteins. Analysis of the acquired data showed us that the various peptide-selection criteria and the various spectral libraries perform very differently and, in many cases, predicted transitions that were either not detected or measured with low intensity. The best performing criteria and libraries were NIST Spectrum Count, PeptideAtlas Spectrum count, and SRM Atlas.

These three have 70-80% overlap with the top three performing transitions. Next we compared the sensitivity of these predicted transitions to the optimized transitions and found 60-70% loss in sensitivity as compared to the optimal transitions. This suggests that while utilizing public spectra libraries is a fast strategy to build SRM assays, this can result in a significant loss of sensitivity, and thus if a lab has access to local spectral data, it will be best to utilize that.

**Tuesday 10:50 am - 12:00 pm**

**Characterization of PTMs, Key Ballroom 5**

**Tues 11:00 - 11:30 am: Targeting Protein Post-translational Modifications for non-invasive Diagnosis of Oral Cancer**

Tim Griffin; University of Minnesota, Minneapolis, MN

Numerous types of protein post-translational modifications drive cancer progression. Given their link to cancer mechanisms, these modifications hold great value as potential biomarkers for early diagnosis and therapeutic targets during the passage of mature proteins by proteases, which is a critical step in cancer invasion and tumorigenesis. Here we will present our work seeking to identify putative peptide products of protease activity in oral cancer in non-invasively collected clinical samples, with a focus on whole saliva as the sample of choice. Not only do these peptides have potential as non-invasively collected biomarkers, but these are also more easily assayed in a high-throughput manner via mass spectrometry. We will also describe our ongoing efforts at developing computational tools for characterization of post-translational modifications from mass spectrometry-based peptidomics and proteomics data. Specifically we will describe our efforts at developing an improved method for label-free quantification of peptidomic data, with a particular focus of its effectiveness for post-translational modification characterization. We will also present an update on work developing a web-based, open source framework called Galaxy-P, which has the potential to transform the way in which researchers implement and disseminate workflows for analysis of complex, mass spectrometry-based datasets.

**Tues 11:30 - 11:45 am: Global analysis of Bacteria-host Interaction by AMPylation using Human Nucleic Acid Programmable Protein Arrays**

Xiaobo Yu; Andrew R Woolley; Phi Luong; Yi Heng Hao; Markus Grammel; Nathan Westcott; Jim Park; Jie Wang; Xiaofang Bian; Gokhan Demirkann; Howard C. Hang; Kim Orth; Joshua LaBae; Arizona State University, Tempe, AZ; University of Texas Southwestern Medical Center, Dallas, TX; The Rockefeller University, New York, NY

AMPylation has recently emerged as a general mechanism employed by infectious bacteria to regulate cell signaling in their hosts. These bacterial AMPylation enzymes, or AMPylators, are secreted into the host cells by bacterial Type III or IV Secretion Systems, and transfer an AMP moiety from ATP to Tyr or Thr residues of host proteins, presumably to benefit the pathogen’s infection. AMPylation disrupts the binding of GTPase to its downstream effectors, e.g. PAK1, and causes cell rounding. Considering the conservation of AMPylation domains in both prokaryotic and eukaryotic organisms, it is hypothesised that protein AMPylation will play important role in the regulation of a wide range of cellular processes. Currently our understanding of AMPylation is limited and determining the variety of substrates modified by AMPylators will help illuminate the functional consequences of AMPylation. Despite intense effort using biochemistry and mass spectrometry, only a few substrates have been identified so far.

Herein we built high-density Nucleic Acid Programmable Protein Arrays (NAPPA)(1) displaying human proteins expressed by a human HeLa cell-free expression system for use with enzymatic post-translational modification (PTM) screens. We developed a high-throughput discovery platform for the identification of proteins modified by AMPylation using an ATP analog containing a nonradioactive alkynyl chemical reporter N6pATP(2). We screened 10,000 unique human proteins with two bacterial AMPylators, VopS and IbpAFic2, from Vibrio parahaemolyticus and Histophilus somni, respectively, yielding many new GTPase and non-GTPase substrates, and generating the first bacteria-host interaction AMPylation network. All the results demonstrated this approach can be extended to identify novel substrates of other AMPylators as well as other PTMs.

References:

**Tues11:45 am - 12:00 pm: Temporal Phosphorylation Dynamics Analysis of TLR Stimulation: Role of MARCKS in TLR Signaling**

Virginie Sjoelund; Iain Fraser; Aleksandra Nita-Lazar; NIAID, NIH, Bethesda, MD

The Toll-like receptors (TLRs) are a family of pathogen recognition receptors that alert the host to the presence of pathogens. Cytokines are among the most important genes to be regulated by TLRs, their role in initiating and giving their role in the orchestration of the inflammatory response, mechanisms of modulating their production have garnered a lot of interest, in particular in the area of the development of therapies for the treatment of chronic inflammatory diseases. We investigated the changes in the phosphoproteome post activation of TLR4, TLR2/1 and TLR7 in C57 macrophages at 5 different time points using triple SILAC. In average, we identified 500 reproducible phosphosites per receptor. We found that most temporal phosphorylation changes were similar between the three different receptor stimulations. Functional bioinformatics analysis identified networks involved in actin cytoskeleton signaling, RhGTPase signaling and phospholipase C signaling, among others. We found 24 phosphosites that were statistically differentially phosphorylated at a given time point between the different TLRs. These proteins are involved in the cytoskeleton, with MARCKS (myristoylated alanine-rich C-kinase substrate) being a particular...
phosphoprotein that is differentially phosphorylated between the 3 different stimulations. By using a PKC inhibitor, the role of MARCKS and its phosphorylation state into the trafficking of the TLR to the endosome, its role in cell migration and signaling in the canonical TLR pathway was investigated.

**Tuesday 3:00 - 3:30 pm**

**Robert J. Cotter New Investigator Award Lecture**

**Key Ballroom 3,4,6**

**Tues 3:00 - 3:30 pm: Cell Surface Chemoproteomics for Capturing States of Human Cardiac Differentiation from Pluripotent Stem Cells**

Subarna Bhattacharya; Sandra Chuppa; Erin Kropp; Rebekah Gundry, Medical College of Wisconsin, Milwaukee, WI

Human pluripotent stem cells (embryonic (ES) and induced (iPS)) provide a valuable tool for studying early stages of development. Recent improvements in our ability to reproducibly and robustly generate cardiomyocytes from ES/iPS has ushered in new opportunities to study early events in cardiac development and to generate cell types relevant for disease modeling, drug testing, and potentially therapy. However, major challenges include the inability to biochemically direct in vitro cardiac differentiation to specific, selected endpoints. While intracellular markers informative of major developmental stages have been identified (e.g. Mesp1, Isl1), these cannot be employed to isolate therapeutically relevant cells (i.e. require genetic modifications) and there are currently limited tools available for specifically tracking the primary heart field, which ultimately gives rise to the left ventricle. The overall goal of this study is to identify cell surface accessible proteins that will allow for the tracking and live cell isolation of ES/iPS-derived cardiac progenitor cells without genetic manipulations. Toward this end, the Cell Surface Capturing (CSC) Technology, an Ab-independent strategy that uses affinity enrichment of cell surface N-glycoproteins and high mass accuracy mass spectrometry to selectively identify cell surface proteins, was applied to human ES and iPS cells and ES-derived cardiomyocytes. In total, >800 cell surface proteins have been identified, including known markers as well as proteins never previously attributed to these cell types. Comparing these data to our in-house Cell Surface Protein Atlas that contains >3000 proteins identified on >70 cell types, reveals >20 proteins that appear to be restricted to the cardiac lineage. Using flow cytometry, we have validated the presence of these proteins during cardiac differentiation and are currently evaluating their ability to sort functionally relevant populations. Moreover, we are using MRM to quantify selected candidates for which there are no antibodies available.

**Wednesday 8:30 - 9:20 am**

**Plenary Lecture, Key Ballroom 3,4,6**

**US-HUPO - EDRN Joint Symposium**

**Wednesday 10:00 - 10:15 am:**

**Proteomic Analysis of Liver Tissue Interstitial Fluid and Its Potential in Biomarker Discovery for HBV Associated HCC**

Zhilei Liu; Wei Sun; Longqin Sun; Ying Jiang; Fuchu He; Beijing Proteome Research Center, Beijing, China

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors with a very high morbidity and mortality in Southeast Asia, particularly in China. We have established the TIF extraction methods from human adult liver tissue, and the organellar proteins’ pollution was evaluated. Based on this, 6 clinical cases of HBV positive HCC were selected to find the differential expressed proteins in the TIF by using 3 kinds of proteomic theologies including iTRAQ, 2D-DIGE and the substract technology in combination with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-TOF MS) and FT-LTQ mass spectrometry analysis. The total differential expressed proteins are 1276. The increased levels of tropomyosin alpha-3 chain (TPM3), chloride intracellular channel protein 1 (CLIC1), transketolase (TKT) and the decreased level of nicotinate nucleotide pyrophosphorylase (GPR7) in tumor TIF was confirmed by Western blot in an independent set of paired TIF samples from 7 HBV positive HCC patients. ELISA was also applied to verify the diagnosis potential of 2 proteins from enlarged clinical serum samples. We found the protein panel had a good area under the curve to distinguish HCC from LC (0.965, 95% confidence), with 96% sensitivity and 85% specificity. At the first time, by using clinical sample, we demonstrated that disease-related proteins with lower abundance were enriched in TIF which is a promising material for HCC biomarker discovery.

**Wednesday 8:30 - 9:20 am: Cancer Genomes and Their Implications for Research and Patients**

Bert Vogelstein, Johns Hopkins University, Baltimore, MD

In the past five years, it has become possible to identify all of the mutations contributing to neoplasia in an individual cancer. This has not only led to an appreciation of the cancer genome landscape, but has also highlighted critical issues that need to be addressed to eradicate these diseases. Many of the issues pertain to the pathways through which the genetic mutations act, which requires proteomics as well as functional studies. In addition to providing understanding, the new genome-wide studies of cancers are contributing to the formulation of strategies to more effectively prevent or manage these diseases.

**Wednesday 9:20 - 10:30 am**

**Cancer Biomarker: Discovery, Key Ballroom 3,4,6**

**US-HUPO - EDRN Joint Symposium**

**Wed 10:00 - 10:15 am:**

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Zhilei Liu; Wei Sun; Longqin Sun; Ying Jiang; Fuchu He; Beijing Proteome Research Center, Beijing, China

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors with a very high morbidity and mortality in Southeast Asia, particularly in China. We have established the TIF extraction methods from human adult liver tissue, and the organellar proteins’ pollution was evaluated. Based on this, 6 clinical cases of HBV positive HCC were selected to find the differential expressed proteins in the TIF by using 3 kinds of proteomic theologies including iTRAQ, 2D-DIGE and the substract technology in combination with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-TOF MS) and FT-LTQ mass spectrometry analysis. The total differential expressed proteins are 1276. The increased levels of tropomyosin alpha-3 chain (TPM3), chloride intracellular channel protein 1 (CLIC1), transketolase (TKT) and the decreased level of nicotinate nucleotide pyrophosphorylase (GPR7) in tumor TIF was confirmed by Western blot in an independent set of paired TIF samples from 7 HBV positive HCC patients. ELISA was also applied to verify the diagnosis potential of 2 proteins from enlarged clinical serum samples. We found the protein panel had a good area under the curve to distinguish HCC from LC (0.965, 95% confidence), with 96% sensitivity and 85% specificity. At the first time, by using clinical sample, we demonstrated that disease-related proteins with lower abundance were enriched in TIF which is a promising material for HCC biomarker discovery.
The increased use of abdominal imaging has led to a higher rate of identifying pancreatic cysts, but currently it is not possible to accurately determine which cysts have high malignant potential and should be removed. Our previous work showed that the glycoforms of specific proteins in the cyst fluid were significantly changed between cysts of different pathological states and may serve as accurate diagnostic biomarkers. Here we further tested this hypothesis using antibody-lectin sandwich arrays in broad screens of protein glycoforms and targeted studies of candidate markers. We initially profiled 16 different glycoforms of proteins captured by 72 different antibodies in cyst fluid from mucinous and non-mucinous cysts (n = 22). Glycan alterations were not widespread among proteins but were mainly confined to the proteins MUC5AC and endorepellin. Specific glycoforms of these proteins, defined by reactivity with wheat-germ agglutinin (WGA) and a blood group H (BGH) antibody, were significantly elevated in mucinous cysts, whereas the core protein levels were not significantly elevated. A three-marker panel based on these glycoforms distinguished mucinous from non-mucinous cysts (n = 22). Glycan alterations were limited to particular proteins and glycans, and they may be highly specific and sensitive biomarkers for the diagnosis of pancreatic cysts.

The use of selected reaction monitoring (SRM) with liquid chromatography and tandem mass spectrometry (LC-MS/MS) has become an increasingly popular method for quantitative analyses of target proteins in biological or clinical samples.

It has been shown that the use of synthetic peptide standards and isotope dilution makes possible accurate quantitation of changes in protein concentrations between multiple laboratories. The ability to multiplex these SRM assays, known as scheduled multiple reaction monitoring (MRM), has allowed quantitation of potentially hundreds of proteins in a single LC-MS/MS analysis. However, the acceptance of MRM assays for protein analysis has been limited due to the difficulty in establishing MRM assays compared to using traditional antibody-based assays. Here we demonstrate that once SRM assays for target peptides were established, it could be transferred from another laboratory and multiplexed to scheduled MRM assays across multiple platforms to be simplified.
All posters will be attended by presenting authors on both Monday and Tuesday. A happy half-hour is hosted with the posters and exhibits on Monday and Tuesday following the formal presentation times listed below. All posters should be set up by 10:00 am on Monday morning and removed Tuesday evening at 6:00 pm.

MONDAY: Odd-numbered posters present 3:30 - 4:15 pm and even-numbered posters 4:15 - 5:00 pm.
TUESDAY: Odd-numbered posters present 4:00 - 4:45 pm and even-numbered posters 4:45 - 5:30 pm.

Poster Topics

Early Career Session Talks

Bioinformatics & Systems Biology.....................................................001 - 021
Biomarker Discovery & Validation..................................................022 - 043
Cancer Biomarkers .......................................................................044 - 052
Glycoproteomics & Glycomics ......................................................053 - 062
Imaging MS .................................................................................063 - 067
Phosphoproteomics .....................................................................068 - 072
Quantitation ..................................................................................073 - 078
Sample Prep ..................................................................................079 - 090
Other ............................................................................................091 - 122

Early Career Talks

Speakers selected for the three Early Career sessions on Monday afternoon may also present a poster of their work in this area. Presenters, titles, and abstracts for these talks are found in the Oral Abstracts on pages 22-28.

Bioinformatics & Systems Biology, 001 - 021

001 Splice Variant Analyses in Two Her2 Positive Breast Cancer Cell Lines; Rajasree Menon; Hogune Im; Michael Snyder; Emma (Yue) Zhang; Shiaw-Lin Wu; William S Hancock; Gilbert Omenn; U of M, Ann Arbor, MI; Stanford University, Palo Alto, CA; Northeastern University, Boston, MA; University of Michigan, Ann Arbor, MI

002 The CRAPome: A Contaminant Repository for Affinity Purification Mass Spectrometry Data; Alexey Nesvizhskii; Dattatreya Mellacheruvu; Zachary Wright; Amber Couzens; Jean-Philippe Lambert; Nicole St-Denis; Tu Li; Yana Miteva; Simon Hauri; Mihaela Sardu; Teck Low; Vincentius Halim; Richard Bagshaw; Nina Hubner; Abdallah al-Hakim; Annie Bouchard; Denis Faubert; Damian Fermin; Wade Dunham; Marilyn Goudreau; Zhen-Yuan Lin; Beatriz Gonzalez Badillo; Tony Pawson; Daniel Durocher; Benoît Coulombe; Ruedi Aebersold; Giulio Superti-Furga; Jacques Colinge; Albert Heck; Hyungwon Choi; Matthias Gstaiger; Shabaz Mohammed; Ileana Cristea; Keiryn Bennett; Michael Washburn; Brian Raught; Robert Ewing; Anne-Claude Gingras; University of Michigan, Ann Arbor, MI; Samuel Lunenfeld Research Institute, Toronto, Canada; Princeton University, Princeton, NJ; Institute of Molecular Systems Biology, ETH, Zurich, Switzerland; Stowers Institute for Medical Research, Kansas City, MO; Utrecht University, Utrecht, The Netherlands; Radboud University, Nijmegen, The Netherlands; Institut de recherches cliniques de Montréal, Montréal, Canada; CeMM Research Center for Molecular Medicine, Vienna, Austria; National University of Singapore, Singapore, Singapore; Ontario Cancer Institute, Toronto, Canada; Case Western Reserve University School of Medicine, Cleveland, OH

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Phosphorylation of the Sae2 Endonuclease Regulates Its Solubility and Activity in DNA Repair; Qiong Fu; Chia-Fang Lee; Maria Person; Tanya Paull; The University of Texas at Austin, Austin, TX
**Poster 001: Splice Variant Analyses in Two Her2 Positive Breast Cancer Cell Lines**

Rajasree Menon, Hoguen Irm; Michael Snyder; Emma (Yue) Zhang; Shiah-Lin Wu; William S Hancock; Gilbert Omenn; U of M, Ann Arbor, MI; 2 Stanford University, Palo Alto, CA; 3 Northeastern University, Boston, MA; 4 University of Michigan, Ann Arbor, MI

Multiple transcripts are produced through the use of alternative promoters, alternative polyadenylation sites, and alternatively spliced exons. To add to the complexity, genes produce different splicing events in different cell types and splicing results in protein isoforms with different biological activities. Hence, it is reasonable to assume that tumor cells, which undergo altered cellular mechanisms and angiogenesis, may have a different variant of a gene than that of the corresponding normal cells. The purpose of this study is to find the splice variants expressed in breast cancer cell line models SKBR3 (Her-2+/- ER-/-PR-, adenocarcinoma) and SUM149 (Her-2+ (not activated)/ER/PR-, inflammatory ductal carcinoma). We integrated the information from RNA-Seq and proteomic mass spectrometry data from these cell line models to identify the splice variants. There were 213 and 235 splice variant transcripts expressed with peptide evidence in SKBR3 and SUM149 cell lines, respectively, that were not found among the transcripts expressed in the normal human mammary epithelial cell line MEC; only 58 of these splice variant transcripts were found in both SKBR3 and SUM149. Pathway enrichment analysis indicated Ribosomes as the leading enriched pathway for the 58 common variants found in SKBR3 and SUM149. Glycolysis/gluconeogenesis was the most enriched pathway among the 213 SKBR3 variants and Adherens junction was the most enriched for the 235 SUM149 variants. We are currently studying the biological annotations and protein-protein interactions of the splice variants expressed in these cell lines. The cell-line specific splice variant proteins may play roles in a variety of mechanisms related to the breast cancer progression and metastasis and specifically in the differences between activated and not activated Her2/neu (ERBB2) or adenocarcinoma vs inflammatory ductal breast cancer.

**Poster 002: The CRAPome: A Contaminant Repository for Affinity Purification Mass Spectrometry Data**

Aleksy Nesyvaszhki; Dattatreya Mellacheruvu; Zachary Wright; Amber Couzens; Jean-Philippe Lambert; Nicole St-Denis; Tuo Li; Yana Miteva; Simon Haant; Mihaela Sardiu; Teck Low; Vincent C. Hanafusa; Richard Bagshaw; Abre Al-Hakim; Annie Bouchard; Denis Faubert; Damien Fermin; Wade Dunham; Marilyn Goudreault; Zhen-Yuan Lin; Brentito Gonzalez; Badillo; Tony Watson; Daniel Durocher; Reni Colombe; Ruedi Aebersold; Giulio Superti-Furga; Jacques Colinge; Albert Heck; Hyungwon Choi; Matthias Gstaiger; Robert Ewing; Zachary Wright; Mike Lunenfeld Research Institute, Toronto, Canada; 2 Princeton University, Princeton, NJ; 3 Institute of Molecular Systems Biology, ETZ, Zurich, Switzerland; 4 Stowers Institute for Medical Research, Kansas City, MO; 5 U of Tandem, Utrecht, The Netherlands; 6 Radboud University, Nijmegen, The Netherlands; 7 Institute for Medical Research Center for Molecular Medicine, Vienna, Austria; 8 National University of Singapore, Singapore, Singapore; 9 University of Michigan, Ann Arbor, MI; 10 Samuel Lunenfeld Research Institute, Toronto, Canada; 11 Case Western Reserve University School of Medicine, Cleveland, OH

Affinity purification coupled with mass spectrometry (AP-MS) is now a widely used approach for the identification of protein-protein interactions. However, for any given protein of interest, determining which of the identified polypeptides represent bona fide interactors versus those that are background contaminants (e.g. proteins that interact with the solid-phase support, affinity reagent or epitope tag) is a challenging task.

While the standard approach is to identify nonspecific interactions using one or more negative controls, most small-scale AP-MS studies do not capture a complete, accurate background protein set. Fortunately, since negative controls are largely bait-independent, we reasoned that the negative controls generated by the proteomics research community could be developed as a resource for scoring AP-MS data.

Here we present the Contaminant Repository for Affinity Purification (The CRAPome), currently containing AP-MS data from 343 control purifications conducted by 11 different research groups (www.crapome.org). Users employ an intuitive graphical user interface to explore the database, by either querying one protein at a time, downloading background contaminant lists for selected experimental conditions, or uploading their own data (alongside their own negative controls when available) and performing data analysis. The CRAPome database scores contaminants vs. true interactors based on semi-quantitative mass spectrometry data (normalized spectral counts) embedded in most mass spectrometry experiments. The Significance Analysis of Interactome (SAINT) scoring scheme, in addition to a simpler Fold Change calculation (FC score) are used to score user-supplied data and return a ranked list of putative interactors. We also describe database structure and composition, provide examples of the use of this resource to filter contaminants with properly chosen controls, and demonstrate the utility of the scoring scheme for identifying bona fide interaction partners. The CRAPome accommodates a variety of purification schemes and, while currently focused on human data, will be expanded to other species.

**Poster 003: Enabling Pathway-directed Targeted Analysis Based on Protein Discovery Data**

Christine Miller; Stefan Jenkins; Norton Kitagawa; Theodore Sana; Steven Fischer; Agilent Technologies, Santa Clara, CA

A key step in proteomics research continues to be identification of biologically significant differential proteins using label-free LC/MS/MS. Techniques such as 2D LC, pl-based fractionation and gel electrophoresis may be employed to increase separation efficiency as a strategy for obtaining more peptide MS/MS spectra and thus increasing the number of proteins identified. Improvements in instrumentation and fractionation techniques now allow the efficient and comprehensive identification of many proteomes. Transforming these protein identifications into a biological context is an essential step in understanding the results from differential expression experiments. A workflow will be described that allows protein identification results to be analyzed using a variety of statistical tools, and then visualized on pathways. Based on this analysis and visualization, lists of proteins of interest can be extracted for subsequent targeted proteomics experiments. In addition, pathway-directed targeted experiments can be developed from multi-omics data. A set of yeast samples was used to demonstrate both the identification of pathways of interest from a classical data-dependent approach and a pathway-directed approach based on metabolomics data. This work demonstrates the effectiveness of this approach in facilitating biological understanding.

**Poster 004: Visualize: A Free and Open Source Multi-tool for Post-Search Quantitative Proteomics Data Analysis**

Brian Halligan; Andrew Greene; Medical College of Wisconsin, Milwaukee, WI

One of the key challenges in Proteomics is the conversion of a set of MS results to biological insight. While many tools will provide a list of proteins that are differentially expressed between individual or multiple sets of MS runs, the critical requirement is the ability to both determine the statistically significant, differentially expressed protein and to annotate the biological consequences of the differences. There are several commercial applications for post processing of proteomics data, but these expensive and license restricted applications lack the ability to carry out sophisticated quantitation and annotation of results. We have developed Visualize, a free and open source application that allows the proteomics scientist to visualize proteomics results at the level of individual scans, proteins, and biological pathways. Visualize accepts data from many search engines (Sequest, Mascot, X!Tandem, OMSSSA, and Comet) as well as from the TPP. Data can be visualized at the level of individual spectra, peptides, proteins, runs or across whole experiments. Quantitation can be
carried out using spectral counting, SILAC SPEcTARA, and mass tags (Itraq, TMT). Data can be compared at the level of proteins, peptides, or spectra, and statistical significance and power analysis can be performed across pairs of samples or groups of samples. The integrated Protein Browser allows the user to visualize the observed peptides in the context of physical and biological features of the protein. Proteins can be annotated either using standard databases such as GO, Wikipathways, David, or KEGG, or using user created lists. Data can be exported into Excel workbooks featuring multiple worksheets and embedded graphs or as high resolution pdfs suitable for inclusion in presentations, proposals and publications. Unlike commercial products, the full version of Visualize can be distributed along with results in a compact format without any license restrictions to customers and collaborators.

**Poster 005: Solving the Bioinformatics Bottlenecks of Massive Storage & Data Distribution, Huge Computational Needs, and Flexible and Fast Reporting**

Christine A Jelinek1; Vidya Venkatraman1; John Tra2; Gautam Saxena1; Scott Kuzdzal1; Jennifer Van Eyk1; 1The Johns Hopkins School of Medicine, Baltimore, MD; 2Johns Hopkins University, Baltimore, MD; 1Integrated Analytic Inc., Bethesda, MD; 2Shimadzu, Columbia, MD

Bioinformatics was a significant bottleneck in the JHU NHLBI lab’s ability to store, process, and analyze the vast amounts of data generated by the MS machines. With today’s modern mass spectrometers easily producing one to two gigabytes of data per hour, it was a critical requirement to have an informatics service that could process the MS data at least 10x faster that the MS could generate it. Further, once processed, the JHU NHLBI lab needed a mechanism for the MDs, clinicians, and biologists to interrogate their data in a sufficiently flexible manner and ensure that queries returned within seconds, thus allowing lab members to identify or discard scientific artifacts quickly. Finally, the lab sought a system that would be accessible from any geographic location and would seamlessly integrate the storage and dissemination needs, the massive computation processing needs, and the reporting/analysis requirements, thus increasing lab efficiency, reducing errors, increasing reproducibility, and ensuring auditability.

Working with Integrated Analysis and Shimadzu Scientific, the lab helped build an integrated informatics service that is available to JHU NHLBI from anywhere in the world, scales to terabytes worth of data, and allows high flexibility for querying and reporting of data. The lab has achieved a 100x fold increase in search speeds; reports that previously crashed our high-end desktops now run in less than 30 seconds from a low-end laptop; and the lab’s collaborators have identified putative biomarkers (some of which were validated by ELISA and are now being commercially validated) using the service’s query and reporting abilities.

Looking towards the future, the lab projects opening up the system to reviewers or the scientific community. The larger community could then easily reprocess and query/report/analyze the existing MS data, thus allowing the wider scientific community to re-mine the MS data many times over.

**Poster 006: Construction of Protein Phosphorylation Networks by Data Mining, Text Mining, and Ontology Integration: Analysis of the Spindle Checkpoint**

Karen E Ross1,2; Cecilia N Arighi1; Jia Ren1; Cathy H Wu1,2; 1University of Delaware, Newark, DE; 2Georgetown University, Washington, DC

Knowledge representation of the role of phosphorylation is essential for the meaningful understanding of a wide range of biological processes. However, such a representation is challenging because proteins can exist in numerous phosphorylated forms with each one having its own characteristic protein-protein interactions, functions, and sub-cellular localization. In this paper, we present a bioinformatics framework for the annotation and knowledge representation of phosphorylated proteins and construction of phosphorylation networks. The integrated approach involves: (i) text mining guided by the Rule-based Literature Mining System for Protein Phosphorylation (RLIMS-P), a tool that identifies phosphorylation-related information in scientific literature; (ii) data mining from curated protein-protein interaction databases; (iii) protein form and complex representation using the Protein Ontology (PRO); (iv) functional annotation using Gene Ontology (GO); and (v) network visualization and analysis with Cytoscape. We used this framework to study the spindle checkpoint, a heavily phosphorylation dependent process that monitors the assembly of the mitotic spindle and blocks cell cycle progression at metaphase until all chromosomes have made bipolar spindle attachments. The phosphorylation networks we constructed, centered on the human checkpoint kinase BUB1B (BubR1) and its yeast counterpart MAD3, offer a unique view of the spindle checkpoint that complements the picture provided by existing network building tools such as the Search Tool for Retrieval of Interacting Genes/Proteins (STRING). Our approach can be easily applied to any biological process that is affected by phosphorylation.

**Poster 007: A Fast Peptide Sequence Search Engine**

Chuming Chen1; Zhiwen Li1; Hongzhan Huang2; Baris E Suzek2; Cathy H Wu1,2; 1University of Delaware, Newark, DE; 2Georgetown University, Washington, DC

Peptide match is an important tool in proteomics. The purpose of peptide match is to quickly locate a given peptide in a protein sequence database. The Protein Information Resource (PIR) has developed a new web-based application with fast peptide sequence search engine based on Lucene to facilitate the peptide match activities: (http://pir.georgetown.edu/pirwww/search_peptide.shtml)

The underlying Lucene-based index structure are designed to quickly retrieve all occurrences for a given query peptide in the UniProtKB protein sequences. The matched results then can be narrowed down to a specific set of organisms by browsing the taxonomy tree or taxonomy group. The web site provides cross-links of the query peptide to three popular peptide spectra databases and libraries (gpmDB, PeptideAtlas and NIST Peptide Library) if the query peptide exists in any of these databases. In addition to the web interface to our search engine, we also provide programmatic access to it via RESTful web services. The web application is updated every four weeks, synchronized with the UniProtKB releases.

**Poster 008: New e-Learning Resources in Proteomics: India’s Virtual Proteomics Initiatives**

Sanjeeva Srivastava; Indian Institute of Technology Bombay, Mumbai, India

In recent years, proteomics and related disciplines have been incorporated as part of academic course curriculums across the globe due to its increasing impact on clinical and industrial research. Since development of various expensive and sophisticated proteomics facilities in academic settings remains challenging due to the shrinking educational budgets, primarily in the developing countries, various e-Learning and open-learning programs are gaining popularity. To this end, our research group at Indian Institute of Technology Bombay has developed a Static Virtual Proteomics Lab and Clinical Proteomics Remote Triggering Virtual Laboratory (available at http://lib.lib.co.in/?sub=41), and other related e-Learning resources for proteomics with a motivation to disseminate high-quality educational contents. It is the opening initiative from a developing country for the establishment of a web-based learning platform in proteomics and best to our knowledge, hitherto there is no existing Virtual Lab dedicated exclusively to online proteomics education, except ours. Our Static Virtual Proteomics Lab endeavor has been included as a tutorial article under the International Proteomics Tutorial Programme (IPTP 14) conducted by HUPO and EuPA. It contains three modules; an overview of gel-based proteomics (module I), MALDI-TOF MS (module II) and bioinformatics (module III), each with their own set of experiments. While the Remote Triggering Virtual Laboratory covered global and differential proteomic analysis and their applications in elucidation of mechanisms of drug action, identification of autoantibody response in diseased samples, studying protein interactions, and PTMs. In a separate web-based
Poster 009: How Much of the Human Proteome Has Been Detected? A PeptideAtlas Perspective

Terry Farrah; Eric W Deutsch; Michael Hoopmann; Janice Hallows; Zhi Sun; Chung-Ying Huang; Robert L. Moritz; Institute for Systems Biology, Seattle, WA

A goal of the Human Proteome Project is to characterize at least one protein product from each protein coding gene. Using nextProt as a reference, we assess our progress as a community toward this goal. The Human PeptideAtlas is uniquely suited to aid in this assessment: it is a compendium of 470 (to date) shotgun proteomics datasets representing a wide variety of sample types and technologies and contributed by diverse laboratories around the world. PeptideAtlas processes each dataset through a uniform software pipeline and combines the results to yield a low-redundancy protein list with a stringent 1% false discovery rate. We recently made an effort to maximize proteome coverage in PeptideAtlas by searching for public data sources that either employed the latest technology or targeted classes of proteins that had been so far under-detected. The resulting 2012 Human PeptideAtlas includes at least one peptide for each of ~12,500 novel protein entries among ~7,500 gene products yet to be curated. Thus, we estimate that about 62% of the predicted human proteome has been confidently detected via shotgun methods.

In our presentation we will discuss the strengths and weaknesses of this assessment. We will also characterize those proteins yet missing from PeptideAtlas in terms of tissue localization, transcript abundance, and Gene Ontology enrichment, propose reasons for their absence, and suggest strategies for detecting them in the future.

Poster 010: Keeping Track of Interactomes Using the ProHits LIMS

Guomin (Frank) Liu1; Hyungwon Choi2; Jianping Zhang; Brian Raught3,4; Mike Tyers5; Alexey Nesvizhskii6; Anne-Claude Gingras7; Samuel Lunenfeld Research Institute, Toronto, Canada; 2National University of Singapore, Singapore, Singapore; 3Ontario Cancer Institute, Toronto, Ontario, Canada; 4IRIC, University of Montréal, Montréal, Canada; 5University of Michigan, Ann Arbor, MI

Affinity purification coupled with mass spectrometry (AP-MS) is a robust technique used to identify protein-protein interactions. With recent improvements in sample preparation, and dramatic advances in MS instrumentation speed and sensitivity, this technique is becoming more widely used throughout the scientific community. To meet the needs of research groups both large and small, we have developed software solutions for tracking, scoring and analyzing AP-MS data. Here, we provide details for the installation and utilization of ProHits, a Laboratory Information Management System designed specifically for AP-MS interaction proteomics that we distribute freely to the scientific community at ProHitsMS.com, and under continuous development. The complete ProHits solution (1) performs scheduled backup of mass spectrometry data and initiates database searches (Mascot, X!Tandem, Comet, SEQUEST and the output from the TransProteomics Pipeline are now supported). It stores search results and enables linking the mass spectrometry data to entries in the relational database module called "Analyst", which is also available as a stand-alone application (including as an easy-to-install virtual machine implementation (2)). ProHits Analyst is organized in a hierarchical manner by project, bait, experiment and sample and also serves as an electronic notebook. When a sample is created, mass spectrometry search results can be uploaded. Search results can be explored using a series of viewers, filtered based on mass spectrometry quality, frequency of detection or background lists, viewed in Cytoscape-Web or exported to text or as a PSI XML format for deposition in interaction databases. Importantly, however, search results can be further analyzed using the SAINt statistical tool which is seamlessly integrated within ProHits to derive interaction confidence scores (3-5). With the integration with a number of open source tools and public repositories, ProHits facilitates transparent analysis and reporting of AP-MS data.

Poster 011: An Integrated Protein Repository for Mining Proteomics Data

Martin Dambsø1,2; Bernard Delanghe1; Christian Ravnsvorbg1,2; 1Thermo Fisher Scientific, Odense, Denmark; 2Thermo Fisher Scientific, Bremen, Germany

Modern proteomics must face the challenge of performing bioinformatics analysis and comparison of large datasets. It is a time consuming and at times nearly impossible task to distinguish known proteins from novel proteins in these data sets without proper annotation and comparison with literature sources. Tools are needed that can handle the complexity of these data including: redundancy (same protein but different accession codes or alleles & fragments), different protein database accession codes or outdated accession codes and protein annotation. To resolve these issues we have developed ProteinCenter - a tool that enables efficient data mining and categorizing of large data sets. ProteinCenter contains public sequence databases to form a complete and consistent superset of 13 million protein sequences derived from over 100 million protein records from GenBank, Refseq, EMBL, UniProt, Trembl, PIR, IPI, PDB, Ensembl etc., including more than 10 million outdated accession numbers. Proteins are richly annotated by consolidated annotation from public databases together with high standards annotation from internal computational enrichment of the sequence data. The integrated database can be further analyzed using the SAINT statistical tool which is seamlessly integrated within ProHits to derive interaction confidence scores (3-5). With the integration with a number of open source tools and public repositories, ProHits facilitates transparent analysis and reporting of AP-MS data. Here, we provide details for the installation and utilization of ProHits, a Laboratory Information Management System designed specifically for AP-MS interaction proteomics that we distribute freely to the scientific community at ProHitsMS.com, and under continuous development. The complete ProHits solution (1) performs scheduled backup of mass spectrometry data and initiates database searches (Mascot, X!Tandem, Comet, SEQUEST and the output from the TransProteomics Pipeline are now supported). It stores search results and enables linking the mass spectrometry data to entries in the relational database module called "Analyst", which is also available as a stand-alone application (including as an easy-to-install virtual machine implementation (2)). ProHits Analyst is organized in a hierarchical manner by project, bait, experiment and sample and also serves as an electronic notebook. When a sample is created, mass spectrometry search results can be uploaded. Search results can be explored using a series of viewers, filtered based on mass spectrometry quality, frequency of detection or background lists, viewed in Cytoscape-Web or exported to text or as a PSI XML format for deposition in interaction databases. Importantly, however, search results can be further analyzed using the SAINt statistical tool which is seamlessly integrated within ProHits to derive interaction confidence scores (3-5). With the integration with a number of open source tools and public repositories, ProHits facilitates transparent analysis and reporting of AP-MS data.
towards the bone resorption. In addition, the differences in the abundance of cytoskeletal proteins suggest that, not surprisingly, the osteoclast-specific function of bone resorption requires cytoskeletal remodeling.

Screening of the osteoclast transcriptome and proteome after enriching multinucleated osteoclasts allowed us to characterize dynamic changes defining the osteoclast functional program. This study demonstrates how the use of modern high-throughput tools and a carefully designed, integrative, systematic approach can rapidly identify processes and molecules critical for a biological system.

Poster 013: Exosomes in the Tumor Microenvironment

Meghan Burke 1; Waeowalee Choksawangkarn 1; Suzanne Ostrand-Rosenberg 1; Catherine Fenselau 1; University of Maryland, College Park, MD; 2UMBC, Baltimore, MD

Myeloid-derived suppressor cells (MDSC) are immature myeloid cells that inhibit both innate and adaptive immunity to cancer cells. Inflammation enhances accumulation of MDSC in the tumor microenvironment. It is postulated that exosomes, 40-100 nm extracellular vesicles, participate in carrying out the functions of MDSC. MDSC were induced in BALB/c mice by the 4T1 mammary carcinoma and developed in either a conventional tumor microenvironment or a microenvironment with increased inflammation due to high levels of the pro-inflammatory cytokine IL-1β. Exosomes shed from the MDSC were purified using ultracentrifugation. Qualitative and quantitative proteomics has been used to identify the protein cargo of these exosomes and to analyze differential effects of inflammation. Qualitative surveys using in-gel tryptic digestion followed by LC-MS/MS analysis have identified 927 proteins in exosomes shed from conventional MDSC and 307 proteins shed by inflammatory MDSC. Protein identifications were made using a meta-search program (Pep2RM) which uses Mascot in addition to six open source search engines. The iTRAQ method has been used to quantitatively determine the effect of inflammation on protein abundances in three biological replicates. Ratios were measured for 226 proteins, determined by Proteome Discoverer. Among these, 103 proteins have reporter ion ratios altered by at least 2 fold. Cellular location and functional ontology have been assigned based on annotation in UniProt Knowledgebase. Proteins of high interest include S100/A9, S100/A8, and neutrophilic granule protein.

Functional studies showed that S100A8/A9 proteins in exosomes mediate MDSC chemotaxis, thereby validating and demonstrating the significance of proteins identified by proteomic analysis.

Poster 014: Identification of Nitrogen Load Sensing Pathways Using Integrated Proteomic and Transcriptional Profiling

Nantaporn Haskins 1; Sandra Kirsch Heibel 1; Waeowalee Choksawangkarn 1; Catherine Fenselau 1; University of Maryland, College Park, MD; 2UMBC, Baltimore, MD

Mice were fed control (CP) or high (HP) protein diets on a 13C6-labeled lysine, and analyzed by mass spectrometry and Integrated Proteomics Pipeline, version 1.0f. RNA from the same mice was purified, reverse transcribed into cDNA, and analyzed using Affymetrix microarrays and Partek analysis software. Transcription and proteome data analysis was conducted using Ingenyway Pathway Assist software.

Expression of genes in the mTOR, AMPK and eIF2 pathways change in response to different protein intake. Upstream of AMPK, PI3K, PP2, and PKA were increased in the HP diet compared to CP. Downstream of AMPK, TSC, which is involved in mTOR regulation, was also increased in the HP diet. Enzymes in fatty acid and steroid synthesis were increased in the CP diet compared to the HP diet. In the eIF2 signaling pathway PKR, PI3K, PKD1, and GSK3β, involved in phosphorylation of eIF2α and eIF2β, were increased in the HP diet. As expected, expression of urea cycle enzymes was higher in mice fed HP diet.

These studies show correlation between activities of mTOR, AMPK and eIF2 pathways, dietary protein and expression of urea cycle enzymes. This may lead to new therapies focused on increasing urea synthesis even in the absence of high protein intake.

Poster 015: Combined-metric, Unsupervised Grouping and Fixed-residue Greedy Approximate to Exact Algorithm to Predict N-linked Glycosylation Sites Using Experimentally Identified N-linked Glycosites

Paul Aiyetan; Hui Zhang; The Johns Hopkins Medical Institutions, Baltimore, MD

INTRODUCTION: The development of glycoproteomic technologies for N-linked glycopeptide together with high resolution mass spectrometers identified thousands of N-linked glycosylation sites that contain the consensus NXS/T sequons. We explored these to predict additional N-glycosylation sites from protein database and additional residues that may drive the co-translational glycosyltransferase reaction and perhaps further help refine the N-glycosylation motif.

METHODS: We performed an ungapped multiple alignment of surrounding amino acid about the identified N-glycosylation sites and performed both parametric and nonparametric (combined-metric) evaluation of peptide residue positional ratios and informational content. We explored the positional ratio variance to elucidate region and residues with extreme selectivity pattern and employed an unsupervised clustering to derive non-canonical (cluster-based) groups of amino acid residues. We statistically modeled enrichment scores, functional scores, and posterior probability estimates given functional scores for each identified peptide. We then implemented a fixed-variable approximate to exact algorithm to elucidate motif for predicting N-linked glycosylation sites.

RESULTS: For residues across our training data set containing 4704 identified N-glycosites, there appears a general enrichment of amino acids W, Y, T, and N. In the -5 to +5 positions, a significant aggregated predilection is observed for the amino acids V, I, F, and Y. No significant predilection for a particular or groups of amino acids appear to be observed outside the peri-glycosite region. Derived motifs represented up to 70 percent of N-glycosylation sites in the training set and over 90 percent when algorithmically combined with empirical nearest canonical physicochemical group residues. The trend was equally observed (over 90 percent) in our validating set of 3329 independent glycosylation sites.

CONCLUSIONS: Glycopeptide determining motif is predominantly dependent on the peri-glycosite region. Empirically derived glycosylated peptide sequences, extracted motifs, together with computed scores add a higher level of confidence to a N-linked glycosylated site prediction.

Poster 016: Improving Mass Spectrometry Data Searching Workflow to Maximize Protein Identifications

Shadab Ahmad 1; Amol Prakash 1; David Sarracino 1; Bryan Krastins 1; Mingming Ning 1; Barbara Frewen 1; Scott M. Peterman 1; Gregory Byram 1; Maryann S. Vogelsang 1; Gouri Vadali 1; Jennifer Sutton 1; Mary F. Lopez 2; Thermo Fisher Scientific, BRIMS, Cambridge, MA; 1Massachusetts General Hospital, Boston, MA

Mass spectrometry has become an established method for protein identification and characterization in recent years. However the number of identified proteins remains less than the actual number of proteins supposed to be present in complex biological samples. Mass spectrometry based peptide/protein identification depends on many factors ranging from sample preparation to database searching method. Undesirably, only a fraction of spectra
Poster 017: A Computational Approach for N-glycopeptide Identification and Quantification Using SILAC
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Stable isotope labeling with amino acids in cell culture (SILAC) is a simple and effective approach for mass spectrometry-based quantitative proteomics. Tandem mass spectrometry (MS/MS) is often employed for peptide identification in a SILAC experiment, since the high complexity of the human proteome makes it impossible to identify peptides using LC-MS data alone. Here, we applied the SILAC method to study N-glycopeptides and proposed a computational approach to identify and quantify N-glycopeptides. We first approached this problem from a theoretical perspective and used numerical simulation to investigate whether it is feasible to identify N-glycopeptides without MS/MS data. The theoretical results showed the significantly reduced complexity of N-glycoproteome, established a relationship between the number of uniquely matched peptides and the instrument’s mass precision, and demonstrated the feasibility of identifying most N-glycopeptides without MS/MS data. Further, we proposed a novel computational approach for N-glycopeptide identification and quantification using SILAC by optimally integrating multiple data sources, including human protein sequence, N-glycosylation sequence motifs, numbers of lysine and arginine residues in each glycopeptide, heavy / light peak pairs, mRNA expression profiles and LC-MS data. We applied this newly developed computational method to our SILAC experiment data on SKOV-3 and OVCAR-3 ovarian cancer cell lines. This method successfully identified and quantified 2151 and 2010 glycopeptides in SKOV-3 and OVCAR-3 ovarian cancer cell lines, respectively, and the respectively and the respectively glycopeptides were further verified by MS/MS data showing high specificity.

Poster 018: Comparative Quantitative Analysis of Spectral Counting vs. Precursor Intensity Measurement of Putative Esophageal Adenocarcinoma (EAC) biomarkers
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Esophageal adenocarcinoma (EAC) arises from glandular cells in the lower third of the esophagus towards the junction with the stomach. While the incidence of squamous cell cancer, another family of esophageal cancer, has remained relatively stable over recent years, the incidence of adenocarcinoma has shown a notable increase and prognosis is currently very poor due to late diagnosis. We used the Precursor Acquisition Independent From Ion Count (PACIFIC) method to generate comparative shotgun proteomic data on differentially regulated proteins between patients’ normal esophageal tissue and EAC tissue. Briefly, normal esophageal tissue and EAC tissue were obtained from each of five patients. Protein was purified by Filter Aided Sample Preparation (FASP) method. Samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a LTQ Orbitrap mass spectrometer by using captive spray ionization (CSI). Use of CSI allowed modification to PACIFIC protocol that decreased data generation time for a single sample to 24 hours from standard 2.5 days using electro spray ionization (ESI). Differences in protein quantity were determined by peptide spectral counting. Proteins showing significant differences by spectral counting were subjected to further validation by peptide area under the curve (AUC) quantification using Skyline. We present utility of Skyline to analyze data independent PACIFIC data.

Poster 019: A Phospho-peptide Spectrum Library for Improved Targeted Assays
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The profound and diverse effect of protein phosphorylation has created a keen interest in their characterization and quantitation in biologically relevant samples. However, they present unique challenges to the mass spectrometer. The fragmentation pattern of phosphorylated peptides is less predictable than that for unmodified peptides, increasing the uncertainty of MS/MS spectra identifications and making more difficult the prediction of reliable fragment ions to monitor in targeted assays. Further, the presence of multiple isoforms that are not chromatographically resolved present an additional complication. The intact peptide mass can be used to distinguish between modified and unmodified forms, but one must rely on the specific fragmentation patterns to distinguish between different modified forms when a peptide contains multiple sites for phosphorylation.

Generating SRM targeted assays requires choosing pairs of precursor/fragment ion masses for each peptide of interest. Knowing which fragment ions will produce a strong signal requires either a good prediction model of fragmentation or prior measurement of fragmentation. Because fragmentation of phospho-peptides is difficult to predict, we turned to empirical observation.

We collected spectra from both synthetic phospho-peptides as well as phospho-enriched samples into a spectrum library. By acquiring spectra from carefully selected mixtures of a few synthetic peptides, we improve the throughput over injecting individual peptides while maintaining the certainty of the peptide identification. Furthermore, by acquiring HCD spectra at several activation energies, we have an unbiased measure of relative intensities of fragment ions under targeted assay conditions. Using the information in the library, we were able to compile instrument methods that out-performed methods constructed based on heuristics alone. One may compute which b- and y-ions will differ between two different modified forms of one peptide, but the library provides empirical evidence of which ions are readily observed and may be used as a sensitive diagnostic for a particular modified form.
Poster 020: Direct Characterization of the Human Polyclonal Antibody Response Following Booster Vaccination Against Tetanus Toxoid

Daniel Boutz; Jason Lawler; Yariv Wine; Andrew Horton; Edward Marcotte; George Georgiou; University of Texas at Austin, Austin, TX

Through adaptive immunity, the vertebrate immune system can maintain a memory of previously encountered pathogens, allowing for a more rapid and robust response to subsequent infections. This immunological memory arises from the sequence variability of antibodies (immunoglobulins) expressed by B cells in the B-cell repertoire and bone marrow. The mechanisms and processes behind how the adaptive immune response functions are incredibly complex, and while much has been elucidated since antibodies were first discovered over a century ago, many important questions remain regarding the selection, regulation, and expression of monoclonal antibodies and the B cells that make them. Experimentally addressing these questions is not easy, as direct characterization of the polyclonal antibody response in serum has remained elusive due to technical complications, such as the non-germline origin of antibody sequences. We recently developed a method to overcome these challenges by combining next-generation (NextGen) sequencing of B cells and high-resolution mass spectrometry, and demonstrated its effectiveness in rabbit. Expanding on this method, we now present the first direct and extensive characterization of the human polyclonal antibody response.

Antigen-specific antibodies were identified from the sera of two individuals prior to and following booster vaccination against tetanus toxoid. The large datasets obtained through NextGen sequencing and MS analysis provide a wealth of information, but also present new problems to standard methods of data interpretation, especially for peptide mapping and antibody clonotyping, which required the development of novel approaches. Our results reveal a persistent population of distinct clonotypes which were present before booster and remained detectable at least nine months after. A smaller subset of clonotypes showed more dynamic turnover following the booster and as the response settled into steady-state. Further characterization of specific monoclonal antibodies and peptides provide additional insights into abundance, binding affinity, and structural epitope specificity of clonotypes composing the antigen-specific repertoire.

Poster 021: Comprehensive Antibody Production and Validation Strategies Enhance Reliability and Reproducibility of Immunonarrays for Functional Proteomics

Wayne A. Speckmann; Robin T. Clark; Alejandra Solache; EMD Millipore, Temecula, CA

Antibodies have become an indispensable tool for life science researchers and are utilized in an increasingly diverse number of immunonarrays. Indeed, characterization of the functionality of antibodies has grown in importance as the pace and demands of research compel scientists to focus on expedient acquisition of results rather than on time-consuming screening for application-specific antibody suitability. Despite this, most commercially available antibodies have not been rigorously tested and have only been analyzed via a single method (typically Western blot). EMD Millipore has developed a high throughput antibody production and validation screening process to ensure that only the highest quality antibodies are offered to our scientific partners. Our proprietary antibody development system utilizes automation that has been customized to facilitate every feasible process in production and validation. This enables the development of a sizable and rapidly expanding catalog of polyclonal and monoclonal antibodies against a diverse array of targets while still allowing testing and validation of each in a variety of applications. These include Western blot, immunohistochrometry, immunocytochemistry, flow cytometry, and immunoprecipitation, all employing an extensive library of relevant sample materials. In conclusion, our rigorous process and attention to detail allow EMD Millipore to consistently produce antibodies that are thoroughly validated, enabling functional proteomics researchers to complete their investigations faster and more cost-effectively.

Poster 022: Generalizability of Discoveries from Clinical Proteomic Studies – How Can We Do Better Under Practical Constraints?

Zhen Zhang; Johns Hopkins University, Baltimore, MD

There are a number of practical and mutually constraining factors affecting how discoveries from clinical proteomic studies will generalize in future independent validations. For example, the desire to push the limit of analytical technologies to look deep into the proteome or sub-proteomes of interest often results in a substantial level of assay analytical variability and at the same time severely limits the number of samples that can be processed within a duration of stable instrument performance. Biologically, recent analysis of TCGA data sets confirmed the extreme diversity of the genomic landscape of cancer. Except for a few well-known genes, cancer phenotypes are more likely to be characterized by a complex combination of a large number of low frequency genomic aberrations and for a particular tumor, often only a few such changes are required. From a statistical point of view, discovery of low-frequency truly phenotype-associated proteomic changes using a single set of high-dimensional expression data from a clinical sample set of limited size and with considerable analytical variability will likely be an “ill-defined” problem. In this presentation, we argue and use real data examples to illustrate that an integrated approach in which existing knowledge and data sources are incorporated into the data analysis process in both statistically and biologically meaningful ways could allow us to sufficiently reduce the solution space to a manageable size by information/data “triangulation” while retaining candidate targets that could have a high potential to generalize their performance in independent validations.

Poster 023: Surrogate Biomarkers Discovery and Validation in Duchenne Muscular Dystrophy

Ramya Marathi; Sree Rayavarapu; Kristy J Brown; Jenny Mak; Kanneboyina Nagaraju; Eric P. Hoffman; Craig M. McDonald; Yetrib Hathout; Children’s National Medical Center, Washington, DC; George Washington University, Washington, District of Columbia; University of California, Davis, CA

Duchenne muscular dystrophy (DMD) is one of the most common and severe form of childhood muscular dystrophies affecting about 1 in 3,500 boys. The disease is due to lack of expression of dystrophin, an essential protein for muscle fiber integrity and function. The need to define surrogate biomarkers to monitor DMD progression and response to treatments is becoming crucial as promising treatment strategies for DMD are entering clinical trials. The most widely used clinical endpoint for DMD in the clinic is the 6 minute walk test. However, this test seems to be not sensitive enough in clinical trials where treatments are administered for a short period of time and also impractical for DMD patients who lost ambulation. Thus, defining meaningful molecular endpoints that are linked to the disease progression and that are sensitive to treatments is highly valuable. We have initiated an integrated “omic” study on serum samples including miRNA, proteomics, metabolomics and cytokine arrays assays. We started with proteomics and metabolomics analysis of serum samples of DMD patients (n = 10) and controls (n = 10). Using label free proteome profiling we identified 14 potential biomarkers that were highly elevated (p value < 0.05) in serum of DMD patients relative to controls. Furthermore, using SILAC mouse strategy we identified 14 additional proteins that were highly elevated (by at least 50 fold) and 6 that were decreased by the same factor in serum of dystrophin deficient mouse model relative to wild type mouse. The elevated proteins were mostly of muscle origin while decreased proteins were enzymes. Of these, 7 were validated in serum samples of DMD patients. Finally metabolomics profiling using UPLC-TOFMS identified 38 metabolites that were highly altered in serum of DMD patients relative to controls. These biomarkers await validation in longitudinal study of a large DMD cohort.
Poster 024: Plasma Biomarker Discovery for COPD by 2-D Gel Electrophoresis and Mass Spectrometry.

Kizhake Soman*; Farrah Kheradmand; John Wiktorskiwicz; Zheng Wu; Matthew J. Bautista; Susan Staalform; Kurosky*

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Chronic obstructive pulmonary disease (COPD) encompasses a wide range of tobacco-related inflammatory lung diseases and is now the third leading cause of death in the U.S. COPD is characterized by progressive deterioration of pulmonary function and airflow limitation that is not fully reversible despite smoke cessation. Recently, we have made progress in investigating the pathophysiology of inflammatory diseases of the airways including COPD, with emphasis on biomarkers. Here we have aimed to identify protein biomarkers for stable and acute-exacerbated forms of the disease using plasma samples from 10 smokers in their stable (COPD) and exacerbation (AECOPD) states, and 10 subjects (seven nonsmokers) without COPD as control. Each sample was separated with the Biofluids Analytical Platform (BAP) - developed at UTMB - into a high molecular weight “protein fraction” and a low molecular weight (<23 kDa) “peptide fraction”. Fourteen highly abundant common proteins were removed from the former to enable detection of low abundance proteins. The samples were analyzed in three parallel streams: the protein fraction by 2-Dimensional Gel Electrophoresis (2-DGE) followed by MALDI-TOF/TOF protein identification, and the peptide fraction by label-free LC-MS/MS and 180/160 stable isotope labeling. To identify candidate biomarkers for the stable and exacerbated forms of the disease, protein abundance comparisons COPD vs. Control, and AECOPD vs. COPD were performed in each analysis stream. Six proteins in the protein fraction showed differential abundance for COPD and five for AECOPD, with fibrinogen, haptoglobin, and hemoglobin being common between COPD and AECOPD. The peptides in the peptide fraction mapped reliably (peptide count ≥2, at least one a proteotypic peptide) to as many as 91 distinct proteins, confirming the proposed importance of proteolysis in COPD pathogenesis. Peptides mapping to 13 proteins were markers for COPD and 6 for AECOPD, with three proteins common between the two groups.

Poster 025: Characterization of Multiple Blood Proteomes in African American Stroke Patients

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We report the results of our initial characterization of three sub-proteomes in the blood of African American (AA) stroke patients; namely, the plasma proteome, the whole peripheral blood mononuclear cell (MC) proteome (wMCp), and the nascent MC proteome (nMCp). The wMCp and nMCp are defined as proteomes consisting of all extractable MC proteins, or only proteins that are newly synthesized during a given period of time, respectively.

Blood specimens were collected from AA patients within 24 hours after an acute ischemic stroke. Controls were age- and gender-matched AAs who were hypertensive with no stroke history. Plasma and live MCs were separated from fresh whole blood by centrifugation. The nMCp was metabolically labeled and isolated from the wMCp using a click chemistry-based approach. Pooled samples were obtained from each of the following four groups: Female control, Female stroke, Male control and Male stroke (n=3-5), and analyzed with a bottom-up, label-free quantitative proteomic approach (mass spectrometry: SYNAPT G2-S HDMS (Waters).

An increase in more than 20 plasma proteins was seen in both female and male stroke patients, predominantly in the categories of blood coagulation and immune response. The wMCp showed an increase in proteins associated with defense and immune responses in stroke patients, whereas changes in the nMCp were primarily those involved in translational activities. Gender-specific changes were observed for all three blood proteomes. Examples include an increase in proteins involved in inflammation in female plasma, regulation of body fluid level in female wMCp and nMCp, and response to hypoxia/oxidative stress in male wMCp and nMCp. The nMCp showed more substantial changes than the wMCp did in stroke, implicating its potential as a novel source of biomarkers for stroke. Hence, characterizing multiple blood proteomes is a viable approach for biomarker discovery for stroke, including gender-specific biomarkers.

Poster 026: The Unrestricted Identification of Post-Translational Modifications Without Enrichment in Urine Proteome

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Research on the human urine proteome may lay the foundation for the discovery of relevant disease biomarkers. Post-translational modifications (PTMs) have important effects on the function of proteins and other potential biomarkers. Identifying PTMs without enrichment adds no extra steps to conventional identification procedures for urine proteomics. The only difference is that this method requires software that can conduct unrestricted identification of PTMs. In this study, routine urine proteomics techniques were used to identify urine proteins. Unspecified PTMs were searched by MODa and PEAKS 6 automated software, followed by a manual search to screen out in vivo PTMs by removing all in vitro PTMs and amino acid substitutions. There were 75 peptides with 6 in vivo PTMs that were found by both MODa and PEAKS 6. Of these, 34 peptides in 18 proteins have new in vivo PTMs compared to the annotation information of these proteins on the Uniprot (http://www.uniprot.org) website. These new in vivo PTMs may have implications for disease biomarkers discovery.

Poster 027: Multi-omic and Functional Network Analysis of Paediatric Urine from Patients Diagnosed with Idiopathic Nephrotic Syndrome

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Idiopathic nephritic syndrome (INS) is the most prevalent glomerular disease in children. In spite of progress, its pathogenesis is still unknown and the therapy options are confined to gross immune modulation. A variety of methods for diagnostic and treatment purposes are available for the patients; however, the lack of understanding regarding the pathogenic mechanisms underlying INS can lead to poor therapeutic response and adverse side-effects. Here, we describe quantitative multi-omics approach to reveal new molecular factors involved in pathogenesis of INS with potential diagnostic and therapeutic significance.

Urine samples were collected from 10 children diagnosed with INS receiving no therapy and 10 healthy children. All samples were purified using spin filters followed by affinity depletion of albumin. The purified proteins were recovered and digested with trypsin overnight. Label-free protein expression data were acquired with SYNAPT G2-S using an ion mobility data independent approach, whereby the collision energy was switched between low and elevated energy state during alternate scans and associate precursor and product ions by means of retention and drift time alignment. The acquired data were processed and searched against a human database that was amended to account for N-terminal processed peptides. Normalized label-free quantitation results were generated using TransOmics software. In a similar fashion the diluted neat urine samples were analysed using a small molecule profiling approach.

The resulting data was also analyzed using TransOmics. Interpretation of the data has shown a significant number of proteins to be over-expressed in the urine from INS patients, which includes a high percentage of glycosylated proteins. Metabolites of
interest showing statistically significant changes include homocysteine, glutamate and uridine. Pathway analysis of the complimentary datasets strongly suggests correlation with the neuronal system disorders network, specifically acute fatigue.

Poster 028: A Review of Relative Protein Abundance Estimation from Label-free LC-MS Proteomics
Melissa Matzke; Joseph Brown; Samuel Payne; Carrie Nicora; Anil Shukla; Thomas O. Metz; Joel Pounds; Karin Rodland; Richard Smith; Katrina Waters; Jason Mcdermott; Bobbie-Jo Webb-Robertson; Pacific Northwest National Laboratory, Richland, WA
With the increased interest in the application of MS-based proteomics to clinically relevant problems, it is not surprising relative protein quantification has come to the forefront of computational proteomics research. In particular, label-free shotgun proteomics is highly effective for the identification of peptides in complex biology samples, and subsequently obtaining a global protein profile of a sample, however it does not directly yield protein quantities. Thus, protein quantities must be inferred from one or more measured peptides. The most commonly employed computational approaches for the estimation of protein abundances are considered in the context of a dilution study using a complex biological sample rather than a protein limited spike-in experiment. The dilution design, although contrived, more closely resembles a real-life clinical dataset, including biological variability in the observed response. We present an overview of the performance of these computational approaches in the presence of common issues (e.g., shared peptides) when dealing with clinical proteomics datasets.

Poster 029: Extracorporeal Shock Wave Biomarker Discovery using iTRAQ-enabled Longitudinal Equine Serum Proteome Analysis
Mary Robinson1; Brigitte Simons2; Jesse Vanderhoef1; John Hekko2; Lawrence Soma;1 University of Pennsylvania, Philadelphia, PA; AB SCIEX, Concord, ON
Extracorporeal shock wave therapy (ESWT) is a treatment sometimes applied illegally to racehorses before competition. A method to evaluate the equine serum proteome for potential biomarkers of ESWT has been developed. Equine serum was collected from horses 24 hours before, immediately before (t = 0), and 24 and 48 hours after application of ESWT (n = 2). Samples were depleted of albumin and IgG using multiple affinity removal columns. Remaining proteins were trypsinized and peptides from each sample were isobarically labeled (AB SCIEX iTRAQ). All peptides were combined into a single sample, separated with online two-dimensional liquid chromatography, and detected with mass spectrometry (AB SCIEX TripleTOF 5600). An ab initio database search with ProteinPilot identified 153 unique proteins (FDR = 5%, p < 0.05) representing 68 protein superfamilies. The effect of ESWT on protein abundance was assessed by normalizing all results to the -24 hour sample. Horse to horse variability was assessed by evaluating the ratio of the -24 hour samples from each horse. Day to day variability was assessed by fold changes between the -24 and 0 hour samples. Some interesting fold changes were observed between -24 and 24 or 48 hours after ESWT treatment that were not observed between -24 and 0 hours. This study demonstrates the advantage of using iTRAQ-labeling to multiplex measurements in several samples. Controlling for horse to horse and day to day variability, while simultaneously measuring thousands of peptides in a single LC-MS/MS experiment, is expected to greatly accelerate discovery of ESWT serum biomarkers.

Poster 030: Development of a Targeted LC-MS/MS Assay For Measurement of Farnesylated Lamin A Peptide: Assessment of a Potential Adverse Effect Biomarker
Ronald Miller; Qian Huang; Tracy Diamond; Daniel Spellman; Francisco Dieguez; Mike Miller; Bonnie Howell; Merck Research Laboratories, West Point, PA
Our goal was to develop a target LC-MS/MS assay for measurement of farnesylated lamin A peptide. Such an assay would provide a tool for testing a leading hypothesis of the mechanism of action behind reported lipodystrophy side-effects coinciding with certain marketed viral protease inhibitor treatments. Measuring the generation of farnesylated lamin A peptide, using an in-vitro prelamin A processing model, involved processing nuclear extracts incubated with immobilized prelamin. However, this approach did not prove degradable due to the cleavage peptide in this model. A mass spectrometry-based multiple reaction monitoring (MRM) assay was developed to measure farnesylated lamin A peptide in vitro to ensure the presence of the C-terminally processed cleavage product peptide which would serve as a biomarker for this adverse event. Results indicated a rapid and thorough degradation of the farnesylated lamin A cleavage product peptide in nuclear extract. Thus, evidence was generated to support that this approach would not serve as a viable pre-clinical biomarker to de-risk lipodystrophy.

Poster 031: Matrix Metalloproteinases in Age-Related Macular Degeneration
Stephanie Ecker; Joshua Hines; Bert Glaser; Ocular Proteomics, Towson, MD
The Diagnostic Vitreous Proteome (DVP) project was developed to investigate the proteome of vision threatening diseases and discover biomarkers that can offer personalized treatment for affected patients. For 5 years we have been screening sequential in-office vitreous samples of patients with wet Age-Related Macular Degeneration (AMD) who are receiving intravitreal anti-VEGF therapy using Reverse Phase Protein Microarrays. Matrix Metalloproteinases (MMPs) were chosen as one of our protein families of interest because it has been proposed that they play a role in the progression of AMD through matrix degradation allowing invasion of pathologic blood vessels leading to retinal neovascularization. Our DVP work has revealed MMP-9, MMP-14 and TIMP-2 as proteins of interest in AMD. Our lab has previously showed that MMP-9 levels are significantly higher in wetAMD patients who have subretinal fluid accumulation, a sign of more progressive disease. Recently, we began studying the difference in the DVP of AMD patients who have neovascularization confined to the choroid (CNV)(n= 109) vs. patients who have neovascularization originating in the retina called Retinal Angiogenic Proliferation (RAP) (n= 128). Recent analysis showed elevation of MMP-14 and TIMP2 levels in patients with RAP lesions, (P= 0.0002 and P= 0.0022, respectively). These findings may suggest that RAP lesions require more invasive activity than CNV lesions.

This work suggests that AMD is a heterogeneous disease on a biochemical level and that MMPs may be used as a disease biomarker, treatment biomarker or new pharmaceutical drug target for patients suffering from AMD.

Poster 032: Over-representation of Proteins Identified as Disease Biomarkers and Their Relation to Post-mortem Events
Ulla Sollenberg1; Kim Kutlima2; Mats Borén1; Marcus Söderquist1; Karl Sköld1;1 Denator AB, Gothenburg, Sweden; 2 Upssala University, Uppsala, Sweden
Recent reports show that a group of protein and peptide biomarkers, regardless of tissue origin or species, is often found expressed differentially in various disease states. Here we have investigated a suspected overlap between these proteins and those found to change post-mortem. Proteins with one or more phosphorylation sites are of particular interest since they appear to be over-represented in this group. Data was collected from a range of proteomic studies in which tissue samples were heat-stabilized or snap-frozen to create a list of 56 unique proteins reported to be changed post-mortem. These were compared with another list of 48 proteins often found in 2DE experiments when studying disease states. The identity of 65% (31 proteins) of the disease-changing proteins subset matched with the post-mortem changing proteins subset. When focusing on phosphorylations 39 (81%) of the 48 disease-changing proteins subset have known phosphorylation sites. In successful post-mortem changing proteins, 26 of the 31 proteins (84%) contain at least one known phosphorylation site. This over-representation of phosphorylation sites, compared to a total average of 30%, suggests that proteins, and in particular their post-translational modifications, change substantially post-mortem. Taken together with a recent report of hyperphosphorylation of proteins in non-heat stabilized tissue and
the elimination of 99.6% kinase activity in heat-stabilized tissue samples, we can conclude that it is highly important to quickly inactivate both phosphatases and kinases to ensure reliable measurement of phosphorylation states without interference from post-mortem events.

**Poster 033: The Urine Proteome of Radiation Exposure**

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Intentional or accidental exposure to ionizing radiation has become one of the potentially devastating public health threats because it can affect large numbers of people and can cause irreparable damage to multiple organ systems. Currently, there is no biomarker for early radiation exposure or radiation injury, which is critical for treatment triage and early intervention. Biofluids such as urine is an ideal medium to identify radiation exposure/injury biomarkers because it is easy to collect and can be collected from the same individual over time to monitor injury progression. We tested the potential of identifying biomarkers from urine collected at early time points from C57BL mice exposed to escalating doses of single fraction whole body irradiation (WBI) using 2D LC-MS/MS. Then, a permutation analysis-of-variance (ANOVA) F-test was performed on each protein to detect proteins with significant abundance difference in urine from the WBI groups compared to the non-irradiated group. Our results revealed consistent and significant differences in urine collected from the non-irradiated group and the group irradiated at the lethal dose (10.4 GY) 24 hours and 72 hours post irradiation. Among the signature of high dose irradiation, we found a small list of proteins that are consistently higher in the urine from irradiated mice as early as 24 hours post irradiation. The presence of these proteins indicate early and persist renal dysfunction, inflammation, and kidney/liver injury and could be used to screen for the exposure of a potential lethal or near lethal dose in patients. To monitor the effectiveness of treatment interventions in patients with a fatal radiation exposure, and to evaluate new mitigating agents against lethal radiation.

**Poster 034: Identification, Prioritization and Evaluation of Glycoproteins for Aggressive Prostate Cancer Using Quantitative Glycoproteomics and Antibody-based Assays on Tissue Specimens**

Jing Chen 1; Jieffeng Xi 1; Yuan Tian 1; George Bova 2; Hui Zhang 1; Johns Hopkins University, Baltimore, MD; 2 University of Tampere, Tampere, Finland

Prostate cancer is highly heterogeneous in nature; while the majority of cases are clinically insignificant, some cases are lethal. Currently, there are no reliable screening methods for aggressive prostate cancer. Since most established serum and urine biomarkers are glycoproteins secreted or leaked from the diseased tissue, the current study seeks to identify glycoprotein markers specific to aggressive prostate cancer using tissue specimens. With LCMS/MS glycoproteomic analysis, we identified 350 glycopeptides with 17 being altered in aggressive prostate cancer. ELISA assays were developed/purchased to evaluate 4 candidates, i.e. cartilage oligomeric matrix protein (COMP), periostin, membrane primary amine oxidase (VAP-1) and cathepsin L, in independent tissue sets. In agreement with the proteomic analysis, we found that COMP and periostin expressions were significantly increased in aggressive prostate tumors while VAP-1 expression was significantly decreased in aggressive tumor. In addition, the expression of these proteins in prostate metastases also follows the same pattern observed in the proteomic analysis. This study provides a workflow for biomarker discovery, prioritization and evaluation of aggressive prostate cancer markers using tissue specimens. Our data suggests increase in COMP and periostin and decrease in VAP-1 expression in the prostate may be associated with prostate cancer.

**Poster 035: Reproducibility in Urine Proteomic Profiling by MALDI-TOF/MS**

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Background: Proteomic profiling using MALDI-TOF/MS could be a valuable technology for biomarker identification. However, its use is laden with lack of reproducibility that confounds scientific inferences and limits its broader use.

Aim: We aimed to estimate a background noise to determine the specific signal detection and evaluate different data normalization strategies to derive the best statistical approaches to utilize MALDI-TOF/MS for proteomics.

Methods: We analysed a urine pooled from 10 samples with a 12.58 pmol of a 1589.9 m/z internal standard peptide. For the inter-assay variability assessment, fourteen aliquots were dialyzed by MALDI-TOF/MS. For the intra-assay study, an aliquot was divided into 20 separate sub-aliquots and analyzed by MALDI-TOF/MS. To estimate the signal detection limit (sLOD), serial dilution of a urine pool up to 1/256 were analysed in triplicate. We evaluated the sLOD and adjusted the data appropriately to reduce its variability. We investigated six data normalization approaches – the mean, median, internal standard, relative intensity, total ion current and linear rescaling normalization. Between-spectrum and the overall spectra variability were evaluated by the coefficient of variation (CV).

Results: Within a mass range of 1000 m/z to 4000 m/z, we identified 129 and 122 peaks for inter-assay and intra-assay results, respectively. Normalization methods performed almost similarly in both studies, except internal standard with an increased CV (78% and 26%, respectively). sLOD showed a marked decreasing trend with increasing m/z. With sLOD adjustment, raw data show a drastic reduction in CVs. Median and mean normalizations performed better, especially in the intra-assay study (up to 21% decrease in CV). Data dispersion was mainly reduced by mean normalization. After sLOD correction, median normalization appears as a preferable choice.

Conclusion: Signal detection limit and normalization strategies can increase label-free data reproducibility, especially with sLOD correction.

**Poster 036: Protein Expression Program of Human Bone Marrow-derived Mesenchymal Stromal Cells: Donor-to-donor Heterogeneity and Proteomic Changes During Cell Propagation**

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Mesenchymal stem cells (MSCs) have recently been the focus of intense investigation primarily for their immunomodulatory properties. Several different groups have studied the role of MSCs in grave virus-host disease, but the results are not always comparable. It is becoming more widely recognized that MSCs exist as a highly heterogeneous population, even when they are derived from a single cell clone. Their quality is a function of various interacting variables including donor attributes, cell propagation conditions, and epigenetic and proteomic reprogramming. Currently, there is no validated bioassay method available to reliably describe cell quality, safety, and efficacy. This work aims to further examine the biochemical machinery of these cell populations using mass spectrometry-based proteomic approaches. We performed a comparative survey of proteins expressed by MSCs obtained from six human donors. The comparison spans more than 7700 proteins including 712 transcription and translation regulators, 384 kinases, and 29 cytokines. The data revealed the extent of donor-to-donor variability in the identity of proteins expressed, which was approximated to be at around 21%. Moreover, the expression reprogramming events initiated by cues from an in vitro cell culture was examined using a label-free quantitative strategy.
The expression dynamics of over 1000 proteins were profiled, 200 of which were selected for protein expression patterns. The patterns were confirmed by flow cytometry studies. Proteins such as galecin-1, a protein highly associated with the immunosuppressive capacity of MSCs; FHL2, a co-regulator of gene transcription and associated with epithelial-mesenchymal transition; CD35 and CD243, have been identified to experience up-regulated expression patterns with cell passing. The results were used to study the functional alterations in MSCs initiated by passaging. Furthermore, the significance of these changes in relation to MSC characterization was described.

**Poster 037: Development of Urinary Biomarkers to Monitor Oligomer Treatment in Duchenne Muscular Dystrophy**

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Phosphorodiamidate morpholino (PMO) is a promising drug for treatment of Duchenne Muscular Dystrophy (DMD). PMO successfully restored dystrophin expression and muscle function in both murine and canine DMD models. The drug is currently in phase II clinical trial. However, in order to obtain dystrophin expression in DMD patients, injections of the drug are needed. Pre-clinical studies showed that PMO accumulates in the kidney after high dose IV injections but resolves after a few days. Our goal is to define specific and sensitive urinary biomarkers that are sensitive to accumulation and clearance of PMO from the kidney. These biomarkers will then be used to monitor PMO dosage and scheduling in human patients. In a preliminary study we used a dystrophin-deficient mouse model to define such biomarkers. We first generated an 15N labeled micley colony from which urine was collected and used as a spike-in standard in urine collected from high dose PMO treated (960mg/kg/wk, IV) and PBS treated mdx mice. Urine was collected hourly then daily until 10 days after injections. Spiked urine samples were processed for SDS-PAGE, LC-MS/MS analysis and proteome profiling. In each urine samples, about 169 proteins were identified and quantified. Proteins that were significantly elevated in urine of the PMO treated mouse relative to the PBS treated mouse included, osteopontin, clustenin, gelsolin, cystatin C, beta-2-microglobulin, ACE and GGT1. Interestingly, some proteins were increased in the first day after injection while others were increased in the fourth day after injection. Albumin remained unchanged. Our data demonstrates some potential urinary biomarkers than can be used as tools to monitor treatment with PMO. These await validation in human samples.

**Poster 038: Quantitative Variability Analysis of Normal Twin Urinary Proteome**

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Analyzing urine samples has become increasingly popular in recent years, due to the non-invasive nature of sample collection and reduced complexity of the proteome compared to blood.

To date, urine proteomic approaches have been examined for their potential to serve as biomarkers for various human diseases including cancer, renal failure, and inflammation. However, variability of the urinary proteome is thought to be much greater than in blood. A quantitative measure of the inter-individual variability from normal urinary proteome is reported to be greater than 45%. Determining the normal fluctuation of individual urinary proteins is important in providing threshold guidelines in biomarker discovery.

For the quantitative variability assessment, monozygotic (MZ) twins could offer additional benefit of controlling for environmental factors by eliminating genetic variables. MZ twin pairs share identical genomes at the DNA level and the differences within pairs could reflect environmentally influenced differences.

Early morning urine samples from nine overnight fasted female MZ twin pairs were obtained for quantitative protein variability analysis. Urine samples were analyzed in duplicates by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Velos ion trap mass spectrometer. Six hour-long LC gradient was used to enhance protein identification and data were obtained by data dependent acquisition (DDA). Differences in protein quantity were determined by peptide spectral counting.

The quantitative degree of urinary proteome variation was assessed by Spearman correlation coefficient and Ward hierarchical clustering analyses. Among the nine pairs, three twin pairs clustered together. When a subset of abundant proteins were subject for a further analysis, protein expressions varied by more than 1X10^2 among the nine twin pairs. These preliminary results indicate that urinary proteome variability exceeds the genetic boundaries of MZ twins, reflecting the impact of non-genetic environmental influences in biological samples.

**Poster 039: MALDI Analysis of Human Tears Samples Collected Before & After LASIK Eye Surgery:**

**A Bioinformatic Analysis**

Kenneth Parker; Marvin Vestal; Michael Mines; Denise Ryan; Rose Sia; Kraig Bower; Ryan Walsh; Jennifer Benson; Abdullah Turjoman; Mark Duncan; SimulTof Systems, Sudbury, MA; Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; Ophthalmology Service, Walter Reed Army Medical, Washington, DC; Anschutz Medical Campus, U. Colorado Denver, Denver, CO

Human tear samples were collected from 20 individuals who underwent LASIK vision correction surgery. Tear was collected directly onto a petri dish with buffer and the samples were stored at -70°C until analysis. Samples were then thawed, the tear diluted with water (4 uL: 1:10), and each sample was subjected to dialysis (water) to remove salts and other low MW contaminants.

The dialysate (1 uL), containing the tear peptides and proteins, was then spotted onto a MALDI target and the sample solution (HCCA, 1 uL) was added. MALDI spectra were collected in linear and reflectron modes. Consistent with previous reports, the most intense peaks in the MALDI spectra correspond to intact lysozyme (MW 14,691), intact lipocerin (MW 17,476), and various peptides derived from loricain-specific proline rich protein (PRR4). Most of the PRR4 peptides were members of a series and were related to each other by successive N-terminal or C-terminal truncations, beginning at various internal peptide cleavage sites, or ending at the C-terminal residue of intact PRR4 protein.

Peptide sequences were assigned either by accurate mass mapping, MALDI MS-MS analysis, or both. Several different degradation pathways were evident in different samples. Monoisotopic masses from m/z 800-5,000 and average masses from m/z 1,200-20,000 were separately resampled into mass bins and submitted to principal component analysis (PCA) to determine which masses were inter-related, and to determine which samples were most similar to one another. In addition, a novel modified peptide mass fingerprinting related program was written to identify series of N-terminal and C-terminal truncated peptides. The relationships between the tear constituents and the changes in tear constituents following surgery will be discussed.

**Poster 040: Asymptomatic African Americans with High-risk APOL1 Genotypes Show Signs of Impaired Angiogenesis**

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African Americans having two high-risk APOL1 alleles, termed G1 and G2, are about 7 times more likely to develop non-diabetic kidney diseases. We sought to identify potential urine biomarkers differentially excreted by young, asymptomatic African Americans having high-risk APOL1 genotypes when compared with WT participants. Thirty-seven previously genotyped participants (5 high-risk G1 homozygotes, 20 WT homozygotes, and 12 heterozygotes) donated mid-stream, first morning urine samples. Three label-free urine samples from each homozygote group were processed for SDS-PAGE, LC-MS/MS analysis and proteome profiling. About 315 proteins were identified.
in each urine sample. Proteins that were elevated in urine in those having high-risk ApoL1 genotypes relative to control participants included: angiotensinogen, haptoglobin, pyruvate kinase isozymes M1/M2, alpha-1-proteinase, lymphatic vessel endothelial hyaluronic acid receptor 1, alpha-1-antichymotrypsin, alpha-1-antitrypsin, vesicular integral-membrane protein VIP36. The following proteins were excreted less by high-risk participants: kalikrein-1, aminopeptidase N, syndecan-4, IST1 homolog, leukocyte-associated immunoglobulin-like receptor μ, mucin-20, leukocyte elastase inhibitor, glutathione peroxidase 3, protein HEG homolog 1, and small proline-rich protein 3. Many of these proteins are associated with angiogenesis and subsequent urinary cytokine analysis with Milliplex Human Cytokine/Chemokine Panel bead assay revealed that four angiogenesis-promoting cytokines are significantly reduced in the high-risk group: TGF-α (P=0.004), PDGF-AA (P=0.016), IL-1α (P=0.032), VEGF (P=0.036). This pilot data indicates that young, asymptomatic African Americans with high-risk ApoL1 genotypes may have impaired angiogenesis. These results are consistent with prior evidence indicating that vascular disease may underlie ApoL1-associated kidney diseases. This study was funded by a CTSA pilot award.

**Poster 041: Urimem, a Simple Economical Urinary Urinary Protein Membrane, Potentially Facilitates biomarker Research**
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Biological samples from patients are invaluable for both medical research and medical practice. Ideally the samples should be preserved for the same period of time as the duration of their corresponding medical records. Urine is a body fluid that can be non-invasively acquired and contains important biological information about the patient. It is an important biological sample that can be preserved for each stage of a disease for each patient. Simple and inexpensive urinary protein sample preservation can be the starting point for comprehensive biological sample storage. Here, we propose a method to adsorb urinary proteins to polyvinylidene fluoride (PVDF) membrane that can then be dried and stored. This membrane that stored urinary proteins is named Urimem. This method is very simple and inexpensive and requires minimal sample handling. It does not use organic solvents, and it is environmentally friendly. More importantly, the proteins on the membrane are dry and are prevented from degradation, which keeps the protein pattern faithfully preserved. The membrane may even be able to be stored at room temperature for weeks. The quantity of eluted proteins from a membrane is sufficient for biomarker validation experiments.

**Poster 042: Biomarkers in Human Cervicovaginal Fluid to Predict Labour**
Jan Heng1; Stella Long2; Michael Permezel2; Gregory Rice3; Megan Di Quinzio4; Harry Georgiou1; 1Samuel Lunenfeld Research Institute, Toronto, ON, Canada; 2University of Melbourne, Melbourne, VIC, Australia; 3University of Queensland, Herston, QLD, Australia

Preterm birth (PTB; birth <37 weeks of gestation) remains the major cause of neonatal morbidity and mortality. Better performing biomarkers to predict PTB compared to current tests (e.g. detection of fetal fibronectin) are required. In pregnancy, the human cervicovaginal fluid (CVF) proteome reflects the local biochemical milieu and is influenced by the physical changes occurring in the cervix and adjacent overlying fetal membranes. As term and preterm labour share a final common pathway of cervical ripening, myometrial activation and membrane rupture leading to birth, we hypothesise that CVF biomarkers predictive of term labour are of utility in the setting of preterm labour.

We performed two separate 2D-PAGE analyses (8-16% and 10% gels, n=9 women for each study) on CVF samples collected from women during late pregnancy and in spontaneous term labour. Thirteen significantly altered proteins associated with term labour were IL1RN, CSTA, SOD1, TRX1, GSTP1-1, PRDX2, FABP5, ALBU, ANXA3, COL4A2, SERPINB1, SERPINB3 and SERPINB4 (p<0.05). Using ELISA, we validated IL1RN, CSTA, SOD1, TRX1 in approximately 300 samples (n=70-120 women); these proteins were robust to the influences of vaginal microflora and unprotected sexual activity.

Subsequently, 2D-DIGE and 2D-PAGE analyses were performed on CVF samples collected 6-23 days prior to preterm premature rupture of fetal membranes (PPROM) and subsequent PTB (n=5) compared to gestation-matched controls (n=10). Nine proteins were differentially expressed: IL1RN, TRX1, FABP5, CSTA, GGCT, ANXA3, VDBP, SERPINB1 and SERPINB3 (p<0.05). In conclusion, common differentially expressed proteins between normal term labour and PPROM supports our hypothesis. These proteins are involved in anti-inflammatory activity, protease inhibition and oxidative stress defence; and provided further insights into the complex and diverse biochemical pathways of human parturition. The future creation of a multiplex biomarker test would improve the prediction of parturition, especially PTB.

**Poster 043: Serum peptide Profiles and Cytokines as Biomarkers of Colon Cancer**
Yaping Tian; Dept. of Clinical Biochemistry, Beijing, China

Colon cancer is a kind of malignant disease originating from the epithelial cells in colon tract and the incidence increased markedly in China in recent years. Dietary epidemiological studies showed that there were correlations between the consumption of red meat and processed meat and cancer of the colon, it might be related with the oxidative damage caused by the biochemistry of heme compounds in meat and their oxidative processes in epithelial cells of colon tract. In clinical work, there are only few tumor markers, such as CEA could help the diagnosis and differential of colon cancer from oxidative and inflammation diseases. Lack of specificity and sensitivity were the main problem of these biomarkers. The purpose of this study was to explore whether there were suitable serum markers with enough sensitivity and specificity and then to combine them together to set up diagnostic model to differential diagnosis of colon cancer from colitis and colon polyps. 33 patients with colitis, 62 patients with colon polyps, 101 cases with colon cancer and 83 healthy people were involved in this study. The serum biochemical tests, tumor markers and cytokines have been measured and the serum proteomics have been studied by MALDI-TOF-MS. The results indicated that the serum CEA, CA199, IL-10, CRP, SA and serum peptides (m/z 2022.34, 2932.56 and 4210.57) have been analyzed simultaneously and then establish diagnostic model by using bioinformatics method will be markedly increase the diagnostic sensitivity and specificity. The AUC will be reach 0.929, which is helpful to efficiently differentiate colon cancer from benign colon diseases.

**Poster 044: Deep Proteome Mapping of HeLa and U2OS Human Cancer Cell Lines**
Vadiraja B. Bhat1; Samantha Peacock2; Cristian Ruse2; 1Agilent Technologies, Wilmington, DE; 2Cold Spring Harbor Laboratory, Bungtown Road, NY

Number of expressing proteins and their post translational modifications in given single human cell type is currently unknown and protein expression may differ from cell line to cell line within a single organism. Latest advancements in LC-MS technologies may help to identify most of the expressing proteins in a given cell or tissue. In this study, we investigated HeLa and U2OS human cancer cell lines by LC-MS/MS analysis using nanoLC coupled to a q-TOF mass spectrometer. Protein samples were reduced, alkylated and trypsinized before OGE fractionation. 5-ml of OGE fractions were analysed on iFunnel 6550 nano HPLC-Chip/q-TOF mass spectrometer using 60 min gradient (3-30% B) and the data was analysed using Spectrum Mill bioinformatics tool. In this study, we demonstrated OGE fractionation and LC-MS/MS approach for deep proteome mapping of human cancer cell lines. All together about 10,800 unique human proteins and about 90,000 unique human peptides were identified in the preliminary analysis of HeLa and U2OS human cancer cell lines. About 9,000 unique proteins in HeLa and about 8,200 unique proteins in U2OS cell lines were identified. In this large scale deep proteome mapping study we think that we identified most of the expressing proteins in these two cell lines. Currently we are evaluating expression difference between HeLa and U2OS cell lines.
Poster 045: Towards Cancer Biomarker Discovery Using Clinical Proteomics: The Case for Immunoaffinity Depletion of Abundant Blood-derived Proteins from Tissue Homogenates DaRue Prieto; Xiaoying Ye1; King Chan; Bih-Rong Wei2; R. Mark Simpson2; Josip Blonder1; 1FNLI/SAIC-Frederekich, Inc, Frederick, MD; 2NCI/CCR, Bethesda, MD
Mass spectrometry (MS)-based profiling of clinical specimens (e.g., tissues, bio-fluids) has been increasingly used in cancer biomarker research to characterize changes in protein expression between tumor and healthy tissue or between the blood of diseased and healthy individuals. These molecular profiles may lead to distinct insights that are not readily evident using in vitro cultured cells or animal models and may facilitate discovery of clinically applicable cancer biomarkers. However, the discovery of valid cancer biomarkers using MS-based proteomics has proven difficult, primarily due to the analytical challenges exemplified in the wide dynamic range of human plasma protein levels (i.e., > 10 orders of magnitude) and the fact that the top 10 most abundant proteins constitute ~ 90% of the total plasma protein mass. These highly abundant proteins create a background that interferes with the identification of low abundant biologically relevant biomarker proteins. Arguably, the most effective approach to reduce the background and address this issue is to remove abundant plasma proteins using immunoaffinity depletion. Immunoaffinity depletion of highly abundant proteins from plasma/serum has been conducted as a mandatory step in virtually all proteomic studies utilizing profiling of human blood specimens for cancer biomarker discovery. To our knowledge, there are no reports on using immunoaffinity depletion of abundant blood-derived proteins from tissue homogenates, despite the fact that fresh-frozen tissue contains entrapped blood/plasma, lymph and interstitial fluid. Herein, we demonstrate that the immunoaffinity depletion of abundant blood-derived proteins from fresh-frozen tissue is of equal importance as immunoaffinity depletion of plasma/serum and that tissue homogenates depletion should be adopted as an essential step in the experimental design of any study using MS-based proteomics for molecular profiling of clinical tissue specimens in the context of cancer biomarker discovery/research.

Poster 046: SpikeTides - Peptide Standards for Cancer Biomarker Screening and Monitoring of Tryptic Digestion Karsten Schnatbaum1; Johannes Zerweck1; Lawrence Eckler1; Holger Wenschuh1; Alexander Schmidt2; Christopher M. Coleman1; JPT Peptide Technologies GmbH, Berlin, Germany; 2Universitat Basel, Biozentrum, Basel, Switzerland; 3Keck Foundation, Yale University School of Medicine, New Haven, CT
SpikeTides have recently been described as a low cost peptide source for efficient development of MRM assays. Furthermore, SpikeTides have been used successfully as heavily labeled, internal peptide standards for absolute and relative quantification of protein expression levels (1, 2). In order to fully exploit the potential of this new synthesis and quantification concept, several applications have been targeted. We report on the development of a ready-to-use kit –TrypCheck Kit – that enables monitoring of both, the efficiency and the reproducibility of tryptic digests of biological samples. Since reliable digestion is essential for all MS-based proteomics approaches, the development of robust and validated protocols is crucial (2). The kit described here can be employed within routine protein digestion workflows without the need of additional equipment. Additionally, the peptides can be used as references for retention time calibration in MRM assays. Furthermore, we describe the design and assembly of a SpikeTides – peptide kit containing 252 light or heavily labeled peptides derived from relevant tumor associated antigens (TAA) deduced from an NCI prioritization approach (3) and available SRM assay conditions (4). Initial results of real-life MRM assays using the SpikeTides set in combination with biological samples are presented. Our results demonstrate the ease of implementing the TAA SpikeTides kit for as a cost-efficient multiplexed detection platform for relative quantification of TAA expression levels in biological samples.

(2) Glatter, T. et al., Large-Scale Quantitative Assessment of Different In-Solution Protein Digestion Protocols Reveals Superior Cleavage Efficiency of Tandem Lys-C/Trypsin Proteolysis over Trypsin Digestion. J. Proteome Res. 2012, 11, 5145-5156.
Poster 049: Characterization of the Retinoic Acid Receptor Responder protein-1 by Mass Spectrometry

Haei Seol; Kristy J. Brown; Joseph M. Devaney; Brennan T. Harmon; Roger Packer; Yetrib Hathout; Children’s National Medical Center, Washington, D.C.

Retinoic acid receptor responder protein-1 (RARRES1) has been described as a single pass type II membrane protein. The exact role of this protein is not known. Previous studies suggested that RARRES1 is a tumor suppressor because of its decreased expression by DNA hypermethylation in some tumors. However, in a recent study we showed that RARRES1 protein is overexpressed in certain tumors such as Schwann cells. Moreover, the protein was actually secreted by these tumor cells and not attached to the cell membrane, as suggested earlier. Examination of the published secretome studies revealed that RARRES1 is also secreted by several other tumor cell lines and adipose tissue. This prompted us to characterize the true identity of the RARRES1 protein. The human malignant Schwann cell line (sNF96.2) was cultured and used to express RARRES1 protein. TaqMan gene expression assay was used to quantify RARRES1 mRNA. Total proteins from both conditioned medium and plasma membrane were collected and further fractionated by SDS-PAGE. The bands corresponding to the area where RARRES1 protein migrated was excised, digested with either trypsin or chymotrypsin, and the resulting peptides characterized by LC-MS/MS using LTQ-Orbitrap instrument. To determine the N-terminal peptide, a database search without specific enzyme was used. To determine potential glycosylation sites, PNGase F was used in combination with trypsin and LC-MS/MS analysis. The sNF92.2 cell line expressed full length RARRES1 mRNA. RRARRES1 protein was exclusively detected in the extracellular milieu and undetectable in plasma membrane or cell pellet fraction. Secreted RRARRES1 migrated at higher MW (~180 kDa) in the SDS-PAGE than its predicted 42 kDa, suggesting potential post-translational modifications of the protein. The N-terminal peptide [AAPAGSGDPPDGQPDAGVPR] started at residue 35 consistent with SignalP prediction. Furthermore, the protein was found to be glycosylated at residue 142. This data suggest that RRARRES1 is a secreted glycoprotein.

Poster 050: Nanoporous-based Method for Peptide Signatures Discovery to Predict Breast Cancer Risk of BRCA1 Mutation Women

Jia Fan; Hung-jen Wu; Ye Hu; The Methodist Hospital Research Institute, Houston, TX

Breast cancer (BC) is the most common cancer occurring in women worldwide, and the second most frequent cause of cancer death. Women born with BRCA1 mutation have 50-70% chance to developing BC. But not everyone carried BRCA1 mutation will develop to BC. This attract us to find specific biomarkers that can predict the cancer risk of those people. So far, most of the studies of biomarkers are at protein level. Actually, protein is also a substrate of proteases, and can be cleaved to generate degradation products (peptides). These peptides offer a potentially rich pool for biomarker discovery. Another reason for less attention of peptides is the technological limitation. The current methods still require multi-steps pretreatment and non high-throughput. To address these challenges, we developed nanoporous silica chips to isolate peptides from serum by size exclusion and surface interaction. In this study, the serum samples from four different groups containing 50 patients were screened by MALDI-TOF MS after processing on nanoporous silica chips (Healthy control, Sporadic BC, BRCA1 carried Healthy control, 4.

BRCA1 carried BC). The data were processed using Flexanalysis and ClinProTools software. The hierarchical clustering was performed to analyze peak mass and relative intensity list. Finally, four peptide signatures were found to show unique level changing (including up and down regulation) in BRCA1 carried Healthy and BC groups, respectively. As we expected, some of them have been identified to be the degradation products of proteins, such as, TFF4, FIBA, FPA, CO3 and APAO1. The changing of peptides also reflect the protease activities in cancer, including protease activator and inhibitor. In summary, we provided a sensitive nano-based platform for peptidomic discovery and the peptide signatures will provide a new perspective for cancer study and have a potential to be used for cancer risk predication of BRCA1 carried women.

Poster 051: Automated Analysis of Immunohistochemical Images Identifies Candidate Location Biomarkers for Cancers

Aparna Kumar; Arvind Rao; Santosh Bhavani; Justin Newberg; Robert Murphy; Carnegie Mellon University, Pittsburgh, PA

We have developed an automated system to quantitatively compare the subcellular location of proteins in immunohistochemistry images. Our method is applied to images from the Protein Atlas that cover hundreds of proteins in four normal and cancerous tissues in order to identify proteins that change location in cancer. The pipeline begins by selecting the subset of available images that pass a quality threshold. Next the images are spectrally unmixed into their hematoxylin and diamino-benzidine components, reflecting the distribution of DNA and protein respectively. Regions of each image are sampled and 650 features are calculated on each region to quantitatively describe staining patterns. The normal and cancer features for each image are compared as a nonparametric test to find proteins whose location patterns change significantly. We show that our method finds proteins already known to undergo such changes, as well as a number of new, potential “location biomarkers” for cancer. In addition we identify a number of proteins whose staining pattern can be used to discriminate normal and cancer images with high accuracy.

Poster 052: Improving Efficiency, Quality and Reproducibility in Monoclonal Antibody Generation through Automation and High Throughput Screening

Arazo Saadat; Rey Dimagiba; Alejandra Solache; EMD Millipore, Temecula, CA

Monoclonal antibodies are powerful reagents for the analysis, validation and quantitation of proteins. Being invaluable tools in many biologic assays they continue to be first choice in the biological research field. However, the conventional technology used for hybridoma generation and screening is time consuming and offers very low throughput. With the increasing demand for monoclonal antibodies, the conventional technology could pose a challenge to quality and production capacity. Here we report a novel semi automated approach to producing high quality mouse monoclonal antibodies combining high throughput hybridoma production with protein microarray based screening and selection. The implementation of this technology has allowed us to standardize our monoclonal development process and leading to consistent results and efficient workflow to maximize resources while producing high quality monoclonal antibodies.
Poster 053: A High Resolution/Accurate Mass Targeted Approach for N-Linked Glycopeptides Screening and Quantitation using a Hybrid Quadrupole Orbitrap Mass Spectrometer

Scott M. Peterman; Amol Prakash; Bryan Krastins; David Sarracino; Mary Lopez; Julian Saba; Ming Ming Ning; Shad; Kevin Yarema; Hui Zhang; Weiming Yang; Shuang Yang; Punit Shah; Shisheng Sun; Jian-Ying Zhou; Brooks Jackson; Hui Zhang; Johns Hopkins School of Medicine, Baltimore, MD

N-linked glycans on glycoproteins play an important role in disease state stratification based on the glycan type and composition. Recent developments in sample preparation, LC, MS and MSn data acquisition strategies, and processing software have dramatically increased glycoprotein/peptide studies. However, the number of different glycans present on the peptide backbone still presents challenges for identification and quantitation by mass spectrometry (MS). Increasing the number of glycan composition per amino acid residue dilutes resulting MS signal and reduces the probability of triggering an MSn event. In addition, the location of the N-linked glycan relative to the preferred site of enzymatic cleavage may reduce the digestion efficiency, further diluting the signal while increasing the complexity of the full scan spectrum. To alleviate the issues with glycan heterogeneity, we have incorporated the use of immunoaffinity capture of target glycoprotein(s) and interaction partners and utilized HR/AM LC/MS and MS/MS data analysis acquisition. IP capture significantly reduces the background matrix facilitating increased protein sequence coverage as well as detection, characterization, and quantitation of modified and unmodified peptides using post-acquisition data processing. Byonics software was first used to identify the peptide sequence and glycan composition per glycopeptide. The resulting glycopeptides list was imported into the Pinpoint software Screening Tool to identify additional glycoforms based on HR/AM MS matching of isotopes, charge states, and retention times. The list of glycopeptides identified in the Screening Tool was directly imported into Pinpoint for quantitation across different biological states. The quantitative analysis was performed on the percent composition per amino acid as well as the distribution of specific glycan structures obtained from targeted glycoproteins and interaction partners from serum. IP-MS results were compared to those of whole serum digests.

Poster 054: Targeted Identification of Cell-surface Sialylglycoproteins in Pancreatic Cancer Cells

Yuan Tian; Ruben Almaraz; Caitlin Cho; Qing Li; Danli Li; Punit Shad; Kevin Yarema; Hui Zhang; Johns Hopkins University, Baltimore, MD

Sialic acid modifications on cell surface glycoproteins play important roles in biological and pathological processes. Altered expression of sialylglycoproteins has been reported to correlate with human cancers and may have prognostic significance. We selectively analyzed cell surface sialylated glycoproteins in a pancreatic cell line using combination of metabolic labeling, mass spectrometry, and lectin microarray. Glycoproteins metabolically labeled with azido-sialic acids were biotinylated using Staudinger ligation, isolated by biotin affinity chromatography, and identified by mass spectrometry. In addition to the identification of 9 previously known cancer-associated glycoproteins, 3 novel sialylglycoproteins were further validated using immunoassays. The strategy for selective enrichment of azido-sialylglycoproteins is useful for characterization of cell surface sialylglycoproteins as biomarkers for disease diagnosis and treatment.

Poster 055: A Fully Automated Workflow for LC-MS Analysis of Labeled and Native N-Linked Glycans Released from Proteins

Julian Saba; Udayanath Aich; Xiaodong Liu; Srinivasa Rao; Chris Poh; Thermofisher Scientific, San Jose, CA; Thermofisher Scientific, Sunnyvale, California

Glycans on proteins play significant roles in many biological processes. However, the complex nature of glycans poses challenges to their characterization. LC-MS is a powerful tool for structural characterization. But, the currently available modes of glycans phase that provide do not sufficient selectivity, resolution prior to MS analysis. Here we present a novel stationary phase that provides superior selectivity and resolution compared to traditional modes. We have characterized the structures of N-linked glycans from various proteins using this new column, hybrid quadrupole-orbitrap mass spectrometer and bioinformatics tools, thereby, enabling true high throughput workflow. Initial experiments were carried out using 2AB labeled N-glycans from bovine fetuin. The column enabled separation of glycans based on charge: neutral, followed by acidic from mono- to penta-sialylated. Due to increased resolution and separation provided by the column we were able to identify more glycans, compared to commercial HILIC columns. Additionally, we were also able to use this column to separate native bovine fetuin glycans. To our knowledge this is also the first time the hybrid quadrupole-orbitrap mass spectrometer has been used for free glycan analysis. The primary advantage of this instrument is the ability to generate HCD fragmentation and detect them within the Orbitrap, providing HR/AM fragment ions. This allows for differentiation of near mass fragment ions which we observed to be useful for correctly assigning branching and linkage.

The variation of collision energy can provide different fragmentation within the mass spectrometer. In order to maximize both glycosidic and cross ring fragments, we incorporated step-collision energy. The use of LC-MS/MS for glycan analysis increases the complexity of data analysis due to the large number of MS/MS spectra. We incorporated bioinformatics tool to simplify data analysis, thus enabling the development of a true high throughput workflow. We extended this workflow to characterize glycans from various biological sources.

Poster 056: Comprehensive Analysis of N- and O-linked Glycosylation in gp120 of Human Immunodeficiency Virus Using Glycoproteomics and Glycomics

Weiming Yang; Weiming Yang; Punit Shah; Shisheng Sun; Jian-Ying Zhou; Brooks Jackson; Hui Zhang; Johns Hopkins School of Medicine, Baltimore, MD

Envelope gp120 of human immunodeficiency virus is heavily armored with a dense glycan shield, which plays critical role in envelope folding, immune-evasion, antigenicity, and immunogenicity of the virus. In-depth characterization of the glycosylation sites and their attached glycans of gp120 is desired to understand the antigenicity in developing neutralizing antibodies. In this study, novel glycoproteomic and glycomic approaches were applied to the comprehensive analysis of glycan shield of gp120. Hydrase chemistry based glycoproteomic identification of N-linked glycosylated sites revealed that 18 out of 20 potential N-linked glycosylation sites were occupied and, by the use of mild beta-elimination followed by Michael addition with dibromoethylamine (BEMA), O-linked glycosylated sites were identified on gp120 for the first time. After sialic acids protection by solid-phase labeling, glycomic analysis detected a striking number of 83 different N-linked glycans, of which 38 (46%) were sialylated. Oligomannose glycans (Man5-9GlcNAc2) accounted for 29% of identified N-glycans. Seven O-linked glycans were identified using beta-elimination. Five-hexose was detected as the main O-linked sugar on the gp120 glycoprotein. These data were further supported by the analysis of intact glycopeptides with glycans attached to the peptides using MS/MS analysis of enriched gp120 glycopeptides. Finally, based on our newly acquired detail information toward the glycans and glycosylation sites, 3D model of gp120 was generated to illuminate the defensive potential of glycans in line with their glycosylation sites. The comprehensive analysis of gp120 and the potential 3D structure have implication of immunogens for developing neutralizing antibodies against the glycosylated gp120 on the native glycan shield of HIV particle.

Poster 057: Characterization of N-glycoproteins from Prostate Cancer Cells

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Introduction: Recent studies pointing to the deficiencies of the PSA test and the increasing number of cases of under/over diagnosis/treatment of prostate cancer (CaP) necessitate an urgent clinical need for reliable and specific biomarkers. Aberrant
glycosylation is known to be a hallmark of cancer and shows strong reciprocity with disease progression.

Hence, glycoproteins and their aberrant glycosylation may be useful as diagnostic and prognostic markers that can reliably distinguish aggressive and non-aggressive forms of the disease.

Design: We suggest the involvement of certain N-glycosylated proteins hypothesized to be imparting an aggressive phenotype to the prostate cancer cells based on the observation that cells show reduced migration upon N-glycosylation inhibition with Tunicamycin (TM). Here, we used whole cells and conditioned medium from TM treated and non-treated LnCap and PC3 cell lines which are androgen-dependent and androgen-independent, respectively. Global-proteomic, glyco-proteomic and glycemic analysis were applied to the characterization of tryptic peptides, N-glycopeptides and N-glycans. Obtained MS spectra were searched against protein database and results were analyzed using Bioinformatic tools.

Result: We identified a total of 577 unique glycopeptides from both cell lines with differential expression in each source of sample. Candidate N-glycoproteins were selected for further verification and clinical validation to serve as potential biomarkers in stratifying cancer.

**Poster 058: Glycoprotomic Analysis of Malignant Ascites Fluid for Metastatic Biomarkers of Ovarian Cancer**

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Malignant ascites can be produced in metastatic ovarian cancer and is a rich source of material for glycoproteomic analyses. After separating the fluid from cells in these samples, glycomic and proteomic analyses were conducted on the fluid. Different methods were used to enrich glycosylated proteins and different abundance of N-linked glycoprotein from the fluid. The most successful was lectin binding with different lectins that enriched for glycosylated proteins based on their glycan content. We used three biomarkers (CA125, MUC1 and fibronectin) to monitor the enrichment using Western blot analysis assuming that other tumor proteins with similar glycosylation would accompany these same biomarkers. N-linked glycoproteins were released by PNGase F from the lectin bound fractions, enriched by solid phase extraction with graphitized carbon cartridges and analyzed by nano-LC Chip/TOF or nano-LC q-TOF mass spectrometry. Proteomic analysis of the same fractions was performed by trypsin digestion and peptide mass fingerprinting. The relative abundance of different N-glycan groups in each sample was calculated as a percentage of the total glycan abundance. This measurement allows us to compare the relative abundance of different glycan groups in each of the lectin bound samples. Many of the abundant glycan groups are likely bound to abundant serum proteins so their removals would reduce the presence of these glycans, especially immunoglobulins (IgG). If the proteomic analysis of the lectin bound fractions did not contain immunoglobulins or other abundant proteins, then the remaining proteins in the fractions would be the source of N-glycans identified in the fraction. In this manner, less abundant N-glycans could be identified along with tumor-associated proteins, not normally identified in normal plasma or serum. This top-down effort helps us to identify the glycosylated proteins specific to ovarian cancer and lets us enrich for the protein source of aberrant glycosylation in ovarian cancer.

**Poster 059: Defects in O-glycosylation Due to a Non-functional bus-4 gene, Protect Caenorhabditis elegans from Bacterial Infections**

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The nematode Caenorhabditis elegans is a useful model to study host-bacteria interactions. Several bacteria that infect humans, including Staphylococcus aureus and Yersinia pestis also infect C. elegans. Worms with a non-functional bus-4 gene are resistant to infection by these bacteria and by the worm specific bacterium, Microcystis nematophilum. We purified oligosaccharides from the C. elegans bus-4 and wild type strains and used mass spectrometry based methods to compare the N- and O- glycan distributions between the two strains. N-glycans were not significantly affected, but there was a notable alteration in the distribution of O-glycan oligosaccharides as well as a change in the distribution of monosaccharides between the two strains. Lectin staining of delipidated nematodes with Agaricus bisporus (ABA) lectin, which is specific for O-glycan core-1 sugars containing Galβ1,3GalNAc-, exhibited a decrease in staining in the rectal region where M. nematophilum establishes infection in C. elegans. These results suggest that bus-4, a homologue of a human galactosyltransferase gene, is required for proper O-glycosylation and that proper O-glycosylation is required for infection by S. aureus, Y. pestis, and M. nematophilum.

**Poster 060: Characterization of Glycan Microheterogeneity in Serum Glycoprotein ITIH4**

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Inter-alpha-trypsin inhibitor heavy chain 4 (ITIH4) is a 120 kDa glycoprotein found in serum. ITIH4 is expressed at a basal level in liver tissue and is implicated in liver development and formation; altered levels of ITIH4 have been observed in multiple cancers. Altered glycosyltransferase expression is also known to occur in cancer cells. Therefore, we believe it is important to characterize ITIH4 glycosylation microheterogeneity in order to identify potential cancer-related changes in ITIH4 glycosylation. First, we sought to characterize ITIH4 from (1) HEK cells with ITIH4 over-expressed and (2) serum from healthy individuals. We confirmed that the 4 predicted ITIH4 N-glycosylation sites (NB1, N207, N517 and N577) are glycosylated by treating ITIH4 tryp tic/GluC glycopeptides with PNGaseF in the presence of O18 water. For fifth non-fucosylated glycosylation site was also discovered at N274 with the rare NNV motif. ITIH4 glycosylation microheterogeneity was observed through identification of both N- and O-glycopeptides by performing CID MS/MS on ITIH4 trypsin/GluC glycopeptides enriched via HILIC chromatography. ITIH4 N-glycopeptides contained both high mannose and complex glycan structures. Three of the four observed O-glycopeptides contained more than one SerThr residue; therefore, the site of glycosylation could not be determined. High mannose and complex N-glycans associated with ITIH4 were also detected through MALDI-TOF MS analysis of detached (PNGaseF), permethylated glycans. We are currently comparing microheterogeneity of glycoforms in the over-expressed ITIH4 with the glycoforms of ITIH4 isolated from serum samples. This characterization of ITIH4 glycosylation provides a baseline for future comparison of its glycosylation with ITIH4 purified from patients with hepatocellular carcinoma.

**Poster 061: Using a Novel Method for the O-GlcNAc Site Mapping of Mitochondrial Proteins**

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As a dynamically regulated post-translational modification (PTM), O-linked β-D-N-acetylglucosamine addition (O-GlcNAcylation) plays critical roles in signal transduction, transcriptional control, cell cycle regulation, protein degradation, and stress responses, among others. Abnormally regulated O-GlcNAcylation has been found to be involved in diseases such as diabetes, Alzheimer disease, and heart failure. Although much progress has been made, the detection of O-GlcNAc is still a challenging task by using standard mass spectrometry methods. Therefore, developing highly efficient and sensitive enrichment, identification, and quantification methods is currently required for O-GlcNAc proteomics. To this end, we have developed a novel method. In this method, O-GlcNAc groups in peptides are tagged with UDP- GalNAc in the presence of galactosyltransferase mutant, the tagged peptides can thus be captured by using a multifunctional method.
reagent (which bears a terminal cyclodextrine, a disulfide bridge and a biotin handle) via copper-free click chemistry followed by neutreadin chromatography. The tagged peptides can be released by reductive cleavage and detected by ETD mass spectrometry. In comparison to previous methods, the newly developed one shows higher selectivity, specificity, and sensitivity. The applicability of this method has been demonstrated by the site mapping of rat liver mitochondrial proteins. Twelve O-GlcNAc sites, corresponding to 11 protein groups, were identified from mitochondria.

Moreover, the O-GlcNAcylation of the electron transport chain proteins has been found to result in the overproduction of reactive oxygen species (ROS), which plays a central role for hyperglycemia-induced diabetes.

**Poster 062: Identification of N-glycopeptides Using N-Glycosite Prediction and Accurate Mass Matching**

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Protein glycosylation has long been recognized as one of the most common post-translational modifications. The aberrant glycosylation is a fundamental characteristic of many cancers and other disease states, and most biomarkers and therapeutic targets used in clinical laboratories are glycoproteins. The tandem mass spectrometry (MS/MS) analysis of glycopeptides coupled with MS/MS spectra of label-free sequences in protein database has become effective approach for large-scale glycopeptide identification in complex samples. However, low abundance and important peptides were not identifiable by the MS/MS-based approach due to the poor or lack of MS/MS spectra. Here we describe a novel LC-MS method for high throughput identification of N-glycopeptides based on glycosite prediction using signature 18O labeling of N-glycosylation sites coupling with accurate mass measurement of SILAC-labeled glycopeptides. The method includes following steps: 1) Potential N-glycopeptides from a cell line were predicted using N-glycosylation motif and the gene expression data of the cell line. 2) Glycopeptides were isolated from a tissue sample and SILAC labeled cells by solid-phase extraction of N-linked glycopeptides. 3) SILAC labeled N-glycopeptides from cells were labeled with 18O during removal of N-glycans by PNGase F and mixed with the N-glycopeptides from the tissue sample. 4) The accurate mass of the mixed glycopeptides were measured by LC-MS. 5) N-glycopeptides from tissue samples were identified by accurate mass matching of all 18O labeled peptides and the SILAC-labeled peptides from cells. The 18O labeling on N-glycosylation sites provided signatures of N-glycosites and reduced false positive identification. The lysine and arginine labeled SILAC peptide pairs increased the matching accuracy. The identified N-glycopeptides using this method were further verified by LC-MS/MS spectra with high specificity. The method was used to the identification and quantification of ovarian cancer tissues and showed a great potential in the high throughput analysis of N-linked glycopeptides.

**Poster 063: Visualisation and Identification of Proteins Directly from a Single On-tissue Tryptically Digested Sections Using MALDI Imaging HDMS**

Emmanuelle Claude; Mark Towers; Kieran Nesson; Johannes Vissers; LeRoy Martin; Waters Corporation, Manchester, UK

Mass spectrometry imaging (MSI) was first reported for the direct analysis of intact proteins from tissue. The need for the identification of proteins and therefore their enzymatic digestion has become an essential step in the discovery of biomarkers from tissue. Structural identification is traditionally carried out after processing untargeted MS image data followed by further manual acquisitions either on the same or consecutive sections. A data independent MALDI imaging acquisition method is presented, where MS and MS/MS information are acquired within a single experiment, without any precursor selection or selection requirements. The precursors and the fragments are correlated post acquisition on the basis of their common drift time, which is further refined utilizing spatial distribution commonality.

**Poster 065: MALDI Imaging HDMS: A Novel Data Independent Technique for the Visualisation and Identification of Lipids Directly from Single Tissue Sections**

Emmanuelle Claude; Mark Towers; Kieran Nesson; Johannes Vissers; LeRoy Martin; Waters Corporation, Manchester, UK

Lipidomics is an emerging application area that is taken advantage of the mass spectrometry developments that enabled the simultaneous analysis of a wide range of analytes. The spatial localization of lipids within tissue microstructures is however often lost during the lipid extraction process and challenges image analysis. MS imaging developments on the other hand allowed for the mapping of lipid species. However, the identification of these lipid species is typically performed after processing the untargeted
MS imaging data, followed by further manual acquisitions either on the same or consecutive sections.

A data independent acquisition method called MALDI Imaging (HDMSE) is presented, where MS and MS/MS information are acquired within the same experiment, without any precursor selection. Post-acquisition, precursors and fragments are correlated on the basis of ionic drift in the gas phase, which is further refined utilizing commonality of their spatial distributions. Proof-of-principal experiments have been carried out using part of a rat whole body section where CHCA matrix was applied evenly to the sample in several coats using a SunCollect nebulising spray device. Data were acquired using a MALDI SYNAPT G2 instrument with tri-wave ion guide optics to separate ions according to their gas phase mobility. Within the same MALDI imaging experiment, the mass spectrometer was set to apply alternate collision energies to the transfer cell between low energy and elevated collision energies, with the latter inducing lipid fragmentation. Since fragmentation occurs post-ion mobility separation, the precursors at low energy have the same drift time as their associated fragments from the elevated energy scan. The dataset was subsequently processed using High Definition Imaging (HDI) MALDI software for enhanced image analysis. Drift time alignment and spatial correlation of the data from both low and elevated energy functions were carried out with the same platform.

**Poster 066: Proteomic Biomarker Discovery and Validation: Coupling MALDI Imaging and LC ESI Tandem Mass Spectrometry to Characterize Radiation-Induced Tissue Damage**

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Integral to the characterization of the radiation-induced tissue damage is the identification of unique biomarkers. Biomarker discovery is a challenging and complex endeavor requiring both sophisticated experimental design and accessible technology. The resources within the National Institute of Allergy and Infectious Diseases (NIAID)-sponsored Consortium, Medical Countermeasures Against Radiological Threats (MCART), allow for leveraging robust animal models with novel molecular imaging techniques.

One such imaging technique, MALDI (matrix-assisted laser desorption ionization) imaging mass spectrometry (IMS), allows for the direct spatial visualization of biomolecules (including proteins) in an unbiased manner. MALDI-IMS acquires mass spectra directly from an intact tissue slice in discrete locations across an x, y grid that are then rendered into a spatial distribution map composed of ion mass and intensity. The unique mass signals can be plotted to generate a spatial map of potential biomarkers that reflect pathology and molecular events.

Key sequelae of the acute radiation syndrome (ARS) and the delayed effects of acute radiation exposure (DEARE) are manifested in hematopoietic, gastrointestinal and pulmonary diseases. As such, these organs are prime targets for evaluating radiation-induced tissue damage. In pursuit of on-going efforts to understand the biological ramifications of radiation-induced tissue damage and for development of MCART, we established an experimental platform for untargeted proteome biomarker discovery using MALDI IMS for both mouse gut and lung tissue. In addition, we coupled our discovery MALDI IMS with traditional liquid chromatography electrospray ionization tandem mass spectrometry (LC ESI-MS/MS) for protein identification.

Our preliminary data indicates MALDI IMS results in visualization of unique ion markers between control and irradiated mouse gut and lung tissue. On-going efforts seek to identify the unique ion markers in order to establish identification and validation of protein biomarkers reflective of radiation-induced tissue damage.

**Poster 067: Novel Approaches to Managing and Contrasting Complex Ion mobility MALDI Imaging Datasets**

_Emmamuelle Claude; Marie Claude Djidja; Mark Towers; Kieran Neeson; LeRoy Martin; Janine Erler; Waters Corporation, Manchester, United Kingdom; Institute of Cancer Research, London, United Kingdom_

Mass spectrometry imaging (MSI) has proved to be a powerful analytical tool for the detection, localization and identification of many analytes, including metabolites, lipids and proteins, originating from complex, biological sample surfaces. MSI experiments generate vast amounts of data, depending on image size and acquisition mass range, which will both directly relate to the number of ions detected, the number of pixels and possibly the addition of ion mobility to improve the specificity of the analysis.

Tissue sections were xenograph tissue, where the rat animals were administrated an anti-cancer drug called Dasatinib at a concentration of 30 mg/kg, and scarified at different time points (1 and 3-hours). In situ digestion was performed with a trypsin solution being sprayed directly on the tissue samples and an overnight incubation. Several layers of matrix, a-cyano-4-hydroxycinnamic acid (CHCA) containing anneal in acentonitrile:water:TFA (1:1:0.1), was also sprayed directly onto the tissue samples.

We are presenting a new approach where HDMS Compare software is used in combination with High Definition Imaging (HDI) software. HDMS compare is a powerful analytical tool that investigates the data by comparing two datasets based on multi-dimensional differences in the m/z and drift time domains. It measure differences between samples that are believed to be very similar and were the difference cannot be detected by MS only. After Comparing, Inspecting and Detecting the 2-D plot images, a peak list was generated where the tryptic peptides were more abundant in the 3-hours vs. the 1-hour post dose tissue sections. The peak list was used to generate ion images of the contrasted tissue sections in the HDI software to confirm the highest intensities and identity of the tryptic peptides in the 3-hour tissue section.

**Poster 068: Dissecting PI3K-AKT Pathway by Phosphoproteomic Profiling of PIK3CA Knock-in Mutants**

_Xinyan Wu; Santosh Renuse; Siddiq Zahari; Nandini Sahasrabuddhe; Raghothama Chaerkady; Morassa Mohseni; Jian Yang; Min-Sik Kim; Jun Zhong; Jian Qian; Heng Zhu; Bert Vogelstein; Ben Ho Park; Akhilesh Pandey; John Hopkins University, Baltimore, MD; Institute of Biometrica, Bangalore, India_

The phosphatidylinositol 3-kinase subunit PIK3CA is frequently mutated in human cancers. PIK3CA mutations are observed in ~30% of breast cancers. Three recurrent oncogenic “hotspot” mutations (E542K, E545K and H1047R) comprise the majority of somatic PIK3CA mutations. Although many studies have implicated PIK3CA mutations with features of transformation, definitive mechanisms describing how these mutations promote cell growth and proliferation have not been fully elucidated.

Two MCF10A (a normal mammary gland epithelial cell line) knock-in cell lines with E545K or H1047R hotspot mutations were used for our phosphoproteome study.

We employed stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative mass spectrometry to study phosphoproteomic alterations caused by PIK3CA onogenic mutations. In order to identify signaling proteins directly involved in PI3K-AKT pathway, a novel PIK3CA specific inhibitor (J124) was used to suppress PIK3CA activity. Phosphopeptides were enriched by TiO2-based strategy after strong cation exchange (SCX) fractionation and analyzed on a high resolution LTQ-Orbitrap Velos mass spectrometer. We identified 8,125 unique phosphopeptides from 1,835 unique proteins. Among these, 2,239 peptides were hyperphosphorylated in MCF10A cell with PIK3CA mutants. Notably, the PIK3CA inhibitor, J124, could specifically suppress the phosphorylation levels of 1,187 hyperphosphorylated peptides. These are likely to be effectors of PI3K although only 34 of them
were previously known AKT1 substrates. By integrating data from protein microarray-based phosphorylation experiments with AKT1, we found 6 novel hyperphosphorylated proteins that were phosphorylated in vitro by AKT1. In addition to identifying novel AKT substrates, we also discovered that oncogenic activation of PI3K/AKT pathway could profoundly modulate the activity of a large number of key metabolic enzymes through phosphorylation, particularly the enzymes involved in glycolysis, glycogen synthesis/degradation and pentose phosphate pathways. Our data suggested the fundamental role of PI3K/AKT pathway in regulating the preference of cancer cells for glycolysis, known as the Warburg Effect.

Poster 069: Quantitative Phosphoproteomics Using Novel Deuterium Incorporated Isobaric Reagents, DIART.
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Protein phosphorylation plays a important role in signal transduction in eukaryotic cells. Kinases and phosphatases that bring about reversible protein phosphorylation account for nearly 2-4% of the proteome and approximately 1/3rd of the eukaryotic proteins are phosphoproteins. Filamentous fungi have enormous impact on mankind. As a model organism A.nidulans remains a key organism for genetic studies in filamentous fungi. Inspite of numerous phosphoproteomic studies in the literature, not a single study has focused on A.nidulans. Using affinity chromatographic resins like TiO2 we demonstrate selective enrichment of phosphopeptides using an A.nidulans cell lysate. Using localization software, we identified the line, threonine and tyrosine phosphorylation sites on these phosphopeptides with high confidence.

Furthermore, using synthetic phosphopeptides and an isobaric tagging reagent DIART, we demonstrate a cheaper and robust method to perform quantitative phosphoproteomics. We further apply this methodology to study the performance of DIART on TiO2 enriched phosphopeptides from A.nidulans.

Poster 070: Screening and Validation of Polyclonal Antibodies Against Phosphorylated Targets
Trinette Chuang; April Fisher; Alejandra Solache; EMDMillipore, Temecula, CA
Highly specific and highly validated antibodies are essential tools in most research projects. This specificity testing can be especially challenging for antibodies specific against post-translational modified targets, e.g. phosphorylation. Although several publications have highlighted the importance of stringent validation for these research reagents, as of yet there are no set universal guidelines or standardization. At EMD Millipore, we have developed internal validation criteria that far exceeds the industry standards. We make it our mission to only release antibodies that meet our strictest internal guidelines. We do not hesitate to discard those antibodies that do not meet these stringent specifications.

Poster 071: A Global Phosphoproteomic Screen to Examine acute Phosphorylation Changes Upon Activation of Mutant EGFR Signaling in Human Lung Adenocarcinoma
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Lung cancer is the leading cause of cancer-related death in both men and women. Mutations in the EGFR kinase domain in lung cancer are associated with sensitivity to tyrosine kinase inhibitors (TKIs). Although patients harboring these mutations respond dramatically to TKIs, they eventually develop resistance. In this study, we sought to identify the immediate direct and indirect targets of mutant EGFR signaling in two human lung adenocarcinoma cell lines, H3255 that harbors the TKI-sensitizing mutation, L858R, and H1975 that is resistant to EGFR-TKI and expresses the most common secondary resistance mutation, T790M. We performed a global phosphoproteomic screen using stable isotope labeling by amino acids in cell culture (SILAC), phosphopeptide enrichment, and tandem mass spectrometry to identify dynamic changes in phosphorylation upon EGFR stimulation and TKI inhibition. A total of 8,468 phosphosites were identified in H3255 and H1975 respectively, which correspond to 2,571 and 2,131 proteins. In H3255 cells, 515 phosphosites exhibited increased phosphorylation upon EGFR stimulation and phosphorylation decreased in 306 sites upon TKI inhibition. However, in H1975, although phosphorylation increased in 470 sites upon EGFR stimulation, only 74 sites were dephosphorylated upon TKI inhibition. Ingenuity pathway analysis (IPA) demonstrated several canonical pathways enriched among specific groups of regulated phosphosites, such as IRS, ERK/MAPK, MTOR, EGF, E44, EGF, P70S6K, and PI3/AKT pathways. A majority of phosphosites that were phosphorylated upon EGFR stimulation and dephosphorylated upon TKI inhibition in H3255, such as MAPK1, MAPK3, and RPS6 had no response to TKI inhibition in H1975, suggesting that H1975 is indeed resistant to erlotinib. Interestingly, several phosphosites in H1975 were downregulated upon EGFR stimulation and upregulated upon TKI inhibition. Such interesting subsets of regulated phosphosites may identify novel targets of mutant EGFR signaling as well as on- and off-target effects of a mutant EGFR-directed TKI.

Poster 072: Top-Down High Resolution Electron Capture Dissociation Mass spectrometry for Characterization of Post-Translational Modifications in Mouse Cardiac Troponin
Senfe Ayaz-Guner; Jeffrey W. Walker; Ying Ge; University of Wisconsin-Madison, Madison, WI
Cardiac troponin I (cTnI, 24 kDa) is the inhibitory subunit of the key thin filament troponin-tropomyosin regulatory complex playing a critical role in Ca2+-mediated regulation of skeletal and cardiac muscle contraction and relaxation. Phosphorylation of cTnI is a critical cell signal regulating contractility that is particularly important in modulating cardiac function. cTnI is known to be phosphorylated by protein kinase A and protein kinase C at five sites, Ser22/Ser23, Ser42/44, and Thr143, primarily based on results from in vitro phosphorylation assays by the specific kinase(s). Herein, we have employed top-down high resolution electron capture dissociation (ECD) mass spectrometry (MS) to characterize cTnI purified from wild-type and transgenic mouse hearts which allows for fine mapping of all possible post-translational modifications to one amino acid with 100% sequence coverage. Our top-down MS data unambiguously identified Ser22/23 as the only two sites basally phosphorylated in wild-type mouse cTnI with full sequence coverage, which was confirmed by the lack of phosphorylation in cTnI-Ala22, transgenic mice where Ser22/23 in cTnI have been rendered nonphosphorylatable by mutation to alanine.

Poster 073: An iSDMS Approach Reveals the Potential Linkage Between VPS4B Depletion and Activation of Fatty Acid β-Oxidation in Breast Cancer Cells
Zhongping Liao1; Stefani Thomas3; Yunhu Wan1; Helen Lin1; David Ann1; Austin Yang1; University of Maryland Baltimore, Baltimore, MD, 1Johns Hopkins University, Baltimore, MD, 3City of Hope Medical Center, Duarte, CA
The endosomal/lysosomal system, in particular the endosomal sorting complexes required for transport (ESCRTs), plays an essential role in regulating the trafficking and destination of endocytosed receptors and their associated signaling molecules. Recently, we have shown that dysfunction and down-regulation of vacuolar protein sorting 4B (VPS4B), an ESCRT-III associated protein, under hypoxic conditions can lead to the abnormal intracellular accumulation of epidermal growth factor receptor (EGFR) and aberrant intracellular EGFR signaling in breast cancer. However, the pathophysiological consequences of this abnormal EGFR accumulation and altered EGFR signaling in response to VPS4B-mediated ESCRT-III dysfunction remain largely elusive. In this study, we decided to use a global and internal standard-assisted synchronous intracellular isobaric mass spectrometry (iSDMS) method to address the effects of VPS4B dysfunction in altering EGFR-mediated protein expression. In contrast to many traditional protein expression profiling analyses, iSDMS directly measures the rates of protein synthesis and
degradation and consequently, dynamic protein expression in response to the abnormal EGFR accumulation and cell signaling. Our initial results indicate that VPS4B down-regulation significantly affects the expression of many proteins involved in energy metabolism by changing their rates of synthesis and degradation. In particular, we found that the expression of several proteins involved in glycolytic pathways were down-regulated, while proteins with roles in mitochondrial fatty acid β-oxidation were up-regulated in VPS4B-depleted cells. This observation is also consistent with our previous finding that the expression of VPS4B is down-regulated under hypoxic conditions, suggesting that the adoption of fatty acid β-oxidation could potentially serve as an alternative energy source and survival mechanism for breast cancer cells in response to hypoxia-mediated VPS4B dysfunction.

Poster 074: Qualitative and Quantitative Characterization of the Metabolome, Lipidome and Proteome of Hepatocytes Transfected with Cytochrome P450 Using Data Independent LC-MS
Suzanne Geenen; Lee Gettings; Christian Cojocaru; Giorgis Isaac; Lucy Fernandes; LeRoy Martin; Robert Tonge; Johannes Vissers; James Langridge; Ian Wilson; AstraZeneca; Macclesfield, UK; 2Waters Corporation, Manchester, UK

Drug toxicity is a major reason for the failure of candidate pharmaceuticals during their development. It is therefore important to realize the potential for toxicity in a timely fashion. Many xenobiotics are bioactivated into toxic metabolites by cytochromes P450 (CYP). However, the activity of these enzymes typically falls in in-vitro systems. Recently, a transformed human hepatocyte cell line (THLE) became available in which the metabolic activity of specific CYP isoforms is maintained. THLE cells could be an ideal system in which to examine the potential toxicity of candidate pharmaceuticals. The baseline effect of the addition of CYP2E1 into THLE hepatocytes has been characterized to better understand the biochemistry of this model system.

Dedicated and independent sample preparation protocols were applied in order to isolate metabolites lipids and proteins. Three independent replicates of THLE null or THLE +2E1 cells were investigated for all analyte classes. Proteins were recovered and digested with trypsin overnight. The same LC-MS Omics Research Platform was used for all experiments and generic, application dependent LC conditions applied throughout. In all instances, MS data were acquired using a data independent analysis (DIA) approach, whereby the energy applied to the collision cell was switched between a low and elevated energy state during alternate scans. For the proteomics experiments, ion mobility separation (IM) was incorporated into the analytical schema (IM-DIA). Multimodal data were processed and searched using Transomics software, allowing for normalized labeling quantitation. Pathway analysis and systems biology experiments were conducted to interrogate the datasets further using various bioinformatics tools.

Comparison of the correlation variance and fold change between the two groups illustrates significant analyte expression. Data Comparison of the correlation variance and fold change between interrogate the datasets further using various bioinformatics tools.

Poster 075: Robust and Sensitive Quantitative Measurement of the Growth Promoting Hormone IGFBP via a Mass Spectrometric Immunoassay
Eric Niederkofler; David Phillips; Urban Kieman; Kemmons Tubbs; Bryan Kraitsins; Mary Lopez; Dobrin Nedelkov; ThermoFisher Scientific, Tempe, AZ; ThermoFisher Scientific BRIMS, Cambridge, MA

Mass spectrometry-based methods are becoming more prominent in the targeted measurement of protein biomarkers in biofluids, nonetheless due to lack of sensitivity, robustness, speed of analysis, and throughput, its adoption in the clinical field has been slow. However, recent technological advances in instrumentation and sample preparation and enrichment have begun to enable MS-based targeted protein assays to become a more preferred alternative to classical ELISAs and other automated immunoassays. The use of these technological advancements in the robust, sensitive, and high-throughput measurement of the growth promoting hormone, insulin-like growth factor 2 (IGF2) is presented.

Albeit the physiological role of IGF2 in growth and development still remains largely unknown, IGF2 exerts endocrine, paracrine, and autocrine effects in virtually all tissues and has been investigated as a potential biomarker in a variety of disease states. Currently IGF2 determination is generally used in the diagnosis of non-islet cell tumor hypoglycemia, however, recent demonstration of IGF2’s role in obesity and metabolic and cardiovascular risks have increased interests in IGF2 determination.

To measure IGF2, a mass spectrometric immunoassay was developed that utilizes an affinity pipette device (MSIA-Tips), containing immobilized anti-IGF2 antibodies, to quickly and efficiently extract and enrich IGF2 from biological milieu for downstream analyses by LC-MS.

Equipped with anti-IGF2 MSIA-Tips on a Versette platform, IGF2 was extracted with high-throughput from human plasma samples. IGF2 des1-6, spiked into samples prior to immunoaffinity extraction, served as internal reference for analytical normalization and IGF2 quantification. Following affinity co-enrichment and co-elution, tryptic surrogate peptides of both IGF2 and IGF2 des1-6 were analyzed by LC-MS. Generated linear standard curves spanned from 5 – 1500 ug/mL, with assay linearity and spiking-recovery studies resulting in 90-100%. In addition, the assay proved to be robust by demonstrating intra- and inter-assay precisions of less than 15%.

Poster 076: Accurate Quantitation of Dystrophin Protein in Human Skeletal Muscle Using Mass Spectrometry
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Duchenne muscular dystrophy (MDM) is the most common and severe form of muscular dystrophy caused by nonsense mutations in the dystrophin gene resulting in the loss of dystrophin protein expression. Dystrophin is predominantly expressed in skeletal muscle and is known to play an essential role in muscle fiber integrity and function. Quantitation of human dystrophin protein in muscle biopsies is a clinically relevant endpoint for both diagnosis and response to dystrophin-replacement therapies for dystrophinopathies. A robust and accurate assay would enable the use of dystrophin as a surrogate biomarker, particularly in exploratory Phase 2 trials. Dystrophin is a fairly low abundance protein, accounting for approximately 0.002% of the total striated muscle protein. Currently available methods to quantitate dystrophin rely on immunoblot or immunohistochemistry methods that are indirect and not reproducible.

We developed a mass spectrometry based approach to accurately quantitate dystrophin protein in a total protein extract from human muscle biopsies. Our approach uses a combination of stable isotope labeled dystrophin as a spike-in standard, gel electrophoresis and high precision mass spectrometry to detect and quantify multiple peptides of dystrophin within a complex protein mixture. We tested two approaches; one method using known amounts of 13C6 and 15N2 labeled synthetic peptides as spike-in standards after tryptic digestion of dystrophin and a second method using full length labeled dystrophin as spike-in before tryptic digestion of the samples. Samples were analyzed using QExactive instrument equipped with Orbitrap. Results: The use of full length 13C6-labeled dystrophin as internal standard was found to be superior to the spike-in strategy with standard peptides. Indeed the former method was highly reproducible from run to run and linear over a wide dynamic range, detecting as low as 5% of dystrophin relative to the normal amount in healthy individuals.
Poster 077: Evaluation of Methods for Protein Relative Quantification in Complex Samples

Yan Wang, Waeowalee Choksawangkam; Avantika Dhabaria; University of Maryland, College Park, MD

One major application of proteomics is to identify proteins with changed expression levels under different conditions (control Vs treatment, wild type Vs. mutant, etc.) in a complex proteome. Over the last decade, dozens of tools, both chemically and computationally, have been developed to help with this kind of analysis. In this presentation, we are attempting to benchmark different methods on their effectiveness in identification of differentially expressed proteins in a model system using a shotgun approach.

When looking for differentiated proteins, the underlying assumption is that expression of the majority of proteins (house-keeping proteins) remains unchanged so that the organism will survive. Based on this assumption, we developed a model system with whole cell lysate as a “base” that does not change, and 11 commercially available proteins spiked in at different levels and ratios as “targets”.

Protein mixtures were digested with trypsin and tryp tic peptides were analyzed in triplicate with a 4-hour gradient by nano LCMSMS using LTQ Orbitrap XL. The ipi human protein database (V3.75) was modified to include the 11 proteins that we spiked in for both search engines (Proteome Discoverer and Mascot) prior to data processing.

Methods are categorized into 3 areas and data analyzed with different software tools available to us and evaluated for number of proteins identified and accuracy in protein relative abundance. While the focus is on the 11 “target” proteins, we will evaluate number of “base” proteins that are identified to have altered levels (false discovery). The three areas are: 1. Amine reacting chemical labeling (iTraq, and DiArt), analyzed by Proteome Discoverer 1.3 and Mascot server 2.3. 2. Label free spectra counting, analyzed by Scaffold Distiller 3.6 and Mascot server 2.3 (emPAI) 3. Chromatography based label-free analysis, analyzed by Proteome Discoverer 1.3 (precursor ion quantitation), and Sieve 1.3.

Poster 078: Proteins and Metabolic Pathways with 18O Isotopic Labeling Associated with Effect of Cocaine in the Plasma of HIV Seropositive Women

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Quantitative proteomics using 18O stable isotope labeling is a very promising approach due to its simplicity, general applicability, absence of secondary by products and low cost. However 18O-labeling is difficult to control and a standard protocol of general applicability is still lacking. We present an integrated approach including 1) optimized 18O-labeling protocol suitable for all kind of samples; 2) an improved algorithm (probability ratio) and a refined method to calculate false discovery rates (FDR) for automated peptide identification; 3) labeling efficiency control of each one of the quantified peptides; 4) a validated statistical model for the null hypothesis; 5) a software platform (QuixToT) for semi-automated quantification and statistical analysis. We aimed to identify proteins and metabolic pathways with isotopic labeling with 18O associated with the effect of cocaine use in the plasma of HIV seropositive women as compared to non-cocaine users. Our working hypothesis is that additional protein pathways are activated in cocaine users that may accelerate disease progression. Our preliminary results of the implementation of this method on 2 out of 12 plasma samples from HIV seropositive women with and without cocaine indicate that a total of 802 proteins were identified (FDR<0.05) and 252 proteins were quantified using a lower FDR<0.01.

Poster 079: Investigation of Pressure-enhanced Trypsin Proteolysis

Vera Gross1; Scott Wainsley2; Greta Carlson1; Alexander R. Ivanov3; Alexey Nevizhskii3; Alexander Lazarev2; 1Pressure Biosciences, Inc, South Easton, MA; 2Barnett Institute, Northeastern University, Boston, MA; 3University of Michigan, Ann Arbor, MI

Inefficient proteolytic digestion continues to present significant obstacles for quantitative proteomic workflows. Attempts to improve efficiency of enzymatic digestion have utilized approaches such as enzyme immobilization, chemical additives or manipulation of thermodynamic conditions such as mechanical or thermal energy and high pressure. Nevertheless, our understanding of factors that can affect proteolytic efficiency rests primarily on isolated empirical observations, leaving open opportunities for improvements in digest efficiency as well as construction of better theoretical models.

We present part of a systematic study to investigate the impact of hydrostatic pressure on trypsin specificity. Our goal is to understand the relationship between cleavage site environment and enzyme activity, as a function of high pressure. Five model proteins were digested using three enzyme-to-substrate ratios, two levels of pressure and four time points. Digests were separated on nanoflow HPLC and analyzed by high resolution MS/MS. A custom data analysis approach built on the NIST MSQC pipeline was applied to generate peptide ion intensities and group them together to form the basis for detection of reproducible peptide abundance, thus accounting for various charge states and/or modifications. Data were then grouped by condition/series and the results were analyzed and plotted using the statistical package R.

Our data suggest that pressure effects on proteolysis are substrate protein-specific, and can result in improvements in digestion and quantitative recovery of proteins that are digested poorly under normal conditions. In addition, for most proteins studied, the relative intensities of several peptides recovered after pressure-enhanced digestion were significantly higher than in controls, supporting the suitability of this approach for quantitative workflows.

Poster 080: Development of NIST Reference Material 8321 - Peptide Mixture for Proteomics

Mark Lowenthal; David Bunk; Ashley Beasley-Green; Karen Phinney; NIST, Gaithersburg, MD

The first peptide-based reference material from the National Institute of Standards and Technology is being developed for proteomics applications. RM 8321 - Peptide Mixture for Proteomics - consists of a mixture of synthetic peptides in a buffered solution for the intended use in both intra- and inter-laboratory instrumental validation and quality control. RM 8321 consists of a heterogeneous mixture of synthetic, tryp tic peptides meant to represent a moderately complex matrix allowing users to test instrumental sensitivity and dynamic range capabilities, as well as chromatographic performance and data analysis workflows. Standard peptides were directly mixed to include peptides of varying concentration ranges, with a broad retention-time elution profile, and with comprehensive m/z's. The peptides were selected based on favorable liquid chromatography-tandem mass spectrometry (LC-MS/MS) characteristics, such as good ionizability and fragmentation behavior. A total of 439 synthetic peptides constitute the final mixture of the RM. A qualitative analysis of RM 8321 is described here using LC-MS/MS separation and detection in both an ion trap mass spectrometer and a triple quadrupole
mass spectrometer. Qualitative peptide/protein identifications were performed using both theoretical and experimental tandem MS libraries for improved confidence. RM 8321 will be the first complex proteomics reference material offered by NIST in an ongoing effort to improve instrumental quality control for the proteomics community.

**Poster 081: New On-Bead Digestion Protocols Improve LC-MS Workflows Of Albumin Depleted Samples**

Swapan Roy1,2; Matthew Kuruc2; Biotech Support Group, Monmouth Junction, NJ; ProFACT Proteomics Inc., Monmouth Junction, NJ

Biotec Support Group LLC introduces a new on-bead digestion method for LC-MS proteomic studies. We have demonstrated that the NuGel® silica-based supports perform optimally when using tryptic digestion of the bound proteins, prior to LC-MS; most commonly called “on-bead digestion” in the literature. Key benefits of on-bead digestion are much simpler workflows, eliminates elution, no precaptations, and shorter preparation time. We report on the adaptation of these protocols to our AlbuVoid product. This product severely depletes albumin, by selectively voiding (or not binding) the albumin from serum/plasma. Consequently, it enriches the underlying low abundance proteins. These proteins can then be proteolytically digested on the bead with comparable or better LC-MS performance then protocols involving eluting the proteins. Such protocols are proposed for extension to the complete line of NuGel and related products for hemoglobin depletion, glycoproteins and kinases.

**Poster 082: Cellular Debrionomics: The Isolation of Proteins from Insoluble Cellular Debris Otherwise Excluded from the Analysis**

Gary Smejkal; William Skea; John-David Herlihy; Covaris, Woburn, MA

The disruption of cells, tissues or microorganisms precedes the analysis of centrifugal supernatants, while typically leaving insoluble pellets of "cellular debris" that are excluded from the analysis. Cellular debrionomics examines the residual proteins isolated from centrifugal pellets and whether their exclusion significantly impacts global proteomics analyses. Adaptive Focused Acoustics (AFA) technology can be used to efficiently lyse cells and tissues, as well as improve the solubilization of materials. AFA, dounce homogenization (DH), and cryofracture (CF) were compared for the isolation of total protein, phosphoproteins, and organellar proteins from cryostabilized mammalian tissues. From murine cardiac muscle, AFA supernatants yielded two times more total protein than DH supernatants as determined by Bradford assay, corroborated by multiplexed staining of SDS-PAGE for phosphoproteins and total protein. Two-dimensional gel analysis (2DE) revealed 29 unique phosphoproteins in AFA supernatants that were not detected in DH supernatants. However, when the insoluble pellets of cellular debris produced by DH were subsequently solubilized by AFA, significant numbers of proteins and phosphoproteins not previously detected in the DH supernatants were recovered. Moreover, 59 proteins were present in AFA solubilized DH pellets at two-fold or greater concentration than in their corresponding DH supernatants. Slot blot analysis showed a ten-fold increase of histone H2B2 from porcine cardiac muscle samples processed by AFA compared to DH and CF samples, suggesting these methods were less effective for the disruption of nuclei and potentially biased towards the cytoplasmic components. The incomplete disruption of cellular organelles by DH is further suggested by ELISA, which showed similar quantiles of the cytosolic proteins AKT and pAKT in murine liver samples processed by either AFA or DH, but showed a two-fold increase in the endoplasmic reticulum proteins rpS6 and phosphorylated rpS6 in AFA samples.

**Poster 083: ProDM® – A Colorimetric Kit for Rapid Monitoring and Standardization of Tryptic Digestion for Improved and Reproducible Shotgun Proteomics**

Kutralanathan Renganathan1; Stella B. Somiari1; Steven Wolfe2; Florentina Mayko2; Stephen Russell2; Richard I. Somiari1; ITS-Biosciences LLC, Johnstown, PA; Windber Research Institute, Windber, PA

Digestion of proteins into peptides is a critical pre-analytical step that can affect the success of shotgun proteomics. Insufficient or excessive digestion may result in a failed experiment or the generation of data that is difficult or impossible to interpret, underscoring the need to optimize this step prior to mass spectrometry. Our goal was to determine if ProDM®, a commercially available kit developed for protein digestion monitoring can be used to monitor and standardize tryptic digestion prior to shotgun proteomics of plasma and serum. We compared ProDM to SDS-PAGE and Agilent Bioanalyzer-Protein 80 kit for monitoring plasma and serum protein digestion in buffer systems containing a) only ammonium bicarbonate (Ambic), b) Ambic + Trifluoroethanol (TFE), and c) Ambic + Deoxycholic acid (DOC). The digested samples were analyzed by nano-LC/MS/MS, and the effect of the different buffer systems and duration of digestion on a) number of proteins identified, b) sequence coverage and c) missed cleavages were compared. ProDM® contains ready-to-use reagents and can be used with any spectrophotometer capable of reading absorbance at 595nm. This study demonstrates that TFE was the best buffer system and, ProDM® and Agilent Bioanalyzer detected the presence of undigested proteins that were not obvious on SDS-PAGE gels. Digests containing more undigested proteins had reduced sequence coverage and fewer numbers of identified proteins. Furthermore, digestion for 24h compared to 8h resulted in 6% less proteins identified and 17% less sequence coverage. This presentation describes the extent to which ProDM can be used to monitor and standardize tryptic digestion; eliminate the guesswork during tryptic digestion and thus improve protein identification, sequence coverage and reproducibility.

**Poster 084: In-situ Digestion of Proteins Separated by Thin Layer Chromatography and Paper Electrophoresis Using MALDI-TOF Detection**

Stephen Hattan; Kenneth Parker; Marvin Vestal; SimulTof Systems, Sudbury, MA

Thin Layer Chromatography and Paper Electrophoresis are simple, inexpensive, separation techniques that can be applied to a variety of different sample types. Outlined here is the application of these fundamental techniques for rudimentary protein separations. However, unique to the current protocol is the inclusion of an immobilized enzyme on the paper strips used as a separation substrate. Paper strips modified to contain trypsin and pepsin are demonstrated. Control of enzyme activity is regulated by appropriately selecting and buffering the pH of the system so that, during the separation process, the bound enzyme is inactive. In this manner, for any given sample, separations may be carried out at the protein level. Once the separation is completed, a buffer exchange is performed along the length of the separation strip initiating enzyme activity and the digestion of proteins into their constituent peptides. After adequate incubation time the strip is dried, coated with matrix, and analyzed directly by MALDI-TOF mass spectrometry. The application of this novel methodology is demonstrated for the analysis of protein standards and complex proteomic samples.
Currently, proteomic analyses of formalin-fixed, paraffin-embedded (FFPE) archived tissue are limited by the overwhelming complexity of the cellular proteome and the challenge of dissecting areas of interest, making disease related changes in protein expression difficult to detect in these histological specimens.

Expression Microdissection (xMD) is an operator-independent dissection technology that enables the enrichment of specific cell populations in FFPE samples. In xMD, tissue sections are stained for specific cellular markers, placed in close contact with a polymer film, and then exposed to a light source. The focal, transient heating of the stain initiates the melting of the EVA film over any of the stained targets. This enables specific capture of areas of interest, while leaving unstained tissue behind.

In this study, we used a custom flashcube microdissection device to microdissect nuclei across an entire FFPE rat brain section in milliseconds; we developed a new method to combine and recover proteins from multiple capture films and procured nuclei for targeted proteomic analyses. Shown in these samples was a significant enrichment in nuclear localized proteins, with an average 18% of proteins localized to the nucleus-versus 11% of proteins in whole tissue scrapes (p<0.0001), and a de-enrichment in cytoplasmic and cytoskeletal proteins (28% of proteins in the flashcube-xMD samples versus 37% of proteins in the whole tissue scrapes, p<0.0001, localizations from UniProt).

Multiple Reaction Monitoring (MRM) shows an average of 5-fold enrichment of histones over cytoplasmic proteins.

We demonstrate that this new microdissection technology allows for increased sequence coverage of identified nuclear proteins as well as the identification of methylated lysines and known single nucleotide polymorphisms (SNPs), which was not observed in the whole slide scraped samples. This is the first report that demonstrates a high-throughput, user-independent subcellular procurement method for fixed tissue compatible with downstream proteomic analysis.

Poster 086: Microwave-assisted Disulfide Bond Reduction and Trypsin Digestion Using Rapid Enzymatic Digestion System

Hyunjung Seo; Bong Kyo Seo; Yongha In; Kyu H. Park; Yangsun Kim; Hudson Surface Technology Inc., Fort Lee, NJ, USA

Proteomic analysis has been extensive and comprehensive, handling a sheer number of samples by high-throughput instruments thereby opening the door to many clinical applications. The sample preparation is one of the rate-determining steps in current proteomic studies. We have recently developed Rapid Enzymatic Digestion System (REDS) that harnesses microwave-assisted enzyme reactions, which has successfully shown to save time.

In this study, REDS was applied to three different sizes of proteins; lysozyme, BSA, and IgG to test the effects of microwave on the disulfide linkage reduction by dithiothreitol (DTT)/iodoacetamide treatment. Three proteins were reduced in various REDS conditions according to the change of concentration, temperature, power, and reaction time. The reduced proteins were subjected to trypsin digestion by REDS at 37 Celsius, 250 W for 30 min and analyzed by MALDI-TOF MS. The sequence coverage of lysozyme was 95% after 3 min REDS at 60 Celsius, 250 W of DTT treatment. The results demonstrated that the sequence coverage after 5 min REDS of DTT treatment was similar to the one from the water-bath based conventional method, though the coverage decreased with the increase of the protein size. Using the optimized REDS sample preparation condition and based on the results, we will digest proteins in human saliva within one hour and identified by LC/MS/MS prior to the comparison with the protein identification by the conventional sample preparation method.

This method would considerably contribute to bottom-up proteomics.

Poster 087: Heat Inactivation Enables Reliable Measurement of Tissue Proteome

Olof Sköld; Marcus Söderquist; Denator AB, Gothenburg, Sweden

Tissue sampling leads to major disturbance of the tissue homeostasis. Proteolytic - and other protein-modifying enzymes rapidly change the composition of protein, peptides and their post-translational modifications (PTMs). Subsequent analytical results reflect loss in vivo and products from post-mortem in vivo processes. Enzyme inactivation and standardization of sample handling eliminate this and enables analyses of undistorted information about the ‘pre-sampling’ state. In this work, a heat stabilization system, utilizing conductive heat, at controlled pressure, has been used to generate rapid, homogenous thermal denaturation of enzymes and thereby stop degradation in different kinds of tissue. The heat-stabilized samples were compared to snap-frozen samples and, in time study manner, compared with different post-sampling intervals. The protein and peptide content, including their PTMs, were examined using mass spectrometry, western blot and RPPA. Heat stabilization eliminates enzymatic activity and maintains the proteome integrity judging from the number, intensity, and identity of the peptides detected by MS. Western blot shows that after heat stabilization, levels of phosphorylated forms of CREB, GSK3β and ERK1/2 remain unchanged after two hours at room temperature, whereas snap-frozen samples showed significantly lower levels after 10 min in room temperature. Further rapid changes in phosphorylation states are demonstrated by RPPA on different proteins (pAKT1, pCREB, pATM/ATR substrate and pGSK3b) only minutes after tissue sampling. We conclude that post-sampling changes, particularly in phosphorylation states, may distort our view of in vivo proteomic profiles. When focusing on analysis of protein phosphorylation states, adequate suppression of both phosphatases and kinases is important.

We believe that the use of heat stabilization, as an alternative or complement to conventional snap-freezing, stops enzymatic activity and enables proteome analyses to reflect the in vivo status as closely as possible.

Poster 088: Optimization of Surface Acoustic Wave Nebulizer Designs for Proteomics

Scott Heron1, 2; Scott Edgar1; Yue Huang1; Young Ah Goo1, 2; Michael Wite1, 2; David Goodlett1, 2; University of Maryland, Baltimore, MD; 3University of Washington, Seattle, WA; 4Deurion LLC, Seattle, WA

Recently we reported surface acoustic wave nebulization (SAWN) (S. Heron et al. Anal. Chem. 2010, 82, 3985-3989) as a novel approach for the generation of multiply-charged ions for mass spectrometry from a planar surface. SAWN is softer than electrospray ionization (ESI) (Huang et al. JASMS 2012) and is easily integrated with both digital microfluidics (DMF) and molded microfluidics due to its planar nature. Despite these advantages SAWN sensitivity remains lower than what is observed with ESI.

It has been shown previously (J. Ju et al. Sensors and Actuators A: Physical. 2008, 570-575), that standing wave devices produce smaller more homogeneous distributions of droplets. Using Doppler phase velocimetry we measured the size and distribution of droplets produced by our original SAWN design and while the majority of droplets are below 10 microns in diameter a smaller distribution of much larger droplets were also produced which make up the majority of the sample volume. Larger droplets require significantly more time or higher temperatures to adequately desolvatate before detection by the mass spectrometer.

In an attempt to improve sensitivity we designed a series of new SAWN transducers based on standing waves that included designs for aonetric rings and higher frequencies, to see which is most efficient at generating smaller more homogenous droplets from peptide samples. Preliminary data from MS readings shows us that we have an increased signal intensity using these devices.
This is backed up by data collected from the MS and doppler phase velocimetry for these new designs.

**Poster 089: Cookie Cutter Proteolysis: Achieving Reproducible, Efficient Digestions for Proteomic Workflows**  
Rachel Lieberman; Brian Feild; Scott Kutzdol; Kevin Meyer; Nick Herold; Shimadzu Scientific Instruments, Columbia, MD; Perfinity Biosciences, Inc., West Lafayette, IN

Protein sample preparation workflows for mass spectrometric analysis that involve proteolysis are often labor-intensive, time consuming and user dependent. These workflows often involve digestion, solid phase extraction, drying, and re-suspension prior to reversed phase separation into the mass spectrometer. The introduction of variability at many of these steps hinders discovery initiatives as well as the ability to convert these discoveries into viable assays. Recently, an automated protein digestion platform was developed which accelerates the digestion process through use of immobilized enzyme reaction (IMER) columns. Furthermore, online digestion is integrated with desalting and reversed phase chromatography to create a seamless workflow to the mass spectrometer once the sample is injected. The benefit of streamlining these processes can be demonstrated by the reproducibility achieved.

This presentation discusses how an automated digestion platform benefits protein sample preparation workflows from the perspectives of increasing efficiency and reproducibility. Peptides resulting from 20 benchtop trypsin digests and 20 IMER trypsin digests will be compared. Proteolysis of cell lysates and blood samples in a trypsin IMER being investigated were typically achieved in less than 4 minutes with high levels of peptide recovery and reproducibility along with low carryover.

**Poster 090: Detection of Emerging Microbial Contaminants in Biological Matrices Using Mass Spectrometry Based Proteomics (MSP) Approach**  
Rabih Jabbour; Samir Deshpande; Mary Wade; Robert Webb; Patrick McCubbin; Edgewood Chemical Biological Center, Apg, MD; Science and technology Corporation, Edgewood, MD; Public Health Commands, Edgewood, US; Optimics Inc., Abingdon, MD

The advancement in mass spectrometry based proteomic techniques are showing promising capabilities in addressing various biological and biochemical applications. Effective food monitoring and detection of emerging threat in food samples from processing to consumer facilities are of vital importance to private industry and government institutes. We are developing a mass spectrometry based proteomic (MSP) approach that is capable of providing rapid detection and reliable identification and characterization of known and emerging microbes without the prior knowledge of the samples nor the utilization of specific primers reagents. In this tech the samples are processed using in-house developed biological sample processing system in which the microbial proteins are isolated, enriched, and then digested with trypsin to obtain tryptic peptides. The result tryptic peptides are then exposed to nano-liquid chromatography mass spectrometry/mass spectrometry (nLC-MS/MS) analysis followed by bioinformatics data processing. This approach was utilized for the analysis of different food samples that were contaminated by microbes that either full sequenced and/or not sequenced yet. The result showed that the MSP was capable of providing the identification of the microbial strains and species present in the food samples as well as the background microbes due to the nature of the food matrices. This MSP was also capable of provide a bioforensic profile of the food sample and its contaminants, which is another useful advantage of utilizing such technique. This presentation will address the comparative proteomics analyses of the food samples and provide discussion on the utilization of the MSP technique.

**Poster 091: The Diagnostic Vitreous Proteome: Development and Commercialization of Ocular Diagnostic Tests Based on Vitreous Humor Sampling**  
Joshua Hines; Stephanie Ecker; Bert Glaser; Ocular Proteomics LLC, Towson, MD

To succeed in transforming healthcare in the 21st century, many areas of medicine will need to move towards a personalized approach. The U.S. population is aging rapidly, as nearly 20% of its population (2.7% people) will be 65 years or older by 2030. Blinding diseases associated with aging including Age-related Macular Degeneration, Diabetic Retinopathy & cataract affect roughly 1 in 7 Americans over age 65. By 2015 the expenditures from these diseases are estimated to increase by 60%, and cost $5.084 B in the U.S. alone! The Diagnostic Vitreous Proteome (DVP) has the potential to help physicians eliminate ineffective treatments, saving functional vision for numerous patients and also save millions of dollars per year.

The DVP incorporates two key pieces of technology: an automated instrument for safe and minimally invasive in-office sampling of 50-100 µl of vitreous humor, and a high-throughput Reverse Phase Protein Microarray (RPPM). RPPMs are capable of analyzing several hundred of protein cytokines and activated receptors for each sample, providing a diagnostic tool for predicting clinical response to therapy. While prior diagnostic tools to monitor these blinding disorders primarily used morphologic changes, the DVP is revolutionary because it is the first tool that analyzes biochemical pathways to better determine the state of these diseases. As we have previously demonstrated, activated receptors are shed into the vitreous in and have the ability to show treatment response much sooner than morphological changes alone. By determining the biochemical profile of the eye, for the first time, physicians will have real-time data that will help determine the appropriate individualized treatment for the patient. The ability to control therapeutic options like frequency and dosage will provide unprecedented avenues for prevention and reversal of vision loss and blindness.

**Poster 092: The Studies on Serum Proteomic Profiling as Possible Diagnostic Tools of Cancer**  
Yaping Tian; Dept. of Clinical Biochemistry, Beijing, China

MALDI-TOF MS is widely applied to analyze serum samples for the diagnosis of human diseases and for the identification of potential biomarkers. The various types of magnetic beads might provide different purification capability for low abundance proteins and peptides in body fluids or blood. The aim of our study was to establish a proteome fractionation technique for choosing magnetic beads for proteomic analysis in cancer research. We had compared different kinds of magnetic beads and finally confirmed that MB-WCX could give the best and stable proteomic pattern. Then we have used the established protocol to study different cancers, such as breast cancer, ovarian cancer, endometrial cancer, gastric cancer and liver cancer etc. The MALDI-TOF MS results have been analyzed by ClinProt-Tools to establish a mini-optimized proteomic profile diagnostic pattern to distinguish cancer from other diseases or healthy controls. The results indicated that that MALDI-TOF MS is a high-throughput blood sample analysis method and a potential useful tool for cancer diagnosis by using blood or serum of human. It also could help to identify the potential biomarker and we were indeed found a number of peptides which is valuable for the further studies, such as peptides from complement component C3, apoprotein A1, fibrinogen beta chain and nucleophosmin respectively.
Poster 093: Serological Autoantibody Profiling of Type 1 Diabetes by Protein Arrays

Xiaofan Bian1, Shane Miersch2, Garrick Wallstrom3, Sahar Sibani2, Tamara Bogdanenko4, Olive Healy5, Desmond Schatz6, Mark A. Atkinson5, Ji Gu5, Joshua LaBaer1, 1Biosense Institute, ASU, Tempe, AZ; 2University of Toronto, Toronto, Canada; 3Millipore Corp, San Diego, CA; 4Tufts Medical Center, Boston, MA; 5University of Florida, Gainesville, FL

The need for identifying biomarkers that can provide early diagnosis of Type 1 diabetes (T1D), trace disease progression, evaluate response to therapy and most importantly illustrate disease pathophysiology is well recognized in the T1D research community. Historically, four known autoantigens have been reported as the major targets of autoantibody (AAB). However, the discovery of these autoantigens was based on the known understanding of T1D pathogenesis and restricted in the landscape of pancreatic islet by radioimmunoassay (RIA) or immunoprecipitation. No systematic screening of individual proteins at the proteome level has been reported to search for new T1D AAB biomarkers yet. In the present work, we established a pipeline to screen for novel T1D associated AAB biomarkers using the innovative protein microarray platform—Nucleic Acid Programmable Protein Array (NAPPA).

We conducted a two-stage, sequential serological immunoreactivity screening study and a validation study. During the first stage of screening, we eliminated uninformative proteins by screening a relatively small number of 50 patient and 20 matched control samples on ~6000 human proteins, and selected around 750 proteins for further testing. These genes were printed in duplicate and challenged with 74 cases and 75 controls in the second stage of screening, resulting in the identification of 26 candidate autoantigens including ZnT8 which is a known autoantigen in T1D that fit our criteria (p<0.005, FDR <10%). 10 such candidate genes were selected for further validation via LIPS assay in an independent serum set with 46 cases and 46 controls. Using this assay, we confirmed the performance of a minor T1D autoantigen with a sensitivity of 36% at the specificity of 98%, thus demonstrating the power of protein microarrays in the search to identify novel T1D AABs. The establishment of this approach may help promote the AAB study in T1D in the long term.

Poster 094: Temporal and Spatial Proteomic Analysis of Human Cytomegalovirus Infection, Assembly, and Egress

Rommel Mathias1, 2, Todd Greco1, Ileana Cristea1, Princeton University, Princeton, NJ; 3La Trobe University, Melbourne, Australia

The betaherpesvirus human cytomegalovirus (HCMV) is among the most common animal viruses, and its viral genome is predicted to encode over 190 viral proteins (strain AD169). The double-stranded DNA genome is housed within the nucleocapsid, surrounded by a layer of tegument, and encompassed by a membranous envelope. Assembly of the infectious virion is an elaborate process of considerable interest that still remains largely undefined. In the nucleus, capsid assembly, maturation, DNA packaging and initial tegumentation is thought to precede nuclear egress, followed by cytoplasmic tegumentation and envelopment. Major gaps governing the latter molecular events still exist. To gain a comprehensive understanding of the time- and space-dependent process of virion assembly, proteomic profiling was performed of lung fibroblast MRC5 cells following HCMV infection (strain AD169). Analyses were performed at different stages of the infectious process by harvesting cells at 0, 24, 48, 72 and 96 hours post infection. Organelles from MOCK- (uninfected) and HCMV-infected cells at all times points were fractionated by density.

Interestingly, the buoyant density of the organelles increased following infection, with significant differences being observed at the late stages of infection. These findings are in agreement with the formation of viral vesicles during the processes of assembly and egress late in infection. The fractionated organelle samples (a total of 186 samples per biological replicate) were analyzed by GeLC-MS/MS using Orbitrap instrumentation. A total of 5050 proteins were identified, including 104 viral proteins, a quarter of which remain uncharacterized. Label-free spectral counting revealed diverse differences in viral protein abundance, time of expression, and cellular localization. These results demonstrate that certain still uncharacterized viral proteins specifically associate with cellular organelles, such as endosomes, in a temporal-specific manner. This is the first study to comprehensively define the changes in organelle-specific proteomes following HCMV infection.

Poster 095: In-depth Proteomic and Bioinformatic Analysis of Plasmodium Falciparum Male and Female Gametocyte Infected human Red Blood Cells

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Plasmodium sexual stages (gametocytes) are the only forms of the malaria parasite that are transmitted from humans to mosquitoes. Critical interactions of gametocyte proteins with compartments of the host human red blood cell (RBC) may influence gametocyte morphology, clustering, sex differentiation and parasite egress during gametogenesis. Although sex-specific gametocyte proteomes are available for the rodent malaria parasite, P. berghei, no such dataset exists for the important human malaria parasite, P. falciparum, and there is concern about the lack of conservation of protein markers between parasite species. We used an assortment of sample preparation methods prior to LC-MS/MS analysis to acquire the proteomes of the normal human RBC and P. falciparum NF54 stage V gametocyte infected RBCs (GiRBC). High abundance proteins in RBCs hinder identification of low abundance proteins, especially since such proteins can interfere with protein identification in the MS data-dependent scan mode.

To overcome this limitation, we used the peptide ligand library technology to balance the protein concentration in the RBC sample, which resulted in a 5-fold increase in the number of high confidence protein identifications, to acquire the complete NF54 GiRBC proteome, we analyzed both soluble and membrane proteins and to support the characterization of the sex-specific proteomes, we also acquired the GiRBC proteome of the P. falciparum Dd2 clone, which only produces mature, female stage V gametocytes. In total, 724 and 864 Plasmodium proteins were identified from Dd2 (female) and mixed-sex NF54 GiRBC samples, respectively. We then identified orthologs from the completed P. berghei sex-partenioned gametocyte proteomes. Doing so added confidence to our identifications of putative falciparum male/female gametocyte markers. We have produced a baseline dataset with ~2000 high confidence RBC and GiRBC proteins, which in turn will help lay the groundwork for understanding the interaction of parasite and host cell proteins for this critical developmental stage.

Poster 096: MMP-9 is Differentially Expressed in the Macrophage Secretome of Hispanic HIV+ Women with Neurocognitive Impairment

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Perivascular macrophages and microglia are the primary cells infected with HIV-1 in the brain. Upon infection, macrophages release proteins that may be related to neuronal degeneration and death. However, not all HIV patients develop neurocognitive disorders. Our hypothesis is that changes in the macrophage secretome play an important role in the emergence of cognitive impairment in HIV-1 seropositive women. Investigating differences in macrophage protein secretion is of primary importance to understand signaling and metabolic pathways that may be altered during the incidence of HIV-1 associated neurological disorders (HAND). Macrophages (MDM) were isolated from the peripheral blood of 9 HIV+ and 3 HIV- women characterized for neurocognitive function by adherence of monocyes in culture for 7 days. Serum-free supernatants were collected for protein isolation and quantification prior to iTRAQ labeling. Protein identification was performed using a LTQ Orbitrap.
Poster 097: Measurement of Membrane Transporter Proteins in Human Lung using Quantitative Targeted Absolute Proteomics (QTPA)

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Introduction: The disposition of drugs in the lung may be modulated by transporter proteins present. We report here the development and application of a QTPA method using multiplexed MRMs via LC-MS/MS to quantify 12 transporters in membrane fractions from human lung and various cell lines (e.g. A549 cells). The 12 transporters selected for quantification were P-gp (MDR1) (ABCB1), BCRP (ABC2), OCT2 (SLC22A2), OCT3 (SLC22A3), OATP2B1 (SLC21A6), OATP1A2 (SLC01A2), MRPA (ABCC12), OATP2A1 (SLCO2A1), OATP4C1 (SLCO4C1), OCTN1 (SLC22A4), OCTN2 (SLC22A5) and PEPT2 (SLC15A2).

Methods: The QTP method employed 13C and 15N labeled peptide standards (C-terminal R or K) (JPT Spike Tides_TQL) using an isotope dilution approach for quantification. Membrane fractions from 5 human lung autopsy tissue and various cell culture samples were prepared by homogenization and sequential centrifugation. Addition of heavy labeled peptide standards (C-terminal R or K) (JPT Spike Tides_TQL) (SLC22A4), OCTN1 (SLC22A4), OCTN2 (SLC22A5) and PEPT2 (SLC15A2).

Results: Optimal digestion for 22 h provided measurable proteotypic peptides for 9 of 12 transporters evaluated, with OATP1A2, MRPA and OCTN2 the only proteins below the LOD of 0.1 pmol/mg membrane protein. Replicate (n=5) analysis from one lung had CVs < 9.5% for 9 peptides measurable, with another peptide having CV=21%. Expression of 6 proteins was quite low (0.1-2 pmol/mg) and variable across the 5 lungs, often varying > 10 fold. OATP2A1 was unique with relatively high expression, from 0.5 to 69 pmol/mg. It had low expression in two lung samples from smokers. These results may assist in understanding drug disposition in the lung.

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

Poster 098: Proteomic Analysis of Integrin Signaling Pathway in Pancreatic Carcinoma Cell lines (BxPC-3) and Potential Targets for Therapeutic Intervention

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Pancreatic cancer is one of the most aggressive human malignancies and ninth leading cause of cancer death in the world. Most patients diagnosed with pancreatic cancer die within 6 months, and only 4% survive 5 years after diagnosis. Early diagnosis and better treatments are desperately needed to improve the survival rate of pancreatic cancer patients. The proteomic analysis of BxPC-3 3 pancreatic cancer cell lines we were able to identify 41 proteins involved in oxidative stress response in pancreatic cancer and among them the presence of SOD2-mitochondrial, peroxiredoxin 2 and 4 and glutathione peroxidase was quite prominent, whereas these proteins were absent in control, suggesting aberrant regulation of novel homeostasis and stress adaptation in cancer cells. The high throughput proteomic analysis of oxidatively stressed BxPC-3 pancreatic cancer cell lines has identified the presence of prominently two integrin signaling pathways which might be involved in triggering PDAC. The existence of Cav-1-Fyn-SOS-cRAF-ERK signaling pathway reveals that oxidative stress might activate aavinol-1 protein in the membrane which in turn activate the downstream Proto-oncogene tyrosine-kinase Fyn and triggers MAPK/ERK pathway and finally activate the transcription of oncogenes. Other key pathway of integrin signaling is Focal Adhesion Kinase (FAK), which diversely interacts with cytoskeletal proteins like actin, talin, vinculin, and Factin and these proteins are important in the formation of focal adhesions, and play role in activation of integrin signaling involving RAC, PAK, MKK and JNK pathway. In approximately two-thirds of the PDAC cases, a defect in integrin signaling has been identified. Interestingly, the inhibition of integrin heterodimer αvβ6 resulted in a significant reduction in cell proliferation and invasion, and it could be proposed as therapeutic target against pancreatic cancer adenocarcinoma.

Poster 099: Comparison of Nanowire Pellicles Used for Plasma Membrane Proteome Enrichment

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The use of nanoparticle pellicles to isolate and enrich plasma membrane proteins was first developed by Jacobson in 1983. We have sought to expand this approach by using nanowires rather than particles to construct our pellicles. The substitution of nanowires for nanoparticles should create a stronger pellicle, which will better enrich our sample for plasma membrane and transmembrane proteins. We have evaluated both silica and iron silicate nanowires with different lengths and densities. All wires were coated with cationic alumina. The anionic surfaces of multiple myeloma cells were coated with the cationic nanowires. Following pellicle formation, cells were lysed via nitroson cavitation and the pellicle/plasma membrane was isolated by centrifugation. Protein extraction and LysC/tryptic digestion were performed prior to analysis by nanoLC-MS/MS on an LTQ-orbitrap. Proteins were classified as plasma membrane or transmembrane based on annotation in the UniProtKnowledgebase Transmembrane proteins were found to be enriched two to three-fold relative to the whole cell lysate (three biological replicates). A higher number of total proteins was identified from samples coated with the more dense iron silicate nanowires in two biological replicates. Cross-linked samples yielded only a slightly higher number of total protein identifications than non-cross-linked. SEM images revealed the tendency for iron silicate nanowires to coat the cells more densely than silica nanowires. This corresponds to a higher number of total protein identifications and suggests that their higher weight may play a positive role. Images obtained by optical microscopy confirm that cells remain intact and are not crushed by pellicle attachment.

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Lipids play essential roles in health and disease. The discovery of novel alterations in lipid levels related to human diseases could lead to the development of novel biomarkers and shed light on the etiology of many human diseases. The challenge with global lipid analysis is the chemical complexity and the large range of concentrations of thousands of lipid species that are present in biological samples. Here we present a robust workflow for global lipid profiling, which employs UPLC, ion mobility mass spectrometry and TransOmics software for high throughput discovery of lipid alterations in biological samples.

Lipid extracts from biological matrices were separated using Charged Surface Hybrid (CSH) C18 UPLC according to acyl chain length and number of double bonds. Major lipid classes were identified using both positive and negative ESI. After chromatographic separation, lipids were analyzed using a hybrid QTOF system upgraded with an Ion Mobility Separation (IMS) cell capable of separating lipids according to their size and shape. For example, differences in the acyl chain length or number of double bonds affect the shape and size of lipid molecules, resulting in characteristic migration times across the drift cell. Therefore, IMS provided an additional degree of separation besides chromatography, improving peak capacity and increasing selectivity over conventional UPLC. Data were analyzed using Transomics software developed for visualization, processing, and interpretation of multi-dimensional MS data.

In conclusion, we present a simple and robust solution for the high-throughput, automated identification of lipid alterations using novel analytical and informatics tools. The use of UPLC coupled to ion mobility provides multiple degrees of orthogonal separation, delivering unprecedented peak capacity required for the confident identification of lipid species in a biological mixture.

Poster 101: Improved Sensitivity and Specificity of Global GC-MS Urinary Metabolomics with Urease Pre-treatment
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A challenge associated with gas chromatography mass spectrometry (GC-MS)-based metabolomics analysis of urine is the high amount of urea. Without any pre-treatment, endogenous levels of urea produce large peaks during GC-MS analysis, which can cause major chromatographic interferences and mask low-intensity metabolite peaks. It has been demonstrated that urea can be effectively removed from urine samples through treatment with urease. However, the effects of urease pre-treatment, if any, on the global metabolome in respect to both metabolite identification and quantification are unknown. We present a series of designed GC-MS metabolomics experiments to evaluate the effects of urease pre-treatment from both a quantitative and qualitative perspective. The results of our first experiment demonstrate that there are both qualitative and quantitative differences with and without urease pre-treatment. At a large volume of urine (100 uL), we found that more metabolites were detected when using urease pre-treatment vs no treatment, and that metabolite abundances were also increased in pre-treated versus untreated samples. Our second experiment demonstrates that metabolite sensitivity with urease pre-treatment increases logarithmically with volume. In particular, both the metabolite identification and quantification were observed to have the largest overall increases with pre-treatment versus no treatment at volumes of 25 uL and higher. Future studies will evaluate the sensitivity of urease pre-treatment to detect candidate metabolite biomarkers of diabetes.
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Poster 104: Analysis of Amyotrophic Lateral Sclerosis Linked Protein FUS in vitro by Tandem Mass Tag Mass Spectrometry
Kirstin Boopie, John Leszyk, Scott Shaffer, Daryl Bosco; University of Massachusetts Medical School, Worcester, MA
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that results from the degeneration of upper and/or lower motor neurons. Currently, there is no effective treatment and patients generally succumb to the disease within 3-5 years of diagnosis. Mutations in the gene encoding Fused in Sarcoma/Translocated in Liposarcoma (FUS/TLS, or FUS) are known to account for a subset of both familial and sporadic ALS cases. FUS is normally localized to the nucleus of the cell, but ALS linked mutations in the C-terminal domain of FUS (specifically within the nuclear localization signal) cause FUS to mislocalize to the cytoplasm. ALS-linked cytoplasmic mutant FUS has also been shown to associate with stress granules, stalled translational complexes that form when cells are undergoing stress. These complexes serve to reconfigure the proteome so that cells can efficiently overcome stress and re-establish homeostasis. Our hypothesis is that ALS-FUS interferes with the function of stress granules, thereby causing cells to die under conditions of stress.

We seek to identify the effect of ALS-linked mutant-FUS on the proteome under conditions of stress. This will allow us to assess whether mutant FUS interferes with the normal function of stress granules. Quantitation by tandem mass tag (TMT) mass spectrometry (MS) of various systems and/or stressors will allow us to accomplish this. We have compared the proteome of HEK 293T cells that contain inducible expression of GFP-tagged WT-FUS and disease causing mutant variants of GFP-tagged FUS under conditions with and without stress, identifying ~1,500 proteins in a total cell lysate. We have identified a subset of proteins that are found to change in abundance under conditions of stress; results that are further corroborated by immunofluorescence and western blot. These studies will help us understand how the recruitment of FUS to stress granules can alter cellular homeostasis and cause ALS.

Poster 105: A Role for Growth Associated Protein 43, a Novel Caspase-3 Substrate, in AMPA Receptor Endocytosis and Long-Term Depression in Neurons
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Caspase-3 is a cysteine protease best known as an executioner protease in apoptosis. Our previous studies suggest that caspase-3 plays an important non-apoptotic role in synaptic plasticity as well.

In hippocampal neurons, caspase-3 activity is specifically required for NMDA receptor-dependent long-term depression (LTD), but not for long-term potentiation and metabotropic glutamate receptor (mGlur)-dependent LTD. Importantly, activation of caspase-3 in LTD promotes AMPA receptor endocytosis instead of cell death. In this study, we propose to address how caspase-3 induces AMPA receptor endocytosis in LTD by identifying substrates of caspase-3 in neurons using an enzymatic N-terminal enrichment method and mass spectrometry. Among the identified putative caspase substrates, we confirmed that Growth associated protein 43 (Gap43), Drebrin (Dbr1), and Brain acid soluble protein 1 (BASP1) are caspase-3 substrates. Interestingly, caspase-3-resistant mutants of Gap43 block AMPA receptor endocytosis and LTD in neurons. Taken together, our data suggest that Gap43 plays an important role in the caspase-3-mediated postsynaptic function and plasticity.

Poster 106: Proteomics in Amyloid and Prion Discovery: global Identification of Detergent-resistant Protein Aggregates by Tandem Mass-spectrometry
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Numerous degenerative diseases, including Alzheimer, Parkinson and Huntington, are associated with the accumulation in affected tissue of misfolded and aggregated proteins in the form of amyloid deposits. Beyond disease states, functional protein amyloids have been recently found in organisms from bacteria to humans. Amyloids share a common ordered filamentous structure composed of beta sheets that lie perpendicular to the fiber axis. The abundance of interlocking hydrogen bonds between beta-sheets (steric zippers) within amyloid fibers ensures extraordinary strength and stability, which manifests as resistance to proteases and strong detergents. Extreme examples of amyloids are prion–amyloid-based infectious proteins. In contrast to the single human prion protein (PrP), multiple amyloid-based prion proteins have been recently identified in fungi. The perservasiveness of prion aggregation in nature is largely unexplored, partly because prion propensity cannot be faithfully predicted by bioinformatic searches.

We present here a novel unbiased proteomic technique that isolates and identifies amyloid-based prions from cellular lysates, primarily by exploiting their extreme detergent resistance. After enrichment, amyloid aggregates are subject to a unique filtration technique, followed by elution of trapped aggregates, detergent removal, tryptic digestion and identification by mass-spectrometry. Only a few large molecular weight protein complexes survive the process, with amyloid aggregates being the most abundant. This strategy was validated on several prion-forming proteins from fungal cells. The described method has potential for discovering new prions, as well as natural and disease-linked amyloids across divergent organisms.

Poster 107: Determination of the Mechanism and Specificity of SAMT Analog Inactivation of HIV-1 Nucleocapsid NCp7 by Mass Spectrometry
Lisa M Jenkins1; Ryo Hayashi1; Deyun Wang2; Pankaj Gupta3; Matthew Hassink2; David E. Ott3; Ettore Appella2; Daniel H. Appella2; National Cancer Institute, NIH, Bethesda, MD; 2NIDDK, NIH, Bethesda, MD; 3SAIC-Frederick, Frederick National Laboratory, Frederick, MD
Although antiretroviral therapy has advanced, there remains a critical need for new prevention strategies, such as topical microbicides, especially those targeted at resistant strains of HIV. The HIV-1 nucleocapsid protein, NCp7, represents a prime target for antiretroviral inhibition; it is composed of two highly conserved zinc-binding domains and plays multiple roles throughout the virus replication cycle. We have developed a class of small molecule inhibitors of NCp7, based upon an S-acyl-2-mercaptobenzamide thioester (SAMT) scaffold, that eject coordinated metal from the zinc-binding domains of NCp7 in vitro and in cells. These compounds display potent antiviral activity without evidence of cytotoxicity in cell models, ex vivo cervical explants, and in animal models. We have used mass spectrometry to investigate the mechanism of viral inactivation, both in vitro and in infected cells, demonstrating that the SAMTs covalently modify NCp7 within the Gag polypeptide, resulting in ejection of zinc. The loss of bound zinc leads to accumulation of aggregated, unprocessed proteins in purified virions. As there are cellular zinc finger proteins with coordination motifs similar to that of NCp7, we have developed an alkyne analog of the SAMTs to use in Click chemistry reactions to pull down protein targets of the SAMT compounds. These studies have further demonstrated the specificity of the SAMT compounds, as few cellular proteins are recovered in these experiments. Moreover, they suggest that development of modified SAMT compounds could be used to target proteins for other disease states.
Poster 108: Discovery of a Potential Therapeutic Target in a Mouse Model of Inflammatory Myopathy Using SILAC Mouse Sree Rayavarapu 1, 2; William Coley 1; Jack Vandermeerunen 1, 3; Erdinc Gackar 4; Kyoto University, Japan; 2; Kathyayini Tappeta 1; Travis Kinder 1; Blythe Dillingham 1; National Medical Center, Washington DC, USA; 3Children’s National Medical Center, Washington DC, USA

Objective: Myositis is characterized by severe muscle weakness and muscle inflammation. Emerging evidence indicates that mechanisms such as endoplasmic reticulum (ER) stress, autophagy, AMPK1 deficiency, and hypoxia play a pathogenic role in myositis. Here we have hypothesized that ER stress activates downstream ubiquitin proteasome pathway (UPP) and degeneration in myositis. The objective of this study is to identify perturbed pathways in the MHC class-I mouse model of myositis.

Methods: An in vivo SILAC mouse (13C6 Lys-labeled) couple with LC-MS/MS analyses was used to identify proteomic modifications. We have performed two comparisons; labeled-C57BL/6 vs. unlabeled myositis mice and labeled-C57BL6 vs. unlabeled single transgenic control mice. Disease specific proteomic modifications were validated using a spike in strategy and further confirmed using immunoblotting. In addition, we evaluated the effect of a proteasome inhibitor, bortezomib, on the disease phenotype using well-standardized functional, histological, and biochemical assessments.

Results: SILAC mouse technique identified 178 proteins to be significantly differentially expressed in myositis muscle. Majority of modulated proteins were the members of ER stress response, UPP, oxidative phosphorylation, glycolysis, cytoskeleton, and muscle contractile apparatus. Myositis muscle showed significantly increased ubiquitination of proteins indicating the activation of UPP. Further, ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1) was found to be specifically present in myositis muscle but not in other dystrophic muscles. Inhibition of UPP significantly improved muscle function as assessed by the % force recovery and also significantly decreased the levels of pro-inflammatory cytokine TNF-a in myositis mice.

Conclusion: UPP is activated in myositis muscle and is a potential connecting link between chronic ER stress and muscle fiber degeneration. UCHL1 is a potential biomarker for disease progression. Inhibition of UPP might be a potential therapeutic strategy for myositis.

Poster 109: Quantitative Comparison of Protein Expression Between Vascular Smooth Muscle Cells of Different Embryonic Lineages of Origin Sarah Parker; Koen Raedschelers; Elena Gallo; Hai Dietz; Jennifer Van Eyk; Johns Hopkins University, Baltimore, MD

The anatomical location of aortic aneurysm is distinctly non-random in Marfan and other genetic syndromes of TGFß signaling-disregulation, with the aortic root by far the most commonly affected site. At this location, there is a potentially key interface between vascular smooth muscle cells (VSMCs) derived from second heart field (SHF) and cardiac neural crest (CNC) embryonic lineages of origin. In the current study, we hypothesize that lineage-specific TGFß signaling-induced protein expression differences between SHF and CNC-derived VSMCs may underlie a vulnerability to aneurysm in the aortic root. Methods: Aortic VSMCs from either the SHF or CNC were isolated from mice engineered with Cre-lox based lineage tracing construct, cultured to confluency then starved overnight and stimulated with either 5ng/ml TGFß1 or 1 vehicle for 24 hours. Experimental groups (SHF/CNC, + / - TGFß1) were run in duplicate, generating 8 samples. Tryptic peptides from 50μg of the soluable fraction of each sample were labeled with iTRAQ reagent, combined, and separated into 4 fractions by strong cation exchange (SCX) chromatography. Each fraction was run separately on an LTQ Orbitrap Velos MS interfaced with a 2D nanoLC system. Data were searched using the XTandem algorithm, with iTRAQ quantification performed using the LIBRA software package. Results: 1447 non-redundant proteins were identified (false discovery rate ≤ 5% and ≥ 2 observed peptides) from 14,878 scans of 9,358 peptides. At baseline, 16 proteins showed >1.5-fold up-regulation in the SHF lineage, and 6 were up-regulated in the neural crest. We detected 6 and 7 up-regulated, as well as 2 and 14 down-regulated proteins by TGFß signaling; stimulation in the SHF and CNC lineages, respectively. Summary: The relevance of differentially abundant proteins to TGFß signaling was assessed via pathway analysis and literature search, identifying one candidate –inhibitor of kappa kinase 2S – which may modulate the TGFß signaling response between these cells.

Poster 110: The Human Eye Proteome Project - Perspectives on an Emerging Proteome Richard Semba 1; Jan Enghild; Vidya Venkatraman; Thomas Dyhrlund 2; Jennifer Van Eyk 3; Johns Hopkins Univ, Baltimore, MD; 4Aarhus University, Aarhus, Denmark; 5Johns Hopkins University, Baltimore, MD

There are an estimated 285 million people with visual impairment worldwide, of whom 39 million are blind. Despite drug treatments, laser photocoagulation, and surgery, many people continue to go blind, and the pathogenesis of many eye diseases remains poorly understood. The human eye is currently an emerging proteome that may provide key insight into the biological pathways of disease. To facilitate future proteomic studies of the eye in health and disease, the Human Eye Proteome Project was organized at the Human Proteome Organization (HUPO) World Congress in September 2012 and recognized as an official initiative by this organization in December 2012. The authors have reviewed the literature and have compiled 4842 non-redundant proteins that currently represent the known proteome of the human eye. There are many subproteomes of the eye that remain unknown. Proteomics may greatly facilitate the identification of new biomarkers for specific eye diseases such as age-related macular degeneration, primary open angle glaucoma, and diabetic retinopathy. Proteomic investigations of the human eye are much in their infancy. The field of vision science would be impacted if the large repertoire of investigative proteomic tools were applied, enhancing both the understanding and physiology of eye diseases that affect sight. The application of modern proteomic tools to the eye and visual science is an audacious goal that is an important key to the cure and prevention of eye diseases.

Poster 111: Quantitative Proteomic Analysis of Ubiquitin-Modified Tissue Proteins Stefani Thomas; Hui Zhang; Robert Cotter; Johns Hopkins School of Medicine, Baltimore, MD

Post-translational modification by ubiquitin is a fundamental regulatory mechanism for protein functions. Several cellular processes including the cell cycle, apoptosis, cell adhesion, angiogenesis, and tumor growth are mediated by the formation of mono- or poly-ubiquitin linkages on proteins via an isopeptide bond between the C-terminal glycine in ubiquitin and the ε-amino groups of specific lysine residues in substrates. The low stoichiometry of ubiquitination presents an analytical challenge for the detection of endogenously modified proteins in the absence of the protein level enrichment of ubiquitin or the over-expression of ubiquitin or its substrates. However, the recent availability of antibodies that recognize peptides with lysine residues that contain a di-glycine ubiquitin remnant (K-ε-GG) has greatly improved the ability to enrich and identify ubiquitination sites from complex protein lysates from cells via mass spectrometry. The majority of these studies were performed using cell lysate.

Here, we employ anti-K-ε-GG antibody-based enrichment and quantitative mass spectrometry to interrogate the ubiquitin-modified proteome at the tissue level. Briefly, 5 mg of tissue homogenate-derived proteins was enzymatically digested and fractionated offline using basic pH reversed phase chromatography followed by anti-K-ε-GG antibody-based enrichment, iTRAQ labeling and LC-MS/MS analysis. Gene Ontology (GO) biological process enrichment revealed distinct classes of up-regulated K-ε-GG modified proteins including those involved in metabolic processes, cell organization and biogenesis, and transport. The relative abundance of ubiquitin linkages (K6, K11, K27, K29, K33, K48, and K63) was determined to elucidate the potential functional

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implications of the ubiquitin modifications. Quantitative global proteomic analysis provided insight into the relationship between protein abundance and the ubiquitin-modified proteome. We have therefore established a workflow for the quantitative assessment of endogenous ubiquitylation in tissues.

**Poster 112: Effect of Post-translational Modifications, Citrullination, on Thin Filament Regulation**

Justyna Fert-Bober, Jennifer Van Eyk, The Johns Hopkins University, Baltimore, MD

Activation of the cardiac sarcomere is a cooperative process that involves complex protein–protein interactions. These interactions ultimately result in the activation of the thin filament and the subsequent interaction between actin and myosin that leads to ATP hydrolysis and myofilament force development. Apart from the translational changes in protein expression, post-translational modifications of myofilament proteins are essential for the regulation of cardiac function both under physiological and pathophysiological conditions. Our preliminary data suggests an important role for citrullination, post-translational modifications on arginine residue that could influence the myofilament apparatus via protein–protein interactions and/or alteration of ATP hydrolysis.

The biological activity of myosin II was determined from its rate of F-actin activated ATP hydrolysis. A standard biological assay for monitoring ATP hydrolysis by myosin ensured that in the presence of F-actin, myosin had a hydrolysis rate greater than in the absence of F-actin. However, citrullination of myosin caused opposite effect in the presence of F-actin mirroring myosin and a lower hydrolysis rate of ATP. Furthermore, functional properties of the citrullinated tropomyosin were defined by actin affinity measured by cosedimentation, troponin T affinity, and regulation of the actomyosin MgATPase. In conclusion, the study has revealed changes in function in the myofilament proteins, actin, myosin and tropomyosin after in vitro induction of citrullination. Numerous specific sites of citrullination in cardiac myofilament proteins have been identified by a methodology based on chemical modification of peptide-bound citrulline residues follow by liquid chromatography/tandem mass spectrometry. These changes could contribute to the altered contractile performance of the heart and lead to cardiac hypertrophy and cardiomyopathies although more study has to be done.

**Poster 113: Identification and Quantification of Cysteine Sulfoxidation Sites**

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Reactive oxygen species (ROS) play important roles in normal biological functions and pathological process. Elevated levels of ROS serve as an endogenous source of DNA-damage agents that promote genetic instability.

ROS such as hydrogen peroxide and superoxide can alter the function of proteins by oxidizing free sulfhydryl groups on cysteines. Cysteine sulfoxidation products include reversible sulfenic acid (＝SOH) and irreversible states, sulfenic (＝SO2H) and sulfonic (＝SO3H) acid. Here we describe an approach to detect cysteine oxidation without additional enrichment steps using a mass spectrometry-based strategy.

The method was initially optimized using in vitro oxidized BSA. Digested samples were resolved via UPLC using a long nano-column and analyzed on the Orbitrap Elite mass spectrometer. In total we identified differential 17 cysteine oxidative modifications on 13 cysteine residues of BSA. Next, overexpressed ATM protein purified from human cells was treated with hydrogen peroxide and multiple oxidation sites were identified. Several were quantitated using MS1 integrated peak areas, which demonstrated some sites increased levels of oxidation while others remained unchanged with increasing oxidant treatment. Minimization of protocol steps is beneficial for limiting extraneous oxidation induced by sample handling. Finally, proteomic analysis was performed on Jurkat cell lystate treated with oxidizing agent, and over 140 cysteine sulfoxidation peptides were identified, among them several known sites. Five modified peptides were selected for quantitative analysis using targeted LC-MS/MS and extracted fragment ion peak areas. Dose concentration curves showed increasing levels of irreversible oxidation upon increased concentration of hydrogen peroxide. This approach allowed us to identify cysteine sulfoxide modified peptides in a complex mixture without prior enrichment, and to quantify selected sites.

**Poster 114: New Nano ESI source Development for Increased Performance Nano LC-MS with Plug-and-Spray Configuration**

Christian Ramsvort1 2, Reiko Kiyonami1 2, Peter Nielsen1 2

Thermo Fisher Scientific, Odense, Denmark; Thermo Fisher Scientific, San Jose, CA

Nano-flow LC-MS is widely used for qualitative and quantitative proteomics studies due to its high sensitivity and specificity; Improper connections often result in leaks and large swept volumes that cause substantial peak broadening and thus poor sensitivity while a poor high voltage connection will yield poor data because of unstable spray. In order to address these common issues, we have developed a new nano-electrospray source and emitter concept in which, a column, column heater, high voltage electrode and an emitter are combined in one ready-made assembly. The performance of this new source concept was evaluated.

EASY-Spray was installed on the Orbitrap Elite mass spectrometer and connected to an Easy-nLC 1000 nanoLC pump. Three column (50 μm id x 15 cm, 2 μm) assemblies were used for evaluating column-to-column and run-to-run reproducibility. Both complex peptide mixtures and simple digest mixture were used. The retention time reproducibility, peak shape, resolution and peak capacity in different temperature ranges were evaluated. In order to test the device flexibility, several flow rates from 150 nl/min to 1000 nl/min were used. A 15 min linear gradient was used for the simple digest sample separation Results: The column assembly is positioned in the source without the need to adjust the X, Y, or Z positions of the emitter. A high spray stability was achieved over all LC runs with three different columns. The obtained chromatographic resolution, sensitivity, and reproducibility matches other state-of-the-art data without need for adjustments (or expert intervention) beyond plugging in the column/sprayer assembly. More than a thousand proteins were identified with increased component detection from the complex protein digest mixtures. The reproducibility of the retention time from column to column for the simple standard mixtures was less than one percent.

**Poster 115: High-Throughput Microfluidic Applications**

Angela Donatini; Jim Murphy; Jay Johnson; Steven Cohen; Giuseppe Asta; Asita Chakraborty; LeRoy Martin; Waters Corporation, Milford, MA

A novel platform was developed for the expansion of microfluidic LC-MS beyond typical nanoscale applications into areas traditionally performed at analytical scales. The material used to fabricate the device allows for pressures of 12,000 psi. The integrated metal electrospray emitter supports flow rates from 100s of nl/min up to 8 μl/min and allows for the use of 150 μm in ID separation channels. Peak widths are on par with commercial ultra-high pressure LC instrumentation and cycle times as low as 10 min. System versatility will be demonstrated by several examples including intact proteins analysis, lipidomics and biopharmaceutical applications.

All experiments were performed using a nanoflow system coupled with an oTOF mass spectrometer fitted with an electrospray ionization source designed to accommodate the microfluidic device. The emitter is connected to a packed channel by zero dead-volume connections and incorporates the use of nebulising gas. The separation channel was 150 μm x 5 cm and packed with sub-2 μm reverse-phased particles of various chemistries.

For intact protein analysis, using Ribonuclease A, Cytochrome C, Holotransferrin and Apomyoglobin, good resolution was achieved using shorter alkyl chain resins. Additionally, light and heavy chains of reduced monoclonal antibodies could be separated. Separations were performed at 80°C, using a 3 μL/min linear gradient from 20% to 55% ACN over 5 min. Applicability for biopharmaceutical use is shown with minute amounts of
Trastuzumab, demonstrating peak widths less than 6 s with 90% sequence coverage. Methionine oxidation and sites of glycosylation were also detected.

The method was also employed for lipid analysis showing improved separation for the major classes, particularly inter and intra forms. The microfluidic system offered high retention time reproducibility with RSD values smaller than 0.2%. This is especially useful for lipidomic analysis, which requires the comparison of a large number of LC-MS chromatograms from multiple sample sets.

**Poster 116: New Chemical Proteomic Methods To Access Drug-Protein Interactions**
Matthew Kuruc; Swapan Roy; ProFACT Proteomics Inc., Monmouth Junction, NJ

Chemical and functional proteomics can help identify, and characterize protein content that binds to or interacts with small molecule compounds under consideration for therapeutic development. After high-throughput screening, there often remains a need to index or catalog protein interactions that might have been overlooked during the initial screen. While some advancements have been made to index off-target proteins using recombinant proteins and yeast 2-hybrids, these platforms may introduce artifact evidence that may not be relevant to natural systems. It is proposed that novel separations can be coupled to LC-MS to provide direct functional annotation of interaction(s) between a drug compound and its complement of proteins. Such annotation requires that the proteins be investigated be retained in their natural, underaged and structurally intact form. In our new separation platform, highly complex proteomes are bound to a stationary support or matrix with low or weak binding energy. Such weakly bound proteins can be displaced by a drug-like compound for release back into the aqueous phase. While these systems have been under investigation for purified proteins, we disclose how they can be adapted to proteomic comparisons or for systems containing complex mixtures of proteins, such as would be contained in cellular extracts or body fluids, to positively enrich the drug interacting proteome from the vast amount of protein content that does not interact with the drug compound(s). These strategies involve multiple weak affinity surfaces working in parallel or as composites, with different binding characteristics. The net result is the compression of protein concentrations, making these systems practical and industrially productive towards most cellular extracts or body fluids without any predefined knowledge or know-how of the protein content contained within those samples.

**Poster 117: On-line Chip based 2D RP/RP LC-MS/MS Approach for Proteomic Analysis**
Xiang Zhu; Christie Hunter; Jenny Albanese; Remco van Soest; Tina Settineri; Eksigent, part of AB SCIEX, Dublin, CA; AB SCIEX, Foster City, CA

Two-dimensional (2D) liquid chromatography is widely used for proteome identification and quantification using the advantage of increasing peak capacity. Herein, we developed a simplified chip based 2D-LC workflow using a high pH/RP first dimensional separation and low pH/RP secondary dimension coupled to mass spectrometer for proteomic analysis.

The 2D LC separation was performed using ekspert® nanocLC 425(Eksigent, part of AB SCIEX) system. Digested E. coli cell lysates were first loaded onto a 200μm x 15cm C18 column at pH 9.8 with a flowrate of 1 μL/min. Step gradient was used to sequentially elute peptide fractions, which were diluted to pH 2.5 before being captured by a 200μm x 6mm C18 chip trap column. Each fraction was then separated with a 75 μm x 15cm C18 chip column at 300 nL/min and analyzed with TripleTOF® 5600 (AB SCIEX). Data was processed with ProteinPilot® Software (AB SCIEX).

The preliminary result suggested the on-line 2D RP-RP method as an easy and competitive approach for proteome discovery. The comparison experiments of 1D, 2D-6 fraction and 2D-10 fraction were performed. Using ~1ug of E. coli digested cell lysates, there are 1.8x and 2.1x increase in the identification numbers for 2D-6 and 2D-10 fractions versus the 1D configuration at the peptide level (5% local FDR). The other advantage of 2D workflow is the larger sample loading capacities on the column. When the loading amount was increased by 10x, the number of detected peptides increased by 3.3x and 4x for the 2D 6 and 10 fraction workflows, respectively, over 1D workflow. The measured retention time of peptides detected in both the 1D 1ug and 2D 10ug experiments showed very good correlation (r2 0.99, slope 1.0). Further optimization of both the first and second dimension is ongoing to further improve the peptide detection rates.

**Poster 118: Discovery of Pro-Angiogenic Endothelial Cell Signalling from Angiotensin-(1-7) through the Mas1 Receptor with Tandem Mass Spectrometry.**
Timothy Stodola; Brian Hoffmann; Jordan Wagner; Andrew Greene; Medical College of Wisconsin, Milwaukee, WI

Angiogenesis in skeletal muscle has been shown to be modulated by the renin-angiotensin system. Angiotensin II (AngII) is well known to work through the angiotensin receptor type I (AT1) to promote angiogenesis and cause vasoconstriction, fibrosis, cellular growth and migration. Recent work in our lab has shown Angiotensin-(1-7) (Ang1-7) to promote skeletal muscle angiogenesis in response to electrical stimulation through the Mas1 receptor independently of AngII acting through the AT1 receptor. Additionally, we show in an ex vivo tube formation assay that Ang1-7 induces an increased angiogenic response in rat microvascular endothelial cells. Since little is known about the role of the Mas1 receptor in angiogenesis, we sought to identify downstream components of the Mas1 receptor signaling pathway in endothelial cells and determine their possible role in an angiogenic response. The Mas1 receptor was immunoprecipitated (IP) after cryolysis with and without stimulation from Ang1-7. IP was validated by immunoblotting for the Mas1 receptor. The receptor was also identified via tandem mass spectrometry (MS), along with other potential components of the Mas1 signaling pathway. Non-treated and Ang1-7 treated IP tandem MS results were compared to eliminate non-specific protein interactions resulting in specific identification of 109 proteins unique to Ang1-7 stimulated Mas1 signaling complexes. Proteins involved in known pathways affected by Mas1 receptor stimulation, such as regulators of nitric oxide, G-protein, and NF-kappaB signaling, and previously unknown affected processes, such as protein synthesis, exocytosis, and cellular reorganization, were detected.

**Poster 119: Quantitative Analyses of Protein-protein Interactions in the B-cell Receptor Signaling Pathway**
Brianne Petritis; Mitch Magee; Benjamin Ober-Reynolds; Justin Saul; Jin Park; Ian Shoemaker; Jason Steel; Kevin Peasley; Joshua LaBaer; Arizona State University, Tempe, AZ

Aberrant protein signaling contributes to the formation and progression of diseases, including Burkitt’s lymphoma, a highly aggressive non-Hodgkin lymphoma that affects B-cells. The comprehensive characterization of protein-protein interactions (PPIs) in the B-cell receptor (BCR) pathway will help elucidate the molecular mechanisms during tumor growth and response to treatment. Here, we coupled nucleic acid programmable protein arrays (NAPPA-SPRi) with surface plasmon resonance imaging (SPRi) to obtain high throughput, quantitative analyses of human PPIs within the BCR pathway. In our initial experiment, 92 proteins in the BCR pathway were displayed on the arrays and queried with 5 purified proteins considered to be central signaling proteins in this network.

NAPPA-SPRi detected and quantitatively measured the strength (i.e., affinity) and interaction rate (i.e., kinetics) for 13 known PPIs as well as 126 novel interactions, which was validated by SPRi to obtain high throughput, quantitative analyses of human PPIs within the BCR pathway. In our initial experiment, 92 proteins in the BCR pathway were displayed on the arrays and queried with 5 purified proteins considered to be central signaling proteins in this network.
CD20) of Burkitt's-like lymphoma cell models following various perturbations (i.e., genetic mutations, cytotoxic treatment). Our high throughput, quantitative approach could be applied toward characterizing other signaling pathways and may help to identify potential therapeutic targets of disease.

**Poster 120: Sphingosine-1-Phosphate Mediated Chemotaxis of Osteoclast Precursors Investigated using Targeted Proteomics via Mass Spectrometry**

Nathan Manes, Eunkyung An, Virginie Spelwini, Jia Sun, Bastian Angermann, Masaru Ishii, Martin Meier-Schellersheim, Ronald Germain, Aleksandra Nita-Lazar, National Institutes of Health, Bethesda, MD; Osaka University, Osaka, Japan

Osteoclasts are monocyte-derived multinuclear cells that attach to the bone matrix and are responsible for bone resorption. Misregulation of osteoclasts has been implicated in numerous skeletal diseases including osteoporosis and osteoarthritis. Our laboratory recently reported that the phospholipid sphingosine-1-phosphate (S1P) regulates bone resorption in mice by mediating both chemotaxis and chemorepulsion of osteoclast precursors through two G-protein coupled receptors (S1PR1 and S1PR2), which antagonize each other in an S1P-concentration dependent manner. In this investigation, transcriptomics, shotgun proteomics, and targeted proteomics are being employed to enable quantitative simulation of the S1P-chemotaxis signaling pathway within osteoclast precursors.

A set of mouse chemotaxis pathway target proteins was constructed from a literature and pathway database search. RNA-Seq of osteoclast precursors (RAW 264.7 cells) was used to identify expressed target proteins and their specific splice isoforms. Shotgun mass spectrometry (nanoLC-Velos-Orbitrap-MS/MS) was used to identify proteotypic peptides. Selection of the target peptides used a wide variety of criteria including peptide proteotypic qualities, sequence uniqueness, and vulnerability to posttranslational modification (e.g., oxidation and deamidation). SPOT synthesis was used to prepare 409 crude, unlabeled, semi-quantitated peptide standards against the 171 target proteins. A spectrum library of the peptide standards was constructed using shotgun mass spectrometry (nanoLC-TSQ-Vantage-QqQ-MS/MS), Proteome Discoverer, Mascot, and Skyline. Targeted mass spectrometry (nanoLC-TSQ-Vantage-QqQ-SRM) of the peptide standards was used to develop SRM assays. SRM of RAW cell samples resulted in the confident identification and semi-quantitation of 208 of the 409 peptide targets. Absolute quantitation of 42 high-priority target proteins was performed using 65 heavy-labeled, quantitated, internal peptide standards. Additionally, a supplementary set of 145 crude, unlabeled peptide standards was prepared to target missed proteins. Quantitative pathway simulation was performed using Simmune.

**Poster 121: Using Chicken IgY Antibodies to Establish Proteotypic Peptide Affinity Capture Reagents**

Shiming Zhang, Shu-Cai (David) Huang, Wei Yan, GeneTel laboratories LLC, Madison, WI; Institute for Systems Biology, Seattle, WA

Good capture reagents are in urgent need for affinity-enriched proteomics to quantitatively analyze human biomarkers, i.e., proteotypic peptides, in clinical samples. Mammalian antibodies have been historically used as affinity capture reagents. However, human proteotypic peptides often fail to elicit strong antibody response in mammalian hosts, mainly due to the closely evolitional relation. Exploring non-mammalian species to generate antibodies against proteotypic peptides is an attractive alternative. We launched a pilot project of generating chicken antibodies against cancer marker peptides. All six randomly picked peptides, with or without modification, stimulated strong antibody response in immunized chickens. The purified antibodies possess high affinity (about 10-9M or better) to the target peptides. The IgY antibodies were conjugated to magnetic beads to generate affinity capture reagents. These capture reagents can efficiently and sensitively capture the target peptides both in buffer solution and in plasma tryptic digestion mixture. The experiments demonstrate that using chicken to produce proteotypic peptide antibodies is a feasible approach for generating affinity capture reagents.

**Poster 122: Phosphorylation of the Sae2 Endonuclease Regulates its Solubility and Activity in DNA Repair**

Qiong Fu, Chia-Fang Lee, Julia Chow, Maria Person, Tanya Pauil, The University of Texas at Austin, Austin, TX

The processing of DNA double-strand breaks (DSB) for homologous recombination (HR) is regulated by cyclin-dependent kinases (CDK) in eukaryotes. One of the critical targets is the Sae2 protein in budding yeast (CtIP in S. pombe and mammalian cells), which functions together with the Mre11/Rad50/Xrs2 (MRX) complex in many aspects of DNA metabolism that involve DSB. In this study we investigated the activity of Sae2 in vivo and in vitro to determine how CDK and Mec1/Tel1 phosphorylation regulate 5’ strand resection and HR through Sae2. We identified sites of phosphorylation and acetylation through mass spectrometry and analyzed these through genetic analysis. Sae2/CtIP has low expression levels under normal growth conditions, and has not been identified in major yeast proteome studies. In order to detect and quantify Sae2, high and low copy overexpression systems were generated. From LC-MS/MS on yeast cells expressing high copy Sae2, we identified Sae2 and selected precursor ions for targeted LC-MS/MS. Using Skyline, extracted fragment ion chromatograms function as pseudo Selected Reaction Monitoring (pSRM) peaks to quantify the level of Sae2 in the low copy system. Differences in Sae2 expression levels are observed in the soluble and insoluble fractions. Surprisingly, the phosphorylation events regulate the solubility of the Sae2 protein in a DNA damage-dependent and dynamic manner. We present a model of Sae2 regulation in which the natural insolubility of this protein provides a strong barrier to its activity, but a barrier that can be breached very quickly and reversibly by transient phosphorylation.
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