Fifth Annual Conference

INTEGRATIVE PROTEOMICS FOR THE FUTURE

February 22 - 25, 2009

San Diego, California

Sixth Annual Conference
March 7 - 10, 2010
Marriott City Center, Denver, Colorado

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Cara Wykowski, *The Proteome Society*
Cathy Wu, *Georgetown University*

PROGRAM OVERVIEW

SUNDAY	MONDAY	TUESDAY	WEDNESDAY
8:00 – 5:00 pm SHORT COURSES 8:00 am – 5:00 pm **Characterization of PTMs**, *Shimadzu, Harbor Rm, 3rd fl* 8:00 am – 12:00 pm **Data Extraction/Analysis for LCMS Based Proteomics** *Plaza Rm, 2nd fl* 1:00 – 5:00 pm **Sample Preparation** *Agilent, Library Rm, 1st fl* 1:00 – 5:00 pm **Techniques for Biomarker Discovery** *GE Healthcare* *Plaza Rm, 2nd fl*	**8:00 – 9:20 am** PARALLEL SESSION A **Cellular Networks** *Pavilion, 4th fl* PARALLEL SESSION B **Organelle Proteomics / Omics of Mitochrondria** *California A, 2nd fl* PARALLEL SESSION C **Standardized Clinical Proteomics Platforms** *Plaza Room, 2nd fl*	**8:00 – 9:20 am** GENERAL SESSION **Era of Systems Medicine** *Pavilion, 4th fl*	**8:00 – 9:20 am** PARALLEL SESSION B **PTM Analyses of Complex Biological Matter** *California A, 2nd fl* PARALLEL SESSION C **Glycoproteomics** *Plaza Room, 2nd fl*
	9:20 – 9:45 am **Coffee Break / Exhibits** *Poster/Exhibit Hall, 2nd fl*	**9:20 – 9:45 am** **Coffee Break / Exhibits** *Poster/Exhibit Hall, 2nd fl*	**9:20 – 9:45 am** **Coffee Break / Exhibits** *Poster/Exhibit Hall, 2nd fl*
6:00 – 7:00 pm GENERAL SESSION **Presidents' Symposium** **Keynote Lecture:** Robert Waterston *Pavilion, 4th fl*	**9:45 am – 12:00 pm** GENERAL SESSION **Proteogenomics** & Panel Discussion *Pavilion, 4th fl*	**9:45 am – 12:00 pm** GENERAL SESSION **Proteomics and Systems Medicine** & Panel Discussion *Pavilion, 4th fl*	**9:45 – 11:20 am** PARALLEL SESSION A **Mass Spectrometry Advancement** *Pavilion, 4th fl* PARALLEL SESSION B **Membrane Proteomics** *California A, 2nd fl* PARALLEL SESSION C **Bioinformatic Analyses of Protein Structure** *Plaza Room, 2nd fl*
	12:15 – 1:30 pm **Vendor Lunch Seminars** Agilent, *Library, 1st fl* GE Healthcare, *Coronado, 3rd fl* Shimadzu, *Harbor, 3rd fl* Thermo, *Plaza Rm, 2nd fl*	**12:15 – 1:30 pm** **Vendor Lunch Seminars** Bio-Rad, *Library, 1st fl* Protein Discovery, *Harbor, 3rd fl* Thermo, *Plaza Rm, 2nd fl*	
7:00 – 9:00 pm **Opening Reception** *Poster/Exhibit Hall, 2nd fl*	**1:30 – 3:30 pm** **Mon. Poster Session & Exhibits** *Poster/Exhibit Hall, 2nd fl*	**1:30 – 3:30 pm** **Tues. Poster Session & Exhibits** *Poster/Exhibit Hall, 2nd fl*	**11:30 am – 1:00 pm** **Lunch Break**
	3:45 – 5:20 pm PARALLEL SESSION A **Bioinformatics of MS** *Pavilion, 4th fl* PARALLEL SESSION B **Chemical Proteomics** *California A, 2nd fl* PARALLEL SESSION C **Systems Analysis for Biomarker Discovery I** *Plaza Room, 2nd fl*	**3:45 – 5:20 pm** PARALLEL SESSION A **Systems Analysis for Biomarker Discovery II** *Pavilion, 4th fl* PARALLEL SESSION B **Databases and Systems Integration** *California A, 2nd fl* PARALLEL SESSION C **Structure & Dynamics of Macromolecular Assemblies** *Plaza Room, 2nd fl*	**1:00 – 3:15 pm** GENERAL SESSION **PTM Analyses** & Panel Discussion *Pavilion, 4th fl* **3:15 – 3:30 pm** **Closing Remarks**
	5:30 – 7:30 pm **Dinner Break**	**5:30 – 7:30 pm** **Dinner Break**	**Closing Event: Dinner Cruise on San Diego Bay** Meet at 6:00 pm in Hotel Lobby
	7:30 – 9:00 pm EVENING WORKSHOPS **Cardiovascular Proteomics**, *Pavilion 4th fl* **Affinity Reagents** *California A, 2nd fl* **Future of Biomarkers**, *Plaza Room, 2nd fl*	**7:30 – 9:00 pm** EVENING WORKSHOPS **SPI**, *Pavilion, 4th fl* **Disease Proteomics**, *California A, 2nd fl*	

EXHIBITORS

USHUPO is pleased to acknowledge the support of conference exhibitors.
Participants are invited and encouraged to visit exhibit booths during the hours below.

Exhibit Booth Schedule

7:00 – 9:00 pm	Sunday evening, Opening Reception
9:20 – 9:45 am	Monday and Tuesday morning breaks
1:30 – 3:30 pm	Monday and Tuesday Poster Sessions

Exhibitors may be present at other times during the day.

AAAS / Science
Booth 7
www.aaas.org

Agilent Technologies
Booth 1
www.agilent.com/chem

American Chemical Society Publications
Booth 2
http://pubs.acs.org/

Bio-Rad Laboratories
Booth 17
www.bio-rad.com

Cambridge Isotope Labs, Inc.
Booth 4
www.isotope.com

Captrotec Bioanalytics GmbH
Booth 9
www.caprotec.com

Dionex Corporation
Booth 24
www.dionex.com

GE Healthcare
Vendor Seminar only
www.ge.com

Genebio SA
Booth 19
www.genebio.com

Integrated Analysis Inc.
Booth 8
www.i-a-inc.com

Ludesi
Booth 3
www.ludesi.com

Monarch LifeSciences, LLC
Booth 22
www.monarchlifesciences.com

Nanoxis
Booth 13
www.nanoxis.com

NuSep
Booth 6
www.nusep.com

Promega Corporation
Booth 5
www.promega.com

Protea Biosciences
Booth 23
www.proteabio.com

Protein Discovery, Inc.
Booth 12
www.proteindiscovery.com

Protein Forest, Inc.
Booth 10
www.proteinforest.com

Proteome Software, Inc.
Booth 18
www.proteomesoftware.com

Proxeon
Booth 21
www.proxeon.com

Rosetta Biosoftware
Booth 16
www.rosettabio.com

Shimadzu Scientific Instruments
Booth 14
www.shimadzu.com

Sigma-Aldrich
Booth 20
www.sigma-aldrich.com

Thermo Scientific
Booth 15
www.thermo.com/ms

Uvic Genome BC Proteomics Centre
Booth 11
www.proteincentre.com

VENDOR LUNCH SEMINARS

The following companies offer lunch seminars to conference participants (seating is limited) on Monday and Tuesday.
Attendees are encouraged to reserve a seat at company exhibit booths.

Monday, 12:15 – 1:30 pm	Tuesday, 12:15 – 1:30 pm
Agilent Technologies, *Library Rm, 1st fl*	**Bio-Rad**, *Library, 1st fl*
GE Healthcare, *Coronado, 3rd fl*	**Protein Discovery**, *Harbor, 3rd fl*
Shimadzu Biotech, *Harbor, 3rd fl*	**Thermo Scientific**, *Plaza Room, 2nd fl*
Thermo Scientific, *Plaza Room, 2nd fl*	

GENERAL INFORMATION AND HOTEL MAPS

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 Poster-Exhibit Hall, 2nd fl

Monday Posters should be set up by 8:00 am on Monday and removed by 9:00 pm Monday evening. See abstracts page 35.

Tuesday Posters should be set up by 8:00 am on Tuesday and removed by 9:00 pm Tuesday evening. See list abstracts 59.

Posters should be attended from 1:30 – 3:30 pm on the scheduled day.

TALKS. All General Sessions and Parallel Session A's are located in Pavilion, 4th fl. Parallel Session B's are in California A, 2nd fl and Parallel Session C's are in Plaza Room, 2nd fl.

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 USHUPO PC computer will use this time to load their files.

INTERNET ACCESS. Free wireless internet access is provided in the lobby area (ground floor). A code card with access code is available at conference registration or hotel front desk.

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

CLOSING EVENT. Join us for a dinner cruise on San Diego Bay. Tickets are required and may be purchased through Monday at 3:00 pm from the conference registration counter. Meet in hotel lobby at 6:00 pm, Wednesday evening to walk to the boat. See your ticket for more details.

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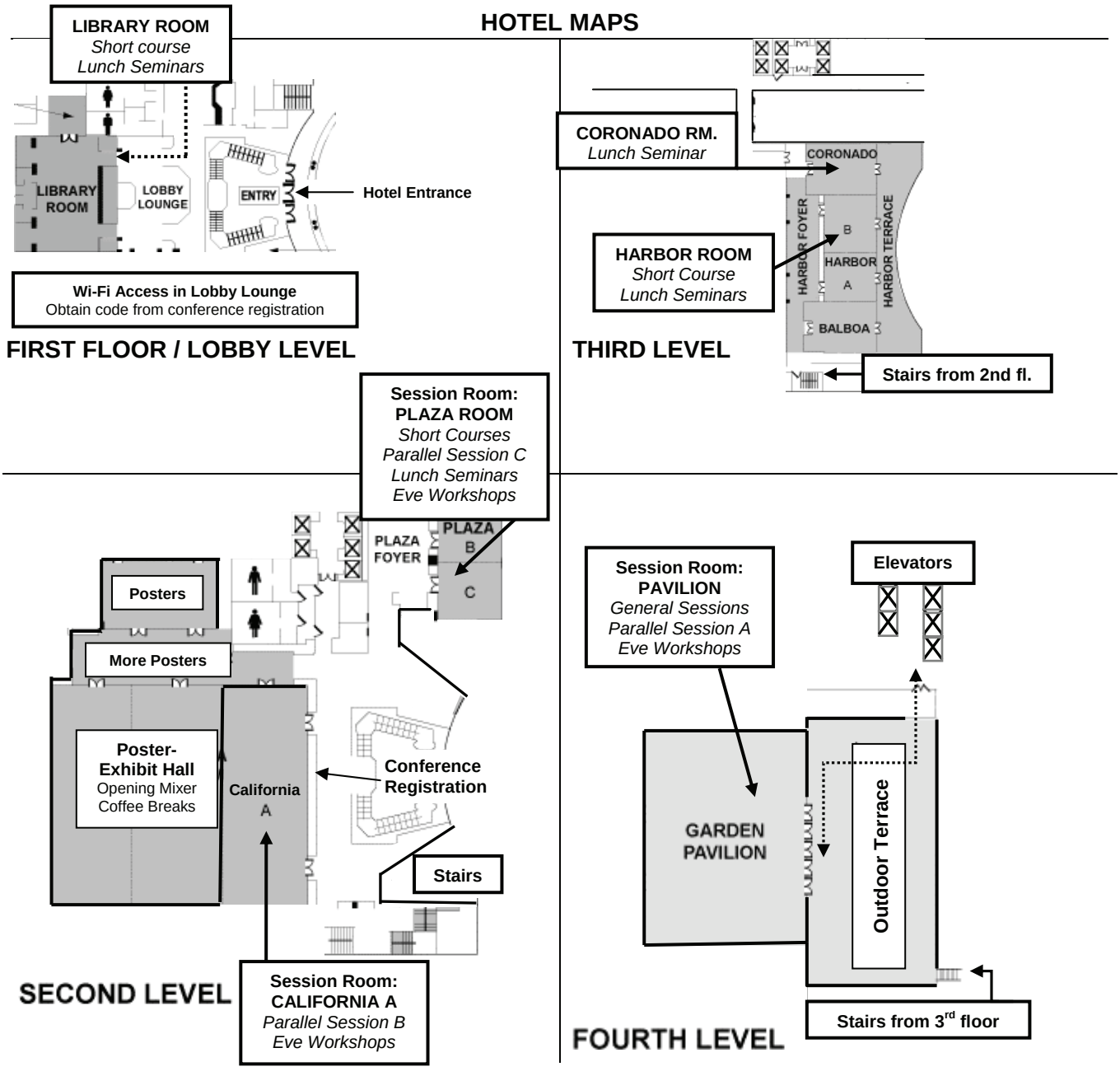
HOTEL MAPS



SUNDAY, FEBRUARY 22

7:00 am: Registration Opens, *Top of the Stairs, 2nd fl*

SHORT COURSES

8:00 am – 5:00 pm **Characterization of PTMs for Proteomics Application and Biomarker Discovery**, *Harbor Rm, 3rd fl*
Sponsored by Shimadzu Biotech

8:00am – 12:00pm **Data Extraction and Analysis for LC-MS Based Proteomics**, *Plaza Room, 2nd fl*

1:00 – 5:00 pm **Techniques for Biomarker Discovery**, *Plaza Room, 2nd fl*
Sponsored by GE Healthcare

1:00 – 5:00 pm **Sample Preparation and Fractionation for Proteomics**, *Library Room, 1st fl*
Sponsored by Agilent Technologies

6:00 – 7:00 PM: PRESIDENTS' SYMPOSIUM (Opening Session), *Pavilion, 4th fl*

Co-Chairs: William Hancock, *US HUPO President* and Gilbert Omenn, *US HUPO President-Elect*

Opening Remarks: Jan Schnitzer, *Organizer US HUPO 2009*

Keynote Lecture: **Robert Waterston**, *Genome Sciences, University of Washington*

7:00 – 9:00 PM: OPENING RECEPTION, *Poster/Exhibit Hall, 2nd fl*

MONDAY, FEBRUARY 23
INTERFACE OF PROTEOMICS & GENOMICS

8:00 – 9:20 AM: PARALLEL SESSION A, *Pavilion, 4th fl*
CELLULAR NETWORKS
Co-Chairs: Roman Zubarev and David States

Abstract Page #

8:00 - 8:25 am **Unbiased Pathway Analysis of Expression Proteomics Data Predicts Signaling Status in Cancer Cells and Biopsy Samples**; Roman Zubarev; *Uppsala University; Uppsala, Sweden*..... 17

8:25 – 8:50 am David States; *School of Health Information Sciences at Houston, University of Texas*

8:50 - 9:05 am **Characterization of Proteomic Changes Induced in Cells and Tissues by Oxidative Stress**; Alexander Ivanov; *Harvard School of Public Health; Boston, MA*..... 17

9:05 - 9:20 am **Quantitative Phosphoproteomic Dissection of Signaling Pathways Applied to T Cell and Mast Cell Signaling**; Arthur R. Salomon; *Brown University; Providence, RI*..... 17

8:00 – 9:20 AM: PARALLEL SESSION B, *California A, 2nd fl*
ORGANELLE PROTEOMICS / OMICS OF MITOCHONDRIA
Co-Chairs: John Yates and Paul Brookes

8:00 – 8:25 am Paul Brookes; *University of Rochester, Rochester, NY*

8:25 – 8:50 am **Exploring the Role of PTMs in Mitochondria in Heart Failure and with Cardiac Resynchronization Therapy;** Jennifer E. Van Eyk; *Johns Hopkins Medical Instit.; Baltimore, MD*..... 18

8:50 - 9:05 am **Quantification of Proteome Changes in L6 Myotubes with Giant Mitochondria Induced by Blocking Autophagy**; Rongxiao Sa; *University of Minnesota; Minneapolis, MN*..... 18

9:05 - 9:20 am **Label-Independent Quantitative Analysis of Skeletal Muscle Mitochondria in Rat Obesity Model**; Nichole Reisdorph; *National Jewish Health; Denver, CO*..... 18

8:00 – 9:20 AM: PARALLEL SESSION C, *Plaza Rm, 2nd fl*
STANDARDIZED CLINICAL PROTEOMICS PLATFORMS
Chair: Dan Liebler

8:00 - 8:25 am **Performance and Optimization of LC-MS/MS Platforms for Proteomic Analyses: Interlaboratory Studies**; Dan Liebler; *Vanderbilt Univ School of Medicine; Nashville, TN* 19

8:25 - 8:50 am **Integration of Chromatography, LC-MS/MS Data Acquisition, and Peptide Identification Performance Metrics into a Proteomics Software Pipeline**; Paul Rudnick; *NIST; Gaithersburg, MD* 19

8:50 - 9:05 am **Reproducibility of Protein MRM-Based Assays: Towards Verification of Candidate Biomarkers in Human Plasma**; Steven C. Hall; *UCSF; San Francisco, CA*..... 19

9:05 - 9:20 am **A Lectin Affinity-Based Biomarker Discovery Protocol Targeting Cancer-Specific Glycopeptides in Human Plasma**; Penelope M. Drake; *UCSF; San Francisco, CA* 20

9:20 – 9:45 am: COFFEE BREAK – EXHIBITS, *Poster/Exhibit Hall, 2nd fl*

9:45 AM – 12:00 PM, GENERAL SESSION, *Pavilion, 4th fl*
PROTEOGENOMICS
Co-Chairs: Gil Omenn and Martin Latterich

9:50 - 9:20 am **Mapping and Measuring Proteomes**; Ruedi Aebersold; *ETH Zurich; Zurich, Switzerland* 20

10:20–10:50 am Patrick Brown; *Stanford University, Palo Alto, CA*

10:50–11:20 am **Cancer Epigenomics: The Bridge between Genomics and Proteomics of Human Cancers**; William G. Nelson; *Sidney Kimmel Comprehensive Cancer Ctr at Johns Hopkins; Baltimore, MD*

11:20 am–12pm **Panel Discussion: Integrated Proteogenomics – Where, How and When?**
Discussion Leader: Pavel Pevzner *Participants:* Robert Waterston, Ruedi Aebersold, Patrick Brown, William Nelson, Gil Omenn, Martin Latterich

12:15 – 1:30 PM: VENDOR LUNCH SEMINARS

Agilent Technologies, *Library Room, 1st fl* **GE Healthcare**, *Coronado Room, 3rd fl*

Shimadzu, *Harbor Room, 3rd fl* **Thermo Scientific**, *Plaza Room, 2nd fl*

See next page for seminar descriptions. Reserve a seat at company exhibit booths.

MONDAY LUNCH SEMINARS

Lunch Seminars are from 12:15 – 1:30 pm. See below for room locations.

AGILENT TECHNOLOGIES, *Library Room, 1st Floor*

New Advances in PTM Analysis and Sample Preparation for Proteomics

Presenter: Dr. Christine Miller, *Agilent Technologies, Inc.*

From protein extraction to fractionation, sample preparation plays a key role in proteomics. This session will address solutions for a variety of challenges including extraction of proteins from formalin-fixed paraffin-embedded tissues, improvements in tryptic digestion, and a variety of fractionation approaches. In addition, a newly introduced microfluidic chip will be described which allows easy automation of phosphopeptide enrichment and subsequent analysis of phosphopeptides. This reusable microfluidic chip has an enrichment column consisting of titanium dioxide particles sandwiched between reverse-phase materials for on-line selective phosphopeptide enrichment. This approach allows robust, easy-to-use and reproducible enrichment of phosphopeptides from complex matrices.

GE HEALTHCARE, *Coronado Room, 3rd Floor*

Improving the Gold Standard: Advances in 2D and DIGE for Improved of Intact Proteins and Protein Isoforms

Speaker: Christine Rozanas, Ph.D.

Gel-based methods for proteomics continue to play a strong role in upstream characterization due to the unparalleled ability to resolve and quantify thousands of protein isoforms from complex lysates. The individual resolved protein spots are then much more amenable to mass spectrometry analysis. Over the past ten years, the use of Difference Gel Electrophoresis (2-D DIGE) has provided new tools for quantitation with statistical confidence of each individual protein isoform, resulting in over 1000 citations and continuing adaptation of the technique to new research areas. With the resurgence of interest in the use of 2D gels with DIGE, GE Healthcare continues to introduce products to improve the 2D workflow, resulting in better spot patterns, quantification of more low abundant proteins and the full use of DIGE, including integrated advanced statistical analysis.

This seminar will describe the current state-of-the-art for DIGE, including workflow improvements. New products for sample preparation, isoelectric focusing improvements, and the latest software will be included. We will also provide examples of where DIGE has been applied to specific sample types and non-2D gel separations.

SHIMADZU, *Harbor Room, 3rd Floor*

Interpreting Organized Chaos: Using Multistage Mass Spectrometry to Characterize Glycopeptides & Glycoproteins

Organizers and Presenters: Faith Hays and Scott Kuzdzal, *Shimadzu Scientific Instruments*

Glycosylation is one of the most common post translational modifications of proteins and plays a vital role in most biological processes. Recently many researchers have utilized Electron Capture Dissociation to cleave amide bonds of glycopeptides preferentially to glycosidic bonds. ECD spectra are complex and often provide ambiguous information. In contrast, laser desorption/ionization time-of-flight (MALDI-QIT-TOF) MS generates fragments resulting primarily from the cleavage of sugar linkages, not peptide-peptide or peptide-sugar linkages. This seminar will review practical approaches to glycan analysis, including N-Terminal ladder sequencing, lectin affinity capture, 2D gels and simplified MSn analyses.

THERMO SCIENTIFIC, *Plaza Room, 2nd Floor*

Identification, Characterization, and Quantitation of Signaling Networks: The Role of Mass Spectrometry in Building Biomarker Assays for Phosphoprotein Cascade Monitoring

Speaker: Michael Moran, Ph.D., Director of the Advanced Protein Technology Centre,
The Hospital for Sick Children, University of Toronto

Monitoring protein expression levels following drug treatment provides a direct measure of that treatment. The biggest challenge is in determining which proteins are included in the signaling pathway, and which peptides qualify as putative biomarkers. This presentation will describe a complete workflow for the characterization and quantitation of protein expression levels from label-free discovery on an LTQ Orbitrap mass spectrometer to SRM-based targeted peptide quantitation using triple quadrupole mass spectrometry.

MONDAY, FEBRUARY 23
INTERFACE OF PROTEOMICS & GENOMICS

1:30 – 3:30 PM, MONDAY POSTER SESSION / EXHIBITS
Posters and exhibits will be attended; Refreshments available

3:45 – 5:20 PM, PARALLEL SESSION A, *Pavilion, 4th fl*
BIOINFORMATICS OF MS
Co-Chairs: Pavel Pevzner and Vineet Bafna

Abstract Page #

3:45 - 4:10 pm **Mass Spectrometry of T.Rex: Treasure Trove of Ancient Proteins or Contamination / Statistical Artifacts?**; Pavel Pevzner; *UC San Diego; La Jolla, CA*

4:10 - 4:35 pm Vineet Bafna; *UC San Diego; La Jolla, CA*

4:35 – 4:50 pm **Integrating Top-Down and Bottom-Up MS Data to Predict Post-Translational Protein Modifications Using Markov Chain Monte Carlo;** Stuart R. Jefferys; *University of North Carolina, Chapel Hill, NC* 20

4:50 – 5:05 pm **Detection of Co-Eluted Peptides Using Database Search Methods**; Gelio Alves; *NCBI/NLM/NIH; Bethesda, MD* 20

5:05 – 5:20 pm **ETD Support in the Trans-Proteomic Pipeline**; David D. Shteynberg; *Institute for Systems Biology, Seattle, WA* 21

3:45 – 5:20 PM, PARALLEL SESSION B, *California A, 2nd fl*
CHEMICAL PROTEOMICS
Co-Chairs: Christoph Borchers and Joseph Loo

3:45 - 4:10 pm **Crosslinking Combined with Mass Spectrometry for Structural Proteomics**; Christoph H. Borchers; *UVic Genome BC Proteomics Centre; Victoria, BC* 21

4:10 - 4:35 pm **Prospects for Defining Protein-Small Molecule Ligand Interactions Using ESI Mass Spectrometry**; Joseph A Loo; *University of California, Los Angeles; Los Angeles, CA* 21

4:35 - 4:50 pm **Artificial Kinase Substrates for MALDI-TOF MS-Based Intracellular Phosphorylation Analysis**; Laurie L. Parker; *Purdue University; West Lafayette, IN* 21

4:50 - 5:05 pm **A Cocktail of Isotopically Labeled Peptide Standards for MRM Based Quantitation of 45 Human Plasma Proteins**; Michael A Kuzyk; *UVic Genome BC Proteomics Centre; Victoria, BC* 22

5:05 - 5:20 pm **Small Molecule Capture Compounds™ - Towards a Targeted Reduction of Proteome Complexity**; Christian Jurinke; *caprotec bioanalytics GmbH; Berlin, Germany* 22

3:45 – 5:20 PM, PARALLEL SESSION C, *Plaza Room, 2nd fl*
SYSTEMS ANALYSIS FOR BIOMARKER DISCOVERY I
Co-Chairs: Helmut Meyer and Paul Tempst

3:45 - 4:10 pm **High Performance Proteomics as a Tool in Biomarker Discovery**; Helmut E Meyer; *Ruhr University; Bochum, Germany* *22*

4:10 - 4:35 pm **Blood-Based Exopeptidase Activities as Biomarkers for Cancer**; Paul Tempst; *Memorial Sloan-Kettering Cancer Center; New York, NY* 23

4:35 - 4:50 pm **Spectral Profiles: A Novel Representation of Tandem Mass Spectra and its Applications for *de novo* Peptide Sequencing and Identifications**; Sangtae Kim; *UCSD; La Jolla, CA* 23

4:50 - 5:05 pm **Low Cost, Scalable Proteomics Data Analysis Using Amazon's Cloud Computing Services and Open Source Search Algorithms**; Brian Halligan; *Medical College of Wisconsin; Milwaukee, WI* 23

5:05 - 5:20 pm **Protein Biomarkers beyond Fluctuations in Concentration**; Chad R. Borges; *Arizona State University; Tempe, AZ* 24

5:20 – 7:30 pm: Dinner Break

7:30 – 9:00 PM: EVENING WORKSHOPS

Cardiovascular Proteomics, *Pavilion, 4th fl* **Affinity Reagents & Detection Arrays**, *California A, 2nd fl*

Future of Biomarkers, *Plaza Room, 2nd fl*

See next page for Evening Workshop descriptions.

MONDAY EVENING WORKSHOPS

Workshops begin at 7:30 pm. See below for room locations.

PROTEOMICS IN CARDIOVASCULAR BIOLOGY AND MEDICINE, *Pavilion, 4th fl*

Chair: Susan Old, *NIH/NCRR*

7:30 – 7:50 pm: Pothur Srinivas, *NIH/NHLBI*
NHLBI Mission in Cardiovascular Proteomics: Opportunities and Challenges

7:50-8:10 pm: Cathy Costello, *Boston University*
Molecular Insights on Cardiovascular Response to Stress

8:10 – 8:30 pm: John Yates, *The Scripps Research Institute*
Application of Quantitative Proteomic Approaches to Advance Biology and Medicine

8:30 – 9:00 pm
Panel Discussion: Robert Balaban, *NIH/NHLBI*; Robert Gerszten, *MGH*; Jennifer Van Eyk, *Johns Hopkins*, and Peipei Ping, *UCLA*

AFFINITY REAGENTS & DETECTION ARRAYS FOR PROTEOMICS, *California A, 2nd fl*

Co-Chair: Josh LaBaer, *Director, Harvard Institute of Proteomics*
Co-Chair: Henry Rodriguez, *Director, Clinical Proteomic Technologies for Cancer, National Cancer Institute*

Affinity Capture Reagents

1. John Chaput *, Biodesign Institute at Arizona State* University
   Synbodies - Applying Chemical Diversity to Mimic Antibody Function
2. Guo-Liang Yu, *President and CEO, Epitomics, Inc.*
   A High Throughput System for the Generation of RabMAbs with Multiple Applications

Affinity Detection Platforms

1. Martin Gleeson, *VP Assay Technology, Genalyte Inc.*
   Silicon Photonic Chip Based Molecular Interaction Detection System for Research & Diagnostic Application
2. Mark S. DiIorio, *President & CEO, MagneSensors, Inc.*
   Rapid, Ultra-Sensitive Bioassays Using Magnetic Detection of Magnetic Nanoparticle Labels

THE FUTURE OF BIOMARKERS, *Plaza Room, 2nd fl*

Organizer: Sudhir Srivastava, *NCI Early Detection Research Network*

7:30 – 7:50 pm: Sudhir Srivastava, *NCI EDRN*
Resources Developed by the NCI Early Detection Research Network: Application to Other Diseases
Discovery of biomarkers, derived from genomics, proteomics, metabolomics and/or glycomics platforms continues to be beset by poor performances and false promises. The quality of samples, the lack of standards for reagents and platforms, and poor study designs are, in part, some of the contributing reasons to false discoveries and subsequent failures to meet the clinical needs. The Speaker will discuss some of the resources that the National Cancer Institute's Early Detection Research Network, is building to systematically address these problems.

7:50 – 8:10 pm: Karin Rodland, *PNNL*
Proteomics and Biomarker Discovery: Challenges and Opportunities
This talk will describe some of the major technical and experimental challenges in biomarker discovery, particularly in the use of proteomics in biomarker discovery. Opportunities for solving these problems, using multidisciplinary approaches that include advanced instrumentation, improved bioinformatics tools, and high quality patient samples will be discussed.

8:10 – 8:30 pm: Michael A. Tainsky, *Karmanos Cancer Institute, Wayne State University School of Medicine*
Autoantibody Diagnostics: A Hub Technology for Convergent Systems Biology
The humoral immune system is an exquisite biosensor of changes in protein composition. Increases in protein expression, mutations, and the production of variant proteins are all inducers of novel antibodies. We will discuss the application of multiple genomic and proteomic systems feeding into protein microarray detection of autoantibodies against tumor-specific biomarkers useful for the early detection of cancer.

8:30 – 8:50: David J. Galas, *Institute of System Biology*
Systems Biology and Biomarkers: Approaching Complexity
This talk will discuss the emerging view of complex functional networks in biological systems and the role of biomarkers in providing information about the states of these networks in health and disease. The talk will address the problems of data integration and inference in the discovery and use of biomarkers and discuss several examples of this research approach.

8:50 – 9:00: **Open discussion**

TUESDAY, FEBRUARY 24, 2009
SYSTEMS MEDICINE

8:00 – 9:25 AM, GENERAL SESSION, Pavilion, *4th fl*
ERA OF SYSTEMS MEDICINE
Chair: Peipei Ping

Abstract Page #

8:00 – 8:05 am Opening Remarks

8:05 – 8:40 am **Mitochondria Proteome Alteration In Disease;** Robert Balaban; *NHLBI*

8:45 – 9:25 am **Proteomic Imaging of Endothelial Caveolae: Pumping Antibodies into Tumors**; Jan E. Schnitzer; *Sidney Kimmel Cancer Center; San Diego, CA* 24

9:20 – 9:45 am: COFFEE BREAK – EXHIBITS, *Poster/Exhibit Hall, 2nd fl*

9:45 AM – 12:00 PM, GENERAL SESSION, *Pavilion, 4th fl*
PROTEOMICS & SYSTEMS MEDICINE
Chair: Salvatore Sechi

9:45 – 9:50 am Opening Remarks

9:50 – 10:30 am Peter Liu; *ICRH-CIHR, University of Toronto; Toronto, Canada*

10:35–11:15 am **Interrogating Human Disease Using Protein Microarrays**; Michael Snyder; *Yale University; New Haven, CT* 24

11:15 am – 12 pm **Panel Discussion: The Reality and Future of Systems Medicine**
Discussion Leader: Susan Old, NCRR *Participants:* Robert Balaban, Jan Schnitzer, Michael Snyder

12:15 – 1:30 PM: VENDOR LUNCH SEMINARS

Bio-Rad Laboratories, *Library Room, 1st fl* **Protein Discovery**, *Harbor Room, 3rd fl*

Thermo Scientific, *Plaza Room, 2nd fl*

See next page for seminar descriptions. Reserve a seat at company exhibit booths.

TUESDAY LUNCH SEMINARS

Lunch Seminars are from 12:15 – 1:30 pm. See below for room locations.

BIO-RAD LABORATORIES, *Library Room, 1st Floor*

Join Bio-Rad Laboratories for lunch and presentation.

PROTEIN DISCOVERY, *Harbor Room, 3rd Floor*

Pierre Chaurand, PhD; *Vanderbilt University School of Medicine, Nashville, TN*

High Throughput Preparation and Analysis of Formalin-Fixed Paraffin Embedded Samples by MALDI MS

The preparation of formalin-fixed paraffin embedded samples for analysis by mass spectrometry is still a developing technique. The chemical and enzymatic techniques for reversing protein cross-linking, digestion and liberating peptides from these samples include many reagents that are detrimental to analysis by mass spectrometry. Electrophoresis-solid phase extraction (eSPE) using the Passport 1200 Sample Prep Station (Protein Discovery, Inc.) offers a unique method for cleaning up samples of this type in a way that is high throughput (96 well format) and directly amenable to mass spectrometry. The unique features of this technology for the concentration, desalting, and detergent removal will be demonstrated along with comparison against traditional sample preparation techniques such as solid-phase extraction.

Michael Ford, PhD; *NextGen Sciences, Ann Arbor, MI*

Removing Matrix Effects and Improving Throughput in Quantitative Peptide Analysis Using Electrophoresis-Solid Phase Extraction

The sensitive and reproducible quantification of proteins at or below the nanogram per milliliter concentration range is necessary in the development of new diagnostic markers and biotherapeutic treatments. The use of liquid chromatography coupled with single or multiple reaction monitoring tandem mass spectrometry provides the selectivity, sensitivity, and throughput required. However, biological samples such as plasma and urine contain salts, lipids and various other components that often limit run-to-run reproducibility and sensitivity and generally contaminate the column, autosampler, and source. Traditional techniques such as solvent precipitation and solid phase extraction assist in removing these matrix effects, but generally suffer from poor selectivity and limit the reproducibility and sensitivity of the assay. Here we report the use of a 96-well format electrophoretic system for the removal of salts, lipids and other interfering compounds and concomitant enrichment of peptides for reproducible, quantitative measurement from human urine. The benefits of electrophoretic sample preparation include longer column lifetime, shorter gradients, reduced matrix effects, reduced instrument downtime. Examples of this workflow will be presented along with recommended procedures and a discussion of the analytical performance achieved.

THERMO SCIENTIFIC, *Plaza Room, 2nd Floor*

Quantitative Cancer Stem Cell Phosphoprotein Profiling by Use of Tandem Mass Tags and LC-HCD-MS/MS in an LTQ Orbitrap

Speaker: Carol Nilsson, M.D., Ph.D.; *Pfizer Global Research and Development, Structural and Computational Biology, San Diego, CA*

Cancer stem cells (CSCs) are hypothesized to provide a repository of cells in tumor cell populations that are refractory to chemotherapeutic agents developed for the treatment of differentiated tumor cells. Knowledge of the signaling process is expected to provide new targets for therapeutic intervention. In this study, a novel quantitative phosphoproteomic workflow was applied to determine pathways of differentiation in an NSC11 glioblastoma cancer stem cell line. Peptides were labeled with isobaric Tandem Mass Tags, combined into one sample, and phosphopeptides were enriched by hydrophilic interaction chromatography and TiO_2 prior to analysis with an LTQ Orbitrap mass spectrometer. Protein identification was performed and relative peptide quantification was performed simultaneously by comparison of the abundance ratios of the reporter ions from the TMT tags.

TUESDAY, FEBRUARY 24, 2009
SYSTEMS MEDICINE

1:30 – 3:30 PM: MONDAY POSTER SESSION / EXHIBITS
Posters and exhibits will be attended; Refreshments served

3:45 – 5:20 PM, PARALLEL SESSION A, *Pavilion, 4th fl*
SYSTEMS ANALYSIS FOR BIOMARKER DISCOVERY II
Co-Chairs: Mark Chance and Gordon Mills

Abstract Page #

3:45 - 4:10 pm **Discovery and Scoring of Protein Interaction Sub-Networks Discriminative of Late Stage Human Colon Cancer**; Mark Chance; *Case Western Reserve University; Cleveland, OH* 24

4:10 – 4:35 pm **Systems Biology Approach to the Discovery and Implementation of Targeted Therapeutics**; Gordon B. Mills; *UT Houston / MD Anderson Cancer Center; Houston, TX*

4:35 – 4:50 pm **Novel Markers of Myocardial Injury**; Robert Gerzsten; *Massachusetts General Hospital; Boston, MA*

4:50 - 5:05 pm **The FGFR3 Network in Multiple Myeloma: A Phosphotyrosine Proteomic Profile Associated with FGFR3 Expression, Ligand Activation, and Drug Inhibition**; Michael F. Moran; *Hospital For Sick Children; Toronto, Canada* 25

5:05 - 5:20 pm **Integrative Approach for Discovery of Diagnostic Serum Biomarkers of Prion Disease in Mice**; Hyuntae Yoo; *Institute for Systems Biology; Seattle, WA* 25

3:45 – 5:20 PM, PARALLEL SESSION B, *California A, 2nd fl*
DATABASES AND SYSTEMS INTEGRATION
Co-Chairs: Martin McIntosh and Cathy Wu

3:45 – 4:10 pm **Interrogating Proteomic Data Using Genomic Databases to Identify Cancer Specific Proteins**; Martin McIntosh; *Fred Hutchinson Cancer Research Center; Seattle, WA*

4:10 - 4:35 pm **Biological Pathway and Network Analysis for Functional Interpretation of Large-Scale Proteomics Data**; Cathy H Wu; *Georgetown University Medical Center; Washington, DC* 25

4:35 - 4:50 pm **RAId_DbS: Mass-Spectrometry Based Peptide Identification with Knowledge Integration**; Yi-Kuo Yu; *NCBI/NLM/NIH; Bethesda, MD* 25

4:50 - 5:05 pm **Proteomics by the Numbers: The Use of Computers to Aid Analysis, Understanding and Prediction in Mass Spectrometry Proteomics**; Lennart Martens; *EMBL-EBI; Hinxton, Cambridge* 26

5:05 - 5:20 pm **Virtual Screening to Identify Signal Transduction Pathway Segments in the APC(Min/+) Mouse**; Gurkan Bebek; *Case Western Reserve University; Cleveland, OH* 26

3:45 – 5:20 PM, PARALLEL SESSION C, *Plaza Room, 2nd fl*
STRUCTURE & DYNAMICS OF MACROMOLECULAR ASSEMBLIES
Co-Chairs: Lan Huang and Ralph Bradshaw

3:45 - 4:10 pm **Investigation of the Structural Dynamics of the 26S Proteasome Complex Using Mass Spectrometry**; Lan Huang; *UC Irvine; Irvine, CA* 26

4:10 - 4:35 pm **Tracking UPS Using Mass Spectrometry – Profiling of Ubiquitylated Proteins to Reveal Proteasome Substrates.**; Thibault Mayor; *University of British Columbia; Vancouver, BC* 26

4:35 - 4:50 pm **Advanced H/DX-MS Strategies for High Resolution Modeling of Large Protein Complexes**; David Schriemer; *University of Calgary; Calgary, Canada* 27

4:50 - 5:05 pm **Structural Mass Spectrometry of a Molecular Machine: Functional Consequences of Conformational Changes in the ClpAP Protease**; Jen Bohon; *Case Western Reserve University* 27

5:05 – 5:20 pm **Mapping Gene Regulatory Pathways by Assembly of Physical and Genetic Interactions**; Trey Ideker; *UC San Diego, La Jolla, CA* 27

5:20 – 7:30 pm: Dinner Break.

7:30 – 9:00 PM: EVENING WORKSHOPS

Statistical Proteomics Initiative, *Pavilion, 4th fl* **Disease Proteomics**, *California A, 2nd fl*

See next page for Evening Workshop descriptions.

TUESDAY EVENING WORKSHOPS

Workshops begin at 7:30 pm. See below for room locations.

STATISTICAL PROTEOMICS INITIATIVE, *Pavilion, 4th fl*
Organizer: Martin McIntosh, *Fred Hutchinson Cancer Research Center*

7:00 – 7:35 pm
Welcome and Introduction: Martin McIntosh, *FHCRC*

7:35 – 8:00 pm
Ann Oberg, *Mayo Clinic* and Olga Vitek, *Purdue University*
Introduction to Statistical Principles in Designing Proteomics Experiments
In this presentation, suitable for the applied laboratory scientists, basic statistical design principles are reviewed and discussed in the context of proteomics experiments.

8:00 – 8:25 pm
Michael MacCoss, *University of Washington*
Some Important Statistical Issues in Mass Spectrometry Proteomics Data Analysis
This presentation will discuss some important unsolved problems in mass spectrometry data analysis that deserve more attention from statistical and computational scientists.

8:25 – 9:00 pm
SPI General Updates, Discussions and New Proposals

DISEASE PROTEOMICS: TRANSLATION OF DISCOVERY INTO CLINICAL PRACTICE, *California A, 2nd fl*
Co-Organizer: Daniel W. Chan, *Johns Hopkins Medical Institutes*
Co-Organizer: Gilbert W. Omenn, *University of Michigan*

The Workshop will begin with a brief 25-minute introduction on Clinical Needs and on Performance Requirements to accelerate the progress on disease biomarkers from discovery to confirmation to validation to commercial and clinical introduction. Next, three interacting panels will discuss and engage participants in discussion of the questions outlined below.

Biomarker developers, users, and regulators from academe, industry and government are invited to join us for this stimulating discussion.

Interacting Panels –

Academic: Bill Hancock (Northeastern University) and Mike Snyder (Yale University)

Government: Jacob Kagan (NCI-EDRN) and Maria Chan (FDA)

Industry: Paul Predki (Invitrogen), Scott Kuzdzal (Shimadzu), Isaac Mizrahi (Beckman Coulter), and Thomas Li (Roche)

Questions –

1. How can we combine a focus on diagnoses with a focus on disease mechanisms? What are good examples? What are good examples of stratification of patients for prognosis and for choice of therapy?
2. Must we measure individual or patient specimens or can properly pooled specimens suffice for disease vs normal, or staging of disease analyses? How do we confidently differentiate disease vs normal if biologic replications of each have quite marked differences, even in the same individual?
3. How can we move from proteomics of diseased organs to detectable biomarkers in plasma or other readily assayed biofluids?
4. What are the core criteria from clinical chemistry that should be met in our studies in order to gain CLIA and FDA approvals and clinical acceptance?

WEDNESDAY, FEBRUARY 25, 2009
PROTEIN STRUCTURE AND MODIFICATION

8:00 – 9:20 AM, PARALLEL SESSION B, *California A, 2nd fl*
PTM ANALYSES OF COMPLEX BIOLOGICAL MATTER
Co-Chairs: Josh Coon and Robert Cotter

Abstract Page #

8:00 – 8:25 am **Why Hybrid Mass Spectrometers with Multiple Analyzers and Dissociation Methods Will Transform Protein Post-Translational Modification Analysis**; Joshua Coon; *University of Wisconsin-Madison; Madison, WI*

8:25 - 8:50 am **Strategies for the Analysis of Lysine Modifications**; Robert Cotter; *Johns Hopkins University; Baltimore, MD* 28

8:50 - 9:05 am **Use of Antibody Microarrays for Ovarian Cancer Biomarker Discovery**; Arturo B. Ramirez; *Fred Hutchinson CRC; Seattle, WA* 28

9:05 - 9:20 am **Quantification of Posttranslationally Modified Peptides Using Tandem Mass Tags and Electron Transfer Dissociation**; Rosa I Viner; *ThermoFisher Scientific; San Jose, CA* 28

8:00 – 9:20 AM, PARALLEL SESSION C, *Plaza Room, 2nd fl*
GLYCOPROTEOMICS
Co-Chairs: Cathy Costello and Nelly Viseux

8:00 – 8:25 am **Proteomic and Glycomic Analyses of E-cadherin Complexes**; Cathy Costello; *Boston University School of Medicine; Boston, MA* 28

8:25 – 8:50 am Nelly Viseux; *Shire Pharmaceuticals; Cambridge, MA*

8:50 - 9:05 am **Glycoproteomics of IgA1**; Matthew B. Renfrow; *University of Alabama at Birmingham* 29

9:05 - 9:20 am **Identification of Cancer Specific Glycosylation Patterns of PSA for Prostate Cancer Diagnosis**; Yan Li; *Johns Hopkins University; Baltimore, MD* 29

9:20 – 9:45 am: COFFEE BREAK – EXHIBITS, *Poster/Exhibit Hall, 2nd fl*

9:45 – 11:20 AM, PARALLEL SESSION A, *Pavilion, 4th fl*
MASS SPECTROMETRY ADVANCEMENT
Co-Chairs: Natalie Ahn and Michael Siu

9:45 - 10:10 am **Absolute Quantification of Potential Cancer Markers in Clinical Tissue Homogenates Using Multiple-Reaction Monitoring on a Linear Ion Trap Mass Spectrometer.**; Michael Siu; *York University; Toronto, ON* 29

10:10 - 10:35 am **Functional Proteomics Identifies Targets of Phosphorylation by B-Raf Signaling in Melanoma**; William Old; *University of Colorado; Boulder, CO* 30

10:35 - 10:50 am **Quantitative Label-Free Proteomics of Human Saliva**; Joseph A Loo; *University of California, Los Angeles; Los Angeles, CA* 30

10:50 - 11:05 am **PAcIFIC: How to Dive Deeper into the Proteomics Ocean**; Alexandre D. Panchaud; *University of Washington; Seattle, WA* 30

11:05 - 11:20 am **Intact Protein Analysis Using Electron Transfer Dissociation Mass Spectrometry**; Zhiqi Hao; *ThermoFisher Scientific; San Jose, CA* 30

WEDNESDAY, FEBRUARY 25, 2009
PROTEIN STRUCTURE AND MODIFICATION

9:45 – 11:20 AM, PARALLEL SESSION B, *California A, 2nd fl*
MEMBRANE PROTEOMICS
Co-Chairs: Mark Baker and John Yates

Abstract Page #

9:45 - 10:10 am	**Comprehensive Coverage of Membrane Proteomes**; Mark Baker; *Macquarie University; Sydney, Australia*	31
10:10-10:35 am	Christine C. Wu; *University of Colorado School of Medicine; Aurora, CO.*	
10:35 - 10:50 am	**Post-Translational Modifications of Integral Membrane Proteins Resolved by Top-Down Fourier-Transform Mass Spectrometry.**; Julian Whitelegge; *UCLA; Los Angeles, CA*	31
10:50 - 11:05 am	**Native Membrane Protein Immobilization on a Solid Surface**; Kenneth Olesen; *Nanoxis; Gothenburg, Sweden*	31
11:05 - 11:20 am	**Conserved Waters Define a Structural and Functional Channel Involved in Activation of the G Protein-Coupled Receptor Rhodopsin**; Mark Chance; *Case Western Reserve Univ.; Cleveland, OH*	32

9:45 – 11:20 AM, PARALLEL SESSION C, *Plaza Room, 2nd fl*
BIOINFORMATIC ANALYSES OF PROTEIN STRUCTURE
Chair: Elizabeth Komives

9:45 - 10:10 am	**Predicting the Peptide Recognition Specificity of the SH3**; Wei Wang; *UCSD; La Jolla, CA*	32
10:10–10:35 am	**Finding Ligand Binding Sites on a Proteome-Wide Scale and its Implications**; Philip Bourne; *San Diego Supercomputer Center; UCSD; La Jolla, CA*	
10:35 - 10:50 am	**Efficient Identification of Chemical Crosslinks Using Label-Free Pattern Comparisons To Deduce Structural Features of Macromolecular Complexes**; David W. Speicher; *The Wistar Institute; Philadelphia, PA*	32
10:50 - 11:05 am	**Protein Structure Determination by Combining Structural Mass Spectrometry Data with Rosetta**; Xiaojing Zheng; *Case Center for Proteomics; Cleveland, OH*	32

1:00 – 3:15 PM, GENERAL SESSION, *Pavilion, 4th fl*
PTM ANALYSES
Chair: Christoph Borchers

1:00 – 1:05 pm	Opening Remarks
1:05 – 1:35 pm	**Innovative Technology for the Study of Protein Post-Translational Modifications;** Donald F. Hunt; *University of Virginia; Charlottesville, VA* 33
1:35 – 2:05 pm	**Lys-N; A Protease Ideal for Proteomics Facilitating the Targeted Analysis of Post-Translational Modifications and *de novo* Sequencing;** Albert Heck; *Utrecht University; Utrecht, The Netherlands* 33
2:05 - 2:35 pm	**New Developments for the Improved Characterization of Post Translational Modifications**; Richard Smith; *PNNL; Richland, WA* 33
2:25 – 3:15 pm	***Panel Discussion:*** **PTM in the Future –What, When and How?** *Discussion Leader:* Christoph Borchers *Participants:* Donald Hunt, Albert Heck, Richard Smith, Cathy Costello, Joshua Coon, Robert Cotter
3:15 – 3:30 pm	Announcement of 2010 conference; Closing remarks.

Conference Closing Event: Dinner Cruise of San Diego Bay *(Ticket required)*

6:00 pm: Meet in Hotel Lobby to walk to dinner cruise.
Please wear comfortable shoes and sweater / jacket.

Monday, 8:00 – 9:20 am
Cellular Networks

MON 8:00 - 8:25 am: Unbiased Pathway Analysis of Expression Proteomics Data Predicts Signaling Status in Cancer Cells and Biopsy Samples

Roman A. Zubarev[1]; Sara Stahl[2]; Johan Lengqvist[2]; Birgitta Mork[2]; Y.M. Eva Fung[1]; Christopher Adams[1]; Bo Stenerlow[1]; Rolf Lewensohn[2]; Janne Lehtio[2]; Kristina Viktorsson[2]
[1]Uppsala University, Uppsala, Sweden; [2]Karolinska Institute, Stockholm, Sweden

Expression proteomics data acquired in an inbiased analysis can be used to generate non-trivial hypotheses on which signaling pathways are activated and des-activated in the sample of interest. The hypothesis generation is performed by a novel software tool Pathway Search Engine (PSE). We have developed a PSE that uses a database of protein-protein and protein-molecule interactions TRANSPATH as well as a pathway analysis tool ExPlain developed earlier for mRNA array analysis. This PSE has been described and its performance characterized using original as well as literature proteomics data with known signaling status (1). Here we report on PSE applications for the analysis of biological systems with a priori unknown activated pathways.

In a prime example, signaling pathways were analyzed in non-small cell lung carcinomas (NSCLC) irradiated by ionizing radiation of different quality. While low linear energy transfer (LET) radiation regularly employed for cancer therapy failed to induce apoptosis in NSCLC U-1810 cells, half the dosage of a high-LET radiation killed the same cells within 48 h. For pathway analysis, cells were harvested 4 h after irradiation, and an unsupervised, unbiased proteomics analysis was performed by a single nano-LC MS/MS run per sample. Protein quantification was performed by the lable-free method which yielded 650 identified proteins (common for all samples) as wellas their relative abundances (unique for each sample). Two replicates of each sample were analyzed. The PSE has identified the JNK-pathway activation (p=6•10-^6) as a key event in response to high-LET IR-induced apoptotic signaling. In addition, the Fas-pathway was reported activated (p=3•10-5) and the p38-pathway was found deactivated (p=0.001) compared to untreated cells. Immuno-based analyses confirmed that high-LET irradiation caused an increase in phosphorylation of JNK, while pharmacological inhibition of JNK blocked high-LET induced apoptotic signaling. We concluded that in contrast to conventional low-LET IR, high-LET IR can trigger activation of the JNK-pathway which in turn is critical for induction of apoptosis in these cells. The immuno-chemical analysis of Fas and p38 pathways have also agreed with the PSE outcome. Thus PSE predictions were largely validated, and the PSE itself was confirmed to be an efficient hypothesis-generating tool.

1. Zubarev, R. A.; Nielsen, M. L.; Savitski, M. M.; Kel-Margoulis, O.; Wingender, E.; Kel, A. Identification of dominant signaling pathways from proteomics expression data, J. Proteomics, 2008, 1, 89-96.

MON 8:50 - 9:05 am: Characterization of Proteomic Changes Induced in Cells and Tissues by Oxidative Stress

Yelena Margolin ; Emily M. Freeman; Alexander R. Ivanov
Harvard School of Public Health, Boston, MA

Cellular response to oxidative stress is accomplished through signal transduction cascades involving post-translational modifications (PTMs) of proteins. Proteomic studies of signal transduction are confounded by low levels, complex dynamics and stoicheometry of protein PTMs in biological samples, as well as suboptimal detection sensitivity of MS-based techniques, and require more efficient methodologies for sample preparation and enrichment of modified peptides.

Fat adipose tissue samples originated from mice of distinct gene lineages were lysed and partitioned using the novel pressure cycling method. The protein extracts were subjected to 2D-gel and gel-LC MS/MS quantitative profiling. The phosphopeptide analysis included trapping both phosphorylated and non-phosphorylated peptides on the pre-column with sequential two elutions onto analytical nano-LC column coupled to LTQ Orbitrap MS. 2D-gel and gel-LC MS/MS quantitative analysis revealed a substantial overlap of differentially expressed proteins in white adipose tissue of genetically different model animals. PTMs including 4-hydroxynonelation, phosphorylation, acetylation, and ubiquitylation of selected proteins were characterized. Correlation of transcriptomic and proteomic expression profiles enabled identification of differentially regulated patterns in signaling pathways and interaction networks. To study changes in protein phosphorylation, we have developed and characterized a new approach for selective enrichment of phosphorylated peptides using a mixed-mode trapping column coupled on-line to LC-MS. We validated the efficiency of this method using phosphopeptide standards and applied it to the study of protein phosphorylation in HepG2 cells induced by oxidative stress. Elucidated alterations in protein abundance and PTMs related to hypoxia, oxidative stress response, energy, lipid and glucose metabolism pathways may be useful in the mechanistic studies of obesity, associated metabolic disorders, and cellular oxidative stress response. A highly efficient automated on-line phosphoenrichment technique was developed and further validated using complex peptide mixtures and whole cell lysates.

MON 9:05 - 9:20 am: Quantitative Phosphoproteomic Dissection of Signaling Pathways Applied to T Cell and Mast Cell Signaling

Arthur R. Salomon
Brown University, Providence, RI

Here we introduce and evaluate a new quantitative approach for phosphoproteomic analysis of signaling pathway structure. Current approaches in phosphoproteomics focus on analysis of the global phosphoproteome in a single cellular state or of receptor stimulation time course experiments, often with a restricted number of time points. Our approach combines genetic analysis of isogenic signaling pathway mutants with a quantitative phosphoproteomic method that examines disruption of downstream phosphorylation events through a time course of receptor activation using recently developed visual pathway analysis tools. This new approach is evaluated in the context of the T cell signaling pathway and a T cell clone lacking the upstream Zap-70 tyrosine kinase and its reconstituted counterpart. In our approach, label free quantitation using normalization to copurified phosphopeptide standards is applied to assemble high density temporal data within a single cell type, either Zap-70 null or reconstituted cells, providing a list of candidate phosphorylation sites that change in abundance after T cell stimulation. Metabolic labeling of proteins using the SILAC method allows for the calculation of ratios used to compare Zap-70 null and reconstituted cells across a timecourse of receptor stimulation, providing direct information about the placement of newly observed phosphorylation sites relative to the critical T cell signaling protein Zap- 70. Quantitative phosphoproteomic signatures indicative of downstream inhibition, downstream activation, and mutant compensation are revealed and validated with proteomic pathway visual analysis tools using the established T cell signaling pathway structure as a scaffold. New predictions of the structure of T cell signaling pathway are provided at the phosphorylation site level. The approach described here seeks to transcend the publication of phosphoproteomic data with minimal biological analysis in favor of a targeted approach that positions the deluge of newly discovered phosphorylation sites relative to canonical signaling landmarks. Our methods are adaptable to any cell culture signaling system in which isogenic wild type and mutant cells have been or can be derived using any available phosphopeptide enrichment strategy.

Monday, 8:00 – 9:20 am
Organelle Proteomics / Omics of Mitochondria

MON 8:25 - 8:50 am: Exploring the Role of PTMs in Mitochondria in Heart Failure and with Cardiac Resynchronization Therapy

Giulio Agnetti; Shengbing Wang; Lesely Kane; David Kass; Simon Sheng; Jennifer E. Van Eyk
Johns Hopkins Medical Institutes, Baltimore, MD

Cardiac resynchronization therapy (CRT) has become an effective clinical treatment for heart failure (HF) patients leading to improvement in heart function, clinical symptoms and survival. Yet, the molecular mechanisms behind the therapeutic benefit of CRT are not known. Our latest data shows that dyssynchrony-induced heart failure (DHF) and CRT affect the mitochondrial subproteome by altering specific key proteins in cellular redox control and oxidative phosphorylation (OxPhos) pathways, manifested as changes in both protein quantity and PTMs within the mitochondria. Cardiac mitochondria isolated from the left ventricle of a large mammalian model of produced by 3 weeks of rapid pacing and compared to control unpaced hearts and hearts that underwent 3 additional weeks of either synchronized or desynchronized pacing. CRT resulted in increase efficient ATP production, reduced ROS production from complex I and increased enzymatic activity of ATP synthase, complex V. These functional improvements have been correlated to a number of novel phosphorylated amino acid residues. Furthermore, specific phosphorylations have now been correlated or causatively linked to different functional aspects. For example, CRT alters the phosphorylation ATP synthase beta subunit, dephosphorylating several of the 5 known phosphorylated residues; one of which acts as a functional switch. While a newly discovered Tyrosine phosphorylation of ATP synthase beta is increased with CRT and appears to control OxPhos supercomplex formation. Target MRM experiments for various phosphorylated residues of ATP synthase beta are now being used to quantify the level of phosphorylation in various animal and human models providing insight into universality of the phosphorylation regulation of the OxPhos pathway.

MON 8:50 - 9:05 am: Quantification of Proteome Changes in L6 Myotubes with Giant Mitochondria Induced by Blocking Autophagy

Rongxiao Sa; Marian Navratil; Edgar A Arriaga
University of Minnesota, Minneapolis, MN

Background:Giant mitochondria are believed to be dysfunctional organelles accumulating in aged or diseased post-mitotic tissue. The mechnism of giant mitochondria formation is not fully understood, but early reports suggest that they accumulate in cells due to deficient autophagy. We have previously determined by immunofluorescence microscopy that, upon inhibition of autophagy, the expression of the mitochondrial fusion protein OPA1 is significantly decreased in giant mitochondria. In order to identify other proteins and molecular processes that may involved in giant mitochondria formation, we carried out a proteomic experiment to study the proteome changes in L6 myotubes containing giant mitochondria.

Experimental Design: In this study, L6 rat myoblast cells were cultured in DMEM medium supplemented with either heavy(13C615N4-Arg and 13C415N2-Lys) or light (12C614N4-Arg and 12C614N2-Lys) amino acids using stable isotope labeling in cell culture(SILAC). After cell differentiation, cells labeled with heavy amino acids were treated with RNAi to knock down the expression of the autophagy related ATG7 gene. The cells labeled with light amino acids were used as control. The treated and control cells were then pooled and fractioned into nuclear, mitochondrial and cytosolic fractions. Proteins in each fraction were separated by SDS-PAGE and cut into 10 slices and then subjected to in-gel tryptic digestion. The peptide mixtures were separated by nano-HPLC and MS analysis was done using a Thermo Finnigan LTQ-orbitrap instrument. The data was searched against a decoy IPI rat database using Mascot. The identified proteins had at least two peptides with the threshold P value set to 0.01, which is equivalent to FDR< 2%. Quantification was done with MSQuant using the ratio of intensities of the peptides with heavy over light isotopes. Proteins with ratios ≠1(P≤ 0.01) were analyzed with the Ingenuity Pathway Analysis software(IPA).

Results: Knocking down the ATG7 gene was confirmed by real-time PCR and the formation of giant mitochondria was confirmed by microscopy. A total of 2600 proteins were identified, 40% changed in expression(ratios ≠1), and 8% had > 50% expression change. In agreement with our earlier studies, OPA1 was downregulated by 23%. Based on the autophagy pathway, blocking of ATG7 could lead to downregulation of downstream proteins in this pathway. For example, we determined that two of those proteins(SCARB2 and LAMP1) were downregulated. As expected, autophagy blockage alters mitochondrial function(IPA, p=2.68×10-7). Fifteen proteins in the oxidative phosphorylation complexes were downregulated suggesting that mitochondrial biogenesis is impaired when blocking autophagy. Not surprisingly, 11 enzymes involved in glycolysis appear upregulated, which would serve as a compensatory mechnism to meet ATP demands. Lastly, protective mechnisms against reactive oxygen species are also altered: mitochondrial SOD increased, while cytosolic SOD decreased; PRDX1 and PRDX4 were upregulated while PRDX2,PRDX3 and PRDX5 were downregulated. Altogether, these results point to altered mechanisms in giant mitochondria and to their possible role in aging and disease. This presentation will further elaborate on the metabolic pathways that are highlighted by IPA.

This work is supported by NIHR01-AG20866

MON 9:05 - 9:20 am: Label-Independent Quantitative Analysis of Skeletal Muscle Mitochondria in Rat Obesity Model

Nichole A Reisdorph[1]; Richard Reisdorph[1]; Roger Powell[1]; Matthew Jackman[2]; Michael Armstrong[1]
[1]National Jewish Health, Denver, CO; [2]University of Colorado, Denver, Aurora, CO

Global analysis of the mitochondrial proteome from skeletal muscle has presented unique challenges, including contamination from high amounts of myosin during the enrichment process. We have applied various protein fractionation techniques to skeletal mitochondrial proteomes from an obesity resistant rat model. Two-dimensional gel electrophoresis studies were confounded by poor representation of the proteome. Fractionation using reversed phase chromatography was complicated by the tendency of myosin to bind to mitochondrial proteins, resulting in poor resolution and heavy myosin contamination. Strategies utilizing molecular weight cut off, strong cation exchange, and antibody-based depletion of myosin were similarly ineffective. We therefore used 1-dimensional SDS-PAGE as a fractionation technique to separate myosin and to resolve muscle mitochondrial proteins. Intact mitochondrial proteins from pooled obesity resistant (OR) and obesity prone (OP) rats were separated on a gradient gel. Gel lanes were cut into 40 slices and subjected to in-gel trypsin digests. Peptides were first analyzed in MSMS mode on a quadrupole time-of-flight (QTOF) mass spectrometer for global protein identification. While our reversed phase method resulted in 330 mitochondrial proteins identified by 2 or more peptides, the SDS-PAGE fractionation technique yields, on average , 15-20 unique mitochondrial proteins per gel slice, for an anticipated total of over 500 proteins following validation. In order to determine quantitative differences between mitochondrial proteins from obesity prone and obesity resistant rats, we employed a label-independent quantitation method using accurate mass and retention time for molecular features. Peptides were analyzed on a QTOF in MS mode (n=3) for quantitative profiling. Molecular features were aligned for mass and retention time in GeneSpring MS. After filtering and statistical analysis, features that passed a two-fold change filter were exported as an inclusion list for targeted MSMS analysis. Quantitative differences are evident between OR and OP rats; for example in three consecutive gel slice sets we found 84 molecular features with changes ranging from 2 fold to over 20 fold. Targeted MS/MS analysis results allowed matching of a subset of these features to 6 proteins identified with 2 or more peptides. This study underscores the challenges inherent in tissue-specific mitochondrial proteomics, including the importance

of fractionation, and illustrates an effective quantitative method for the mitochondrial proteome.

Monday, 8:00 – 9:20 am
Standardized Clinical Proteomics Platforms

MON 8:00 - 8:25 am: Performance and Optimization of LC-MS/MS Platforms for Proteomic Analyses: Interlaboratory Studies

Daniel C. Liebler[1]; Amanda G. Paulovich[2]; David L. Tabb[1]; Paul A. Rudnick[3]; Karl R. Clauser[4]; Dean D. Billheimer[5]; Ronald K. Blackman[4]; Amy L. Ham[1]; Helene L. Cardasis[6]; Jacob D. Jaffe[4]; David M. Bunk[3]; Mehdi Mesri[7]; Mehdi Mesri[7]; Christopher R. Kinsinger[7]; Thomas A. Neubert[6]; Mehdi Mesri[7]; Tony G. Tegeler[8]; Birgit Schilling[9]; Asokan Mulayath Variyath[10]; Lorenzo Vega-Montoto[10]; Pei Wang[2]; Mu Wang[8]; Jeffrey R. Whiteaker[2]; Lisa J. Zimmerman[1]; Steven A. Carr[4]; Bradford W. Gibson[9]; Susan J. Fisher[11]; Fred E. Regnier[12]; Henry Rodriguez[7]; Cliff Spiegelman[10]; Paul Tempst[13]; Stephen E. Stein[3]

[1]Vanderbilt University, Nashville, TN; [2]Fred Hutchinson Cancer Research Center, Seattle, WA; [3]National Institute of Standards and Technology, Gaithersburg, MD; [4]Broad Institute of MIT and Harvard, Cambridge, MA; [5]University of Arizona, Tucson, Arizona; [6]New York University, New York, NY; [7]National Cancer Institute, Bethesda, MD; [8]Monarch Life Sciences, Indianapolis, IN; [9]Buck Institute for Age Research, Novato, CA; [10]Texas A&M University, College Station, TX; [11]University of California at San Francisco, San Francisco, CA; [12]Purdue University, West Lafayette, IN; [13]Memorial Sloan-Kettering Cancer Center, New York, NY

Shotgun proteomics platforms based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) are employed for unbiased biomarker discovery, yet their performance has been poorly defined. A series of interlab studies (part of the National Cancer Institute-funded Clinical Proteomics Technologies Assessment for Cancer (CPTAC) Network) evaluated ion trap-based LC-MS/MS platforms for shotgun proteomics and employed standard protein mixtures, evolved standard operating procedures (SOPs), evaluated sources of variability and detection of proteins at different abundances in complex mixtures. Analyses were performed on Thermo LTQ and LTQ-Orbitrap instruments. We describe 44 system performance metrics for monitoring chromatographic performance, electrospray source stability, MS1 and MS2 signals, dynamic sampling of ions for MS/MS and peptide identification. These metrics display consistent, reasonable responses to controlled system perturbations and can reveal even subtle differences in performance of system components. Analyses of data from interlaboratory studies using SOPs identified outlier data and provided clues to specific causes. Interlaboratory variation in the metrics indicates which system components provide the greatest challenges in standardization. SOPs required progressive refinement and practice to produce comparable results. While identifications of peptides were associated with low repeatability and reproducibility, these values for protein identification were far greater. Peptide variation was high, with different subsets of peptides identified in each analysis from a simple protein mixture. Protein variation was low, however, because different subsets of peptides may equivalently identify each protein. We describe large-scale production of the yeast Saccharomyces cerevisiae reference proteome, which is now offered to thecommunity. Using metrics described above to characterize LC-MS/MS performance, we provide a historic reference dataset demonstrating reasonable performance of platforms in expert laboratories. The results provide a basis for laboratories to benchmark their own performance, to improve upon current methods, and to evaluate new technologies. Additional studies used a 48 human protein mixture spiked into the yeast proteome to estimate the power of standardized LC-MS/MS platforms to detect proteins at defined levels in a yeast proteome extract. This approach can be used to benchmark the power of shotgun platforms for detection of differentially expressed proteins at different levels of concentration in a complex matrix, thereby providing a tool to evaluate proteomic platforms for biomarker discovery.

MON 8:25 - 8:50 am: Integration of Chromatography, LC-MS/MS Data Acquisition, and Peptide Identification Performance Metrics into a Proteomics Software Pipeline

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Analytical variability is an often overlooked reality for most proteomics workflows. Failure to identify and measure it can undermine biomarker discovery efforts. In this report, we describe a panel of performance metrics targeting 6 specific areas of LC-MS/MS proteomics platforms. These categories include liquid chromatography, ion source, dynamic sampling, MS, MS/MS and peptide identification. Variability in each these categories is dissected with specific and quantitative metrics which can be used to evaluate technical repeatability (within a series) and reproducibility (between series). These metrics have been implemented in a freely available software pipeline. The pipeline directly processes Thermo Fisher .raw files (from LTQ and LTQ-hybrid mass spectrometers) and includes: (1) a converter/feature-finding algorithm for extracting peak lists, and approximating peak widths and peak heights, (2) a peptide identification engine (SpectraST or OMSSA), (3) a program that calculates all of the metrics using the output files, and (4) a program generating summary statistics reported in a text file. The programs are implemented in C or C++ and the pipeline script is written in Perl. An example application using increasing loading amounts of a yeast reference material will be presented. This software represents a new tool for assessing technical variability and optimizing performance in shotgun proteomics.

MON 8:50 - 9:05 am: Reproducibility of Protein MRM-Based Assays: Towards Verification of Candidate Biomarkers in Human Plasma

Steven C. Hall[1]; Terri Addona[2]; Susan E. Abbatiello[2]; Steven J. Skates[3]; David M. Bunk[4]; Birgit Schilling[5]; Clifford H. Spiegelman[6]; Lisa J. Zimmerman[7]; Amy-Joan Ham[7]; Hasmik Keshishian[2]; Simon Allen[1]; N. Leigh Anderson[8]; Ronald K. Blackman[2]; Christoph H. Borchers[9]; Charles Buck[10]; Helene L. Cardisis[11]; Michael P. Cusack[5]; Nathan G. Dodder[4]; Bradford W. Gibson[5]; Jason M. Held[5]; Tara Hiltke[12]; Angela Jackson[9]; Eric B. Johansen[1]; Christopher R. Kinsinger[12]; Jing Li[7]; Denkanikota R. Mani[2]; Medhi Mesri[12]; Thomas A. Neubert[11]; Richard K. Niles[1]; Amanda G. Paulovich[13]; Trenton C. Pulsipher[3]; Henry Rodriguez[12]; Paul A. Rudnick[4]; Derek Smith[9]; David L. Tabb[7]; Tony J. Tegeler[14]; Asokan M. Variyath[6]; Lorenzo J. Vega-Montoto[6]; Asa Wahlander[11]; Sofia Waldemarson[11]; Mu Wang[16]; Jeffrey R. Whiteaker[13]; Susan J. Fisher[1]; Daniel C. Liebler[7]; Fred E. Regnier[10]; Paul Tempst[15]; Steven A. Carr[2]

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Verification of candidate protein biomarkers requires high throughput analytical methodology that is quantitative, sensitive and specific. In addition, robust standard operating procedures (SOPs) for sample preparation, data acquisition and analysis must be strictly followed in order implement these types of studies across multiple sites with minimum variation in the results. Currently, validation of biomarkers is accomplished by employing immunoassays. However, development of immunoassays for protein biomarkers depends on suitable antibodies that, if not

available, can result in considerable time and expense to produce. Recently, the utility of multiple reaction monitoring (MRM) coupled with stable-isotope dilution mass spectrometry (SID-MS) for quantification of proteins in human plasma and serum has been demonstrated. However, reproducibility and transferability of protein-based MRM assays across multiple laboratories has yet to be shown as feasible. Towards this goal, we describe a study that was implemented to assess intra- and inter-laboratory performance of an MRM-SID-MS assay for quantitating seven target proteins that were spiked into human plasma.

MON 9:05 - 9:20 am: A Lectin Affinity-Based Biomarker Discovery Protocol Targeting Cancer-Specific Glycopeptides in Human Plasma

Penelope M. Drake[1]; Michael T. Lerch[1]; Eric B. Johansen[1]; Richard K. Niles[1]; Birgit Schilling[2]; Haichuan Liu[1]; Bensheng Li[2]; Simon Allen[1]; Steven C. Hall[1]; H. Ewa Witkowska[1]; Fred E. Regnier[3]; Bradford W. Gibson[2]; Susan J. Fisher[1]

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Glycans are an important class of post-translational modifications. Typically found on secreted and extracellular molecules, glycan structures signal the internal status of the cell. Glycans on tumor cells tend to have abundant sialic acid and fucose moieties. We propose that these cancer-associated glycan variants be exploited for biomarker development aimed at diagnosing early-stage disease. Accordingly, we developed a mass spectrometry-based workflow that incorporates chromatography on affinity matrices formed from lectins, proteins that bind specific glycan structures. The lectins Sambucus nigra (SNA) and Aleuria aurantia (AAL), which bind sialic acid and fucose, respectively, were covalently coupled to POROS beads (Applied Biosystems) and packed into PEEK columns for high performance liquid chromatography (HPLC). Briefly, plasma was depleted of the fourteen most abundant proteins using a multiple affinity removal system (MARS-14; Agilent). Depleted plasma was trypsin digested and separated into flow through and bound fractions by SNA or AAL HPLC. The fractions were treated with PNGaseF to remove N-linked glycans, and analyzed by LC-MS/MS on a QStar (Excel and Elite models). Data were analyzed using Mascot software. The experimental design included positive controls—fucosylated and sialylated human lactoferrin glycopeptides—and negative controls—high mannose glycopeptides from Saccharomyces cerevisiae—that were used to monitor the specificity of lectin capture. Key features of this workflow include the reproducibility derived from the HPLC format, the positive identification of the captured and PNGaseF-treated glycopeptides from their deamidated Asn-Xxx-Ser/Thr motifs, and quality assessment using glycoprotein standards. Protocol optimization also included determining the appropriate ratio of starting material to column capacity, identifying the most efficient capture and elution buffers, and monitoring the PNGaseF-treatment to ensure full deglycosylation. Future directions include using this workflow to perform mass spectrometry-based discovery experiments on plasma from breast cancer patients and control individuals.

Monday, 9:45 am – 12:00 pm
Proteogenomics

MON 9:50 - 9:20 am: Mapping and Measuring Proteomes

Ruedi Aebersold

ETH Zurich, Zurich, Switzerland

The human genome project has taught us that a complete map -in the case of the genome project the complete genomic sequence – along with computational tools to navigate the map - represent invaluable resources for experimental and theoretical biologists. A main consequence of such a complete map is that all the biological processes have to be explainable with the components that constitute the map. Proteomics has not reached the stage that complete maps are available but the urgent need for their generation is now widely recognized.

In this presentation we will discuss experimental and computational challenges related to the generation of complete proteomic maps. We will also discuss recent technical advances towards complete proteome analysis and describe software tools and data resources that will transform proteomics from perpetual proteome mapping to accurate proteome measurement.

Monday, 3:45 – 5:20 pm
Bioinformatics of MS

MON 4:35 - 4:50 pm: Integrating Top-Down and Bottom-Up MS Data to Predict Post-Translational Protein Modifications Using Markov Chain Monte Carlo

Stuart R. Jefferys; Morgan C. Giddings

University of North Carolina, Chapel Hill, Chapel Hill, NC

Protein function often depends on chemical post-translational modifications (PTMs). While mass spectrometry (MS) is one of the best tools to investigate PTMs, examining a protein's modifications usually requires integrating data from multiple MS approaches. As no comprehensive, automated means for combining top-down (intact mass) and bottom-up (peptide) data exist, this process is manual and can be time consuming. We have developed a system using Markov chain Monte Carlo (MCMC) to integrate data from multiple sources and automatically produce ranked predictions of protein modification states (PTM scenarios).

There are two key challenges to automated PTM characterization: the enormous search space of possible modification configurations, and the heterogeneous and sometimes conflicting data available, including top-down and bottom up mass spectrometry, protein databases, and PTM prediction programs. Most of the possible PTMs configurations are unlikely, so our approach uses MCMC optimization to search for the most probable configurations based on the data available. MCMC can be used to find rare high scoring configuration states while only exploring a miniscule fraction of the entire search space. As part of a JAVA-based implementation, we use a modular scoring function to integrate intact mass data with peptide fragment data and evaluate possible configurations. Scoring can be easily modified to include additional experimental or context based evaluation.

Our prototype system integrates top down (intact mass) and bottom up (MS/MS peptide) data, along with other information such as the likelihood of modifications from protein databases and prediction programs, to propose PTM scenarios consistent with these data. When using only top-down data, the program selects from an almost infinite number of possible PTM scenarios to generate sets of PTMs compatible with the target input mass. The inclusion of bottom up data significantly enhances the search by constraining the matches to those consistent with the available peptide fragmentation data.

When run in single answer mode and using theoretical peptide MS/MS results, correct modification scenarios are reproducibly obtained. When run in distribution mode, both optimal and sub-optimal answers are generated. This distribution provides information about the nearly-best answers, adding tolerance for imperfect data. A distribution of answers allows relative probabilities to be assigned to different answers, differentiating situations with only one solution versus those where multiple answers are highly supported. New experiments can then be performed and their data can be used to produce a new, refined distribution of answers.

MON 4:50 - 5:05 pm: Detection of Co-Eluted Peptides Using Database Search Methods

Gelio Alves[1]; Aleksey Y. Ogurtsov[1]; Siwei Kwok[2]; Wells W. Wu[3]; Guanghui Wang[3]; Rong-Fong Shen[3]; Yi-Kuo Yu[1]

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Current experimental techniques, especially those applying liquid chromatography mass spectrometry, have made high-throughput proteomic studies possible. The increase in throughput however also raises concerns on the accuracy of identification or quantification. Most experimental procedures select in a given MS scan only a few relatively most intense parent ions, each to be fragmented (MS/MS) separately, and most other minor co-eluted

peptides that have similar chromatographic retention times are ignored and their information lost.

We have computationally investigated the possibility of enhancing the information retrieval during a given LC/MS experiment by selecting the two or three most intense parent ions for simultaneous fragmentation. A set of spectra is created via superimposing a number of MS/MS spectra, each can be identified by all search methods tested with high confidence, to mimick the spectra of co-eluted peptides. The generated convoluted spectra were used to evaluate the capability of several database search methods - SEQUEST, Mascot, X!Tandem, OMSSA, and RAId_DbS - in identifying true peptides from superimposed spectra of co-eluted peptides. We show that using these simulated spectra, all the database search methods will gain eventually in the number of true peptides identified by using the compound spectra of co-eluted peptides.

MON 5:05 - 5:20 pm: ETD Support in the Trans-Proteomic Pipeline

David D. Shteynberg[1]; Christine Carapito[2]; Bruno Domon[2]; Jimmy K. Eng[1]; Luis Mendoza[1]; Natalie Tasman[1]; Eric W. Deutsch[1]; Ruedi Aebersold[2]
[1]Institute for Systems Biology, Seattle, WA; [2]ETHZ, Zurich, Switzerland

Electron Transfer Dissociation (ETD) is an alternative fragmentation technique to Collision Induced Dissociation (CID). In CID the predominant fragment ions formed in MS/MS spectra are b and y ions. On the other hand, c and z ions dominate ETD generated fragmentation spectra. Furthermore, in ETD even relatively labile modified amino acid side chains are left intact and it is possible to sequence longer peptides and peptides that are in higher charge states than those currently analyzed with CID methods.

The Trans-Proteomic Pipeline (TPP) is an open source suite of tools that facilitates and standardizes the analysis of LC-MS/MS data. The TPP includes software tools for MS data representation, MS data visualization, peptide identification and validation, protein identification, quantification, and annotation, data storage and mining, and biological inference. Until now TPP supported analysis of CID data only.

Here we introduce new functionality in the TPP that allows for the analysis of ETD data. We show how this data can complement CID data on the same samples to improve the confidence and coverage of identified proteins. We analyze the performance of the TPP on ETD data generated by different search algorithms including OMSSA, Sequest, and Tandem and compare the performance. The features introduced here will be available as part of a future TPP software suite release.

The TPP software suite is available for download under an open source LGPL software license at tools.proteomecenter.org, and can be installed on common operating systems including: Windows, Linux, and Macintosh (this platform is not currently officially supported although it has been reported to work well by the user community). Free email support for the installation and operation of these tools is also available, as is a searchable knowledge base through Google Groups.

Monday, 3:45 – 5:20 pm
Chemical Proteomics

MON 3:45 - 4:10 pm: Crosslinking Combined with Mass Spectrometry for Structural Proteomics

Christoph H. Borchers
Uvic Genome BC, Victoria, BC, Canada

Crosslinking combined with mass spectrometry is an emerging approach for studying protein structure and protein-protein interactions. Unambiguous mass spectrometric identification of crosslinked peptides derived from proteolytically digested crosslinked proteins is still challenging, however. Here, we describe the use of novel chemical crosslinkers and novel crosslinking straegies, that promise to overcome many challenges associated with other crosslinking reagents and techniques. These crosslinkers are distinguished from others by a unique combination of properties including the capability of being isotopically-coded, fluorescent and cleavable either chemically or through dissociation in the mass spectrometer. Furthermore, some of the crosslinkers have affinity tags for selective enrichment of crosslinked peptides. Novel strategies are focused on the specific enrichment of inter-crosslinked peptides which are most informative for characterizing protein-protein interaction. Beyond proof-of-principle on model complexes the usefulness of these novel crosslinkers and strategies for biological samples are already shown. For example, using our fluorescent and photo-cleavable crosslinker BiPS we provided the first direct evidence describing the docking site of a phosphorylated G-protein coupled receptor C-terminus on the multi-functional adaptor protein -arrestin. This clearly demonstrates the broad potential and application of these novel crosslinker in structural and cellular biology.

MON 4:10 - 4:35 pm: Prospects for Defining Protein-Small Molecule Ligand Interactions Using ESI Mass Spectrometry

Joseph A. Loo
University of California-Los Angeles, Los Angeles, CA

The application of ESI-MS for studying protein-ligand complexes has utility in biology, biochemistry, and biomedical research. Proteins and other biomolecules such as metal ions, nucleotide cofactors or with other proteins interact to form functional molecular machines. They interact through electrostatic, hydrophobic, and hydrogen bonding interactions. The assessment of protein interactions with small molecule ligands can address the functional role of proteins and protein complexes. Potentially ESI-MS could be used as a tool for screening small molecule compound mixtures to identify low-to-medium affinity ligands. Developments in intact protein MS and tandem MS (MS/MS) to define protein-ligand complexes and the sites of ligand bonding will be presented. All forms of ion activation, including collisionally activated dissociation (CAD), infrared multiphoton dissociation (IRMPD), and electron capture dissociation (ECD), coupled with tandem MS of gas phase protein complexes can yield structural information for protein-ligand interactions. MS/MS is developed further as a means to determine the specific sites of small molecule ligand binding. These methods have been applied to determine the binding sites of metals to metalloproteins and ATP to protein kinases. Nucleotide-binding proteins have important biological function in a number of cellular processes, as nucleotides are co-factors or substrates for enzymes, regulators of protein function, or structural binding motifs. Nucleotide-binding proteins, such as ATPases and G-proteins are essential components of signaling pathways. Protein kinases represent the largest mammalian enzyme family, with more than 500 members in the human proteome; the ATP-binding sites of protein kinases are highly conserved. ESI-MS/MS can be used to deliver a “signature” for ATP-binding that can be used to identify new protein kinases.

MON 4:35 - 4:50 pm: Artificial Kinase Substrates for MALDI-TOF MS-Based Intracellular Phosphorylation Analysis

Laurie L. Parker[1]; Jean Y. Wang[3]; Stephen B. H. Kent[2]; Stephen J. Kron[2]
[1]Purdue University, West Lafayette, IN; [2]University of Chicago, Chicago, IL; [3]University of California, San Diego, San Diego, CA

Cellular signaling is a major contributor to cell decision-making, and needs to follow the correct program in order for all the cells of an organism to cooperate and do their jobs keeping the organism alive. Changes in phosphorylation signaling are directly associated with cancer via their control over proliferation and differentiation of cells, and provide potential biomarkers for analyzing onset, stages of progression and treatment. Most if not all cancer-related kinase pathways involve multiple combinatorial events, where phosphorylation of one protein often stimulates another, triggering binding interactions and turning kinase activities ‘on’ or ‘off’, with the overall effect of a host of thresholds being crossed and resulting in cellular messages going haywire. Monitoring the overall profile of a number of phosphorylation signals would provide a more sophisticated model for understanding when kinase activities go wrong on a global level, however the current limitations of protein analysis technology make it very difficult to study

phosphorylation signaling more comprehensively than just a few kinases at a time. Mass spectrometry allows for incredible sensitivity and molecular detail in protein analysis and has theoretically unlimited multiplex capabilities. Unfortunately however, dynamic range problems associated with modern mass spectrometers make it very difficult to perform proteomics experiments that are translatable to the clinic.

We use synthetic chemical biology to address the challenge of finding these needles in the haystack. We are designing artificial protein sensors that can be put into cells and pulled back out again for analysis using matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI-TOF MS) to get an overall report on the internal states of cancer-related kinase activities. Using peptide synthesis and native chemical ligation, we are constructing artificial proteins that will mimic natural kinase substrates using modules that have specificity for adaptor domain interaction and phosphorylation by particular kinases, and modules for cell-permeation capabilities, localization signals, and affinity tags for rapid isolation from cells. Our preliminary work focuses on methods for synthesizing biosensors and better understanding the analysis of phosphopeptides with MALDI-TOF MS, initially aimed at studying Src-family kinase signaling in chronic myelogenous leukemia.

MON 4:50 - 5:05 pm: A Cocktail of Isotopically Labeled Peptide Standards for MRM Based Quantitation of 45 Human Plasma Proteins

Michael A. Kuzyk[1]; Derek Smith[1]; Tyra J. Cross[1]; Juncong Yang[1]; Angela M. Jackson[1]; Darryl B. Hardie[1]; N. Leigh Anderson[2]; Christoph H. Borchers[1]

[1]UVic-Genome BC Proteomics Centre, Victoria, Canada; [2]Plasma Proteome Institute, Washington, DC

Mass spectrometry-based multiple reaction monitoring (MRM) quantitation of proteins can dramatically impact the discovery and quantitation of biomarkers via rapid, targeted, multiplexed protein expression profiling of clinical samples. A cocktail of 45 peptide standards, easily adaptable to common plasma proteomics workflows, has been created to permit absolute quantitation of 45 endogenous proteins in human plasma trypsin digests. All experiments were performed on simple tryptic digests of human plasma without prior affinity depletion or enrichment. Proteotypic tryptic peptides containing isotopically-coded amino acids ($[^{13}C_6]$Arg or $[^{13}C_6]$Lys) were synthesized for all 45 proteins. Peptide purity was assessed by capillary zone electrophoresis, and the peptide quantity was determined by amino acid analysis. For maximum sensitivity and specificity, instrumental parameters were empirically determined in order to generate the most abundant precursor ions and y-ion fragments. Concentrations of individual peptide standards in the cocktail were optimized to approximate endogenous concentrations of analytes, and to ensure the maximum linear dynamic range of the MRM assays. Excellent linear responses (r >0.99) were obtained for 43 of the 45 proteins with attomole-level limits of quantitation (<20% CV) for 27 of the 45 proteins. Analytical precision for 44 of the 45 assays varied by <10%. LC-MRM/MS analyses performed on 3 different days, on different batches of plasma trypsin digests, resulted in CV's of <20% for 43 of the 45 assays. Concentrations for 39 of the 45 proteins are within a factor of 2 of reported literature values. This cocktail of internal standards has many uses and can be applied to the characterization of trypsin digestion kinetics and plasma protein expression profiling since 31 of the 45 proteins are putative biomarkers of cardiovascular disease.

MON 5:05 - 5:20 pm: Small Molecule Capture Compounds™ - Towards a Targeted Reduction of Proteome Complexity

Erik Duelsner; Aysel Alici; Christian Jurinke; Hubert Koester

caprotec bioanalytics GmbH, Berlin, Germany

The isolation and identification of proteins from complex biological samples is a challenging task in proteomics. Therefore, a targeted isolation of enzyme families would be highly desirable.

This requires the functional separation of sub proteomes from the whole biological sample in a form which allows the identification by state-of-the-art bioanalytics and bioinformatics.

Separation by physical properties like molecular weight and charge can reduce the complexity of protein mixtures but lacks any selectivity concerning protein function. Affinity chromatography and chip based technologies achieve functional separations by immobilized selectivity functions but struggle from surface effects and steric hindrance.
Here we present a novel technology, called Capture Compound Mass Spectrometry (CCMS), which allows the isolation of sub proteomes based on their interaction with synthetic small molecules, called Capture Compounds (CC).

Capture Compounds combine three different functionalities within one individual molecule: 1) a selectivity function for the specific affinity driven interaction with proteins, 2) a reactivity function for the photo cross-linking of Capture Compound and protein, and 3) a sorting function, biotin, for the isolation of captured proteins.

The data and experiments presented here, demonstrate the application of this novel technology for the analysis of three different interactomes: 1) the SAH interactome, 2) the cAMP interactome and 3) the staurosporine interactome. The CCMS technology is compared to pull down experiments. In contrast to pull down assays the data demonstrate a higher yield of identified proteins out of complex samples analyzed by gel electrophoresis and mass spectrometry.

We conclude that CCMS is a promising, novel technology for the functionality directed reduction of complexity and a higher enrichment of low abundant proteins compared to other proteomic approaches. The gel-free LC-MS analysis of captured protein complexes is unique for the CCMS technology. The versatility of the method allows a straightforward synthesis of novel Capture Compounds for many different applications and areas of life sciences and drug discovery. Capture Compounds can be used for the isolation, identification and profiling of novel and known proteins within complex biological samples.

Monday, 3:45 – 5:20 pm
Systems Analysis for Biomarker Discovery I

MON 3:45 - 4:10 pm: High Performance Proteomics as a Tool in Biomarker Discovery

Helmut E. Meyer[1]; Barbara Sitek[1]; Kathy Pfeiffer[1]; Bence Sipos[2]; Günther Klöppel[2]; Christian Mölleken[3]; Wolfgang Schmiegel[3]; Kai Stühler[1]

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Since more than 10 years, the word PROTEOME catches the attention of more and more researchers in the life science field. At about the same time the term high throughput proteome analysis came up with the intention to analyse all proteins in a complex protein mixture in parallel. Thus, a huge amount of data is produced from a single sample and the following analysis and validation becomes the time limiting step.

However, the limited number of available biomarkers for diagnosis, status of the disease, therapy control and prediction of the course of the disease demands for new efforts in finding new ones. Especially, proteomics raises high expectations in finding new and reliable biomarker for human diseases.

By applying micro dissection combined with saturation DIGE technology and mass spectrometry we could succeed in finding new biomarker candidates for liver cirrhosis caused by HCV

infection. This technology allows us to analyze quantitatively the proteome of just a few thousand cells from individual patient samples and to get reproducible proteomics data pointing to new biomarker candidate proteins for liver cirrhosis. Validation of these candidate biomarkers could be demonstrated by immunohistochemistry. By Western blotting and Elisa test first candidate biomarkers could be identified and quantified in the sera of patients suffering for liver cirrhosis at different stages of the disease. Thus, high performance proteomics is the basic principle for reliable results which allows us to discover new biomarker candidates for liver cirrhosis using minute amounts of patients' material.

MON 4:10 - 4:35 pm: Blood-Based Exopeptidase Activities as Biomarkers for Cancer

Paul Tempst
Memorial Sloan-Kettering Cancer Center, New York, NY

The concept of enzymatic activities as disease markers has a long-standing precedent (Gutman et al, J Clin Invest 1938; 17:473; Schwartz, Clin Chem 1973; 19:10). Yet, in most present-day biomarker discovery studies, enzymes are simply looked at as 'proteins' that are (or aren't) physically present and may undergo changes in concentration as the result of disease. Many enzymes are also present below the mass spectrometric detection limits and therefore 'invisible' in traditional screens. The case could be made, though, that given enough substrate, time, and optimal assay conditions, catalytic or synthetic products may accumulate to levels that become detectable on a complex-proteome background. This may be particularly true for proteolytic enzymes as they generate peptide products that are tailor-made for all MS-based proteomic read-outs.

Human cells produce some 550 proteases that share one principal functionality, the lysis of peptide bonds, but differ greatly in their biological roles. Proteases have also been implicated in disease, notably in cancer where they promote both tumor progression or suppression. Examples of peptide degradation have unexpectedly been observed in recent serum oncopeptidomics studies whereby subsets of peptides provided class discrimination between patients with different types of solid tumors and control individuals without cancer. All relevant peptides sorted into nested sets of sequences, each the result of exopeptidase activities that confer cancer-type-specific differences superimposed on the proteolytic events of ex vivo clotting. We therefore speculated that exopeptidase activities might serve as biomarkers for cancer. There exist an estimated 91 human exopeptidases, 72 of which could potentially contribute to the observed serum peptide degradation patterns.

We will describe an exopeptidase activity assay that measures *de novo* peptide breakdown in large numbers of biological samples. The full array of metabolites is quantitated (c.v. ≤15% over multiple replicates) and the results can be subjected to kinetic and/or statistical analysis. Preliminary analyses of patient samples and mouse models of prostate cancer have been carried out to test prospective uses in diagnostics. Second, initial results of a comprehensive biochemical analysis of extracellular exopeptidases using cell culture models of invasive and non-invasive prostate cancer will be presented.

MON 4:35 - 4:50 pm: Spectral Profiles: A Novel Representation of Tandem Mass Spectra and its Applications for *de novo* Peptide Sequencing and Identifications

Sangtae Kim; Nuno Bandeira; Pavel A. Pevzner
University of California, San Diego, La Jolla, CA

Recent advances in *de novo* peptide sequencing enabled new tag-based peptide identification tools (e.g., Inspect and Paragon) that are orders of magnitude faster than the traditional MS/MS database search approaches. However, reliable full-length *de novo* peptide sequencing remains an elusive goal and even the most accurate *de novo* tools correctly reconstruct only 30-50% of peptides. We argue that accurate full length *de novo* peptide sequencing may be an unattainable goal for many spectra since they do not provide enough information to disambiguate between correct and incorrect reconstructions. Spectra often have variable local quality (along the peptide length) making some regions not amenable to *de novo* sequencing. In such cases, it makes more sense to reconstruct a gapped peptide (e.g., D[186]AAENTDAQK) rather than a contiguous peptide. While it may appear that gapped peptides are less useful than full-length peptides, we argue that in most applications there is little difference between these two representations. Indeed, in most applications, *de novo* peptide sequencing is not the final goal in analyzing a spectrum but rather a prelude to error-tolerant database searches and other applications like metaproteomics. We argue that long gapped peptides are nearly as good for such applications as full-length *de novo* reconstructions. For example, the gapped peptide D[186]AAENTDAQK has 9 continuous amino acids and thus, for most practical applications, is as useful as any peptide of length 9. Gapped peptides occupy a niche between peptide sequence tags and full-length reconstructions: they are nearly as accurate as short tags and, at the same time, typically have a unique match in the protein database.

We introduce the notion of a spectral profile that enables accurate *de novo* sequencing of gapped peptides and reveals the variable spectral quality along the peptide length. Spectral profile is a novel representation of tandem mass spectra with "intensities" of all masses varying from 0 to 1. The intensity of mass x measures how often it is used as a prefix mass among all high-scoring interpretations of the spectrum. Thus, the spectral profile compactly represents information about all high-scoring *de novo* reconstructions (spectral dictionary) even if there are billions of such reconstructions. The spectral profiles are conceptually similar to the motif profiles that are used in various areas of bioinformatics (e.g., in regulatory genomics). Similarly to diverse applications of motif profiles, spectral profiles have a multitude of applications. Spectral profiles can be used for gapped peptide generation. For the ISB Standard Protein Mix database, our MS-Profile tool correctly reconstructs 65% of gapped peptides as compared to 46%, 28% and 26% correct reconstructions of full or nearly full-length peptides by PepNovo+, MS-Dictionary, and PEAKS. They can also be utilized for database filteration in the same way as peptide sequence tags in InsPecT. For database filtering, MS-Profile shows two orders of magnitude better filtering efficiency than InsPecT while retaining comparable accuracy. MS-Profile also intuitively visualize high scoring *de novo* reconstructions, revealing poor quality spectra or poor quality regions within long peptides that other methods have difficulties analyzing.

MON 4:50 - 5:05 pm: Low Cost, Scalable Proteomics Data Analysis Using Amazon's Cloud Computing Services and Open Source Search Algorithms

Brian D Halligan; Joey F Geiger; Andrew K Vallejos; Andrew S Greene; Simon N Twigger
Medical College of Wisconsin, Milwaukee, WI

One of the major difficulties for many laboratories setting up proteomics programs has been obtaining and maintaining the computational infrastructure required for the analysis of the large flow of proteomics data. We describe a system that combines distributed cloud computing and open source software to allow laboratories to set up scalable virtual proteomics analysis clusters without the investment in computational hardware or software licensing fees. Additionally, the pricing structure of distributed computing providers, such as Amazon Web Services (AWS), allows laboratories or even individuals to have large-scale computational resources at their disposal at a very low cost per run. We provide detailed step by step instructions on how to implement the virtual proteomics analysis clusters as well as a list of current available preconfigured Amazon machine images containing the OMSSA and X!Tandem search algorithms and sequence databases which is also available on the Medical College of Wisconsin Proteomics Center website (http://proteomics.mcw.edu/vipdac).

Distributed computing models such as AWS allow the user to select different computer configurations to run the same machine image. This makes the testing and benchmarking of different computer configurations running OMSSA and X!Tandem very simple. We present the analysis times and costs associated with

various combinations of different instance types for the head and worker nodes, as well as for virtual clusters with different numbers of worker nodes. Using this data, the user can determine what is the most cost effective combination instance sizes and number of worker nodes to analyze their data within a time frame that fits best with their requirements. We also examine the relative overlap of peptide and protein identifications produced by OMSSA and X!Tandem both at the level of proteins identified by the complete analysis as well as at the level of individual spectra. From this, we demonstrate the value of analyzing the spectral data with multiple algorithms and combining the results.

MON 5:05 - 5:20 pm: Protein Biomarkers Beyond Fluctuations in Concentration

Chad R. Borges; Randall W. Nelson
Arizona State University, Tempe, AZ

In addition to fluctuations in protein concentration, protein biomarkers of disease include genetic changes (resulting in protein sequence alterations) and qualitative / quantitative changes in protein posttranslational modifications that must be monitored in comprehensive "all-the-time, every time" fashion if they are to be tapped into as sources of diagnostic information. When employed with proper sample preparation, mass spectrometry provides a clinically viable key to such comprehensive analyses.

As part of the effort to ease the increasing burden of type 2 diabetes upon society, this study presents verification (from the analysis of over 100 human plasma samples) of glycemic control and oxidative stress panels of protein biomarkers (analyzed by mass spectrometric immunoassay) from outside the realm of fluctuations in protein concentration that can be used together to produce a near-perfect healthy vs. diabetic cohort discriminant with promising characteristics for early-onset detection, risk prediction, and utilization as adjunct tools in monitoring therapy.

A unique application of principle component analysis to the two most robust protein markers of hyperglycemia and oxidative stress produced a single numerical descriptor that yields near-perfect cohort discrimination based on traditional receiver operating characteristic curve analysis (ROC AUC = 0.99 ± 0.0008 standard error). Subsequent work resulted in the production of a single multiplexed assay for use in the routine analysis of large numbers of human plasma samples.

Tuesday, 8:00 – 9:20 am
Era of Systems Medicine

TUES 8:45 - 9:25 am: Proteomic Imaging of Endothelial Caveolae: Pumping Antibodies into Tumors

P. Oh; P. Borgstrom; J. Testa; Y. Li; J. Yu; A. Chrastina; J.E. Schnitzer
Sidney Kimmel Cancer Center, San Diego, CA

Cancer and other disease biomarkers and targets may provide key diagnostic, prognostic and therapeutic opportunities including clinical trial surrogates and screens for patient treatment assignment. Drugs, gene vectors, and nanoparticles may benefit greatly from improved *in vivo* delivery through homing to specific disease biomarkers. Yet *in vivo* barriers limit access to most disease targets including cancer. We have developed novel systems biology approaches that integrate nanotechnology-based subcellular fractionation, quantitative organellar & subtractive proteomics, bioinformatic interrogation, antibody generation, expression profiling, and various *in vivo* imaging modalities to quickly identify and validate target candidates for pre-clinical and clinical testing. Analysis of rodent and human tumor samples have been compared to focus on clinical meaningful targets and to understand model relevance to human disease. Tissue and tumor microenvironmental influences on endothelial cell expression are extensive. We have developed quantitative proteomic analysis using a new spectral intensity index to identify proteins specific to tumor vs. normal endothelium as well as concentrated in caveolae. Novel targets in caveolae enable antibodies to penetrate deep into solid tumors and single organs and were utilized to improve tissue-specific imaging and treatment. Caveolae not only express tissue-specific proteins but also function to rapidly and actively pump select antibodies across the endothelial cell barrier to reach the inside of the tissue. This targeted penetration occurs within seconds to minutes in normal tissues and with in minutes to a few hours in various tumor models. The pervasive access inside the tumor improves the efficacy of radioimmunotherapy in destroying both stromal and tumor cells and in treating a wide variety of solid tumors. Various rodent tumors are imaged rapidly and specifically after intravenous injection of specific monoclonal antibodies. This tumor penetration and treatment efficacy requires the presence of caveolae on the tumor endothelium and only occurs when the antibodies deliver their attached radionuclides directly and rapidly into the tumors. This work represents a novel discovery, validation and delivery strategy that so far provides promising results. Testing in humans and similar proteomic mapping of the human vasculature is now necessary to understand limitations and possibilities for clinical translation to imaging and treating human disease.

Tuesday, 9:45 am – 12:00 pm
Proteomics and Systems Medicine

TUES 10:35 - 11:15 am: Interrogating Human Disease Using Protein Microarrays

Michael Snyder
Yale University, New Haven, CT

We have profiled the spectrum of autoantibodies produced in patients with ovarian cancer, asthma and other disease. Human sera from disease and healthy individuals were used to probe protein microarray containing large numbers of human proteins and a number of reactive proteins were identified. In many cases, the expression of these antigens was examined further. A summary of these results will be presented at the meeting.

Tuesday, 3:45 – 5:20 pm
Systems Analysis for Biomarker Discovery II

TUES 3:45 - 4:10 pm: Discovery and Scoring of Protein Interaction Sub-Networks Discriminative of Late Stage Human Colon Cancer

Rod K Nibbe; Mark R Chance
Case Western Reserve University, Cleveland, OH

We employed a systems biology approach to identify and score protein interaction sub-networks whose activity patterns are discriminative of late stage human colorectal cancer (CRC) versus control in colonic tissue. We conducted two gel-based proteomic experiments to identify significantly changing proteins between normal and late stage tumor tissues obtained from an adequately sized cohort of human patients. A total of 67 proteins identified by these experiments were used to seed a search for protein-protein interaction sub-networks. A scoring scheme based on mutual information, calculated using gene expression data as a proxy for sub-network activity, was developed to score the targets in the sub-networks. Based on this scoring, the sub-network was pruned to identify the specific protein combinations that were significantly discriminative of late stage cancer versus control. These combinations could not be discovered using only proteomic data, or by merely clustering the gene expression data. We then analyzed the resultant pruned sub-network for biological relevance to human CRC. A number of the proteins in these smaller sub-networks have been associated with the progression (CSNK2A2, PLK1, IGFBP3) or metastatic potential (PDGFRB) of CRC. Others have been recently identified as potential markers of CRC (IFITM1), and the role of others is largely unknown in this disease (CCT3, CCT5, CCT7, GNA12). The functional interactions represented by these signatures provide new experimental hypotheses that merit follow-on validation for biological significance in this disease. Overall, the method outlines a quantitative approach for integrating proteomic data, gene expression data, and the wealth of accumulated legacy experimental data to discover significant protein sub-networks specific to disease.

TUES 4:50 - 5:05 pm: The FGFR3 Network in Multiple Myeloma: A Phosphotyrosine Proteomic Profile Associated with FGFR3 Expression, Ligand Activation, and Drug Inhibition

Michael F. Moran[1]; Paul Taylor[1]; Jiefei Tong[1]; Jonathan St-Germain[2]; Lily Jin[2]; Suzanne Trudel[3]
[1]Hospital For Sick Children, Toronto, Canada; [2]University of Toronto, Toronto, Canada; [3]Princess Margaret Hospital, Toronto, Canada

Signaling by activated growth factor receptor tyrosine kinases is manifest through networks of proteins that are phosphorylated by, and/or bind the autophosphorylated receptors. FGF Receptor-3 (FGFR3) is a drug target in a subset of human multiple myeloma tumors, and is mutationally activated in some cervical and colon and many bladder cancers, and certain skeletal dysplasias. In order to determine the FGFR3 network in myeloma, mass spectrometry was used to identify and quantify protein phosphotyrosine (pY) sites associated with FGFR3 activation and inhibition. Myeloma cell and tumor proteins were subjected to label-free quantification of Orbitrap MS ion currents, and by using a triple quadrupole instrument for selective reaction monitoring (SRM) with tandem MS. This indicated modulation of FGFR3 kinase activity by phosphorylation of tandem tyrosine residues in the FGFR3 kinase domain activation loop. A set of 45 protein pY sites associated with FGFR3 activation by FGF were sensitive to inhibition by the FGFR3-directed inhibitor PD173074, and constitute an FGFR3 signaling network. The network features transactivation of additional tyrosine kinases including JAK1 and IGF1R, and phosphorylation of the cytoplasmic tail of Syndecan-1 (CD138), which is a biomarker and target in myeloma that modulates tumor-bone marrow interactions. Therefore, tumor analysis by phospho-proteomics enabled the identification and pharmaco-dynamic monitoring of drug target and target network activities. This may be a generally applicable approach to facilitate drug development and stratify tumors.

TUES 5:05 - 5:20 pm: Integrative Approach for Discovery of Diagnostic Serum Biomarkers of Prion Disease in Mice

Hyuntae Yoo[1]; Inyoul Y. Lee[1]; Daehee Hwang[3]; David Baxter[1]; Rose Pitstick[2]; George A. Carlson[2]; Leroy Hood[1]
[1]Institute for Systems Biology, Seattle, WA; [2]McLaughlin Research Institute, Great Falls, MT; [3]POSTECH, Pohang, Korea

Prion disease is a fatal neurodegenerative disease with potential infectivity and long presymptomatic incubation times. In order to discover serum biomarkers for early diagnosis of prion disease, brain and serum samples were collected from prion-infected mice and age-matched control mice throughout the incubation time. Global transcriptome analysis was performed on brain samples from five independent mouse models throughout the incubation times followed by novel statistical analysis, where 46 genes were identified as potential diagnostic marker candidates. Also, a direct shotgun proteomics approach was applied to serum samples at ten time points from one of the five mouse models, resulting in identifying 13 candidate proteins. For directed quantification of 54 candidate proteins identified from the two approaches, a novel targeted proteomics approach based on synthetic spike-in peptides and iTRAQ labeling was developed and applied to multiple mouse serum samples. Fifteen candidates were confirmed to change significantly in serum samples from prion-infected mice as compared to those from control mice. Validation of the candidates is in progress.

Tuesday, 3:45 – 5:20 pm
Databases and Systems Integration

TUES 4:10 - 4:35 pm: Biological Pathway and Network Analysis for Functional Interpretation of Large-Scale Proteomics Data

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

Many bioinformatics tools have been developed for the management and analysis of proteomics data. However, functional analysis and interpretation of large-scale proteomic data remain challenging and require effective use of advanced bioinformatics methods and databases for data integration, mining, and comparative analyses. We have developed the iProXpress expression analysis system with integrated databases and analytical tools for functional and pathway analysis and expression profiling of proteomics and microarray data. The system consists of (i) a data warehouse with rich annotation and functional descriptions for individual proteins integrated from about 100 molecular databases, (ii) software modules for comprehensive protein ID mapping and sequence analysis and functional profiling, as well as (iii) a web-based graphical user interface for searching, browsing and cross comparisons of multiple datasets and experiments. The iProXpress system further connects with text mining tools to identify relevant protein-protein interaction and protein phosphorylation information from the scientific literature, with visualization tools that overlay pathways or protein networks with functional attributes (such as correlated gene expression), and with pathway and network analysis results from other tools (such as the Ingenuity Pathway Analysis tool).

We have applied this approach to several studies for biological pathway and network analysis, here illustrated with a study to identify early signaling proteins associated with estrogen-induced apoptosis in breast cancer cells. The proteomics data were generated using immunoprecipitation/1D-gel and MS/MS to identify proteins phosphorylated at tyrosine (pY-IP) or interacting with AIB1 (AIB1-IP), a transcription coactivator of nuclear receptors that has been shown to play important roles in estrogen-induced anti-apoptotic effects. Our results showed that AIB1 interacts with an enriched group of proteins specifically in estrogen-induced apoptotic cells, including those involved in RNA metabolism and transcription, and with functions of chromatin interaction and transcriptional regulation. Pathway mappings suggest that proteins in GPCR pathway may be involved in estrogen-induced apoptosis. We further generated an AIB1 interaction network based on literature and protein-protein interaction databases, and analyzed correlated genes from DNA microarray of NCI-60 panel of cancer cells. The AIB1 network allows close examination of its functional association with other genes and formulation of hypothesis on its potential roles in estrogen-induced apoptosis. The integrated bioinformatics approach thus supports biological pathway and network analysis and facilitates hypothesis generation and target identification in the systems biology context.

TUES 4:35 - 4:50 pm: RAId_DbS: Mass-Spectrometry Based Peptide Identification with Knowledge Integration

Gelio Alves; Aleksey Y. Ogurtsov; Yi-Kuo Yu
NCBI/NLM/NIH, Bethesda, MD

Existing scientific literature is a rich source of biological information such as disease markers. Integration of this information with data analysis may help researchers to identify possible controversies and to form useful hypotheses for further validations. In the context of proteomics studies, individualized proteomics era may be approached through consideration of amino acid substitutions/modifications as well as information from disease studies. Integration of such information with peptide searches facilitates speedy, dynamic information retrieval that may significantly benefit clinical laboratory studies. We have integrated from various sources annotated single amino acid polymorphisms, post-translational modifications, and their documented disease associations (if they exist) into one enhanced database per organism. We have also augmented our peptide identification software RAId_DbS to take into account this information while analyzing a tandem mass spectrum. In principle, one may choose to respect or ignore the correlation of amino acid polymorphisms/modifications within each protein. The former leads to targeted searches and avoids scoring of unnecessary polymorphism/modification combinations; the latter explores possible polymorphisms in a controlled fashion. To facilitate new discoveries, RAId_DbS also allows users to conduct searches permitting novel polymorphisms as well as to search a knowledge database created by the users. We have finished constructing enhanced databases for 17 organisms. The web link to RAId_DbS and the enhanced databases is

http://www.ncbi.nlm.nih.gov/CBBResearch/qmbp/raid_dbs/index.html.
The relevant databases and binaries of RAId_DbS for Linux, Windows, and Mac OS X are available for download from the same web page.

TUES 4:50 - 5:05 pm: Proteomics by the Numbers: The Use of Computers to Aid Analysis, Understanding and Prediction in Mass Spectrometry Proteomics

Michael Mueller; Lennart Martens
EMBL-EBI, Cambridge, United Kingdom

Proteomics research has so far been primarily exploratory and data-driven. As a result, the necessity to analyze and interpret the large amounts of obtained data have been the main focus of bioinformatics efforts in proteomics, with the majority of computational work being done after data acquisition.

Two developments are now shifting the focus of the field towards more targeted analyses however. The first of these relates to the successful application of Single Reaction Monitoring (SRM) to track a particular (set of) protein(s) across different samples or time points. The second results from the availability of an increasing amount of exploratory proteomics data in dedicated, publicly accessible repositories. Meta-analyses of these data are providing insights into the influence of experimental conditions on protein recovery, as well as the influence of peptide sequence on fragmentation characteristics.

As the field is thus starting to take up targeted analysis, the role of bioinformatics is changing as well. Rather than focussing on data processing after analysis, proteomics bioinformatics is increasingly used to predict optimal targets, moving the focal point of computational work prior to data acquisition.

Here we present two relevant analyses we have recently carried out in support of targeted proteomics. The first analysis investigated whether existing technological platforms are sufficiently powerful to resolve individual protein isoforms, and the second analysis then proceeded to determine the maximal coverage (both of the genome and the proteome) that is theoretically achievable using present-day technologies.

Furthermore, we have created a database of peptide transition signatures that can be used to reliably and uniquely identify a specific protein isoform against the background of the entire proteome. With the field of proteomics increasingly geared towards targeted analyses, we aim to supply the required bioinformatics tools to optimally support the field in these new endeavours.

TUES 5:05 - 5:20 pm: Virtual Screening to Identify Signal Transduction Pathway Segments in the APC(Min/+) Mouse

Gurkan Bebek; Mark Chance
Case Western Reserve University, Cleveland, OH

Integration of various levels of cellular signaling, e.g., pathways, regulatory networks, protein-protein interactions, will provide a way to broadly analyze biological networks. Flexible and extensible models are needed to exploit data indicating the dynamics of genes and proteins to identify major pathways that would differentiate healthy and disease states.

In this work we adopt a data mining approach to identify biologically significant pathway segments and identify how cellular responses are disturbed in colon cancer. In an earlier proteomics study 38 proteins were observed to have differential expression levels in APC(Min/+) mice, a model of APC-related cancer susceptibility, compared to wild type. Assuming that these target proteins are correlated with each other in terms of tumorigenic phenotype, the effect should be reflected at the level of expression as well as in the interaction patterns of these proteins. Hence, if these proteins are statistically associated with available high-throughput data, we should observe these relationships and eventually form hypotheses about the functional relationships of these proteins in terms of pathways.

To achieve this goal, first functional annotations of known pathway proteins are collected. We map known pathway proteins to their functional annotations to capture underlying characteristics of pathways. These characteristics are then used to search for possible (unknown) pathway segments. Association rule mining, a procedure to collect data attributes that are statistically related in the underlying data, is used to discover patterns from known pathways.

Next, a protein-protein interaction network is created from known protein-protein interactions (PPI). In this work, reliability scores to each PPI are assigned by integrating microarray expression levels, network topology, and protein subcellular localization data with a logistic regression model. Using the weights, false positive PPIs, proteins that are not likely to interact, are discarded.

We also attempt to recover false negative PPIs. Additional interactions are inferred based on protein family relationships derived from an examination of multiple species. Given proteins from this extended and purified network, we utilize the acquired association rules, and search for possible signaling pathway segments connecting the identified target proteins. We count association rules on each path connecting the target proteins and identify significant paths to form hypotheses for colon cancer signaling.

This new approach will further improve our understanding of normal and disease states by establishing signaling paths among target proteins. The hypotheses formed can be further refined through multiple cycles of experimental verification.

Tuesday, 3:45 – 5:20 pm
Structure and Dynamics of Macromolecular Assemblies

TUES 3:45 - 4:10 pm: Investigation of the Structural Dynamics of the 26S Proteasome Complex Using Mass Spectrometry

Xiaorong Wang; Yingying Yang; Lan Huang
University of California, Irvine, Irvine, CA

The 26S proteasome is a multicatalytic protein complex essential for ATP/ubiquitin-dependent protein degradation in both the nucleus and cytosol. It consists of two subcomplexes, the 20S core particle (CP) and the 19S regulatory complex (RP). The 20S CP is responsible for various catalytic activities, while the 19S RP is involved in several biochemical functions including recognition and unfolding of polyubiquitinated substrates, assisting in opening the gate of the 20S chamber and subsequently translocating unfolded substrates into the 20S for degradation. Selective degradation in cells is tightly controlled and plays an important role for regulating cell cycle progression, signal transduction and maintaining genome stability, etc. Although intensive studies were carried out to understand ubiquitin-proteasome degradation pathway, the regulation of the 26S proteasome function remains to be elusive. Towards this goal, we have employed affinity purification and SILAC-based quantitative mass spectrometry to determine the structural dynamics of the 26S proteasome complex and elucidate its regulatory mechanisms under various physiological conditions.

TUES 4:10 - 4:35 pm: Tracking UPS Using Mass Spectrometry – Profiling of Ubiquitylated Proteins to Reveal Proteasome Substrates

Christopher Hao Pu; Neng Fang; Thibault Mayor
CHiBi, University of British Columbia, Vancouver, Canada

The ubiquitin proteasome system (UPS) is the main cellular pathway for proteolysis. Targeted proteins are first covalently attached to poly-ubiquitin chains and then recognized and degraded by the proteasome a large protein complex. A formidable challenge to deciphering the biology of ubiquitin is to map the networks of substrates and ligands for components of the UPS. Several different receptors guide ubiquitylated substrates to the proteasome, and neither the basis for specificity nor the relative contribution of each pathway is known. To address how broad of a role the ubiquitin receptor Rpn10 (S5a) plays in turnover of proteasome substrates, we implemented a method to perform quantitative analysis of ubiquitin conjugates affinity-purified from experimentally-perturbed and reference cultures of Saccharomyces cerevisiae that were differentially labeled with 14N and 15N isotopes. Shotgun mass spectrometry coupled with relative quantification using metabolic labeling and statistical

analysis based on q-values revealed ubiquitylated proteins that increased or decreased in level in response to a particular treatment. We first identified over 225 candidate UPS substrates that accumulated as ubiquitin conjugates upon proteasome inhibition. To determine which of these proteins were influenced by Rpn10, we evaluated the ubiquitin conjugate proteomes in cells lacking either the entire Rpn10 (rpn10Δ) or only its UIM poly-ubiquitin binding domain (uimΔ). Twenty seven percent of the UPS substrates accumulated as ubiquitylated species in rpn10Δ cells, whereas only one-fifth as many accumulated in uimΔ cells. These findings underscore a broad role for Rpn10 in turnover of ubiquitylated substrates, but a relatively modest role for its ubiquitin-binding UIM domain. This approach illustrates the feasibility of systems-level quantitative analysis to map enzyme-substrate networks in the UPS.

TUES 4:35 - 4:50 pm: Advanced H/DX-MS Strategies for High Resolution Modeling of Large Protein Complexes

David C. Schriemer
University of Calgary, Calgary, Canada

A frontier in the development of stuctural biology involves the high resolution modeling of structures for complex, muliprotein systems in both static and dynamic terms. Current successes in single-crystal diffraction and cryo-electron microscopy and impressive but few in number. New methodology is required that can overcome current technical barriers in the field, particularly those that relate to sample requirements. While it may be possible to engineer sufficient quantities of single proteins, it is more problematic to do so with multiprotein systems. Thus, appealing characteristics for any new approach would include the ability to generate structural data in a mass-unlimited fashion, from trace-level material (i.e. endogenous) reflecting the post-translational modifications often critical to the function and makeup of the complex. No such technology currently exists, however methodologies incorporating structural mass spectrometry data and computational routines offer considerable promise in this regard. In this presentation, we will describe our efforts to improve hydrogen/deuterium (H/D) exchange methods for use in data-directed generation of pseudoatomic models of multiprotein systems. Specifically, this includes extending the dynamic range of H/D methods, to support both lower sample amounts and higher molecular weights, using “deuterium label compression” methods and the MS/MS domain. New software for high-throughput mining of MS/MS data for structural MS purposes will be presented in this context. To derive high resolution structures from H/D data, we will demonstrate data-mining strategies that involve the generation of ambiguous interaction restraints for use in protein-protein docking. We present this strategy as a means of overcoming one of the key limitations of the H/D method, namely, the inherent difficulty in delineating interfaces from induced adaptations of structure. Examples will be drawn from protein systems involved in the assembly and function of the cytoskeleton (e.g., tubulin) as well as host-pathogen interactions.

TUES 4:50 - 5:05 pm: Structural Mass Spectrometry of a Molecular Machine: Functional Consequences of Conformational Changes in the ClpAP Protease

Jen Bohon[1]; Laura D. Jennings[2]; Christine M. Phillips[2]; Stuart Licht[2]; Mark R. Chance[1]
[1]*Case Western Reserve University, Cleveland, OH;*
[2]*Massachusetts Institute of Technology, Cambridge, MA*

ATP-dependent proteases are highly-regulated molecular machines that perform essential cellular functions in all organisms. The most notable of these functions are the dissolution of protein aggregates and the degradation of unwanted proteins, processes which are required for cell growth, mediation of stress responses, and protein quality control. Understanding the conformational changes that enable these proteases to manipulate their substrates is important in providing an experimental basis for the design of agents to modulate physiology by activating or inhibiting these molecular machines. In this study, we have used a combination of synchrotron protein footprinting, enzymatic assays and mass spectrometry to study such conformational changes in the *E. coli* ATP-dependent protease ClpAP.<P>Protein footprinting probes the solvent accessibility of side chains in the macromolecule, allowing protein interaction sites and conformational changes occurring upon complex formation to be mapped to specific areas of the protein. Synchrotron protein footprinting works via the generation of large quantities of hydroxyl radicals by direct irradiation of a protein solution over milliseconds. These radicals oxidatively modify protein side chains when they react with solvent-exposed protein regions, while areas of the protein not exposed to solvent are protected from these modifications. Protease digestion of the sample followed by liquid-chromatography-coupled mass spectrometry (LCMS) and tandem mass spectrometry (MSMS) allows identification of affected regions and often the specific residue that is modified. Changes in modification rate with the addition of complex components identify interprotein interactions and conformational changes.</P>ClpAP is comprised of a double-ringed tetradecameric ClpP protease core flanked by a hexameric ClpA ring on one or both ends. The ClpA chaperone is required for substrate recognition, unfolding and translocation of the unfolded substrate to the ClpP active sites for subsequent degradation. We have investigated two aspects of ClpAP; the ATP-driven formation of the ClpA hexamer and the role of the N-terminus of the ClpP protease in substrate interaction and processing. Synchrotron footprinting experiments were performed in the absence of nucleotide, where ClpA is disassembled, and in the presence of ATPγS, where it is hexameric; the results were compared to solvent-accessibility predictions based on two existing hexameric computational models and the ADP-bound monomeric crystal structure. The data suggest conformational changes upon hexamer formation requiring modification of the models and also provide structural support for a previously proposed mechanism for substrate translocation via the ClpA D2 loop. Surprisingly, footprinting experiments also revealed that the N-terminus of ClpP (peptide 1-12) becomes significantly more solvent-exposed upon interaction with ClpA and formation of the ClpAP complex. These footprinting studies and parallel biochemical experiments using both wild type and an N-terminal mutant of ClpP reveal the importance of the ClpP N-terminus in both substrate gating and enzymatic activity in the ClpAP molecular machine.

TUES 5:05- 5:20 pm: Mapping Gene Regulatory Pathways by Assembly of Physical and Genetic Interactions

Trey Ideker
University of California, San Diego, La Jolla, CA

Common modifications to lysine residues on proteins include acetylation, mono-, di and trimethylation, ubiquitylation and SUMOylation, with multiple modifications found on lysine rich proteins such as histones, histone acetyl transferases (HATs) and deacetylases (HDACs). We have developed several strategies for characterizing and quantitating these modifications, and for distinguishing isomeric forms. Deuteroacetylation of purified histones produces tryptic cleavages at arginine residues and an opportunity to quantify hyperacetylation states from chemically similar peptide fragments. Using this approach, MS/MS analyses with data-dependent data acquisition have been used to distinguish the sixteen isomeric species of a hyperacetylated histone H4 fragment containing four lysine sites. N-terminal sulfonation is being used to improve *de novo* sequencing as a means for PTM characterization and has been used to determine ubiquitylation sites. This method, used initially for CID mass spectra to produce y-ion spectra, has been extended to electron transfer dissociation analyses, and shows some value for *de novo* sequencing when used with doubly-charged ions. Similar approaches are used to determine SUMOylation sites following digestion with trypsin and chymotrypsin. Both the deuteroacetylation and N-terminal sulfonation methods have been extended to larger peptide fragments as part of a “middle-down” strategy.

Wednesday, 8:00 – 9:20 am
PTM Analyses of Complex Biological Matter

WED 8:25 - 8:50 am: Strategies for the Analysis of Lysine Modifications

Robert J Cotter; Dwella M Nelson; Omoruyi Osula; Steve Swatkoski
Johns Hopkins School of Medicine, Baltimore, MD

Common modifications to lysine residues on proteins include acetylation, mono-, di and trimethylation, ubiquitylation and SUMOylation, with multiple modifications found on lysine rich proteins such as histones, histone acetyl transferases (HATs) and deacetylases (HDACs). We have developed several strategies for characterizing and quantitating these modifications, and for distinguishing isomeric forms. Deuteroacetylation of purified histones produces tryptic cleavages at arginine residues and an opportunity to quantify hyperacetylation states from chemically similar peptide fragments. Using this approach, MS/MS analyses with data-dependent data acquisition have been used to distinguish the sixteen isomeric species of a hyperacetylated histone H4 fragment containing four lysine sites. N-terminal sulfonation is being used to improve *de novo* sequencing as a means for PTM characterization and has been used to determine ubiquitylation sites. This method, used initially for CID mass spectra to produce y-ion spectra, has been extended to electron transfer dissociation analyses, and shows some value for *de novo* sequencing when used with doubly-charged ions. Similar approaches are used to determine SUMOylation sites following digestion with trypsin and chymotrypsin. Both the deuteroacetylation and N-terminal sulfonation methods have been extended to larger peptide fragments as part of a "middle-down" strategy.

WED 8:50 - 9:05 am: Use of Antibody Microarrays for Ovarian Cancer Biomarker Discovery

Arturo B. Ramirez; Christian M. Loch; Yuzheng Zhang; Yan Liu; Martin W. McIntosh; Paul D. Lampe
Fred Hutchinson Cancer Research Center, Seattle, WA

In the United States, ovarian cancer annually affects 21,000 women and leads to 15,000 deaths. Due to the anatomical characteristics of the ovary, symptoms of this disease are vague and can be confused with other ailments, leading to a delay in diagnosis and decreased survival rates.

Some ovarian cancer diagnostic markers have been previously reported but their effectiveness in early detection screening is limited because of insufficient sensitivity and specificity. There is a great need for the discovery of ovarian cancer markers before it spreads to other organs in order to increase survival rates of this disease.

We hypothesize that proteins produced by ovarian cancer tumors are present in serum and can be used to diagnose this disease at earlier stages and lead to higher survival rates.

Our strategy for biomarker discovery utilizes antibody microarrays to probe cancer sera to recognize differences in its proteomic profile compared to normal sera. We created our own microarray platform containing 5000 recombinant single chain and full length antibodies. Our array was incubated with serum samples from individuals with and without ovarian cancer to identify antibodies that bind proteins differentially expressed in serum from ovarian cancer. We have run 300 microarray experiments using ovarian cancer serum, ovarian cancer cyst and ascites fluid samples, ovarian cancer cell line lysates and normal controls.

Statistical analysis identified those antibodies which can predict ovarian cancer status. Our array platform confirmed that previously published markers for ovarian cancer (CA125, Mesothelin, HE4) could discriminate disease status and were significantly up-regulated in cancer serum. We found additional novel proteins in serum that can discriminate disease status as well (or better) than the previously published markers in two separate serum cohorts with partially distinct sample sets.

We validated the microarray results by showing higher levels of the proteins in individual cancer serum samples using western blotting. Using immunoprecipitation and mass spectrometry techniques, we have begun the identification and characterization of the corresponding novel antigens.

Although further validation of these results is ongoing, this antibody microarray platform constitutes a high-throughput and highly sensitive method for the discovery of cancer biomarkers in biological fluids.

WED 9:05 - 9:20 am: Quantification of Posttranslationally Modified Peptides Using Tandem Mass Tags and Electron Transfer Dissociation

Rosa I Viner; Terry Zhang; Tonya Second; Vlad Zabrouskov
Thermo Fisher Scientific, San Jose, CA

The relative quantification of proteins in cells, tissues, and body fluids is of great interest in proteomics. Over the past decade, numerous mass spectrometric quantification strategies have been developed. One of these strategies - mass spectrometry-based multiplex quantitative methods (including TMT and iTRAQ) has been successfully employed for quantitative proteomics and biomarker discovery using CID fragmentation. However, analysis of some PTMs, such as glycosylation, sulfonation and phosphorylation, is difficult with CID since these modifications are labile and preferentially lost over peptide backbone fragmentation, resulting in little to no peptide identification and quantitation. Compared to CID, Electron Transfer Dissociation (ETD) preserves labile PTMs, and allows direct mapping of peptide/protein modifications.

In this study we assessed the utility of combining multiplexed tandem mass isobaric tag (TMT) labeling and ETD for relative quantification of O-glycosylation and other labile modifications containing peptides. Alpha crystallin was chosen as a model protein because of the well known presence of O-GlcNAc in addition to other PTMs (glycation, phosphorylation, acetylation). The samples were digested, labeled with TMT6 tags, mixed in different ratios and analyzed on a hybrid linear ion trap–orbitrap mass spectrometer equipped with ETD.

ETD analysis of both labeled and unlabeled peptides pinpointed at least one O-GlcNAc-containing peptide in each of the protein chains in addition to a multitude of other PTMs including glycation, phosphorylation, and acetylation. In addition, ETD of TMT6 labeled peptides produces four unique reporter ions that could be used for relative quantification. TMT reporter ion ratios measured by ETD had similar accuracy and precision as those obtained by conventional CID techniques. When applied to glycosylated or other modified peptides, ETD was often the only dissociation which reliably provided confident identification, PTM location and quantitative information, all in the same spectrum. This suggests that ETD-based workflows can be complementary to traditional CID approaches when used for simultaneous qualitative and quantitative analysis of modified peptides.

Wednesday, 8:00 – 9:20 am
Glycoproteomics

WED 8:00 - 8:25 am: Proteomic and Glycomic Analyses of E-cadherin Complexes

Krystyn Blackmon-Ross[1]; Mihai Nita-Lazar[2]; Maria A. Kukuruzinska[2]; Catherine E. Costello[1]
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Changes in intercellular adhesiveness is a regulated and dynamic process in the development and maintenance of organs and tissues. Alterations in cell-cell adhesiveness, when unregulated, allows cancer cells to cause destruction into surrounding cellular stroma, from cell adhesion to cell growth. Cadherins have been shown to be critical in normal mammalian development, and have a role in signaling pathways that regulate cellular proliferation and differentiation. Defects in E-cadherin mediated adhesion can promote cancer and abberant glycosylation is a hallmark of many cancers. The relationship between cancer and glycosylation is of great interest, but is difficult to study as glycosylation patterns are numerous and, unlike phosphorylation, may not directly modulate protein function. Utilizing non-malignant and malignant cell

cultures, we have investigated the hypothesis that the N-glycosylation status of E-cadherin is inversely correlated to the strength of its adhesion junction complex. Preliminary studies have found that hyper-N-glycosylated E-cadherin is found in cells with loose cell-cell contacts, while hypo-N-glycosylated E-cadherin is detected in stable cell-cell contacts. We have used glycomic, proteomic and biochemical tools to investigate the glycosylation of E-cadhedrin at different growth stages of normal tissues and in malignant cells, as well as the components of the E-cadhedrin complex as a function of glycosylation state.

This research is supported by NIH grants P41 RR10888 and S10 15942 (CEC) and R01 R01 DE10183 and R01 DE15304 (MAK).

WED 8:50 - 9:05 am: Glycoproteomics of IgA1

Kazuo Takahashi; Stephanie B. Wall; Hitoshi Suzuki; Zina Moldoveanu; Bruce A. Julian; Jiri Mestecky; Jan Novak; Matthew B. Renfrow

University of Alabama at Birmingham, Birmingham, AL

IgA Nephropathy (IgAN) is the most predominant form of glomerulonephritis in the world with 20-40% of all IgAN patients needing dialysis or kidney transplant. IgA1 in the circulation of patients with IgA nephropathy (IgAN) is aberrantly glycosylated; the hinge-region *O*-linked glycans are galactose (Gal)-deficient. These Gal-deficient IgA1 molecules are recognized by anti-glycan IgG or IgA1 antibodies, and immune complexes (IC) are formed. IgA1 contains several *O*-glycosylation sites per heavy chain. It is not known whether the glycosylation defect occurs randomly or preferentially at specific sites. Also, it is not known what combination of *O*-glycan chains result in the pathogenic form(s) of IgA1. Our group has undertaken the task of performing glycoproteomic analysis of IgA1 from a variety of sources including from serum (both healthy and disease), serum isolated immune complexes, IgAN patient derived B-cell lines, and IgA1 isolated from patients with multiple myeloma. Identifying the pathogenic forms of IgA1 is complicated by the fact that IgAN is a progressive disease which causes damage to the kidney over the course of 20-30 years. As a result, pathogenic IgA1 molecules are masked by the "normal" population of *O*-glycosylated IgA1 isoforms. Additionally, locating and characterizing the entire range of *O*-glycan attachment sites is analytically challenging due to clustered Serine and Threonine residues in the IgA1 heavy chain hinge region where the *O*-glycans are attached. We have established protocols for the analysis of IgA1 *O*-glycan heterogeneity and site-specific *O*-glycan chain analysis with a combination of glycan specific lectin western blots, LC-FT-ICR mass spectrometry, and tandem mass spectrometry (ECD and ETD) towards the goal of understanding the pathogenesis of this disease. In this work, we present results which make significant strides in overcoming the analytical challenge of locating and characterizing each site of *O*-glycosylation in all the glycoforms of IgA1 within a single sample. This includes IgA1 HR preparations for ECD by use of IgA1 specific proteases and trypsin which overcome the observed suppression of ECD type fragmentation around sites of glycosylation. Also, we will present results which reflect our continued efforts to properly sample the population of IgA1 molecules from patients with IgAN and to properly assess the heterogeneity of IgA1 *O*-glycan isoforms compared to normal healthy controls.

WOA 9:05 - 9:20 am: Identification of Cancer Specific Glycosylation Patterns of PSA for Prostate Cancer Diagnosis

Yan Li; Danni L. Meany; Lori J. Sokoll; Daniel W. Chan; Hui Zhang

Johns Hopkins University, Baltimore, MD

Prostate specific antigen (PSA) is the best-known serum marker for prostate cancer diagnosis and monitoring. However, one of the challenges it faces is its high false positive rate resulting in many unnecessary biopsies for cancer patients in the diagnostic grey zone. One possible method to improve the detection specificity of prostate cancer using PSA is to use the glycosylation patterns of PSA. If the PSA glycosylation pattern is associated with prostate cancer development, the specific glycosylation forms can be potentially used to improve the specificity of cancer detection.

Two immunoassay-based technologies: a high-density lectin microarray and an electrochemiluminescent (ECL)-based lectin-antibody immunoassay were used to analyze glycosylation patterns of PSA in this study. Both methods are able to provide a sensitive, reproducible, and high though-put analysis. We first extracted PSA proteins from tissue and serum samples from normal and cancer patients. The same amount of PSA protein was used to probe high-density lectin arrays containing 94 lectins to globally profile PSA carbohydrates. The lectins that have different binding ratios to PSA were then selected as targeted marker candidates. An ECL-based immunoassay was developed to validate the glycosylation change. The normal and cancer tissue samples with the same amount of PSA protein were used in ECL-based assay to measure targeted glycan level. The assays were applied to 26 normal sera and 26 cancer sera with matched PSA level (2.5-10ng/mL). The performance of the assays was evaluated using a ROC curve.

The results showed that the performance of SNA-1 PSA ECL-based immunoassay has improved cancer detection within the diagnostic grey zones of PSA (4-10ng/ml) and the free PSA to total PSA ratio (10-25%). It also provided strong evidence that the glycosylation of PSA was associated with prostate cancer development. A larger sample size will be needed to validate this finding In the future.

Wednesday, 9:45 – 11:20 am
Mass Spectrometry Advancement

WED 9:45 - 10:10 am: Absolute Quantification of Potential Cancer Markers in Clinical Tissue Homogenates Using Multiple-Reaction Monitoring on a Linear Ion Trap Mass Spectrometer

Leroi V. DeSouza[1]; Alexander D. Romaschin[2]; Terence J. Colgan[3]; K.W. Michael Siu[1]

[1]York University, Toronto,Canada; [2]St. Michael's Hospital, Toronto, ON, Canada; [3]Mount Sinai Hospital, Toronto, Canada

Multidimensional liquid chromatography with tandem mass spectrometry with iTRAQ-labeling typically used for differential expression analysis in biomarker discovery does not always detect peptides from these biomarkers in all samples analyzed. We will present the results of targeted analyses using multiple reaction monitoring (MRM) on a linear ion-trap tandem mass spectrometer. The MRM approach when combined with the newly released mTRAQ reagent, a non-isobaric variant of the iTRAQ tag available in two versions, enables absolute quantification of peptides and proteins via isotope-dilution mass spectrometry. This approach was applied to clinical endometrial tissue homogenates in an effort to quantify two endometrial cancer biomarkers, pyruvate kinase (PK) and polymeric immunoglobulin receptor (PIGR). We successfully demonstrated the feasibility of this approach on 20 individual samples and further verified the differential expressions of these two biomarkers in endometrial carcinoma. PK was determined to be present at an average concentration of 58.33 pmol/mg of total proteins and in the range of 9.13 – 87.66 pmol/mg in the soluble fraction of the normal proliferative endometrium homogenates. By contrast, the average concentration of PK in the cancer sample homogenates was 237.2 pmol/mg of total proteins and in the range of 66.10 – 570.9 pmol/mg. PIGR was found to be expressed at an average concentration of 8.85 pmol/mg of total proteins with a range of 1.02 – 49.61 pmol/mg in the normal proliferative control samples, and an average concentration of 200.2 pmol/mg with a range of 7.63 – 810.4 pmol/mg in the cancer samples (Fig 3B). This study confirmed qualitatively the differential expressions previously observed, but also showed that the actual relative differential expressions in these samples were much higher than those reported in the discovery study. These results validated earlier observations of dynamic compression in iTRAQ-labeling with hybrid quadrupole / time-of-flight mass spectrometry (DeSouza, L.V. et al. J. Proteome Res. 2008, 7, 3525-3534).

WED 10:10 - 10:35 am: Functional Proteomics Identifies Targets of Phosphorylation by B-Raf Signaling in Melanoma

William M. Old[1]; John B. Shabb[3]; Stephane Houel[1]; Hong Wang[1]; Kasey L. Couts[1]; Chia-yu Yen[1]; Elizabeth S. Litman[1]; Carrie H. Croy[1]; Karen Meyer-Arendt[1]; Jose G. Miranda[1]; Robert A. Brown[1]; Eric S. Witze[1]; Rebecca E. Schweppe[4]; Katheryn A. Resing[1]; Natalie G. Ahn[2]

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Melanoma and other cancers harbor oncogenic mutations in the protein kinase B-Raf, which leads to constitutive activation and dysregulation of MAP kinase signaling. In order to elucidate molecular determinants responsible for B-Raf control of cancer phenotypes, we present a method for phosphoprotein profiling, using negative ionization mass spectrometry to detect phosphopeptides based on their fragment ion signature caused by release of PO3–. The method provides an alternative strategy for phosphoproteomics, circumventing the need for affinity enrichment of phosphopeptides and isotopic labeling of samples. Using this method we identified a number of targets of oncogenic B-Raf signaling, based on their responses to treating melanoma cells with MKK1/2 inhibitor. Regulated phosphoproteins included known signaling effectors and cytoskeletal regulators. We investigated FAM129B, a target belonging to a protein family with unknown category and function, and established the importance of this protein and its MAP kinase-dependent phosphorylation in controlling melanoma cell invasion into 3-dimensional collagen matrix.

WED 10:35 - 10:50 am: Quantitative Label-Free Proteomics of Human Saliva

Iain Campuzano[1]; Pinmanee Boontheung[2]; John B. Hoyes[1]; Joseph A. Loo[2]

[1]Waters Corporation, Manchester, UK; [2]University of California, Los Angeles, Los Angeles, CA

High-throughput proteomic measurements using mass spectrometry has been facilitated largely by the ability of the LC-tandem mass spectrometer to acquire data in the data dependant mode. To further increase the level of proteome coverage for proteome analysis, we are exploring an approach to mass spectrometry-based protein identification that facilitates the simultaneous acquisition of qualitative and quantitative information in a data independent fashion without the use of stable isotope labeling. A data independent mode of acquisition differs from an LC-MS/MS data acquisition, as no ion transmission window is applied with the first mass analyzer prior to collisionally activated dissociation (CAD). Alternating the energy applied to the collision cell between low and elevated energy on a scan-to-scan basis provide accurate mass precursor and associated product ion spectra from every ion above the limit-of-detection of the mass spectrometer. The method therefore provides a near 100% duty cycle, with an inherent increase in signal intensity due to the fact that both precursor and product ion data are collected on all isotopes of every charge-state across the entire chromatographic peak width. The correlation of product to precursor ions, after deconvolution, is achieved by using reconstructed retention time and chromatographic peak shapes. Thus, this MS-E concept retains the high mass measurement accuracy property of quadrupole time-of-flight (QTOF) analyzers with high duty cycle, multiplexed LC-MS measurements for high protein sequence coverage and protein identification.

Using this strategy, we have quantified (relative and absolute) over 200 proteins from human whole saliva. Saliva is the fluid that irrigates the mouth and oral cavity. It has a rich proteome that is derived from the salivary glands, the linings of the oral cavity, and blood. Saliva is readily available and easier to collect compared to other body fluids, e.g., CSF, tears, and urine. Compared to plasma, with highly abundant albumin, transferrin, and IgGs present, the salivary proteome presents far fewer challenges for accessing the entire protein complement. For these reasons, the use of saliva for diagnostic purposes presents an attractive potential option.

Data was acquired on a QTOF mass spectrometer incorporating a new high field pusher device and dual stage reflectron. This arrangement enables a resolution of greater than 10,000 FWHM to be achieved with a flight tube 35-cm in length. A saliva trypsin digest was analyzed using the data-independent method of acquisition. Over 200 non-redundant proteins were identified from an approximate sample load of 300-ng. We also demonstrate the ability for absolute quantification of protein levels in saliva by the addition of an internal protein standard, to which the data set is normalized. Protein levels span over 3-orders of magnitude as measured from a single LC-MS/MS run. Our study to explore the diagnostic potential of saliva for human diseases could significantly impact efforts to discover early detection markers for diseases such as pancreatic cancer.

WED 10:50 - 11:05 am: PAcIFIC: How to Dive Deeper into the Proteomics Ocean

Alexandre D. Panchaud; Alexander Scherl; Scott A. Shaffer; Priska von Haller; David R. Goodlett

University of Washington, Seattle, WA

The vast majority of shotgun proteomics experiments are conducted by using data-dependent precursor ion selection that mean; ions, more precisely peptides, are selected for further fragmentation based on their intensity. While very popular and successful, this approach suffers major drawbacks in terms of dynamic range and under-sampling of low abundant peptides as a selection bias is created towards species with the highest signal.

Here, we demonstrate the superior performance of a data-independent acquisition method that we named Peptide Acquisition Independent From Ion Count (PAcIFIC). PAcIFIC acquires tandem mass spectra at every m/z value (i.e. m/z "channel") without regard for whether a precursor ion is observed or not. In fact no precursor ion scans are even conducted. Collision induced dissociation (CID) spectra are taken every 1.5 m/z across a range of 15 m/z to 30 m/z per LC-MS analysis, thus acquiring 10 to 20 CIDs per cycle depending on the chromatography resolution. Repeated injections are preformed in an identical fashion until the desired m/z mass range is achieved, typically 400-1400 u.

To assess the potential of PAcIFIC on a challenging, "timely" sample to the world of proteomic biomarker discovery, we analyzed a tryptic digest of human plasma proteins. We show that PAcIFIC provides unique performance for analysis of human plasma in terms of number of proteins identified (746 at FPR ≤ 0.5% to 5382 at FPR ≤ 5%) and achieved dynamic range (seven orders of magnitude at FPR ≤ 0.5% to nine at FPR ≤ 5%), everything without any extensive fractionation other than immuno-depletion of the seven most abundant proteins. Dynamic range is highly increased with our PAcIFIC strategy due to the fact that very narrow m/z ranges are concentrated in the ion trap regardless of whether a precursor ion is present thus maximizing ion concentration prior to CID.

WED 11:05 - 11:20 am: Intact Protein Analysis Using Electron Transfer Dissociation Mass Spectrometry

Zhiqi Hao; Jae C Schwartz ; Andreas FR Hühmer

ThermoFisher Scientific, San Jose, CA

Electron transfer dissociation (ETD), compared to collisional activation, is relatively insensible to the size, the amino acid composition and post-translational modifications of peptides or proteins. The fact that ETD randomly cleaves backbone bonds makes it an advantageous tool for large peptide and intact protein analysis. ETD of intact proteins performs with high efficiency, generating very informative, yet extremely complex spectra which contain highly charged product ions that are difficult, or even impossible to resolve at unit resolution. ETD was recently implemented in a hybrid linear ion trap - Orbitrap mass spectrometer. The high resolution and accurate mass of the Orbitrap would greatly facilitate the analysis of intact proteins using ETD. For unit resolution instruments, proton transfer reaction (PTR) following ETD was developed to reduce spectral complexity. PTR removes protons from the multiply charged product ions, generating a simplified spectrum that contains product ions of resolvable charge states at unit resolution. PTR has recently implemented in LTQ XL under instrument control software. In this

study, ETD was applied to proteins top-down analysis both in hybrid linear ion trap and in unit-resolution linear trap. ETD combined with accurate mass and high resolution was employed to study optimized conditions for intact protein sequencing. The performance of PTR for ETD spectra simplification was evaluated. The utility of ETD-PTR approach for intact protein analysis in unit-resolution linear trap was also investigated.

Using ETD with accurate mass and high resolving power, standard intact proteins ranging in size from 8 kDa to 46 kDa were analyzed. The resulting spectra are information rich, containing multiply charged c/z type of product ions which are well resolved. The optimized ratio of analyte cation and ETD reagent anion was investigated for maximum sequence coverage. The optimized ETD reaction time for maximum sequence coverage was found to be significantly shorter than ETD reaction time for peptide fragmentation. Longer ETD reactions lead to decreased overall sequence coverage while N- and C-terminal sequence coverage is increased.

When ETD of intact proteins was performed in unit-resolution instrument, multiply charged product ions in the resulting spectra could not be resolved adequately. Thus, reducing product ion charge state to produce simplified spectra is necessary for data interpretation. Charge reduction can be achieved either by extended ETD reaction, or by PTR. Our results indicate that c/z type ions generated by extended ETD reaction contain one or more extra hydrogen than expected, due to charge reduction through multiple electron transfers. These ions are excluded from identification by database search software due to the unexpected mass shift. Furthermore, extended ETD reaction generated secondary fragmentation products which interfere with data analysis. PTR, which reduces product ion charge state by subtracting protons, generated c/z type ions of expected mass for data analysis software. Our data from intact proteins up to 30 kDa indicates that PTR following ETD in linear trap significantly improves sequence coverage when compared to ETD alone. The utility of ETD-PTR in unit resolution linear trap for intact protein analysis, as well as its limitations, will be discussed.

Wednesday, 9:45 – 11:20 am
Membrane Proteomics

WED 9:45 - 10:10 am: Comprehensive Coverage of Membrane Proteomes

Mark Baker[1]; Joel Chick[2]; Albert Lee[2]; Rohit Saldanha[1]; Iveta Slapetova[2]; Graeme Robertson[3]; Paul Haynes[2]; Nicolle Packer[2]
[1]Australian Proteome Analysis Facility (APAF), Macquarie University, Australia; [2]Chemistry & Biomolecular Sciences, Macquarie University, Australia; [3]Concord Hospital, Sydney, Australia

Proteomics promises (but has yet) to deliver comprehensive coverage of any mammalian membrane proteome - primarily because of problems associated with membrane protein solubility issues and the low copy number of many important membrane proteins. The Membrane Proteome Initiative (MPI) aims to undertake large scale plasma proteome analyses of “standard” liver membrane preparations. This talk will summarise our recent work on the initiative, related glycoproteomics developments and membrane protein:protein interactomics studies in cancer. We report data evaluating membrane protein immunoprecipitation, fractionation and digestion methods combined with new separation e.g., peptide immobilized pH gradient isoelectric focusing (peptide IPG-IEF) and mass spectrometry on human cancer cell1,3 or mammalian (MPI) membranes2,4,5 with a view to increasing the depth of mammalian membrane proteome coverage. Using a mouse EHS cancer model shotgun proteomics methods and peptide IPG-IEF have recently been compared and shown (by us and others) to reproducibly, reliably and quickly separate trypsin degraded peptides from liver membranes for membrane protein identification. In addition, methanol-assisted (0%, 40% & 60%) trypsin digestion of membrane proteins resulted in optimal peptide coverage and release of ectodomain-containing membrane proteins when deployed with peptide IPG-IEF. Peptides concentrated into 3 main pI regions and ~95% were in 2 fractions. In total, ~2,000 non-redundant membrane proteins were identified with a very high level of coverage (including 42 rat CYP450 protein family members). Integral membrane proteins were identified and 513 were predicted to have 1->19 transmembrane segments. As expected, biochemical ontology indicated origin mainly from microsomal origin (e.g., ribosomal/structural proteins; 17%), mitochondrial (e.g., electron transport chain; 15%), cell membrane (12%), endoplasmic reticulum including CYP450s (11%) or either Golgi, secretion pathways, endosomes, peroxisomes or cytoplasmic vesicles (<3%). Addition of pI as a filtering tool enhanced reliability of identifications. An alternate method for membrane protein identification, in which protein glycosylation was targeted as an enrichment strategy has been developed, in which different subsets of membrane proteins were found to be enriched by lectins and hydrazine chemistry. Other approaches such as immunoprecipitation and MS identification of interacting proteins from cancer cell lines has enabled identification of specific proteins that are involved in the epithelial cancer cell “metastasome’ around the lynchpin protein uPAR (i.e., urokinase receptor).

Collectively, our data demonstrate that a combination of methods including peptide IPG-IEF, glycoprotein enrichment and immunoprecipitation can be used for a comprehensive coverage of the membrane sub-proteome. We provide deeper (more comprehensive) mammalian membrane proteome coverage than has been previously accomplished. Significantly, we report changes in liver membrane proteomes from cancer-bearing mice – a major consideration not previously considered when examining plasma biomarker profiles or the “seed and soil” hypothesis of metastasis.

1. Saldanha RG, et al., J Proteome Res. 2007 6; 1016-1028; 2. Chick JM, et al., J Prot. Res. 2008, 7; 1036-45; 3. Saldanha RG, et al., J. Prot. Res., 2008, 7; 4792-806; 4.Chick JM, et al., J. Prot. Res., 2008, 7; 4974-4981; 5. Lee, A, et al., J. Prot. Res ,submitted October, 2008.

WED 10:35 - 10:50 am: Post-Translational Modifications of Integral Membrane Proteins Resolved by Top-Down Fourier-Transform Mass Spectrometry

Julian Whitelegge
The Pasarow Mass Spectrometry Lab, UCLA, Los Angeles, CA

Integral membrane proteins remain a challenge to proteomics because they contain regions of polypeptide with physicochemical properties poorly suited to today’s bottom up protocols. These transmembrane regions may potentially contain important post-translational modifications of functional significance and there is interest in development of protocols to improve coverage in these domains. One way to achieve this goal is by using top-down mass spectrometry whereby the intact protein including its transmembrane regions is subjected to mass spectrometry and dissociation. Here we describe top-down high-resolution Fourier-transform ion cyclotron resonance mass spectrometry experiments of a number of post-translationally modified integral membrane proteins including both polyhelix bundle and transmembrane porin motifs with molecular weights up to 35 kD (bacteriorhodopsin, subunits of the cytochome b6f complex and the murine voltage-dependent anion channel, VDAC). Analysis of the intact protein effectively provides complete coverage of transmembrane domains because modifications that change mass can be detected wherever they are. While sequence coverage in transmembrane domains can be very high, this is not always so and consequently post-translational modifications are not always localized as precisely as is desirable. Strategies toward improving precision are discussed.

WED 10:50 - 11:05 am: Native Membrane Protein Immobilization on a Solid Surface

Kenneth Olesen; Jennie Wikstrom; Max Davidson
Nanoxis, Gothenburg, Sweden

The LPI™ technology is based on the use of proprietary surfaces and sample preparation protocols designed to retain the natural environment around the membrane proteins. The membrane proteins can be subjected to a variety of protocols in a highly controlled manner, to provide effective and precise preparation of samples for downstream analysis. LPI™ FlowCell is a single use device that allows for immobilization of intact proteoliposomes

directly produced from membrane preparations of a wide variety of cells and tissues. Proteins are kept in their native cell membrane with retained structure and function. LPI™ FlowCells are used in applications such as ligand binding studies, epitope identification, quantification, biomarker identification and results are presented.

WED 11:05 - 11:20 am: Conserved Waters Define a Structural and Functional Channel Involved in Activation of the G Protein-Coupled Receptor Rhodopsin

Thomas E Angel; Sayan Gupta; Beata Jastrzebska; Krzyszyof Palczewski; Mark R Chance
Case Western Reserve University, Cleveland, OH

Agonist-induced allosteric activation of G-protein coupled receptors (GPCRs) likely is conserved among the members of this superfamily, but the molecular basis of activation is not fully understood. We exposed the GPCR rhodopsin to millisecond high flux x-rays to generate hydroxyl radicals from structural water molecules embedded in the transmembrane segment. These radicals reacted with adjacent protein side chains to form stable modification products. Quantitative mass spectrometry analysis of the modified and unmodified peptides was conducted. Differences in the modification rates allowed us to assess the details of bound water structures, dynamics and exchange with bulk water as a function of rhodopsin photoactivation and transformation to the inactive, ligand-free opsin state. The results define an allosteric water channel that mediates communication between the retinal and the cytoplasmic face of the receptor during transient formation of the activated state.

This work demonstrates that radiolytic footprinting can be used to probe water structure and dynamics for membrane proteins. This methodology together with other approaches also has the potential to define activation channels for other family A GPCRs, transmembrane signaling proteins and ion channels.

Wednesday, 9:45 – 11:20 am
Bioinformatic Analyses of Protein Structure

WED 9:45 - 10:10 am: Predicting the Peptide Recognition Specificity of the SH3

Wei Wang
UC San Diego, La Jolla, CA

Protein-protein interactions play critical roles in almost every biological process. Understanding the mechanism of protein recognition and how the interaction specificity is achieved can greatly help reconstruct protein interaction network and develop therapeutic inhibitors. I will discuss our recent efforts on deciphering the protein recognition code using free energy calculation and classification analyses. We have applied this method to studying the interactions between Src homology 3 (SH3) domain and peptides. These interactions are weak and transient, and they are difficult to be detected by high throughput techniques. We characterized the domain-peptide interaction interface using molecular dynamics simulation and free energy component analysis. We then used a classification algorithm to predict the binding peptides for a given SH3 domain. We assessed the performance of the method using both computational and experimental measurements. Our method achieved significantly higher sensitivity, specificity and prediction accuracy than many methods. We believe the integration of computer modeling and statistical analyses provides a general framework to decipher the protein recognition code.

WED 10:35 - 10:50 am: Efficient Identification of Chemical Crosslinks Using Label-Free Pattern Comparisons to Deduce Structural Features of Macromolecular Complexes

David W. Speicher; Donghai Li; Hsin-Yao Tang
The Wistar Institute, Philadelphia, PA

Identification of chemical crosslinks in protein complexes can provide unique insights into intra-molecular structure, protein-protein interactions, and protein docking surfaces. The specific distance constraints defined by crosslinks can be used to test and refine homology-based structural models and inter-molecular docking models. Crosslinking data can also complement NMR and crystallographic studies when molecular rearrangements occur that have not been captured in high resolution structural determinations. The availability of highly sensitive, high mass accuracy mass spectrometers has greatly enhanced the ability to identify low stoichiometry crosslinks with high confidence. However, extensive use of crosslinking to probe protein structure and macromolecular interactions has been inhibited by limitations of available software tools. Typically, only a tiny portion of the MS/MS spectra in an LC-MS/MS analysis result from crosslinked peptides and MS/MS spectral quality can often be low. Simply identifying the specific crosslinked peptide spectra from the thousands of spectra in a single LC-MS/MS run is tedious and imprecise. Similarly, interpreting these spectra and assigning sequences to crosslinked peptides involves substantial manual data interpretation with the risk of incorrect assignments and overlooked crosslinks. The complexity of the problem increases rapidly as the size of the protein complex increases. We have developed a strategy to greatly facilitate interpretation of crosslinked peptides and applied it to various protein complexes, including crosslinks of the monomer and dimer forms of a 90 kDa protein that undergoes major dynamic rearrangements. A key step in our analysis pipline is label-free LC-MS pattern comparisons using Rosetta Elucidator software to quickly identify the small subset of peptide signals that are possible crosslinked peptides. This approach is also used to identify qualitative and quantitative differences in crosslinks of proteins undergoing major structural rearrangements. Peptide masses identified as possible crosslinks in the LC-MS pattern comparisons are compared to all possible theoretical crosslink masses and spectra that match uncrosslinked peptides with good scores are eliminated. Targeted LC-MS/MS analysis on an Orbitrap mass spectrometer is then used to obtain high mass accuracy MS/MS spectra for all putative crosslinks including peptides that did not trigger an MS/MS spectra in the initial analysis or where spectral quality was low. This approach increases the speed of data interpretation and identifies substantially more crosslinks. The high mass accuracy of both the precursor ion and fragment ions greatly increase confidence of sequence assignments even when low quality sparse spectra are obtained. In addition, the label-free pattern comparison is well suited for comparison of experimental conditions that induce conformational rearrangements.

WED 10:50 - 11:05 am: Protein Structure Determination by Combining Structural Mass Spectrometry Data with Rosetta

Xiaojing Zheng[1]; Robert M Vernon[2]; David Baker[2]; Mark R Chance[1]
[1]Case Center for Proteomics and Bioinformatics, Cleveland, OH; [2]University of Washington, Seattle, WA

Despite the obvious importance of protein structural information, the challenges to obtaining it are considerable using current technologies such as X-ray and NMR. Computational tools have been applied to protein structure determination, using both ab initio and homology modeling approaches, and accuracy has improved considerably over the past several years. However, if a model is not available (with sequence similarity of 30% or more), ab initio methods are required where millions of initial “decoy” structures have to be assembled and refined. After refinement, it is often the case that many low energy structures are generated, with difficulties inherent in choosing among them.

In this study, we are trying to develop a computational protein structure prediction method using Rosetta that incorporates structural mass spectrometry data. Hydroxyl-radical-mediated footprinting coupled with mass spectrometry (FP-MS) provides information on protein side chain solvent accessibility, thus defining exposed surface and buried hydrophobic core residues within a target protein. The structural information generated from footprinting is used to provide restraints for structure sampling and permits filtering of decoys that are inconsistent with experimental data. Since the generation and filtration of decoy structures is the most computationally expensive process for ab initio prediction, we can expect an increase in the speed of modeling, which will allow larger proteins to be effectively modeled and we expect an increase in final model accuracy as well.

Our preliminary data has demonstrated that this novel approach is very successful and efficient as applied to ubiquitin. For the test case with ubiquitin, implementation of "filtering" sped up computation by 50-500 fold as false-positive decoys were eliminated in the early stages. In addition, backbone rmsd was improved from 1.5 Å (best with the standard Rosetta protocol) to below 1.0 Å. Currently, we are carrying out large-scale calibration experiments: 15 targets from the protein structural initiative with solved structures were selected for calibration and testing purposes for modeling/footprinting. These initial results will be presented. Our long-term goal is to provide routine structure determination for protein domains (up to 200 aa in length) with an accuracy equal to that of NMR using an optimized Rosetta and footprinting data alone.

Wednesday, 1:00 – 3:15 pm
PTM Analyses

WED 1:05 - 1:35 pm: Innovative Technology for the Study of Protein Post-Translational Modifications

Donald F. Hunt
University of Virginia, Charlottesville, VA

This lecture will focus on a combination of enrichment technology and mass spectrometry instrument development that facilitates identification of posttranslational modifications on proteins and peptides. Development of a new ion source that facilitates simultaneous generation of positive and negative ions by electrospray ionization and chemical ionization, respectively, now makes it possible to record collision activated dissociation (CAD) and electron transfer dissociation (ETD) mass spectra on the high resolution LTQ-FT instrument. Parent ion and fragment ion mass spectra can be obtained at resolutions in excess of 40,000 and with mass measurement accuracy in the high ppb range. Applications of this instrumentation for the characterization of class I MHC phosphopeptides, histone post-translational modifications and O-GlcNacylated proteins will be described. In humans, O-GlcNAcylation, the addition of N-acetylglucosamine to Ser and Thr residues on proteins, is catalyzed by a single enzyme and removed by a single GlcNAse. Discovered by Gerry Hart at Johns Hopkins University in 1984, O-GlcNAcylation is now thought to rival phosphorylation in its abundance and play an equally important role in cell signaling. The O-GlcNAc modification is labile and easily lost under CAD collisions but stable to ETD. Peptides, with and without, the modification also co-elute under conventional HPLC conditions. Like phosphorylation, O-GlcNAcylation is usually found to be substoichiometric and ionization of the modified peptide is suppressed in the presence of the unmodified peptide. Technology for enriching O-GlcNAcylated peptides from complex mixtures will be discussed and results from recent studies will be presented. If time permits, a role for proteolytic enzymes acting on chromatin during stem cell differentiation will also be discussed.

WED 1:35 - 2:05 pm: Lys-N; A Protease Ideal for Proteomics Facilitating the Targeted Analysis of Post-Translational Modifications and *de novo* Sequencing

Albert J.R. Heck
Utrecht University, Utrecht, The Netherlands

In mass spectrometry based proteomics trypsin is still the most-widely used protease to generate proteolytic peptides from proteins. Here, we describe the utility of a relatively little explored metalloendopeptidase with Lys-N cleavage specificity. We show that when Lys-N generated proteolytic peptides are subjected to low-pH SCX fractionation profiles can be obtained in which peptides from different functional categories are well separated. The four categories we are able to separate to near completion are I) acetylated N-terminal peptides, II) singly phosphorylated peptides containing a single basic (Lys) residue, III) peptides containing a single basic (Lys) residue and IV) peptides containing more than one basic residue.

Analysing these peptides by LC-MS/MS using collision induced dissociation and electron transfer induced dissociation provides unique optimal targeted strategies for proteome analysis. For the phosphorylated peptides in II and the "normal" single Lys containing peptides in III ETD provides unique straightforward sequence ladders of c'-ions, from which the exact location of possible phosphorylation sites can be easily determined. Overall, the combination of Lys-N proteolysis, low-pH SCX and RP separation, with CID and ETD induced fragmentation, adds a new very powerful method to the toolbox of proteomic analyses, especially facilitating the targeted analysis of post-translational modifications and *de novo* sequencing.

WED 2:05 - 2:35 pm: New Developments for the Improved Characterization of Post Translational Modifications

Richard D. Smith; Ljiljana Pasa-Tolic; Yufeng Shen; Tyler H. Heibeck; Weijun Qian; Feng Yang; Mikhail E. Belov; Gordon A. Anderson; Joshua N. Adkins; Saiful M. Chowdhury; Daniel Lopez-Ferrer; Konstantinos Petritis; Vladislav A. Petyuk; Ronald J. Moore; Nikola Tolic
Pacific Northwest National Laboratory, Richland, WA

This presentation will describe new developments that help to increase the coverage of protein PTMs, and improved informatics tools of their identification. Improved high resolution LC separations have been developed for the characterization of intact proteins. New informatics approaches have been developed that decrease false discovery rates and that use "unique sequence tags" for improved identification. The presentation will also illustrate these developments using a new platform based upon the combination of high speed ion mobility separations with accurate mass time of flight mass spectrometry.

MONDAY POSTERS

Posters to be set-up by 8:00 am Monday. Posters should be attended 1:30 – 3:30 pm at Monday Poster Session. Remove posters by 9:00 pm Monday eve.

Bioinformatics, 01 - 11

Mon Poster 01: Molecular Structure and Heavy Metal Specificity of DMT-1

Fintan Gonsal; Harinda Rajapaksha; Mangalika Wicramasinghe; Sarath Bandara
Faculty of Agriculture University of Peradeniya, Peradeniya, Sri Lanka

Metal ions are essential cofactors for a range of biological processes including gene regulation, free-radical homeostasis and phosphorylation. Divalent metal transporter-1 (DMT-1) which is located at the apical membrane of enterocytes lining the small intestine has been known to transport Fe and other divalent metals across the biological membrane is known to its nonspecific in human than in prokaryotes. There are various mechanisms used by prokaryotes to overcome toxicity in their environment which humans are lacking and are yet to be discovered. Thus homology modeling was employed to generate two models for human divalent metal transporter-1 (DMT-1) and the prokaryotic divalent heavy metal transporter-1 (DHMT-1). Best models for DMT-1 and DHMT-1 were selected after evaluating 30 homology models derived from matching PDB entries 1AR1, 2NQ2 and 1V54 for DHMT-1, 1PW4, 2A65 and 3DH4 for DMT-1. The best models were subjected to a short molecular dynamics refinement with GROMACS program package to obtain equilibrium.

Mon Poster 02: Combinatorial Deconvolution Algorithm for Tandem Mass Spectra of Large Intact Protein

Yuval Inbar[1]; Pieter Dorrestein[2]; Puneet Souda[3]; Julian P Whitelegge[3]; Vineet Bafna[1]; Pavel A Pevzner[1]
[1]Computer Science and Engineering, UCSD, La Jolla, CA; [2]Pharmacology, Chemistry and Biochemistry, UCSD, La Jolla, CA; [3]Chemistry and Biochemistry, UCLA, Los Angeles, CA

Tandem mass spectrometry of intact proteins is an emerging technology in proteomics. Spectral deconvolution is the first step of spectrum interpretation. The deconvolution methods focus on grouping spectral peaks into isotopic envelopes, from which they derive a list of mono-isotopic masses. The main challenges in spectral deconvolution include (1) clustering peaks in peak-rich m/z regions of the spectrum, (2) detecting envelopes with borderline fit to theoretical isotopic distributions, and (3) resolving overlaps between different isotopic envelopes. All these challenges are amplified as the size and the charge state of the intact protein increases.

We present MS-DECONV, a combinatorial algorithm for spectral deconvolution. The algorithm generates a large set of candidate envelopes and efficiently selects a high scoring subset of them. In difference from previous approaches, the algorithm scores combinations of candidate envelopes, rather then evaluating each separately. By doing so, the algorithm is more likely to both correctly interpret the peak-rich regions of the spectrum, and to select envelopes with poor fit to the theoretical isotopic distribution. We approach the signal overlap challenge by introducing an intensity-split scoring model that allows peaks to be assigned to multiple envelopes. When a set of envelopes is evaluated, the intensity of a peak is distributed between all envelopes that share the peak, according to the isotopic pattern of the contributing envelopes.

We compared MS-DECONV with Thrash (run through ProSight-PC) on a data set of six spectra from known unmodified proteins. We classified a peak as true-positive if its mono-isotopic mass is within 5 PPM from the theoretical mass of an abundant ion of the protein. We demonstrate highly improved performance of at least 15% higher sensitivity in each of the test cases at comparable specificities, with over 100% improvement in some cases. Moreover, our predictions have a higher accuracy: The PPM accuracy of our predctions was between 1.3× to over 2.3× better than Thrash. For a spectrum with more than 1500 peaks and 26 different charge states, MS-DECONV takes ~30 seconds on a standard single processor Linux machine.

MS- DECONV is available as a web-service at http://bix.ucsd.edu/msdeconv.

Mon Poster 03: Corra and TIQAM: Computational Software Suite for Discovery and Verification of Biomarker Candidates

Mi-Youn Brusniak[1]; Kelly Cooke[1]; Francisco Esteva[2]; Simon Letarte[1]; Carey Sheu[1]; Aysegul Sahin[2]; Julian Watts[1]; Ruedi Aebersold[3]
[1]Institute for Systems Biology, Seattle, WA; [2]The University of Texas M. D. Anderson Cancer Ctr., Houston, TX; [3]Institute of Molecular Systems Biology, Zurich, Switzerland

We introduce a complete software suite named Corra:TIQAM as open source software tools for discovery and verification of biomarker candidates, as well as other proteomic applications in which multiple samples are being quantitatively compared. The Corra software component is for generating target candidate lists via label-free LC-MS-based quantitative proteomics. The TIQAM software component is for assisting the selection of candidate transitions and their validation for Selected Reaction Monitoring (SRM) proteomic experiments. The candidate peptide or protein lists for TIQAM can be generated either directly from Corra, or via any other independently generated candidate list in FASTA format. Corra extends and adapts existing algorithms used for LC-MS-based proteomics and statistical algorithms to select significantly differentially abundant LC-MS detected peptide features, in a form compatible with subsequent sequence identification via targeted tandem mass spectrometry. Such identified candidate proteins can then be further verified and quantified in additional biospecimens of interest using a more sensitive SRM LC-MS proteomic approach. For the success of accurate quantification, it is critical to choose optimal SRM transitions that can be identified and validated. To support this process, we developed TIQAM as a desktop application. TIQAM-PeptideAtlas interfaces with the public observed MS/MS database, PeptideAtlas. TIQAM-Digestor integrates TIQAM-PeptideAtlas output, pepXML files and other data, to select transition lists according to user-defined priorities. TIQAM-viewer provides a visual aid to analyze SRM-triggered MS/MS experiments. To illustrate the workflow for Corra:TIQAM, we show the application of the tools to a case study for human disease biomarker discovery and verification. In the discovery phase, matched pairs (i.e. from the same patient) of normal and diseased tissue were used. N-glycosite peptides were isolated from the tissue samples and LC-MS analyses were performed in triplicate. Corra was used to identify peptide features that were differentially abundant between the disease and normal tissue samples. These features were then subsequently targeted for sequence identification via LC-MS/MS. Since we are ultimately interested in identifying new candidate markers for human disease, we next wanted to verify whether these candidate peptides/proteins could additionally be measured in blood plasma. A subset of the identified proteins was processed by TIQAM to design and assist SRM experiments. The optimized transitions generated by TIQAM were then used to measure the candidate proteins in a new set of plasma samples obtained from normal and diseased patients. Here, we introduce and illustrate the complete Corra:TIQAM software suite, applied to the discovery and verification of biomarker candidates, and include a brief discussion of planned future work.

Mon Poster 04: A New Algorithm for the Analysis of Intact Proteins

Keith Waddell; David Horn; Xiangdong Li; Christine Miller
Agilent Technologies, Santa Clara, CA

Currently, the most common algorithm for intact protein mass determination is maximum entropy deconvolution. This method transforms a mass spectrum in m/z units into a mass spectrum containing the zero-charge representation of the intact protein (in Dalton units). Complex data produces false positive “overtone” peaks, which correspond to masses calculated from randomly dispersed peaks from the raw data. Also, if proteins do not perfectly coelute, it is difficult to choose one or more averaged spectra from the chromatogram that produces high quality results for these multiple proteins.

Here we describe a new algorithm called “Large Molecule Feature Extraction” (LMFE) for the determination of the masses of large molecules in complex mixtures. This approach first produces

extracted ion chromatograms for all peaks in the raw LC/MS data and subsequently groups the peaks with the same retention time and elution profile into "coelution groups". The peaks within a given coelution group will contain the different charge states of the same protein, which are subsequently grouped together by algebraic charge state deconvolution. While algebraic deconvolution is generally untenable for very complex spectra, the coelution grouping greatly simplifies the peak spectra that are used during algebraic deconvolution making the process much more robust.

For a mixture of all E. coli cytosolic proteins, the base peak chromatogram was integrated and peak spectra extracted from each integrated peak. Subsequently, maximum entropy deconvolution was performed on the 90 averaged spectra using a mass range of 6 to 70 kDa. This required 90 minutes of analysis time and resulted in 140 protein compounds. Using LMFE, 682 protein masses were found in 15 minutes, which is almost 5 times as many compounds in 1/6th the amount of time. Thus, for complex mixtures, LMFE greatly outperforms maximum entropy deconvolution.

Mon Poster 05: New Functionality for the Trans-Proteomic Pipeline: Tools for the Analysis of Proteomics Data

Luis Mendoza[1]; David Shteynberg[1]; Natalie Tasman[1]; Brian Pratt[2]; Jimmy K. Eng[3]; Henry Lam[4]; Alexey I. Nesvizhskii[5]; Eric W. Deutsch[1]; Ruedi Aebersold[6]
[1]Institute for Systems Biology, Seattle, WA; [2]Insilicos LLC, Seattle, WA; [3]University of Washington, Seattle, WA; [4]Hong Kong University of Science and Technology, Hong Kong, China; [5]University of Michigan, Ann Arbor, MI; [6]Institute for Molecular Systems Biology (ETH), Zurich, Switzerland

High throughput LC-MS/MS is capable of simultaneously identifying and quantifying thousands of proteins in a complex sample. Consistent and objective analysis of large datasets is challenging and time-consuming. Over the past four years, we have developed and made improvements to the Trans-Proteomic Pipeline (TPP), an open source suite of tools that facilitates and standardizes such analysis. The TPP includes software tools for MS data representation, MS data visualization, peptide identification and validation, protein identification, quantification, and annotation, data storage and mining, and biological inference. We present an overview of the TPP and describe newly available functionality.

We have recently introduced several new features in the TPP. We provide a new validation tool (iProphet) that combines peptide identifications from multiple runs, experiments, and search strategies (e.g. search engines) to determine more accurate post-PeptideProphet probabilities. We have expanded support for the mzML version 1.0 data format in our converters and parsers. Support for spectral library searching has been enhanced with the inclusion of the latest version of SpectraST, which allows the user to create custom spectral libraries from previous search results, as well as search against them; we have also integrated library download and management utilities in the GUI. Several bug fixes and improvements to the user interface have also been made.

All of the TPP software tools are available for download under an open source software license at tools.proteomecenter.org, and can be installed on several popular operating systems. Free email support for the installation and operation of these tools is also available, as is a searchable knowledge base.

Mon Poster 06: Correlation Statistics Analysis for MS Peaks from Protein Mixtures

Dariya I. Malyarenko[1]; William E. Cooke[1]; Dennis M. Weaver[2]; Eugene R. Tracy[1]; Christine L. Gatlin-Bunai[1]; Dennis M. Manos[1]
[1]College of William and Mary, Williamsburg, Virginia; [2]Langley Center, St. Leo University, Hampton, Virginia

In comparative MS proteomics applications high-dimensional data are routinely produced due to the typically much smaller number of samples than variables (observed peaks.) The presence of strongly correlated signals in such data may present a challenge for analysis, e.g., for the discovery of diagnostic patterns. To develop automated tools for MS dimension reduction, correlation statistics need to be analyzed for standard mixtures with known components. In this work, MALDI-TOF mass spectra were collected for multiple replicates of a protein standard mixture (56 replicates) and pooled serum samples (88 replicates). Following signal processing, peak detection and alignment, pair-wise correlation statistics were analyzed between detected peak signals. The correlation histogram indicated the presence of high between-peak correlations of non-random origin. Even after using total ion normalization, strong (> 0.8) long-range correlations in m/z were observed between ionization states of the same molecule. Strong correlations between nearby peaks in m/z for the protein standard mixture were ascribed to adducts and neutral losses. Strong correlations in pooled serum samples were observed between modified forms of known apolipoproteins. Different normalization methods for correlation histograms were explored to set the thresholds for automated detection of ionization adducts and protein modifications and results will be reported.

Mon Poster 07: Label-Free, Normalized Quantification of Complex Mass Spectrometry Data for Proteomics Analysis

Jingyi Yu*; Noelle M Griffin*; Fred Long; Phil Oh; Sabrina Shore; Yan Li; Jim Koziol; Jan E Schnitzer
Sidney Kimmel Cancer Center, San Diego, CA

Comprehensive proteomic profiling of tissue, cells, organelles or even biological fluids can benefit greatly from replicate MS measurements and multiple analytical methods. However, such data include inherent biases and variations of all mass spectrometric measurements and thus present computational and statistical challenges, especially for quantitative comparative analysis of samples. Quantifying protein expression differences remains a key challenge still requiring systematic assessment and validation. Current label-free abundance features such as spectral counting and chromatographic peak area measurements are widely used but both have their limitations including lack of precision at low peptide number and computationally demanding algorithms for peak area calculations. To circumvent these shortcomings, we develop and extensively validate a novel normalized label-free quantitative method termed the normalized spectral index, SIN, which combines three prominent mass spectrum abundance features: peptide and spectral count with fragment ion (ms/ms) intensity. We clearly demonstrate the benefit to be gained from combining these features over using them in isolation. Both relative abundances and estimated actual protein levels can be calculated. With proper normalisation, SIN largely eliminated variances between replicate MS measurements, regardless of protein load, thereby permitting: i) quantitative reproducibility, ii) unsupervised clustering of distinct samples and iii) highly significant quantification of thousands of proteins detected in replicate MS measurements of the same and distinct samples. This method was further validated with both comparative immunoblotting and densitometry. Quantitative and comprehensive analysis is necessary to compare different samples and to accurately identify the differences. Thus, this normalized spectral index method may advance further the utility of shotgun proteomic analysis in systems biology and biomarker/target discovery by allowing powerful quantitative analysis and comparison between replicate datasets and even with pre-existing data.

Mon Poster 08: ReSASC: A Simple Resampling-Based Algorithm to Determine Differential Protein Expression from Spectral Count Data

Kristina M. Little[2]; Klaus Ley[1]
[1]La Jolla Institute for Allergy and Immunology, La Jolla, CA; [2]University of Virginia, Charlottesville, VA

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the leading method used to determine the protein composition of complex biological samples. While stable-isotope labeling (ICAT, ITRAQ, and SILAC) methods are widely used to measure relative protein abundance, there are several difficulties associated with their use, including increased costs and time required for sample processing. They have also been shown to reduce the sensitivity of peptide detections. Accordingly, spectral

counting has become a ubiquitous alternative metric for protein abundance quantification. While spectral counts are theoretically simple and have been shown to increase linearly with protein abundance levels, a statistical framework has been lacking. Spectral counts are not normally distributed—discounting parametric tests—and non-parametric tests (eg, Wilcoxon rank-sum test) generally do not have sufficient power when accounting for multiple comparisons. Another caveat to consider when using spectral count data is the difference between detection and identification—that is, differentiating between proteins that are present in the sample but not detected by MS and proteins that are not present, both of which will have spectral counts of zero. To address the difficulties associated with analysis of spectral count data, we have developed ReSASC (Resampling-based Significance Analysis for Spectral Counts), which is conceptually similar to local-pooled error for gene chips. ReSASC pools similarly-expressed proteins and samples from this pool to create a hypothetical set of spectral counts for each protein. A second p-value, p', is obtained from this analysis and corresponds to the ratio of hypothetical counts with non-significant Wilcoxon p-values (eg, p' = (number of hypothetical SC sets with p>0.05)/(total number of hypothetical sets taken). We have applied this procedure to two data sets: seven replicate MS runs of human plasma that were randomly separated into two groups, and a group of duplicate MS samples from 13 control patients and 7 patients with abdominal aortic aneurysm (AAA). Using ReSASC, no proteins were found to be differentially expressed among the seven replicates, while 66 of the 1102 identified proteins were found to be differentially expressed in AAA patients. This method outperforms standard nonparametric statistical methods and is simpler to understand and operate compared to other recently developed methods for spectral count data.

Mon Poster 09: Novel Software for Iterative Development of SRM-Based Targeted Assays

Amol Prakash[1]; Scott Peterman[1]; Taha Rezai[1]; David Sarracino[1]; Bryan Krastins[1]; Michael Athanas[2]; Mary F. Lopez[1]
[1]Thermo Fisher Scientific, Cambridge, MA; [2]Vast Scientific, Wayland, MA

Selective reaction monitoring (SRM)-based assays are rapidly becoming a preferred technology for many applications including diagnostics, screening and therapeutic drug monitoring. SRM assays can deliver high sensitivity, selectivity and throughput, and when taken together, these parameters provide a breakthrough quantification methodology. However, developing efficient assays is not trivial since multiple refinement steps, each of which is quite complex and involved, are required. In this report, we present novel software that is designed to facilitate multiple iterative development steps. Once a protein is chosen for assay development, the SRM workflow is initiated by the selection of peptides and transitions (fragment ions) that are designated as suitable targets for quantification. The described software algorithm enables intelligent application of empirical and heuristic-based approaches to define the first set of transitions. Further algorithmic steps refine and add to the list of transitions by utilizing acquired data, assessing quality and then applying the information to build the next iterative transition set. In addition, the described software provides novel algorithms and validation strategies for the analysis of complex biological samples such as plasma or serum. Each iterative set of transitions may include some or all transitions and peptides from the previous set and some additional. All the iterative data are tracked by the software thus automating the entire process. When developing multiplexed SRM assays for multiple proteins, the software employs intelligent computational resource management to maximize hardware utilization and deliver optimal results. In this report, we present an overview of the entire workflow including assay building, data analysis and calibration, as well as in-depth details on each stage of the process.

Mon Poster 10: Critical Assessment of Experimental Data Compilation in the NIST Reference Peptide Library

Qian Dong; Jeri Roth ; Paul Rudnick ; Niksa Blonder; Xinjian Yan; Stephen Stein
National Institute of Standards and Technology, Gaithersburg, MD

The NIST Reference Peptide Library was built with the primary aim of improving the reliability and consistency of protein identification in biological samples. Based on experience from producing the NIST/EPA/NIH Mass Spectral Library, widely used for EI chemical analysis for over 25 years, it was envisioned that this same approach could be applied to help biologists and other researchers in proteomics. Enhanced by empirically determined peak information and full annotation, spectral matching enables significantly faster and more reliable peptide identification compared to sequence matching, as is done in current practice.

Since its initiation in 2004, the NIST Peptide Mass Spectral Reference Library has been under rapid development, facilitated by a software infrastructure consisting of various computer evaluation algorithms and a centralized information management system. The latest release of the publicly accessible library, Release 2, has shown substantial improvement in both quality and coverage. It currently contains fully evaluated reference spectra for human, mouse, rat, yeast, and E. coli, totaling 475,000 spectra from 271,000 unique peptide sequences obtained from over 500 experiments.

However, building more complete libraries remains a major challenge due to the need to collect and evaluate gigabytes to terabytes of complex and high-dimensional proteomics data generated from an enormous variety of platforms, techniques, methodologies, and sources. As a result, the current proteome coverage in the libraries is hard to assess, estimated 22% for yeast and 14% for human, with respect to the limitation of the mass spectrometers and a given sample of interest. Thus, a targeted strategy is needed in order to more effectively collect data and increase proteome coverage. A statistical analysis of the current experiments compiled in the library is required to understand and quantify the overall status of the collection. One objective of this analysis is to give new insight into how data have been collected based on biological information such as species of origin, tissue, sub-cellular location (if applicable) and disease state. The analysis will also explore the experimental conditions, such as chromatography, instrument type and labeling, of the collected data. Another objective is to track the number of uniquely identified peptides contributed by each experiment. In addition, it would be beneficial to work closely with those conducting proteomics experiments to identify which particular types of proteomics experiments might be most useful. By combining the above efforts, guidelines can be developed to help researchers at NIST improve their collection strategy by selecting targeted data sets to be included in the new library releases.

The result of the analysis will be reported in detail with hopes to provide a summary and critical assessment of the NIST data collection currently available

Mon Poster 11: Identification of Serum Peptides Common to and Unique to Ovarian and Breast Cancer

Andrea Sacconi[1]; Gordon Whiteley[2]; Simona Colantonio[2]
[1]Regina Elena Cancer Inst/SAIC-Frederick, Inc.,NCI, Rome, Italy; [2]SAIC-Frederick, Inc., NCI-Frederick,, Frederick, MD

As epidemiologic studies clearly showed, family history plays a crucial role in both ovarian and breast cancer studies and genes such as BRCA I and BRCA II have been associated to an increased susceptibility of both diseases. We expected to find a similar correlation between the two diseases also in proteomic profiling studies.

We analyzed serum samples from two different data sets: 200 serum samples from University of Padova, Italy for the breast cancer set (normal vs breast cancer) and 171 sera collected at the Northwestern University, Illinois for the ovarian cancer set (high risk vs ovarian cancer).

Data were obtained from high resolution mass spectrometry analysis (PrOTOF) after reverse phase fractionation on C18

magnetic beads as previously described. Each serum sample was run in duplicate with the entire serum sample preparation procedure performed by a Hamilton Star Robot in order to increase reproducibility.
The bioinformatic analysis procedure was entirely built using Matlab. Replicates with a low Pearson coefficient were removed. The spectra were binned and normalized using Total Ion Current as previously described. Different statistical tests were applied to determine the peaks with the smallest p-values between groups and the features were extracted to build three different and independent classifiers: K-nearest neighbor support vector machine and decisional tree. The classification was performed with a 10-fold cross validation.
The classification results of the ovarian cancer data set obtained using the first 74 peaks with the smallest p-values gave a sensitivity of 100% and a specificity of 94%; the sensitivity and specificity values were slightly reduced using a pattern of only 12 down-regulated peaks common to the breast cancer set and were 95% and 88%. When we used the same 12 down-regulated peaks to classify the breast cancer set we obtained similar results: sensitivity 97%, specificity 81% respectively. Impressive results for the breast cancer were obtain using a larger proteomic pattern: either top 81 peaks with smallest p-value or even 16 peaks (common 12 down-regulated peaks and 6 up-regulated peaks) classification procedures gave sensitivity of 100% and specificity of 100%. The peak with the absolute smallest p-value in the breast cancer set was up-regulated in cancer and was able to give a sensitivity of 93% and specificity of 100% in the breast cancer samples set. However, this peak did not show discrimination in the ovarian cancer set indicating its specificity for breast cancer.
The presence of 12 common down-regulated peaks leads to the conclusions that there are common pathways for the two diseases, while the presence of specific peptides in the two different diseases shows the possibility to discriminate the two diseases using a proteomics approach. The identity of the peaks should help clarify the mechanisms involved in the biology of the two diseases.

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Cellular Networks, 12 - 15

Mon Poster 12: Global Quantitative Analysis of the IR Response of p53 Post-Translational Modification Mutant Thymocytes

Lisa M. Miller Jenkins[1]; Sharlyn J. Mazur[1]; Yang Xu[2]; Ettore Appella[1]
[1]National Cancer Institute, NIH, Bethesda, MD; [2]University of California-San Diego, La Jolla, CA

p53 is a sequence-specific transcription factor with crucial roles in apoptosis, cell cycle arrest, and DNA repair. It is maintained at low levels in unstressed cells, but stabilized and activated through extensive post-translational modification (PTM) upon DNA damage. To elucidate functions of specific PTMs, knock-in mice were generated containing mutations of Ser18 (Ser15 in humans) to alanine in both alleles of endogenous p53, preventing phosphorylation in the N-terminal transactivation domain. Global proteomics analyses were performed to determine the change in the response of thymocytes to ionizing radiation (IR) in mutant as compared with wild type (wt) mice. This quantitative analysis identified both lower-abundance and higher-abundance proteins, as well as proteins involved in a range of molecular functions and biological processes. While many proteins were found to be significantly affected by IR in the wt thymocytes, there was a decrease in the number of affected proteins in the p53(S18A) thymocytes. Additionally, pro-apoptotic proteins that increased in the wt after IR were not significantly affected in p53(S18A) mice. Proteins affected by the S18A mutation suggest a decrease in p53 activity, as well as effects on other signaling pathways. These studies help identify important roles for p53 in DNA damage responses and how those roles are modulated by post-translational modifications.

Mon Poster 13: Examining the Molecular Network behind Enterocyte Differentiation In Mouse Models of Colon Cancer

Vishal Patel[1]; Mark R. Chance[1]
[1]Case Center for Proteomics and Bioinformatics, Cleveland, OH; [2]Case Western Reserve University, Cleveland, OH

Colorectal cancer is thought to arise from an accumulation of mutations in the colonic crypts, the proliferative source of cells in the intestine. The young enterocytes in the crypt continue dividing as they migrate upwards towards the epithelial surface. This migratory process results in a compartmentalization of the epithelium that is even more pronounced in the small intestine, which has finger-like outpouchings of the epithelium called villi. As the villi perform absorptive and metabolic functions in the small intestine, the cells migrating up from the crypts undergo a reprogramming, thereby morphing into villus cells. Mouse models of colon cancer develop tumors in the small intestine, and understanding how the crypt-villus axis is dysregulated could yield insight into the development of colorectal cancer.

2D DIGE and microarray experiments have been conducted on the crypt and villus cells from mice carrying mutations in APC and p21, as these mice develop different rates of small intestinal cancer and serve as models for a more and less severe disease process, respectively. To understand how the crypt cell transitions to a villus cell, the microarray data have been mapped onto a protein-protein interaction (PPI) network developed from publicly available databases. Using theories of avalanche cascades, the flow of mRNA has been mapped from sources in the crypt cells to sinks in the villus cells, resulting in a diffusion matrix. The diffusion matrix is then mined for high traffic pathways to find the "transition subnetwork": a subset of proteins that mediates the cellular reprogramming in the crypt-to-villus transition. The functional role of the transition subnetwork will be examined in Caco-2 cells, a unique colorectal cell line that has the ability to differentiate in vitro into a mature villus-like cell. A combination of siRNA knockdown and stable transfections will be used to decrease the activity of the subnetwork, in an attempt to prevent this terminal differentiation of the cell line.

Mon Poster 14: Quantitative Mass Spectrometry Reveals Targets of the Cytotoxic Response to DNA Damage

Aaron Aslanian[1]; John R. Yates[2]; Tony Hunter[1]
[1]Salk Institute for Biological Studies, La Jolla, CA; [2]The Scripps Research Institute, La Jolla, CA

The DNA damage response performs a critical function as a barrier to the development of cancer. Moreover, the majority of current chemotherapeutic treatments produce cytotoxicity through the induction of DNA damage. Although the core of the DNA damage response is known, the full scope of the molecular events controlled by the DNA damage response remains unclear. A systems biology approach is required to elucidate additional proteins that function as part of the DNA damage response. The information to be gained through the identification of novel components of the DNA damage response will be invaluable to the development of improved cancer prevention and treatment.

Alkylating agents are an important class of chemotherapeutics that modify DNA bases resulting in base mispairing and a DNA replication block. They have been used to treat lymphomas, chronic leukemia, multiple myeloma and solid tumors. The alkylating agent methyl methanesulfonate (MMS) was used to induce DNA damage in this study. Two different doses were examined: a weak dose that elicits a DNA damage response but no cytotoxicity and a strong dose that elicits both DNA damage and cytotoxicity. Two time points were examined: an acute time point immediately following treatment and a cytotoxic time point several hours after treatment.

A proteomics approach was used to identify proteins whose abundance is altered in response to DNA damage. Quantitative mass spectrometry was performed using stable isotope labeling with amino acids in cell culture (SILAC) in combination with multi-dimensional protein identification technology (MudPIT). Protein-DNA interactions are important for both the recognition and repair of DNA damage. Consequently, cellular fractionation of samples

prior to analysis will provide additional key information about changes in protein localization in response to DNA damage. Therefore, a chromatin-enriched nuclear component and a cytoplasmic component were prepared. Biological duplicates of all samples were analyzed on an LTQ-Orbitrap. Spectra identification was performed using Sequest, data filtration was performed using DTASelect and protein quantification was performed using Census.

Quantification of the relative levels of over 1500 proteins was achieved for each condition examined. The abundance of several hundred proteins was altered greater than two-fold in response to MMS treatment. Comparison of the chromatin-enriched nuclear component and the cytoplasmic component of cells suggest that some changes were due to altered cellular localization. Intriguingly, many of the proteins identified as being up- or down-regulated were components of protein complexes, with several members showing parallel alterations in protein abundance. This indicates a coordinated response to DNA damage that can be detected using quantitative mass spectrometry.

Mon Poster 15: Dynamics of the MAPK Insulin Signaling Pathway Using a Label-Free Platform Based on MS/MS Average TIC

John M Asara[1]; Adam Friedman[2]; Pengyu Hong[3]; Jason Locasale[2]; Norbert Perrimon[2]
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Large-scale proteomics experiments similar to those first introduced by Gavin et. al. are needed in order to interrogate protein-protein interactions in cellular signaling pathways. We approached this question by performing TAP-MS experiments on 15 key nodal bait proteins in the MAPK pathway in drosophila SR+ cells with and without insulin and EGF (Spitz) stimulation. Data dependent LC/MS/MS experiments were run using a Proxeon EasynLC coupled to a Thermo LTQ-Orbitrap XL for at least two replicates of each bait condition for a total of 94 LC/MS/MS experiments including TAP vector controls. The data was searched against the reversed Flybase protein database due to its completeness using Sequest within Thermo's Proteomics Browser Software suite. In order to quantify changes in protein levels between bait conditions, we developed a software suite called NakedQuant v1.0 based on the "Spectral TIC" approach that averages the TIC values from each identified MS/MS spectrum (spectral count) per protein identified. The method was previously compared to SILAC and spectral counting in a proteomics screen (Asara et. al., Proteomics, 2008). The software utilizes several features including protein grouping across experiments, normalization as well as fold change and ratio calculations based on spectral counts, TIC sum and TIC average. From the output, different networks were assembled based on the dynamic average TIC signal change using several different criteria including simple fold changes between basal and stimulated conditions using common identified proteins and pairwise changes in signal between basal and stimulated conditions where at least two or more proteins need to change together. The network revealed many canonical interactions and several novel interactions in the MAPK signaling pathway. Several of these novel interactions have been verified biochemically and correlate with a previously published RNAi screen using pERK as the readout under basal and insulin treatment (Friedman and Perrimon, Nature, 2007). These data show that novel interactions in signaling pathways through protein-protein interaction studies can be effective using label-free mass spectrometry approaches.

Chemical Proteomics, 16 - 17

Mon Poster 16: A Quantitative Mass Spectrometry Platform for Activity-Based Protein Profiling

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Activity-based protein profiling (ABPP) utilized active-site directed chemical probes to monitor the functional state of enzymes directly in native biological systems. Identification of the specific sites of probe labeling on enzymes remains a major challenge in ABPP experiments. We describe an advanced ABPP platform that utilizes a tandem orthogonal proteolysis strategy (TOP) coupled with mass spectrometric analysis to simultaneously identify probe-labeled proteins together with their exact sites of probe modification. This strategy, termed TOP-ABPP, relies on a TEV-protease cleavable linker that selectively releases probe-modified peptides for mass spectrometry analysis. Identification of sites of probe-labeling provides fundamental insights into the molecular basis of specific probe-protein interactions. In order to quantitatively interrogate protein activities from multiple different proteomes, we have developed a variant of the TOP-ABPP method that incorporates isotopic labels into the clickable TEV-biotin tag for TOP-ABPP to yield isotopically-tagged probe-labeled peptides for MS analysis. Importantly, this approach will be universally compatible with any alkyne-containing activity-based probe. Two biological samples will be treated with either 'light' or 'heavy' variants of the TEV-biotin tag and mixed together for MS analysis. Fragmentation of the isotopically-labeled peptides will provide peptide IDs using the SEQUEST algorithm and quantitation will be achieved through integrating the mass-ion intensity for the 'light' and 'heavy' variants of the parent ion in MS1 mode. The quantitative analysis of activity-changes in a variety of proteomes will yield valuable insight into enzyme activities and signaling networks implicated in disease.

Mon Poster 17: Large-Scale Profiling of Protein Acylation using Click Chemistry

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S-palmitoylation is a reversible covalent post-translational attachment of fatty acids on cysteines that mediates the association of diverse proteins with membranes via a labile acyl-thioester linkage. Many proteins have been identified as targets of palmitoylation, most notably G proteins, which are often palmitoylated proximal to prenylation or myristoylation sites. Other diverse classes of palmitoylated proteins include ion channels, receptors, cytoskeletal proteins, and kinases, although the extent and dynamics of protein palmitoylation are poorly understood. Traditionally, proteins are identified as targets of palmitoylation by metabolic labeling with 3H-palmitate. A recent method has been developed to replace acyl-thioesters with affinity tags for enrichment and proteomic identification in yeast, yet this relies on the fidelity of the exchange reaction and is hampered by numerous false positives. We demonstrate that the commercially available compound 17-octadecynoic acid (17-ODYA) can serve as a bioorthogonal, click chemistry probe for in situ labeling, identification, and verification of palmitoylated proteins in human cells. After enrichment and trypsin digestion, peptides are analyzed using an LTQ ion-trap mass spectrometer using multidimensional protein identification technology (MudPIT). We identified over 500 predicted palmitoylated proteins from human Jurkat T-cells, including G proteins, receptors, and a family of uncharacterized hydrolases whose plasma membrane localization depends on palmitoylation. We also provide a technically straightforward gel-based method to rapidly validate palmitoylated proteins identified by large-scale proteomic screens.

Databases and Systems Integration, 18 - 20

Mon Poster 18: Evaluation of Efficiency of Cross-Species Comparisons using ProteinCenter

Martin Damsbo; Morten Bern; Hans Jespersen
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With the advent of genome-wide sequencing projects, large scale proteomics studies became visible for more varieties of different species. As a result, modern proteomics has to face the challenge of cross-species data comparison. This is a time-consuming, and sometimes almost impossible, task. Currently the field of proteomics lacks sophisticated software tools to perform comparisons of large-scale proteomics data derived from different species. Here we present a new development within the software

tool, ProteinCenter, that enables complex comparisons of proteomics datasets at several levels. For example, a comparison can be made at the homology level as defined by the level of sequence identity, by shared peptides and peptide-based extraction of unique entries and as a peptide-sharing algorithm, with the added requirement that group members must also share the same Sim60 group (60% sequence homology).
Using HUPO Brain Proteome projects deposited in PRIDE database, we investigated the efficiency of the cross-species comparison based on the homology, peptide evidence and peptide-sharing algorithm based on Sim60 grouping. The results of human and mouse cross-species comparison as well the efficient data mining and categorization of large data sets of proteins sets will be presented.

Mon Poster 19: A New Approach to Achieve a Fast In-Depth Biological Overview of Proteomics Data

John Lindsay; Peter Venø; Morten Bern
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Modern proteomics must face the challenge of performing bioinformatics analysis and comparison of large datasets. It is a time consuming and sometimes almost impossible task to distinguish known proteins from novel 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.
To resolve these issues we have developed ProteinCenter - a tool that enables efficient data mining and categorising of large data sets. The ProteinCenter database is build using the Sun Java technology and the Microsoft mySQL database technology for optimal performance. ProteinCenter contais public sequence databases to form a comprehensive and consistent superset of 10 million protein sequences derived from over 40 million protein records from GenBank, Refseq, EMBL, UniProt, Swiss-Prot, Trembl, PIR, IPI, PDB, Ensembl etc., including more than 5 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 is updated regularly depending on its source, enabling tracking of outdated accession keys. All the information in ProteinCenter can be accessed by a web user interface which allows for import and comparison of database dependent search results. Also complex quires and statistical calculation based on the information kept in the database can be performed on imported search results. We show here that protein sequences change accession key so often that a 2½ year old database dependent search result from Mascot (searched against the IPI database) of a mouse brain plasma membrane proteome data set (1) contains 37 % outdated (861 of all 2356 proteins) accession keys. It is essential to keep track of outdated accession key in order to compare data sets across time.

(1) Nielsen, PA., et al., Proteomic mapping of brain plasma membrane proteins. Mol Cell Proteomics, 2005. 4(4): p. 402-8.

Mon Poster 20: An Ultra-Structure System for Heterogeneous Data Management in Proteomic and Systems Biology Research

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Background: High-throughput proteomic experiments generate copious, richly-interconnected data sets that present substantial management and integration challenges. A further challenge comes from the dynamic nature of proteomic research; new data sources regularly become available, new experiments generate new types of data, and techniques are constantly being modified and refined. Maintaining information systems in the face of such complexity and rapid change is daunting, particularly for the individual researcher.

The Ultra-Structure design methodology, proposed by Long in 1995, presents a solution to this problem by reformulating how databases are applied. Designed to deal with complexity and change in heterogeneous information systems, it represents both data and processes as formal rules within a database. In doing so, an Ultra-Structure system enables users to explicitly store domain knowledge and data in both a machine- and human-readable form. End users themselves can then change the system's capabilities by altering database contents; no computer code or schemas need be touched. The methodology has been successfully applied in areas from business management to automated document declassification for the US government. Its application to proteomic and systems biology research promises to substantially advance the ability to successfully organize and analyze the large data sets being produced.

Results: We are in the midst of a proteogenomic mapping project for translational annotation of the human genome, and we have all of the aforementioned challenges to deal with. These include handling large, complex, heterogeneous data sets, managing rapidly changing methods and protocols, and having a readily reconfigurable analysis pipeline for the integration and analysis of these data. While the system originally used a traditional entity-relationship database design for all data management, it rapidly became difficult to manage as the number of tables grew for representing distinct data types. We transitioned the system the Ultra-Structure methodology, finding a substantial simplification of the resulting schema. The Ultra-Structure system accommodates tandem mass spectra, spectrum/peptide mappings generated by search engines, multiple genome drafts, multiple gene annotation sets, ontology, and chemical structures, all within a small and general framework that is not specifically tied to proteomic research. It represents core concepts common to many areas of biological research, such as networks of biological/data components, ontology, locations, procedures, and provenance, and can be readily used for other areas. General software procedures driven by user-modifiable rules can perform tasks such as logical deduction and location-based computations.

Conclusion: The Ultra-Structure approach has simplified and unified our proteogenomic data management into a powerful and flexible framework. With Ultra-Structure, diverse information sources can be integrated into a common system, allowing for the possibility of systems-scale analyses, while enabling end-users to easily and directly modify the information system.

Mass Spectrometry Advancement, 21 - 25

Mon Poster 21: Simple and Fast 2D LC-MS Combined with Confident Data Interpretation

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Analysis of complex biological samples often requires several separation steps. One of the commonly used technologies in the field of proteomics is automated two-dimensional Liquid Chromatography coupled with Mass Spectrometry. (2D LCMS) based on on-line strong cation exchange (SCX) / reverse phase (RP) separations, often called MuDPIT for Multi-Dimensional Protein Identification Technique.

Here we describe a simple, automated SCX/ RP on-line 2D separation strategy achieved on a split-free 1D nano LCMS system followed by confident data interpretation for the evaluation of the difference between the 1D and 2D separation approache.

To evaluate the system performance we analyzed the proteome of mouse placental cells. The cytosolic fraction was subjected to cysteine reduction and alkylation with iodoacetamide followed by trypsin digestion. The lysate was purified and analyzed by 1D and 2D methods on a split-free nano LCMS system (EASY-nLC from Proxeon Biosystems, Odense, Denmark) coupled to an LTQ-Orbitrap (Thermo-Fisher, Bremen, Germany). To perform the 2D separation a salt gradient was injected in 10 steps, varying from 0.05 to 0.5 M NH4Acetate.

By using the auto-sampler component and standard sample injection programs we were able to accomplish the salt delivery using a split-free nano LCMS system and therefore transforming the system into 2D system.

As a result a 1D analysis yielded about 500 protein identifications whereas the 2D analysis identified approximately 1500 proteins. Using ProteinCenter (Proxeon A/S) we were able to demonstrate that the 2D separation approach enabled the identification of more low abundant proteins. These results clearly demonstrate the significant improvement which can be achieved by using a more comprehensive protocol to achieve the 2D behavior.

Since no hardware changes are necessary, one can obtain much more information out of a single sample as easily as a traditional 1D run and making use of the same 1D nano LCMS setup.

Mon Poster 22: Intelligent Scheduling to Facilitate Building High-Throughput, Targeted SRM Assays for Protein Quantification

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Targeted SRM assays for protein quantification are robust and selective, even in complex matrices. These assays are increasingly applied in the areas of disease prognostics and therapeutic drug monitoring. In order to develop effective assays for diseases or pathologies with multiple intersecting metabolic and/or signaling pathways, multiplexed assays that can monitor panels of peptides will have to be developed. . Unfortunately, designing effective SRM assays still remains a challenge, because optimization of sensitivity, selectivity and throughput by algorithmic and prediction methods alone may lead to improving the performance of one parameter at the expense of others.

We present novel ideas to resolve this problem. By exploiting the physical properties of the peptides/proteins to be monitored, we separate them in a hydrophobicity gradient. This separation affords greater efficiency since each transition is not monitored across the entire LC separation, but only at the moment that elution is predicted. Thus, the number of analytes measured in a single run can be increased by a factor of 10, leading to a significant increase in throughput. For optimum results, sophisticated data processing is required to ensure the highest sensitivity and selectivity. In order to achieve this, the described strategy employs previously generated, discovery-based data to facilitate and enhance method-building and data-verification. The approach capitalizes on the typical proteomics workflow where SRM experiments are typically preceded by discovery-based experiments in order to develop a list of target proteins and peptides. These discovery data provide empirical information on the elution order, fragmentation pattern and charge state of individual target peptides. The information is invaluable for the optimization of throughput, sensitivity and selectivity.

Methods: Trypsin digested maternal serum samples from Trisomy 21 and normal first trimester pregnancies were analyzed on an Orbitrap instrument with CID and HCD as the fragmentation modes. Label-free differential analysis was performed using SIEVE, and the top 50 proteins with the optimum p-values, expression ratios and other filtering parameters were selected. Using CID and HCD MS2 spectra generated on the Orbitrap, time-scheduled SRM assays were created using novel SRM Workflow algorithms. The resulting SRM assays were run on a TSQ Vantage using the same serum samples. Spectra were processed with SRM Workflow algorithms, and LOQ, LOD and elution times were calculated.

Preliminary Data: In this study, we investigated the quantitative protein and peptide differences in maternal serum from normal and Trisomy 21 pregnacies. Using the knowledge generated by the discovery experiments, we are able to make time-scheduled SRM assays for monitoring a panel of peptides in a 30 minute LC gradient with high sensitivity and selectivity. This result presents a quantum leap over previous research limits.

Mon Poster 23: Analysis of SILAC Data by Spectral Counting for High-Throughput Quantitative Proteomic Analysis of Primary Cell Cultures

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One of the major goals of mass-spectrometry based proteomics is the relative quantification of a large number of differentially expressed proteins between two or more biological samples. To this end, Stable Isotope Labeling of Amino Acids in Cell Culture (SILAC) has become a widely used technique in quantitative proteomics experiments, even though well-known limitations in its analytical power exist (i.e., throughput, signal-to-noise requirements). Spectral counting is a high throughput label-free quantification technique that bypasses many of the limitations of SILAC data analysis, however since spectral counting requires that samples be analyzed in independent mass spectrometry runs it is subject to higher technical variability. We report a hybrid approach, SILAC Peptide Count Ratio Analysis (SPeCtRA), which involves analyzing SILAC data sets by separately identifying and then counting the heavy and light peptide spectra from proteins detected within the same LC MS/MS run. We show that SPeCtRA is an accurate and sensitive technique for high-throughput quantitative proteomic analysis. We used SPeCtRA to compare vascular endothelial cell protein abundance between high (20 mM) and low (11 mM) glucose culture conditions in a line of primary cells isolated from rat skeletal muscle. Among the 154 proteins that were quantified in our experiment, we detected a network of differentially abundant proteins that are all linked to a transcription factor, NRF2, which is activated by cellular oxidative stress. Our results demonstrate that SPeCtRA is high throughput quantification technique capable of detecting biologically relevant differences in protein abundance.

Mon Poster 24: Elimination of Matrix Effects in Quantitative Peptide LC-SRM/MS Assays using High Throughput Electrophoretic Sample Preparation

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The sensitive and reproducible quantification of proteins at or below the nanogram per milliliter concentration range is necessary in the development of new diagnostic markers and biotherapeutic treatments. The use of liquid chromatography coupled with single or multiple reaction monitoring tandem mass spectrometry provides the selectivity, sensitivity, and throughput required. However, biological samples such as plasma and urine contain salts, lipids and various other components that often limit run-to-run reproducibility and sensitivity and generally contaminate the column, autosampler, and source. Traditional techniques such as solvent precipitation and solid phase extraction assist in removing these matrix effects, but generally suffer from poor selectivity and limit the reproducibility and sensitivity of the assay.

Here we report the use of a 96-well format electrophoretic system for the removal of salts, lipids and other interfering compounds and concomitant enrichment of peptides for reproducible, quantitative measurement from human urine. Synthetic exogenous peptides (WWGGQPLWITATK, VWNYFQR, LSELIQPLPLER and YIFSDDSSQLTIK) were spiked into human urine at varying concentrations. Performance characteristics of offline electrophoretic sample preparation using 30 uL of spiked urine were compared to offline SPE to determine the applicability of the technique for quantitative bioanalysis. Following preparation, samples were injected into a reverse phase LC/MS/MS using a Thermo TSQ tandem mass spectrometer. Both the technique of electrophoretic concentration and SPE provide ng/mL sensitivity for the model peptides examined in this study. However, after only 8 consecutive injections of the SPE processed samples, significant retention time drift (~30s) was observed requiring extensive washing and column conditioning. Electrophoretic sample preparation allowed 16 consecutive injections with no observable

retention time drift. Further benefits of electrophoretic sample preparation include longer column lifetime, shorter gradients, reduced matrix effects, reduced instrument downtime. Examples of this workflow will be presented along with recommended procedures and a discussion of the analytical performance achieved.

Mon Poster 25: Gas-Phase Fragmentation of Singly-Protonated Peptides in CID and MALDI Conditions. Revisiting the Aspartic Acid Effect

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Peptides were synthesized to systematically study effects on fragmentation of amino acid composition, peptide length, relative positions of arginine (R) and aspartic acid (D), replacement of these amino acids for others, and other factors. MS/MS spectra recorded in CID and MALDI conditions with different instruments and different excitation conditions for most peptides show similar fragmentation pathways. However, certain spectral differences with practical consequences will be discussed. Protonated peptides containing R and D and no “mobile protons” cleave preferentially at the C-terminus of D. Dissociation of peptides of different size and numbers of residues between D and R do not show distance dependence, confirming the ‘charge-remote’ nature of this fragmentation. Two additional fragmentations are found to have significant contributions: (a) scission of the peptide bond of R to form y1 or b1 ion, almost independent of peptide size or structure, and (b) neutral loss of ammonia, more pronounced with N-terminal R but significant also with C-terminal R (much less with non-terminal R). Relative rates of these fragmentation channels were estimated from their energy-dependence. It is noted that, as expected, peptide ions with N-terminal R and D at various positions show intense b-ion peaks from fragmentation at the C-terminus of D, whereas tryptic peptides, i.e. with C-terminal R, fragment not only to the expected y-ions but also generate significant amounts of the corresponding b-ions. These ions arise from a different mechanism involving a mobile proton and ‘unpeeling’ of the ion from the carboxyl end, stopping upon reaching the aspartic acid.

Omics of Mitochondria / Organelle Proteomics, 26 - 28

Mon Poster 26: Quantitative Proteomic Analysis of Ovarian Cancer Cells Identified Mitochondrial Proteins Associated with Paclitaxel Treatment

Yuan Tian ; Aik-Choon Tan ; Xiaer Sun ; Matthew T. Olson; Natini Jinawath ; Daniel W. Chan; Ie-Ming Shih; Zhen Zhang; Hui Zhang
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Paclitaxel has been widely used as an antimitotic agent in the treatment of human cancer and adds substantial efficacy to the first-line chemotherapeutic regimen for most ovarian cancers. However, the frequent occurrence of paclitaxel resistance limits its function for the ovarian cancer treatment. The molecular mechanisms controlling the paclitaxel resistance are poorly understood. Using genomic approach, we have previously identified NAC1 gene associated with paclitaxel resistance in ovarian cancer. In the attempt to better understand the cellular responses to paclitaxel treatment and resistance, we used quantitative proteomics to identify protein changes associated with paclitaxel treatment and NAC1 function. The SKOV-3 ovarian cancer cells with inducible expression of dominant negative NAC1 were applied to inactivate the endogenous NAC1.

SKOV-3 cells with and without NAC1 were treated with paclitaxel and quantitative proteomic analyses was performed using iTRAQ labeling and mass spectrometry. To verify the quantitative changes identified using iTRAQ and 2D-LC-MALDI-TOF/TOF system, the proteins from ovarian cancer cells were further analyzed by additional two label-free quantitative proteomic methods using peak intensity from Q-TOF system and spectral count using LTQ. We have quantified 1372 proteins, and among these, 32 proteins showing changes by iTRAQ method were also validated by at least one of the label-free methods, and 6 protein changes were validated by both methods. Moreover, tubulin and mitochondrial proteins were the major cellular components with changes associated with paclitaxel treatment. This suggests that mitochondria may play a role in response to paclitaxel treatment and resistance.

To characterize the altered mitochondrial metabolism associated with treatment of chemotherapeutic drugs in ovarian cancer cells, we purified the mitochondria from cells with and without drug treatments. After the purification, mitochondrial proteins were analyzed using iTRAQ and 2D-LC-MALDI-TOF/TOF. The mitochondrial proteins involved in the mitochondrial metabolism in ovarian cancer cells treated with chemotherapeutic drugs were identified by their altered abundances.

We found co-regulation of tubulin and mitochondrial proteins after paclitaxel treatment. Tubulin is a well-known target for paclitaxel function and responsible for paclitaxel induced cell death and was observed of up-regulated after paclitaxel treatment. One of the mechanisms of paclitaxel function is believed to induce cell death by altering microtubule assembly through the binding to the microtubule polymer so as to stabilize microtubules; as a result, it disrupts the normal re-assembling of microtubule network which is required by mitosis and cell proliferation. Another protein, cytochrome c, was also identified up-regulated in cancer cells treated with paclitaxel. Cytochrome c was reported previously of released from mitochondrion thus inducing cell apoptosis upon paclitaxel treatment. Several other mitochondrial proteins including mitochondrial Enoyl-CoA hydratase, mitochondrial trifunctional enzyme, mitochondrial ATP synthase, cytochrome c, and mitochondrial malate dehydrogenase were also identified with up-regulation in paclitaxel treated cells. This study represents the first proteomic study to discover the association of paclitaxel treatment and mitochondria protein changes in cancer cells, which offers a new direction for studying the mechanism of drug resistance of cancer cells. The identified proteins will be useful for elucidation of the molecular mechanism of paclitaxel treatment and paclitaxel resistance.

Mon Poster 27: Comprehensive Proteomic Analysis of Lung Endothelial Caveolae Identify Novel Functions for these Membrane Micro-Domains

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Caveolae are membrane micro-domains which are suggested to be involved in vesicular transport across the plasma membrane. We performed comprehensive comparative proteomic analysis of the luminal endothelial cell (EC) plasma membranes (P) and their caveolae (V) isolated from rat lung tissue. The aim was to identify a caveolar proteome functionally enriched in a subset of EC proteins in order to develop a better understanding of these transport vesicles. A total of 16 MS measurements using four different MS techniques (RP-MS/MS-LCQ, RP-MS/MS-LTQ, 2D-LC-MS/MS-LCQ, 2D-LC-MS/MS-LTQ) were performed separately on the P and V fractions. The resulting caveolar (V) proteome consisted of over 1000 proteins with two or more peptides, including 100 proteins whose peptides were not detected in P. As the V fraction is physically isolated intact from the P fraction, the protein composition of V is deemed a subset of the proteins present in P prior to V isolation. We used the SIN method, a normalized label-free quantitative method, recently developed in our lab to identify proteins enriched in V over P. Proteins that were identified with two or more peptides in V and one or more peptide in P were included in the analysis (>1300 proteins). We calculated a Spectral Index (SI) for each protein and a ratio based on the SI of the same protein in both fractions (V/P ratio). Proteins with V/P ratio ≥1.0 were considered enriched in caveolae (>600 proteins). A significant number of these proteins were validated by Western blot analysis and electron microscopy. We performed a comprehensive bioinformatics analysis of these caveolar proteins, including Pfam domain analysis, gene ontology and network analysis as well as broad review of the literature relating to our

caveolar proteins. Our analysis showed Z-score correlation for expected functions of our caveolar proteins including membrane trafficking, signalling, mechanotransduction, with negative z-scores for nucleotide metabolism and transcription factors. We have also identified a number of novel functions not previously ascribed to the caveolae. This is the first large-scale MS data on the proteome of caveolae as they exist *in vivo*.

Mon Poster 28: A Proteomic Study of the Molecular Architecture of a Human Dense Core Secretory Vesicles

Steven J Bark; Jill L Wegrzyn; Laurent Taupenot; Daniel O'Connor; Vivian Hook
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Neuropeptides and catecholamines are primary regulators of diverse physiological processes including pain response, depression, behavior, energy metabolism, vascular tone, and neuronal and neuroendocrine function. Because of the critical role of these secretory molecules in physiology, their production is compartmentalized into specific dense-core secretory vesicles and secretion is highly regulated. Therefore, understanding the protein components of the dense-core secretory vesicle is a necessary step in defining the important factors involved in regulation of neuropeptide and catecholamine secretion in cell-to-cell communication.

In this work, we report the systematic study of the protein components of human dense-core secretory vesicles. These vesicles were isolated from a human pheochromocytoma of the adrenal medulla. Freeze-thaw lysis and ultracentrifugation provided membrane and soluble protein component fractions, which were separated by 1-D gel electrophoresis. Gel lanes were cut into molecular-weight regions, subjected to in gel trypsin digestion and analyzed by LC-MS/MS using an Agilent XCT Ultra Ion Trap Mass Spectrometer. High-confidence identification of >1500 proteins in the membrane fraction and >1000 proteins in the soluble fraction enabled a systems-wide analysis of important proteins and their suborganellar localization. Importantly, quadruplicate technical replicates for all samples enabled statistical evaluation of quantities for >500 proteins. Functional and pathway analyses were performed using custom pipeline programs and DAVID (NIAID and NIH). A custom Excel script employing the NSAF method reported by Zybailov, et. al. was used for spectral counting and required protein identifications to be present in a minimum of 3 replicates.

This human data is highly consistent with, but much more expansive than our previous data on bovine dense core secretory vesicles. These data demonstrate a remarkable conservation of proteins across species and include SNAREs, Rabs GTPases membrane transporters and cytoskeletal proteins. From literature, these proteins are likely components of all secretory vesicles. However, several proteins appear to be dense-core secretory vesicle specific and include neurohumoral factors and proproteins, proteases with protease inhibitors specific for neuropeptide processing and neurotransmitter enzymes specific for adrenal tissues. Because of our previous knowledge of bovine dense-core secretory vesicles, we were able to evaluate current identification and quantitation limits in reference to this human data. This was effectively demonstrated by identification of several known low abundance proteins such as Cathepsins B and L, which indicate identifications in the range of ~10(e5) to ~10(e6) below the most abundant proteins present in these vesicles. In summary, this study (1) provides a qualitative and quantitative basis for future comparative analysis of dense-core secretory vesicles in particular and other secretory vesicles in general; and (2) introduces the first concrete data of human secretory vesicle protein components involved in cell-to-cell communication.

Protein Arrays and Antibodies, 29 - 33

Mon Poster 29: HaloTag Protein Arrays: An Integrated Biomolecular Interaction Analysis Platform

Marjeta Urh[1]; Nidhi Nath[1]; Robin Hurst[1]; Brad Hook[1]; Kate Zhao[1]; Dieter Klaubert[2]; Bob Bulleit[1]; Keith Wood[1]
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Protein arrays are poised to become a central proteomics technology providing means to investigate protein interactions and functions as well as protein expression level and protein modifications. We are seeing an explosive progress and interest in development of protein array technology, however lack of high-throughput method for protein expression and purification remains a significant bottleneck in advancing this technology. Here we present a new integrated approach for creating protein arrays that combines in-vitro protein expression system with covalent, site specific and oriented immobilization method which allows for rapid and specific capture of proteins onto a glass slide surface directly from a complex protein mixtures (e.g. cell lysates) without any prior purification. The method is based on HaloTag fusion protein which was engineered using different methods of mutagenesis to covalently and rapidly bind to a specific chloroalkane ligand either free in solution or attached onto a surface. In parallel we developed a specific surface based on hydrogel-coated glass slides composed of cross-linked PEG functionalized with chloroalkane. Protein fusions were created using HaloTag vectors and synthesized in the in vitro protein expression system. We show that multiple fusion proteins can be rapidly synthesized in less than 2 hours and immobilized directly form the lysate in parallel for detection of protein-protein and protein-nucleic acid interactions. In addition, capture of functional enzymes and their modulation by small molecules is reported. Arrayed enzymes are observed to be stable for up to a month when stored at -20ºC in PBS buffer containing 50% glycerol. Enzymes fused to HaloTag and expressed in vitro were stably stored and exposed to multiple freeze-thaw cycles, enabling print-on-demand arrays for longitudinal studies. In conclusion, we show that HaloTag technology in combination with cell-free expression provide a simple and rapid method for multiplexed protein interaction studies.

Mon Poster 30: Antibody-Array Interaction Mapping (AAIM): Discovery and Study of Serum Amyloid P Component Interactions with Kininogen in Human Serum

Derek Bergsma[1]; John Buchweitz[1]; Songming Chen[2]; Robert Gerszten[3]; Brian Haab[1]
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We have developed antibody-array interaction mapping (AAIM) to enable the sensitive and precise measurement of multi-protein interactions in biological samples. By probing potential interactions among a set of 48 proteins in serum, we found that the innate immune system protein serum amyloid P (SAP) interacts in normal human serum with the inflammatory protein kininogen (KNG). We therefore used AAIM in a variety of formats to gain insights into the regulation and function of this interaction. The interaction depends on the presence of calcium and zinc, and it does not inhibit the fundamental ligand recognition properties of SAP, indicating that KNG may co-localize to sites where SAP binds. Furthermore, experiments using human plasma revealed that SAP interacts with complement C1q in complexes that are distinct from the SAP-KNG complexes, suggesting separate contexts for SAP's respective roles in immune clearance and inflammation. The fact that SAP-C1q complexes appeared only in patients with moderately or highly elevated c-reactive protein (CRP) suggested that SAP is involved in immune clearance even at moderate CRP elevations. These studies demonstrate the value of a novel technology for probing protein-protein interactions and provide new information about innate immunity and inflammation which could have relevance in pathological situations involving SAP binding.

Mon Poster 31: Discovery and Characterization of Lung Cancer Specific Monoclonal Antibodies

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A novel method has been developed previously at Biosystems International for generating disease specific monoclonal antibodies (mAbs) against a mixture of immunogens. Here, this method is adapted to cancer with the immunization of glycoproteins isolated from the plasma of patients with advanced non-small cell lung cancer. The major plasma proteins were reduced using a multi-step depletion protocol, and the depleted plasma was used to immunize mice. A high throughput ELISA screening assay was developed at Biosystems International, and it was used to identify cancer specific IgG-producing hybridomas that discriminated lung cancer from matched controls. A procedure was implemented at the Barnett Institute to identify the cancer specific antigens using a workflow that combined immunoprecipitation, SDS-PAGE, Western blotting and LC-MS/MS (or MALDI-TOF) analysis. In this presentation, we report on the approach to generate cancer specific mAbs that discriminate lung cancer plasma from matched normal plasma. The identification of the antigen for 3 cancer specific mAbs harvested from ascites fluid, two to different epitopes of haptoglobin and one to complement factor H, is presented. A sandwich ELISA assay was developed for the two mAbs of haptoglobin and, the concentration of the antigen in plasma from cancer and normal individuals was measured. In addition, we will show that a mAb to haptoglobin was generated to the native form in plasma (based on surface plasmon resonance analysis) and to be affected by glycosylation.

Mon Poster 32: Enzyme-Linked Immuno PCR

Alex Belyaev; Katherine Felts; Craig Monell
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Immuno PCR (IPCR) can provide for significant increases in sensitivity compared to standard ELISAs using the same antibodies. However, traditional IPCR applications face several hurdles including cumbersome reagent manufacturing, limited compatibility with existing ELISA formats, and a complex intellectual property landscape. We describe enzyme-linked immuno PCR (EL-IPCR), a pioneering immuno PCR approach that combines the high sensitivity of immuno PCR, convenient reagent production processes, and applicability to existing ELISA kits. Similar to ELISA, an enzyme (a polymerase for example) is linked to a detection antibody. However, instead of adding chromagenic, chemiluminescent, or fluorogenic detection substrates as in traditional ELISA, a modified oligonucleotide substrate is added. The substrate contains 2' O-Methyl modified RNA (2'OMe) and deoxyuridine (dU) modified nucleotides. The linked polymerase, such as Klenow fragment of DNA polymerase I, creates a copy of the substrate using normal A, C, T, G nucleotides resulting in a DNA strand that lacks modifications. The copy, but not the modified oligonucleotide, is amplified in QPCR using a Pfu polymerase that can easily amplify the copy, but not the original modified substrate. We demonstrate that the modified oligonucleotide template can be copied in solution by an anchored Klenow probe. We also show that the Klenow probe and a modified oligonucleotide probe can be effective when acting in proximity, both in solid phase and homogenous immuno-QPCR assays.

Mon Poster 33: Quantitative Multiplex Immuno-QPCR

Katherine Felts; Bilan Hsue; Craig Monell
Agilent Technologies, La Jolla, CA

A technique for antigen detection, called immuno-PCR, was developed by Sano et al. in 1992. It combines the molecular recognition of antibodies with the exponential DNA amplification capability of PCR. The procedure is similar to conventional enzyme-linked immunosorbent assays (ELISA) but allows for greater sensitivity, smaller sample volumes, and multiplex applications. Instead of an enzyme, a DNA molecule is linked to the detection antibody which serves as a template for PCR. Real-time or quantitative PCR (QPCR) provides accurate quantitation over a large dynamic range and has exquisite sensitivity. When applied to immuno-PCR, QPCR makes it possible to quantify very small amounts of DNA-coupled detection antibody with high accuracy. Success using this method is dependent on consistent production of antibody-DNA conjugates, background reduction measures, and optimization of the DNA amplification conditions. Here we present immuno-QPCR methods for the detection of protein analytes using biotinylated antibodies, mouse antibodies, and rabbit antibodies in monoplex and multiplex applications.

Proteogenomics, 34 - 37

Mon Poster 34: Proteomic and Genomic Analysis of High-Density Lipoprotein (HDL) Particles in Obese Individuals

Lisamarie A. Collins[1]; Shama P. Mirza[1]; Tesfaye B. Mersha[1]; Lisa Martin[2]; Ahmed H. Kissebah[1]; Michael Olivier[1]
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Low HDL cholesterol levels have been shown to be correlated with increased risk for cardiovascular diseases, due to the anti-atherogenic and anti-oxidative properties of HDL particles. Obese individuals are particularly prone to cardiovascular co-morbidities, even with normal HDL cholesterol levels. This risk is primarily mediated by a shift to small, dense HDL particles (HDL3). HDL3 particles in obese individuals appear to be functionally defective as they lack their potent anti-oxidative and anti-inflammatory properties, and enhance atherogenic plaque formation.

In our Metabolic Risk Complications of Obesity Genes (MRC-OB) study, we analyzed the lipoprotein particle profile in 532 individuals from our cohort, and identified a quantitative trait locus affecting HDL median particle diameter on human chromosome 12p13 (LOD = 3.15). The interval contains 144 annotated genes, none of which have a known role in lipid or lipoprotein metabolism.

We pursued a proteomic approach to identify potential candidate genes in this genomic region that lead to an altered composition of HDL particles in obese individuals, and may mediate the observed shift in median particle diameter. We isolated HDL fractions from human serum samples of six obese and lean sibling pairs from our cohort using non-denaturing fast protein liquid chromatography to identify quantitative differences in the HDL proteome that could potentially mediate this effect. Proteins were isolated from HDL particles by chloroform extraction and quantified using isotopic labeling and tandem mass spectrometry.

We quantified a total of 57 proteins, 54% of the previously reported HDL proteome. Of these, six proteins were significantly altered in obese individuals with small median HDL diameter (APOA2, C3, C4A, GC, HP, TTR). However, none of these genes is located on human chromosome12. Therefore, we identified gene interaction networks between the altered proteins in HDL and the genes in the QTL interval using Ingenuity Pathway and Gene Ontology Enrichment analyses. We identified five genes (C1S, VWF, C1RL, CD163, PTPN6) that could be linked to the altered proteins. For our initial genetic analysis, we analyzed 35 SNPs spanning the von Willebrand factor (VWF) gene and detected significant association with HDL median particle diameter for four SNPs ($p = 0.004$-0.045). One of these SNPs, rs216321, alters the amino acid sequence (Arg852Gln) of the protein.

This study illustrates the usefulness of proteomic analyses to identify genetic predispositions to quantitative traits such as HDL particle diameter. Further studies will determine the functional mechanism by which VWF and other genes identified in our analysis influence HDL particle size in obese individuals.

Mon Poster 35: Global Proteomic Profiling Analysis of Human Fibroblast Cells in Proteus Syndrome

Tesfaye Mersha Baye; Shama Mirza; Michael Olivier
Medical College of Wisconsin, Milwaukee, WI

Proteus syndrome is a rare but severe type of segmental overgrowth in a variety of tissues. The cause for the abnormal growth is unknown and no treatment options are currently available

for afflicted individuals. We isolated two fibroblast cell lines from surgically removed overgrowths from a female patient with Proteus Syndrome, and performed comprehensive mass spectrometry (MS) based global proteomic analysis to analyze and quantify differentially expressed proteins from diseased and healthy fibroblast cells. The cell lysate was sub-fractionated into soluble (cytosol, sol), mitochondrial (mito) and membrane (mmb) protein fractions before analysis. A total of 129 cytosolic, 157 mitochondrial, and 109 membrane proteins were identified. All proteins were confirmed by three MS runs. By intersecting these three protein sets, we discovered a common set of 15 (between mito vs. mmb), 34 (between mito vs. sol) and 15 (between mmb vs. sol) proteins whose expression levels were significantly different, whereas 9 proteins were common among all three sub-fractions. We used an integrated proteogenomic approaches including Gene Ontology, IPA and cluster analysis to characterize functional commonalities among these proteins.

Mon Poster 36: The Protein Structure Initiative (PSI) Knowledgebase Technology Portal

Lester G Carter
Lawence Berkeley Laboratories, Berkeley, CA

The NIH-funded Protein Structure Initiative (PSI) is a consortia of research centers applying high-throughput techniques to the determination of protein structures. Many of the technologies and techniques developed by these centers are also of great potential use the scientific community in general. The aim of the PSI knowledgebase technology portal is to assist in dissemination of this information. The portal can be accessed either through the main PSI knowledgebase website, or directly via: https://isswprod.lbl.gov/PSIKPortal/Browse/Default.aspx

Mon Poster 37: A Novel Array-Based Approach for the Characterization of DNA-Bound Proteins

Shama P. Mirza; Yi Zhang; Michael Olivier
Medical College of Wisconsin, Milwaukee, WI

Interaction of proteins with DNA is vital for regulating cellular processes including transcription, replication, and recombination. Comprehensive characterization of proteins that bind to DNA is required for understanding these cellular mechanisms. Traditionally, DNA-protein interactions have been investigated using electrophoretic mobility shift assays (EMSA). The assay is based on the differential electrophoretic mobility of free DNA vs. DNA-protein complexes in a gel. Despite its common use, the EMSA assay does not provide the identity of the DNA binding protein(s). To improve the methodology, we explored a novel approach based on surface binding of proteins to oligonucleotides on arrays, followed by protein identification using mass spectrometry.

To characterize proteins bound to oligonucleotides on arrays, we used DNA sequences in the promoter region of the human insulin-induced gene 1 (INSIG-1) gene previously shown by EMSA to bind proteins from nuclear extracts. A single nucleotide polymorphism (SNP rs2721) affects the binding, resulting in differential EMSA patterns for the two alleles of the SNP. We covalently linked a 5'-amine- modified double-stranded oligonucleotide to an aldehyde-terminated self-assembled monolayer. To validate differential protein binding, nuclear extract from human liver HepG2 cells was loaded on the surface and incubated. The bound proteins were reduced, alkylated, and digested with trypsin. Tryptic peptides were collected, desalted, and analyzed on an ion trap mass spectrometer. A full range MS spectrum was acquired followed by MS/MS of the six most abundant ions from the full MS scan, followed by database searching using the SEQUEST algorithm. Differential protein binding of human liver HepG2 nuclear extract proteins to different allelic nucleotides is observed. Of the proteins captured on the T-allelic array, proline and glutamine rich splicing factor (SFPQ) was identified as the most abundant protein. SFPQ is a well known DNA and RNA binding protein involved in various nuclear processes.

This new technology allows the unbiased analysis of proteins binding to DNA and offers new opportunities for genomic research and the analysis of DNA sequence variants.

PTM Analyses of Biological Matter, 38 - 43

Mon Poster 38: Chip-Based Enrichment and Identification of Phosphopeptides

Ning Tang; Martin Vollmer; Karsten Kraiczek; Christine Miller; Keith Waddell
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Comprehensive identification of protein phosphorylation is a challenging application in the proteomics field due to the complexity and low abundance of phosphorylation events. Researchers have tried various enrichment approaches to simplify and enrich the phosphopeptides before analyzing with LC/MS/MS. Recent advances in microfluidic technology have provided an opportunity to allow easy automation of phosphopeptide enrichment and subsequent analysis of phosphopeptides. We have developed a re-usable microfluidic chip with an enrichment column consisting of titanium dioxide particles sandwiched between reverse-phase materials for on-line selective phosphopeptide enrichment. This approach allows robust, easy-to-use and reproducible enrichment of phosphopeptides from complex matrices. The chip also gives the user options of analyzing the unbound peptides. Specific enrichment of phosphopeptides from Casein, MAP kinases and complex biological samples will be demonstrated.

Mon Poster 39: Phosphoproteomics of Mutant EGFR Signaling in Glioma

Vaibhav Chumbalkar; Rebecca Maywald; Khatri Latha; YeoHyeon Hwang; Anupama Gururaj; Marta Rojas; Oliver Bogler
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Aberrant EGFR signaling is a major contributing force to glioma progression and treatment resistance. The most prevalent mutation, EGFRvIII, is an inframe deletion of the extracellular domain, which occurs in the about 40% of glioblastomas and promotes growth and survival of cancer cells. More recently, new point mutations in the extracellular domain of EGFR have been identified by The Cancer Genome Atlas. We are investigating the signaling of these abnormal receptors to identify the basis of their oncogenicity.

We have analyzed the EGFRvIII specific signal using shotgun phosphoproteomics based on recovery of tyrosine-phosphopeptides by Phosphoscan and mass spectrometry using an ion trap mass spectrometer equipped with collision induced and electron transfer induced dissociation capabilities. Glioma cell lines expressing EGFRvIII and wild-type EGFR, and with different PTEN backgrounds were compared by this approach. In triplicate runs of two separate sets of cell lines, we obtained over 100 and 150 tyrosine phosphorylated proteins, respectively. Spectral intensity and count was used in a label-free quantification, and revealed several EGFRvIII-related phosphorylations, which showed a significantly greater level of phosphorylation when compared to cells expressing wild-type EGFR.

The signaling of EGFRvIII is ligand-independent, does not involve receptor dimerization, and is of low intensity. This low intensity signal has made it challenging to uncover whether there are components of EGFRvIII signaling that are distinct from wild-type EGFR signaling. We have created a chimeric EGFRvIII that can be dimerized experimentally, using a variant FKBP12 domain and cognate small molecule, a process termed chemically induced dimerization (CID). CID increases EGFRvIII signaling several fold in intensity, without leading to ubiquitination and degradation, and is allowing us to investigate the nature of the EGFRvIII signal in greater depth than before, using shotgun phosphoproteomics as above.

Another possible mechanism behind EGFRvIII's impact on glioma biology is differential cellular localization and we are investigating whether it partitions to the nucleus with different kinetics than

EGFR, and whether it interacts with different targets in this location.

Mon Poster 40: Disulfide Bonds Mapping in Salmon Egg Lectin 24 K Using MALDI MS and MS/MS

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The carbohydrate-binding properties of lectins and their lack of enzymatic activity make this class of compounds "biological adhesives" of great importance. Lectins are invaluable for cell-cell and cell-matrix interactions. Lectins also play significant roles in the fertilization of eggs in higher animal species. There is mounting evidence that egg lectins provide a block to polyspermy via binding to glycoprotein ligands. Block to polyspermy is vital to ascertain proper procreation of species.

Because of its superior sensitivity, mass spectrometric detection of peptides has become one of the most popular analytical methods in proteomics. Mass spectrometry allows not only the detection of peptides in minute quantities, but also the structural analysis of post-translational modifications such as phosphorylation, glycosylation, and oxidative disulfide bond formation.

The disulfide bond pattern in the salmon egg lectin (SEL24K) from the Chinook salmon Onchorhynchus tshawytscha is presented. The disulfide bond pattern was established with a multi-enzyme digestion strategy in combination with MALDI-MS & MS/MS mass spectrometry. The disulfide bond pattern was found to be symmetrical in the tandem repeat sequence of SEL24K and is fully consistent with symmetrical bond patterns found in egg lectins from closely related fish species with highly conserved locations of cysteines. All cysteine residues were disulfide linked.

Mon Poster 41: Identification and Characterization of 3'-Nitrotyrosine Modified Proteins in Cerebrospinal Fluid

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The invasion of the central nervous system (CNS) by the human immunodeficiency virus (HIV) during the early stages of the systemic infection induces anti-viral responses that contribute to neuronal damage and toxicity. HIV dementia (HIVD) is the most common form of HIV-associated neurological dysfunction and has been associated with the up-regulation of various nitro-oxidative stress pathways. Previous studies have linked the neuronal damage observed in HIVD individuals to the oxidative damage of proteins via the action of reactive nitrogen species (RNS). Elevated levels of RNS interact with neuronal proteins and lead to the selective nitration of tyrosine residues. Tyrosine nitration alters protein structure and function, affects biological half-life, and prevents the phosphorylation of key tyrosine residues for signal transduction pathways. Due to the impact of the nitrotyrosine modification, we plan to employ a 'bottom-up' proteomics approach to identify nitrated proteins and characterize the modification site on the nitrated proteins in the CSF of individuals diagnosed with HIVD. The protein composition of the CSF reflects the pathological condition of the central nervous system (CNS); therefore, the CSF provides a unique opportunity for the investigation of the development and clinical manifestations of HIV-associated neurological dysfunction.

To enrich the nitrotyrosine-containing sub-proteome, we utilize a 3'-nitrotyrosine (3-NT) antibody in a Protein G-based Co-Immunoprecipitation (Co-IP) technique. The 1-dimensional gel electrophoresis is used to validate the enrichment of the 3'-nitrotyrosine modified proteins via the 3-NT Co-IP procedure. As a means of protein identification, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and electrospray ionization mass spectrometry (ESI-MS) is utilized in both an off-line and on-line proteomics approach to identify the nitrotyrosine-containing proteins. In addition to MALDI-MS and ESI-MS (LCQ), the enhanced sensitivity and mass accuracy of the LTQ-Orbitrap hybrid mass spectrometer (ThermoFisher, San Jose, CA) is utilized to characterize the location of the nitro-modified tyrosine residue in the proteins isolated by the anti-nitrotyrosine antibody. An in vitro nitrated bovine serum albumin (n-BSA) model is used to validate the experimental design. From the in vitro n-BSA model, we were able to isolate the modified proteins via the 3-NT antibody and following tryptic digestion the location of the modified tyrosine residues were characterized via the LTQ-Orbitrap. The enriched nitrotyrosine-containing proteins from both the Non-HIVD and HIVD CSF samples were enzymatically digested and subjected to multiple rounds of both low-energy collision-induced dissociation (CID) and electron transfer dissociation (ETD) fragmentation via the LTQ-Orbitrap. The additional ion-ion chemical ionization-induced fragmentation produced as a result of ETD enables the analysis of larger peptides, the fragmentation of peptides with multiple basic residues, and the identification of labile post-translational modifications. Both the MS and MSn spectra are transferred to the Orbitrap mass analyzer for high resolution analysis, thus leading to the identification of the nitrotyrosine-modified proteins and characterization of the nitro-modified tyrosine residues.

Mon Poster 42: Cellular Glycolipids in Cultured Glioblastoma Multiforme Brain Tumor Cells

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Glioblastoma multiforme (GBM) cells are characterized by high invasivity and resistance to apoptosis. Galectin 1 (gal 1) is highly expressed in GBM and has been identified as a therapeutic target for treatment of glioblastoma (Puchades et al. J. Proteome Res., 2007, 6: 869-875). GBM is infamous for the inevitable relapse after surgical extirpation, which maybe accounted for by the presence of cancer stem cells (CSC). NS11, a CSC cell line, undergoes distinctly induced phenotype changes following different treatments. The biochemical changes that occur in CSCs are largely unknown. However, the underlying biochemical modifications are rarely studied.

Gal 1 binds to terminal galactose residues on proteins and lipids. The polar lipids GM1 and asialo-GM1 glycosphingolipid (GSL) are known to bind to gal 1. To study the changes in these potential gal 1 ligands, we developed an efficient method for the analysis of polar lipids in GBM after gene and/or chemotherapy and CSC after induced differentiation.

Log phase cultures were extracted with 1:1 methanol:chloroform and incubated (48 oC) overnight. The supernatant was collected and phase separated with additional chloroform and H2O. The aqueous phase contained the polar lipids. The polar lipid fraction was separated by nLC in a self-packed 80 mm X 50 µm phenyl hexyl column. The gradient was 15%A/85%B to 2%A/98%B (A: 98% H2O, 2% methanol, 10 mM NH4OAc; B: 98% methanol, 2% H2O, 10 mM NH4OAc) in 4 min. at 400 nL/min. Effluent was on-line analyzed by negative-ion microelectrospray with a modified hybrid LTQ 14.5 T FT-ICR MS. Structural assignment was based on accurate mass and tandem mass spectrometry. Polar lipid abundance was based on signal magnitudes (He, H. et al. Anal. Chem., 2007, 79: 8423-8430).

Our unique method permits isolation, separation and identification of ~1,000 polar lipids of various polar head groups and nonpolar tails at biological levels. High resolution and high mass accuracy provided by FT-ICR MS along with low-resolution MS/MS greatly enhance the identification of the polar lipids. Often MS/MS is necessary to determine sugar sequence to identify isobaric species (i.e. iGB4/GB4 vs. Asialo GM1). Unique polar lipid profile changes are seen after gene/chemo-therapy in both GBM and CSC. Differences in the polar head group identity and length, degree of unsaturation, and hydroxylation of the non-polar ceramide tail were identified under various treatment regimes. These lipidomic analyses are a major portion of our inter-institutional, multidisciplinary, systems biological approach to the

study of glioblastoma, which correlates phenotypic, genomic, transcriptomic, proteomic and glycomic data sets. The systems biological approach is essential to discover new biomarkers and identify novel therapeutic targets for treatment of glioblastoma.

This work was supported by National High-Field Fourier Transform Ion Cyclotron Resonance Mass Spectrometry ICR Facility at the National High Magnetic Field Laboratory (NSF DMR 00-84173), the Falk Foundation and the John C. Merchant Foundation.

Mon Poster 43: Enrichment and 12-plex Profiling of Phosphoproteins Involved in Cellular Proliferation and Death with Affinity Chromatography, SILAC, and Tandem Mass Tags

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Protein phosphorylation constitutes one of the most widely studied post-translational modifications. It is estimated that as much as 30% of all cellular proteins are transiently phosphorylated on serine, threonine, and tyrosine residues at any given time. Reversible protein phosphorylation regulates nearly all intracellular biological events including signal transduction, protein-protein interaction, protein stability, protein localization, apoptosis, and cell cycle control. Deregulation of protein phosphorylation is a hallmark of numerous human diseases including cancer, metabolic, and immune disorders. Using phospho-proteomics to study the etiology of human disease can be a difficult task because of the transient or labile state of the phosphate group and the low phosphoprotein abundance. Phosphoprotein expression profiling by MS is further limited by the technical difficulty and reproducibility of the sample preparation workflow and by the analysis of an inadequate number of replicates. We have addressed these issues with a combination of phosphoprotein enrichment, automated phosphopeptide enrichment with TiO2-coated magnetic beads, and 12-plex multiplexed quantitative analysis by combining SILAC and Tandem Mass Tag (TMT) reagents with novel MS acquisition methods on an LTQ-Orbitrap.

We employed this phospho-proteomics enrichment strategy to trace specific phosphorylation events in the phosphatidylinositol-3-kinase (PI3K)-Akt signaling pathway in response to chemical inhibitors of PI3K (LY294002) and mTOR (rapamycin). Enrichment of phosphoproteins using an IMAC-based Phosphoprotein Enrichment Kit and of phosphopeptides with TiO2 coated magnetic beads, in combination with quantitative mass spectrometry, provided high resolution replicate data of the action of these drugs in insulin responsive 293T cells. Of specific interest was the quantitative reduction in phosphorylation of the retinoblastoma protein, Rb, on residue T373 in response to rapamycin. Reduction in Rb (T373) phosphorylation coincides with altered phosphorylation and kinase activity of PKA (Ser99), Akt (Ser473), GSK3α (Ser9), and GSK3β (Ser21), ultimately regulating cyclin D stability, cyclin-dependent kinase 4/6 activity, and cell proliferation. Interestingly, we also observed a reduction in phosphorylation of the Death Inducer Obliterator protein, DIDO1 (S1456). Both DIDO1 Ser1456 and PKA Ser99 are substrates for Aurora Kinase A, which has been shown to be over-expressed in many human cancers and more recently implicated in activation of Akt. Taken together these results provide important insights into the mechanism of action of PI3K and mTOR inhibitors on cell cycle control and apoptosis.

In summary, we demonstrate an efficient enrichment of phosphoproteins belonging to the cell death and proliferation machinery using complementary phosphoprotein and phosphopeptide affinity approaches. We demonstrate quantitative reproducibility across technical replicates using a highly multiplexed quantitative analysis, and we identified differential phosphorylation cascades affected by these activators of cell proliferation or cell death. These results demonstrate the ability to quantitatively profile complex phosphoprotein mixtures with high statistical confidence using a combination of protocol and reagent enhancements.

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Mon Poster 44: ProteoMiner: A Novel Tool for Differential Clinical Proteomics

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Blood samples are the major source for clinical proteomics approaches, which aim to identify diagnostically relevant or treatment-response related proteins. But, the complexity of serum with the presence of high-abundant proteins and an enormous dynamic range of protein concentrations hinder a comprehensive analysis of the low-abundant proteins. A novel tool to overcome these barriers of serum analysis, utilizes combinatorial hexapeptide ligand libraries (ProteoMiner™). In contrast to most common approaches based on the principle of immune-depletion, this technology concentrates low abundance proteins and concurrently reduces high abundance species. Thus the dynamic range will be compressed and low abundance proteins become more easily detectable. Here, we demonstrate that ProteoMiner™ can also be employed for comparative and quantitative analysis of complex biological samples, an imperative prerequisite for large scale clinical proteomics applications. First, we spiked human serum samples with another complex proteome (E. coli lysate) in increasing concentrations ranging from 3 µg to 300 µg, respectively. Then, we processed the mixed samples with ProteoMiner™ and the eluted proteins were examined by quantitative image analyses of 2D-gels. We found, that the concentration of the spiked bacteria proteome, reflected by the maintained proportional spot intensities, was not altered by ProteoMiner™ treatment. Therefore, we conclude that the ProteoMiner™ technology can be used for quantitative analysis of low abundant proteins in complex biological samples.

Mon Poster 45: Illustra triplePrep Kit, A Rapid Method to Extract DNA, RNA and Proteins from a Single Undivided Sample

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GE Healthcare recently developed a novel method (illustra triplePrep Kit) to isolate high quality genomic DNA (gDNA), total RNA and total denatured proteins from a single undivided sample in less than one hour. It allows researchers to correlate DNA, RNA and protein data directly from exact same sample and to generate more data from as little as 1 mg tissue. The performance of triplePrep was tested with a wide range of animal tissues and cultured cells in various downstream applications such as PCR, restriction digestion, sequencing and array CGH (gDNA); RT-qPCR and gene expression analysis (RNA) and SDS-PAGE, Western Blotting, 2-D DIGE and LC-MS (Protein). When compared with commonly used sample preparation methods, triplePrep not only generates higher yields of high quality DNA, RNA and proteins from small samples but also provides a faster and more flexible workflow.

Mon Poster 46: Building a Robust and Reliable Proteomics Biomarker Pipeline: NCI's CPTAC Network

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Better biomarkers are urgently needed to improve diagnosis, guide molecularly targeted therapy, and monitor activity and therapeutic response across a wide spectrum of disease. Proteomics methods hold special promise for the discovery of novel biomarkers that might form the foundation for new clinical blood tests, but to date their contribution to the diagnostic armamentarium has been largely unsuccessful. While the scientific literature annually reports

many biomarkers, few prove sufficiently resilient, reproducible, and real to translate into clinically useful assays. Advances in methods and technology now enable conceptualization and evaluation of a comprehensive biomarker pipeline from the following essential process components: candidate discovery in tissues or proximal fluids, prioritization of candidates using targeted screening approaches, verification of candidates by quantitative assays in patient (100s) samples, biomarker validation and clinical assay development in large population studies, and commercialization.

The primary goal of NCI's Clinical Proteomics Technology Assessment for Cancer (CPTAC) is to establish an integrated pipeline that will analyze cancer-relevant proteomic changes in human clinical samples. To do this, CPTAC has focused on bridging the gap between candidate discovery and clinical validation. The first step toward completing this bridge is to develop technology platforms that reliably detect changes in the human proteome. These technologies must be reproducible both within a lab and across labs. CPTAC has explored mass spectrometry and affinity-based platforms and developed reference materials and metrics to evaluate instrument performance. These materials and metrics are transferable across proteomic technologies, thus enhancing multiple components of the biomarker pipeline. The second step is to employ these technologies in the evaluation of each platform's ability to detect cancer-relevant changes. This larger step involves analyte (biomarker candidate) prioritization, clinical sample collection, assay development, and assay evaluation.

CPTAC has recently completed the first phase of technology evaluation and standardization. Multiple inter-laboratory studies have been able to demonstrate that specificquantitative proteomic assays are reproducible between labs. CPTAC is implementing this overall strategy to verify biomarker candidates for their ability to discriminate breast cancer prior to biopsy. Such a pipeline will either provide credentialed biomarker candidates for a large clinical validation study or provide an efficient means of attrition indicating that an analyte can not be effectively verified using current technology. If successful, implementation of this pipeline will streamline the biomarker development process, shorten the timeline, and improve the success rate of clinical validation for biomarkers of any disease type.

Mon Poster 47: Biomarker Discovery by Time-Course Statistical Analysis of Proteomic Data

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Although much of the differential proteomic expression data from biological phenomena and diseases are temporal in nature, traditional statistical methods such as two-factor ANOVA are still commonly applied in their analyses. These "static" methods ignore the sequential nature of time-course data and the magnitude of the time intervals. Methods specifically designed to extract differential protein expression by recognizing the dynamic nature of the data are more appropriate in such cases. Moreover, it has been demonstrated that time-course analytical methods are capable of discovering more differential expression than static methods are, in time-course genomics data. We have applied time course statistical analysis to different kinds of proteomic data and compared the differential expression obtained with those obtained by static methods. To our knowledge, such analyses of proteomic data have not been reported to date. We describe here our application of the program EDGE to a proteomic study of cardiogenesis in murine hematopoietic stem cells, and report the temporal differential expression detected. EDGE was developed by John Storey and coworkers for the Extraction of Differential Genomic Expression [Storey et al., 2005, Proc. Natl. Acad. Sci. USA 102, 12837-12842]. Our procedure consisted of 2-D gel image analysis with Nonlinear Progenesis SameSpots 3.1 to obtain protein abundance levels ("normalized volumes") for all spots, followed by analysis with EDGE. Proteomic expression was recorded in three sample groups: (1) mouse embryonic stem cells (mESM), (2) in the absence of leukemia inhibitory factor (LIF) whose removal induces differentiation in murine embryonic stem cells, and (3) with Fidarestat (an aldose reductase inhibitor, ARI), each at four time points 4 , 8 , 16 , and 24 h. The data set consisted of a total of 20 gels, each with an initial set of approximately 1400 spots. EDGE detected 28 spots displaying temporal differential expression which were subjected to protein identification by mass spectrometry and further investigated for their role in cardiogenesis. This yielded considerably more differential expression than was detected by the static analysis method two-factor ANOVA. Multivariate analyses of the data by hierarchical clustering (HC) and Principal Components Analysis (PCA) revealed three major groups in terms of the pattern of protein expression, which we are investigating further. These results will be described in detail and their biological implications discussed.

Mon Poster 48: Automating the Retention Time Determination for Targeted Peptide Detection/Quantitation: Building Flexibility into Scheduled SRM Assay Development

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The role of targeted protein/peptide detection via SRM-based assays has increased over the past year from primarily quantitation to discovery and verification of putative biomarkers. The increased sensitivity and selectivity provided via selected reaction monitoring (SRM) results is an effective approach to addressing biological complexity of the background matrix while probing for proteotypic peptides. To maximize experimental results as a function of sample consumption, researchers are employing timed acquisition strategies for monitoring thousands of SRM transitions in one experiment. The difficulty associated with method development lies in compiling the necessary experimental information for constructing SRM tables that consist of precursor-product ion information, Q2 collision energies, and retention time information. Previous attempts to building timed SRM experiments simply transfer the entire experimental method used from discovery experiments acquired on a different mass spectrometer. This approach requires precise duplication of the LC experimental conditions or the use of wide retention time windows to ensure that the targeted peptide is detected in the timed SRM experiment. Additionally, any deviation in experimental details must be eliminated without the need to acquire the samples on the second instrument using the desired conditions, which limits the flexibility to change the chromatographic gradient conditions to increase throughput. Our approach is to use a trainer set of peptides to provide a means of determining the relationship between Krokhin hydrophobicity factors and retention times for the peptides used in the trainer set. The mathematical expression can then be applied to the list of targeted peptides to identify retention times.

A set of eleven tryptic peptides from yeast enolase was used to characterize the chromatographic set employed for targeted peptide quantitation of C. elegans proteins. The selection of the eleven peptides provides a range of hydrophobicity factors from 7 to 38 providing a marker for most enzymatically cleaved peptides used in a targeted list. The trainer set was analyzed in triplicate using an SRM assay on a triple quadrupole mass spectrometer to determine reproducibility. From the initial experiments, the linear equation of hydrophobicity factor vs. retention times were calculated and applied to a list of targeted peptides from C. elegans digest. A set of 200 C. elegans peptides was used to test the agreement with predicted and experimentally measured retention times. Integrated peak areas and %CV's were calculated and compared to results obtained using non-timed SRM tables to determine the success rate. Comparisons were made for three different chromatographic gradient lengths.

This paper presents a strategy to build timed SRM assays for targeted proteotypic peptides in an automated fashion for any HPLC condition. A linear equation determined from the analysis of a peptide trainer set provides a means of relating the calculated

Krokhin hydrophobicity factors for each targeted peptide to a predicted retention time window used to increase the detection efficiency of a SRM assay. Initial results on 200 proteotypic peptides from C. elegans digest shows over an 85% success rate of reproducibly detecting and quantifying the peptide signals from a timed SRM experiment.

Mon Poster 49: Identification of Increased Levels of Circulating Insulin-Related Biomarkers in Schizophrenia Patients

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Diagnosis of schizophrenia is subjective due to the absence of empirical tests. We found increased circulating levels of insulin, proinsulin, chromogranin A and other insulin secretory granule constituents in normo-glycemic, first onset schizophrenia subjects using targeted two-site immunoassays, liquid chromatography tandem mass spectrometry and Mutli-Analyte Profiling (MAP). These results are indicative of a general increase in secretory output from pancreatic beta cells, possibly as compensation for the increased prevalence of insulin resistance observed in schizophrenia. The finding that there was no difference in circulating insulin-related peptides in subjects with bipolar disorder, suggested that the effect is not a common feature of neuropsychiatric disorders. These studies suggest that hyperinsulinemia may play a role in the onset of schizophrenia and measurement of insulin-related peptides may have utility in diagnosis or stratification of patients prior to antipsychotic treatment. Most importantly, drugs which improve insulin signaling may represent an attractive novel treatment strategy for this debilitating disorder.

Cell and Tissue Proteomics, 50 - 56

Mon Poster 50: Evaluation of Occurrence of Alternatively Spliced Proteins Detected by Large Scale Proteomics Projects

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Introduction

Alternative splicing has been widely recognized as one of the most significant cell mechanisms in eukaryotes for generation of protein diversity. Currently splicing detection is prerogative of genomic technique in particular DNA microarrays and RT-PCR sequencing as well as computational prediction methods. According to computational estimations more than 60% of human genes undergo alternative splicing. However, alternative mRNA isoforms not always translate into different protein isoforms and can be involved in regulation of initiation of translation, RNA editing and other processes. In the current study we examined the occurrence of alternatively spliced proteins detected by large scale proteomics projects. All currently available HUPO liver and brain proteome projects were under investigation. The data was downloaded from PRIDE database in XML format from http://www.ebi.ac.uk/pride/. Bioinformatical analysis was performed on datasets containing at least 50 protein identifications. Proteins with shared peptides were clustered into groups as indistinguishable proteins using ProteinCenter software package (www.proxeon.com) that allows performing automated and interactive comparison of lists of proteins which take into account all of the differences arising from allelic or splicing variations as well as dealing with fragment vs. full-length sequences. Using examples from HUPO brain and liver proteomics studies we observed that more than 30% of reported proteins belong to alternatively spliced sequences. At the same time not all of them can be claimed as splice variants due to absence of unique peptides corresponding to the splice sequence. Using ProteinCenter we performed clustering of large scale proteomics datasets in order to distinguish variable isoforms of proteins. For example eight different isoforms were observed for human intermediate filament binding protein – plectin 1 in HUPO Liver projects with a total of 141 different peptides sequenced (see cluster alignment). A Plec1 gene is known to contain 33 exons and detailed analysis of exon coverage by peptides will be presented. With the development of large scale proteomics techniques it is becoming obvious that mass spectrometry based methods will bring another quality level in characterization of splice variants due to the ability to detect a peptide corresponding to one or another splice variant. Furthermore the targeted quantitative analysis can lead to the creation of the depository of unique detectable peptides corresponding to the alternatively spliced proteins.

Mon Poster 51: Long-Term Proteome Preservation in Bone Marrow Particle Blocks

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Background- Preservation of intact macromolecules is a challenge in clinical environment. Today there is no economical method available to preserve bone marrow samples for molecular studies. We have studied feasibility of preserving DNA, RNA and protein in bone marrow aspirate particle preparation using a novel molecular friendly tissue processing system.

Material and Methods- Bone marrow aspirates particle preparation were fixed in Molecular Fixative™ and processed using Tissue-Tek® Xpress™ (Sakura Finetek, Torrance, CA). Paraffin blocks were kept at room temperature for at least 18 months. DNA, RNA and protein were extracted from the freshly isolated samples, 50 micron thick sections of the paraffin blocks immediately after processing and after 18 months. Commercial kits (Bio-Rad and Calbiochem) were used to isolate various subcellular compartments (nuclear, cytoplasmic organelles, cytoplasmic membrane) from the paraffin-embedded tissue. Isolated extracts were further separated on a fluid phase isoelectric focusing chamber (Bio-Rad Micro-Rotofor) and run on SELDI-TOF mass spectrometry on different protein-chip chemical surfaces. 2D gel electrophoresis with further spot excision and trypsin digestion was used to identify proteins. Immunohistochemistry was performed on paraffin-embedded tissue section for 20 major hematopoietic proteins. DNA and RNA were analyzed using gel electrophoresis and PCR for large amplicons of GAPDH (DNA-450bp) and beta actin (RNA-705 bp). Real-time PCR was performed for several house keeping genes.

Results- Protein profiling using mass spectrometry demonstrated similar pattern to fresh samples. Similar 2D gel pattern were obtained from samples with adequate yield. Immunohistochemistry was successful for all studied proteins. High molecular weight DNA and RNA were preserved in paraffin blocks. PCR for large fragments was successful in all samples.

Conclusions- It is feasible to use a novel formalin-free bone marrow particle preparation for preservation of high molecular weight macromolecules. This can serve as a cost-effective method for bio-repository purpose. Furthermore, preservation of bone marrow micro-architecture opens door for in situ molecular studies.

Mon Poster 52: Protein Phosphorylation in Human Embryonic Stem Cells

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Human embryonic stem cells (hESCs) combine the unusual characteristics of self-renewal and pluripotence, making them ideal for identifying the key players in these important cellular processes. While hESCs have been extensively analyzed for global mRNA and microRNA expression, very little is known about the proteins involved in their signal transduction pathways. Protein phosphorylation is a key post-translational modification that controls activity of many signal transduction pathways. We are profiling hESC-specific phospho-proteins in order to understand how these proteins may be implicated in regulation of self-renewal, pluripotence and differentiation. Here we present a preliminary phospho-proteomic profile of hESCs that suggests an important role for protein modification in maintenance of the undifferentiated state. Lysates of WA09 hESCs were enriched for phospho-proteins by PMAC enrichment (Clontech). These phospho-proteins were

then digested by trypsin and the phospho-peptides were further isolated. The phospho-peptides were identified by mass spectrometer (MS/MS). More than 200 phospho-peptides were identified. Among them, we observed phosphorylation of various hESC-associated proteins. The reprogramming factors SOX2 and LIN28 and the transcription factor UTF1 were found to phosphorylated in hESCs. In addition, DNMT3b, a DNA methyltransferase, was found to be phosphorylated. DNA methylation is an epigenetic modification that regulates gene expression and is crucial for embryonic development. Our future studies will compare hESC lines to their differentiated derivatives and to induced pluripotent stem cells (iPSCs). The outcome of this characterization will help us understand the mechanisms that control pluripotency and differentiation and may lead to new methods for inducing pluripotency in differentiated cells.

Mon Poster 53: Characterization of Mesenchymal Stem Cell Neuronal Differentiation by High Content Analysis, SILAC, and Targeted Protein Quantification with Mass Tag SRMs

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Multipotent stem cells hold great promise for regenerative medicine and as important tools for biological research. Adipose-Derived Human Mesenchymal Stem Cells possess the ability to differentiate into multiple cell lineages and are readily available in sufficient quantity, making them an attractive tool to study differentiation. However, a full understanding of the regulation of differentiation at the protein level remains unknown. The goal of this study was to use proteomics and cell-based screening tools to characterize stem cells during neuronal differentiation. We first used high content image analysis to assess neurite outgrowth and temporal-spatial expression changes of known neuronal markers during neuronal differentiation. Stable Isotope Labeling of Amino acids in Cell culture (SILAC) with subsequent proteomic analysis by mass spectrometry was used to discover proteins differentially regulated during differentiation. Protein changes observed by mass spectrometry were verified by Western blots and a novel Selective Reaction Monitoring (SRM) strategy. As a complementary alternative to Western blots, Tandem Mass Tag 0 (TMT0) tagged peptides from recombinant proteins were first characterized by MS, then spiked into TMT6 labeled cell lysates from undifferentiatied and neuronal differentiated mesenchymal stem cells. Specific MS/MS transitions of the digested recombinant protein standards labeled with TMT0 and th 5 Da heavier cellular peptides labeled with TMT6 were used for targeted quantification of protein and phosphopeptide levels. Here we report the significant regulation of many gene ontologies identified by SILAC and verified by complementary quantitative proteomic methods. These protein changes coincided with an increase in both neuronal morphology and levels of protein markers MAP2 and Beta-3-tubulin, as determined by high content analysis. This model demonstrates how cell-based and proteomic profiling of stem cells offer valuable insights into the mechanisms of cellular differentiation.

Mon Poster 54: 2-D Analysis of Leaf Protein Samples Depleted of RuBisCo under Denaturing Conditions

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One of the fundamental challenges in proteome analysis is the fact that samples are often dominated by a relatively small number of high-abundance proteins whose presence can obscure less abundant proteins and limit the capacity and resolution of the separation technique(s) employed. This is very apparent for serum and plasma, where some 20 proteins constitute more than 98% of the protein mass. It also applies to the proteome of leaves and other photosynthetic tissues, where over 50% of the protein typically consists of the single enzyme Ribulose Bisphosphate Carboxylase (RuBisCo). The removal of highly abundant proteins is addressed by two fundamentally different approaches. First, immunodepletion was used to remove, in the case of serum or plasma, species-specific proteins. Alternatively, one can use a library of combinatorial hexapeptides to bind all possible proteins in a complex mixture. In this approach, which is independent of the sample, the concentration of high abundant proteins is reduced because of the limited binding sites for them.

This study was initiated in order to examine the applicability of ProteoMiner protein enrichment kits for the reduction of RuBisCo and enrichment of medium and low abundance proteins from leaf-derived protein samples. Effective application of ProteoMiner requires soluble protein at high concentration and this presents a challenge for plant-derived samples since 1) plant extracts typically have low protein concentrations and 2) much of the protein in plants is not soluble in the absence of chaotropes or detergents. We have developed effective methods for ProteoMiner enrichment of spinach leaf samples prepared both under native and denaturing conditions that circumvent these constraints.

Mon Poster 55: Rapid Method for Biomarker Evaluation and Validation using Step-Wise Density Gradient Extraction and Statistical Analysis

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In recent years, the role of biomarkers in drug development and diagnostics has expanded across a broad range of applications from research tools during early discovery to surrogate endpoints in the clinic. Due to the development of high-throughput proteomics, including high sensitivity mass spectrometry and automation of protein identification, the number of publications on experimental biomarkers has extended into the several thousands each year. However, only a small number of these markers will achieve clinical endpoints. Biomarker evaluation and validation constitute critical steps in biomarker development. In this study, we demonstrate a rapid method for biomarker evaluation and validation using, A: Edge™ (Enhanced Density Gradient Extraction) technology, a density based extraction method for simple and reproducible fractionation of tissue homogenates/cell lysates, B: a unique statistical method for evaluation of potential biomarkers based on the relative percentage distribution of the marker across several fractions. The utility of the method in biomarker evaluation was shown using rat models of high fat diet, since high fat diet has a profound impact on brain function and apoptosis risk. Twenty rats were separated into two groups of 10, one group fed with special high fat diet for 6 weeks and one group fed with regular low fat diet for the same time. To assess the effect of high fat diet on brain synaptic dysfunction and apoptosis, rat brain and heart tissues were homogenized then fractionated using the Edge 200 separation system. Fractions were subjected to western blot analysis using the synaptic function marker p-synapsin I and the oxidative stress markers GRP75 and HSP70. Results were analyzed statistically giving clustered data for each of the markers. The results show that Edge technology combined with a proprietary statistical analysis represents a unique method for easy and rapid evaluation of any potential biomarker.

Mon Poster 56: Immunoaffinity Enrichment and Targeted Mass Spectrometry for Quantitation of Carbonic Anhydrase 12 Protein as a Biomarker of Renal Cell Carcinoma

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The incidence of renal cell carcinoma (RCC) is increasing in the United States while novel targeted therapies present a new set of clinical questions. A reliable panel of blood RCC biomarkers of disease activity could guide therapeutic and preventive interventions and serve as surrogate end-points in clinical trials. Inactivation of the Von Hippel Lindau (VHL) tumor supressor gene and the resulting constitutive up-regulation of hypoxia inducible factor (HIF) are hallmarks of the majority of clear cell RCC and the earliest known signal transduction defect in this cancer. We took

advantage of the availability of human RCC cell lines deficient in VHL to interrogate the global gene expression changes linked to loss of VHL function as a first step in RCC biomarker discovery. We identified a number of specific genes that are upregulated by loss of VHL. We found that these genes or Cell line Derived Biomarkers (CDBs) are also upregulated in all patient RCC tumors examined when compared to matched normal renal parenchyma obtained from the same individual. Furthermore, we investigated the correlation of protein and gene expression profiles of the CDBs. In this study, we have developed a proteomics platform consisting of protein immunoaffinity enrichment and targeted mass spectrometry to detect and validate upregulation of carbonic anhydrase 12 (CA12) as a circulating biomarker of tumor activity. Carbonic anhydrase was enriched first through an antibody immobilized to magnetic beads and digested with trypsin. A candidate peptide of CA12 was quantitated using multiple reaction monitoring coupled with stable isotope dilution mass spectrometry (MRM/SID-MS). We demonstrated sufficient throughput, good recovery and reproducibility to enable robust detection and quantitation of the candidate biomarker proteins at the low ng/mL levels in human plasma of RCC patients before and after curative nephrectomy. Similar analysis of other CDBs is underway.

Disease Proteomics, 57 - 76

Mon Poster 57: US National Heart Lung and Blood Institute Proteomics Initiative

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In September 2002 the National Heart, Lung, and Blood Institute (NHLBI), a component of the US National Institutes of Health, initiated a multidisciplinary Proteomics Technology Development Initiative providing support for seven years to ten interactive Proteomic Centers operating within US research institutes and universities. The aim is to enhance and develop innovative proteomic technologies and apply them to major biological questions relevant to heart, lung, blood, and sleep disorders. The NHLBI Proteomics Initiative is intended to complement and enhance NHLBI's research programs in human diseases and disorders involving heart and vascular, lung, and blood systems and resources. This poster will summarize recent work being conducted in the ten centers and highlight several tools or resources being developed within the Initiative for open access and dissemination to the scientific community. The participating centers are: the Cardiovascular Proteomics Center at Boston University, directed by Catherine Costello, PhD; the Proteomics Center at Johns Hopkins University, directed by Jennifer Van Eyk, PhD; the Proteomics Center at the Medical College of Wisconsin, directed by Andrew Greene, PhD; the Cardiovascular Proteomics Center at the Medical University of South Carolina, directed by Daniel Knapp, PhD; the Seattle Proteomics Center of the Institute for Systems Biology, directed by Ruedi Aebersold, PhD; the Proteomics Center at Stanford University, directed by Garry Nolan, PhD; the Center for Medical Proteomics at the Uniformed Services University, directed by Harvey Pollard, MD, PhD; the Proteomics Center at the University of Texas Medical Branch at Galveston, directed by Alex Kurosky, PhD; the Southwestern Center for Proteomics Research at the University of Texas Southwestern Medical Center, directed by Thomas Kodadek, PhD; and the Proteomics Research Center at Yale University, directed by Kenneth Williams, PhD. The NHLBI Proteomics Initiative maintains a web site at www.nhlbi-proteomics.org.

Mon Poster 58: Proteomic Characterization of Cerebrospinal Fluid in Tuberculous Meningitis for Potential Biomarkers

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Tuberculous meningitis (TBM) is a serious extra pulmonary manifestation of tuberculosis caused by Mycobacterium tuberculosis group of bacteria. The incidence of TBM is increasing worldwide especially in children and AIDS patients and most of these patients are localised in developing and under-developed countries especially Asia, Sub-Saharan Africa and South-America with little access to quality healthcare .Presently the fastest tool of diagnosis of TBM is Acid-Fast bacilli (AFB) smear by microscopy which has sensitivity of < 10% and PCR testing of CSF which has sensitivity of <62.5%. Culture of TB bacilli is also 100% specific but is takes few weeks and is not sensitive enough. With this challenge of early diagnosis of TBM and the need to initiate therapy at earliest to prevent any neurological deficit, we started on this project to discover viable biomarkers for TBM.

2-Dimensional Difference Gel Electrophoresis(2-D DIGE) followed by Decyder 6.5 software analyses were employed to assess differentially expressed proteins in CSF of TBM patients in comparison to normal individuals.All the patients were HIV negative and PCR and culture positive for TBM while controls had no neurological problem and no active tuberculosis.15 spots were identified by Decyder analyses as having singficant ($p<0.05$) variation in their level of expression. Their identification by ESI Q TOF MS/MS will be reported and discussed.

Mon Poster 59: The Cellular and Proteomic Changes by Oxidative Stress in Frataxin Homologue (YFH1) Deletion Mutant Related to Friedreich's Ataxia Disease

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Although biological markers of oxidative stress have been described in many disorders the molecular mechanism of stress propagation and the stage(s) at which it is most relevant for these disorders remains unclear. Friedreich ataxia (FA) is a rare but dramatic disease with an estimated prevalence of 1 per 50,000. FA is an autosomal recessive disease caused by a 6% - 30% reduction in the concentration of the mitochondrial protein frataxin (FXN). This condition is clinically characterized by progressive gait and limb ataxia, sensory loss and muscle weakness often associated with cardiomyopathy, diabetes mellitus, and scoliosis. The gene responsible for FA is located on chromosome 9, consists of 7 exons and is called the *FRDA* gene. More than 95% of the FA patients are homozygous for large expansions of a GAA triplet repeat sequence located in the first intron. Early studies in *S. cerevisiae* implicated the yeast homologue of frataxin (Yfh1p) in iron homeostasis and respiratory function. Moreover, Yfh1p deficiency results in alterations in protein functions associated with oxidative damage. This model system therefore, provides a unique opportunity to target oxidative stress to mitochondria and to study the molecular mechanism(s) of this phenomenon in a biological system. The cell response to oxidative stress was evaluated by addition of H_2O_2 to the *S. cerevisiae* wild type or ΔYFH1 mutant strains by suppression of frataxin synthesis. Based on the nature of the pro-oxidant mechanism of action, we can expect differences in cellular damage (particularly location) and response at least in early stages of oxidative stress. As expected growth of the wild type arrested immediately after H_2O_2 exposure and recovered in 2 hours. Mutant, on the other hand, initially had a significant increase in growth relative to the wild type followed by a decline in growth 4 hours following H_2O_2 addition. To investigate this surprising behavior, we measured yeast viability, carbonyls content, and levels of ROS under identical conditions. While no change in viability was observed in wild type the number of viable mutant cells significantly decreased after 4 hours and eventually reached zero. In mutant, both the carbonyls and ROS levels reached their maximum at 3 hours following H_2O_2 treatment. The impact of oxidative stress on protein function in wild type and mutant was also evaluated by proteomic techniques. Damage to proteins by free radical oxidation is generally linked to formation of carbonyl groups in amino acid side chains, and is most prevalent on arginine, lysine, proline, and threonine residues. Biotin hydrazide was used to derivatize carbonyl groups in protein extracts. Oxidized proteins were selected by avidin affinity chromatography and following trypsin digestion proteins were identified by RPLC-MS/MS. Overall 53 and 55 proteins was identified in wild type and mutant, respectively. Most of the proteins were found in both wild type and mutant strain, and were related to cellular metabolism. The unique proteins found in the mutant were

found to be involved in protein synthesis, transcription and frataxin function, suggesting a different mechanism of oxidative stress propagation in the mutant strain.

Mon Poster 60: Extending the Clinical Applications of MALDI Mass Spectrometry to Microbiological Routine Diagnostics

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MALDI-TOF MS / SARAMIS is a straightforward, rapid, robust, and, inexpensive method for the routine identification of bacteria and fungi in clinical microbiology laboratories. Automated identification systems are widely-used in medium-to-high-throughput clinical microbiology laboratories. However, such systems are relatively slow because they depend on bacterial growth and metabolic activity. Bacterial identification by MALDI-TOF mass spectrometry provides a promising way to accelerate pathogen identification, since it can be performed in a few minutes from small samples. In this study we compared with 1,400 clinical routine samples the performance of MALDI-TOF MS coupled to SARAMIS (Spectral ARchiving And Microbial Identification System, AnagnosTec, Germany) with established methods (VITEK2/API, BioMérieux) in the clinical microbiology routine diagnostics.

Mon Poster 61: Characterization of HIV-Associated Dementia Proteins in Cerebrospinal Fluid(CSF)

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Advanced HIV infection is commonly associated with progressive immune suppression and the development of HIV-associated neurocognitive disorders (HAND), which can progress to profound functional disability and even death. Currently there is no specific biomarker test for HAND. The discovery of reliable, predictive, prognostic biomarkers at an early stage would enable us to make appropriate therapeutic decisions in a timely manner and to better understand the disease pathogenesis

To characterize the putative biomarkers, we performed an in-depth proteomic analysis of cerebrospinal fluid (CSF) derived from HIV-associated Dementia patients and non-Dementia subjects (controls) to determine the differences in expressed proteins. A non-gel based multi-dimensional protein identification technology (MUDPIT) coupled with the iTRAQ quantitation method was used to perform global differential proteomic analysis of the CSF samples. High abundance proteins including transferrin, IgG and albumin were first removed using the multiple affinity removal system (MARS). The remaining lower abundant proteins were enriched and tagged with different iTRAQ reagents after tryptic digestion. Strong cation exchange (SCX) and reversed phase chromatography were used to further separate the resulting peptides and analyzed using nanospray tandem mass spectrometry (LC-MS/MS).

In this study, an inclusive HIV-associated CSF protein database containing about 500 non-redundant CSF proteins was established from pooled HIV-infected individuals. Among them, numerous secreted membrane and glycoproteins were identified to be specifically associated with HAND. The detection of specific CSF proteins in the HIV protein database reflects the disease physiological process that occurs in brain, which will have a significant impact on future efforts to find biomarkers by using generic proteins. Our pilot analyses of the dementia samples also revealed several proteins with significant differences between the dementia and the non-dementia groups of samples, which allow us to further discover the disease onset or progression through the potential biomarkers of HAND.

Mon Poster 62: Automated, Efficient TiO2 Magnetic Bead Capture of Isobaric Tandem Mass Tag (TMT6)-Labeled Phosphopeptides Coupled with Mass Spectrometric Detection

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The accurate measurement of phosphorylated proteins and peptides is crucially important due to their central role in cell signaling pathways. Unfortunately, phosphopeptides are typically present at very low levels in cell lysates. An efficient and robust pre-analytical enrichment step for phosphopeptides facilitates the understanding of these fundamental biological pathways. Coupled with phosphopeptide enrichment, mass tag labeling technologies allow the simultaneous quantitative monitoring of phosphopeptides in multiple samples with mass spectrometric detection. In this study, we compared quantitative profiles of cancer stem cell phosphoproteins and phosphopeptides by coupling enrichment and tagging technologies. Cell lysate proteins from six different treatments were initially enriched with phosphoprotein enrichment columns. The recovered phosphoproteins were digested with trypsin and subsequently labeled with isobaric Tandem Mass Tags (TMT6) at their amino termini and lysine residues. The six samples were pooled and the phosphopeptides were enriched by a combination of hydrophilic interaction (HILIC) chromatography and automated TiO2-enrichment on magnetic beads. The eluted phosphopeptides were analyzed on an LTQ-Orbitrap mass spectrometer and proteins were identified with Proteome Discoverer software. Relative peptide quantification resulted from the comparison of reporter ion abundance ratios from the TMT tags. Over 200 proteins were quantified and differential site phosphorylation was observed.

Mon Poster 63: Quantitative Analysis of Epidermal Growth Factor Receptor (EGFR) Phosphorylation in Response to Erlotinib Treatment

Guolin Zhang; Bin Fang; Umut Oguz; Richard Z Liu; Elizabeth R Remily; John M Koomen; Steven Eschrich; Eric B Haura
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EGFR is an important signaling molecule and drug target in solid tumors such as lung and colon cancer. Qualitative and quantitative of phosphorylation patterns of EGFR would increase the understanding of EGFR biology and responses to small molecule inhibitors. Using in-gel digestion and LTQ-Orbitrap- MS based proteomics in combination with immunoaffinity purification, we identified total 56 EGFR phosphosites including 13 phosphotyrosines, 27 phosphoserines and 16 phosphothreonines from different sources of EGFRs. This includes recombinant EGFR, immunoaffinity purification, immunoprecipitated EGFR and straight cell lysate. Twenty eight (28) phosphosite identifications (10Y, 13S, 5T) have over 80% peptide probability. Our result also shown that immunoprecipitation can increase the sequence coverage of protein identification. To monitor the phosphorylation of EGFR response to erlotinib, a small molecule EGFR tyrosine kinase inhibitor, we examined sites on EGFR using MS. We initially extracted the Extracted Ion chromatogram (EIC) from LTQ-Orbitrap-MS and liquid chromatogram multiple reaction monitoring (LC-MRM) raw data of IP and recombinant EGFR samples. Quantitative curves with good linearity for functional phospho- and non-phosphopeptides were then constructed. We employed this quantitative strategy to measure the change of EGFR phosphorylation in HCC827 lung cancer cells in response to increasing concentrations of erlotinib. Our results revealed the changes of the ratio of all previous profiled phosphopeptides to their non-phosphorylated counterparts. Some phosphosites were validated with immunoblotting using phosphospecific antibodies for EGFR. Additional results were validated by LC-MRM. In summary, we have established a strategy to profile and quantitate EGFR phosphorylation sites using MS. This strategy could be

useful in examining EGFR in tumor tissues and correlated with outcomes.

Mon Poster 64: Large-Scale Differential Proteome Analysis in Plasmodium Falciparum under Drug Treatment

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Proteome studies contribute markedly to our understanding of parasite biology, host-parasite interactions, and mechanisms of drug action. For most antimalarial drugs neither mode of action nor mechanisms of resistance development are fully elucidated although this would be important prerequisites for successfully developing urgently required novel antimalarials.

Here, we establish a large-scale quantitative proteomic approach to examine protein expression changes in trophozoite stages of the malarial parasite Plasmodium falciparum following chloroquine and artemisinin treatment. For this purpose SIL (stable isotope labeling) using 14N-isoleucine and 13C6,15N1-isoleucine was optimized to obtain 99% atomic percent enrichment. Proteome fractionation with anion exchange chromatography was used to reduce sample complexity and increase quantitative coverage of protein expression. Tryptic peptides of subfractions were subjected to SCX/RP separation, measured by LC-MS/MS and quantified using the novel software tool Census. In drug treated parasites, we identified a total number of 1,253 proteins, thus increasing the overall number of proteins identified in the trophozoite stage by 30%. A relative quantification was obtained for more than 800 proteins. Under artemisinin and chloroquine treatment 41 and 38 proteins respectively were upregulated (>1.5) whereas 14 and 8 proteins were down-regulated (<0.5). Apart from specifically regulated proteins we also identified sets of proteins which were regulated as a general response to drug treatment. The proteomic data was confirmed by Western blotting.

The methodology described here allows for the efficient large-scale differential proteome analysis of P. falciparum to study the response to drug treatment or environmental changes. Only 100 µg of protein is required for the analysis suggesting that the method can also be transferred to other apicomplexan parasites.

Mon Poster 65: Discovery of Blood Plasma Proteins Associated with Severe Burn Injury by Applying Large-Scale Quantitative Proteomics

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Quantitative proteomics analysis represents a unique challenge to clinical proteomics applications where large specimen cohorts are necessary to address patient-to-patient variations. Here, we present the first large-scale proteomic discovery of blood plasma proteins associated with severe burn injury by applying a recently reported quantitative methodology utilizing a stable isotope 18O-labeled "universal" reference sample for relative quantitation. The 18O-labeled "universal" reference was generated by pooling across all patient specimens and then spiking an equal amount of pooled reference into each unlabeled patient sample. The peptide/protein abundances could then be quantified based on 16O/18O abundance ratios following mass spectrometry analysis. The application of a stable 18O-labeled reference sample effectively enabled the large-scale quantitative study of a set of 10 healthy subjects and 15 severely burned patients (both at the early and later time points). We were able to confidently quantify 388 plasma proteins with at least two different peptides per protein. 110 of those quantified proteins had significant abundance changes in response to burn injury. The regulation of ~35 proteins observed in our study was in good agreement with previous inflammation literature, such as haptoglobin (HP) and histidine-rich glycoprotein (HRG) involved in the acute phase response pathway. We also identified ~50 proteins not previously known to be associated with burn response or inflammation. Significant canonical pathways represented by these proteins include acute phase response signaling, the complement system, and coagulation system. The elucidation of the proteins involved in the response to severe burn injury may help reveal novel biomarkers for predicting multiple organ failure and survival of burn patients.

Mon Poster 66: Candidate Cytokine Biomarkers for Major Depressive Disorder in Patient CSF Are Gender Specific: Implications for Biology and Therapeutics

Ofer Eidelman[1]; Harvey B. Pollard[1]; David Jacobowitz[1]; Meera Srivastava[1]; Catherine Jozwik[1]; Jonathan Savitz[2]; Paul Carlson[2]; Peixiong Yuan[2]; Wayne Drevets[2]; Husseini Manji[2]
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Major Depressive Disorder (MDD) costs the U.S. healthcare system between $233-$360B per year, and affects up to 15% of the U.S. population at least once in their lifetime. Since some symptoms of MDD have been correlated with "cytokine sickness", and certain proinflammatory cytokines have been reported to be in CSF or serum of MDD patients, we have searched for objective MDD biomarkers to complement the clinical diagnosis of MDD. Validated candidate cytokine biomarkers may indicate a rationale for novel MDD therapeutics. We have measured specific cytokine levels in cerebrospinal fluid (CSF) from MDD patients and healthy controls using Searchlight® and ELISA methods. We have found that the distributions of cytokine levels can be gender dependent in addition to being MDD status dependent. We have also found that panels of several cytokines are better predictors of MDD status, and that these panels are gender dependent.

Mon Poster 67: The Human Platelet Signaling Proteome Is Gender-Specific

Catherine E. Jozwik[1]; Ofer Eidelman[1]; Meera Srivastava[1]; Wei Huang[1]; Stephen W. Rothwell[1]; David M. Jacobowitz[1]; Jerry Wright[2]; Gregory P. Mueller[1]; Harvey B. Pollard[1]
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The incidence of cardiovascular diseases is ten-times higher in males than females, although the biological basis for this gender disparity is not known. However, based on the fact that anti-platelet drugs are the mainstay for prevention and therapy, we hypothesized that the signaling proteomes in platelets from normal male donors might be more activated than platelets from normal female donors. We tested this hypothesis using conventional 2-D gel electrophoresis and mass spectrometry to detect high abundance proteins, and a quantitative, large scale antibody microarray platform to detect low abundance proteins. Few high abundance proteins differed by gender. However, using data from the antibody microarrays, we found that platelets from male donors express significantly higher levels of signal transduction and intracellular signaling cascade proteins than platelets from female donors. In silico connectivity analysis showed that the 24 major hubs in platelets from male donors focused on pathways associated with megakaryocytic expansion and platelet activation. Examples included STAT3, RAC1, ERK1 and others. By contrast, the 11 major hubs in platelets from female donors were found to be either negative or neutral for platelet-relevant processes. Examples included c-MYC, p53, and the glucocorticoid receptor (GCR-alpha). Gender-specific expression levels of selected proteins were validated in specific instances by western blots. These validated proteins included IκBα; protein kinase C α, β & δ; p38; ERK1; beta integrin; STAT3; and caspase 7. The discovery of gender-specificity for the platelet signaling proteome is completely unprecedented, and suggests a fundamentally new biological mechanism for gender discrimination in cardiovascular disease.

Mon Poster 68: Changes in the Mitochondrial Proteome during Anesthetic Preconditioning and Ischemia

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Brief exposure to a volatile anesthetic such as isoflurane protects the myocardium from ischemia and reperfusion injury. We successfully used LC-MS/MS and enzymatic 18O labeling to define cellular mechanisms that may contribute to the protective effect of isoflurane on cardiac mitochondria during myocardial ischemia. Instrumented Westar rats were subjected to a 30-min occlusion and 15-min reperfusion. Isoflurane was administered for 30-min before a 15-min washout period and occlusion (isoflurane-ischemia) while control animals did not receive isoflurane (ischemia). Additional animals were subjected only to isoflurane without ischemia (isoflurane) or received no treatment at all (control). Cardiac mitochondria were isolated by differential centrifugation. Cellular proteins were isolated from lysed mitochondria and labeled with 18O. Pairs of samples were analyzed by tandem MS.

A total of 366 proteins were confidently identified, using the SEQUEST algorithm, from an average of three replicate experiments. Of the 366 proteins identified, 107 are currently annotated as mitochondrial proteins in the UniProt database. Using ZoomQuant software analysis tools, 32% of proteins annotated as mitochondrial proteins were quantified. A protein was considered to have a change in regulation if its ratio was at least two standard deviations away from the mean calculated from trials mixing two equal amounts of control mitochondrial proteins. A total of 31 mitochondrial proteins were consistently up or down-regulated during ischemic and preconditioned ischemic events in cardiac mitochondria. Interestingly after isoflurane treatment, subunits of the ATP complex were up-regulated after ischemia, in contrast to untreated mitochondria after ischemia. An up-regulation of the ATP complex could help to restore ATP levels faster after an ischemic event. Isoflurane also lead to a differential up and down-regulation of NADH-ubiquinone oxidoreductase (complex I of the electron transport chain) subunits in the absence of ischemia. Such distortion of complex I subunits could contribute to the production of ROS reported to be necessary for signaling during preconditioning. Finally heat shock protein 70 (hsp70) was significantly reduced in mitochondria after isoflurane exposure. Hsp70 has been demonstrated to be an important contributor to protection in preconditioning. Our data support the possibility that a translocation from mitochondria to the cytosol may be required for Hsp70 to have a protective effect.

Overall we have demonstrated that LC-MS/MS and enzymatic 18O labeling provides a powerful tool to monitor changes of mitochondrial proteins under various conditions of ischemia and reperfusion injury.

Mon Poster 69: Proteomic Fingerprints in CSF and Serum/plasma of HIV-infected Patients with Neurocognitive Disorders

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Introduction of Highly Active Anti-retroviral Therapy resulted in decreasing of numbers HIV-associated dementia (HAD) cases from 30% to 7%. Nevertheless, percentage of HIV-infected individuals suffering from neurocognitive impairments (CI) remains unchanged due to increasing percentage of milder forms of CI. Therefore, there is an urgent need for suitable biomarkers in cerebrospinal fluid (CSF) and sera to predict the onset and tempo of HIV associated cognitive disorders (HAND) which can be linked directly to advanced HIV infection. Recent advances of proteomics allowed systematic studies of CSF and serum/plasma which opened new ways to study viral-host interactions and which may provide new insight into treatment and disease monitoring. During recent years, we have used various proteomic approaches to study proteomes in clinical samples such as CSF and serum/plasma. To facilitate analysis of low abundance proteins, we removed the 6 most abundant proteins: albumin, IgG, IgA, 1-Antitrypsin, Haptoglobin, and Transferrin from CSF samples and 12 most abundant proteins from sera samples: 6 proteins listed above and additional 6: fibrinogen, a2-macroglobulin, IgM, orosomucoid, apolipoprotein A-I, apolipoprotein A-II. Most of our analyses were performed employing 2-dimensional electrophoresis (2DE) with Difference Gel Electrophoresis (DIGE). Most recently we used a combination of SELDI-TOF, weak cation exchange (WCX) chromatography and 1-dimensional electrophoresis (1DE). Protein identification has been performed using nano-liquid chromatography tandem mass spectrometry (nano-LC-MS/MS). Our primary validation method is quantitative western-blot analysis. In concurrent experiments we investigated a secretome of HIV-1 monocyte derived macrophages (MDM). Underlying rationale for these analyses was to link function of cells which serve as reservoirs of the virus in the brain with proteomic profile of CSF and/or serum/plasma.

We now report an overview of results of these studies. Within the CSF, proteins with regulatory functions (complement C3 and its fragments, neuronal cell adhesion molecule (NrCAM), cystatin C, vitamin D binding protein, clusterin, gelsolin, procollagen C-endopeptidase enhancer) were found to be differentially expressed and have been validated by Western-blot analysis. Within serum/plasma, proteins with regulatory functions such as complement C3, ceruloplasmin, afamin, prealbumin and gelsolin were found to be differentially expressed. Although cohorts of samples used for discovery and validation phases were too small to draw conclusions about utility of these proteins as diagnostic biomarkers, they provide new insights into host's response to the viral infection.

This work was supported, in part, by National Institute of Health Grants 1 R21 MH075662-01, 20RR 15635 from the COBRE program from the NIRR.

Mon Poster 70: Multi-Modal Proteomic Target Discovery and Orthogonal Confirmation of preclinical Diabetic Retinopathy Drug Development Biomarkers

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As a leading cause of adult onset blindness, diabetic retinopathy represents one of the most profound complications of diabetes. While recent advances have been made in pharmacotherapies for diabetic retinopathy, preclinical testing of potential therapies is hampered by the lack of reproducible biomarkers to monitor disease models and therapeutic efficacy. We have previously demonstrated duration-dependent retinal vascular permeability, apoptosis, and genomic changes with diabetes. The aim of this study was to identify proteomic alterations associated with functional dysregulation of the retina with diabetes that could eventually be used a surrogate endpoints in preclinical drug testing studies. A multi-modal approach of antibody (Luminex), electrophoresis (2-DIGE), and LC-MS (iTRAQ) quantitation methods was used to provide the broadest proteome coverage. The different technologies proved complimentary with limited or no overlap in the coverage between methods. Crystallin family proteins were the largest magnitude changes observed in this discovery work. These proteins were confirmed by orthogonal methods and were demonstrated to be regulated in a statistically significant manner in multiple sets of animals. For some changes, reversal/prevention with insulin treatment was demonstrated by immunoblotting. With the targets initially indentified and confirmed in this study, the resulting candidate biomarkers can undergo further validation and eventually be used in preclinical drug development to gauge pharmacoefficacy.

Mon Poster 71: Halogenated-Peptides as Internal Standards (H-PINS) for Liquid Chromatography Mass Spectrometry

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As the application for quantitative proteomics in the life science has grown in recent years, so has the need for more robust and generally applicable methods for quality control and calibration.

The reliability of quantitative proteomics is tightly linked to the reproducibility and stability of the analytical platforms, which are typically multi-component (e.g. sample preparation, multi-step separations, and mass spectrometry) with individual components contributing unequally to the overall system reproducibility. Variations in quantitative accuracy are thus inevitable, and quality control and calibration become essential for the assessment of the quality of the analyses themselves. Towards this end, the use of internal standards can not only assist in the detection and removal of outlier data acquired by an irreproducible system (quality control), but can also be used for detection of changes in instruments for their subsequent performance and calibration.
Here we introduce a set of halogenated peptides as internal standards. The peptides are custom designed to have properties suitable for various quality control assessments, data calibration and normalization processes. The unique isotope distribution of halogenated peptides makes their mass spectral detection easy and unambiguous when spiked into complex peptide mixtures. In addition, they were designed to elute sequentially over an entire aqueous to organic LC gradient, and to have m/z values within the commonly scanned mass range (300 to 1800 Da). In a series of experiments in which these peptides were spiked into an enriched N-glycosite peptide fraction (i.e. from formerly N-glycosylated intact proteins, in their de-glycosylated form), we show the utility and performance of these halogenated peptides for sample preparation and LC injection quality control, as well as for retention time and mass calibration. Further use of the peptides for signal intensity normalization and retention time synchronization for selected-reaction monitoring (SRM) experiments is also demonstrated.

Mon Poster 72: 2DE Analysis of Nasopharyngeal Proteins from Respiratory Syncytial Virus-Infected Infants

Yashoda M Hosakote; Paul Jantzi; Dana L Esham; Heidi Spratt; Alexander Kurosky; Antonella Casola; Roberto P Garofalo
The University of Texas Medical Branch, Galveston, Texas

Respiratory syncytial virus (RSV) is the most important cause of lower respiratory tract infections in infants and young children. Proteome analysis of nasopharyngeal secretions may be useful in the identification of biomarkers which discriminate (and thus predict) infants with different severity of RSV-induced respiratory illness. In this study, children ≤ 2 years of age with symptoms of respiratory infections were enrolled. Children were assigned a clinical diagnosis of upper respiratory infection (URI) alone or bronchiolitis, with or without hypoxia. Nasopharyngeal secretions (NPS) were analyzed for viral pathogens. Separation and analysis of NPS proteins was performed using high-throughput two-dimensional gel electrophoresis (2DE) coupled with mass spectrometry to identify differentially expressed proteins. Among more than 150 proteins identified, about 60 of them showed significant differences in abundance (with statistical variance of the spot volume within 95% confidence level) between RSV-infected infants with different severity of clinical symptoms. The majority of identified proteins were cytoskeletal proteins, as well as functional and regulatory proteins which are normally present in nasal cavity. We identified 15 proteins involved in free radical scavenging, molecular transport and cell death and 12 proteins involved in infectious disease and cancer. Interestingly, enzymes involved in detoxification and anti-oxidant activity, including superoxide dismutase and catalase, were expressed at lower abundance in NPS of patients with more severe disease. The differential protein expression in URI vs bronchiolitis indicate that multiple cellular pathways were involved in the process of RSV-mediated disease, suggesting that multiple protein targets for an effective therapeutic strategy.

Mon Poster 73: System-Wide Phosphoproteomics Reveals Signal Transduction Pathways Activated by Oxidized Phospholipids

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One of the major contributors to heart disease and stroke is atherosclerosis, a chronic inflammatory disease of the vessel wall characterized by accumulation of LDL, entry of monocytes, cell death and, in the final stage, thrombosis caused by plaque rupture. LDL minimally modified by oxidation, membranes of apoptotic cells, and atherosclerotic lesions, all contain active components of Ox-PAPC (oxidized products of the phospholipid 1-palmitoyl-2-arachidonyl-sn-3-glycero-phosphorylcholine). We previously identified Ox-PAPC as: i) an activator of endothelial-monocyte interactions, and ii) capable of regulating more than 1,000 genes in human aortic endothelial cell (HAEC) culture. Moreover, a recent report correlated levels of oxidized phospholipids in blood with the risk of artery disease. Despite the advances in understanding the role of oxidized phospholipids in atherosclerosis and other inflammatory diseases, the key signal transduction effectors activated by Ox-PAPC remain poorly characterized.

The goal of this study is to gain insight into the signaling events initiated by Ox-PAPC in aortic endothelial cells through phosphoproteomic profiling. To identify changes in phosphorylation induced by Ox-PAPC, we employed low passage bovine aortic endothelial cells (BAEC). We used antibody- and chemical affinity-based phosphopeptide enrichment strategies followed by mass spectrometry analysis with a LTQ-Orbitrap. We applied our custom software to locate and integrate both sequenced and unfragmented phospho-peptide peaks for chromatography alignment and peptide quantification. We also found that co-treatment with phosphatase inhibitors substantially facilitated the identification of otherwise transient phosphorylation events specific to Ox-PAPC activation.

Our results identified previously unreported changes in phosphorylation induced by Ox-PAPC, including phosphorylation events in receptor tyrosine kinases, transcription factors, and cytoskeletal proteins. We also identified specific signal transduction pathways activated by Ox-PAPC, including the coordinated increase in many phosphorylation events associated with tight-junction proteins. Immunoblot-based validation experiments confirmed many of the identified changes were conserved in both HAEC and BAEC. Furthermore, we found vascular endothelial growth factor receptor 2 (VEGFR2) to be responsible for the regulation of inflammatory and sterol metabolism genes induced by Ox-PAPC, revealing a new role of this receptor tyrosine kinase in the initiation of the atherosclerotic plaque.

Mon Poster 74: Assessment and Application of MaxQuant Software for the Quantitative Analysis of Breast Cancer Cells with Chemotherapeutic Agents using SILAC

Melanie Flint[1]; Brian Hood[1]; Torsten Ueckert[2]; Michael Rosenblatt[3]; John Rogers[3]; Thomas Conrads[1]; Bernard Delanghe[2]
[1]University of Pittsburgh, Pittsburgh, PA; [2]Thermo Fisher Scientific, Bremen, Germany; [3]Thermo Fisher Scientific, Rockford, IL

Breast cancer is one of the most frequently diagnosed malignancies in women and more than 50% of patients receive chemotherapy as part of the clinical management of this disease. Although a number of different options exist, selection of the most appropriate chemotherapeutic agent depends on many factors such as the type/stage/grade of the tumor, lymph node status and the age of the patient. An informed selection of the chemotherapeutic agent is particularly critical to achieve maximum efficacy in the therapeutic regimen. Two of the most common chemotherapeutics prescribed are doxorubicin and paclitaxel, each of which induce breast cancer cell death through different mechanisms of action; doxorubicin, an anthracycline antibiotic, intercalates DNA and prevents DNA replication whereas paclitaxel,

which is commonly used to treat metastatic breast cancer, is a plant alkaloid that binds beta-tubulin and hyper-stabilizes microtubules to induce cell cycle arrest . In this study, we sought to examine the proteomic response in MDA-MB-231 breast cancer cells exposed to doxorubicin and paclitaxel. This experiment employed a three-way stable isotope labeling by amino acids in cell culture (SILAC) strategy to enable this three arm comparison. Three different cultures of MDA-MB-231 cells were grown according to the following: no chemotherapeutic, DMEM supplemented with 12C6-Lys 12C614N4-Arg; doxorubixin-treated, DMEM supplemented with 13C8-Lys 13C614N4-Arg; paclitaxel-treated, DMEM supplemented with 13C6-Lys, 13C615N4-Arg. After a 48 h exposure, samples were combined in a 1:1:1 ratio and digested with trypsin. The peptide digest was analyzed by nanoflow reversed-phase liquid chromatography (nanoRPLC)-tandem MS with an LTQ-Orbitrap MS. To evaluate software alternatives and identify optimal data analysis settings, we assessed two software packages for the quantitative analysis of mixtures of control cell lysates grown in each of three isotope incorporation conditions and combined at specific ratios. We applied these applications and optimal settings for the analysis of the proteomic response of MDA-MB-231 breast cancer cells to these two chemotherapeutic drugs. Our data demonstrated that these two chemotherapeutic drugs that differ in their mode of action result in a dramatically different alteration of several proteins within breast cancer cells, including significant changes in proteins such as beta-tubulin isoforms and actin regulators.

Mon Poster 75: Proteomic Signature for Kidney Allograft Rejection in Serum and Urine

Meera Srivastava[1]; Ofer Eidelman[1]; Wei Huang [1]; Yelizaveta Torosyan[1]; Catherine Jozwik[1]; Harvey Pollard [1]; Roslyn Mannon[2]
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Introduction: Renal transplantation has become the preferred method of management for end-stage renal disease, with long term allograft loss being the major obstacle. The leading cause for renal allograft dysfunction is graft rejection, and potential treatments are based on a histological diagnosis. However, clinical experience indicates that graft rejection is prognostically challenging, even with intensive treatment. The problem is that markers for predicting graft rejection are limited in number and quite non-specific. A more useful approach to treating graft rejection would appear to be early, non-invasive identification of graft rejection.

Approach: Our new approach has been to use a high throughput, multiplexed diagnostic protein array platform for analyzing serum and urine samples from male and female patients. The patients were categorized based on their kidney function, as well as the presence and status of their kidney allograft.

Hypothesis: We hypothesize that proteins released from transplant cells and tissue can be detected in serum and urine, and used to create a set of stage-specific candidate serum and urine protein biomarkers for graft rejection.

Methods: Pooled sera or urine from normal patients, and patients with either stable function, acute rejection, or chronic rejection of kidney allograft (5-7 patients in each category) were labeled with the fluorescent dye Cy3, and assayed on antibody Microarrays. Robustness of comparisons of data from multiple spots on many microarrays was assured by mixing each sample with a Cy5-labeled internal control made of pooled normal sera or urine. Based on the antibody microarrays, dozens of serum and urine proteins were found to be specifically associated with the severity of the disease. We were also able to validate many of these biomarkers using a quantitative Reverse Capture Protein Microarray platform. To use this platform, serum or urine samples were spotted individually in serial dilutions on a glass slide, and all are probed simultaneously with a specific antibody predicted by the antibody microarray platform.

Results: We find that MUPP1, JAM1, integrin beta1 and Neurexin 1 are highly expressed in the serum of the stable function allograft recipients. By contrast, loss of SCARB1 and TIAL1 are characteristic of a failing allograft. However, urine levels of integrin beta 1 decreased, whereas of integrin beta 3 increased in acute and chronic types of rejection. Additionally, reduced excretion of the acidic phospholipid-binding annexin 11, and the lipid activated protein kinase C-like 2 (PKN2) protein emphasize the possibility that phospholipid signaling is activated in the failing allografts. We also linked several potential biomarkers in acute and chronic rejection of human kidney transplant to the tight junction pathway (KREGG pathway) using the ingenuity pathway analysis program, which ties together cell adherence, migration, and proliferation.

Conclusion and Interpretation: The present data suggests that numerous biomarkers in serum and urine can be linked to kidney allograft rejection. We conclude that detection of acute rejection by protein microarray technology offers a promising non-invasive tool for the surveillance of renal allograft recipients, and, potentially, for guiding the treatment for graft rejection.

Mon Poster 76: A Bioinformatics Suite for Clinical Proteomics

Julius C-Zar; Kiprotich Chemweno; Catherine Kimani; Edwin Mulwa; Michael Mwangi; Patrik Njougu; Gautam Saxena
Integrated Analysis Inc, Bethesda, MD

We report a bioinformatics suite for proteomics which addresses three areas: (1) integration, aggregation, and statistical derivations of heterogeneous proteomics data and the ad-hoc querying and visualization of such data; (2) storage and retrieval of terabytes of sensitive data in a secure, robust and managed manner on a hosted server; and (3) automation, management, and maintenance of peptide/protein searches on a hosted, high-end multi-processor Linux server.

(1) From Data Integration through to Data Presentation: Through Johns Hopkins University, Expression Pathology Inc., and a large children's hospital in Arizona, we have developed over the last two years a proteomics data warehouse software product called "Proteomics Analyzer Software System™" (PASS™) which we license to others. PASS:

(a) Intelligently summarizes search output data from multiple replicates, dozens of fractions, and thousands of search output files. This Summarizing includes derivations of relevant statistics and other calculations.

(b) Provides interface for entering sample annotation data

(c) Integrates tremendous amount of protein annotation data from public protein repositories

(d) Powerful PTM and point mutation analysis

(e) Powerful ad-hoc querying, reporting, and visualization, all through Excel

(2) Hosted Data Storage, Retrieval, and Management: We provide a hosted, secured data storage server which allows end-users to upload or download terabytes of valuable data (usually MS raw files) at up to 100 Mb/sec. Authorized users can upload/download data from any machine with an internet connection, but no end-user can overwrite or delete previously uploaded files. Further, to minimize data theft, we limit the number of individuals granted permission to download files. Finally, all transfers are logged and can be audited, and the transfer process is verified to ensure that all files were transferred successfully.

(3) Automating and Managing the Peptide/Protein Searches on a Hosted High-End Linux Server: We host a peptide/protein search solution on a high-end, multi-processor Linux server that is accessible via the internet. We have developed a graphical interface that allows end-users to submit multiple (e.g., 100s) MS raw file and associate them with a set of search parameters that they (or one of their colleagues) had previously saved. Equally important, the interface allows end-users to associate their MS raw files with a sample ID, run number, and fraction number, thus providing contextual information to the final search outputs. This contextual information is later used by PASS. The entire process is managed and maintained, thus ensuring that the latest search algorithms, database FASTA files, and other components are up-to-date.

All three of the above solutions can be used separately or together, as they are seamlessly integrated into a comprehensive bioinformatics suite for proteomics.

TUESDAY POSTERS

Posters to be set-up by 8:00 am Monday. Posters should be attended 1:30 – 3:30 pm at Monday Poster Session. Remove posters by 9:00 pm Monday eve.

Bioinformatics, 01 – 13

Tues Poster 01: Intensity Normalization for Label-Free Mass Spectrometry Data: A Case Study in Biomarker Discovery

Cindy Lou Chepanoske[1]; Timothy P. Bonnert[1]; Daniela M. Schlatzer[2]; Serge Ilchenko[2]; David Maahs[3]; Janet Snell-Bergeon[3]; Marian Rewers[3]; Mark R. Chance[2]

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The breadth of biomarker discovery studies employing mass spectrometry proteomic profiling techniques has expanded as the instrumentation platforms reach new heights of accuracy, specificity and dynamic range. The scientific rationales underlying this proteomics revolution require ever more complex experimental designs that must be mirrored by a corresponding increase in the analytical and informatics capabilities of public-domain or commercial software solutions. Indeed, many studies have recently been published in peer-review journals which demonstrate validated approaches for the statistical treatment of data to support discovery of peptide and protein targets for further verification. Label-free studies in particular, pose specific data analysis challenges as these studies are not limited in study size or design complexity, as can be the case with some labeling techniques. Also, as the data are represented as intensity measurements and not as intrinsic ratio values, run-to-run normalization is usually required to allow these data to be accurately compared.

The variety of sample matrices which may undergo various separation techniques, ranging from one-dimensional separation to two- and three-dimensional separation techniques prior to analysis, makes it difficult to impose a 'one size fits all' approach to intensity normalization. Here, we demonstrate the comparison of a variety of normalization procedures using label-free samples taken from a pilot study to discover urinary markers of diabetic complication which analyzed samples collected from the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study. This study contained 3 groups of clinical samples: non diabetic (Non DM), type 1 diabetic without microalbuminuria (T1DM) and type 1 diabetic with microalbuminuria (T1DM MA). Each of the 3 phenotypic groups contained 3 biological replicates and was analyzed using reverse phase separation label-free LC-MS/MS. A constant amount of yeast enolase was added to each sample prior to digestion with Lys C. The expression level of peptides derived from enolase is used to evaluate the effects of the intensity normalization prior to Analysis of Variance (ANOVA) with respect to false-positive detection. Using the Rosetta Elucidator® System, it is possible to rapidly compare multiple normalization methods, including variations in statistical cuts (trimming) of the data prior to normalization, in one analysis pipeline. Incorporation of a variety of intensity normalization routines, including methods used in the microarray field and implemented as BioConductor R libraries, in addition to built-in methods available in the Elucidator System will be presented.

Tues Poster 02: Run-to-Run Variability in LC/MS Based Proteomics

Xinjian Yan; Bhaskar Godugu; Stephen Stein

National Institute of Standards and Technology, Gaithersburg, MD

LC/MS based proteomics (LMBP) is now widely used in the field of life science. However, the combined effects of biological and technical variability, cause LMBP to suffer greatly from the problem of poor reproducibility. For the same sample, run in different laboratories can often produce results significantly different from one another. In our continuing effort to address this issue, and to develop standards and measures of variability, we are examining technical variability of LMBP. Here, we report measures of the under-sampling, ineffective sampling and mixture sampling (multiple components) of the eluting ion components.

The objective of this study is to find and describe the inconsistency of identified peptide ions in multiple runs by analyzing data of component clusters, sampling and identification. Component clusters are formed by analyzing m/z and retention values of MS peaks acquired from widely used instruments (LTQ or LTQ/Orbitrap are examined here). Ideally, one cluster corresponds to only one chemical component, which can be a peptide, modified peptide, contaminant, or any chemical additive used in the preparation of the sample. However, in reality, there are many mixed cases – a cluster may relate to two or more components. In this study, peptides are identified through matching LC/MS/MS spectra to NIST peptide spectral library by using SpectraST (an open-source spectra-matching library search tool).

For example, in one experiment a chicken egg yolk sample was examined nine times with the same experimental setting. Each of the nine runs identifies 1107 to 1164 unique peptide ions. The total unique peptide ions identified from the 9 runs are 1396. The number of these peptide ions occurred in all the 9 runs is 868 (62.2%). The other 528 (1396 – 868, 37.8%) ions are identified from 1 to 8 runs. The total missed identification number is 2282 (18.16%, 2282/(9*1396)) in the nine runs. Our analysis result shows that the 2282 missed identifications could be attributed to 1. mixture sampling, 351 (15%); 2. ineffective sampling 667 (30%); and 3. under-sampling 1254 (55%). These statistical data enable the development of quantitative measures of this inconsistent identification, its cause and distribution of identified peptide ions in multiple runs. Considering that many peptide ions are generally not even sampled in the measurement, the number of identifiable, but unsampled peptide ions is even larger, and estimates will be presented based on distributions of identifications as a function of signal strength. This work was supported in part by the National Cancer Institute of the National Institutes of Health.

Tues Poster 03: Quantitation of Protein Abundances in LC-MS Proteomics

Yuliya Karpievitch[1]; Jianhua Huang[1]; Jeff Stanley[1]; Navdeep Jaitly[2]; Wei-Jun Qian[2]; Thomas Metz[2]; Joshua Adkins[2]; Charles Ansong[2]; Richard Smith[2]; Alan Dabney[1]

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In tandem mass spectrometry, protein abundance measurements must be translated from MS peak heights (ion counts) of constituent peptides. However, this translation is complicated by many factors. MS intensities (peak heights or areas) are related to ion counts, but ion counts do not necessarily reflect absolute abundance levels. Furthermore, ion counts for different peptides of the same protein can vary greatly. Finally, many peptides that are observed in some samples are not observed in others.

A peptide that is seen in one sample may not be seen in another. In fact, out of all peptides identified in a collection of samples, it is common to have 20 − 40% of all attempted recordings missing. To the extent that a missing peak occurs randomly, without depending on what the peak's height would have been if observed, this problem can be easily addressed. However, in our data, the dominant mechanism leading to unobserved peaks appears to be "censoring", where an unobserved peak is unobserved due to its falling below detection limits. Failing to account for this censoring leads to biased abundance estimates, by overestimating means and underestimating variances. Standard statistical tools are available for producing unbiased estimates.

We present here tools for assigning protein-level abundance estimates in tandem mass spectrometry experiments. A statistical model is formulated that expresses protein abundance in terms of peptide level intensities. The model accounts for the fact that many peptide measurements will be unobserved, due to both random missingness and censoring. Model parameters are estimated using maximum likelihood. Emphasis is placed on the problem of comparing protein abundances between two or more groups.

Tues Poster 04: Evaluation of Partial Tryptic Cleavages Observed after In-Gel and In-Solution Digestion using High Mass Accuracy LC-MS/MS Analysis

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LC-MS/MS data from tryptic digests can be searched against pertinent protein sequence databases using full trypsin, partial trypsin or no-enzyme specificity. Use of full trypsin specificity minimizes search space and search times compared with partial tryptic and no-enzyme specificity, and it can result in reduced numbers of false positive identifications due to random chance. But if large numbers of partial tryptic peptides are actually present in the sample, use of full tryptic constraints will result in false positive identifications for all partial tryptic peptides, and some of these false positive identifications will yield high scores and appear to be true positives. Partial tryptic peptides can result from "ragged ends" caused by partial proteolysis in vivo or during sample workup. Another potential source of partial tryptic peptides is contaminating proteases present during tryptic digestion. The very high sensitivity of linear ion trap mass spectrometers has increased the dynamic range of LC-MS/MS detection to the point where even very minor additional proteolysis can result in detection of large numbers of partial tryptic peptides. We have observed large numbers of partial tryptic peptides are often produced by contaminating proteases present during the digestion. The use of hybrid ion trap mass spectrometers with a high mass accuracy detector, such as the LTQ FT Ultra and the LTQ Orbitrap, enables very high confidence identifications of both full and partial tryptic peptides after a partial tryptic database search. Secondary cleavages due to contaminating proteases present during the trypsin digestion can be distinguished from protein "ragged ends" by digesting the intact form of recombinant proteins isolated on 1-D gels. A systematic analysis of yields of partial tryptic fragments using in-gel digestion was used to evaluate multiple lots of sequence grade trypsin, different commercial sources, time of digestion, protein loads, and amount of trypsin used. Although low levels of chymotryptic-like cleavages have frequently been previously reported, the predominant secondary cleavages we observed in in-gel digestions were indicative of N-aminopeptidase activity. Although the yields of partial tryptic peptides after in-gel digestion were usually less than 5% the yield of full tryptic fragments, large numbers of partial tryptic peptides can usually be observed for abundant proteins present in either in-gel or in-solution digests. In addition to the substantial secondary cleavages that occur during trypsin digestion, samples such as plasma and serum have extensive numbers of ragged ends due to in vivo proteolysis or proteolysis during sample processing. When LC-MS/MS data from such samples are searched using full trypsin specificity, large numbers of apparent low abundance proteins are identified with good scores that are actually partial tryptic fragments of high abundance proteins. It is therefore generally advisable to use partial tryptic rather than full tryptic specificity when analyzing LC-MS/MS data to minimize high scoring false positive identifications. Such data can be subsequently filtered using full trypsin specificity if the minor partial tryptic peptides resulting from ragged ends or contaminating proteases are not of interest.

Tues Poster 05: MassQuest: A Comprehensive Proteomic Pipeline, Based on Robust Nonparametric Methods, for Biomarker Discovery in Different MS Platforms

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Using mass spectrometry (MS) for proteomic analysis is still challenging due to the large dynamic range and the high complexity of the molecules of interest. In addition, there are problems related to variations between the biological samples, resolution and accuracy of the MS instruments, and limitations in finding low-abundance molecules. In particular, independent MS studies to date have failed to reproduce the same biomarkers. All of these problems can be greatly reduced by improving existing analytical tools.

We present MassQuest, a Java package that provides a comprehensive pipeline for the analysis of large-scale proteomic obtained from different MS platforms. It is important to note that by different platforms we also mean low- and high-resolution 1D MS as well as 1D and 2D MS. The main theme in this pipeline is to use statistical and data analysis approaches that rely upon robust nonparametric methods when simple assumptions cannot be made. This allows the incorporation of prior knowledge about the data and mass spectrometer when applicable.

MassQuest includes multiple components with different algorithms. First, low-level signal processing component can be applied on 1D data in all MS platforms. Second, peptides are extracted in 1D by a model-based, platform-independent feature extraction approach, together with their m/z, intensity, and charge information. Finally, matching of the corresponding peptides across multiple MS data is performed by clique finding and optimization method. If LC-MS data is analyzed, the feature extraction step is followed by a robust estimation clustering method for LC-MS feature extraction. Local alignment based on a nonparametric kernel-type regression estimator is used to align the multiple LC-MS maps prior to matching.

We tested the algorithms implemented in MassQuest on different data and MS platforms and compared the results with the current state-of-the-art software. Using our approach, we found more true features in 1D MS platforms such as SELDI, MALDI and even tandem MS. In 2D MS, we found more features, more matches, and better correlation between replicated LC-MS experiments.

Tues Poster 06: New Developments in the ProteoWizard Open Source Software Library

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The ProteoWizard open source software project has two components: 1) a set of C++ libraries to facilitate cross-platform proteomics tools development and 2) a set of cross-platform data analysis tools created using those libraries. Since its initial release a year ago, the project has undergone significant development, with major contributions from the Spielberg Family Center for Applied Proteomics at Cedars-Sinai Medical Center, the Vanderbilt University Mass Spectrometry Research Center, Insilicos Software, and the Seattle Proteome Center at the Institute for Systems Biology.

The ProteoWizard libraries provide a current implementation of the HUPO-PSI mzML format; many software tools use this implementation for mzML support, including the Trans-Proteomic Pipeline (TPP). ProteoWizard currently supports many other formats, including Thermo
RAW, Waters RAW, Bruker FID/YEP/BAF, mzXML, MGF, with support for more formats under active development. ProteoWizard also now fully supports all the major operating system platforms with native compilers, including Microsoft Visual Studio on Windows, XCode on OSX, and gcc on Linux. The ProteoWizard API allows the proteomics software developer to write analysis code that is insulated from the data format and operating system (subject to the availability of data access libraries provided by the various instrument vendors).

New analysis modules have been added to ProteoWizard, including data smoothing, peak picking and charge state calculation, intensity thresholding, precursor recalculation, chromatogram calculation, and ms1 feature finding. All new modules include general interfaces to facilitate use and comparison of multiple algorithms.

The ProteoWizard tools incorporate all the new features of the library. For example, MSConvert is a general data format conversion tool that also allows filtering and processing for subsets

of spectra. MSPicture supports static visualization of ms1 data with demarkation of pepXML ms2 identification information. MSAcesss allows scriptable command-line access to spectra and metadata. SeeMS is a Windows program that provides straight-forward browsing and visualization of any data format supported by ProteoWizard.

In summary, ProteoWizard provides a mature and open source framework for proteomics tools development and is being actively used and extended by a community of developers internationally. The ProteoWizard libraries and tools can be found at http://proteowizard.sourceforge.net

Tues Poster 07: Mass Spectrometric Identification of the Arginine and Lysine deficient Proline Rich Glutamine Rich Wheat Storage Proteins

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Tandem mass spectrometry (MS/MS) of enzymatic digest has made possible identification of a wide variety of proteins and complex samples prepared by such techniques as RP-HPLC or 2-D gel electrophoresis. Success requires peptide fragmentation to be indicative of the peptide amino acid sequence. The fragmentation information is searched against the predicted fragmentation pattern of peptides generated from an in silico protein sequence database. Contrary to expectations, only a small number of the likely peptides are observed. Factors contributing to this include: incomplete enzymatic cleavage, few cleavage sites for the chosen enzyme, ion suppression in the mass spectrometer, composition of the database, and the search engine(s) used. Particularly challenging are proteins that are members of highly similar families containing repetitive sequence motifs and proteins with few tryptic cleavage sites. The wheat gluten proteins display these characteristics. The gluten proteins are of great commercial value and play a major role in human nutrition because their viscoelastic properties lend them to the production of a wide variety of food products. They are composed of the monomeric gliadins (&alpha, &gamma and &omega types) and polymeric glutenins. The glutenins are composed principally of the low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS). Homologs of these proteins originate from the three genomes of hexaploid bread wheat, whose family members are similar and have repetitive protein sequences with an abundance of glutamine and proline. To increase MS/MS protein sequence coverage of the wheat gluten proteins, we used several enzymatic cleavages (chymotrypsin and thermolysin in addition to trypsin), constructed a database containing wheat sequences derived from more four different wheat EST assemblies, analyzed spectral data with multiple search engines and used a commercially available software package (Scaffold) to combine and visualize all results.

The fewest peptide fragments and identified proteins were obtained with trypsin. The effectiveness of the different enzymes varied depending upon the protein type fragmented. Chymotrypsin was more effective than thermolysin in cleaving &alpha, &gamma and &omega gliadin proteins. However, thermolysin produced a greater number of peptides for the LMW-GS than did chymotrypsin or trypsin, while the HMW-GS were effectively cleaved by both thermolysin and trypsin. Database searching with X!Tandem and Mascot version 2.1 found 41 unique proteins using chymotrypsin alone, 45 with thermolysin and with trypsin 25. The effectiveness the different enzymes varied depending upon the protein type fragmented. Chymotrypsin was more effective than thermolysin in cleaving alpha gliadin proteins, gamma gliadins or the Omega gliadins. In contrast, thermolysin produced a greater number of peptides of the LMW-GS than did chymotrypsin or trypsin. However the HMW-GS were effectively cleaved by both thermolysin and by trypsin. Chymotrypsin cleavage often resulted in protein identifications with more peptide fragments than either of the other two enzymes. However thermolysin was much more effective than trypsin in producing protein identifications and a large number of fragments per protein. Combining the search results for all enzymes and search engines increased the overall sequence coverage and the number of proteins identified to 56.

Tues Poster 08: A Ranking-Based Scoring Function for Peptide-Spectrum Matches

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In order to analyze the large volumes of tandem mass spectrometry (MS/MS) proteomics data that is being generated these days we need to rely on automated algorithms that identify peptides from their mass spectra. An essential component of these algorithms is the scoring function used to evaluate the quality of peptide-spectrum matches (PSMs). We argue that since this problem is at its core a ranking task (especially in the case of de novo sequencing), it can be solved effectively using machine learning ranking algorithms.

We developed a new discriminative boosting-based approach to scoring that uses the RankBoost machine learning algorithm (Freund et al. 2003). Our scoring models draw upon a large set of diverse feature functions that measure different qualities of PSMs (e.g., the number of annotated peaks, the score of the peptide's path in a spectrum graph, the offset of peak m/z values from their expected location, etc.) Though many of the features used in our models are, each on their own, only slightly helpful at indicating if a PSM is correct or not, the RankBoost algorithm combines them into a powerful discriminatory scoring function for PSMs. The RankBoost algorithm also has the advantage that it only incorporates into its models features that are useful (i.e., they help it make fewer ordering mistakes on the training data); essentially performing its own internal ``feature selection''.

Our method improves the performance of our de novo sequencing algorithm beyond the current state-of-the-art; when making a single sequence prediction, our results are 10%-20% more accurate than the current high-performance algorithms PepNovo (Frank & Pevzner 2005) and the commercial software Peaks (Ma et al. 2003). Our algorithm also greatly enhances the performance of database search programs by both increasing the efficiency of tag filtration and improving the sensitivity of PSM scoring. When searching spectra from a human sample against the IPI sequence database, these improvements lead to a 20% increase in the number of peptide identifications compared to InsPecT (Tanner et al. 2005). Furthermore, our advanced PSM scoring function makes it practical to perform large-scale MS/MS analysis, such as proteogenomic search of a six-frame translation of the human genome (in which we achieve a reduction of the running time by a factor of 15 and a 60% increase in the number of identified peptides, compared to InsPecT). Our scoring function is incorporated into PepNovo+ which is available for download or can be run online at: http://bix.ucsd.edu.

Tues Poster 09: Shared Peptides in Mass Spectrometry Based Protein Quantification

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In analyzing the proteome using mass spectrometry, the mass values help identify the molecules, and the intensities help quantify them. The relative abundance of a peptide across samples is a proxy for the relative abundance of the parent protein. This is acceptable only when the peptide sequence is unique to the protein. By contrast, when a peptide is shared across proteins (Ex: homologues and isoforms), its abundance (and relative abundance) depends upon contributions from multiple proteins. For this reason, shared peptides have been traditionally disregarded in protein-level quantification analysis. However, this may significantly decrease the number of proteins for which abundance estimates can be obtained. While often unreported, a significant portion of the data is ignored.

In this work, we investigate the use of shared peptides which are ubiquitous (~50% of peptides) in mass spectrometric data-sets. In many cases, shared peptides can help compute the relative

amounts of different proteins that share the same peptide. Also, proteins with no unique peptide in the sample can still be analyzed for relative abundance across samples. Our work is the first attempt to use shared peptides in protein quantification, and makes use of combinatorial optimization to reduce the error in relative abundance measurements. We describe the topological and numerical properties required for robust estimates, and use them to improve our estimates for ill-conditioned systems. We also propose an extension to our approach for using shared peptides for detectability computation, and point to the importance of detectability values in extending the scope of shared peptide analysis in protein quantification.

Extensive simulations validate our approach even in the presence of experimental error. We apply our method to a model of Arabidopsis root-knot nematode infection, and elucidate the differential role of many protein family members in mediating host response to the pathogen.

Tues Poster 10: Peak Reassembly in Elucidator's® Image Processing Pipeline Corrects for Chromatographic Misalignments

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Label-free LC-MS analysis is gaining acceptance as a validated approach for molecular profiling and biomarker discovery in which multiple treatment groups may be analyzed together. However, as a component of systems medicine, LC-MS proteomics must be made more robust and the variability associated with liquid chromatography must be overcome.

Differential proteomics of LC-MS data relies significantly on the time-alignment of chromatograms across a set of mass spectrometric data. Successful time-alignment of chromatographic peaks allows for a quantified comparison of experimental treatment groups. This time alignment may not always compensate for strong variations in chromatography or changes in peptide elution order. Consequently, a single peptide ion may represent multiple peaks at different retention times. Conversely, small residual misalignment may complicate identification of unique peptide ions that have similar mass-to-charge ratios and retention times. In this case, the sample analysis may errantly combine distinct peptide ions with proximate chromatographic retention times.

Rosetta Biosoftware's Elucidator creates a composite representation of overlaid sample intensity images bounded by the dimensions of mass-to-charge ratio and retention time. Peak detection identifies regions of significant intensity that may contain all or some of the (mis-) aligned peaks from a single peptide ion. Detected peaks are subjected to an intelligent comparison of underlying data files (Henle and Hunt, U.S. Provisional Patent Application No. 61/052,584), which reveals corroboration and differentiates between distinct and misaligned peptide ions. Neighboring peaks are grouped into a combined set when the algorithm determines that peaks originate from the same peptide ion. Peak reassembly results in a revised set of peaks that represents the expression profile of unique peptide ions across the entire data set.

Tues Poster 11: Minimizing LC-MS Alignment Shifts through Quantitative Best Master Selection

Ernst S. Henle; Brandon T. Hunt

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Label-free LC-MS analysis is gaining momentum as a valid method for molecular profiling and biomarker discovery. However, if these analyses are to become true contributors to systems medicine, then there must be an effort to make these analyses more robust and eliminate the variability associated with liquid chromatography.

Rosetta Biosoftware's Elucidator® system enables label-free differential proteomics by facilitating a quantified comparison of experimental treatment groups represented by LC-MS data. Corresponding peaks amongst LC-MS runs are related to each other by their common m/z and retention time values. (Weng et al., Discover biological features using composite images. USPTO Application No.: 20070211928). Chromatographic retention times are not sufficiently reproducible and must be aligned for accurate quantification across LC-MS data sets.

Alignment proceeds by time-warping one LC-MS data set to a master LC-MS data set such that peaks on the time-warped LC-MS data set have the same retention times as those on the master LC-MS data set. Alignments are repeated for each LC-MS data set such that all data sets are time-adjusted to the designated master. Alignment fidelity depends on identifying the best data set that acts as master. We define the best master to be the master which requires the least amount of alignment from the other LC-MS runs. The Elucidator system carries out a trial alignment using a randomly selected master data set. The resulting trial time shifts at each retention time of each data set are offset corrected such that the median shift of each LC-MS data set is zero. The median shift values of all the offset-corrected LC-MS data sets constitute a best alignment shift curve. The LC-MS data set whose offset-corrected time alignment shifts most closely resemble this best shift curve is designated the master LC-MS data set.

Tues Poster 12: "The Australian Proteomics Computational Facility" Utilising a Large Computing Resource for Your Proteomics Research

Robert L. Moritz; Simon G. Michnowicz; Jagan Kommineni

Australian Proteomics Computational Facility, Ludwig Institute , Australia

Undoubtedly, the Achilles' heel of current proteomic analyses is the processing and validation of the vast volumes of MS/MS spectra obtained from current mass spectrometry instrumentation. Our approach for addressing these needs has been met by the construction of the Australian Proteomics Computational Facility (APCF) in 2007. The APCF is both a hardware and software solution for rapid, high volume proteomics mass spectrometry data analysis at a single online internet site. This shared national resource strategy addresses the needs of large scale automated mass spectrometry data analysis allowing access to multiple industry-standard algorithms installed on an advanced high-performance multi-processor computing cluster. To date, the facility has met with wide acceptance from the proteomics community with over 60,000 individual data searches being performed by many groups both in Australia and around the world, independent of distance from the facility based in Melbourne.

The APCF's large computational power of over 1000 CPU cores is managed by multiple interactive systems that direct resource allocation and act as gateway systems to the computational nodes. The APCF has constructed a sophisticated queuing system, which allows multiple concurrent MS/MS databases searches to be performed, without competition, using best utilization of computational resources combined with fault-tolerance to ensure the integrity of each submitted search. Specially designed web based software provides a unified interface to receive jobs from a range of tools including the APCF designed automated interface "UNITE", Mascot Daemon and standard web pages in a highly secure manner through 128 bit SSL encryption.

The APCF constructed interface "UNITE" is a world's-first tool that allows users to seamlessly access multiple industry-standard MS/MS databases searching algorithms (Mascot, X!!Tandem and OMSSA) remotely and simultaneously at the APCF. In addition, a unique result file download utility Mass Extraction Download Information Centre (MEDIC) allows any number of result files to be downloaded to the user's PC for subsequent downstream analysis. UNITE is a complete automation utility where raw instrument files are automatically converted from many different instrument manufacturers before database searches are performed by single algorithm searches or simultaneously submitting them to multiple algorithm searches at the click of a button. APCF also provides access to many regularly updated sequence databases, including SwissProt, IPI and the LudwigNR, a non-identical database of over 7.5 million entries.

This unique world's first integrated approach to sharing of proteomics computing and databases has placed Australia at the

forefront of efforts to identify the proteins associated with the early detection of major human diseases as well as many other research programs including plants, animals and microbes requiring proteomic analysis. In addition, the APCF gathers together expertise to provide leadership for proteomic data interpretation on locally generated data. This data can also be used in contributions to other world-wide large-scale proteomic efforts. The APCF can be accessed by a secure user account which can be obtained from the APCF at www.apcf.edu.au The APCF is open to all Australian and New Zealand researchers as well as expanding the usage to other countries.

Tues Poster 13: MSProcess – Mass Spectrometry Identification and Annotation Pipeline

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The advancements of proteomics technologies have led to a rapid increase in the size of datasets as well as the rate at which they are generated. We rely on strong bioinformatic platforms to provide accurate identifications for analysis in a high-throughput environment. We have developed MSProcess, a platform for the processing and analysis of proteomics LC-MS/MS data. This analysis pipeline provides a full solution to the computational challenges resulting from large-scale proteomic experiments. In an effort to promote data exchange and release within the proteomics community, the database back-end is consistent with the Proteome Experimental Data Repository (PEDRo) schema and the analysis follows guidelines determined by the Proteomics Standards Initiative (PSI). The pipeline is organized into five modules: 1) organization and standardization of the results from the search engines including SEQUEST and Spectrum Mill; 2) peptide and protein validation, 3) clustering and isoforms determination of proteins; 4) full annotation utilizing BLAST results, ontologies, protein domain, and pathway resources; and 4) quantification using the Normalized Spectral Abundance Factor (NSAF).

Databases and Systems Integration, 14 – 17

Tues Poster 14: The Protein Structure Initiative Structural Genomics Knowledgebase

Margaret J. Gabanyi[1]; John D. Westbrook[1]; Wendy Tao[1]; Raship Shah[1]; Andrei Kouranov[1]; Torsten Schwede[2]; Konstantin Arnold[2]; Florian Kiefer[2]; Lorenza Bordoli[2]; Michael Podvinec[2]; Jurgen Kopp[3]; Paul D. Adams[4]; Lester G. Carter[4]; Wladek Minor[5]; Rajesh Nair[6]; Joshua La Baer[7]; Helen M. Berman[1]
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The Protein Structure Initiative (PSI) has produced more than 3300 protein structures in a high throughput manner. In order to achieve this, new technologies for all aspects of the structure determination and analysis pipeline have been developed. The strategies used for target selection by PSI centers have resulted in the structural determination of many novel sequences, and an increase in the structural coverage of protein families.

The PSI Structural Genomics Knowledgebase (PSI SGKB) gives centralized access to the structures, annotations, models, protein production protocols, materials and technologies catalyzed by the PSI efforts. In collaboration with the Nature Publishing Group, the PSI SGKB expanded to become the Structural Genomics Gateway that provides a research library, editorials about new research advances and technologies, news, and an events calendar to present a broader view of structural biology and structural genomics. A description of how this resource can be used for enabling biological research will be given.

The PSI SGKB is funded by the NIGMS.

Tues Poster 15: NCBI Peptidome: A New Data Repository for Mass Spectrometry Proteomics Experiments

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Building on extensive experience in creating biological scientific data repositories to provide an integrated approach to the use of gene and protein sequence information, the National Center for Biotechnology Information (NCBI) has created a new project to collect and distribute mass spectrometry peptide and protein identification data. Results and conclusion-level information are captured, together with sufficient raw data and descriptive information to enable understanding of the experiment and analysis of the underlying data. The results are organized into Studies and Samples. A Sample describes all results common to a given biological sample, while a Study organizes Samples into the meaningful datasets which make up an experiment. In addition to data storage, web-based interfaces are available to help users query, browse and download individual peptides, proteins, or entire studies. The goal is to make data submission as simple as possible, while encouraging a high level of experimental annotation. The burden of data submission is minimized by accepting common file formats from which required information is extracted. Data submission involves filling out a spreadsheet with basic descriptive information and packaging it along with original peak list files and output files from the search engine analysis programs. Results are integrated with other NCBI data using the Entrez cross-database search system, allowing dissemination of information beyond the MS Proteomics community. In addition, NCBI and the European Bioinformatics Institute, together with the other ProteomExchange members, are also working on data sharing and distribution.

Tues Poster 16: Improved Support for Targeted Proteomics Workflows in PeptideAtlas

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The PeptideAtlas is a publicly accessible resource containing observed peptides and associated annotations derived from a large number of contributed tandem mass spectrometry (MS/MS) datasets. The data are processed through a consistent and rigorous analysis pipeline, which permits robust identifications and the computation of reliable false discovery rates. Currently there are public PeptideAtlas builds for 7 different organisms, as well as several builds that contain a related subset of data, such as data from glycocapture experiments, from a specific biological source such as plasma, or from selected reaction monitoring (SRM) triggered MS2 experiments obtained solely from triple-quad instruments.

SRM, or MRM (multiple reaction monitoring), especially scheduled SRM, allows researchers to see more deeply into the proteome by allowing them to focus their MS analysis cycles on peptide ions of interest. Recently we have focused on providing tools to allow researchers to leverage the considerable amount of data in PeptideAtlas in order to help design and carry out SRM experiments. One such feature allows users to extract potential SRM candidates from high-quality consensus libraries. By entering information such as amino acids to be included or excluded, precursor mass limits, protein accessions, etc, users can tailor the list of transitions to their needs. Another feature allows for the storage and retrieval of sets of user-validated and other high quality precursor/fragment (Q1/Q3) ion pairs. In addition, various experimental information is also stored with these sets of transitions, such as instrument type, collision energy,
retention time, and relative peak intensity. Finally, we have built various features into the peptide- and protein-level PeptideAtlas interfaces that help users determine which
peptides are the most suitable for SRM experiments, notably the ability to align and visualize closely related groups of proteins, which can help establish which peptides are truly proteotypic.

Collectively, these new features enhance the value of PeptideAtlas as a resource for researchers conducting targeted proteomics experiments. To maximize this benefit we are working to integrate the PeptideAtlas transition database with software tools used for SRM analysis, and providing the transition information in a useful and standardized format.

Tues Poster 17: The Protein Information and Property Explorer: A Rich-Client Web Application for the Management and Functional Exploration of Proteomic Data

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Mass spectrometry experiments in the field of proteomics produce lists containing tens to thousands of identified proteins. With the Protein Information and Property Explorer (PIPE) the biologist can acquire functional annotations for these proteins and explore the enrichment of the list, or fraction thereof, with respect to functional classes. These protein lists may be saved for access at a later time or different location. The PIPE is interoperable with the Firegoose and the Gaggle, permitting wide-ranging data exploration and analysis. The PIPE is a rich-client web application which uses AJAX capabilities provided by the Google Web Toolkit, and server side data storage using Hibernate.

Glycoproteomics, 18 - 21

Tues Poster 18: Rapid Bioinformatics Analysis of Glycopeptides from Diabetes Type 1 Patients

Keld Poulsen[3]; Flemming Pociot[2]; Niels Heegaard[3]; Martin R Larsen[1]; Lene Jakobsen[1]
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Strategies for biomarker discovery increasingly focus on protein and peptide expression patterns in biological fluids. Post-translational modifications (PTM) contribute significantly to pattern complexity and thereby increase the likelihood of obtaining specific biomarkers for diagnostics and disease monitoring. Glycosylation is a common PTM that plays a role e.g. in cell adhesion as well as cell-cell and receptor-ligand interactions. Abnormal protein glycosylation has important disease associations and the glycoproteome is therefore a target for biomarker discovery.

We have recently developed a simple and highly selective strategy for purification of sialic acid-containing glycopeptides (the sialiome) from complex peptide mixtures (Larsen MR et al., MCP 2007, 6(10), 1778-1787). The approach utilizes a highly selective, high affinity of sialic acids for titanium dioxide under specific buffer conditions. In the current study this approach was applied to plasma samples from Diabetes Type 1 patients.

Here we show how ProteinCenter™, a new bioinformatics tool, can be used to analyze glycopeptides from the sialiome of patients with early onset of Diabetes Type 1. Peptides and proteins were identified from plasma samples using tandem mass spectroscopy and Mascot database search. The results were processed in ProteinCenter™ and a total of 109 unique peptides were identified giving 83 unique proteins. ProteinCenter™ revealed that more than 85 % of the proteins either were annotated in GO as extracellular or membrane-associated or contained predicted signal peptides. Moreover, 69 of the 83 identified proteins had at least one deamidation and 75 deamidated Asn-residues were in an N-linked glycan motif (NxS or NxT).

Statistical GO analysis with ProteinCenter™ showed a significant raise in molecular function (MF) and biological process (BP) terms associated with the sialiome and Diabetes Type 1: glycoaminoglycan binding (MF), lipid transporter activity (MF), acute inflammatory response (BP) and B cell mediated immunity (BP).

These findings could potential lead to a more targeted search for Diabetes Type 1 biomarkers.

Tues Poster 19: Cell Surface and Secreted Protein Profiles of Human Thyroid Cancer Cell Lines Reveal Distinct Glycoprotein Patterns.

Bruce Macher; Arthur Arcinas; Ten-Yang Yen; Nicole Haste; Angela Castanieto
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Cell surface proteins are important therapeutic targets. Shed forms of these proteins and secreted proteins can serve as biomarkers for diseases, including cancer. Thus, identification of cell surface and secreted proteins as therapeutic targets and biomarkers has been a prime area of interest in the proteomics field. However, technical difficulties have hampered efforts to effectively isolate and identify cell surface and secreted proteins. Many cell surface and secreted proteins are known to be glycosylated therefore, a proteomics strategy targeting these proteins was applied to obtain proteomic profiles from various thyroid cancer cell lines that represent the range of thyroid tumor types. In this study, we oxidized the carbohydrates of secreted proteins and those on the cell surface with periodate and enriched them via coupling to hydrazide resin. The glycoproteins were identified from tryptic peptides and N-linked glycopeptides released from the hydrazide resin. Analyses of the complex mixtures of peptides were carried out by two-dimensional liquid chromatography-tandem mass spectrometry in combination with the gas phase fractionation. Cell surface and secreted proteins were isolated and characterized from five well characterized thyroid cancer cell lines derived from papillary thyroid cancer (TPC-1), follicular thyroid cancer (FTC-133), Hürthle cell carcinoma (XTC-1), and anaplastic thyroid cancer (ARO and DRO-1). An average of 150 glycoproteins were identified per cell line, of which approximately 60 percent are known cell surface or secreted glycoproteins. The usefulness of the approach for identifying thyroid cancer associated biomarkers was validated by the identification of glycoproteins (e.g. CD44, galectin 3 and metalloproteinase inhibitor 1) that have been found to be useful markers for thyroid cancer. Among the glycoproteins identified are ones that are commonly expressed by all of the cell lines, ones that are expressed in more differentiated thyroid cancer cell lines (follicular, Hürthle cell and papillary), ones that are expressed by cell lines derived from tumors of the most poorly differentiated and fatal forms of thyroid cancer (i.e. anaplastic), and those that are exclusively expressed by a specific cell line. Antibodies to glycoproteins that are associated with these four categories of glycoproteins are being evaluated with lysates from the cell lines by Western blot. Our objectives are to independently validate the results obtained by mass spectrometry, and to select antibodies that can be used to evaluate these potential glycoprotein biomarkers in normal, benign and malignant thyroid tissues.

Tues Poster 20: Identification of Human Breast Cancer Marker Candidates in Plasma Based on Selection by Glycan Targeting Antibodies and Lectins

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It is well known that glycoprotein modifications can be aberrant in cancer patients. Moreover, the glycans involved play a role in the loss of cellular adhesion, metastasis, and secondary tumor colonization. Some of the more prominent forms of modification implicated are in the addition of Lewis x (Le^x) antigen, sialylated Le^x (sLe^x) antigen, α(1,6)-fucosylation, N-linked β(1,6)-branching, and the appearance N-acetylgalactosamine terminal oligosaccharides. The focus of this paper is on the use of glycan targeting antibodies and lectins to select, enrich, and identify glycoproteins carrying these abnormalities from a small population of breast cancer patients. Glycoproteins thus selected were identified and cancer associated changes in their concentration quantified through multiple, independent methods involving either reversed phase chromatographic fractionation of the glycoproteins, shotgun proteomics, iTRAQ labeling, and label free analysis of tryptic peptides by liquid chromatography, MALDI-MS/MS, and ESI-MS/MS. Approximately 1/3 of the glycoproteins selected directly from plasma without abundant protein removal through antibody targeting were been found to change three fold or more in

breast cancer patients. This very high hit ratio validates the efficacy of targeting known cancer associated glycans as a way to recognize glycoprotein marker candidates. Of the targeting agents examined, anti-Le^x (IgM), anti-sLe^x (IgM), *Helix pomatia agglutinin* (HPA), *Lycopersicon esculentum lectin* (LEL), *Aleuria aurantia lectin* (AAL) and *Lens culinaris agglutinin* (LCA) were among the more efficacious. In total, 21 breast cancer biomarker candidates were identified through this glycan targeting process. Validation of these marker candidates and determining their mechanistic role in breast cancer is being undertaken in a larger, more diverse patient population.

Tues Poster 21: In-Depth Urinary N-Glycoproteome Profiling

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Due to its simpler complexity compared with blood, urine is a desirable body fluid for diagnosis and classification of diseases, particularly of the prostate and bladder. However, an in-depth understanding of the urinary proteome remains elusive due to technical limitations. We recently developed an approach for urinary N-glycoproteome profiling, which combined hydrazide-based N-linked glycopeptide enrichment, Off-Gel electrophoresis (OGE) fractionation, and high-performance LTQ-Orbitrap hybrid mass spectrometry.

In this study, proteins from 3 pooled urine samples (normal male, prostate cancer patients, and normal female respectively, 200-300 ml of each) were prepared by TCA precipitation, and digested into peptides using trypsin. N-linked glycopeptides were enriched via the standard hydrazide-based glycopeptide capturing approach developed in our group. The enriched N-linked glycopeptides were further fractionated into 24 OGE fractions (pH3.0—10.0) and analyzed on a LTQ-Orbitrap hybrid mass spectrometer. Acquired MS/MS spectra were searched against the International Protein Index (IPI) human protein database using SEQUEST.

In this way, we identified a total of 1,440 unique N-linked glycoproteins (i.e. containing one or more N-X-S/T N-glycosylation motifs) from 3 pooled urine samples, using a PeptideProphet cut-off score of ≥0.9 (corresponding to a false-discovery rate at the peptide level of ~1.3%). And 96 of them are identified only in prostate cancer patient urine. This data set thus represents the largest urine N-glycoproteome data set published to date.

Among these proteins, 507 were identified in previous urine proteome studies—including the largest and most comprehensive study of the human urinary proteome to date (Adachi et al., 2006, Genome Biol., 7: R80; 1,543 proteins in total). On the other hand, the overlap of this urine N-glycoproteome dataset with the current human plasma build of PeptideAtlas (http://www.peptideatlas.org, as of 2007-04, which includes 16,100 distinct IPI entries) is 737 proteins (i.e. ~51% of our urine glycoproteome dataset). Thus these relatively low overlaps for the new data with the other pre-existing urine/plasma proteome data suggests that the newly identified N-linked glycoproteins in our data are more likely to be enriched for lower abundance urine proteins. These data also indicate that the complexity and dynamic range of the urinary glycoproteome may be much higher than previously thought.

We assume that the candidate biomarkers most relevant to prostate cancer will likely come from the pool of extracellular and membrane proteins that can be detected and quantified in urine. Thus we performed Gene Ontology (GO) cellular component (CC) analyses to evaluate biological sources for the proteins identified from our data. This revealed, as was expected, that there was a significant enrichment for extracellular, cell surface, secreted, and membrane-associated proteins in our new urinary glycoproteome data (significance cut-off $P<0.05$).

The 96 N-linked glycoproteins which we identified only in prostate cancer patient urine give us a very good starting point to profile candidate biomarkers relevant to prostate cancer. Further evaluation on these promising findings may help to assist in building a urine-based screening method to improve the diagnostic approaches for prostate associated cancer diseases.

Mass Spectrometry Advancement, 22 – 25

Tues Poster 22: Improved In-Gel and In-Solution Protein Digestion with the Mass Spectrometry-Compatible Surfactant

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Promega Corporation, Madison, WI

Reliable mass spectrometry based protein identification requires complete protein solubility, good protease digestion and efficient peptide recovery prior to analysis. Optimization of these basic sample preparation steps is critical to ensuring consistent data quality. In effort to improve sample preparation processing we have developed an acid- and thermo-labile surfactant; Sodium 3-((1-(furan-2-yl)undecyloxy)carbonylamino) propane-1-sulfonate (ProteaseMAX™) that demonstrates improved protein solubilization, protease digestion and peptide recovery. In contrast to common surfactants such as SDS this surfactant does not inhibit protein digestion and does not interfere with mass spec analysis or liquid chromatography.

Use of the surfactant for in-gel protein digestion enabled significant improvements in procedure and quality of mass spec data. In-gel digestion normally involves overnight incubation followed by laborious peptide extraction. In the presence of the surfactant, digestion required no longer than one hour. In addition, during the digestion process the surfactant facilitates peptide extraction eliminating the need for post-digest extraction and drying. As a result, protein digestion and peptide recovery is complete in a single one hour step. In-gel digestion data quality is significantly improved by the recovery of longer peptides that typically remained trapped in gel under conventional conditions. With surfactant aided digestion and extraction peptides in the range of 2500 – 4000Da are routinely recovered in quantities sufficient for MS/MS analysis. An investigation of an assortment of proteins of mouse or human origin showed that this simplified protocol provided reliable and robust protein identification with increased sequence coverage in many cases.

The surfactant also appears beneficial for in-solution protein sample preparation. The primary benefits being: its ability to solubilize and denature complex mixtures of hydrophobic proteins (i.e. membrane proteins) under mild conditions while providing a milieu for efficient digestion and subsequent mass spec or LC analysis. While the surfactant is useful for complex mixtures on its own we have also investigated its use with chaotrophes such as Urea and found that the surfactant provides complimentary results and thus increases proteome coverage. Use of both agents for protein sample preparation allowed increased coverage of a mouse heart membrane protein extract by 70% as compared to coverage achieved with use of either agent alone. Experiments with model peptides and protein showed that the surfactant also minimized protein and peptide loss due to absorption to laboratory plastic ware.

Tues Poster 23: Moving from Biomarker Discovery to Validation: Automated Optimization of Triple Quadrupole Parameters for Nanoflow LC/MS

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Biomarker discovery approaches rely on being able to detect peptides by LC/MS/MS, then using database searching to identify the proteins. This leads to a set of putative protein candidates that must then be validated by performing targeted quantitative analyses with patient samples to assess the validity of these potential biomarkers. The most common MS/MS approach has been to generate a highly specific and sensitive triple quadrupole MRM-based assay looking for those same peptides seen in the discovery process. Typically one or more peptides from each

candidate protein will be targeted and selected. The appropriate transitions are then optimized for the selected peptides, which can be a time-consuming process. Because the traditional discovery approach has typically used ion trap based methodology, the ability to use the peptide fragment information from the discovery process in order to generate the MRM-based assay has been somewhat limited. If the MS/MS and collision energy information obtained in the discovery process is available and relevant for the MRM-based assay, this can greatly facilitate the optimization; however, this approach requires that the discovery and validation steps be performed on mass spectrometers that produce similar tandem mass spectra.

This work examines a nanoflow chromatography-based optimization process that uses the information available from a biomarker discovery experiment run on a QTOF to facilitate forming a validation MRM-based workflow on a triple quadrupole system. A comparison of automated and manually-obtained optimization values will be presented for selected peptides. In addition, the impact of retention time based MRM on the LOQ will be examined with a panel of peptides.

Tues Poster 24: Isoelectric Focusing on Digital ProteomeChip Coupled with High Throughput, In-Gel Trypsin Digestion and On-Line Peptide Extraction for Rapid MS Analysis

David Sarracino[1]; William Skea[2]; Russell Garlick[2]; Jim Dasch[2]; Malcolm Pluskal[3]; Bryan Krastins[1]; Taha Rezai[1]; Amol Prakash[1]; Mary F Lopez[1]

[1]ThermoFisher BRIMS, Cambridge, MA; [2]Protein Forest, Lexington, MA; [3]Consulting Services, Acton, MA

A novel advance in separation technology provides for the discrete separation of proteins and peptides by their isoelectric point (pI). The Digital Proteome Chip (dPCTM) separates proteins into individual pI fractions, each in a separate gel plug. A dPC™ consists of 41 hydrogel features, each at a specific pH. During the separation process, charged proteins or peptides migrate in an electric field perpendicular to the chip and become trapped in gel plugs with a pH at or near their pI. Once the separation is complete, the gel plugs can be processed for identification by a variety of detection methods, including Western blot and mass spectrometry. Advantages of the technology include speed of separation (30-60 min), resolution and high-throughput. In this report, we describe a sample processing method that streamlines and automates the downstream processing of the gel plugs prior to MS. Using this method, in-gel trypsin digestion and desalting steps are carried out in a 96 well plate processed with an autosampler coupled to the mass spectrometer. In addition, the protein capacity of the dPC gel plugs (0.5-2 g) is calibrated to interface with nanospray LC-MS.

Tues Poster 25: Electrophoretic Enrichment and Fractionation of Low Molecular Weight Proteins for Bottom-Up Proteomics

Wes E. Steiner; Ben B. Katz; Jeremy L. Norris

Protein Discovery, Inc., Knoxville, TN

The analysis and characterization of complex biological samples using mass spectrometry is contributing greatly to our understanding of the biological processes underlying human health. These techniques are increasingly becoming commonplace in the study of many biological problems. In spite of this increasing use, the sample preparation of biological samples for bottom-up proteomics still remains a significant bottleneck. Many chromatographic techniques, including immunoaffinity depletion columns, ion-exchange, and reversed-phase chromatography, are used in a variety of configurations to mitigate the problems of limited dynamic range and ion suppression that prevent a comprehensive analysis of these important samples. The reduction of sample complexity, removal of interfering species (e.g. high mass protein, salts, detergents, and etc.) and the concentration of low abundant analytes are all required in order to maximize the quality of resultant proteomic data. To that end, this study describes the use of an electrophoretic sample preparation system that rapidly (< 1 hr) depletes high molecular weight proteins, enriching the low molecular weight fraction (< 40 kDa) of the proteome of up to 96 simultaneous complex biological samples for high-throughput bottom-up mass spectrometric proteomic analysis. The advantages and limitations of this approach were investigated.

The low molecular weight proteins of of bovine liver extracts were electrophoretically enriched using the Passport™ 1200 Sample Prep System and Cartridge Kits (Protein Discovery, Inc., Knoxville, TN) according manufacturers protocol. The fractions were digested using trypsin. Samples were analyzed in triplicate using an Agilent 6300 ion trap mass spectrometer. The resulting data was searched using Spectrum Mill.

By controlling the electrophoretic conditions of the sample preparation, high molecular weight proteins such as albumin (~ 66 Da) were controllably depleted from samples, enriching the samples for small proteins. Bovine liver proteins prepared with the Passport 1200 sample preparation system showed 3x increase in the number of identified peptides originating from proteins of molecular weight below 40 kDa. The advantages of electrophoretic sample enrichment include: tunable depletion of high molecular weight proteins, femtomole level sensitivity, and reproducible protein coverage. The applications of this approach to the analysis of low molecular weight proteins will be presented along with protocols for use.

Membrane Proteomics, 26 – 30

Tues Poster 26: Functional Proteomics Analysis of Lung Endothelial Plasma Membranes Identifies GPCRs Which Cause Ligand Induced Budding of Caveolae

Noelle M Griffin; Phil Oh; Sabrina Shore; Halina Witkiewicz; Jan E Schnitzer

Sidney Kimmel Cancer Center, San Diego, CA

Caveolae are membrane micro domains, which are involved in vesicular transport across the plasma membrane. Caveolae bud from plasma membranes (PM) to form free carrier vesicles through a "pinching off" or fission process driven by GTues hydrolysis. Recent work suggests that caveolae are involved in receptor-mediated endocytosis and/or transcytosis of molecules and contain the molecular machinery apparently necessary for regulated, receptor-mediated endocytosis and/or transcytosis of select ligands via vesicle budding, docking, and fusion. Furthermore, we previously demonstrated that Gq is required for the fission process. Therefore we preformed a comprehensive proteomic analysis of rat lung endothelial cell PM and their caveolae to identify G-protein coupled receptors (GPCRs) that may be involved in ligand-induced budding.

Isolated PM and caveolar proteins were separated by SDS-PAGE gel electrophoresis. The entire gel line was cut into approximately 60 bands, trypsin digested and peptides analyzed by 2D-LC-MS/MS analysis using both LCQ and LTQ mass spectrometers. 95% analytical completeness of each sample was reached though multiple replicate analysis such that additional replicate measurements contributed non significant protein identifications. Raw ms data was searched against a rat and human database using the Sequest algorithm. Identified proteins, based on optimized Sequest scores and a minimum of 2 unique peptides per protein, were compared to the GPCR datasets downloaded from www.expasy.org (July 08 version, containing 2963 entries, 19 species) to facilitate identification of such receptors in our dataset. Localization of the receptors to the PM and/or caveolae was confirmed by western blot and electron microscopy. To test for the functional significance of receptor localization in caveolae, receptors were activated by the addition of known ligands for receptors to an in vitro- reconstituted, cell-free assay developed for examining the budding of caveolae from the PM.

Our comprehensive proteomic analysis of both the lung luminal endothelial cell PM and caveolae resulted in the identification of 6 GPCRs in the PM and 9 GPCRs in the caveolae with 2 peptides. As GPCRs are notoriously difficult to detect by mass spectrometry methods, we expanded our search to include GPCRs identified with 1 peptide. This resulted in the identification of 82 GPCRs in

the PM and 62 GPCRs in caveolae, a significant number of which were validated by manual sequencing of the ms/ms spectra and further confirmed by western blot analysis. We found a significant number of these receptors enriched in the caveolae compared the PM. The addition of known ligands for a number of these receptors resulted in the "pinching off" of caveolae directly from PM in our cell-free budding assay. Interestingly, only the receptors that were deemed enriched in the caveolae over the PM had the ability to induce this budding effect, indicating a functional significance for their localization in caveolae.

In summary, comprehensive proteomic profiling of PM and caveolae from rat lung luminal endothelial cells resulted in the identification of GPCRs that when activated by their ligands cause the budding of caveolae from the PM to form free discrete carrier vesicles through a fission process requiring GPCR activation.

Tues Poster 27: Isolation of Caveolae using Detergent And Detergent Free Approaches Identifies Specific Proteins Unique to each Method Yet Localized to Caveolae

Noelle M Griffin; Phil Oh; Sabrina Shore; Halina Witkiewicz; Jan E Schnitzer

Sidney Kimmel Cancer Center, San Diego, CA

Caveolae are membrane micro-domains, which are involved in vesicular transport across the plasma membrane (PM). Two major structural components of caveolae are known, caveolin and dynamin. Dynamin is present around the neck of caveolae when they are attached to the PM but lost after the caveolae bud to form free transport vesicles. We find that dynamin is solubilized by Triton-X-100 and thus caveolae can be sheared away from PM more easily after Triton X-100 treatment than without it. As dynamin is a major component of caveolae we speculated that we may be loosing additional proteins from caveolae by using detergent. Thus, we also purified the caveolae in the absence of detergent, to prevent dynamin solubilization. Consequently, the caveolae are more difficult to shear from the PM and the yield of caveolae is significantly lower using this method. Nevertheless, without detergent, we expect to increase the number of detergent soluble proteins detected in the caveolae. We performed a comprehensive proteomic analysis on the caveolae fractions that were isolated in the presence or absence of Triton X-100 to determine the proteins unique to each method as well as the overlapping proteins in order to expand the caveolar proteome.

Caveolar proteins isolated from rat lung luminal endothelial cells in the presence or absence of Triton X-100 were separated by SDS-PAGE gel electrophoresis. The entire gel line was cut into approximately 60 bands, trypsin digested and analyzed by 2D-LC-MS/MS analysis using an LTQ mass spectrometer. 95% analytical completeness of each sample was reached though multiple replicate analysis such that additional replicate measurements contributed non significant protein identifications. Raw ms data was searched against a rat and human database using the Sequest algorithm. Proteins were deemed identified based on optimized Sequest scores and a minimum of 2 unique peptides per protein. We performed extensive bioinformatics analysis on both datasets to get an overview of the proteins purified by each method.

Our comprehensive proteomic analysis of caveolae isolated with or without Triton X-100 resulted in the identification of a significant number (>1000) of proteins between the two methods, 47% of which were common to both. Furthermore, 28% of the identified proteins were unique to the detergent free preparation and 43% were unique to the preparation with Triton X-100. We confirmed the presence of a number of these proteins from both methods by western blot analysis and electron microscopy verifying that both preparations resulted in the isolation of caveolar proteins. We found that the proteins unique to each isolation procedure had distinct characteristics which may explain their preferential purification by one method over the other.

In summary, the combination of detergent and detergent free methods for the isolation and purification of caveolae resulted in the generation of a comprehensive dataset of proteins that would not have been identified by either method in isolation. We confirmed the localization of a number of these proteins identified by either method to caveolae confirming the isolation and extraction procedures as well as validating the proteomics methodology.

Tues Poster 28: Towards Comprehensive Mapping of Lipid-Embedded Proteins for Endothelial Plasma Membranes *in vivo*

Yan Li; Jingyi Yu; Yipeng Wang; Noelle M. Griffin; Fred Long; Sabrina Shore; Phil Oh; Jan E. Schnitzer

Sidney Kimmel Cancer Center, San Diego, California

Lipid membranes structurally define the outer surface and internal organelles of cells. The multitude of proteins embedded in lipid bilayers are clearly functionally important, yet remain poorly defined. Even today, integral membrane proteins represent a special challenge for current large-scale shotgun proteomic methods. Here, we use endothelial cell plasma membranes isolated directly from lung tissue to test the effectiveness of four different mass spectrometry-based methods, each with multiple replicate measurements, to identify membrane proteins. In doing so, we substantially expand this membranome to 1,833 proteins, including >500 lipid-embedded proteins. The best method combined SDS-PAGE prefractionation with trypsin digestion of gel slices to generate peptides for seamless and continuous 2D-LC/MS/MS analysis. This 3-dimensional separation method outperformed current widely used 2-dimensional methods by significantly enhancing protein identifications including single- and multiple-pass transmembrane proteins; >30% are lipid-embedded proteins. It also profoundly improved protein coverage, sensitivity, and dynamic range of detection, and substantially reduced the amount of sample and the number of replicate mass spectrometry measurements required to achieve 95% analytical completeness. Such expansion in comprehensiveness requires a trade-off in heavy instrument time but bodes well for future advancements in truly defining the ever-important membranome with its potential in network-based systems analysis and the discovery of disease biomarkers and therapeutic targets. This analytical strategy can be applied to other subcellular fractions and should extend the comprehensiveness of many future organellar proteomic pursuits.

Tues Poster 29: Connecting Proteomics and Biological Functions: Evaluation and Systems Analysis of the Lung Endothelial Cell Plasma Membranome

Yan Li; Huiluen Hung; Sabrina Shore; Phil Oh; Halina Witkiewicz; Noelle M. Griffin; Jingyi Yu; Kerri A. Massey; Jan E. Schnitzer

Sidney Kimmel Cancer Center, San Diego, California

Mass spectrometry-based high throughput proteomics is a proven core technology to identify and quantify the protein content of biological systems, particularly cellular organelles. When combined with systems level analysis, proteomics provides a key means to assign individual proteins to specific molecular complexes, pathways and networks. Using mass spectrometry and normalized spectral index, we have quantified 1833 proteins identified in luminal endothelial plasma membrane isolated from rat lung We performed a series of analyses to validate these proteins using alternative approaches, and have analyzed and defined the plasma membrane proteome using systems level analysis. As expected, the majority of proteins identified (~70%) were enriched in the plasma membrane in comparison with total tissue homogenates. Accordingly, ~70% of the annotated proteins were assigned by current public databases to either plasma membrane or plasma membrane-associated subcellular localization(s). The data showed that cytoskeletal, caveolar and membrane trafficking proteins, as well as certain protein translation factor were among the most abundant proteins (from 400 to 4500 ng/mg of the membrane). RT-PCR confirmed 37 novel genes were indeed expressed in rat lung endothelial cells, 3 of which were further validated as plasma membrane proteins by multiple immunoassays. Combined computational analyses revealed that many known plasma membrane-associated functions were significantly over-represented in the membranome, typically

transport, cell adhesion, signal transduction, and mechanotransduction/mechanosignaling. Most importantly, these analyses also revealed certain vital endothelial cell plasma membrane-associated functions, including blood vessel maturation and angiogenesis. Direct protein-protein interaction and pathway analyses indicted that the molecules found in the membranome were indeed grouped into over-presented functions. Hence, combining proteomics with bioinformatics tools has helped us to define both the molecular and functional components of the endothelial cell plasma membranome. We believe that integrating individual protein functions at the systems level enables us to understand how a living cell or tissue interacts with its environment and improves our understanding of pathological processes. Furthermore, the information gained from these studies provides us with new insights into the biological functions of the plasma membrane and may lead to the discovery of novel biological functions and disease targets.

Tues Poster 30: A TAP-Tagging Approach to identify Neuroligin-2 Interacting Partners Specifying Induction of Inhibitory Synapses

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Development of appropriate synaptic connections among nerve cells is a prerequisite process for brain function. Synaptic specializations mediating functional neurotransmission are specialized cell-cell junctions controlling vesicle fusion and neurotransmitter release at the presynaptic membrane and neurotransmitter reception at the postsynaptic membrane. One of the most important aspects of synaptogenesis is the precisely balanced formation and maintenance of the correct ratio between two major types of synapses, excitatory glutamatergic and inhibitory GABAergic. Neurexins and Neuroligins, cell adhesion molecules whose extracellular domains bind to each other, are involved in this process. When expressed on non-neuronal cells or coated on beads, interaction of Neurexins and Neuroligins is able to trigger postsynaptic differentiation in dendrites and presynaptic differentiation in axons, respectively. Importantly, Neurexins and Neuroligins can induce both excitatory and inhibitory synapse formation, with different variants contributing selectively, suggesting that differential complexes of Neurexin/Neuroligin could control the ratio of excitatory and inhibitory synapses. The finding that Neuroligin 2 localizes specifically to inhibitory synapses suggests that Neuroligin 2 itself may help specify formation of a GABAergic synapse. The major components of GABAergic synapses identified to date, neuroligin-2, GABAA neurotransmitter receptors, and gephyrin scaffolding protein are thought to interact indirectly via as yet unidentified partners. Moreover, numerous scaffolding molecules and regulatory enzymes have been identified at glutamatergic postsynaptic sites, whereas identification of such proteins at inhibitory synapses remains relatively unexplored.

To identify neuroligin-2 interacting partners and components of inhibitory synapses, we employed an in vivo Tandem Affinity Purification (TAP) tagging approach known to allow rapid and efficient recovery of a specific protein interactome from a complex mixture. The targeting construct contained the neuron-specific Thy1 promoter driving expression of the fusion protein His6 – Flag – EYFP- Neuroligin 2 (HFY-Nlg2) , so that His6 and Flag tags can be used for facilitating biochemical purification and EYFP for live imaging. Proper synaptic localization of the tagged Neuroligin 2 was tested initially by transfection into cultured hippocampal neurons and then by confocal analysis of the transgenic mice. Multiple transgenic lines were obtained showing widespread punctate EYFP in brain co-localizing with gephyrin and other inhibitory synaptic markers. HFY-Nlg2 puncta were highly expressed in hippocampus, striatum, entpeduncular nucleus, and moderately expressed in cortex, thalamus and amygdala. Overall expression level of the tagged Neuroligin-2 was 10-19% relative to endogenous Neuroligin 2. Detergent solubilized brain extracts from the transgenic mice were subjected to Ni-metal affinity column purification and selective enrichment of the tagged Neuroligin 2 confirmed by western blot. Co-enrichment of endogenous neuroligin-2 and gephyrin is being assessed, and potential additional purification using the Flag or EYFP tags explored. Affinity purified interacting proteins with Neuroligin 2 will be analyzed and identified with mass spectrometry and characterized further.

Protein Arrays and Antibodies, 31 – 34

Tues Poster 31: Identification and Characterization of Novel Protein Complexes

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We are using a combined immunochemical/ms approach to identify and characterize novel multiprotein complexes in the mitochondrion and to develop simple immunoassays for rapid quantitative, high-throughput measurement of these and other key mitochondrial proteins. The assays measure not only protein levels, but also enzymatic activities and levels of post-translational modifications such as phosphorylation, acetylation, and markers of oxidative stress, e.g., nitration and carbonylation. In related work, panels of these protein expression assays that define metabolic pathways are being used to investigate cellular metabolism under changing physiologic and pathologic conditions and to identify biomarkers characteristic of disease states.

We first proved utility of the technique by identifying previously characterized mitochondrial enzyme complexes in the oxidative phosphorylation and fatty acid oxidation pathways, and by making simple protein quantitation and enzyme activity immunoassays specific for each of these large well-known enzyme complexes. More recently we have discovered new multiprotein complexes, including a 6-protein complex containing both inner and outer mitochondrial membrane proteins which may be a cristae-junction complex important for maintenance of mitochondrial morphology, a complex of prohibitin-1, prohibitin-2 and monoamine oxidase B suggestive of an apoptotic function, and a complex of five UDP-glucuronosyltransferases (UGT2B1-5), each of which were previously shown to play roles in conjugation and elimination of drugs and toxic endogenous cellular by-products. For each of these three novel complexes, multiple lines of evidence were used to confirm the protein-protein associations, including the use of multiple independent mAbs to immunocapture each complex and measurement of the total complex molecular weight in the absence of immunocapture antibody.

Identification of these novel protein complexes provides insight into protein interactions and coordinated action of functionally related enzymes. The development of simple immunoassays to measure the levels and functioning of these complexes, and to affinity purify them routinely will facilitate additional detailed studies of their functional roles.

Tues Poster 32: iMALDI+: A Targeted Proteomics Approach to Clinical Diagnostics

Brinda R. Shah; Christoph H. Borchers

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Introduction: Low abundance proteins often play a critical role in the development of many diseases. The use of antibodies is an effective way of enrichment of these low abundance proteins from complex samples. Recent trends in immunoaffinity have shifted to the enrichment of proteolytic peptides instead of proteins, as peptides offer more stability and manageability without compromising the information obtained from their protein counterparts.

We have previously developed a method termed iMALDI (immunoMatrix Assisted Laser Desportion Ionization) which combines immunoaffinity with mass spectrometry for quantitative

analysis of peptides in complex samples. While the use of antibodies provides enrichment of low abundance peptides, mass spectrometry provides sensitivity, specificity and quantitation. To further improve this approach, we are now developing iMALDI-MRM (Multiple Reaction Monitoring) which we term iMALDI+. The targeted approach of MRM in combination with antibody enrichment and MALDI MS allows for much greater sensitivity, specificity, and an increased dynamic range of quantitation.

Upon developing the analytical parameters of this approach, we will apply this technique to the detection of EGFR (Epidermal Growth FActor Receptor), a known oncogene in breast cancer. We will also use this approach to create a multiplex assay to characterize not only EGFR, but also EGFR isoforms and EGFR pathway proteins for greater confidence in diagnosis. Breast cancer tumour tissues that show elevated levels of EGFR activity often have worse prognosis, greater chance of recurrence, and a poorer response to therapy in comparison to those who do not over express it. Early detection of EGFR in the tissue is imperative to proper administration of treatment and increase in survival rate of the patient.

Results: We have previously shown that the comnination of immuno-affinity and MALT-TOF/TOF mass spectrometry allows for detection of mid attomole levels of target peptides out of tumour tissue samples, a 1000 fold improvement over out of solution detetion of target peptides by MALDI. Additionally, the incorporation of an internal standard allows for absolute quantitation of target peptides in clinical samples.

Here we report that, with the combination of our iMALDI approach with MRM on a 4000 QTrap mass spectrometer, we are able to significantly improve upon the previous results to obtain low attomole levels of sensitivyt and much greater specificity through gating of the target peptide. And, we anticipate a 100-fold increase in dynamic range of quantitation of the MRM based method. This technique will also be developed for a comprehensive multiplexed analysis of EGFR isoforms and pathway proteins. The detection of other markers beyond EGFR for specific detection, improved sensitivity, and absolute quantitation of peptides in a multiplexed manner from complex biological samples holds promise for application in clinical diagnostics.

Tues Poster 33: Extracellular Protein Interaction Screening using Protein Microarrays

Lino Gonzalez; Sree Ramani; Irene Tom

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Now that the human genome is complete, one of the next steps is to understand the interactions between all transmembrane and secreted proteins, which represent about one-quarter of all human genes. This knowledge would greatly aid in characterizing the biological function of many receptor/ligand pairs and their potential as therapeutic targets. A significant number of these extracellular proteins still remain orphans. In order to identify candidate partners, we have utilized Genentech's SPDI (Secreted Protein Discovery Initiative) protein library, consisting of over 1,000 purified, secreted proteins. In the past, our lab has used surface plasmon resonance (SPR) and bio-layer interferometry (BLI) technology to screen on the order of hundreds of interactions per day. Here we report the development and validation of a SPDI Protein Microarray that serves to increase our screening capacity to the thousands of interactions per day. Our goal is to utilize these protein arrays to identify novel receptor interactions that can be evaluated for their potential as future therapeutic targets for fighting disease.

Tues Poster 34: Monoclonal Antibody Sequencing: A de Novo-Mediated Database Approach

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An antibody's preference and efficiency in the detection and removal of encountered antigens is heavily dependent on its amino acid sequence. Oftentimes, an antibody's sequence may be determined early in its lifetime by sequencing the DNA of the source cell line. However, few direct protein sequencing options exist when the source is unavailable or for independently verifying antibody integrity. Somatic hypermutation and chemical post-translational modifications confound tandem mass spectrometry-based peptide identification methods; both database search tools and de novo sequencing. We present a hybrid approach that draws on the strengths of these methods, combining a database search against known antibody sequences and a guided de novo sequencing of regions which cannot be annotated using the database.

In the database phase of the method, regions of the antibody consistent with the germline sequence are identified using InsPecT. The protein database contains all germline immunoglobulin genes, as well as a splice graph representation permitting the identification of peptides spanning splice junctions. The regions identified in the database phase are then used as anchors to guide subsequent de novo sequencing.

We demonstrate the accuracy of this method by sequencing an antibody designed against the B- and T-cell lymphocyte attenuator molecule. The efficiency and throughput of our method greatly exceeds the capabilities of Edman degradation, the traditional method of antibody sequencing, transforming days of work into a few hours. We believe this method can be generalized to a variety of other applications, such as the discovery of splice junctions and fusion genes.

Protein Structure and Modifications, 35 – 38

Tues Poster 35: Discovering Human Protein Diversity

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The need to systematically analyze protein variants across human populations is indisputable. With recent technological advances such protein diversity studies are becoming reality, and form the foundation of Population Proteomics - the large-scale investigation of human proteins across and within populations to define and correlate protein variations. Such studies of protein diversity also explore the association of protein modifications with specific diseases, facilitating the discovery and validation of novel protein biomarkers. Presented here are the fundamentals of population proteomics, the results from several studies investigating human plasma protein microheterogeneity across the healthy population in the United States, and the correlation of specific protein variants with the presence of disease.

Tues Poster 36: Multistage-Fragmentation Analysis Is Superior to Neutral Loss Scanning for Phosphopeptide Identification

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Identification of phosphoproteins by mass spectrometry (MS) has remained a challenge due to signal suppression and facile loss of phosphate group during fragmentation. Advanced methods such as electron-transfer dissociation and electron-capture dissociation have been developed for gentle fragmentation keeping the modified moiety intact. However, these methods require additional instrumentation. The standard neutral loss scanning methods are successfully employed for phosphopeptide identification on quadrupole and ion-trap instruments. However, preferential loss of other groups over phospho-loss leads to ambiguous spectra that are not identified. We developed a new strategy, multistage-fragmentation analysis (MSF), wherein fragmentation is performed on a peptide at multiple stages on the most abundant fragment ion at each stage. We demonstrated that the identification of protein phosphorylation can be significantly improved on an ion-trap instrument including multiple modifications on proteins. In addition to posttranslational modifications, protein coverage is increased with our method due to improved peptide identification. We

analyzed alpha-casein, maspin and vascular endothelial membrane proteins to compare the two methods.

Multistage-fragmentation analysis and neutral loss (NL) scanning methods were compared for phosphopeptide identification. Analysis of alpha-casein identified ten and seven peptides using MSF and NL methods, respectively. The MSF method identified all four phosphopeptides expected to be identified by mass spectrometry (www.expasy.org). Using the NL method, only three phosphopeptides were identified. A multiply phosphorylated peptide (QMEAEpSIpSpSpSEEIVPNSVEQK) was identified only by the MSF method. Moreover, identification of one of the non-phosphorylated peptides (HIQKEDVPSER) was possible only by the MSF method. Analysis of membrane proteins from endothelial cells by the two methods also demonstrated that the number of phosphopeptides identified by MSF analysis is higher than that of NL approach. However, the total number of proteins identified is higher when NL method is used. This is due to more number of scans; thereby the time spent on a peptide during MSF analysis is more compared to that in NL scanning.

Overall, the MSF approach appears to be superior to NL scanning in its ability to identify phosphopeptides. Furthermore, the method was found to be especially useful for the identification of multiply phosphorylated peptides. During the NL scanning of multiply phosphorylated peptides, the richness of daughter-ion peaks does not improve even after the phospho loss due to the loss of other phospho-groups in the next stage fragmentation, making the peptide identification difficult. Moreover, the neutral loss of water, amine, salts or solvent adducts are known to be facile fragmentations leading to an intense ion suppressing other daughter ions. These ions will not be further fragmented if the neutral loss ion is fixed for phospho-groups in the MS method. Hence, sequential fragmentation of the most abundant peak is more beneficial in identifying these multiply phosphorylated peptides.

Tues Poster 37: Production of Polypeptides, 3000 to 10000 Da, and Middle-Down Analysis by LC-MSMS

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Alternative cleavage agents to trypsin are required or preferable in a variety of circumstances. When too many arginine and lysine residues are present, tryptic digestion will produce short peptides that are difficult to place in the sequence, and when no arginine or lysine residues are present, no cleavage will occur. Trypsin is also sub-optimal in research strategies that require longer peptides, such as efforts to identify multiple modifications that are linked or associated to modulate function. This laboratory has advocated high temperature acid cleavage as an alternative to trypsin. Our microwave accelerated acid reaction has been shown to cleave selectively on either side of aspartic acid, whose rare occurrence in the proteome, compared to the combined occurrence of the tryptic substrates arginine and lysine, accounts for the production of peptide products that are longer, on average, than the products of tryptic digestion. The increased range of peptide sizes allows improved chromatographic resolution of the peptide products, however, collisional activation of peptides with masses between 3000 and 10,000 Da provides spectra with fewer fragment ions. The challenges of characterizing polypeptides based on these MSMS spectra resemble those being addressed in top-down computational strategies. We are developing a middle-down strategy to characterize the longer peptide products produced by acid cleavage and Glu-C, Lys-N and other endoproteases that cleave with a lower frequency than trypsin. High resolution analysis of both precursor and fragment ions is provided by an HPLC-LTQ-Orbitrap system on a chromatographic time-scale. Tandem mass spectra are processed by Distiller (Matrix Science), and ProSightPC (Thermo Fischer) is used as the search engine. We have incorporated a customized database of the potential products of Asp selective acid cleavage into ProSightPC to facilitate this analysis, resulting in E-values as low as 1e-50 for some spectra. We will demonstrate the feasibility of a robust middle-down proteomics workflow for peptides in the range 3-10 kDa.

Tues Poster 38: A Novel Chip Based Electroelution System for Rapid and Efficient Recovery of Intact Proteins from Polyacylamide Gels

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Proteomics has become an important tool for the discovery of protein biomarkers and drug targets. These experiments are primarily conducted by enzymatic digestion of proteins and subsequent identification of the protein by peptide mass fingerprinting or tandem mass spectrometry (bottom-up). These experiments employ mature technologies and have demonstrated the ability to identify several hundred to thousands of proteins from complex mixtures. The bottom-up approach, however suffers from several limitations, namely that it only provides information on a small portion of the entire protein. This makes it difficult to determine isoforms, alternative splicing products and post translational modifications.

To overcome these limitations, many researchers have begun to employ top-down proteomics experiments. The top-down approach introduces intact, full length proteins to the mass spectrometer. In this approach an accurate intact mass can be measured and the entire sequence is available for determination. One of the biggest limitations to the top-down approach has been the difficulty in separating individual proteins from complex mixtures. Separations can be carried out by multiple chromatographic steps, but this is time consuming and rarely produces a high level of purity. In addition, it is difficult to resolve isoforms, eliminating one of the major advantages of the top-down approach.

Two-dimensional electrophoresis largely overcomes these limitations, with it ability to resolve several thousand proteins in a single gel. In addition, it generally has the ability to resolve isoforms and modifications making it appear to be the logical choice for separations of proteins prior to top-down experiments. It has however, not been widely employed due to the difficulty of removing proteins from polyacylamide gels and the time consuming post recovery clean-up necessary to make the protein amenable to mass spectrometry.

In this paper we describe a novel chip based system to greatly improve the process of extracting proteins from polyacylamide gels and the subsequent analysis by mass spectrometry. This system uses a proprietary micro-channel capillary electrophoresis chip that rapidly and efficiently recovers proteins from gels. The novel design employs a combination of electrophoretic force and hydrodynamic force to de-couple the protein extraction from the electrophoretic path. The system allows 8 proteins to be extracted simultaneously in less than 20 minutes. In addition, the electroelution buffer was designed using an anionic acid labile surfactant that can be cleaved in less than 10 minutes for easy clean-up prior to mass spectrometry. We show the ability to routinely obtain intact mass measurements from proteins recovered from polyacylamide gels from less than 1 ug loadings.

PTM Analyses of Complex Biological Matter, 39 – 44

Tues Poster 39: A Proteomic Study of Steps That Lead to Ciprofloxacin Drug Resistance in Pseudomonas Aeruginosa

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Introduction: Antimicrobial resistance is an acute problem for those suffering from chronic infectious disease such as cystic fibrosis (CF). The opportunistic pathogen Pseudomonas aeruginosa is a key etiological agent responsible for CF patients' chronic lung infection. During repeated exposure to antibiotic treatments with the drugs ciprofloxacin and tobramycin, significant increases in drug resistance quickly develop in P. aeruginosa. While many mechanisms of high-level drug resistance have been investigated after-the-fact, the steps leading to the development of drug resistance are not known, which limits the ability to prevent drug resistance from occurring. We are applying a systems biology examination of the formative steps of ciprofloxacin resistance in P. aeruginosa, with a substantial focus on proteomics.

Methods: We compared naïve wild-type P. aeruginosa populations to those that underwent short-term ciprofloxacin exposure. We applied multiplexed 2D-gel electrophoresis with both total protein stain and phosphoprotein-specific stain to identify proteins putatively associated with drug resistance. Proteins showing differences in expression were identified by tandem mass spectrometry (MS/MS). We then assayed the phenotype and growth patterns of cellular populations of P. aeruginosa PA01 with the genes encoding the differently-expressed/modified proteins knocked out, to ascertain their putative role in drug resistant phenotypes.

Results: Three proteins were initially identified with repeatable differences between wild-type Pseudomonas and drug-tolerant strains. Two of these exhibited a difference in phosphorylation are associated with amino acid metabolism and/or energy production. The third protein showed a difference in total abundance, and is listed as hypothetical. Each of the three strains displayed a reduced initial resistance to ciprofloxacin. While the wild-type strain shows more than a 50-fold increase in the minimal inhibitory concentration (MIC) after 48 hrs exposure, the knockout strains show a range from 5 to 12 fold increase in MIC after 48 hr exposure. Each of the knockout strains were below the "break point" considered for clinical resistance, whereas wild-type was above that level. We also assayed two control strains with other genes knocked out, and those displayed resistance behavior much closer to wild-type, with 30-fold or more increase in MICs.

Discussion: Ciprofloxacin resistance studies to date in P. aeruginosa have primarily focused on mutation of the drug target (DNA gyrase) and/or up-regulation of efflux pump expression. In contrast, we have found evidence suggesting that the initial steps of drug resistance are not so simple as a single mutation, but that multiple steps and mechanisms may be involved. The evidence to date is that there may exist a functional class comprised of both known and several unknown proteins that are directly involved in mediating the first steps of the antibiotic resistance process.

Tues Poster 40: Effect of Oncogenic Tyrosine Kinase Bcr-Abl Signaling on Global Serine/Threonine Phosphorylation Patterns

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Bcr-Abl is an oncogenic fusion protein that possesses aberrant tyrosine kinase activity that is central to the pathogenesis of chronic myelogenous leukemia (CML). Previous studies have linked changes in Bcr-Abl kinase activity to global changes in the phosphorylated tyrosine (pY) profile and to the CML disease state. These changes are believed to be critical events in CML transformation, drug resistance, and disease progression. Because Bcr-Abl is a hyperactive tyrosine kinase and because pY profiling methods matured prior to phosphoserine/phosphothreonine (pSpT) profiling methods, prior global phosphoprofiling efforts have focused on pY events activated by Bcr-Abl. However, previous studies clearly indicate that the impact of Bcr-Abl tyrosine kinase activity is not restricted to tyrosine signaling pathways.

Using strong cation exchange fractionation, TiO2 affinity chromatography and an LTQ-Orbitrap mass spectrometer, we globally enriched for and identified phosphopeptides differentially expressed due to Bcr-Abl activity. The relative degrees of phospho-signaling were determined using our custom software that quantitates both sequenced and unsequenced peaks by global chromatography alignment and peak area integration. The quality of the phosphorylated peptide peak specification and integration was additionally evaluated by an in-house peak assessment algorithm.

The global profiling results show that some pSpT events increase and some decrease in Bcr-Abl expressing cells versus the BaF3 untransformed parental cell line. We additionally perturbed the network to further study the coupled effects between pY and pSpT. In addition to further accentuating the effects of Bcr-Abl on the global phosphosignaling profile, these perturbations also show that pSpT signaling pathways can affect pY signaling in the Bcr-Abl system that is driven by an overactive tyrosine kinase. These results highlight the complex and intertwined nature of phosphotyrosine, phosphoserine, and phosphothronine signaling in cellular networks.

Tues Poster 41: Site-specific Phosphorylation Kinetics Following Kinase Activation using High Resolution Selective Reaction Monitoring (h-SRM)

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Phosphorylation of the hydroxyl amino acids tyrosine, serine and threonine by specific kinases represents a major signaling mechanism in eukaryotes. It has been estimated that as many as one third of all cellular proteins undergo phosphorylation. Over 100 different kinases have been linked to human cancers, making them a prime target for pharmaceutical intervention. The mass spectrometer plays a central role in the detection and characterization of this cellular process using various experimental strategies.

The stoichiometry of phosphorylation needed to propagate cell signaling at any one amino acid residue is generally not known. Stoichiometries of phosphorylation are not typically measured in proteomics studies, but are generally thought to be low relative to the unmodified state. To overcome this relative scarcity many phospho-peptide enrichment strategies have been employed including coordination with metal ions (IMAC and TiO2) and immunoprecipitation with site-specific antibodies. While these methods facilitate the discovery of the phospho-sites, the removal of the unphosphorylated cognate peptides removes any link to the real stoichiometry of the modification. High resolution triple quadrupole mass spectrometers allow for the measurement of these phospho-peptide sites in complex mixtures without phospho-peptide enrichment so that the level of the same peptides in unmodified form can be determined in the same experiment.

In this study we measure the levels of phosphorylation at several sites on the human epidermal growth factor receptor (EGFR) at various time points following stimulation by its specific ligand (EGF) by high resolution selective reaction monitoring (h-SRM).

Tues Poster 42: Global Mapping of the Topography and Magnitude of Proteolytic Events in Biological Systems

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Proteases play central roles in numerous biological and pathological processes. Their importance is underscored by the fact that roughly 2% of the genes in the human genome encode proteases. Despite their essential functions, even the

most well-studied proteolytic cascades remain only partially understood, and a large portion of human proteases are wholly uncharacterized with respect to endogenous substrates and biological function.

Various methods have been devised to identify protease substrates. These methods can generally be placed into three categories: (1) DNA/RNA display, (2) 2-dimensional gel electrophoresis (2D-GE) and (3) N-terminal labeling strategies. These methods have all been applied, with varying degrees success, to identify protease substrates in numerous biological contexts. While protease substrates can, indeed, be identified using these methods, they all suffer from serious drawbacks. Iterative display techniques such as DNA- or RNA-display, rely on ex-vivo expression of proteins and, therefore, often result in a high rate false-positives due to expression of substrates at superphysiological concentrations. 2D-GE is conceptually straightforward, but is severely limited by sensitivity and reproducibility. Furthermore substrates identified by iterative display or 2D-GE require laborious follow-up experiments involving mutagenesis and immunoblotting to identify the topography and sites of proteolysis. N-terminal labeling approaches, in which nascent N-termini are chemically captured and enriched, always identify sites of proteolysis, but detection of cleavage relies on proteomic identification of a single peptide which is frequently difficult or ambiguous.

Here, we present a novel proteomic method for the global identification and description of proteolytic events in native biological systems. Termed PROTOMAP, for PROtein TOpography and Migration Analysis Platform, this system relies on comparitive 1D SDS-PAGE fractionation followed by 1D LC-MS/MS to identify proteomie-wide shifts in gel-migration. These data are assembled into “peptographs” which enable intuitive visualization of the topography and magnitude of proteolysis for each protein. We applied PROTOMAP to the intrinsic apoptotic pathway in Jurkat T-cells and identify hundreds of cleaved-proteins, the majority of which were not previously reported. Furthermore, temporal analysis of the stability of cleavage-products revealed the generation of many long-lived intermediates, suggesting that generation of active effectors may be a primary function of apoptotic proteolytic cascades.

Tues Poster 43: Discovering Rare Unexpected Modifications in High-throughput Comparative Proteomics

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How can one efficiently and reliably identify single-event unexpected modifications on datasets containing over 16 million tandem mass (MS/MS) spectra from multiple species? Most existing methods analyze each MS/MS spectrum in isolation and thus rely on large numbers of multi-site identifications to statistically reject spurious unexpected modifications. In contrast, Spectral Networks blindly combine uninterpreted spectra from overlapping peptides or modification variants of the same peptide to reliably identify unknown modifications but face significant large-scale challenges. First, the construction of spectral networks by aligning every possible pair of spectra becomes a serious bottleneck for the analysis of millions of spectra: the non-optimized construction of spectral networks for 16 million spectra would require the computation of ~128·10^12 pairwise spectral alignments (~31,000 CPU-years). We show how to address this challenge in just a few days by capitalizing on the observation that most pairs of spectra that can be reliably aligned also tend to share small sequence tags. But most importantly, ensuring the reliable identification of rare modifications on large-scale high-throughput experiments turned out to require more complex methods than those applicable to simpler samples. While previous methods would perform poorly at this scale (two mistakes out of every three predictions), we now show how further algorithmic developments can decrease the false positive rate to below one mistake out of every ten predictions. We demonstrate our approach on multiple Shewanella whole-cell lysates to reliably identify thousands of rare and single-event modifications. In addition, the significant alignment of many unidentified spectra further suggests the presence of many putative novel peptides.

Tues Poster 44: Evolution of a Histone H4-K16 Acetyl-Specific DNA Aptamer

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The development of high quality affinity reagents to human proteins represents a major challenge in basic and applied biomedicine. Many large-scale biological assays rely on the use of antibodies to interrogate the nature and function of the human proteome. Unfortunately, only a small number of human proteins have antibodies that are available for use in routine molecular and cellular biology assays. Even less common are antibodies with high affinity and specificity to specific post-translational modifications (PTMs). Aptamers, which are pieces of single-stranded DNA or RNA that fold into structures with binding sites that are complementary in shape and charge to target antigens, provide an attractive alternative to traditional antibodies. Because these molecules can be produced in vitro using test-tube evolution methods, their recognition and binding properties can be tailored to almost any molecular target. Aptamers have been selected to bind ions, small molecules, drugs, peptides, proteins, and even whole cells. Despite these advances, very few aptamers have been selected to bind specific PTMs. Here we describe the evolution of a DNA aptamer selected to bind histone H4 proteins acetylated at lysine position 16. When compared to a standard chip-quality antibody raised to the same target, the best aptamer shows similar affinity, but dramatically higher specificity (2,400-fold). This result suggests that aptamers could be used as tools to identify specific PTMs in complex biological samples.

Structure and Dynamics of Macromolecular Assemblies, 45 - 46

Tues Poster 45: The Dynamic Analysis of Nuclear Receptor – Ligand – Coactivator Complexes towards Establishing Structure Activity Relationships of Anti-cancer Rexinoids

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Mass spectrometry has emerged as an indispensible player in the field of structural biology, remarkable in its ability to provide both structural and dynamic information. Hydrogen-deuterium exchange coupled with high resolution mass spectrometry (HD X MS) has become key in elucidation of protein/protein, protein/DNA and protein/ligand interactions. Proteins have intrinsic molecular motions and molecular interactions are dynamic. HD X MS is conducted in-solution allowing preservation of protein fluidity.

In the present study, we employ HD X MS by use of the high resolving and high mass accuracy capabilities of the hybrid LTQ-FT mass spectrometer to characterize ligand-induced conformational dynamics in retinoid x receptor (RXR) ligand binding domain (LBD) bound to two structurally distinct anticancer drugs, Targretin and 9-cis UAB30. While both RXR specific ligands (rexinoids) show efficacy against breast cancer, Targretin is dose limited due to associated toxicities. In contrast, 9-cis UAB30 is unique in its low level of associated toxicities, deeming it a candidate for chemoprevention. Examination of co-crystal structures of RXR LBD in complex with Targretin or 9-cis UAB30 shows no significant differences. However, HD X MS analyses of these ligands in complex with RXR LBD do show regions of distinguishing dynamic motions within the LBD. This includes &alpha-helices 3, 10, and 11, regions involved in the ligand binding pocket and coactivator binding. Agonist binding drives the removal of complexed corepressors while promoting interactions with coactivators. Ensuing transcriptional activation is of therapeutic interest for a wide range of diseases. To this end, we have also monitored ligand-induced RXR LBD dynamics in the presence of coactivator peptide (GRIP-1). Our results demonstrate the

cooperative nature of ligand and coactivator binding, while providing evidence for region-specific coactivator binding in the absence of ligand. These assembled protein ligand complexes provide us a means of analyzing the structural mechanism of RXR transcriptional activation in the context of cancer chemoprevention. They also provide the basis for establishing structure activity relationships for a variety of anticancer rexinoids.

Tues Poster 46: Assessment of Binding Capabilities of Transcription Factor EGR1 to DNA

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The transcription factor Early Growth Response 1 (Egr1) responds to a diverse array of extracellular signals within minutes, and has been implicated in the growth of a large number of tumor types. We have used nanoporous optical interferometry to determine the binding affinity of this transcription factor containing three zinc finger motifs to its cognate DNA sequence. Interferometry allows the direct, label-free determination of the binding kinetics between macromolecules. The technique relies on porous silicon and the pattern of light refraction on its surface. Proteins or DNA can be selectively bound to the porous silicon, white light is shined on the surface and an interference pattern obtained through a multiband spectrophotometer. The test protein or DNA is then interacted with the bound target and the alteration of the interference pattern is monitored over time. The optical path difference (OPD) in the interference patterns reflects the binding interaction.

To perform our binding assay, EGR1 binding site (as a 25 mer oligo) are synthesized with a biotin label and then interacted with a streptavidin-coated porous silicon (PoSI) chip. A complementary oligo (a 25 mer reverse complement) is added to generate a double-stranded oligo through hybridization. The binding event can readily be observed on the instrument. The PoSi chip is now primed for measuring the interaction of the double-stranded DNA with its transcription factor. The control utilized was a randomized sequence of the cognate DNA binding site of EGR1. Adding Egr1 (1uM), we noted a specific interaction to the double stranded oligo with Egr1 as compared to the randomized oligo control. We are now in the process of evaluating the presence of multiple binding sites within a double-stranded oligo, the effects of substituting certain base pairs in the recognition sequence, the contribution of the length of the oligo as well as testing transcription factors utilizing leucine zipper or homeodomain structures as DNA recognition components.

The method is easily adaptable to monitor the interaction of numerous transcription factors with their specific DNA sequences as well as evaluate the impact of specific DNA substitutions in the recognition sequence. The results of such experiments could assist in the building of system biology models of transcriptional control and regulation.

Systems Analysis for Biomarker Discovery, 47 – 51

Tues Poster 47: Virtual Discovery of Sub-Networks in Human Disease

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The enthusiasm for systems biology approaches for discovering new disease genes is driven by the increasing volume of results from high dimensional experiments in genomics and proteomics, and high-confidence databases of protein-protein interactions (PPIs). Evidence is emerging which indicates that small sub-networks within PPIs can significantly discriminate and classify disease phenotype. In some cases these are shown to be quantitatively superior to traditional single target approaches. Taken together, this suggests new methods are needed to extend the usefulness of data resulting from genomic and proteomic profiling experiments in disease.

For example, the significant targets changing between normal and tumor may be used to "seed" a search of PPIs for small sub-networks that are significant with respect to the seed. The hypothesis is that proteins in the network neighborhood of the seed targets may be good candidates for validation by wet-bench experiments. Further, sub-network activity can be estimated by mRNA values obtained by microarray, and used to discriminate normal from tumor by certain statistical measures, e.g. mutual information. However, it is an outstanding question as to which kind of seed (e.g., proteomic, transcriptomic, genomic) is most likely to discover significant sub-networks with a putative role in disease, and how the topological rank of these sub-networks compares to their rank in discriminating disease phenotype.

We will present and discuss three computational methods based on information flow for discovering topologically significant sub-networks within a human PPI (HPRD) starting with three different seed types. The topological characteristics of these networks will be discussed with respect to the seed type. Further, we will score these sub-networks in terms of their ability to discriminate a colon cancer phenotype, and finally, we will compare and contrast the topological rank to the discriminative score of each of the sub-networks.

Tues Poster 48: Systematic Proteomics Analysis of Human Oral Fluids and Tissues for Salivary Protein Biomarker Discovery of Oral Cancer Progression

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With early detection and treatment, oral cancer survival rates improve significantly. Unfortunately, lacking now is a reliable and non-invasive way of distinguishing oral lesions that will transition from a pre-malignant state to a malignant state. Particularly valuable would be non-invasively-collected biomarkers, such as proteins from whole saliva. To identify such biomarkers, we have sought to discover and validate proteins in whole saliva showing abundance changes associated with this transition, via a systematic analysis of both human oral fluid and tissue samples. Starting with an analysis of whole saliva, we used an advanced, quantitative mass spectrometry-based proteomics method to analyze both the cellular and soluble fractions of whole saliva collected from three subject groups: healthy, pre-malignant oral dysplasia, and malignant oral squamous cell carcinoma (OSCC). Our proteomics method included iTRAQ reagent labeling of protein digests, followed by a three-step fractionation (isoelectric focusing-stong cation exchange liquid chromatography-reverse phase microcapillary liquid chromatography) and analysis using a linear ion trap mass spectrometer. Our analysis identified several hundred proteins from both the soluble and cellular fraction showing abundance changes between pre-malignant and malignant saliva samples. Bioinformatic analysis of these proteins using the Ingenuity Pathway Analysis (IPA) revealed a high representation of proteins involved in inflammatory response, although these proteins were deemed of limited value as candidate biomarkers due to their involvement in a wide variety of other conditions. Therefore, to further prioritize candidate biomarkers for validation, we focused on those proteins showing abundance changes that were found in both the soluble and cellular saliva fractions, resulting in about 70 proteins. Using quantitative western blot analysis in independent saliva samples, we have confirmed the ability of several of these candidate biomarkers to distinguish between pre-malignant dysplasia and OSCC. These proteins participate in diverse biochemical functions, including amino acid metabolism, transport and proteolysis. To complement these salivary studies, we are currently analyzing via proteomics transudate fluid and tissues, including non-invasively collected brush biopsies, sampled directly from oral lesions, with the objective of confirming the source of already identified salivary biomarker proteins, and identifying additional candidate biomarkers. In summary, we have undertaken systematic proteomics studies of human oral fluid and tissue samples, identifying protein biomarkers in whole saliva capable of distinguishing between subjects with pre-malignant dysplastic

lesions and subjects with OSCC, having promise for non-invasive, early detection of oral cancer development in the clinic.

Tues Poster 49: Quantitative Analysis of Candidate Cancer Serological Biomarkers using Gel-Based Fractionation and Label-Free Multiple Reaction Monitoring

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Novel serological biomarkers capable of detecting early stage cancers are highly desirable but their discovery and validation are hampered by numerous biological and technological (inability to comprehensively analyze proteomes) challenges. To minimize these difficulties in discovery mode, we employed xenograft SCID mouse models of cancers coupled with extensive multi-dimensional protein profiling consisting of major protein immunodepletion, solution IEF separation and 1-D SDS gel electrophoresis prior to LC-MS/MS analysis. Using this approach we discovered more than a hundred candidate biomarkers for each of several different types of solid tumors. Hence the next challenge is to validate large numbers of low abundance candidate biomarkers in human serum or plasma in a time and cost effective manner. In the current study, we describe a high sensitivity multiple reaction monitoring (MRM)-based strategy to verify the presence and level of selected candidate biomarkers in serum of cancer patients. Serum samples of patients with early, benign and late-stage cancers and controls were first subjected to immunodepletion of 20 high abundance proteins, and the depleted fraction was further separated on 1-D SDS gels using identical conditions to those employed in discovery mode. Gel slices containing the candidate biomarkers were digested with trypsin and analyzed using MRM analysis. In contrast to the more typical approach of using in-solution proteolysis prior to MRM analysis, the use of a gel-based strategy allows clear distinction among different forms of a protein such as changes in glycosylation or proteolysis. In some cases such size-related shifts may be the disease relevant change rather than changes in the absolute amount of that protein. In addition, consistency in the distribution pattern of different peptides among adjacent gel slices for a given protein offers additional confirmation that the correct peptides are being targeted. To confirm identities of previously unobserved peptides for candidate biomarkers, we compared the sensitivity of MRM-initiated detection and sequencing (MIDAS) on the 4000 Q-Trap mass spectrometer with targeted analysis on the LTQ-Orbitrap mass spectrometer using a pool of late-stage cancer samples. We found that targeted analysis on the LTQ-Orbitrap more consistently detected additional peptides for low abundance proteins. MRM quantitative assays for candidate biomarkers that were confirmed by targeted analysis were subsequently developed using pooled cancer samples. In separate analyses using standard proteins spiked into fractions from depleted plasma samples, we typically obtained linear MRM responses ranging from 50 attomoles to 500 femtomoles with average coefficients of variation of 16%. Confirmed candidate biomarkers that displayed a lower level in control/benign versus late-stage sample pools were further subjected to expanded MRM quantitative analyses on individual samples. Pilot studies using an ovarian cancer model showed that many high priority candidates could be verified by targeted analysis of the late-stage serum samples, and these proteins could be detected and quantified by MRM. In summary, the methods presented here offers a promising, cost effective strategy for identification and validation of relatively large numbers of novel cancer biomarkers without the need to synthesize isotopically-coded peptide standards for each peptide to be quantified.

Tues Poster 50: Use of Censored Regression Models for Relative Quantification in Global Mass Spectrometry Data

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Mass spectrometry (MS) coupled with 2D separation protocols is a powerful technology for study of the human proteome and has the potential to lead to a non-invasive screening mechanism of proteins in easily accessible body fluids. The iTRAQ isobaric labeling protocol is capable of reducing machine duty cycle and increasing laboratory throughput by analyzing up to eight samples simultaneously. The paired nature of this protocol reduces experimental noise between labeled samples within a single iTRAQ experiment since relative abundance estimates and peptide identification occur within the same MS/MS scan. However, analytical techniques that address normalization and differential expression from multiple iTRAQ experiments in a single comprehensive analysis have been challenging. One challenge comes from the incomplete nature of the resulting data matrix form multiple iTRAQ experiments making the use of standard microarray techniques non-trivial. Unlike microarray experiments where a known set of genes are interrogated across all experimental runs, the exact number of peptides within a single iTRAQ experiment is unknown and varies across different iTRAQ experiments. This variability results in peptides being identified and quantified in some iTRAQ experiments, but not all. One contributing factor to this variability is the data dependent acquisition protocols used during the survey scan of the MS spectra. As a sample elutes from the liquid chromatography (LC) column into the MS machine, the largest 3 to 5 parent ions are selected for further interrogation with MS/MS in gated time frames. Whether a peptide is selected or not depends on the elution profile of that peptide through the LC column and the abundance of other peptides eluting at the same time. If we view a peptide that is not observed in one of the iTRAQ experiments as being a truncated, then censored survival modeling techniques can be employed. Simulations show that use of censoring models improves estimation of biases due to experimental run effects resulting in better normalization of the data than non-censored models. However, little is gained using censored models when it comes to differential expression analyses primarily due to the balanced nature of run effects across the labeled iTRAQ samples. Further study is underway for scenarios with unbalanced or non-linear run effects.

Tues Poster 51: Use of Multiplexed Discovery Phase Protein Results for the Development of Targeted MRM Assays for Quantification of Low Abundance Proteins

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Discovery phase proteomic profiling of complex biological mixtures using multiplexed LC-MSE strategy, on a Q-Tof mass spectrometer, produces a list of candidate proteins. Clearly to assess the viability of these protein expression changes requires the analysis across a larger number of samples, preferably in a targeted fashion. Specific peptides from these proteins can be targeted as surrogate markers for that protein, in a screening assay using a tandem quadrupole mass spectrometer operating in the multiple reaction monitoring (MRM) mode.

The data independent approach to the discovery phase, IdentityE [1] produces a comprehensive inventory of precursor and fragment ion information which provides a qualitative and quantitative record of the proteins present in each sample. Proteins are confidently identified from their tryptic peptides, as fragment ions are aligned to their related precursor ions in chromatographic space by retention time and chromatographic peak shape.

The inclusion of an internal standard enables the calculation of the amount of identified proteins present in a sample, since recent studies have shown that the absolute quantitation of proteins is possible by label-free techniques [1,2] by simply averaging the intensity of the top three best ionizing peptides from each identified protein, and normalizing to an internal standard. proteins'
Furthermore the LC-MSE data which includes precursor and fragment ion information, and ion intensities, can be systematically and consecutively filtered using a series of criteria to allow the empirical selection of 'proteotypic' peptides, and their optimum

transitions for subsequent analysis by Multiple Reaction Monitoring (MRM). MRM is a powerful method for quantitatively screening protein markers in biological samples, but the prediction of MRM peptide transitions is challenging. Such empirical determinations of optimum transitions are made possible by the nature of the of IdentityE data and the VerifyE software and the output includes MRM methods built automatically for implementation on a tandem quadrupole mass spectrometer

We will describe the complete workflow, from protein identification through to peptide quantification using a variety of samples including E coli samples and CYP450 enzymes.

1. Gerber, S. et. al. Proc. Natl. Acad. Sci. U.S.A. 2003, 6940-6945.
2. Silva, J. et. al. Mol. Cell. Proteomics, 2006, 144-156.

Cell and Tissue Proteomics, 52 – 59

Tues Poster 52: Alternative 2D Electrophoresis – OFFGEL Electrophoresis Combined with High Sensitivity Microfluidic On-Chip Protein Analysis

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Two dimensional gel electrophoresis (2D-GE) employs isoelectric focusing in the first dimension and a separation of the proteins according to their molecular weight in the second dimension. The gels are then stained using silver stain to visualize the protein pattern. This method is unrivalled in terms of resolution but is a tedious and time-consuming procedure. Here we present a combination of two easy methods that separate proteins in analogy to 2D-GE according to their isoelectric point (pI) and molecular weight (kDa).

For the first dimension, OFFGEL electrophoresis was used. This newly developed method takes advantage of the impressive resolving power of immobilized pH gradient gel based isoelectric focusing (IPG IEF) but in contrast to conventional isoelectric focusing delivers sample in liquid phase thus avoiding sample recovery from the gel. For the second dimension, a microfluidic high sensitivity on-chip protein sizing method was employed. This method allows separating proteins from 5 to 250 kDa and offers a sensitivity equivalent or better than silver staining and a linear dynamic range across four orders of magnitude.

Our data demonstrates that it is possible to easily detect a 1 % change in protein expression.

Tues Poster 53: Specific Detection of Proteins by Immonoprecipitation Combined with High Sensitivity Protein Sizing on Microchips

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Today, immunoaffinity is a crucial tool for the targeted analysis of proteins in complex samples. Techniques like Enzyme Linked Immunosorbent Assay (ELISA) and Western Blotting are widely used for a wide range of applications such as biomarker candidate verification in body fluids or clone selection for recombinant protein expression. Here we present a new method that combines the specificity of an immunoprecipitation approach with the high sensitivity of protein detection on microchips using the High Sensitivity Protein 250 assay for the Agilent 2100 bioanalyzer. Initially, sample proteins are derivatized with a fluorescent dye. After incubation with the specific target antibody, the immunocomplexes are captured with Protein A/G coated magnetic beads, washed and eluted by heat denaturation in the presence of SDS. Samples are then directly loaded on microchips and analyzed automatically with the bioanalyzer for protein size and quantity. The final on-chip analysis takes about 30 min for 10 samples and yields digital data. Together with the sample preparation steps, the total assay time is about 3 hours. Results are compared to Western Blotting and pros and cons' of both methods are discussed.

Tues Poster 54: Heat Stabilization of the Tissue Proteome: a New Technology for Improved Proteomics

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Immediately after sampling, proteases and other protein-modifying enzymes change proteome composition. The results from subsequent analyses reflect a mix of in vivo proteome and degradation products. Important information about the 'pre-sampling' state of the tissue may be distorted or destroyed, leading to reduced reproducibility between samples and even faulty conclusions. This problem is addressed by rapid sample inactivation in a novel tissue stabilization system (Stabilizor T1, Denator AB) which halts post-sampling modifications irreversibly by heat induced protein denaturation. After treatment tissue samples were analyzed with downstream techniques such as western blotting, MALDI-MS or Nano-LC-MS.

When looking at the low mass content (< 10 kDa), the results show a large number of detected peptides in the untreated samples identified as protein degradation fragments from highly expressed proteins such as hemoglobin, dynamin, NADH dehydrogenase. In contrast, the peptides detected in the stabilized samples were identified as known neuropeptides, endogenous peptides and small proteins. The inhibition of phosphorylase is shown to be improved compared to a common chemical inhibitor. After stabilization treatment the levels of phosphorylated CREB, GSK and MAPK were maintained up to 2 hours in room temperature treatment whilst the levels in untreated tissue decreased.

Tues Poster 55: Relative Peptide Quantitation in Time Course Reactions using Scheduled MRM

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Peptide quantitation with multiple-reaction monitoring (MRM) using liquid-chromatography coupled to mass spectrometry (LC-MS/MS) is rapidly becoming a widely-accepted technique because of its high sensitivity and specificity. The need for verification and validation of the high numbers of protein targets generated from discovery data is driving the development of new and innovative instrumentation, software tools, and complete workflows for relative and absolute quantitation experiments. Higher multiplexing of MRM experiments is needed to carryout quantitation of multiple protein targets for cell signaling pathway profiling. This has been made possible with a "scheduled" MRM approach, which greatly enhances duty cycle and data quality, enabling the analysis of over 2000 MRM transitions in a single experiment.

The inner medullary collecting duct (IMCD) of the kidney is a promising cell type that can be isolated in a pure form and demonstrates fast cell signaling responses to extracellular hormonal stimuli. The regulation of the IMCD water channel, aquaporin 2 (AQP2) is through vasopressin-mediated protein phosphorylation and subsequent recruitment of cytoskeletal machinery required for cellular trafficking of recycling endosomes containing phospho-AQP2 to the plasma membrane. Using the MIDAS™ Workflow for simultaneous MRM and peptide sequencing, over 500 scheduled MRM transitions were designed to target AQP2 and endosome associated proteins, such as Ras-related RAB proteins and syntaxins, and such sMRMs were monitored in a vasopressin time course experiment. Post acquisition sMRM quantitation was performed using MultiQuant™ Software to compare the phosphorylation profile of AQP2 and the abundance of endosome trafficking proteins in fractionated IMCD digests treated with vasopressin in a 0 to 30 min time course experiment

Tues Poster 56: Optimized Immunoprecipitation and Co-Immunoprecipitation using Dynabeads

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Immunoprecipitation and co-immunoprecipitation are classical methods used to isolate specific proteins or protein complexes from biological samples. Methods use the antibody-antigen reaction principle to identify a protein that reacts specifically with an antibody in a mixture of proteins so that its quantity, physical characteristics or interaction partners can be determined.

Traditionally, sepharose and agarose slurries have been used, but more recently magnetic beads have gained popularity due to shorter and simpler protocols. Magnetic Dynabeads are ideal for immunoprecipitation. The rapid procedure permits the isolation of labile composites that might otherwise dissociate during long incubation times (or be damaged by proteases), there is no upper size limit for the complex to be isolated (ideal for complex pull-down) and the surface properties give very low non-specific binding. With Dynabeads there is no fear of losing beads (as with spun down resin) and you can scale down the procedure to reduce the consumption of expensive antibodies. Here we show data of improved immunoprecipitation and co-immunoprecipitation protocols, and demonstrate the usefulness of combining GFP-tagged fusion proteins with Dynabeads to enable the combined visualization and pull-down of proteins.

Tues Poster 57: Identification of Subtype-Specific Extracellular Proteins from Ovarian Tumors

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Ovarian cancer is the most lethal gynecologic malignancy with epithelial ovarian tumors comprising ninety percent of ovarian tumors in adult women. The origin of epithelial ovarian tumors are heterogeneous and can be subclassified histologically into serous, mucinous, endometrioid, clear-cell, transitional-cell types, squamous cell, mixed, and undifferentiated subtypes. Different subtypes of ovarian tumors have differences in clinical outcome and response to chemotherapy. Understanding the molecular basis of these subtypes of ovarian tumors is increasingly important in understanding and predicting responses to targeted biological therapeutic agents. Therefore, the direction of ovarian cancer biomarkers discovery for therapy would be toward the subtypes with early stage diagnosed cancers.

In this study, we identified glycoproteins specific for different subtypes of ovarian tumors and determined their tissue-specificity. The glycoproteins from seven subtypes of ovarian tumors and normal control ovarian tissues were isolated from three individuals separately using solid-phase extraction of glycopeptides isolation method. The isolated glycopeptides were analyzed by quantitative proteomic analysis method using the LC-MS and LC-MS/MS data generated by ESI-QSTAR. In addition, spectral count quantitation from LC-MS/MS data generated by multiple analyses using LTQ was also employed to increase the confidence of identification and quantification. To determine which of the ovarian tumor proteins were specific for ovarian tissues, the glycoproteins identified from specific subtype of ovarian tumor were further compared with several other major cancer types in women, such as breast cancer, bladder cancer, liver cancer, etc.

The results from this study allowed us to determine around 1000 N-linked glycosylation sites from cancer tissues and identify subtype-specific glycoproteins for ovarian tumor. This will facilitate the understanding of molecular mechanism of ovarian cancer subtypes and differentiate them in molecular level. The results also provide the candidate glycoproteins as molecular basis for detection and treatment of different ovarian tumors and further development and validation are required for clinical usage.

Tues Poster 58: Secretome Analyses from Staged Pancreatic Cancer Cell Lines

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Pancreatic ductal adenocarcinoma has the lowest 5-year survival of any cancer; its aggressive nature and late onset of physical symptoms lead to poor prognoses. Pancreatic cancer primarily occurs in the exocrine portion of the organ, with fewer occurrences in the endocrine section. Approximately 95% of cancerous exocrine tumors (carcinomas) are derived from ductal cells, while a smaller fraction of tumors are from acinar cells. Recognition of biomarkers is clearly an imperative undertaking for the disease. To tackle this problem, we are attempting to evaluate the secreted protein profile of pancreatic cancer cell lines derived from different stages. Critical to the success of this workflow is the derivation of cells that are sustainable without overt morphological changes in peptide- and protein-free media conditions or dramatic necrosis over a 72 hr profile. We have successfully adapted ten human pancreatic cancer cell lines into low serum environments and pursue collection of conditioned media by a combination of ultracentrifugation, molecular weight cutoff, protein precipitation, gel-based separation, and tryptic digestion followed by MALDI-ToF-MS protein identification. Preliminary data suggested significant cellular autolysis that liberated cytosolic proteins such as beta-actin. To minimize cytosolic contamination, cell lines were subsequently cultured from low-serum to Matrigel™ in a serum-free media to phenocopy the ductal environment typical for these cells. Consistent with the model that advanced stage cancer has increased secretory function, earlier stage pancreatic cancer cell lines showed generally lower protein secretion while later stage cell lines showed generally increased secretion. A catalog of proteins is being compiled in ProteinScape (Bruker Daltonics) and validated biochemically including positive identifications for Glutathione S-Transferase pi (GSTP1) and Galectin-3 (GAL3)—two proteins that have been correlated with tumor secretions from prostate and breast cancer, respectively. Taken together, our reproducible workflow demonstrates the utility of assessing the secretome fraction from cultured cancer cells.

Tues Poster 59: Targeted LC/MS/MS Identification of Selected Proteins in Undepleted Plasma

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The ability to identify specific proteins at low abundance in plasma samples is hampered by the high dynamic range of proteins present (12 orders of magnitude). Using a mixture of test proteins found in the ABRF 2008 test sample, including PSA, hCG beta and rabbit phosphorylase A and B, we sought to develop a rapid means for targeting these proteins in undepleted plasma. Using the digital ProteomeChip (dPC™) and a western blotting method called isoelectric Western blotting (iWB™), we were readily able to target the presence of these proteins to a specific set of dPC gel features within the 6.00-8.00 pH range dPC. The iWB method is a quantitative approach to transferring the contents of the dPC to PVDF. We have demonstrated that the addition of backing layer in combination with the PVDF membrane yields quantitative recovery of the proteins of interest on the PVDF without the “blow through” typically associated with standard Western blotting.

The test samples were then subjected to dPC fractionation and then those gel features identified as protein positive by iWB were extruded for in gel trypsin digestion and LC/MS/MS. Using a Thermo Fisher LTQ mass spectrometer with C18 reverse phase nanocolumn (75 nm ID), we identified all three proteins by this targeted approach. This approach is amenable to targeted identification of any protein so long as an antibody is available. Despite the presence of highly abundant proteins such as albumin or immunoglobulins in the plasma samples, we can enrich for proteins of interest that have differing isoelectric points compared to those abundant proteins and avoids the need for depletion for many analytes.

Disease Proteomics, 60 – 77

Tues Poster 60: Serum Peptide Biomarkers are Altered with Disease States of Mammary Carcinogenesis

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Early detection and prognostic profiling of cancers has the potential to increase lifespan and quality of life. The "field effect" hypothesis that motivated this investigation suggests that there are cellular changes that occur both within and around tumor cells that could be detectable in serum. These changes may be detectable before the disease is histologically identifiable using the current testing methods. This valuable information could potentially come from serum where early stages of tumorigenesis lead to changes in the serum peptidome. An experiment testing this idea was carried out using a rat model of mammary carcinoma. Samples were collected at different stages of progression to determine if MALDI-TOF mass spectrometry could provide a proteomic profile that could identify disease. MALDI-TOF spectra were obtained on an Applied Biosystems Voyager-DE PRO Biospectrometer and analyzed using peak picking computer algorithms and logistic regression models. MALDI-TOF analysis was performed on sera taken from control and carcinogen-treated at each necropsy time point. The peak 4253m/z revealed a monotone change in the intensity difference that was statistically significant between the treated and untreated rats over weeks 2, 3, 4, and 5. The corresponding band was excised from a gel and possible identifications were determined via electrospray ionization (ESI). One biologically plausible candidate for this band was Dermcidin, a protein previously linked to breast cancer. Due to the biological plausibility that this protein could be important we are now carrying out additional experiments examining Dermcidin levels in the samples. We plan to further characterize the role that Dermcidin plays in rat mammary carcinogenesis and investigate any correlation with human breast carcinogenesis.

Tues Poster 61: Proteomics-Level Identification of Kinetically Stable Proteins by Diagonal 2D SDS-Page and Biomarker Discovery in Human Plasma

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Most soluble proteins are in equilibrium with partially and globally unfolded conformations. In contrast, kinetically stable proteins (KSPs) are trapped by an energy barrier in a specific state, unable to transiently sample other conformations. Kinetic stability (KS) may be a feature used by nature to preserve the native folding of a protein under harsh conditions. The biological and pathological significance of KS remains poorly understood due to the lack of simple experimental methods to identify this property. Based on our previous correlation between KS and a protein's resistance to the denaturing detergent SDS, we show here the application of a diagonal two-dimensional (D2D) SDS-PAGE assay to identify KSPs in complex mixtures. We applied this method to the lysate of E. coli and identified structural and functional features that may bias proteins in favor or against KS. We also applied this method to human plasma, and clearly identified several abundant proteins as KSPs. One of them is transthyretin (TTR). TTR is an abundant protein in human plasma that is linked to several amyloid diseases, including familial amyloid polyneuropathy (FAP). The mutation-induced loss of TTR KS has been implicated in the pathological mechanism of FAP. D2D SDS-PAGE of samples containing WT TTR or FAP-related TTR mutants with compromised KS has shown clear differences in their migration pattern. Thus, our results suggest that D2D/SDS-PAGE may be used to screen human plasma for KSP, and may be apply to biomarker discovery for diseases pathologically linked to the abnormal gain or lost of protein kinetic stability.

Tues Poster 62: Proteomics as a Tool to Identify Potential Prognostic Biomarkers in Chronic Lymphocytic Leukemia

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Chronic Lymphocytic Leukemia (CLL) is the most common adult Leukemia in the UK, Western Europe and America. It is a malignancy of naive B-cells. The clinical course of patients with CLL is heterogeneous; some patients survive for years without treatment, others die of a chemotherapy resistant disease within two years of presentation. Genomic studies have found little variation in patients showing differing prognosis, suggesting that it is the same disease but with varying outcomes. At present there is no cost effective, reliable and routine clinical test which can distinguish patient prognosis and a "watch and wait" strategy is currently in clinical use.

Studies have shown that patients who express mutated IgVH genes on the B-cell receptor (BCR) have a good prognosis, whereas patients who express unmutated IgVH genes have a poor prognosis. However IgVH gene mutational status is an expensive and time consuming test and is not practical for routine clinical practice. If the B-cell has not been sensitised to a specific antigen (i.e. unmutated IgVH genes on BCR) it is hyper-response to stimulation through the BCR by antigen. Stimulation of the BCR may prevent apoptosis of malignant cells; therefore a hyper-responsive BCR is linked to bad prognosis.

By artificially stimulated cells and using proteomic techniques we have investigated signaling pathways activated by the BCR to gain a greater understanding of the anti-apoptotic nature of the malignant B-cells and to find potential prognostic biomarkers related to a hyper-active BCR.

Protein was extracted from stimulated and unstimulated cells, from CLL patients categorised as having a poor prognosis (unmutated IgVH genes and hyper-responsive BCR). The extracts were separated using conventional two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The gels were stained with coomassie blue total protein stain and analysed with statistical software. Proteins with a two-fold ($p<0.05$) change in expression between stimulated and unstimulated samples were excised from the gels and analysed by matrix assisted laser desorption/ionisation with time of flight mass spectrometry (MALDI-TOF-MS).

Changes in protein expression were detected in response to prolonged BCR stimulation. Many of the proteins have had no previous connection with BCR signaling or leukemia and give a greater insight to the mechanisms of the BCR. Targets found include ones which are associated with the activation of anaplastic lymphoma kinase (ALK), the plasma kallikrein-kinin system (KKS), the AKT-1 pathway, the MAPK pathways, the adenylate kinase system and involvement in the CD40-dependant activation of B-CLL cells. One of the protein targets found was increased by over two-fold in three independent clinical samples after sustained BCR stimulation. Confirmation work on this protein is presently being undertaken in a number of clinical samples. If we understand more about the BCR signaling pathways then we may be able to identify potential prognostic biomarkers and novel targets for therapeutic intervention that may inhibit survival of the malignant B-cells.

Tues Poster 63: Application of Label-Free Quantitative Differential Analysis for Biomarker Discovery in Tuberculosis Mycobacterium

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Mycobacterium tuberculosis (Mtb) has evolved specialized secretion systems for the transport of proteins across their complex cell walls. One of these, the ESX1 secretion system, is critically required for the virulence of Mtb. Indeed, the primary attenuating deletion in BCG, the live attenuated vaccine used

against tuberculosis, is loss of the genes encoding the ESX1 system. The ESX1 structural components are thought to form a multisubunit cell-envelope spanning structure. However, the molecular function of the five known ESX1 substrates is unclear. It has been speculated that individual ESX1 substrates independently act as effectors to disrupt host cell functions and alternatively, that they interact to form an extracellular virulence machine. In order to comprehensively identify substrates of the ESX1 secretion system, we coupled high resolution LC-MS/MS with novel label-free differential analysis software to analyze M. tuberculosis strains lacking the ESX1 locus. Bioinformatic analysis was carried out using SIEVE. The SIEVE iterative workflow includes chromatographic alignment, a global intensity-based feature extraction and aggregate protein identification assignment. Chromatographic alignment is based upon the pairwise MS full scan comparison of all experimental MS runs with respect to a chosen reference MS run. Overlapping correlation sub-matrices (tiles) are computed using a novel scalable adaptive tile algorithm. An optimal path through each tile is determined using dynamic programming and a final alignment score is calculated. Subsequent to chromatographic alignment, potentially interesting features are exposed based upon high-intensity peaks found in the aligned collective data set. Individually, these peaks define frames ie well defined rectangular regions in the full scan (M/Z versus retention time) plane. Reconstructed ion chromatograms are calculated for each frame to assess relative expression ratio and supporting statistics. After framing, MS2 fragment scans associated with each frame are processed with SEQUEST. A consensus protein report is constructed by statistically aggregating frame information to construct peptides and then peptide information to build proteins. Results from the SIEVE analysis confirmed the identification of the five previously identified secreted proteins as well as other differentially expressed proteins across the mutant strains. Future planned experiments include the development of selective reaction monitoring (SRM) based assays for target peptides in the secreted proteins.

Tues Poster 64: Development of a Multiplexed SRM Assay for Osteoarthritis Biomarkers

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Osteoarthritis (OA) is a prevalent, poorly understood disease whose management would be significantly advanced by the development of assays for early diagnosis and/or disease prognosis. Previous discovery work has identified candidate protein biomarkers for OA (1) including afamin and proteoglycan 4. Recently, emphasis has been placed on advancing proteomics studies from discovery and/or relative quantitation to validated quantitative methods in an effort to establish clinical assays. Targeted selective ion monitoring (SRM) assays provide a vehicle for cost effective, high-throughput quantification and monitoring of specific disease biomarkers. In this study, we applied novel software to integrate information from MS/MS discovery spectra generated on an LTQOrbitrap™ platform to facilitate the development of osteoarthritis specific SRM assays on a TSQVantage™ triple quadrupole mass spectrometer. The SRM assays were used to interrogate synovial fluid patient samples and demonstrate the applicability of this approach for potential OA screening.

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Tues Poster 65: Efficient Biomarker Discovery Coupled with SRM Assay Development in Maternal Serum from Normal and Trisomy 21 First Trimester Pregnancies

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Women over 35 years old constitute about 15% of pregnancies in most developed countries worldwide. The risk for many chromosomal defects increases with maternal age. Down's Syndrome (DS) occurs at a rate of 1 in 600-800 pregnancies and prompts most prenatal diagnoses that involve invasive procedures such as amniocentesis or chorionic villous sampling. These procedures carry a risk of miscarriage and therefore are only applied to women in a high risk groups (1). Over the past several years, there has been an effort to develop less invasive screening methods that can identify aneuploidy early in the pregnancy. Maternal blood tests that measure serum protein markers associated with DS such as free b-hCG, PAPP-A, alpha fetoprotein and inhibin A, combined with the measurement of nuchal translucency have garnered increased acceptance for first trimester screening (2) but the sensitivity and specificity of these markers could still be improved. Previous proteomic studies have identified other putative serum markers (3), however, the need remains for further discovery and confirmation in this area. In this study, we coupled high resolution LC-MS/MS (LTQ-Orbitrap™) with novel, label-free differential analysis software to analyze a cohort of maternal blood samples from first trimester DS and normal pregnancies. Bioinformatic analysis was carried out using SIEVE™ software. The SIEVE iterative workflow includes chromatographic alignment, global intensity-based feature extraction and aggregate protein identification assignment using SEQUEST. Subsequent to the discovery experiments, candidate differentially expressed target proteins were selected and quantitative selective reaction monitoring (SRM) assays were developed on a triple quadrupole mass spectrometer (TSQ Vantage™). Novel SRM development software facilitated the efficient selection of signature peptides from the targeted proteins and the resulting SRM analyses monitored predicted precursor and fragment transition ion pairs. Heavy-isotope internal standards allowed for simultaneous quantification. The quantitative SRM assays were applied to the original cohort samples to verify putative biomarker abundance ratios. Future experiments will test the SRM marker panel on other sample cohorts. The described workflow demonstrates an approach that may in general be applied to link biomarker discovery studies with subsequent candidate verification assays.

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Tues Poster 66: Ludesi´s 2D gel image Analysis Enabled Discovery of Potential Therapeutic Targets for Solid Tumors

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The acknowledged problems of reproducibility and resolution inherent in the 2-DE technology present a challenge for any image analysis software. As a result, in nearly all cases, the extracted data is partly incorrect and incomplete. As a consequence, researchers have to deal with two serious problems in image analysis - false positives and false negatives. Both are costly, not just in terms of the resources spent on downstream analysis of false hits but perhaps more importantly, by impeding a true understanding of the underlying biological system.

Knowing what a challenge it is to identify those proteins of biological interest that are truly differentially expressed between different physiological conditions, while minimizing false positives

and false negatives, many researchers spend substantial amounts of subjective work on the image analysis to compensate for the many errors that accumulate during the analysis. However, even using sophisticated software packages and spending time on manual intervention is not a guarantee for achieving greater accuracy of the analysis. In particular larger data sets and more challenging samples such as tumor tissue that generate complex spot patterns are often particularly difficult to analyze using conventional software.

As a way of enhancing the data set of solid tumors from a mouse model of melanoma, Ludesi was used to generate a robust image analysis that gave higher correctness and less bias. Coupled with multiple statistical approaches, this gave high confidence in the proteins that were selected as being differentially expressed in the tumor mass during specific stages of tumor growth in a mouse model of melanoma. This proteomic approach visualized protein expression changes within an evolving tumor that reflect in vivo processes of tumor progression. Some of these proteins were identified as potential molecular targets for solid tumors.

This work was supported by the Intramural Research Program of the NIH, National Eye Institute.

Tues Poster 67: Proteomics Exploration of Virulence in Salmonella Typhimurium

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Non-typhoidal salmonellae (NTS) are common causal agents of acute food-borne disease worldwide, with the bacterium Salmonella Typhimurium one of the leading causes. Salmonella Typhimurium infections can be life-threatening in elderly, very young, and immunocompromised patients. For example, in AIDS patients a defect in the immune response allows Salmonella Typhimurium to cross the mucosal barrier of the gut and enter the bloodstream triggering NTS bacteremia, a life-threatening complication (Baumler et al. Nature Med. 2008). It is estimated that about 50% of all HIV-positive African adults who become infected with NTS die. Because of the increasing prevalence of antibiotic-resistant isolates, an understanding of the growth and pathogenesis of NTS, specifically Salmonella Typhimurium, is important for developing new therapeutic agents.

To understand the systems involved in virulence of Salmonella Typhimurium, comprehensive and quantitative label-free LC-MS-based proteomics studies using the AMT tag approach are being conducted on samples covering different aspects of pathogenesis and virulence. This approach to proteomics allows for the design and execution of experiments that would otherwise not be feasible using chemical/labeling methods due to time and cost considerations.

It is generally believed that proteins coordinately regulated by Salmonella regulatory proteins required for virulence will represent the best targets for therapeutics. As a first step in understanding how virulence is regulated, we constructed in-frame non-polar deletions of 84 regulators thought to play a role in Salmonella Typhimurium virulence and tested them for virulence. Proteomics profiling was carried out for each of the 15 most attenuated mutants under growth conditions that mimic the intra-macrophage condition to identify proteins coordinately regulated by Salmonella regulatory proteins.

A number of key down-stream proteins that are highly regulated have been revealed. About 1/3rd of the putative targets have been previously implicated in virulence and the rest provide novel targets for therapeutic interventions. These observations have been validated in macrophage cell-lines and Western Blot analysis.

Tues Poster 68: Identification of Serum Peptides Correlating with Ovarian Cancer

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Ovarian cancer is a disease often diagnosed in later stages where survival is poor compared to early stages. As a result, there have been many attempts to discover biomarkers for early stage diagnosis. Mass spectrometry profiling in association with bioinformatic tools, can be useful for the purpose of achieving this aim.

We analyzed 395 serum samples from two different institutions (Northwestern University, IL and University of Brescia, Italy), consisting of 205 biopsy confirmed ovarian cancer samples and 190 high risk samples from women with a first degree relative with ovarian cancer, women who had other cancers, or women who had pelvic masses. Profiling was done using an optimized robust method under controlled laboratory conditions. Briefly, serum samples were fractionated with C18 reverse phase magnetic beads and then analyzed with the prOTOF high resolution MALDI mass spectrometer. The spectra obtained were binned to reduce the number of data points and normalization and noise smoothing techniques were used as previously described. The classification was based on the majority voting of three standard machine learning tools: Support Vector machine, C5 decision tree and Partial Least Method. The mass spectrometer data were randomly split into two subsets, training and testing set. The classification results of the test group showed a sensitivity of 82.1% and a specificity of 86.2%. Bioinformatics identified a list of down-regulated peaks and five that showed the highest discrimination underwent further analysis to identify their amino acid sequence. After concentration and isolation of the investigated peaks with progressively more selective reverse phase chromatographic separation techniques, the sequences of the five peaks were identified using LC-MS/MS (Agilent, 1200 and LCQ, Thermo) and MALDI-TOF/TOF (Ultraflex III, Bruker).

The results confirmed that the five peaks – all down-regulated – were correlated and they were part of the same fragment of fibrinogen alpha chain leading to the hypothesis that the cleavage of the precursor protein – in this case fibrinogen – might be characteristic of the disease. Confirmation using synthetic peptides and corresponding antibodies are in progress.

Acknowledgements: Funded in part by NCI Contract No. HHSN261200800001E

Tues Poster 69: Mood Stabilizer-Induced Changes in the Postsynaptic Density Proteome

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Increasing evidence supports the hypothesis that bipolar disorder arises from abnormalities in cellular plasticity cascades, leading to aberrant information processing in synapses and circuits. In the context of this hypothesis, mood stabilizers such as lithium and valproic acid are thought to exert their therapeutic effects via actions on systems involved in synaptic plasticity. The postsynaptic density (PSD) is an elaborate cytoskeletal and signaling complex that provides anchors for synaptic proteins close to the region of presynaptic neurotransmitter release, and therefore mediates signaling in a host of divergent signal transduction pathways. The goal of this project is to understand the temporal and spatial dynamics of the PSD in the treatment of mood disorders; it is our contention that – in addition to manipulating key "candidate molecules" – these studies are critical to elucidate the mechanisms of synaptic regulation and of mood stabilizer action. The experiments presented here take advantage of proteomic techniques to identify and quantify proteins in PSDs subjected to mood stabilizer treatment. These experiments aim to identify brain region-specific changes in PSD protein levels in response to

treatment, focusing on brain areas that have been implicated in bipolar disorder, including prefrontal cortex, hippocampus, and striatum. In preliminary studies, protein composition of pooled PSD hippocampus preparations from12 rats was analyzed in triplicates by 2-D liquid chromatography and tandem mass spectrometry. Peptides from trypsin digested PSD preparations were first separated off-line by strong cation-exchange chromatography into 36 fractions, followed by reverse phase-tandem mass spectrometry analysis on Eksigent-LTQ-orbitrap system. Quantitative differences between treatment conditions were analyzed using both extracted ion current and spectral count methods. Our data shows that there are networks of signaling proteins that are twofold higher or lower in valproate and lithium treated animals. Our studies identify PSD proteins that are regulated by mood stabilizers in a region-specific manner, with the goal of determining their role in plasticity and behavior and further elucidating the mechanism of mood stabilizer action.

Tues Poster 70: Self-Perpetuating CF-Inflammation with IL-8 Hypersecretion as a Side-Effect of Aberrant TNF/NFKB(p65) and p53 Cell Survival Control in ΔF508-CFTR Cells

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Introduction: Excessive IL-8 secretion in CF lung is generally ascribed to constitutively activated NFκB. However, transcriptional and signaling proteomes that can influence the IL-8 driven CF hyperinflammation are not fully elucidated. Therefore, we undertook large-scale protein expression and biosynthesis profiling (>500 proteins) that included major transcriptional and signaling cascades.

Material and Methods: 35[S]-methionine labeling was used to follow biosynthesis and degradation of proteins in juxtaposition to corresponding IL-8 levels in parental IB3-1 (ΔF508-CFTR) and repaired IB3-1/S9 ([WT]CFTR) cells. An antibody microarray platform (Clontech) and the Ingenuity Pathways Analysis (IPA, Ingenuity Systems) software were used to identify molecular signatures in cells. The protocol involved subjecting cells to a 35[S]-methionine pulse (30-60min), followed by a wash and then a chase with unlabeled methionine (2-4-6h). Derived transcriptional profiles were then analyzed in juxtaposition to IL-8 promoter analysis (Genomatix).

Results: In parental CF (but not repaired) cells, autocrine IL-8 secretion (6h) was increased (~10-fold) and that was accompanied by significant alterations in the cell death/proliferation signaling including upstream NFkB pathway: activation of pro-survival PI3K and downregulation of pro-apoptotic TNF-alpha/TRADD. Following the stressful low methionine "pulse" part of the experiment which exacerbated IL-8 hypersecretion in CF (but not repaired) cells, we chased the trajectory of changes in protein levels and biosynthesis as both the CF and repaired cells attempted to reset their IL-8 associated signaling programs. The repaired IB3-1/S9 ([WT]CFTR) cells with low IL-8 levels responded by distinct dynamics in major IL-8 associated signaling pathways such as TNF/TRADD, IκB/IKK/NFκB(p65), Calcium, Glutamate, and MAPK that was preceded by the p53 upregulation [-log(p-value)≥2]. On the other hand, in the CF IB3-1 cells with the prior PI3K activation, we detected an alternating expression and biosynthesis of STAT3 and NFAT, succeeded by an increase in Sp1 expression. Neither Sp1 nor Acute Phase Response STAT3 was activated in the repaired IB3-1/S9 cells. Instead, biosynthesis rate of Ca-dependent NFAT was gradually increased, whereas the expression of RelA/p65 was eventually downregulated that was consistent with the overlapping NFAT and NFκB binding elements in the immediate IL-8 promoter. Synexpression profile of ER-associated HSPs, calnexin and ITPR3 in the CF IB3-1 cells suggested that ER retention of the ΛF508-CFTR-calnexin complex with subsequent alterations in the Ca-signaling and phagocytosis of dying cells might be directly involved in the excessive IL-8 secretion in CF-cells.

Conclusion and interpretation: Excessive activation of IL-8 in the CF IB3-1 cells results from oscillations of NFAT and STAT3 that are associated with the PI3K activation and aberrant TNF/TRADD and NFκB(p65)-mediated cell survival signaling. By contrast, in the [WT]CFTR IB3-1/S9 cells with maintained ER homeostasis, the cell cycle regulator p53 controls the cell proliferation associated signaling including NFkB(p65), while IL-8 expression appears to be physiologically regulated by the Ca-mediated TNF/NFAT. Thus, IL-8 driven CF-inflammation can result from primary defects in cell death/proliferation due to ΛF508-CFTR, and, consequently, the reinstatement of cell survival control can be a novel approach to CF treatment.

Tues Poster 71: A Workflow for Identification and Confirmation of Protein Biomarkers for Diseases

Ruth A VanBogelen; Richard Jones; Michael Pisano; Michael Ford

NextGen Sciences, Ann Arbor, MI

The ability to routinely test validated biomarker levels is the goal for researchers and clinicians. However, assay development has been a major barrier to reaching this goal. In many cases substantial resources have been expended to generate a list of putative, biologically relevant protein biomarkers. However assay development has always been a rate limiting step to the confirmation and later validation and acceptance of these molecules. The excitement of new biomarkers often fades as the limitations of ELISA/sandwich assays restrict the number of biomarkers that can move through development. We developed a mass spectrometry based workflow to rapidly progress biomarkers to the validation phase without a requirement for expensive and time consuming antibodies. The biomarker discovery approach taken by NextGen Sciences generates information which is crucial to the development of accurate, precise and robust assays. This accelerates the development of multiplexed relative quantitation assays (Stage 1 biomarker assay) to weeks and allows the confirmation of all the putative protein biomarkers in record time. Once a set of biomarkers is confirmed using the Stage 1 assay, a multiplexed absolute-quantitation assay (Stage 2 assay) is the next step in the workflow. This Stage 2 assay can go through vigorous assay validation steps (fit for purpose) so that data from clinical samples can be submitted to regulatory agencies. This presentation will describe how this workflow has worked for disease biomarkers.

Tues Poster 72: Identification of Novel Splice Variants in Mouse Models for Breast Cancer

Rajasree Menon; David J. States; Gilbert S. Omenn

University of Michigan, Ann Arbor, MI

Alternative splicing plays a major role in protein diversity without significantly increasing genome size. Aberrations in alternative splice variants are known to contribute to a number of diseases. The several alternative splice databases now publicly available differ in their annotation and modeling methods and contain many transcripts not present in reference resources like Ensembl or Refseq. The ECgene database is one of the largest alternative splice variant databases [Kim P, et al. Genome Research 2005; 15(4): 566-76]. In this study of potential biomarkers for breast cancer, we have used mass spectrometric data to interrogate a custom-built, non-redundant database created with three-frame translations of mRNA sequences from ECgene and Ensembl to find alternative splice variants. The mzXML files from LC-MS/MS analyses of tumor and normal mammary tissue from a HER2/Neu-driven mouse model of breast cancer [Whiteaker et al, JPR 2007; 6 (10), 3962-3975] were downloaded from PeptideAtlas [http://www.peptideatlas.org/repository/]. These files were searched against the database using X!Tandem software. We identified 3898 distinct peptides with X!Tandem expect score < 0.001 at a false discovery rate < 1.7%. The peptides were analyzed using NCBI blastp and UCSC blatp. We found 7 novel peptides that occurred only in tumor samples; these peptides did not match completely to any known mouse protein sequence and were identified by more than one spectrum. Six of these peptides either matched to intronic sequences of known genes or partially

matched to known protein sequences. For example, 'RGQKPPAMPQPVPTA', a novel peptide identified by 3 distinct spectra, had five amino acids missing when compared with the known peptide sequence 'QKGGKPEPPAMPQPVPTA' from ribosomal protein S3. The known peptide has a functional motif 'GGKPEPP' that is involved in protein-protein interaction mediated by SH3 domains. This motif is missing in the novel peptide we found. We found a novel peptide from the intronic region of Rogdi gene that had a phosphopeptide motif which directly interacts with the BRCT (carboxy-terminal) domain of the breast cancer gene BRCA1 with low affinity. Another novel peptide identified by 3 distinct spectra did not match to any known protein sequence. However, its sequence matched to Mus musculus chromosome 7, clone RP23-49M22 by NCBI tfblast and the peptide sequence had signal peptide and trans membrane regions when analyzed by EBI software InterPro Scan. These data suggest that alternative splice variants play functional roles in tumor mechanisms and are potentially rich sources of candidate biomarkers. More detailed analyses on these proteins are being done.

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Tues Poster 73: Proteomics of Chlamydia Trachomatis Infection

J. Will Thompson[1]; Laura G. Dubois[1]; Hector A. Saka[2]; Yadunanda Kumar[2]; M. Arthur Moseley[1]; Raphael Valdivia[2]

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Chlamydia trachomatis is an obligate intracellular bacterial pathogen that infects ocular and genital epithelial surfaces in humans, and can lead ultimately to blindness, pelvic inflammatory disease, and infertility (Schachter, 1999). Raphael Valdivia in the Duke Department of Molecular Genetics and Microbiology and the Duke Proteomics Core Facility have collaborated to investigate the proteomics of Chlamydia infection. Using HeLa cells and C. trachomatis serovar LGV-L2 as a model of infection, three Chlamydia proteomics projects are currently ongoing in the Proteomics Core Facility looking at differential expression and modification of both human and Chlamydia proteins. Differential expression analysis is being performed using bottom-up LC-MS/MS and LC-MSE with Waters nanoAcquity and Synapt HDMS instrumentation. Database searching for protein ID is being performed using Mascot and IdentityE algorithms, and quantitative analysis is being performed in Rosetta Elucidator. Two projects address protein expression as a function Chlamydia infection in lipid droplets, plasma membrane and membranes of the parasitic vacuole ("inclusion"). Another project is focused on Chlamydia protein expression in the two bacterial developmental forms: elementary bodies and reticulate bodies. From initial data, 684 proteins have been confidently identified and quantified across these studies, including 585 human proteins and 99 Chlamydia proteins. 128 of these proteins were identified in at least two of the three studies. Peptide-level expression data obtained has allowed for the verification of data from a recent publication (Kumar, Valdivia 2008) showing that the bacterial protease CPAF cleaves the n-terminal domains of the human membrane proteins vimentin, cytoskeletal keratin 8 and cytoskeletal keratin 18. In addition, proteomic data has revealed two additional bacterial protease substrates, cytoskeletal keratins 7 and 17. Cleavage of the n-terminus of these substrates has been confirmed by the apparent downregulation of n-terminal peptides in infected cells, and upregulation of n-terminal peptides with semi-tryptic specificity. Time-course experiments can help to reveal primary versus secondary Chlamydial protease substrates, and semi-tryptic peptides are being investigated as heretofore unknown cleavage sites of these proteases.

Schachter, J. (1999) Infection and disease epidemiology. In Chlamydia: Intracellular Biology, Pathogenesis and Immunity, R.S. Stephens, ed. (Washington, DC: ASM), pp. 31; Kumar, Y and Valdivia, R (2008) Actin and Intermediate Filaments Stabilize the Chlamydia trachomatis Vacuole by Forming Dynamic Structural Scaffolds. Cell Host and Microbe, 4, 159-169.

Tues Poster 74: Differential Proteomic Profiling of Serum from Rheumatoid Arthritis Patients Incorporating Two-stage Abundant Protein Depletion

Shanhua Lin; Hua Lin; Ted Jones; Jing Wang; Christopher H. Becker

PPD Biomarker Discovery Sciences, Menlo Park, CA

A two-step immnuoaffinity-removal chromatography system was applied to discovery of biomarkers for rheumatoid arthritis (RA). Human serum samples from RA patients (n=9) and healthy controls (n=9) were arranged in a block-randomized run order and first passed through a 14-antibody (IgY-14) immunodepletion column and then a "SuperMix" immunodepletion column (both manufactured by GenWay Biotech). This two-stage depletion approach removes abundant proteins thus enhancing the detection of lower concentration proteins. The flow-through fraction (containing the lower concentration proteins) and the bound-eluted fraction (containing the proteins captured by the SuperMix column) were separately digested by trypsin. The tryptic peptides of the depleted flow-through fraction were further separated by strong-cation-exchange chromatography (SCX) into four fractions prior to capillary on-line LC-MS analysis; this represents two-dimensional liquid chromatography for the peptides in addition to the two-dimensional protein immunodepletion. More than one hundred proteins were found to be differentially expressed with statistical significance, with quantification of protein concentrations as low as 100 - 1000 pg/mL. A few low abundance proteins include enzymes (cathepsin D and ACE), progesterone binding protein (uteroglobin), endoglin, matrix metalloproteinase-9, cell adhesion molecules (ICAM-1, VCAM-1, L-selectin, and cadherin-5) and growth factors/receptors (hepatocyte growth factor-like protein, macrophage colony-stimulating receptor and inhibin beta C chain). The list of putative biomarkers confirms proteins which have been previously reported to differentiate the serum of RA patients, such as CRP, S100A8, S100A9, and monocyte differentiation antigen CD 14, but also includes interesting new findings of putative biomarkers such as intercellular adhesion molecule 1, vascular cell adhesion protein 1, dipeptidyl peptidase 4, macrophage colony-stimulating factor 1 receptor, and hepatocyte growth factor activator.

Tues Poster 75: Ethnic Differences in 46 Candidate Proteomic Markers of Vascular Disease: The Mayo Clinic Vascular Proteomics Program

Charles X. Kim; Allison A. Ellington; Guanghui Liu; Kent R. Bailey; George G. Klee; Iftikhar J. Kullo

Mayo Clinic, Rochester, MN

Background: Cardiovascular disease (CVD) is the leading cause of death and morbidity in the US. Although it is well known that CVD susceptibility differs between ethnic groups, this variability is poorly understood and not entirely explained by differences in conventional CVD risk factors.

Objectives: We assessed differences in circulating levels of 46 candidate proteomic markers of CVD between African-Americans (AAs) and non-Hispanic Whites (NHWs) as part of the Mayo Vascular Proteomics Program, which is investigating associations of proteomic markers in pathways of inflammation, thrombosis, lipoprotein metabolism and blood pressure regulation with subclinical CVD measures.

Methods: Participants (1,324 AAs, mean age 63.5 y, 71% women; 1,237 NHWs, mean age 58.9 y, 57% women) belonged to sibships ascertained on the basis of hypertension. Solid-phase immunoassays and immunoturbidometric, clot-based, chromogenic, electrophoretic and spectrophotometric assays were used to measure the 46 proteomic markers in plasma (EDTA / citrate) or serum. Missing data (<10% for each marker) was imputed using PROC MI in SAS. Marker levels were log transformed and outliers were adjusted to within 4 SD. To identify markers independently associated with ethnicity, we employed multivariable regression analyses (stratified by sex) that adjusted for conventional risk factors (age, total and HDL cholesterol, body mass index, systolic BP, diabetes, history of smoking), prior history of CVD, medication use (statin and hypertension medications, and

estrogen (in women)) and lifestyle factors (physical activity, alcohol consumption and years of education). Generalized estimating equations were used to correct for intrafamilial correlations.

Results: AAs were older, had higher systolic blood pressures, higher diabetes prevalence, lower physical activity scores, and lower statin and aspirin use than NHWs. For 35 of the 46 proteomic markers, AA ethnicity was a significant, independent predictor of proteomic marker levels in one or both sexes. AAs had higher levels of inflammatory markers (CRP, MCP-1, P-selectin, HSP27, myeloperoxidase, matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2), lipoprotein (a), leptin and vasoconstrictor peptides (pro-arginine vasopressin, pro-endothelin), markers of calcification (osteoprotegerin and osteocalcin), and thrombotic biomarkers (Factor II, V, VIII, vWF, D-Dimer and fibrinogen). AAs had lower levels of the inflammatory markers ICAM, VCAM, IL-18, TNF receptor I, receptor for advanced glycation endproducts, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, lipoproteins (ApoB, ApoCIII, LDL particle size, oxidized-LDL, LpPLA2 activity and mass), adiponectin, and vasodilator peptides (NT-proBNP, proANP, pro-adrenomedullin).

Conclusions: We noted significant ethnic differences in the circulating levels in 35 of 46 candidate proteomic markers of vascular disease. For 23 markers, AA ethnicity was associated with potentially unfavorable levels, including higher levels of inflammatory markers, higher leptin and lower adiponectin levels, higher calcification and thrombosis markers, lower vasodilator-natriuretic peptides and higher vasoconstrictive-antidiuretic peptides. Our findings may help explain the higher propensity of AAs for adverse CVD events and may be useful in developing ethnicity-specific biomarker panels for CVD risk stratification.

Tues Poster 76: Far Upstream Binding Proteins (FUBPs) are Potential Regulators in HBV-Related Hepatocellular Carcinoma

R.M. Zubaidah; G.S. Tan; S. Tan; S.G. Lim; Q.S. Lin; M.C.M. Chung

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Hepatocellular carcinoma (HCC) is a major cause of cancer worldwide. It is often characterized by aggressive tumour behavior and poor prognosis. One of the major etiologies is hepatitis B or C virus infections, with the former being more prevalent in Asia. A 2-dimensional difference gel electrophoresis (2-D DIGE) systematic analysis was performed on moderately- and poorly-differentiated human HBV-related HCC tissues with the aim to elucidate the molecular mechanisms involved in the progression of this cancer. Our results showed that 52 and 26 proteins were found to be differentially regulated in moderately- and poorly-differentiated HCC tissues respectively. We identified a novel protein family, far upstream binding proteins (FUBPs), in both stages of HCC and this was confirmed by western blots. Over-expression of FUBPs indicated a possible role in carcinogenesis, particularly in its modulation of the transcriptional activity of the c-myc proto-oncogene. Interestingly, we also observed elevated c-myc levels in the tissues used in this study by western blot analysis. It has generally been accepted that c-myc plays an important role in HCC progression but its exact activators remains poorly understood. Preliminary data using FUBP siRNA transfection on Hep3B, a HBV antigen positive cell line led to cell proliferation arrest. This effect was however not observed in HepG2, a cell line that is devoid of HBV in the genome. We therefore proposed that the FUBP family of proteins may be one of the possible upstream players that are involved in HBV-related HCC tumorigenesis.

Tues Poster 77: Biomarker Discovery in Early Stage Breast Cancer Using A Label-free Quantitative Proteomics Technology

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Breast cancer is the most common cancer diagnosed among women with approximately 200,000 new cases reported each year and the second leading cause of cancer-related deaths, according to the American Cancer Society. Diagnosing breast cancer as early as possible greatly improves the survival rate. However, mammography, the most common method used to detect breast tumors, has intrinsic limitations. Thus early diagnostic biomarkers are critically important for detection, diagnosis, and monitoring disease progression in breast cancers.

Recently, liquid chromatography (LC) mass spectrometry (MS)-based label-free protein quantification method has become a popular tool for biomarker discovery due to its high-throughput feature and unlimited sample size for quantitative comparison under different biological conditions. In this study, we applied this technology with inclusion of statistical analysis to detect the differential protein expression levels in the plasma samples from the early-stage breast cancer patients. Using combined protein classification and pathway analysis, a panel of potential protein biomarkers has been identified.

The results from this study showed that LC/MS-based label-free protein quantification technology along with bioinformatics analysis provides an excellent opportunity to better determine disease biomarker candidates for future validation studies and development of new strategies for early diagnostics and disease treatment.

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