Fourth Annual Conference

Proteomics & Beyond

March 16 – 19, 2008

North Bethesda, Maryland

Fifth Annual Conference
February 22 - 25, 2009
Westin Horton Plaza, San Diego, California

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PROGRAM OVERVIEW

Sunday	Monday	Tuesday	Wednesday
8:00 – 5:00 pm **Short Courses** 7:00 – 7:45 pm **Opening Session / Keynote Lecture** **Peter Agre** *Salon D* 7:45 – 9:00 pm **Opening Reception** *Poster/Exhibit Hall, Salons E-H*	8:30 -9:30 am **Keynote Lecture** **Leigh Anderson** *Salon D* 9:30 – 10:30 am Parallel Sessions **Technologies for Clinical Proteomics** *Salon D* **Viral Proteomics** *Salon C, B* 11:00 am – 12 noon Parallel Sessions **Cancer Proteomics** *Salon D* **Linking Protein, RNA, DNA and Data** *Salon C, B* 12:15 – 1:30 pm **Vendor Seminars** *Lower-level meeting rooms* 1:00 – 2:30 pm **Poster Session** *Salon E-H* 2:30 – 3:30 pm Parallel Sessions **High Performance MS Technologies** *Salon D* **New Methods for Systems Proteomics** *Salon C, B* 4:00 – 5:00 pm Parallel Sessions **Proteomics and Cardiology** *Salon D* **Computational Proteomics** *Salon C, B* 6:15 – 7:00 pm **Beer & Wine Mixer** *Preceding workshops* *Salon E-H* 7:00 pm **Evening Workshops** **SPI**, *Salon D* **NHLBI**, *Salon C,B* **NIAID**, *Salon A*	8:30 – 9:30 am **Keynote Lecture** **Jeremy Berg** *Salon D* 10:00 am – 12 noon **Funding Agency Roundtable** *Salon D* 12:15 – 1:30 pm **Vendor Seminars** *Lower-level meeting rooms* 1:00 – 2:30 pm **Poster Session** *Salon E-H* 2:30 – 3:30 pm Parallel Sessions **Proteomic Technologies for PTMs** *Salon D* **Systems Biology** *Salon C, B* 4:00 – 5:00 pm Parallel Sessions **Phosphorylation Proteomics** *Salon D* **Statistical Proteomics** *Salon C, B* 7:00 pm **Evening Workshops** **AACC**, *Salon D* **CVI**, *Salon C,B* ***NCRR***, *Salon A*	8:30 – 9:30 am **Keynote Lecture** **Charles Cantor** *Salon D* 9:30 – 10:10 am **Poster Award Presentation** *Salon D* 10:30 am – 12 pm Parallel Sessions **Methods & Instrumentation for Glycoproteomics** *Salon D* Protein Networks in Mitochondria ***Salons C, B*** 1:15 – 2:45 pm **Parallel Sessions** **Disease Proteomics** *Salon D* **Bioinformatics for Systems Biology** *Salons C, B* 2:45 – 3:00 pm **Closing Remarks / Presentation of 5th Annual Conference** *Salon D*

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

7:45 – 9:00 pm	Sunday evening, Opening Reception
10:30 – 11:00 am	Monday and Tuesday morning breaks
1:00 – 2:30 pm	Monday and Tuesday Poster Sessions
3:30 – 4:00 pm	Monday afternoon break

Exhibitors may be present at other times during the day.

21st Century Biochemicals, Inc.
Booth 3
www.21stcenturybio.com

Agilent Technologies
Booth 1
www.agilent.com/chem

American Chemical Society Publications
Booth 11
http://pubs.acs.org/

Applied Biosystems
Vendor Seminar only
www.appliedbiosystems.com

Bruker
Booth 13
www.bruker.com

Denator AB
Booth 20
www.denator.com

Digilab Genomic Solutions
Booth 15
www.genomicsolutions.com

Dionex Corporation
Booth 14
www.dionex.com

Eksigent Technologies
Booth 5
www.eksigent.com

EMD
Booth 4
www.emdbiosciences.com

GE Healthcare
Booth 2
www.ge.com

GenoLogics
Booth 21
www.genologics.com

Hitachi High Technologies America, Inc.
Booth 6
www.hitachi-hta.com/lcms

Integrated Analysis Inc.
Booth 25
www.i-a-inc.com

Invitrogen
Booth 19
www.invitrogen.com

Ludesi
Booth 24
www.ludesi.com

Mass Tech, Inc.
Booth 8
www.apmaldi.com

Promega Corporation
Booth 17
www.promega.com

Protein Discovery, Inc.
Booth 7
www.proteindiscovery.com

Protein Forest, Inc.
Booth 26
www.proteinforest.com

Proxeon
Booth 10
www.proxeon.com

Shimadzu Scientific Instruments
Booth 12
www.shimadzu.com

Sigma-Aldrich
Booth 9
www.sigma-aldrich.com

Syngene
Booth 16
www.syngene.com

Thermo Scientific
Booth 18
www.thermo.com/proteomics

Uvic Genome BC Proteomics Centre
Booth 23
www.proteincentre.com

Waters Corporation
Booth 22
www.waters.com

VENDOR SEMINARS

The following companies offer lunch seminars to conference participants (seating is limited) on Monday and Tuesday.
All seminar rooms are located on the lower level of the conference center.

Monday, 12:15 – 1:30 pm	Tuesday, 12:15 – 1:30 pm
Agilent Technologies, *Brookside Room*	**Applied Biosystems**, *Forest Glen Room*
Invitrogen, *Forest Glen Room*	**Bruker Daltonics**, *Glen Echo Room*
Shimadzu Biotech, *Glen Echo Room*	**Thermo Scientific**, *Brookside Room*

GENERAL INFORMATION

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

POSTERS. Posters are located in Grand Ballroom, Salon E-H.

Monday Posters should be set up by 8:30 am on Monday and removed by 9:00 pm Monday evening. See list page 19.

Tuesday Posters should be set up by 8:30 am on Tuesday and removed by 9:00 pm Tuesday evening. See list page 25.

Posters should be attended from 1:00 – 2:30 pm on the scheduled day.

TALKS. All keynote talks and parallel sessions will take place in Grand Ballroom, Salon D or Salon C,B.

All Speakers must appear ½ hour 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 or Mac computer will use this time to load their files.

INTERNET ACCESS. Wireless Internet access is provided in the Poster/Exhibit Hall (Grand Ballroom, Salon E-H) on Monday and Tuesday.

PARKING. Special parking rate of $5/day for USHUPO participants in the self-parking lot located behind the Bethesda North Conference Center. See USHUPO Registration Desk if you need more information.

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

.

SUNDAY, MARCH 16

7:00 AM: Registration Opens, Lower Level Registration Area

SHORT COURSES

8:00 – 5:00 PM: Tandem Mass Spectrometry*, Glen Echo Room*
Sponsored by Shimadzu Biotech

8:00 AM – 12:00 PM: Sample Preparation and Fractionation for Proteomics, *Brookside Room*
Sponsored by Agilent Technologies

1:00 – 5:00 PM: Techniques for Biomarker Discovery*, Forest Glen Room*
Sponsored by GE Healthcare

1:00 – 5:00 PM: Data Extraction and Analysis for LC-MS Based Proteomics, *Brookside Room*
Sponsored by USHUPO

7:00 – 7:45 PM: KEYNOTE LECTURE, Grand Ballroom – Salon D
Peter Agre, *Duke University Medical Center*;
Aquaporin Water Channels – from Atomic Structure to Clinical Medicine

7:45 – 9:00 PM: OPENING RECEPTION, Grand Ballroom – Salon E-H

MONDAY, MARCH 17

7:30 AM: Registration opens

8:30 – 9:30 AM: KEYNOTE LECTURE, Grand Ballroom - Salon D
Leigh Anderson, *Plasma Proteome Institute*; Sifting Wheat from the Chaff: The Critical Role of Biomarker Verification in Expanding the Clinical Diagnostics Portfolio

9:30 – 10:30 AM: PARALLEL SESSION
TECHNOLOGIES FOR CLINICAL PROTEOMICS, Grand Ballroom - Salon D
Chair: Sanford Markey, *NIMH, NIH*

Abstract Page #

9:30 - 10:00 am **Molecular Imaging and Profiling of Tissues Sections using Mass Spectrometry: Applications in Biological and Clinical Research**; Richard M Caprioli; *Vanderbilt University, Nashville, TN* 31

10:00 - 10:15 am **Strategies for Reducing Biofluid Complexity in Clinical Proteomics**; Niels H. H. Heegaard; *Statens Serum Institut, Copenhagen, Denmark* 31

10:15 - 10:30 am **Mapping Human Protein Microheterogeneity: Implications for Clinical Diagnostics**; Randall W. Nelson; *Arizona State University, Tempe, AZ* 31

9:30 – 10:30 AM: PARALLEL SESSION
VIRAL PROTEOMICS, Grand Ballroom - Salon C,B
Chair: Fatah Kashanchi, *George Washington University*

9:30 - 10:00 am **Proteomic Identification of Brd4 as a Functional Partner of the Papillomavirus E2 Protein**; Jianxin You[1]; Peter M. Howley[2]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Harvard Medical School, Boston, MA* 31

10:00 - 10:15 am **Virion-wide Protein Interactions of Kaposi's Sarcoma–associated Herpesvirus**; Ramona Rozen; Sathish Naranyanan; Yan Yuan; *University of Pennsylvania, Philadelphia, PA* 32

10:15 - 10:30 am **Potential Biomarkers of HIV/AIDS Non-Progressor Patients**; Rachel Van Duyne[1]; Rebecca Easley[1]; A Mendonsa[1]; Zachary Klase[1]; M Young[2]; Fatah Kashanchi[1]; *[1]The George Washington University Medical Center, Washington, DC; [2]Georgetown University, Washington, DC* 32

10:30 – 11:00 AM: MORNING BREAK, Grand Ballroom – Salon E-H
Break, Poster Viewing, Exhibits

11:00 AM – 12:00 PM: PARALLEL SESSION
CANCER PROTEOMICS, Grand Ballroom - Salon D
Chair: Lance Liotta, *George Mason University*

11:00 - 11:30 am **Elucidating Tumor Establishment and Growth Using 2D Electrophoresis**; Donita Garland[3]; W. D. Culp, Jr. [1]; P. Pisa[2]; *[1]University of North Carolina, Chapel Hill, NC; [2]Cancer Center Karolinska, Stockholm , Sweden; [3]University of Pennsylvania, Philadelphia, PA* 32

11:30 - 11:45 am **A Systematic Approach for the Identification of Tumor-associated Membrane Antigens**; Francina C Chahal; *Viventia Biotech Inc., Winnipeg, CANADA* 32

11:45 am - 12:00 pm **Label-Free Based Proteome Profiling of Formalin Fixed Paraffin Embedded Pediatric Brain Stem Gliomas**; Javad Nazarian[1]; Brian Halligan[2]; Mariarita Santi[1]; Tobey J. MacDonald[1]; Yetrib Hathout[1]; *[1]Children's National Medical Center, Washington, DC; [2]Medical College of Wisconsin, Milwaukee, WI* 33

11:00 AM – 12:00 PM: PARALLEL SESSION
LINKING PROTEIN, RNA AND DNA DATA, Grand Ballroom - Salon C,B
Chair: Ron Beavis, *University of British Columbia*

Abstract Page #

11:00 - 11:30 am **Defining the Proteome on ORFs and Orphans**; David J Lipman; *NIH, Bethesda, MD*

11:30 - 11:45 am **Comprehensive Analysis of White Fat Adipose Tissue using Detergent-Free Protein Extraction by Pressure Cycling and High Resolution Tandem Mass Spectrometry**; Emily Freeman[1]; Vera Gross[2]; Gary Smejkal[2]; Alexander Lazarev[2]; Haiming Cao[1]; Gokhan Hotamisligil[1]; Roman Zubarev[3]; Alexander R. Ivanov[1]; *[1]Harvard School of Public Health, Boston, MA; [2]Pressure BioSciences, Inc, Woburn, MA; [3]Uppsala University, Uppsala , Sweden* 33

11:45 am - 12:00 pm **Molecular Mechanisms of Stem Cell Growth and Differentiation using Bioinformatics Anaylsis of Quantitative Differential Expression of Proteins and Phosphoproteins**; Yingxin Zhao; Sigmund J. Haidacher; Margaret Howe; Jiangang Zhao; Ronald Tilton; Randall Urban; Larry Denner; *University of Texas Medical Branch Galveston, Galveston, TX* 33

12:00 – 1:00 PM: Lunch on your own

12:15 – 1:30 PM: VENDOR LUNCH SEMINARS
Agilent Technologies, *Brookside Room, Lower Level* **Invitrogen**, *Forest Glen Room, Lower Level*
Shimadzu Biotech, *Glen Echo, Lower Level*
See next page for seminar descriptions.

1:00 – 2:30 PM: MONDAY POSTER SESSION
Grand Ballroom - Salon E-H

Lunch Seminar Descriptions

AGILENT TECHNOLOGIES, *Brookside Room*

Biomarker Discovery in Rat Muscle Mitochondria

Presenter: Dr. Nichole Reisdorph, National Jewish Medical & Research Ctr.

Biomarker discovery based on combining retention time with the high mass accuracy of current TOF-based mass spectrometric systems is a powerful approach for profiling compounds present in complex biological matrices. In this work, we profile samples using accurate mass and retention time with targeted MS/MS of differentially expressed potential markers in rat muscle mitochondria. Informatics approaches to facilitate rapid differential analysis of samples for profiling applications are also described.

Approaches for Putative Biomarker Validation using MRM Triple Quadrupole Technology

Presenter: Dr. Chris Miller, Agilent Technologies, Inc.

Confirmation of putative protein biomarkers in complex biological samples requires an instrumental method that is both highly selective and sensitive. This work shows how a triple quadrupole mass spectrometer, in combination with a microfluidic HPLC-Chip, provides extremely specific biomarker confirmation at the low-attomole level.

INVITROGEN, *Forest Glen Room*

Protein Analysis Technologies for Biomarker Discovery

Presenters: Dawn Mattoon, *R&D Manager, Invitrogen***;** Mahbod Hajivandi, *Sr. Research Scientist, Invitrogen***;** Erlend Ragnhildstveit, *Research Area Manager, Invitrogen Dynal***;** Vandi Bharucha, *Technology Sales Specialist, Invitrogen*

Biomarker discovery requires the identification and quantitation of low levels of proteins in the proteome. Therefore it requires highly sensitive and reproducible technologies capable of providing a broad dynamic range for highly heterogeneous sample types. This seminar will introduce you to the following techniques that will facilitate your biomarker discovery work:

1. Autoantibody biomarker identification using human protein array technology.
2. Sample fractionation using isoelectric focusing or magnetic bead based enrichment.
3. Mass spectrometry-based protein quantitation.
4. Accelerated western detection protocols.
5. Post translational modification analysis using PTM-specific dyes and click chemistries.
6. Antibody-based techniques.

SHIMADZU BIOTECH, *Glen Echo Room*

Shimadzu's Biotechnologies for Proteomics Applications

Shimadzu's seminar will discuss recent developments of proteomics research and also introduce new instrumentation and software to address your research challenges.

Speakers and Topics:

- Scott Kuzdzal, *Shimadzu Biotech*; Welcome and Introduction of MultiNA - microchip and automatic analysis technologies that Shimadzu will bring about revolutionary changes in life science lab;
- Paul Pevsner, *NYU Medical School*, "Colorectal Carcinoma - MALDI imaging (IMS) identification of Field Defects in Satellite Tissue";
- Rachel Martin, *Shimadzu Biotech*, "Unraveling the Complexities of Post Translational Modifications using MALDI Ion Trap TOF Technology"

2:30 – 3:30 PM: PARALLEL SESSION
HIGH PERFORMANCE MS TECHNOLOGIES, Grand Ballroom - Salon D
Chair: Peter O'Connor, *Boston University*

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2:30 – 3:00 pm **Roman Zubarev**; *Uppsala University*

3:00 - 3:15 pm **Enhancing Linear Ion Trap Mass Spectrometry ETD Performance using Supplemental Activation**; Zhiqi Hao; Jae C Schwartz; John E P Syka; Andreas FR Hühmer; *ThermoFisher Scientific, San Jose, CA* 34

3:15 - 3:30 pm **Taking Benefit from High Mass Accuracy Data**; Alexander Scherl; Scott A. Shaffer; Shannon Tsai; David R. Goodlett; *University of Washington, Seattle, WA* 34

2:30 – 3:30 PM: PARALLEL SESSION
NEW METHODS FOR SYSTEMS PROTEOMICS, Grand Ballroom - Salon C,B
Chair: Joshua LaBaer, *Harvard Medical School*

2:30 – 3:00 pm **Michael Snyder**; *Yale University*

3:00 - 3:15 pm **Class Imaging: Classification of Breast Cancer Sections by MALDI Tissue Imaging**; Detlev Suckau[2]; Martin Schürenberg[2]; Arne Fütterer[2]; Marc Gerhard[2]; Axel Walch[1]; Sören-Oliver Deininger[2]; Catherine Stacey[3]; *[1]GSF-Institut für Pathologie, Neuherberg, Germany; [2]Bruker Daltonik GmbH, Bremen,, Germany; [3]Bruker Daltonics, Billerica, MA* 34

3:15 - 3:30 pm **Exploring the Capabilities of the Protein Identification by LC/MALDI/on-target Digestion Approach**; Melkamu Getie-Kebtie; Peter Franke; Robert Aksamit; Michail Alterman; *FDA/CBER/OCTGT/TVBB, Bethesda, MD* 35

3:30 – 4:00 PM: AFTERNOON BREAK, Poster Viewing, Exhibits; Grand Ballroom – Salon E-H

4:00 – 5:00 PM: PARALLEL SESSION
PROTEOMICS AND CARDIOLOGY, Grand Ballroom - Salon D
Chair: Jennifer Van Eyk, *Johns Hopkins University*

4:00 - 4:30 pm **Revealing the Dynamics of Cardiac Proteasome Complexes: Molecular Organization, Function, and Regulation**; Peipei Ping; *UCLA School of Medicine, Los Angeles, CA* 35

4:30 - 4:45 pm **Cardioinductive Network Guiding Stem Cell Differentiation Revealed by Proteomic Cartography of TNFα Primed Endodermal Secretome**; D. Kent Arrell; Nicolas J. Niederlander; Randolph S. Faustino; Atta Behfar; Andre Terzic; *Mayo Clinic, Division of Cardiovascular Diseases, Rochester, MN* 35

4:45 - 5:00 pm **Differential Proteome Analysis of Aging in Rat Aorta:Identification and Characterization of MFG-E8 as a Key Regulator in Aging**; Zongming Fu[1]; Mingyi Wang[2]; Marjan Gucek[1]; Liqun Jiang[2]; Jing Zhang[2]; Robert N Cole[1]; Robert O'Meally[1]; Simon Sheng[1]; Yurong Guo[1]; Edward Lakatta[2]; Jennifer Van Eyk[1]; *[1]Johns Hopkins University School of Medicine, Baltimore, MD; [2]National Institute on Aging, Baltimore, MD* 36

4:00 – 5:00 PM: PARALLEL SESSION
COMPUTATIONAL PROTEOMICS, Grand Ballroom Salon - C,B
Chair: Nathan Edwards, *University of Maryland*

4:00 - 4:30 pm **The Generating Function of Tandem Mass Spectra: a Strike Against Decoy Databases**; Pavel Pevzner; *University of California at San Diego, San Diego, CA* 36

4:30 - 4:45 pm **PhosphoScan: A Probability-based Method for Phosphorylation Site Prediction using MS2/MS3 Pair Information**; Yunhu Wan[1]; Diane Cripps[1]; Stefani Thomas[1]; Patricia Campbell[1]; Nichlas Amublos[1]; Ting Chen[2]; Austin Yang[1]; *[1]Univerisity of Maryland Baltimore, Baltimore, MD; [2]Univerisity of Southern California, Los Angeles, CA* 36

4:45 - 5:00 pm **Automatic in Silico Interpretation of Native N-glycopeptide Stopflow MS2 CIDspectra Acquired from a Mixture of Unknown Glycoproteins**; Sakari Joenväärä[4]; Ilja Ritamo[3]; Hannu Peltoniemi[4]; Risto Renkonen[2]; *[1]Helsinki University, Helsinki, Finland; [2]Haartman Institute, Helsinki, Finland; [3]Finnish Red Cross Blood Service, Helsinki, Finland; [4]Medicel Ltd, Helsinki, Finland* 36

5:00 – 7:00 PM: Dinner on your own

MONDAY, MARCH 17

6:15 – 7:00 PM: BEER & WINE MIXER, Grand Ballroom E-H

7:00 PM: EVENING WORKSHOPS

USHUPO STATISTICAL PROTEOMICS INITIATIVE, Grand Ballroom – Salon D
Co-Chairs: Samir Hanash and Martin McIntosh, *FHCRC*

1. Welcome: Martin McIntosh and Sam Hanash
2. Review of action items from last year and review of progress *(Martin McIntosh)*
3. Updates on SPI Subgroups
    a. Protein and peptide identification.
    b. Quantitative proteomics.
    c. Experimental design.
    d. Discussion of new proposed groups: e.g. Data resources or other nominations.
4. Presentation and discussion of proposed USHUPO SPI activities:
    a. CAMSI: Critical assessment of mass spectral identifications *(Alexey Nesvizhskii and Stephen Master)*
5. Breakout sessions.
6. Report of breakout sessions and open-discussion of goals of SPI and milestones for the coming year.

NHLBI PROTEOMICS INITIATIVE, Grand Ballroom – Salon C,B

Cardiovascular Medical Proteomics – Moderated by Susan Old, Ph.D., *National Heart, Lung and Blood Institute*

Proteomics Evidence for Oxidative Stress in Cardiovascular Disease
Catherine E. Costello, Ph.D., *Boston University School of Medicine*

Approaches to Post-translational Modification Analysis
Robert J. Cotter, *Johns Hopkins University School of Medicine*

Analysis of Cellular Signaling Complexes by Mass Spectrometry
Andrew S. Greene, Ph.D., *Medical College of Wisconsin*

Pharmaco-proteomics for Cystic Fibrosis
Harvey B. Pollard, M.D., Ph.D., *Uniformed Services University of the Health Sciences*

Discussion

BIODEFENSE FUNCTIONAL AND STRUCTURAL PROTEOMICS, Grand Ballroom – Salon A
Organizer: Cathy H. Wu, *Georgetown University*

The Biodefense Proteomics Research Program is an initiative developed by the National Institute of Allergy and Infectious Diseases (NIAID) to provide the scientific community with newly discovered proteomic information about pathogens and hosts to identify potential targets for the next generation of vaccines, therapeutics and diagnostics. New research and novel proteomic approaches are being developed at the Proteomics Research Centers, and data and reagents are being publicly disseminated through the Resource Center (http://www.proteomicsresource.org/).

The Protein Structure Initiative developed by the National Institute of General Medical Sciences (NIGMS) is experimentally determining the three-dimensional structure of proteins in pursuit of its overall goal of making the three-dimensional atomic-level structures of most proteins easily obtainable from knowledge of their corresponding DNA sequences (http://www.nigms.nih.gov/Initiatives/PSI/).

This workshop will present and discuss the current state-of-the-art research, technologies and resources generated by the synergistic interactions and collaborations of both initiatives, as an outreach to the broad scientific community. The topic areas will be of general interest to researchers in proteomics, functional and structural genomics, bioinformatics, and systems biology, as well as developers of vaccines, therapeutics and diagnostics.

***Continued* - BIODEFENSE FUNCTIONAL AND STRUCTURAL PROTEOMICS**, Grand Ballroom – Salon A
Organizer: Cathy H. Wu, *Georgetown University*

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Moderator: Maureen Beanan, *NIAID*

NOTES

TUESDAY, MARCH 18

7:30 AM: Registration opens

8:30 – 9:30 AM: KEYNOTE LECTURE, Grand Ballroom - Salon D
Jeremy Berg, *NIGMS, NIH*

9:30 – 10:00 AM: MORNING BREAK, Grand Ballroom – Salon E-H
Break, Poster Viewing, Exhibits

10:00 AM – 12:00 PM: FUNDING AGENCY ROUNDTABLE, Grand Ballroom – Salon D

MODERATORS
Gil Omenn, *University of Michigan*
Michael Snyder, *Yale University*

ROUNDTABLE PARTICIPANTS

Department of Energy (DOE)
Susan K. Gregurick, *Program Manager, Computational Biology, OBER/DOE*
Marvin Stodolsky, *DOE*

National Cancer Institute (NCI)
Sudhir Srivastava, *Chief: Cancer Biomarkers Research Group, Div of Cancer Prevention, NCI*

National Center for Research Resources (NCRR)
Marjorie Tingle*, Health Scientist Administrator, Div of Biomedical Technology, NCRR*
Doug Sheeley, *Div of Biomedical Technology, NCRR*

National Heart, Lung and Blood Institute (NHLBI)
Susan E. Old, *Acting Deputy Director, Div of Cardiovascular Diseases, NHLBI*

National Institute of Allergy and Infectious Disease (NIAID)
Maureen J. Beanan, *Program Officer, Div of Microbiology and Infectious Diseases, NIAID*

National Institute on Drug Abuse (NIDA)
Christine Colvis*, Program Director, Genetics & Molecular Neurobiology Branch, NSC, NIDA*

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Salvatore Sechi, *Director Proteomic Program, Div of Diabetes, Endocrinology & Metabolic Diseases, NIDDK*

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Charles G. Ed*monds, Program Director, Cell Biology and Biophysics Div, NIGMS*

National Institute of Neurological Disorders and Stroke (NINDS)
Danilo Tagle*, Program Director, Neurogenetics Cluster, NINDS*

National Institute of Standards and Technology (NIST)
Thomas Wiggins, *Project Manager, Technology Innovation Program (TIP), NIST*

National Science Foundation (NSF)
Peter McCartney, *Division of Biological Infrastructure, NSF*

12:00 – 1:00 PM: Lunch on your own

12:15 – 1:30 PM: VENDOR LUNCH SEMINARS
Applied Biosystems, *Forest Glen Room, Lower Level* **Bruker**, *Glen Echo Room, Lower Level*
Thermo Scientific, *Brookside Room, Lower Level*
See next page for seminar descriptions.

1:00 – 2:30 PM: TUESDAY POSTER SESSION
Grand Ballroom - Salon E-H

Lunch Seminar Descriptions

APPLIED BIOSYSTEMS, *Forest Glen Room*

The 4800 *Plus* MALDI TOF/TOF™ Analyzer is the market leading MALDI platform for biomarker discovery, ID and quant. Powered with the patented OptiBeam™ on axis irradiation technology and more capable QuanTIS™ precursor ion selection this system offers the most efficient path to biological answers in an easy-to-use LC-MALDI capable system, while offering the highest sensitivity and depth of coverage than any other competing mass spectrometry system. Come to our Lunchtime seminar and hear what the 4800 *Plus* MALDI TOF/TOF™ can do for you.

SPEAKERS:
Matthew Willets, Ph.D. *Applied Biosystems Framingham, MA*: "New Solutions for Molecular Imaging Using Mass Spectrometry"

Bruce A. Stanley, Ph.D., *Pennsylvania State University College of Medicine*: "Using iTRAQ® Reagents to Find Same Patient Serum Marker Changes Following Bariatric (weight-loss) Surgery"

BRUKER DALTONICS, *Glen Echo Room*

Get the Whole Picture in Proteomics!

Bruker Daltonics Seminar highlights current topics in proteomics research. Whether you are involved in detecting potential biomarkers in tissue sections or by differential analysis of many samples, or you need to examine intact proteins for modifications, we have instrumentation and software to address your research challenges.

Our speakers, applications scientists from Bruker Daltonics, will present recent results in the areas of molecular histology as an extension to MALDI imaging, label-free quantitation with accurate mass LC/MS and ETD/PTR fragmentation of proteins in spherical ions trap instruments.

THERMO SCIENTIFIC, *Brookside Room*

Profiling Multiple Myeloma: Analysis of Clinical Samples using a Proteomics Discovery Platform
Speaker: Ricky D. Edmondson, PhD*; Director, The Nancy and Stephen Grand Laboratory for Myeloma Proteomics, Associate Professor of Medicine, Associate Professor of Medicine, Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences*

Multiple Myeloma is an incurable cancer that affects plasma cells primarily in the bone marrow. We have taken a label free proteomics approach using nano-LC/MS and a LTQ Orbitrap to obtain protein profiles from isolated plasma cells. Plasma (myeloma) cells are purified from bone marrow aspirates of patients suffering from various stages of Multiple Myeloma; these plasma cells are then digested and analyzed by nanoscale LC/MS/MS. The peptide/protein profiles across hundreds of patients are compared and correlated to gene expression profile data and clinical outcome.

Investigation of Tissue Interstitial Fluid from Kidney Cancer using High Resolution Differential Mass Spectrometry and SRM-Based Protein Confirmation
Speaker: Susan E. Abbatiello, Ph.D., Postdoctoral Associate, Department of Pharmacology, University of Pittsburgh Cancer Institute, Clinical Proteomics Facility Magee Women's Research Institute

Renal cell carcinoma (RCC) is the predominant form of kidney cancer, responsible for over twelve thousand deaths in the US in 2007. Prognosis depends on stage at diagnosis and due to a dearth of symptoms, most patients are diagnosed at advanced stages of the disease. The usual course of treatment begins with total removal of the kidney, or radical nephrectomy. We have designed label-free proteomics experiments using high resolution mass spectrometry to analyze the changes in peptide and protein profiles in tissue interstitial fluid (TIF) from RCC and adjacent normal kidney (ANK) tissues. Tumor and ANK tissues were diced and incubated in PBS to collect secreted and shed proteins followed by trypsin digestion and nanoflow reversed-phase liquid chromatography (nRPLC) coupled to an LTQ-Orbitrap mass spectrometer. Data analysis using SIEVE provided statistical significance of the changes in peptides observed between the RCC and ANK samples, resulting in several protein candidates to target by selected reaction monitoring (SRM) using a triple quadrupole mass spectrometer.

2:30 – 3:30 PM: PARALLEL SESSION
PROTEOMIC TECHNOLOGIES FOR PTMs, Grand Ballroom Salon - D
Chair: Daniel Chan, *Johns Hopkins University*

Abstract Page #

2:30 - 3:00 pm **Surveying Signaling Space Using Antibody-Directed Proteomics**; Michael Comb; *Cell Signaling Technology, Danvers , MA* 38

3:00 - 3:15 pm **Targeted Detection of Prostate Tissue Proteins in Serum Using MALDI-TOF/TOF**; Yan Li[1]; Lori J. Sokoll[1]; John Rush[2]; Daniel W. Chan[1]; Hui Zhang[1]; [1]*Johns Hopkins University, Baltimore, MD;* [2]*Cell Signaling Technology, Danvers, MA* 38

3:15 - 3:30 pm **Post-translational Modifications of Lysine Residues**; Robert J Cotter[1]; Dwella Nelson[1]; Wendell Griffith[2]; [1]*Johns Hopkins School of Medicine, Baltimore, MD;* [2]*University of Toledo, Toledo, OH*..... 39

2:30 – 3:30 PM: PARALLEL SESSION
SYSTEMS BIOLOGY, Grand Ballroom Salon – C,B
Chair: Michael Snyder, *Yale University*

2:30 - 3:00 pm **ErbB Signaling Networks: Quantitative Analysis and Biological Implications**; Forest White; *MIT, Cambridge, MA*..... 39

3:00 - 3:15 pm **Antibody-array Interaction Mapping: The Discovery and Study of New Interactions in Cancer Sera**; Derek Bergsma[1]; Songming Chen[1]; Robert Gerszten[2]; Randall E. Brand[3]; Brian B. Haab[1]; [1]*Van Andel Research Institute, Grand Rapids, MI;* [2]*Massachusets General Hospital, Boston, MA;* [3]*University of Pittsburgh, Pittsburgh, PA*..... 39

3:15 - 3:30 pm **Systems Biology Approach to Studying Muscle's Response to Glucocorticoids**; Erica K.M. Reeves; Zuyi Wang; Yetrib Hathout; Eric P. Hoffman; *Children's National Medical Center, Washington, DC*..... 39

3:30 – 4:00 PM: AFTERNOON BREAK, Grand Foyer

4:00 – 5:00 PM: PARALLEL SESSION
PHOSPHORYLATION PROTEOMICS, Grand Ballroom Salon – D
Chair: Timothy Veenstra, *NCI, NIH*

4:00 - 4:30 pm **Innovative Technology for the Study of Cell Signaling**; Donald Hunt; *University of Virginia, Charlottesville, VA*..... 40

4:30 - 4:45 pm **A Novel Approach for Protein Phosphorylation Sites Mapping**; Ming Zhou[1]; Zhaojing Meng[1]; Andy G. Jobson[2]; Yves Pommier[2]; Timothy D. Veenstra[1]; [1]*SAIC-Frederick, Inc., Frederick, Maryland;* [2]*National Cancer Institute, Bethesda, MD*..... 40

4:45 - 5:00 pm **Quantitative Phosphoproteomics by Mass Spectrometry for Systems Level Analysis of Cellular Signaling**; Nathan C. Tedford; James R. Graham; Amy B. Hall; Neal F. Gordon; Jeffrey A. Radding; *Epitome Biosystems, Inc., Waltham, MA*..... 40

4:00 – 5:00 PM: PARALLEL SESSION
STATISTICAL PROTEOMICS, Grand Ballroom Salon – C,B
Chair: Martin McIntosh, *FHCRC*

4:00 - 4:30 pm **Significant Analysis for Label Free LC-MS/MS Data**; Pei Wang; *Fred Hutchinson Cancer Research Center, Seattle, WA* 41

4:30 - 4:45 pm **CAMSI: Critical Assessment of Mass Spectral Identifications**; Stephen R Master[1]; Alexey I Nesvizhskii[2]; Lukas Kall[3]; William Stafford Noble[3]; [1]*University of Pennsylvania, Philadelphia, PA;* [2]*University of Michigan, Ann Arbor, MI;* [3]*University of Washington, Seattle, WA*..... 41

4:45 - 5:00 pm **Improving the Reliability of Peptide Identifications using Machine Learning**; Xue Wu; Chau-Wen Tseng; Nathan Edwards; *University of Maryland, College Park, MD*..... 41

5:00 – 7:00 PM: Dinner on your own

TUESDAY, MARCH 18

7:00 PM: EVENING WORKSHOPS

All conference participants are encouraged to participate in the workshops.

AACC PROTEOMICS DIVISION, Grand Ballroom – Salon D
Chair: Daniel W. Chan, Ph.D., *Johns Hopkins University*

Speakers/Topics:

1. Daniel W. Chan, Ph.D., *Johns Hopkins University*
   Plasma Proteomic Biomarker Discovery, Validation and Translation

2. Gil Omenn, *University of Michigan*
   The Next Phase of the HUPO Plasma Proteome Project: Linking Organ-Based Proteomes and Biomarker Discovery Initiatives with the Plasma Proteome

3. Glen Hortin, M.D, Ph.D. *NIH Clinical Chemistry*
   Diagnostic Applications of Urinary Proteomic Analysis

4. Craig A. Gelfand, Ph.D. *BD Diagnostics*
   Sample Collection and Handling Variables in Plasma Proteomics

HUPO CARDIOVASCULAR INITIATIVE (CVI), Grand Ballroom – Salon C,B
Co-Chairs: Professor Peipei Ping and Professor Jennifer Van Eyk

Bio-Rad Laboratories is pleased to host refreshments for the CVI workshop.

7:00 - 7:20 pm **New Technologies for Proteomic Analysis in Development in the NHLBI Proteomics Initiative**; Professor Daniel Knapp, *Medical University of South Carolina*

7:25 - 7:45 pm **Intracellular Organellar Proteomics: Uncovering the Basis for Cardiac Phenotype**; Professor Peipei Ping, *University of California, Los Angeles*

7:50 - 8:10 pm **Biomarkers Derived From Planned Myocardial Injury**; Professor Robert Gerszten, *Harvard University, Mass General Hospita*l

8:15 - 8:35 pm **Proteomics of the Lipid Raft-like Domains in Cardiovascular Disease**; Professor Jennifer Van Eyk, *Johns Hopkins University, Bayview Proteomics*

8:40 - 9:00 pm **Panel Discussion** chaired by Professor Gerszten
Participants: Kumar Bala (Bio Rad), David Balshaw (NIEHS), Robert Gerszten, Daniel Knapp, MingMing Ning (Harvard, Brigham), Peipei Ping, Pothur Srinivas (NHLBI), Jennifer Van Eyk, and Thomas Vondriska (UCLA)

NIH RESEARCH RESOURCES FOR PROTEOMICS, Grand Ballroom – Salon A
Moderator: Dr. Douglas Sheeley, National Center for Research Resources (NCRR)

Two programs, the NIH Roadmap National Technology Centers for Networks and Pathways (http://ntcnp.org) and the NCRR Glycomics and Glycotechnology Resources, develop leading edge technologies that can complement and enhance proteomics research. Each program, while somewhat outside the proteomics mainstream, provides access to technologies that integrate proteomics with other fields. Researchers from each program will briefly describe some of the work going on in these programs and discuss the resources they make available to the community.

Speakers:

Dr. Catherine E. Costello, *Boston University School of Medicine*

Dr. Ron Orlando, *Complex Carbohydrate Research Center, University of Georgia*

Dr. Akhilesh Pandey, *Johns Hopkins School of Medicine*

Dr. Robert F. Murphy, *Carnegie Mellon University*

WEDNESDAY, MARCH 19

7:30 AM: Registration opens

8:30 – 9:25 AM: KEYNOTE LECTURE, Grand Ballroom - Salon D
Charles Cantor, *Sequenom, Inc.;* What Proteomics Can Learn from Genomics

9:25 – 10:10 AM: POSTER AWARD PRESENTATION

10:10 – 10:30 AM: MORNING BREAK, Grand Foyer

10:30 AM – 12:00 PM: PARALLEL SESSION
METHODS & INSTRUMENTATION FOR GLYCOPROTEOMICS, Grand Ballroom Salon - D
Chair: David Lubman, *University of Michigan*

Abstract Page #

10:30 - 11:00 am — **Glycoproteins Associated With Breast Cancer**; Fred E. Regnier; Wonryeon Cho; Kwanyoung Jung; Jiri Adamec; *Purdue University, West Lafayette, IN* 42

11:00 - 11:30 am — **Mapping Expression Patterns of Extracellular Proteomes**; Hui Zhang; *Department of Pathology, Johns Hopkins University, Baltimore, MD* 42

11:30 - 11:45 am — **IDAWG: A Novel Quantitative Method for Glycomics**; Ron Orlando; Gerardo Alvarez-Manilla; Lei Cheng; Kelley Moremen; Stephen Dalton; Michael Tiemeyer; Lance Wells; *CCRC/UGA, Athens, GA* 42

11:45 am - 12:00 pm — **Markers of Cancer using a Lectin Glycoarray Approach**; Yinghua Qiu; Tasneem Patwa; Diane M Simeone; Kerby Shedden; Dean Brenner; David Beer; David M Lubman; *University of Michigan, Ann Arbor, MI* 42

10:30 AM – 12:00 PM: PARALLEL SESSION
PROTEIN NETWORKS IN MITOCHONDRIA, Grand Ballroom Salon – C,B
Chair: Salvatore Sechi, *NIH*

10:30 – 10:55 am — **Automated Proteome-Wide Determination and Modeling of Subcellular Location for Systems Biology**; Robert F. Murphy; *Carnegie Mellon University, Pittsburgh, PA* 43

10:55 – 11:20 am — **Jennifer Van Eyk**, *Johns Hopkins University*

11:20 - 11:40 am — **Systematic Construction and Analysis of a Mitochondrial Protein Atlas**; Sarah E. Calvo; David J. Pagliarini; Betty Chang; Sunil A. Sheth; Steven A. Carr; Vamsi K. Mootha; *Broad Institute of MIT/Harvard, Cambridge, MA* 43

11:40 am - 12:00 pm — **Quantitative Mitochondrial Proteomics during Cell Differentiation: Bioinformatic Aspects**; Daniel F. Bogenhagen; Jermel Watkins; Siddhartha Basu; Chun Zhou; *Stony Brook University, Stony Brook, NY* 43

12:00 – 1:15 PM: Lunch on your own

1:15 – 2:45 PM:: PARALLEL SESSION
DISEASE PROTEOMICS, Grand Ballroom Salon – D
Chair: Emanuel Petricoin, George Mason University

Abstract Page #

1:15 - 1:45 pm **Tissue MALDI Mass Spectrometry Imaging (MALDI-MSI) to Identify Diagnostic/Prognostic Biomarkers for Prostate Cancer**; Lisa Cazares; Raymond Lance; Savvas Mendrinos; MaryAnn Clements; Richard Drake; Paul Schellhammer; O. John Semmes; *Eastern Virginia Medical School, Norfolk, VA*..... 43

1:45 - 2:15 pm **Tissue Proteomics: Application of High-Resolution Mass Spectrometry and Label-Free Differential Analysis for Cancer Biomarker Investigations**; Thomas P. Conrads[1]; Brian L. Hood[1]; Jennifer N. Sutton[2]; Susan E. Abbatiello[1]; Rajiv Dhir[3]; *[1]University of Pittsburgh Cancer Institute, Pittsburgh, PA; [2]BRIMS Center, Thermo Fisher Scientific, Cambridge, MA; [3]University of Pittsburgh, Pittsburgh, PA*..... 43

2:15 - 2:30 pm **Phosphorylated VEGF Receptor in the Vitreous of Wet AMD: a New Class of Biomarkers for Predicting Treatment Timing and Response**; Geetanjali Davuluri[1]; Virginia Espina[2]; Emanuel F. Petricoin, III[2]; Mark Ross[2]; Jianghong Deng[2]; Bert M. Glaser[1]; Lance A. Liotta[2]; *[1]National Retina Institute, Towson, MD; [2]George Mason University, Manassas, VA*..... 44

2:30 - 2:45 pm **Autoantigen Biomarker Discovery through Immunological Profiling with Functional Protein Microarrays**; Dawn R Mattoon[1]; Michael G. Smith[1]; Gengxin Chen[1]; Mary Brodey[1]; David Alcorta[2]; Ronald Falk[2]; Dhavalkumar Patel[2]; *[1]Invitrogen, Branford, CT; [2]University of North Carolina, Chapel Hill, NC*..... 44

1:15 – 2:45 PM:: PARALLEL SESSION
BIOINFORMATICS FOR SYSTEMS BIOLOGY, Grand Ballroom Salon – C,B
Chair: Cathy H. Wu, Georgetown University

1:15 - 1:45 pm **Understanding Protein Function on a Genome-scale using Networks**; Mark Gerstein; *Yale University, New Haven, CT*..... 45

1:45 – 2:15 pm **Protein Interaction Networks from Bacteria to Man**; Peter Uetz; *J. Craig Venter Institute, Rockville, MD*..... 45

2:15 - 2:30 pm **PeptideAtlas: Resource for Target Selection for Emerging Targeted Proteomics Workflows**; Eric W Deutsch[1]; Henry Lam[1]; Ruedi Aebersold[2]; *[1]Institute for Systems Biology, Seattle, WA; [2]Institute for Molecular Systems Biology,ETH Zurich, Zurich, Switzerland*..... 45

2:30 - 2:45 pm **Proteogenomic Mapping for the Human Genome: Technologies and Challenges for Identifying Protein-Coding Sequences**; Jainab Khatun; Christopher Maier; Morgan Giddings; *The University of North Carolina at Chapel Hill, Chapel Hill, NC*..... 46

2:45 – 3:00 PM: CONFERENCE CLOSING REMARKS, Grand Ballroom Salon – D

MONDAY POSTER LIST

Posters should be set up by 8:30 am on Monday morning. Posters should be attended from 1:00 – 2:30 pm during Monday Poster Session. All posters should be removed by 9:00 pm Monday evening.

Cancer Proteomics

Cancer Proteomics, *continued*

Board # **Abstract page**

Computational Proteomics

Disease Proteomics

Disease Proteomics, *continued*

Board # **Abstract page**

High Performance MS Technologies

Methods and Instrumentation for Glycoproteomics

New Methods for Systems Proteomics

Protein Networks in Mitochondria

Proteomics and Cardiology

Technologies for Clinical Proteomics

Board # **Abstract page**

Viral Proteomics

Viral Proteomics, *continued*

Board # **Abstract page**

Other

TUESDAY POSTER LIST

Posters should be set up by 8:30 am on Tuesday morning. Posters should be attended from 1:00 – 2:30 pm during Tuesday Poster Session. All posters should be removed by 9:00 pm Tuesday evening.

Bioinformatics for Systems Biology

Board # **Abstract page**

01 **Calibrating E-values for MS/MS Database Search Methods**; Gelio Alves[1]; Aleksey Y. Ogurtsov[1]; Wells W. Wu[2]; Guanghui Wang[2]; Rong-Fong Shen[2]; Yi-Kuo Yu[1]; *[1]NCBI/NLM/NIH, Bethesda, MD; [2]Proteomics Core/NHLBI/NIH, Bethesda, MD* 71

02 **Protein-Centric Search and Analysis at the NIAID Biodefense Proteomics Resource Center**; Peter McGarvey[1]; Raja Mazumder[1]; Hongzhan Huang[1]; Chengdong Zhang[2]; Stephen Cammer[2]; Margaret Moore[3]; Bruno Sobral[2]; Cathy Wu[1]; *[1]Protein Information Resource, Washington, DC; [2]Virginia Bioinformatics Institute, Blacksburg, VA; [3]Social & Scientific Systems, Silver Spring, MD* 71

03 **Connecting NCI Proteomics Repository to the Cancer Biomedical Informatics Grid (caBIG)**; Denny Chan[1]; Carl Blake[1]; Carl Schaefer[3]; Peter Hussey[2]; Liming Yang[3]; *[1]SAIC, Rockville, MD; [2]Labkey Software, Seattle, WA; [3]National Cancer Institute, Rockville, Maryland* 71

04 **A Phylogenetic Paradigm for Dynamic Systems Biology: An Integrative Modeling of the Heterogeneities of the Omics Data and Disease**; Mones Abu-Asab[1]; Mohamed Chaouchi[2]; Hakima Amri[2]; *[1]Laboratory of Pathology, National Cancer Institute, Bethesda, MD; [2]Georgetown University Medical Center, Washington, DC* 71

05 **The Benefits of Measuring Spectral Pattern Reproducibility in Complex Spectra**; Matthew T Olson; Paul S Blank; Dan L Sackett; Alfred L Yergey; *NICHD/NIH, Bethesda, MD* 72

06 **Protein-Centric Integration and Functional Analysis of Cancer Omics Data**; Zhang-Zhi Hu; Hongzhan Huang; Benjamin Kagan; Anna Riegel; Anton Wellstein; Anatoly Dritschilo; Cathy Wu; *Georgetown University Medical Center, Washington, DC* 72

07 **A Pre-Clinical Data Resource for AIDS Research**; Talapady N Bhat[12]; *[1]NIST, Gaitherburg, MD; [2]NIST, Gaitherburg, MD* 73

Cancer Proteomics

08 **Isolation of Proteins from Subcutaneous Tumor and Adipose Tissues using a Pressure Cycling Technology**; Gary B. Smejkal[1]; Deena Small[2]; Sumithra Urs[3]; Ada T. Kwan[1]; *[1]Pressure BioSciences, Woburn, MA; [2]University of New Hampshire, Durham, NH; [3]Maine Medical Center Research Institute, Scarborough, ME* 73

09 **Analysis of the Effects of Ultrafiltration Conditions on Enrichment of Low Molecular Weight Serum Proteins**; Yanming An[1]; Gregory Bascug[1]; Steve Drake[2]; Radoslav Goldman[1]; *[1]Georgetown University Medical Center, Washington, DC; [2]National Institute of Health, Bethesda, MD* 73

10 **Proteomic Identification of Therapeutic Response Biomarkers in A431 Tissue**; Kian Kani[1]; Vitor Faca[2]; Babak Shahbaba[3]; Roland Leuthy[1]; Wenxuan Zhang[1]; Sharon Pitteri[2]; Qing Zhang[2]; Jonathan Erde[1]; Jonathan Katz[1]; Anjali Jain[1]; Parag Mallick[1]; Sam Hanash[2]; David Agus[1]; *[1]Cedars Sinai Medical Center, Los Angeles , CA; [2]FHCRC, Seattle, WA; [3]Stanford University, Stanford, CA* 73

11 **Biomarker Identification in Prostate Cancer Cell Lines using EGFR Targeted Treatment: A Proteomics Approach to Individualized Therapy**; Kian Kani[1]; Vitor Faca[2]; Lindsey Hughes[1]; Babak Shahbaba[3]; Roland Leuthy[1]; Wenxuan Zhang[1]; Sharon Pitteri[2]; Qing Zhang[2]; Jonathan Erde[1]; Jonathan Katz[1]; Anjali Jain[1]; Parag Mallick[1]; Sam Hanash[2]; David Agus[1]; *[1]Cedar's Sinai Medical Center, Los Angeles, CA; [2]FHCRC, Seattle, WA; [3]Stanford University, Stanford, CA* 74

12 **A Label-Free Quantitative Proteomics Study To Identify Candidate Breast Cancer Biomarkers In Response To Hypoxia Treatment**; Safia Thaminy[1]; Mi-Youn Brusniak[1]; Jingchun Chen[1]; Yong Zhou[1]; Simon Letarte[1]; Carey Sheu[1]; Julian D. Watts[1]; Ruedi Aebersold[2]; *[1]Institute for Systems Biology, Seattle, WA, USA; [2]Institute of Molecular Systems Biology, ETH, Zurich, Switzerland* 74

13 **Regulation of Mucin Expression and Glycosylation by Pro-Inflammatory Stimuli in Pancreatic Cancer Cells**; Yi-Mi Wu[1]; Adam Granger[2]; David Nowack[3]; Gilbert S. Omenn[4]; Brian B. Haab[1]; *[1]Van Andel Research Institute, Grand Rapids, MI; [2]University of California, San Francisco, CA; [3]Andrews University, Berrien Springs, MI; [4]University of Michigan, Ann Arbor, MI* 74

14 **Proteomic Strategies for the Identification of Potential Drug Targets within the T Cell mTOR-Raptor Mediated Signal Transduction Pathway**; Christine A. Jelinek; Greg M. Delgoffe; Thomas P. Kole; Dawn Chen; Jonathan D. Powell; Robert J. Cotter; *Johns Hopkins Medical Institute, Baltimore, MD* 75

Cancer Proteomics, *continued*

Board # **Abstract page**

Computational Proteomics

Disease Proteomics

High Performance MS Technologies

New Methods for Systems Proteomics

New Methods for Systems Proteomics, *continued*

Board # **Abstract page**

Phosphorylation Proteomics

Proteomic Technologies for Analysis of Protein Modifications

TUESDAY POSTER LIST

Statistical Proteomics

Board # **Abstract page**

60 **Normalization Methods for LC-MS Spectral Peaks Based on a Large-Scale Calibration Experiment**; Yuliya V. Karpievitch; Alan R. Dabney; *Department of Statistics, Texas A&M University, College Station, TX* 88

61 **Shotgun Sequencing by MS/MS to Discover Candidate Biomarkers from Spectral Counts using Non-Parametric Techniques**; Kristina M. Little[1]; David M. Smalley[1]; Nancy Harthun[1]; Klaus F. Ley[2]; *[1]University of Virginia, Charlottesville, VA; [2]La Jolla Institute of Allergy and Immunology, San Diego, CA* 89

62 **Peptide Identification by Spectral Matching of Tandem Mass Spectra using Hidden Markov Models**; Xue Wu; Chau-Wen Tseng; Nathan Edwards; *University of Maryland, College Park, MD* 89

63 **Global Bioinformatical Analysis of human Liver Proteome**; Ole Vorm; Mortem Bern; Christian Ingrell; Alexandre Podtelejnikov; *Proxeon A/S, Odense , Denmark* 89

64 **Using False Discovery Rates in Protein Identification**; Tom Blackwell; *University of Michigan Medical School, Ann Arbor, MI* 89

65 **A Predictive Model for Identifying Proteins by a Single Peptide Match**; Roger Higdon; Eugene Kolker; *Seattle Childrens Hospital, Seattle, WA* 90

66 **New Functionality for the Trans-Proteomic Pipeline: Improving the PeptideProphet Classifier**; Eric Deutsch[1]; Hyungwon Choi[3]; James Eddes[4]; Jimmy Eng[2]; Johan Malmstroem[4]; Luis Mendoza[1]; Alexey Nesvizhskii[3]; David Shteynberg[1]; Joshua Tasman[1]; Ruedi Aebersold[4]; *[1]Institute for Systems Biology, Seattle, WA; [2]University of Washington, Seattle, WA; [3]Department of Pathology, University of Michigan, Ann Arbor, MI; [4]Institute for Molecular Systems Biology (ETH), Zurich, Switzerland* 90

Systems Biology

67 **Unified Sample Preparation Approach using Hydrostatic Pressure Cycling: Simultaneous Isolation of Proteins, Nucleic Acids and Lipids from a Single Sample;** Vera S. Gross; Greta Carlson; Gary B. Smejkal; Ada T. Kwan; Timothy Straub; Alexander V. Lazarev; *Pressure BioSciences, Inc., Woburn, MA* 90

68 **Identification of Novel Interacting Partners of Protein Kinase CK2: Disclosing Unanticipated Links Between CK2 and a Variety of Biochemical Events**; Giorgio Arrigoni[1]; Mario Pagano[2]; Stefania Sarno[2]; Luca Cesaro[2]; Peter James[1]; Lorenzo Pinna[2]; *[1]University of Lund, Lund, Sweden; [2]University of Padova, Padova, Italy* 90

69 **Development of BIATECH-54 Standard Mixtures for Assessment of Protein Identification and Relative Expression**; Eugene Kolker; Roger Higdon; *Seattle Childrens Hospital, Seattle, WA* 91

70 **Protein Families at the Postsynaptic Density**; Anthony J. Makusky[1]; Ayse Dosemeci[2]; Sanford P. Markey[1]; *[1]National Institute of Mental Health, Bethesda , MD; [2]National Institute Neurological Disorders Stroke, Bethesda , MD* 91

71 **Comparative Modeling of Human Metallothionein Isoform-3 (MT-3)**; Kolin Harinda Rajapaksha; Sarath Bandara; Tushara Chaturanga Bamunuarachchige; Prasanna Kumara Aberathne; Amal Nayanagith Senavirathne; *Faculty of Agriculture Univerasity of Peradeniya, Peradeniya, Sri Lanka* 91

Technologies for Clinical Proteomics

72 **Absolute Quantification using Fluorescent and Isotope Labeled Concatenated Peptide Standards: human serum albumin**; Dhaval M. Nanavati; Aria C. Attia; Sanford P. Markey; *Laboratory of Neurotoxicology, NIMH, Bethesda, MD* 91

73 **Integrating Peptide Identification Data with Clinical and Biological Annotation Data to Support Query, Reporting, and Data Visualization**; Gautam Saxena; Edwin Mulwa; Innocent Ndibatya; *Integrated Analysis Inc., Bethesda, MD* 92

74 **Early Markers of Kidney Transplant Rejection: Quantitative Proteomic Workflows for Discovery and the Development of Non-Invasive, Targeted Assays**; David Sarracino[1]; Waichi Wong[2]; Emmanuel Zorn[2]; Bryan Krastins[1]; Michael Athanas[3]; Mary F Lopez[1]; *[1]ThermoFisher Scientific BRIMS, Cambridge, MA; [2]Massachusetts General Hospital, Harvard, Boston, MA; [3]Vast Scientific, Wayland, MA* 92

75 **Expression Profiling and Identification of Serum and Plasma Proteins using Immobilized Trypsin Beads with LIFT-MALDI-TOF/TOF**; Izabela D. Karbassi; Gaurav Basu; Stefan Gravenstein; Yuping Deng; O. John Semmes; Richard R. Drake; *Eastern Virginia Medical School, Norfolk, VA* 92

Technologies for Clinical Proteomics

Other

Monday 9:30 – 10:30 am
Technologies for Clinical Proteomics

Mon 9:30 - 10:00 am: Molecular Imaging and Profiling of Tissues Sections using Mass Spectrometry: Applications in Biological and Clinical Research

Richard M Caprioli
Vanderbilt University, Nashville, TN

The spatial and temporal aspects of molecular processes in cells and tissues play an enormous part in the biology that defines living systems. Profiling and Imaging MALDI MS provides an effective means to measure and assess these dimensions on a molecular basis, including peptides, proteins, lipids, metabolite, drugs, etc. The technology is extraordinarily high throughput with high molecular specificity. Using a raster of the tissue surface by a laser beam, images of samples are produced in specific m/z values, or ranges of values. Each spot on the sample irradiated by the laser can be varied within the range of the size of 30-200 microns in diameter, and the subsequent mass spectrum typically covers the m/z range 1000-100,000. Individual m/z values can then be assembled from the mass spectra to produce selected m/z images. Sections obtained from any tissue type can be imaged to locate tissue specific molecules in X, Y coordinates of the tissue. 3-D images of organs and whole animal body images may also be generated.

We have employed MALDI imaging mass spectrometry (IMS) technology in studies of a variety of diseases, including several types of cancers, neurodegenerative diseases, and kidney diseases, comparing proteins differentially expressed in diseased tissue with those in the corresponding normal tissue. This will be illustrated with studies of breast tumor biopsies and also those for human glioblastomas. In the latter, MS patterns have also been correlated with patient outcomes. This has been applied in a process termed histology-directed molecular analysis of tissue and biopsy specimens. In addition, 3-D peptide and protein images of mouse head, brain, and uterus have been generated from spatially registered serial sections and have been correlated with MRI and PET images. IMS has also been applied to drug targeting and metabolic studies with analysis of specific tissues after systemic drug administration. Whole animal sagittal sections have been imaged to measure molecular changes in proteins in multiple organs and correlating this with drug concentrations in these same organs. Finally, we have begun to image lipids in biological tissues and have employed ion mobility as an integral part of the imaging instrumentation for these studies.

Mon 10:00 - 10:15 am: Strategies for Reducing Biofluid Complexity in Clinical Proteomics

Niels H. H. Heegaard
Statens Serum Institut, Copenhagen, Denmark

Multianalyte approaches such as mass spectrometric proteomics promise to accelerate the discovery of diagnostic biomarkers through the ability to screen a multitude of analytes in one operation and through pattern analysis capable of uncovering covariability. In practice, however, whole proteomes cannot be analyzed in one simple operation and reduction of sample complexity is usually necessary when studying biofluids such as plasma or serum. The application of different subfractionation strategies in our laboratory will be illustrated by the following serum/plasma proteome projects: (1), development of diagnostic markers for ovarian cancer; (2), disease activity markers for rheumatic disease; and (3), development of markers for dialysis-related amyloidosis. In the ovarian cancer study we subfractionate sera on three different magnetic bead-matrices, i.e., ion-exchange, metal chelate, and reversed-phase prior to MALDI-TOF mass spectrometry. This approach is based on physico-chemical parameters with no direct bearing on pathology. As indicated by our results, however, the approach may succeed especially when information from all the different fractionation methods are combined and when samples and study design are of high quality. In the rheumatic disease study we focus on biofluid subfractions that are likely to directly reflect pathological processes. One such subfraction in blood is the population of microparticles, and we hypothesize that microparticle proteomes will directly reflect normal and pathological processes. Microparticles are membrane-bound vesicles released from apoptotic, stressed, damaged, or activated tissues and cells. Surface markers identify their cellular origin while their profile of modifications (e.g. protein PTMs such as phosphorylation patterns) are likely to reflect the events that led to their release. The focusing provided by isolating the microparticle fraction of plasma for proteome analysis is illustrated by the fact that the total protein content of such particles is estimated to be <0.1% of total plasma protein. Characterization of the particles in blood from patients suffering from systemic lupus erythematosus and rheumatoid arthritis illustrate the potential of diagnostic development using microparticles and modification-specific proteomics in intelligent disease proteomics. Finally, ultrafocused mass spectrometric biomarker discovery may be provided by combining the selective power of immunoreagents (antibodies) with the resolution of mass spectrometry. This is a highly efficient approach for the comprehensive characterization of families of proteins that may be pinpointed based on knowledge of e.g. disease pathology. Thus, using on-line immunoaffinity-ESI-TOF mass spectrometry we discovered a circulating cleaved variant of beta-2-microglobulin in the blood of patients in chronic hemodialysis, a condition known to be associated with the deposition of beta-2-microglobulin molecules in osteoarticular structures. Conclusively, there are several options for reducing sample complexity in biomarker discovery studies and as shown here it is worthwhile to analyze the nature of the pathology and the type of samples involved to choose tailored sample preparation steps and thereby ensure proteome analyses targeted at the most information-rich fractions of the biofluids.

Mon 10:15 - 10:30 am: Mapping Human Protein Microheterogeneity: Implications for Clinical Diagnostics

Randall W. Nelson
Arizona State University, Tempe, AZ

Critical to using mass spectrometry platforms for disease diagnostics is the need to transcend from the general profiling of proteins to the targeting of specific panels of proteins. The value of such targeted approaches lies in the ability to identify and characterize variants forms of the protein(s) under investigation (i.e., gene, translational and posttranslational modifications) and to generate data on only the specific molecular determinants relevant to the disease. This talk will focus on using targeted mass spectrometric immunoassays to investigate microheterogeneity in human plasma and urinary proteins in healthy and disease cohorts. Results illustrating the ability to detect low-level protein variants relevant to cardiovascular disease and type 2 diabetes will be presented, and how these findings are subsequently used to develop advanced assays for disease diagnosis and monitoring.

Monday 9:30 – 10:30 am
Viral Proteomics

Mon 9:30 - 10:00 am: Proteomic Identification of Brd4 as a Functional Partner of the Papillomavirus E2 Protein

Jianxin You[1]; Peter M. Howley[2]
[1]*University of Pennsylvania School of Medicine, Philadelphia, PA;*
[2]*Harvard Medical School, Boston, MA*

Cervical carcinomas are associated with high-risk human papillomaviruses (HPV) such as HPV-16 and -18. Papillomaviruses (PVs) establish a persistent latent infection by maintaining the viral genome as autonomous replicating plasmid in infected cells. In order to maintain the viral genome inside the nuclei of dividing host cells during disassembly and reassembly of the nuclear envelope, the virus has evolved a mechanism for faithful partitioning to daughter cells. The PV E2 protein accomplishes this feat by tethering the viral genome to host chromosomes during mitosis to ensure long-term episomal maintenance of viral genomes within replicating cells. However, how the E2 and viral genome attach to mitotic chromosomes was previously unknown.

Utilizing a proteomic approach to systematically characterize cellular proteins that associate with E2 in vivo, we identified the cellular protein Brd4 (bromodomain-containing protein 4) as a major interacting protein for the bovine papillomavirus (BPV) E2. Brd4 is a member of the double bromodomain family of proteins and binds to histones on mitotic chromosomes. It has also been shown to bind to

acetylated chromatin with preferential binding for acetylated histones.

In our studies, we have found that Brd4 functions as the major cellular receptor for E2 and viral genome on mitotic chromosomes. Blocking the E2-Brd4 interaction prevented the mitotic chromosomal targeting of both E2 protein and the viral genome. Blocking the binding of E2 to Brd4 inhibited transformation of mouse C127 cells by cloned viral BPV DNA. In mouse cells that carry BPV genomes exclusively as viral episomes, expression of a dominant negative inhibitor to break the E2-Brd4 interaction could lead to the dissociation of BPV episomes from host mitotic chromosomes, a loss of the viral episomes in the infected cells after passages and a reversion of the transformed morphology of the infected cells to the nontransformed phenotype.

Further studies uncovered additional function of Brd4 in E2 mediated viral transcription and showed that Brd4 is also targeted by another episomal DNA virus, Kaposi's sarcoma-associated herpesvirus (KSHV). We demonstrated that Brd4 also associates with the HPV-16 E2 protein indicating that interaction with Brd4 is not specific for BPV E2 and may be a general property of all PV E2 proteins. These results suggested that the binding of E2 to Brd4 might provide a novel target for the development of antiviral therapeutics against episomal latent viral infections.

Mon 10:00 - 10:15 am: Virion-wide Protein Interactions of Kaposi's Sarcoma–associated Herpesvirus

Ramona Rozen; Sathish Naranyanan; Yan Yuan
University of Pennsylvania, Philadelphia, PA

Herpes virus virions are highly organized structures built through specific protein-protein interaction. Thus, revelation of protein interactions among virion proteins will shed light on the processes and the mechanisms of virion formation. Recently, we identified 24 virion proteins of KSHV using a proteomic approach (Zhu et al., J. Virol. 79:800-811). In the current studies, a comprehensive analysis of protein-protein interaction between KSHV virion proteins were carried out by using yeast two-hybrid (Y2H) and co-immunoprecipitation (co-IP) approaches. Every pairwise combinantion between KSHV tegument and capsid proteins, between tegument and envelope proteins and among tegument proteins were tested for possible binary interaction. More than thirty protein-protein interactions were identified by both Y2H and co-IP analyses. The results revealed interactions between tegument and capsid proteins such as ORF64 interacting with ORF25 (MCP), ORF62 (TRI-1) and ORF26 (TRI-2). Many interactions were detected among tegument proteins. ORF64 was found to be able to associate with several tegument proteins including ORF11, ORF21, ORF33, ORF45, ORF63, ORF75 and ORF64 itself, suggesting that ORF64 may serve as a hub protein and play a role in recruiting tegument proteins during tegumentation and virion assembly. Our investigation also revealed redundent interactions between tegument proteins and envelope glycoproteins. These interactions are believed to contribute to final envelopment in virion assembly. Overall, this study allows establishment of a virion-wide protein interaction map, which provides an insight into the architecture of KHSV virion and sets up a foundation for exploring functions of these proteins in viral particle assembly.

Mon 10:15 - 10:30 am: Potential Biomarkers of HIV/AIDS Non-Progressor Patients

Rachel Van Duyne[1]; Rebecca Easley[1]; A Mendonsa[1]; Zachary Klase[1]; M Young[2]; Fatah Kashanchi[1]
[1]The George Washington University Medical Center, Washington, DC; [2]Georgetown University, Washington, DC

The search for cancer and disease biomarkers within human peripheral fluids has been a staple of preventative therapeutics throughout the past few years. The comparison of normal versus disease states can indicate an overabundance or lack of expression of critical genes and proteins where illness has afflicted the patient. In particular, the analysis of virally infected serum is desirable due to the ease and non-invasive methods of collecting samples, but also applicable in the determination of involvement of virus with infected cells/tissue. We report in this study the identification of low abundance serum proteins in latently infected long term non-progressors both untreated and those recieving HAART therapy. The difficulty associated with analyzing serum is the presence of high abundance proteins (i.e. albumin, IgG, etc.) which mask potential low abundance biomarkers. Here, we utilize the ProteomeLab IgY serum depletion kit which removes 12 of the most abundant proteins in serum, followed by two-dimensional gel electrophoresis to isolate proteins from the depleted fractions. Comparison of 2DGE signatures between normal and disease states and the isolation of differential proteins were examined by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry in order to identify these proteins. The usage of complementary techniques on the variable proteins and specificity of protein-protein interactions in the context of HIV-1 viral infection serves as validation of these identified proteins. We intend to identify normal cellular proteins which, are altered significantly in the early stages of HIV-1 infection to serve as early detection markers.

Monday 11:00 am – 12:00 pm
Cancer Proteomics

Mon 11:00 - 11:30 am: Elucidating Tumor Establishment and Growth using 2D Electrophoresis

Donita Garland[3]; W. D. Culp, Jr. [1]; P. Pisa[2]
[1]University of North Carolina, Chapel Hill, NC; [2]Cancer Center Karolinska, Stockholm , Sweden; [3]University of Pennsylvania, Philadelphia, PA

A major application of proteomics is the search for biomarkers that can be used for diagnosis, prognosis, and targets for treatment of disease. The goal and greatest challenge is being able to identify proteins of biological interest that are truly differentially expressed between different physiological states, while minimizing false positives and false negatives. 2-D electrophoresis, regardless of the inherent technical issues, remains a key and powerful technique for this endeavor. Multiple statistical approaches have been used in our studies to analyze the gel data increasing the confidence in identifying differentially expressed proteins. This approach was used to study protein expression during tumor growth in the B16-F10 mouse model of melanoma. Six kinetic patterns of protein expression were identified demonstrating the dynamics of protein expression during tumor growth. Using ANOVA techniques the expression of 44 proteins identified a clear biological switch during the in vivo tumor growth. Proteins of interest were identified by mass spectrometry and validated by Western blot. In the same tumor model, expression of the pro-tumorgenic, pro-angiogenic and pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) was reduced in the B16-F10 tumor cells using RNAi technology to elucidate MIF's role in regulating the growth of this tumor. The establishment of the tumors generated by these cells was significantly delayed. 2D-electrophoresis, mass spectrometry and microarray analyses were used to identify those proteins that may have a role in controlling tumor establishment. Comparisons between the B16-F10 tumor cell line with MIF knocked down and control cells revealed 70 differentially expressed proteins with greater than 1.5 fold change ($p<0.05$). The results suggest that the delay in tumor establishment is related to the upregulation in the expression of the anti-angiogenic factor, thrombospondin-1 and defines a novel function of MIF as a key regulator of thrombospondin-1 expression.

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Mon 11:30 - 11:45 am: A Systematic Approach for the Identification of Tumor-associated Membrane Antigens

Francina C Chahal
Viventia Biotech Inc., Winnipeg, CANADA

The concept of cancer immunotherapy is based on the existence of tumor-associated antigens (TAAs) against which the host is capable of mounting an immune response. While immune reactivity to cell surface TAAs is well documented, the complexity of membrane antigens has made their identification difficult. Viventia's product development platforms, Hybridomics™, ImmunoMine™, and UnLock™, have been designed to identify tumor-specific antibodies and their cognate antigens. To navigate the diverse array of membrane antigens that represent targetable molecules for

immunotherapy, we have developed an algorithm for antigen identification. Using human antibodies developed from our discovery and screening platforms we have been able to "mine" the surface of tumor cells for TAAs. Depending on the nature of the antigen as identified through a pre-screening process, we have been able to isolate and identify antigens using standard immunoprecipitation techniques followed by LC-MS/MS. However, for the more complex antigens, we have developed a novel approach based upon two-dimensional liquid chromatography. This is comprised of a ProteomeLab™ PF-2D system in tandem with the LC-MS/MS, (equipped with a nanosource), for purification and identification of membrane-associated membrane TAAs. The results of these studies are discussed with examples of some of the tumor-associated antigens identified to date.

Mon 11:45 am - 12:00 pm: Label-Free Based Proteome Profiling of Formalin Fixed Paraffin Embedded Pediatric Brain Stem Gliomas

Javad Nazarian[1]; Brian Halligan[2]; Mariarita Santi[1]; Tobey J. MacDonald[1]; Yetrib Hathout[1]

[1]Children's National Medical Center, Washington, DC; [2]Medical College of Wisconsin, Milwaukee, WI

Pediatric brain stem gliomas (BSG) are known as one of the most difficult cancers to treat mainly due to their inoperability and diffused lesions. Brain stem gliomas, which develop in children between the ages of six to nine, account for 10-20% of all pediatric CNS tumors. However, almost all available biological BSG brain tissues are formalin fixed paraffin embedded (FFPE) samples. Due to crosslinking, extraction of intact proteins from FFPE tissues is cumbersome. However, peptides are extractable using enzymatic digestion assays, with the limitation being the inability to measure protein concentrations. In an attempt to discover potential BSG markers for therapeutic targeting, as well as the molecular signature of the tumor, we used FFPE tissues from pediatric brain stem glioma for protein identification and quantification. In our label-free protein profiling approach extracted peptides were identified, and using the visualize software, were normalized to an internal standard and quantified. Our approach presents a reproducible method for extraction and quantification of peptides from FFPE samples, as well as providing a list of potential BSG biomarkers.

Although preliminary, our analyses show the feasibility of profiling proteins from FFPE brain tissues and obtaining qualitative and quantitative molecular insights into gliomas. Our approach may lead to the identification of targets that should improve our understanding of the biology and perhaps treatments of BSGs. Furthermore this assay can be applied to the diverse and rich archival PFPE tissues available to the scientific community.

Monday 11:00 am – 12:00 pm
Linking Protein, RNA and DNA Data

Mon 11:30 - 11:45 am: Comprehensive Analysis of White Fat Adipose Tissue Using Detergent-Free Protein Extraction by Pressure Cycling and High Resolution Tandem Mass Spectrometry

Emily Freeman[1]; Vera Gross[2]; Gary Smejkal[2]; Alexander Lazarev[2]; Haiming Cao[1]; Gokhan Hotamisligil[1]; Roman Zubarev[3]; Alexander R. Ivanov[1]

[1]Harvard School of Public Health, Boston, MA; [2]Pressure BioSciences, Inc, Woburn, MA; [3]Uppsala University, Uppsala , Sweden

Fat adipose tissue plays a key role in energy metabolism, lipid synthesis and secretion of signaling proteins linked to obesity, insulin resistance, inflammation and other physiological complications. Efficient proteomic analysis of adipose tissue is highly valuable for studies of type II diabetes, obesity, cancer and many other manifestations of metabolic syndrome. Fat adipose tissue contains up to 80-90% lipids my mass, which makes conventional detergent-based protein solubilization and extraction methods inefficient as they tend to produce highly variable results, especially affecting important hydrophobic membrane proteins localized in organelles and the plasma membrane. Abundant sample-derived lipids tend to sequester detergents into micelles, thus interfering with protein extraction. This study was enabled by the use of alternating hydrostatic pressure (Pressure Cycling Technology, or PCT) and specialized organic solvents for disruption of cells, micelles and membrane fragments and efficient protein recovery from lipid-rich adipose tissue. Adipose tissue samples from obese (ObOb) mice were used to optimize the conditions for protein extraction. When the efficiency of protein extraction was determined, it confirmed overall higher protein recovery by the novel method. Differential proteomic analysis using optimized extraction techniques followed by 1D- and 2D-SDS-PAGE and protein identification by liquid chromatography and high mass accuracy high mass resolution electrospray tandem mass spectrometry have been performed to identify adipose tissue proteins specific to several genetically distinct model animal lines. Several unique protein species were identified in the extracts of adipose tissue. Post-translational modifications including phosphorylation, acetylation and ubiquitylation of selected proteins detected in multiple isoforms were characterized. Comparative proteomic analysis of adipose tissue isolated from several genetically different murine model lines on different diets, has revealed differences in protein expression. The results of proteomic analyses were compared to transcriptomic expression profiles and submitted to functional pathway analysis. Differentially regulated energy and glucose metabolism pathways identified in this way may be useful in the mechanistic studies of obesity and associated disorders such as Type 2 diabetes and non-alcoholic fatty liver disease.

Mon 11:45 am - 12:00 pm: Molecular Mechanisms of Stem Cell Growth and Differentiation Using Bioinformatics Anaylsis of Quantitative Differential Expression of Proteins and Phosphoproteins

Yingxin Zhao; Sigmund J. Haidacher; Margaret Howe; Jiangang Zhao; Ronald Tilton; Randall Urban; Larry Denner

University of Texas Medical Branch Galveston, Galveston, TX

Proteomics approaches provide an invaluable understanding of stem cell identify, growth, differentiation, and multipotency. We have used quantitative mass spectrometry-based proteomics to build a broad knowledge base of the molecules and signaling networks regulating stem cell growth and differentiation. In particular we have studied growth control mechanisms of umbilical cord blood CD133+/CD34+ stem cells. We initially used 2D gel electrophoresis to compare differential protein expression between five conditions. As the starting point, we collected freshly isolated cells (1). Since we were interested in changes that underlie differentiation-dependent loss of the CD133 marker during expansion in culture, we cultured cells for seven days and then used immunomagnetic separation techniques to separate CD133+ (2) from CD133- (3) cells. Finally, we evaluated the effects of the hyperglycemic environment on cells by culturing in the presence normal (4) or high (5) glucose. Five replicate gels were run for each of the five conditions. Gels were stained with Sypro Ruby and fluorescent images captured. Replicate images were then analyzed using Progenesis to find 636 proteins common to all conditions. Statistical analysis indicated that, depending on the comparisons between the five conditions, 40-83 proteins were differentially expressed by at least 2-fold with a $p < 0.05$. MALDI-TOF led to the identification of spots that were generally related to metabolic function.

Since we are also interested in proteins that mediate self-renewal of CD133 cord blood stem cells, we next treated cells with valproic acid which broadly affects chromatin remodeling-dependent changes in gene expression and TEPA which suppresses differentiation-dependent gene expression related to CD38. These treatments increase expansion of CD133+ population and decrease differentiation-dependent expansion of CD133- cells. Extracts from these two conditions were trypsinized and peptides labeled in the presence of heavy 18O-water or normal 16O-water. The two mixtures of peptides were then combined, fractionated by strong cation exchange chromatography, and analysed by tandem LC-MS/MS with zoom scan to quantify expression of peptides and proteins in the two populations of cells. Nearly 25% of these proteins were differentially expressed by more than our 20% statistical limit of detection. These were analyzed using bioinformatics tools to identify biological and molecular functions from Gene Ontology. In addition, differentially expressed proteins

were submitted to Ingenuity Pathway Analysis to identify highly significant signaling networks. These networks were predominantly related to cell growth control and led to the identification of key convergent signaling intermediates. Finally, we used gallium-based IMAC to isolate phosphopeptides, LC-MS/MS to identify and quantify phosphopeptides, and identified phosphorylation-regulated signaling pathways that integrate diverse types of information flow.

These approaches to quantification, identification, and bioinformatics analysis of differentially regulated phosphoproteins and proteins will help define the global molecular mechanisms of stem cell growth and differentiation.

Monday 2:30 – 3:30 pm
High Performance MS Technologies

Mon 3:00 - 3:15 pm: Enhancing Linear Ion Trap Mass Spectrometry ETD Performance using Supplemental Activation

Zhiqi Hao; Jae C Schwartz; John E P Syka; Andreas FR Hühmer
ThermoFisher Scientific, San Jose, CA

Electron Transfer dissociation (ETD) is drawing more and more attention as mass spectrometry based methods for peptide or protein identification and analysis of post-translational modifications. ETD randomly cleaves the backbone bonds of multiply charged peptides and proteins generating extensive c and z type of product ions. This process of ETD is generally indifferent to peptide amino acid composition and post-translational modifications. However, Precursor ion charge is an important factor that affects the dissociation efficiency of ETD. ETD is known to be relatively inefficient on doubly charged precursor ions, which are the most predominant charge type of traditional proteomics: tryptic peptides of a bottom-up approach. To overcome such a problem, a low-energy collisional activation method (supplemental activation, SA) has recently been implemented in the LTQXL with ETD system to enhance ETD performance on doubly charged peptide precursor ions, as well as on precursor ions of higher charge states. The supplemental activation targets the non-dissociated electron transfer precursor ions to generate c and z type of product ions, thus enhance ETD efficiency on peptide precursor ions which do not contain enough charges. In this study, the effect of supplemental activation on ETD performance in LTQ XL is characterized using standard peptides as well as complex protein digests. The utility of ETD with supplemental activation for enhanced peptide identification and protein sequence coverage is investigated.

Our results indicate that the efficiency of ETD on peptides is highly affected by number of charges carried by precursor ions. While higher charged precursor ions can be extensively fragmented by ETD, the lower charged precursors of the same peptide may have very limited fragmentation, thus remaining mostly as the non-dissociated, charge reduced, electron transfer precursor ion species When collisionally activated, the non-dissociated electron transfer species fall apart into product ions. However, under regular condition for collision activation, product ions generated from non-dissociated precursors contain both b/y type and c/z type ion series, leading to a complex spectrum containing two different ions series. To eliminate b/y type ions, the supplemental collision activation was performed at low energy utilizing low activation Q. Spectra obtained under these conditions contain almost exclusively c/z type ions. Supplemental activation was implemented in LTQ XL such that after ETD, the non-dissociated precursor ions was activated without an additional isolation step, thus c and z ions generated from initial ETD remained in the final spectrum. Results obtained using standard peptides indicated that with supplemental activation ETD efficiency was significantly improved on doubly charged precursor ions as well as on precursor ions of other charge states. Peptides which were not identified due to poor ETD fragmentation can now be identified by ETD with supplemental activation. Supplemental activation in LTQ XL was found to be compatible with labile post-translational modifications such as phosphorylation and glycosylation. Use of supplemental activation in data-dependant MS/MS approach for protein identification from complex sample was investigated and optimized instrument methods for enhanced protein identification and sequence coverage will be discussed.

Mon 3:15 - 3:30 pm: Taking Benefit from High Mass Accuracy Data

Alexander Scherl; Scott A. Shaffer; Shannon Tsai; David R. Goodlett
University of Washington, Seattle, WA

Modern MS instruments utilized in proteomics are capable of acquiring data with high mass accuracy. Maximum mass deviations below 10 ppm are routinely achieved on commercial instruments. However, despite the popularity of such instruments and the ease of accessing accurate mass data, high measured mass accuracy is rarely used as a definitive parameter during database searches that seek to match tandem mass spectra to peptide sequence. As recently suggested, the combined approach of high measured mass accuracy on precursor and fragment ions allows one to turn mass spectrometry into a "digital science" (Zubarev and Mann, Mol. Cell Prot. 2007), with a clear line between true and false-positive identifications of peptides and associated modifications. Here we provide further proof of this proposal.

High measured mass accuracy on precursor ions is almost never used in data-dependent proteomics experiments due to the difficulties associated with detecting those ions in an accurate manner. Often, the wrong isotope is labeled as precursor ion, or the signal to noise of the precursor ions is insufficient for accurate peak detection. In a typical data-dependent LC-MS/MS experiment of a complex sample, more then 10% of the spectra matched to peptides have mis-assigned precursor ions. This experimental reality obliges users to use much wider precursor ion tolerances (typically greater then 1 Dalton) or to insert single-ion monitoring analysis at high duty cycle cost. Our approach to solve this problem takes into account precursor ion masses over the entire chromatographic peak. Peak detection is improved and precursor-ion mass value is then corrected post acquisition. This step allows reducing the number of inaccurately detected precursor ions by a factor of 2. In addition, database searching with narrow precursor ion tolerance (typically 5 – 10 ppm) reduces drastically the number of potential peptide candidates to be searched, and thus reducing the false-positive ratio and database search time. Combined, these two effects lead simultaneously to an increasing number of peptide identifications, to a lower false-positive ratio and faster database search time.

High measured mass accuracy of fragment ions has been shown to increase the specificity and speed of the database search, especially in the cases of large databases, potentially modified peptides and atypical protein digestion/cleavage methods (Shaffer et al., ASMS 2007, Scherl et al., ASMS 2007). Here, we show that high measured fragment ion mass accuracy allows clear separation between true and false positive peptide identifications containing unexpected modifications. In contrary, data acquired with higher mass deviation (such as acquired in ion-trap type instruments) does not display a clear differentiation between true and false positive matches.

Monday 2:30 – 3:30 pm
New Methods for Systems Proteomics

Mon 3:00 - 3:15 pm: Class Imaging: Classification of Breast Cancer Sections by MALDI Tissue Imaging

Detlev Suckau[2]; Martin Schürenberg[2]; Arne Fütterer[2]; Marc Gerhard[2]; Axel Walch[1]; Sören-Oliver Deininger[2]; Catherine Stacey[3]
[1]GSF-Institut für Pathologie, Neuherberg, Germany; [2]Bruker Daltonik GmbH, Bremen, Germany; [3]Bruker Daltonics, Billerica, MA

MALDI imaging is a novel technique providing unique molecular information to histological tissue sections. We applied MALDI imaging to a set of tissue sections from breast cancer patients to develop automatic tissue classification routines.
The analysis of larger sample cohorts, such as breast cancer patients, requires statistical analysis of the resulting MALDI imaging data. Here we applied different statistical tools, such as unsupervised principal component analysis (PCA) and supervised classification algorithms to access information from the MALDI imaging data.

Tissue cryosections were thaw-mounted on conductive coated glass slides and the MALDI matrix was applied using vibrational

vaporization. Data were acquired on a MALDI-TOF mass spectrometer in linear mode with image resolution up to 50 µm. Statistical analyses were performed and images were generated either vased on selected protein masses, PCA coefficients or supervised classification results using a support vector machine (SVM) algorithm.

Unsupervised PCA-Analysis allowed the direct visualization of the variance in the MALDI imaging datasets. In most cases the PCA results were in good correlation with the histological examination of the sections. In some cases, however, the results of the PCA did not correlate with the histology. This was due to intensive signals from compounds such as beta-defensins, which originated from contaminations from blood. Exclusion of such peaks from the PCA gave the expected results. Unfortunately, the PCA resulted in a high variance if tumour sections came from different patients. Therefore, the PCA reflected largely the variation across patients rather than variation across tissue types. In contrast, SVM gave direct access to molecular species that were characteristic for specific tissue types.

The visualization of the classification results as 2D-image (Class Imaging) also facilitated the comparison with immuno histostaining. Using the supervised classification approach, it was possible to create a software model for the classification of Her2 positive cancer, Her2 negative cancer and connective tissue. It was possible to apply this model to unknown tissue sections to obtain the correct, simultaneous classification of both tumour types as confirmed by the inspection of the pathologist! However, larger studies are required to provide a final validation of this approach.

Mon 3:15 - 3:30 pm: Exploring the Capabilities of the Protein Identification by LC/MALDI/On-target Digestion Approach

Melkamu Getie-Kebtie; Peter Franke; Robert Aksamit; Michail Alterman

FDA/CBER/OCTGT/TVBB, Bethesda, MD

Tryptic digestion of proteins continues to be a workhorse of proteomics. Traditional tryptic digestion requires several hours to generate adequate protein digest. Increased digestion speed for the generation of the same-day proteomic data is highly desirable. Several factors influence the results of proteolysis, including time, temperature, denaturant, protease concentration, and buffer. A number of enhanced accelerated digestion protocols were developed in recent years. Nonetheless, a need still exists for new digestion strategies that meet the demands of proteomics for high-throughput and rapid detection and identification of proteins.

In this research we performed a detailed evaluation of direct tryptic digestion of proteins on a MALDI target plate and the potential for integrating RP HPLC separation of protein with on target tryptic digestion in order to achieve a rapid and effective identification of proteins in complex biological samples. To this end we utilized a Tempo HPLC/MALDI target plate deposition hybrid instrument (ABI).

We demonstrated that direct deposition of proteins on a MALDI target after reverse-phase HPLC separation and subsequent tryptic digestion of the proteins on the target followed by MALDI TOF/TOF analysis provides substantial data (intact protein mass, peptide mass and peptide fragment mass) that allows a rapid, clean, and effective identification of proteins. In addition, this type of intact protein separation excels the peptide separation-based approach in that, during the separation, each peptide does not lack its association to the protein from which it is originated. That makes the identification of proteins much more reliable and the assignment of multiple locations of posttranslational modification or sequence variation to a single protein species less problematic.

Overall, a method that integrates separation of proteins using RP-HPLC with on-target enzymatic digestion of the proteins for subsequent MALDI-MS analysis has been demonstrated with a mixture of standard soluble proteins, membrane hydrophobic proteins and MRC5 cell lysate. The rapid protein separation and direct deposition of fractions on a MALDI target plate provided by the RP HPLC combined with off-line interfacing with the MALDI MS is a unique platform for rapid protein identification with improved sequence coverage. This simple and robust approach also significantly reduces the sample handling and potential loss in large-scale, bottom-up proteomics experiments incurred between complex HPLC protein separations and MALDI-MS peptide analyses. In addition to applying peptide fragment fingerprinting to confirm the identifications and for structural analysis of proteins, this approach also enables the use of intact protein analysis that provides protein MW information.

"The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy."

Monday 4:00 – 5:00 pm
Proteomics and Cardiology

Mon 4:00 - 4:30 pm: Revealing the Dynamics of Cardiac Proteasome Complexes: Molecular Organization, Function, and Regulation

Peipei Ping

UCLA School of Medicine, Los Angeles, CA

Proteasome complexes serve as a major protein degradation machinery in mammalian systems. Recently studies implicated an essential role of this organelle in many cellular processes and diseases, in particular, cardiovascular diseases. However, the molecular organization, function, and regulation of this organelle in the heart remain largely unknown. Using a proteomic approach, we characterized the various multiprotein complexes constituting these large protease complexes in mammalian tisues; the assembly of proteasome complexes and the functional regulation of this organelle were defined; our results offered mechanistic insights on the proteome biology of proteasomes in health and diseases.

Mon 4:30 - 4:45 pm: Cardioinductive Network Guiding Stem Cell Differentiation Revealed by Proteomic Cartography of TNFα Primed Endodermal Secretome

D. Kent Arrell; Nicolas J. Niederlander; Randolph S. Faustino; Atta Behfar; Andre Terzic

Mayo Clinic, Division of Cardiovascular Diseases, Rochester, MN

In the developing embryo, instructive guidance from the ventral endoderm secures cardiac program induction within the antero-lateral mesoderm. Endoderm-guided cardiogenesis, however, has yet to be resolved at the proteome level. Here, through cardiopoietic priming of the endoderm with the reprogramming cytokine TNFα, candidate effectors of embryonic stem cell cardiac differentiation were delineated by comparative proteomics. Differential two-dimensional gel electrophoretic mapping revealed that over 75% of protein species increased >1.5-fold in the TNFα primed versus unprimed endodermal secretome. Protein spot identification by linear ion trap quadrupole (LTQ) tandem mass spectrometry (MS/MS), and validation by shotgun LTQ-FT MS/MS following multidimensional chromatography, mapped 99 unique proteins from 153 spot assignments. A definitive set of 48 secretome proteins was deduced by iterative bioinformatic screening using algorithms for detection of canonical and non-canonical indices of secretion. Protein-protein interaction analysis, in conjunction with respective expression level changes, revealed a non-stochastic TNFα -centric secretome network with a scale-free hierarchical architecture. Cardiovascular development was the primary developmental function of the resolved TNFα anchored network. Functional cooperativity of the derived cardioinductive network was validated through direct application of the TNFα primed secretome on embryonic stem cells, potentiating cardiac commitment and sarcomerogenesis. Conversely, inhibition of primary network hubs negated the pro-cardiogenic effects of TNFα priming. Thus, proteomic cartography establishes a systems biology framework for the endodermal secretome network guiding stem cell cardiopoiesis.

Mon 4:45 - 5:00 pm: Differential Proteome Analysis of Aging in Rat Aorta:Identification and Characterization of MFG-E8 as a Key Regulator in Aging

Zongming Fu[1]; Mingyi Wang[2]; Marjan Gucek[1]; Liqun Jiang[2]; Jing Zhang[2]; Robert N Cole[1]; Robert O'Meally[1]; Simon Sheng[1]; Yurong Guo[1]; Edward Lakatta[2]; Jennifer Van Eyk[1]

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To understand the effect of aging on the vascular proteome, comprehensive comparative proteomic analyses (2DE and ITRAQ) were carried out on aortas obtained from young (8 months) and old (30 months) rats. Aortas were isolated from healthy young (n=7) and old (n=7) rats. Protein were extracted and subjected to 2-D differential gel electrophoresis (2-D DIGE) (pH4-7 and 6-11; 10%bis-TRIS) to detect protein spots with significant abundance change. Gels were visualized with silver staining (or fluorescent labeling) and the gels images were acquired using Silverfast imager (or Typhoon scanner) and analyzed using Decyder software with protein identification by mass spectrometry. Three hundred fourteen proteins have been identified from 2-D gels and 18 proteins differed in quantities between the two age groups. To expand proteome coverage, Isobaric Tag for Relative and Absolute Quantitation (iTRAQ™) was carried out on the same samples. Six hundred proteins have been identified and 45 proteins differed in abundance between young and old animals. Western blotting and immunohistochemistry were performed on proteins of interest for validation. Taken together, over seven hundred proteins were identified, 50 proteins displaying different abundance that were associated with aging. The proteins having different abundance have been implicated in apoptosis, structure, metabolism, and anti-oxidant defense. MFG-E8, an important apoptosis factor, was elevated level in aorta in old rats and further study also demonstrated its level is also elevated in aged monkeys and human aorta compared with their young counterparts. Function studies indicate MFG-E8 regulates inflammation and cell cycle progression suggesting that this novel protein could be a key regulatory agent involved in aging.

Monday 4:00 – 5:00 pm
Computational Proteomics

Mon 4:00 - 4:30 pm: The Generating Function of Tandem Mass Spectra: a Strike Against Decoy Databases

Pavel Pevzner

University of California at San Diego, San Diego, CA

A key problem in computational proteomics is distinguishing between correct and false peptide identifications. We argue that evaluating the error rates of peptide identifications is not unlike computing generating functions in combinatorics or partition functions in statistical mechanics. We show that the generating functions and their derivatives (like spectral energy) represent new features of tandem mass spectra that significantly improve peptide identifications. The generating function approach improves the sensitivity-specificity trade-off of existing MS/MS search tools, addresses the notoriously difficult problem of “one-hit-wonders" in mass spectrometry, and often eliminates the need for decoy database searches. We therefore argue that the generating function approach has the potential to halve the search time, and simultaneously, to increase the number of peptide identifications in MS/MS searches. Finally, we describe our MS-Dictionary software that enables proteogenomic searches in very large databases , e.g., a 6-frame translation of the human genome.

This is a joint work with Sangtae Kim, Nitin Gupta, and Nuno Bandeira.

Mon 4:30 - 4:45 pm: PhosphoScan: A Probability-based Method for Phosphorylation Site Prediction using MS2/MS3 Pair Information

Yunhu Wan[1]; Diane Cripps[1]; Stefani Thomas[1]; Patricia Campbell[1]; Nichlas Amublos[1]; Ting Chen[2]; Austin Yang[1]

[1]University of Maryland Baltimore, Baltimore, MD; [2]University of Southern California, Los Angeles, CA

Large-scale proteomic methods, coupled with bioinformatics, have proven useful for the identification of protein sequences. With a number of programs available for accurate prediction of peptide and protein sequences, scientific interest is shifting to the identification of post-translational modifications (PTMs). The identification of phosphopeptides can be a crucial aspect of many biological studies, as phosphorylation of proteins is one of the most important natural biological switches within the eukaryotic cell; however, phosphopeptide identification and phosphorylation site localization have remained challenging problems. Furthermore, multiple phosphorylations of peptides make site localization even more difficult. This paper details a probability-based method to detect the exact phosphorylation sites within phosphopeptides using MS2/3 pair information. Our method combines scores corresponding to the prediction of phosphopeptide sequences with probability scores for the localization of phosphorylation sites. This method can rapidly and accurately identify phosphopeptides with one or two phosphorylation sites when a data-dependent neutral loss scan is performed. Furthermore, this method could potentially eliminate the need for cumbersome manual validation to confirm phosphorylation sites. Our program, PhosphoScan, successfully identified 61 of 63 MS2/3 pairs within a data set containing numerous doubly-phosphorylated peptides. A comparison test was performed with SEQUEST and MASCOT predictions using a spectral data set from a synthetic doubly-phosphorylated peptide and the results showed that PhosphoScan analysis yielded a 63 percent phosphopeptide localization improvement compared with SEQUEST and a 57 percent improvement compared with MASCOT.

Mon 4:45 - 5:00 pm: Automatic In Silico Interpretation of Native N-glycopeptide Stopflow MS2 CIDspectra Acquired from a Mixture of Unknown Glycoproteins

Sakari Joenväärä[4]; Ilja Ritamo[3]; Hannu Peltoniemi[4]; Risto Renkonen[2]

[1]Helsinki University, Helsinki, Finland; [2]Haartman Institute, Helsinki, Finland; [3]Finnish Red Cross Blood Service, Helsinki, Finland; [4]Medicel Ltd, Helsinki, Finland

We used mass spectrometry and in house build in silico workflows to characterize intact N-glycosylated glycopeptides from tryptically digested glycoproteins.

N-Glycopeptides were enriched with size exclusion chromatography and mass spectra were acquired with Waters QTOF Ultima Global in stop flow mode. Glycopeptide amino acid sequences were searched against in-house generated N-glycopeptide consensus sequence database and false positive rate was estimated, potential glycan compositions permutated and fitted to the experimental spectra with Medicel Integrator N-glycopeptide Suite tools and workflows.

Traditionally the glycoprotein analysis often needs enzymatic release of the glycan from the peptide moiety. Analysing glycans and peptides separately is much easier compared to the analysis of native intact glycopeptides because analysis methods are already far more advanced. The drawback is loss of information when after analysis it is not possible to go back to the intact glycopeptides and biology of the glycoproteins.

So far there is plenty of software available for glycosylation analysis, but not single one of them is supporting simultaneous analysis of amino acid sequence and glycan fragmentation in the intact native glycopeptides. With the existing software it is possible to analyze the glycan composition in the glycopeptide if the mass of the peptide moiety is known (GlycoMod), but the analysis is based solely on the glycan total mass. It is also possible to generate theoretical N-glycopeptide CID spectrum and compare the empirical spectrum against it (Sweet Substitute), but with this approach you need to have a suspect glycopeptide which will be used to generate the theoretical spectrum and there is no database related search of amino acid sequence. There are softwares to analyze separately the released glycans. The simplest of these use only MALDI ionization and precursor ion mass to calculate the potential glycan compositions (Cartoonist). More advanced softwares (STAT, FragLib, StrOligo) are using the MS2 data to match the glycan structures, but in all of these cases the information about the protein where these glycans were attached will be lost.

As a proof of principle we first analyzed human serotransferrin and then applied the methods to human plasma sample. For selected serotransferrin 150 glycopeptide spectra automated workflow generated 105 putative interpretations from > 109 theoretical glycopeptides. After scoring 62 glycopeptide spectra obtained validated interpretation with concomitant amino acid sequences, glycan compositions and structures. Up to 12 different glycans at the same site as well as three different glycosylation sites were observed. When applying this method to human plasma specimen we could identify 80 glycopeptides with their glycan compositions or structures.

Monday Evening Workshop 7:00 – 9:00 pm
Biodefense Functional and Structural Proteomics

Mon Wrkshp 7:00 - 7:10 pm: NIAID Proteomics, Structural Genomics, and Related Programs

Maureen Beanan; Malu Polanski
Nat'l Institute of Allergy and Infectious Diseases, Bethesda, MD

The primary goal of the seven NIAID (National Institute of Allergy and Infectious Diseases) Biodefense Proteomics Research Centers (PRCs) is to characterize the pathogen and/or host cell proteome, including the identification of proteins associated with innate and adaptive immune responses to infectious agents. PRC research is focused on the proteomic evaluation of NIAID Category A-C priority pathogens and microorganisms responsible for emerging and re-emerging infectious diseases. It is anticipated that the PRCs will discover targets for the next generation of vaccines, therapeutics, and diagnostics using existing or newly developed proteomics technologies. All data generated by the PRCs is being consolidated and made available to the scientific community via the related Biodefense Proteomics Administrative Center. This publicly accessible Web site contains data and technology protocols generated by each PRC and serves as a central information source for reagents and validated protein targets (www.proteomicsresource.org). The primary goal of the two Structural Genomics Centers is to apply state-of-the-art, high throughput structural biology technologies to experimentally characterize the three-dimensional atomic structure of targeted proteins from pathogens in the NIAID Category A-C priority lists and organisms causing emerging and re-emerging diseases. The centers will accept proposals for pathogen targets by external investigators, NIH, and other Government Agencies (http://www3.niaid.nih.gov/research/resources/sg/).

In addition to these programs, NIAID has established the Pathogen Functional Genomic Resource Center to provide scientists with free-of-charge genomic resources and reagents, such as microarrays, protein expression clones, genotyping resources, and bioinformatics services (http://www.niaid.nih.gov/dmid/genomes/pfgrc/). The Microbial Sequencing Centers (MSCs) provide rapid and cost efficient resources for producing high quality genome sequences of pathogens and invertebrate vectors of infectious diseases (http://www.niaid.nih.gov/dmid/genomes/mscs/). The primary goal of the eight Bioinformatics Resource Centers (BRCs) is to provide scientists with genomic and related data for NIAID Category A-C priority pathogens, pathogens causing emerging and re-emerging infectious diseases, and invertebrate vectors of infectious diseases (http://www.niaid.nih.gov/dmid/genomes/brc/).

Mon Wrkshp 7:10 - 7:20 pm: Protein Structure Initiative: Progress and Plans

Ravi Basavappa
NIGMS/NIH, Bethesda, Maryland

The Protein Structure Initiative (PSI) is a U.S. structural genomics program that was initiated in 1999 by the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health. The mission of the PSI is to make the three-dimensional atomic level structures of most proteins easily available from knowledge of their corresponding DNA sequences. Nine pilot research centers established in 2000 tested strategies for high-throughput structural determination, developed methodology and technology for lowering costs and increasing success rates, developed structural genomics pipelines, and solved more than 1,300 structures. The NIGMS has also supported technology development for high-throughput structural biology data collection, including the construction and operation of three state-of-the-art beamlines at the Advanced Photon Source at Argonne National Laboratory. The second phase of the PSI began in July 2005. This phase includes four large-scale research centers focused on the production of a large number of unique protein structures that, combined with computational models, will permit broad structural coverage. During the first year of PSI-2, the centers determined more than 450 protein structures, developed additional new methods, and jointly devised a target selection process to maximize structural coverage and biomedical relevance of the structures. PSI-2 also includes six specialized centers that are focusing on technical problems associated with challenging proteins, such as membrane proteins, small complexes, and proteins from eukaryotes. Two additional centers were just funded to improve the accuracy of comparative modeling. Also, a new materials repository center will store and distribute expression clones. In the near future, a knowledgebase will be organized to serve as a centralized information, analysis, and dissemination center. A description of the PSI pilot research centers as well as the goals, requirements, and progress of the initiative will be presented. Background information, summaries of PSI workshops, and program announcements are available on the NIGMS Web site at: http://www.nih.gov/nigms/funding/psi.html .

Mon Wrkshp 7:20 - 7:40 pm: Functional and Structural Proteomics of SARS-CoV: Defining a Paradigm for a Rational Therapeutic and Prophylactic Response to Emerging Infectious Diseases

Jeremiah S. Joseph
Scripps Research Institute, NIAID PRC, La Jolla, CA

Rapid rational therapeutic and prophylactic responses are of the highest priority when faced with newly discovered infectious diseases. The emergence of the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and the high mortality rates and economic impact associated with it, afforded the scientific community an opportunity to test the utility of state-of-the-art post-genomic technologies to characterize and combat this virus. While virus identification and complete genome sequencing took mere weeks, they have been tough acts to follow for drug and vaccine development. We have undertaken a multi-pronged initiative to understand and address precisely this bottleneck. We are using a structural and functional proteomics approach involving bioinformatics, structural biology (X-ray crystallography, NMR, cryo electron microscopy), genetic approaches (site-directed mutagenesis, antisense functional mapping), and macromolecular interaction studies (ligand fishing techniques, mass spectrometry) to generate a structure-function-interaction map of the entire proteome of the SARS-CoV and its interactions with the host cell. This presents an exciting and comprehensive set of targets for rational, structure-based drug and vaccine design, and defines a paradigm that can be successfully adopted for any emerging infectious disease. We also discuss the primary challenge in this project: most SARS-CoV proteins are natively involved in intimate protein-protein, protein-membrane, or protein-RNA interactions which must be understood for a complete description of its biology.

Mon Wrkshp 7:40 – 8:00 pm: Structural Genomics for System Biology Discovery of Novel Drug Targets

Adam Godzik
Joint Center for Structural Genomics (JCSG), NIGMS PSI

Genomics projects can identify all the information needed to sustain an organism, but to fully understand how it works on the molecular level, we also need to know three dimensional structures of all its components. Structural genomics initiatives, initiated in early 2000s, set to obtain such information by developing high throughput protein structure determination technology. At the Joint Center for Structural Genomics, we targeted a hyper-thermophilic bacterium, *Thermotoga maritima* [Lesley, 2002]. After solving structures of over 150 proteins from *T. maritima* and using molecular modeling techniques to predict high accuracy models of additional several hundred proteins, we achieved a full structural coverage of proteins forming a metabolic network in *T. maritima*, opening a way for

genome wide simulations to test essentiality of individual genes and docking studies to test effects of new drugs or therapies. The same approach can be extended for pathogens.

Mon Wrkshp 8:00 - 8:20 pm: Target Selection Strategies for Biodefense Structural Proteomics

Andras Fiser

Albert Einstein College of Medicine, Bronx, NY

I will present a lecture on the potential of combining proteomics and structural genomics efforts for the benefit of both programs. Our proteomics efforts aim at identifying potential drug targets in a waterborne parasite, Toxoplasma gondii. T.gondii has long been recognized as an important pathogen of both immune competent and immunocompromised hosts. Membrane proteins play key roles on the process of invasion and infection from this parasite. Only a very limited number of membrane proteins have been reported and characterized in T.gondii. Recently, we have performed very comprehensive proteomic analysis of T.gondii membrane proteins. Mass spectrometry data were correlated with four gene predictions in this organism. Currently, about one thousand membrane proteins have been identified and annotated. Additional experimental data was obtained in form of full genome microarray expression data and full genome Chip-on-Chip experiments. These, in combination with other in silico data on protein interactions and comparative genomics are used to compile a list of potential drug targets in T. gondii.

Mon Wrkshp 8:40 - 9:00 pm: Integrated Bioinformatics for Biodefense Functional and Structural Proteomics

Cathy H Wu[1]; Margaret Moore[2]; Bruno Sobral[3]

[1]Georgetown University Medical Center, Washington, DC; [2]Social and Scientific Systems, Silver Spring, MD; [3]Virginia Bioinformatics Institute, Blacksburg, VA

The NIAID Biodefense Proteomics program aims to identify targets for potential vaccines, therapeutics, and diagnostics for agents of concern in bioterrorism, including intracellular bacterial, parasitic, and viral pathogens. The program consists of seven Proteomics Research Centers, which are generating host-pathogen data, covering diverse data types ranging from mass spectrometry data, protein interaction data, protein structures and transcriptional profiles to biological reagents such as clones. Meanwhile, the Resource Center (www.proteomicsresource.org) has developed an integrated bioinformatics framework for public dissemination of the data and reagents and to allow for a systems approach to knowledge discovery. We employ a protein-centric approach to organize and map all genetic, functional, structural, pathway and experimental results as a network of attributes of pathogen or host proteins. Underlying this approach is a comprehensive set of protein/gene-related ID mappings contained in the iProClass data warehouse and name mappings maintained in the BioThesaurus. Program and experiment-specific attributes are created to summarize experimental conditions, results and publications. Select proteins are annotated with controlled comments to flag key experimental findings or additional validation. The availability of the host-pathogen data in an integrated system with analytical tools allows comparisons across different experiments and organisms, revealing the functional significance of expressed proteins and their hidden relationships. Here we show examples of analysis of proteins across different data sets in the context of protein structure, function, evolution and pathway for potential target identification.

Tuesday 2:30 – 3:30 pm
Proteomic Technologies for PTMs

Tues 2:30 - 3:00 pm: Surveying Signaling Space Using Antibody-Directed Proteomics

Michael Comb

Cell Signaling Technology, Danvers , MA

Until recently, signaling molecules and pathways have been analyzed one at a time, making it difficult to survey across the complex circuitry controlling cell function. One goal has been to develop the technology needed to survey across many different signaling spaces to identify activated pathways associated with cell function and disease. Our approach has been to develop antibodies broadly reactive against phosphorylated motifs that allow analysis of specific subsets of the phosphoproteome. For example, substrates of tyrosine kinases, MAPKs, or DNA repair kinases. Combining immunoaffinity purification (IAP) of phosphopeptides with tandem mass spectrometry has proven a powerful analytical approach, advancing our understanding of many different signaling spaces. This talk will illustrate how the approach can be applied to several problems in cancer biology by identifying activated signaling pathways and tracing them to novel fusion proteins driving disease. Signaling networks assembled by oncogenic EGFR and c-Met will be discussed and novel actions of EGFR and c-Met inhibitors will be presented. In addition, we have successfully applied the approach to explore and advance our understanding of other protein modifications beyond phosphorylation including protein acetylation and ubiquitination.

Tues 3:00 - 3:15 pm: Targeted Detection of Prostate Tissue Proteins in Serum Using MALDI-TOF/TOF

Yan Li[1]; Lori J. Sokoll[1]; John Rush[2]; Daniel W. Chan[1]; Hui Zhang[1]

[1]Johns Hopkins University, Baltimore, MD; [2]Cell Signaling Technology, Danvers, MA

Prostate cancer develops over a period of several years and is characterized by molecular changes in cancer cells or prostate tissue prior to noticeable symptoms. Only if proteins from cancer tissue can be detected in patients' blood and the abundance of these proteins in blood is associated with tumor development, can these proteins be used for specific detection of prostate cancer using blood tests.

We have previously identified glycoproteins isolated from pooled prostate cancer tissues using solid-phase extraction of glycopeptide (SPEG) and LC-MS/MS, and these glycoproteins represent a rich source of potential disease markers in serum. First, most cell-surface and secreted proteins are glycosylated, and disease-associated glycoproteins (secreted by cells, shed from their surface, or otherwise released from the cancer tissue) are likely to enter the bloodstream. Second, aberrant glycosylation is a fundamental characteristic of oncogenesis and tumor progression. Third, the reduction in complexity achieved by focusing on the glycoprotein subproteome in both tissues and serum translates into favorable limits of detection, thus increasing the likelihood that the same polypeptide will be detectable in both tissue and serum. Finally, specific mass-spectrometry-based methods and affinity reagents can be developed for the specific and sensitive detection of the identified peptides from tissues in serum.

To targeted detection and quantitative analysis of prostate tissue proteins in serum, 98 glycopeptide sequences identified from prostate cancer tissues were selected and heavy-isotope-labeled peptides were synthesized. We used a platform that combined glycopeptide capture, heavy-isotope-labeled-peptide standards, and a 2D-HPLC-MALDI-TOF/TOF-MS to determine which of the identified glycopeptides from prostate cancer tissues can be detected in sera. Biopsy-positive and negative sera with prostate-specific antigen concentrations less than10ng/ml were selected and analyzed in the study. Using heavy-isotope-labeled-peptides as internal standards, 46 out of 98 glycopeptides identified from prostate cancer tissues were detected in patient' sera. The ng/ml detection limit for this platform provided direct identification of low abundant tissue proteins in patients' sera. A quantitative result of the 46 identified peptides was also obtained using this method.

Our screening approach have successfully transformed proteomic analyses from a traditional data-dependant discovery phase into a validation and targeted analysis by directly focusing on biologically relevant peptides/proteins for identification and quantification. In this present study, we demonstrated that certain subsets of glycopeptides originating from prostate cancer tissues could be detected in patients' sera using mass spectrometry-based technologies. The serum detectable glycopeptides could be used as candidate serum markers for development of assays to improve the speed of analyses for large scale validation and clinical usage.

Tues 3:15 - 3:30 pm: Post-translational Modifications of Lysine Residues

Robert J Cotter[1]; Dwella Nelson[1]; Wendell Griffith[2]
[1]Johns Hopkins School of Medicine, Baltimore, MD; [2]University of Toledo, Toledo, OH

As part of a multi-investigator effort, the Technology Center for Networks and Pathways (TCNP) of Lysine Modifications, our laboratory has developed several novel approaches for determining post-translational modifications of lysine residues, including acetylation, ubiquitylation, and SUMOylation. Histones are generally hyperacetylated, as are the histone acetyl transferases (HATs), the enzymes which acetylate these molecules. In addition to determining the acetylation sites, we have developed a method for absolute quantification of acetylation at these sites by deutero-acetylating purified histones prior to digestion with trypsin, which then cleaves only at arginine residues. To determine the distribution of acetylation at these sites for peptides containing multiple acetylated lysine residues, MS/MS spectra were obtained on a unique tandem time-of-flight mass spectrometer, equipped with a "curved-field reflectron" designed in our laboratory. Additionally, we determined the relative amounts of hyperacetylated histone isoforms having the same mass using a high resolution orbitrap mass spectrometer. For the tryptic peptide H3(4-17) with the sequence: GHKGGKGLGKGGAKR there are 16 possible acetylated isoforms. Ubiquitylation is another modification to lysines for which we can screen among a peptide digest mixture using an N-terminal sulfonation tag. Because the tryptic peptide from a ubiquitylated protein carries a GlyGly branch attached to the ε-nitrogen in lysine, two N-terminal tags are attached so that the peak corresponding to the loss of both tags (430u) distinguishes these from peptides containing only a single tag (215u). Similar approaches are being developed for SUMO (small ubiquitin-like modifier), using a dual enzyme digestion procedure. Finally, we have utilized a combination of N-terminal sulfonation and the very high energy collisions in the tandem TOF instrument to determine several labile post-translational modifications found on histone serine residues, primarily phosphorylation and O-glycosylation.

Tuesday 2:30 – 3:30 pm
Systems Biology

Tues 2:30 - 3:00 pm: ErbB Signaling Networks: Quantitative Analysis and Biological Implications

Forest White
MIT, Cambridge, MA

Signal transduction mediated by protein phosphorylation regulates many cellular biological processes. Aberrations in protein phosphorylation due to kinase (or phosphatase) mutation or overexpression lead to dysregulation of cellular signaling and has been linked to a variety of pathologies, including cancer, autoimmune, and metabolic disorders. Quantification of specific phosphorylation sites regulating signaling pathways involved in these pathological disorders should enable a better understanding of the genesis and progression of the disease state, providing targets for more effective therapeutic intervention.

To effectively monitor protein phosphorylation events governing signaling cascades, we have developed a methodology enabling the simultaneous quantification of tyrosine phosphorylation of specific residues on dozens of key proteins in a time-resolved manner. We have recently extended our analysis of the EGFR signaling network to interrogate the effects of increased expression of HER2 in mammary epithelial cells and EGFRvIII in human glioblastoma cells, as both of these proteins are EGFR family members whose over-expression has been correlated with poor prognosis in several cancer sub-types. For both systems, application of bioinformatic tools resulted in identification of several cohorts of tyrosine residues exhibiting self-similar temporal phosphorylation profiles, operationally defining dynamic modules in the EGFR signaling network. For the HER2 overexpression system, we have also measured biological response to stimulation, generating a quantitative phenotypic data set describing the proliferation and migration rates. To identify the phosphorylation sites most strongly correlated to biological response, partial least squares regression analysis of the phosphoproteomics and phenotypic data sets was performed, resulting in a weighted scoring for each phosphorylation site. For the EGFRvIII glioblastoma system, the phosphoproteomics dataset highlighted a novel crosstalk pathway, leading to identification of a novel potential therapeutic target in glioblastoma.

The combination of mass spectrometry-based analysis of protein phosphorylation with phenotypic measurements and computational modeling has enabled the identification of sections of the signaling network that correlate strongly with biological response to cell perturbation. This approach should yield novel insights into the regulation of biological decisions on the network scale.

Tues 3:00 - 3:15 pm: Antibody-array Interaction Mapping: The Discovery and Study of New Interactions in Cancer Sera

Derek Bergsma[1]; Songming Chen[1]; Robert Gerszten[2]; Randall E. Brand[3]; Brian B. Haab[1]
[1]Van Andel Research Institute, Grand Rapids, MI; [2]Massachusets General Hospital, Boston, MA; [3]University of Pittsburgh, Pittsburgh, PA

Protein-protein interactions are major determinants of protein function, regulation, and activity, and alterations in certain interactions can play roles in diseases. We have developed a new method, called antibody-array interaction mapping (AAIM), for the efficient and sensitive measurement of interactions among a set of proteins in biological samples. Advantages of the method are that it is rapid, sensitive, and can be used with native, biological samples, so that complex, multi-protein associations can be detected. A study of interactions between 48 different cytokines, inflammatory mediators, and glycoproteins in the sera of pancreatic cancer patients revealed many known and new interactions, as well as evidence of clusters of interactions. A particularly intriguing new interaction was between the acute-phase reactant C-reactive protein (CRP) and the inflammatory/coagulation protein kininogen. This interaction was confirmed using a variety of methods. We used AAIM to study the interaction in cancer and control serum samples and found that only CRP expressed in a basal, non-acute-phase state binds kininogen, whereas CRP expressed in the acute phase does not. Also, using multiple antibodies against CRP, we also found evidence for structural differences between the CRP that is bound to kininogen and CRP that is not. These findings provide information about the functions and mechanisms of CRP and have implications for better understanding and treating diseases impacted by inflammation, including atherosclerosis and cancer. These studies also show that AAIM is a valuable new tool to discover and study protein-protein interactions.

Tues 3:15 - 3:30 pm: Systems Biology Approach to Studying Muscle's Response to Glucocorticoids

Erica K.M. Reeves; Zuyi Wang; Yetrib Hathout; Eric P. Hoffman
Children's National Medical Center, Washington, DC

Exogenous corticosteroids (particularly Prednisone and Dexamethasone) are used to treat a wide range of conditions and disorders due to their potent anti-inflammatory and immunosuppressive properties. Corticosteroids have multiple side effects including causing rapid muscle loss. Though the catabolic effects of corticosteroids on muscle atrophy have been known for more than 30 years, the underlying molecular mechanism has been elusive. While Prednisone has a catabolic effect on muscle leading to muscle weakness and atrophy in normal individuals, interestingly, in boys with Duchenne Muscular Dystrophy there is an increase in strength. Just as the catabolic response of normal muscle to corticosteroids is poorly understood, so are the molecular mechanisms leading to the increased strength in DMD patients.

We used a data integration approach to define molecular networks associated non-transcriptioal and transcriptional response of muscle to Prednisone. We studied gene expression data from muscle biopsies from 117 patients representing 11 types of neuromuscular disease relative to normal controls totaling 234 microarrays. A novel bio-informatic approach was implemented to identify molecular networks associated with progressive wasting and weakness. The transcripts from both these clusters were entered into Ingenuity Knowledge base. Two clusters came back with networks nucleated on transforming growth factor beta 1 (TGFB1) but there was no overlap in other network members other than TGFB1. These two networks were then studied in normal muscle regeneration (mouse; 27 time points), where we found majority of the network members in the second pattern are regulated during normal muscle regeneration in a temporally-specific manner. This led us to the model that dystrophic myofibers in different stages of regeneration show inappropriate cross-talk, leading to induction of fibrosis, failed regeneration, and muscle wasting.

Glucocorticoids are a common therapy in certain muscular dystrophies. We looked to see the effect of GCs on the second network. A bolus of methyl-prednisolone was given to an adrenalectomized rat and data collected for 17 time points. GC-regulated transcripts were compared to the second network and results showed that key members of the pathology/regeneration-associated network were regulated by glucocorticoids in a 24 hr cycle. This suggests a model for efficacy of steroids, where prednisone functions by re-synchronizing the regenerative process, thereby decreasing inappropriate network cross-talk, and permitting resolution of the tissue damage (effective repair). The model also suggests that diurnal fluctuations of glucocorticoids in all normal individuals may serve to re-synchronize tissue repair systems body-wide. This model was further supplemented by incorporating a quantitative proteomic time series looking the acute translocational non-transcriptional response of Prednisone treated C2C12 muscle cells (5min, 15min, 30min). Significantly changed proteins were also compared and a number of them mapped to the second network.

When taken together, the integration of these 4 large gene expression and proteomic studies have allowed us to develop a systems model of both normal muscles' response to glucocorticoids as well as model the potential molecular mechanism underlying the beneficial response of dystrophic muscle to steroid treatment.

Tuesday 4:00 – 5:00 pm
Phosphorylation Proteomics

Tues 4:00 - 4:30 pm: Innovative Technology for the Study of Cell Signaling

Donald Hunt

University of Virginia, Charlottesville, VA

This lecture will focus on the use of electron transfer dissociation (ETD) to identify peptides and proteins and to characterize their post-translational modifications on a chromatographic time scale. In this experiment, proteins are fractionated by nano-flow HPLC, converted to gas-phase, positive ions by electrospray ionization, and allowed to react with fluoranthene radical anions inside a linear trap mass spectrometer. Electron transfer to the multiply charged protein promotes random fragmentation of amide bonds along the protein backbone. Multiply charged fragment ions are then de-protonated in a second ion/ion reaction with the carboxylate anion of benzoic acid. MS and ETD-MS/MS spectra are recorded every 500 msec. The m/z values for the resulting singly, doubly, and triply charged ions are used to read a sequence of 15-60 amino acids at both the N and C termini of the protein. This information, along with the measured mass of the intact protein, is used to identify unknown proteins, to confirm the amino acid sequence of a known protein, to detect post-translational modifications, and to determine the presence of possible splice variants.

Presented first will be results of studies to characterize post-translational modifications that involve acetylation, methylation, phosphorylation, and O-GlcNAcylation.

Part two of the presentation will focus on signaling between cancer cells and cytotoxic killer cells by the class I, antigen-processing pathway. Since signal transduction pathways in cancer cells are highly dysregulated, we hypothesized that this might manifest itself in the presentation of unique phosphopeptides by the cancers to the immune system in association with Class I MHC molecules. By using a combination of IMAC, stable isotope labeling, and nano-flow HPLC-tandem mass spectrometry, we are able to detect cancer-specific, Class I phosphopeptides present at levels as low as 1-5 copies per/cell. Recent studies show that the Class II antigen processing pathway also presents phosphopeptides. Results of studies on melanoma, ovarian, breast, and lymphoma cancers will be described.

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Tues 4:30 - 4:45 pm: A Novel Approach for Protein Phosphorylation Sites Mapping

Ming Zhou[1]; Zhaojing Meng[1]; Andy G. Jobson[2]; Yves Pommier[2]; Timothy D. Veenstra[1]

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Protein phosphorylation is the one of the most important cellular regulatory mechanisms. Phosphorylation site mapping traditionally relies on methods that utilize radioactive isotope labeling. Here, we present a novel stable-isotope labeling approach that utilizes adenosine triphosphate in which four oxygen-16 atoms attached of the terminal phosphate group are substituted with oxygen-18 [gamma(18O4)-ATP]. Phosphorylation of various proteins was conducted through in vitro kinase reactions in the presence of a 1:1 mixture of gamma(18O4)-ATP and normal isotopic abundance ATP (ATP). The tryptic peptide fragments of these proteins were then analyzed using mass spectrometry (MS). The phosphopeptides can be easily identified using MS owing to the presence of doublets apart by 6.01 Da, which represent the mass difference between a -18O3PO and a –PO4. The identity of these doublets as phosphorylated peptides could be subsequently confirmed using tandem MS. This method provides an unambiguous method by which to recognize phosphopeptides within a complicated spectrum containing large numbers of unmodified peptides.

Tues 4:45 - 5:00 pm: Quantitative Phosphoproteomics by Mass Spectrometry for Systems Level Analysis of Cellular Signaling

Nathan C. Tedford; James R. Graham; Amy B. Hall; Neal F. Gordon; Jeffrey A. Radding

Epitome Biosystems, Inc., Waltham, MA

Protein phosphorylation plays a crucial role in mediating signaling events that dictate cell phenotype in both normal and disease states, most notably in cancer. While compounds targeting kinases receive significant attention in drug development, the vast majority of promising lead molecules fail. The inherent complexity of cell signaling complicates the prediction of efficacy and avoidance of undesirable toxicity in compound development. Signaling networks, and in turn phosphorylation events, being highly dynamic and interconnected, present many challenges to traditional drug discovery efforts, and require a systems-level approach to gauge therapeutic effects.

Proteomics based approaches can provide highly valuable functional data at the network level, but must be powered by careful sample preparation and rigorous data analysis to glean relevant and meaningful conclusions. We have developed a quantitative systems-oriented network-activity relationship analysis, Q-SONAR™, that combines sample preparation, phosphotyrosine enrichment, LC/MS/MS measurement, and rigorous site-specific and systems level analysis to link quantitative signaling network data with cell phenotype and drug effects. We applied this approach to the analysis of differential network perturbations that result from PI3K, MEK and Src inhibitors, alone and in combination, in EGF stimulated A431 cells with upregulated EGFR expression, a common phenotype of many cancers.

Phosphorylation at hundreds of tyrosine sites was measured quantitatively, including 7 sites on EGFR as well as sites on other RTKs such as HER2 and 3 and the HGF receptor. Phosphorylation of proteins implicated in processes known to be mediated by EGF stimulation such as cell growth (Erk 1 and 2, PI3K, SHC), adhesion and migration (Integrins, Fak, Vinculin, PLCγ and endocytic trafficking (CBL, Dynein, Transferrin Receptor) have also been quantitatively measured at multiple sites. This analysis has elucidated nodes that are expected to be regulated by compounds based upon known activities, but also revealed less intuitive and novel targets not previously thought to take part in EGF signaling. These findings may indicate new interactions and roles for signaling molecules that become more apparent when a larger subset of the network is sampled. Regulation of phosphorylation was also shown to be differentially affected on some proteins with multiple phosphorylation sites, demonstrating the importance of making mechanistic conclusions at the site specific level rather than looking at total protein phospho measurements alone.

Q-SONAR analysis has demonstrated extensive coverage and sensitivity in measuring tyrosine phosphorylation levels in a model that is relevant to cancer signaling. The acquisition of quantitative changes in tyrosine phosphorylation at this level of resolution and sensitivity enables the construction of network models of cellular behavior and compound activity. This analysis can be used to determine the mechanism of action for a candidate drug and on and off target compound effects, profile the unknown signaling activity of a phenotype of interest, and drive hypotheses for follow-up validation and novel targets. The increased sensitivity provided by this approach can aid in bridging the gap between in vitro assays and more physiologically relevant in vivo models where tissue can be in limited supply or present lower endogenous signaling levels.

Tuesday 4:00 – 5:00 pm
Statistical Proteomics

Tues 4:00 - 4:30 pm: Significant Analysis for Label Free LC-MS/MS Data

Pei Wang
Fred Hutchinson Cancer Research Center, Seattle, WA

In this talk, we introduce a statistical model for identifying proteins differentially expressed between case and control group based on label free LC-MS/MS experiment results.

For discrete data where no intensity is available, to decide whether one protein is differentially expressed between the tumor group and the normal group is equivalent to test whether the protein is observed significantly more frequently in tumor tissues than in normal tissues, assuming that the probability of a protein being observed in one LC-MS/MS experiment is proportional to its expression level in the sample.

Compare with other similar studies in the microarray literature (searching for differentially expressed genes), the current problem has three new challenges: (a) we only observe peptides in the experiment, while we need to make inference for the proteins; (b) the identifications of peptides/proteins by matching CID spectra to peptides/proteins database have different levels of error; (c) due to the limitation of the total number of tandem MS scans in one experiment, only a partial of peptides can be observed in one

LC-MS/MS run, which may also result in artificial differences (unrelated to biology factors) in the total number of identified peptides between normal and tumor samples. To address these challenges, an Expectation-Maximization (EM) model is implemented to make inferences of proteins based on peptides' observations, in which peptide prophet scores are used to account for the errors from database search algorithms and re-scaling conditioned on total number of identified CID is employed to remove the artificial effect due to experimental limitation. Then the sample labels are permuted to generate the null distribution of the test statistics.

Tues 4:30 - 4:45 pm: CAMSI: Critical Assessment of Mass Spectral Identifications

Stephen R Master[1]; Alexey I Nesvizhskii[2]; Lukas Kall[3]; William Stafford Noble[3]
[1]University of Pennsylvania, Philadelphia, PA; [2]University of Michigan, Ann Arbor, MI; [3]University of Washington, Seattle, WA

Sensitive and reliable identification of database peptide sequences from tandem mass spectra presents an ongoing challenge for mass spectrometry-based proteomics. A number of packages and approaches are now available to address this problem, and recent advances in mass spectrometry have raised the possibility of improved solutions that utilize the information contained in high mass-accuracy spectra. We therefore believe that a direct comparison of current algorithms and best practices provides a useful benchmark for the field as a whole.

To address this issue, we have organized the CAMSI (Critical assessment of mass spectral identifications) competition. Three data sets of varying mass accuracy (low-accuracy MS1 and MS2, high-accuracy MS1 with low-accuracy MS2, and high-accuracy MS1 and MS2) have been provided in mzXML format. Additionally, since this round of assessment focuses on database-driven methods as opposed to ab initio strategies, we have provided anonymized FASTA databases containing a mixture of known and scrambled protein sequences. The primary endpoints for comparison of search results for each algorithm are the number of peptides correctly identified at defined decoy fractions (decoy identifications/true identifications) of 0.5%, 1%, and 5%. Data sets and scoring scripts are available at master04.med.upenn.edu/~webuser/camsi.

We will describe results from this competition (entry deadline December 7, 2007), including direct method comparisons with and without accurate-mass data as well as assessments of the degree of overlap in identifications from a wide variety of approaches.

Tues 4:45 - 5:00 pm: Improving the Reliability of Peptide Identifications using Machine Learning

Xue Wu; Chau-Wen Tseng; Nathan Edwards
University of Maryland, College Park, MD

As the speed of mass spectrometers, sophistication of sample fractionation, and complexity of experimental designs increase, the volume of tandem mass spectra requiring reliable automated analysis will continue to grow. Software tools that quickly, effectively, and robustly determine the peptide associated with each spectrum with a high degree of confidence are sorely needed.

Currently available tools that post-process the output of sequence-database search engines use three types of techniques to distinguish the correct peptide identifications from the incorrect: statistical significance re-estimation, supervised machine learning scoring and prediction, and combining or merging of search engine results. We present a unifying framework that encompasses each of these techniques in a single model-free machine-learning framework that can be trained in an unsupervised manner. The predictor is trained on the fly for each new set of search results without user intervention, making it robust with respect to different instruments, search engines, and search engine parameters.

We show that this approach outperforms machine-learning techniques applied to a single search engine's output, and that combining search engine results provides additional benefit. We demonstrate the performance of the technique using mixtures of known proteins and by using reverse databases to estimate false discovery rates. On an ion-trap LC-MS/MS dataset derived from a mixture of 8 standard proteins, our machine-learning approach is able to increase the search sensitivity from 62% (based on

X!Tandem E-value) to 97% at the 10% false positive rate; while on a QTOF LC-MS/MS dataset derived from a mixture of 17 standard proteins, the search sensitivity was increased from 76% to 95% at 10% FPR. In addition, we show that the performance of the commercial Mascot tool can be bested by the machine-learning combination of two open-source tools X!Tandem and OMSSA, but that the use of all three search engines boosts performance further still. We show that this technique works well on spectra from a variety of ESI and MALDI instruments. We demonstrate our open-source tool PepArML (Peptide Arbiter by Machine Learning) and show how it can be easily extended to support additional search engines and novel peptide-spectrum match metrics.

Wednesday 10:30 – 12:00 pm
Methods & Instrumentation for Glycoproteomics

Wed 10:30 - 11:00 am: Glycoproteins Associated With Breast Cancer

Fred E. Regnier; Wonryeon Cho; Kwanyoung Jung; Jiri Adamec
Purdue University, West Lafayette, IN

This paper focuses on the use of lectins and glycan targeting antibodies (GTA's) to rapidly probe differences in protein glycosylation associated with breast cancer. One approach used to recognize disease associated aberrations in the glycosylation of proteins was by affinity selection of glycopeptides from tryptic digests of plasma. After removal of the glycan with PNGase F, the selected peptides were identified by RPC-MS/MS. An attractive feature of this approach is that proteins and glycosylation sites are identified simultaneously. A second strategy examined was to carry out lectin selection from plasma at the protein level. Glycoproteins thus selected were identified in several ways. One was to subject them to further fractionation by RPC or SDS-PAGE before proteolysis and MS/MS identification. A second was to tryptic digest the glycoproteins immediately after affinity selection and either examine the peptides directly by MALDI-MS/MS or after RPC by ESI-MS/MS. Mixtures were 30-50 times simpler using the glycopeptides affinity selection strategy but some glycoproteins were missed because only 1-2 signature peptides are obtained from each protein. Protein level affinity selection resulted in slightly higher levels of glycoprotein identification. Quantification was achieved by in vitro stable isotope coding. Isolation of glycoproteins with lectins and GTA's provided 1) a rapid method for enrichment of disease associated glycoproteins from plasma without removal of abundant proteins, 2) substantial reduction in the number of plasma by examined by MS/MS, and 3) a means to easily recognized cancer associated glycoforms. Based on results from this work it can be concluded that in the case of many glycosylated marker proteins, glycan structure is strongly associated with particular types of cancer and disease progression.

Acknowledgements: Financial and technical support from the National Cancer Institute "Clinical Proteomics Technology Assessment for Cancer"

Wed 11:00 - 11:30 am: Mapping Expression Patterns of Extracellular Proteomes

Hui Zhang
Department of Pathology, Johns Hopkins University, Baltimore, MD

Proteins expressed in extracellular surface of plasma membrane, including cell surface proteins, transmembrane proteins, and secreted proteins, are most easily accessible as drug targets or protein markers for molecular imaging or protein tests to improve diagnosis, prognosis, and monitoring therapeutic response for a specific disease. Proteomic analyses of tissues and body fluids hold special promise for the discovery of extracellular proteins associated with the specific disease. However, the quantitative proteomic analysis of proteins from different tissues or organs is challenging due to a high complexity and dynamic range of proteins, as well as, the technical obstacles or lack of robust and producible methods for analysis of a large number of complex biological samples.

If the extracellular proteins can be specifically isolated and analyzed, the complexity would be reduced while improving sensitivity and quantification. Extracellular proteins are very frequently modified by oligosaccharides. The population of glycopeptides therefore represents the majority of extracellular proteomes at very low redundancy and provides a tag for specific analysis of extracellular proteome.

Here, we determined the expression patterns of extracellular proteins from different organs, disease states, and cell types by specific analysis of glycoproteins using LC-MS and LC-MS/MS. Over 95% of proteins identified were from extracellular proteins. The expression patterns for glycoproteins were used for the discovery of tissue and disease-specific extracellular proteins from tissue and body fluids.

Wed 11:30 - 11:45 am: IDAWG: A Novel Quantitative Method for Glycomics

Ron Orlando; Gerardo Alvarez-Manilla; Lei Cheng; Kelley Moremen; Stephen Dalton; Michael Tiemeyer; Lance Wells
CCRC/UGA, Athens, Ga

One of the major challenges in the –omics field is the development of technologies that allow for quantitative analysis between samples. In proteomics, stable isotope approaches, such as SILAC, have been developed to address this need. Here we report a methodology that takes advantage of stable isotope labeling of glycans in cell culture for performing relative quantitative glycomics. This methodology termed IDAWG, isotopic detection of aminosugars with glutamine, relies on the hexosamine biosynthetic pathway that uses the side-chain of glutamine as its sole donor source of nitrogen for aminosugars in the production of sugar nucleotides. Thus, introduction of heavy glutamine (15N) into Gln-free media allows for all aminosugars to become labeled and shifted in mass by +1 dalton. Here we demonstrate that this methodology allows for rapid and nearly complete incorporation of 15N into GlcNAc, GalNAc, and sialic acids of N-Linked and O-linked glycans in various mammalian cell culture systems. Besides aiding in the assignment of structures via LC-MSn approaches, this method allows us to determine whether the glycans isolated from a sample result from cellular processes or serum glycoproteins. Importantly, this method also allows us to compare in a quantitative manner the glycans between two cell populations. Furthermore, half-life studies can be performed on glycan structures by switching a cell population from heavy to light labeling conditions and harvesting and analyzing the glycans by LC-MSn approaches at multiple time points afterwards. Thus, the IDAWG approach is an easily applied and powerful new tool in the glycomics toolbox.

Wed 11:45 am - 12:00 pm: Markers of Cancer Using a Lectin Glycoarray Approach

Yinghua Qiu; Tasneem Patwa; Diane M Simeone; Kerby Shedden; Dean Brenner; David Beer; David M Lubman
University of Michigan, Ann Arbor, MI

A glyco-microarray approach will be used to search for early detection biomarkers of colon, pancreatic and esophageal cancer in human plasma. We use a multi-dimensional liquid phase fractionation of intact N-linked plasma glycoproteins previously isolated by lectin affinity columns. The multi-dimensional fractionation will involve nonporous chromatography to separate the glycoproteins and liquid capillary isoelectric focusing to separate protein isoforms, thus providing a means to collect isolated glycoforms in the liquid phase for further analysis. UV absorption detection will allow profiling of changes between cancer versus control. These fractions will be spotted on nitrocellulose-coated microscope slides to produce a natural glycoprotein microarray, and will be interrogated by various fluorescently-labeled lectins to probe each microarray spot for the presence of different glycan moieties. Plasma samples from cancer and normal patients and patients with inflammatory lesions will be analyzed to search for changes in patterns that reveal specific glycan structural changes that occur during cancer progression. Glycoproteins that reveal such changes will be analyzed by QIT-TOF (MALDI-MSn) mass spectrometry to examine the detailed changes in glycan structure that may serve as biomarkers of cancer. Initial analysis has been performed on a training set of 10 samples each from cancer and normal patients and from patients with related inflammatory lesions or early stages of cancer to identify such potential markers. Novel software has been used to analyze both these arrays and the testing sample set.

Based upon PCA analysis these methods provide a means to identify glycoprotein based markers for early detection of cancer. While these biomarkers may be glycoproteins of relatively high abundance in plasma, it is shown that the biomarkers may themselves be very specific based upon changes in glycan structure. Further work on lectin gel blots for prevalidation studies will be described. It is shown that panels of these biomarkers can be used together in order to increase the sensitivity and specificity of the methodology.

Wednesday 10:30 am – 12:00 pm
Protein Networks in Mitochondria

Wed 10:30 – 10:55 am: Automated Proteome-Wide Determination and Modeling of Subcellular Location for Systems Biology

Robert F. Murphy
Carnegie Mellon University, Pittsburgh, PA

An important challenge in the post-genomic era is to identify subcellular location on a proteome-wide basis. A major source of information for this task will be imaging of tagged proteins in living cells using fluorescence microscopy. We have previously developed automated systems to interpret the images resulting from such experiments and demonstrated that they can perform as well or better than visual inspection. Recent work demonstrates that these methods can be applied to large collections of images from yeast (the UCSF yeast GFP localization database), human tissues (the Human Protein Atlas), and randomly GFP-tagged mouse 3T3 cell lines. A distinct but related task is learning from images what location patterns exist (rather than classifying them into pre-specified patterns). In this regard, we have obtained reasonable results on clustering mouse proteins into subcellular location families that share a statistically indistinguishable pattern. In order to be able to capture and communicate the pattern in each family, we have developed approaches to learning generative models of subcellular patterns from images. These can be used to synthesize images that in a statistical sense are drawn from the same underlying population as the images used for training. The models can be communicated in compact XML files that are compatible with cell model descriptions captured in SBML. We anticipate combining these models to construct cell models containing all expressed proteins in their proper locations. We are currently working to integrate our tools with existing cell modelling systems to permit accurate, well-structured information on subcellular location to be

Wed 11:20 - 11:40 am: Systematic Construction and Analysis of a Mitochondrial Protein Atlas

Sarah E. Calvo; David J. Pagliarini; Betty Chang; Sunil A. Sheth; Steven A. Carr; Vamsi K. Mootha
Broad Institute of MIT/Harvard, Cambridge, MA

Although mitochondria house many core metabolic and signaling pathways, much of the mitochondrial proteome as well its diversity across tissues remain uncharacterized. To produce a high quality atlas of mitochondrial proteins, we performed mass spectrometry of mitochondria isolated from fourteen tissues, assessed protein localization through large-scale GFP tagging/microscopy, and integrated these results with six other genome-scale datasets of mitochondrial localization, using a Bayesian approach. The resulting atlas, which consists of 1097 genes and their protein expression across 14 tissues, provides a powerful resource to address key problems in mitochondrial biology. First, we reveal new metabolic pathways and disease genes, such as MMACHC, associated with the organelle. Second, in an exploration of tissue diversity, we find that mitochondria obtained from two different tissues typically share ~75% of their proteins, though remarkably, the amount of mitochondria can vary as much as 30-fold. Third, using comparative sequence analysis, we rediscover nearly all transcription factor-binding motifs known to control mitochondrial gene transcription, and additionally implicate NF-Y and two novel cis-motifs in this program. Finally, we chart the ancestry of the mitochondrion and observe that nearly 3/4 of its components have bacterial origins. Our large-scale analysis represents the most comprehensive characterization of the mitochondrial proteome to date and provides a foundation for systematically exploring the organelle's contribution to both basic cellular biology and human disease.

Wed 11:40 am - 12:00 pm: Quantitative Mitochondrial Proteomics during Cell Differentiation: Bioinformatic Aspects

Daniel F. Bogenhagen; Jermel Watkins; Siddhartha Basu; Chun Zhou
Stony Brook University, Stony Brook, NY

We conducted a quantitative proteomic study of changes in protein abundance accompanying differentiation of mouse P19 embryonal carcinoma cells to neuron-like cells using LC-MS/MS analysis of peptides labeled with isobaric tags. We documented a significant increase in mitochondrial biogenesis during this developmental program influenced by dramatic upregulation of the transcription co-activator PGC-1 . We also noted that neuronal differentiation involved close associated of mitochondria with cytoskeletal proteins. This introduced an apparent discrepancy between quantitative proteomic results obtained with total cell lysates as compared to mitochondrial lysates. These results illustrate the need to develop a better understanding of the nature and extent of protein associations with the outer mitochondrial membrane. The MiGenes database of mitochondrial proteins has been developed to support proteomic studies. The status of this database will be described to highlight current deficiencies in gene ontology annotation of mitochondrial proteins in key model organisms.

Wednesday 1:15 – 2:45 pm
Disease Proteomics

Wed 1:15 - 1:45 pm: Tissue MALDI Mass Spectrometry Imaging (MALDI-MSI) to Identify Diagnostic/Prognostic Biomarkers for Prostate Cancer

Lisa Cazares; Raymond Lance; Savvas Mendrinos; MaryAnn Clements; Richard Drake; Paul Schellhammer; O. John Semmes
Eastern Virginia Medical School, Norfolk, VA

Diagnosis and prognosis of cancer is based on pathologic morphologic evaluation. The ability to localize disease-specific molecular changes in tissue would be of tremendous benefit toward diagnostic accuracy. Direct profiling of proteins in tissue sections using MALDI mass spectrometry imaging (MALDI-MSI) provides a platform for revealing molecular detail with possible diagnostic and prognostic implications. Using this approach, we have identified specific peptide expression changes that correlate with presence or absence of cancer. The expression of a single m/z accurately defined cancer from normal tissue. The examination of 26 prostate tissue sections (13 cancer and 13 benign) resulted in correct classification of 10 of 13 PCa and 12 of 13 benign tissue. Two of the cancer tissues that failed to express this marker were obtained from hormone-responsive patients post treatment. In a second series of experiments we identified a specific m/z pattern in the primary tumor that was indicative of the presence of metastatic disease. These markers allowed for detection of micrometastatic disease in 5 of 6 target cases. Our results suggest that histology guided MSI is a promising strategy for identification of prostate cancer specific biomarkers that can be utilized to improve cancer diagnosis, enable individualized care, and stratified patient risk for metastatic disease.

Wed 1:45 - 2:15 pm: Tissue Proteomics: Application of High-Resolution Mass Spectrometry and Label-Free Differential Analysis for Cancer Biomarker Investigations

Thomas P. Conrads[1]; Brian L. Hood[1]; Jennifer N. Sutton[2]; Susan E. Abbatiello[1]; Rajiv Dhir[3]
[1]University of Pittsburgh Cancer Institute, Pittsburgh, PA; [2]BRIMS Center, Thermo Fisher Scientific, Cambridge, MA; [3]University of Pittsburgh, Pittsburgh, PA

Conventional protein biomarker discovery investigations are predominantly performed with samples such as serum and plasma. Although such investigations lead to the identification of many candidate biomarkers, seldom do such biomarkers find a place in the clinic arising in part from low sensitivities and specificities for disease diagnosis. In many cases this low diagnostic power is reflective of their origin, for example in the case of inflammation markers in the case of cancer, which indicate the presence of

cancer, but do not accurately indicate the site of malignancy. While such markers may be useful to indicate the presence of disease, they are not often sufficiently sensitive or specific to provide for clinically useful diagnoses. This presentation will discuss applications of laser capture microdissection, high-resolution Fourier transform mass spectrometry, and label-free differential bioinformatic analysis from proteomic investigations from pathological tissue sections for identification of proteins for cancer early detection. Finally, a new discovery workflow based on harvesting proteins from tissue interstitial fluid will be presented. This novel workflow enables direct detection and identification of shed and secreted proteins from the tumor microenvironment and speeds translation to blood-based biomarker assays.

Wed 2:15 - 2:30 pm: Phosphorylated VEGF Receptor in the Vitreous of Wet AMD: A New Class of Biomarkers for Predicting Treatment Timing and Response

Geetanjali Davuluri[1]; Virginia Espina[2]; Emanuel F. Petricoin, III[2]; Mark Ross[2]; Jianghong Deng[2]; Bert M. Glaser[1]; Lance A. Liotta[2]
[1]National Retina Institute, Towson, MD; [2]George Mason University, Manassas, VA

Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness over age 60. Targeted treatment of the vascular endothelial growth factor (VEGF) has been shown to delay vision loss. We explored phosphorylated VEGF receptor and other growth factor receptors in the vitreous, and correlated levels with treatment response. Vitreous-borne phosphoproteins are presented as a new class of biomarkers.

Methods: Sixteen vitreous samples were acquired by fine-needle aspiration. Reverse phase protein microarrays were used to quantify phosphorylated forms of angiogenesis related kinases. Eleven patients underwent sampling prior to intravitreal bevacizumab injection for wet AMD (n=10) or idiopathic choroidal neovascularization (n=1). Two patients had samples collected prior to intravitreal injection and one month later. Five controls were collected prior to surgery for: macular hole, epiretinal membrane, or retinal detachment.

Results:
The patients who received bevacizumab treatment were characterized as non-responders (n=6) and responders (n=5) based on ophthalmic clinical parameters.

The phosphorylated form of vascular endothelial growth factor receptor (VEGFR Y1175) was detectable in the vitreous along with other angiogenesis related proteins (PDGFRβ Y716, PDGFRβ Y751, VEGFR Y996 and c-KIT Y703). There was a statistically significant difference ($p<0.0064$) in VEGFR Y1175 between the responders (mean 10.70, SEM 1.07) and non-responders (mean 4.63, SEM 0.55) and between responders and controls (mean 5.24, SEM 0.0.66). There was no statistical difference between the non-responders and controls. In addition there was a statistically significant difference in VEGFR Y996 ($p<0.0366$) and PDGFR β Y751 ($p<0.0024$) for the responder group. PDGFRβ Y716 ($p<0.9597$) and c-KIT Y703 ($p<0.0544$) were not found to be significantly associated with treatment response categories.

In two eyes (patients 2 and 7), VEGFR-Y1175 was measured from vitreous samples taken just prior to and one month following intravitreal injection of bevacizumab. Patient 2, characterized as a responder had an initial high level of VEGFR Y1175 similar to others in the responder group. There was a 33 percent decrease in VEGFR-Y1175 levels after treatment with bevacizumab.

Patient 7, characterized as a non-responder had an initial low level of VEGFR-Y1175, similar to the levels in control eyes and non-responder eyes. This patient exhibited an increase in VEGFR Y996, c-KIT Y703 and PDGFRβ Y716 post-treatment. This is in direct contrast to Patient 2, who responded to treatment, and exhibited reduction in these same proteins.

Conclusion:
The novel use of reverse phase protein microarrays as a proteomic technology in ocular disease is described. Our data has revealed the previously unknown existence of phosphorylated forms of VEGFR and other growth factor receptors in the vitreous. These activated signal pathway proteins presumably originate from retinal or sub-retinal cells. We showed significant differences in the VEGFR levels between patients with neovascular and non-neovascular disease as well as between responders and non-responders to anti-VEGF therapy. Measurement of specific signal pathway proteins in the vitreous could become the basis for early detection, prognostic determinations and individualized timing of therapy for AMD patients, as well as offering the potential to identify other therapeutic targets.

Wed 2:30 - 2:45 pm: Autoantigen Biomarker Discovery through Immunological Profiling with Functional Protein Microarrays

Dawn R Mattoon[1]; Michael G. Smith[1]; Gengxin Chen[1]; Mary Brodey[1]; David Alcorta[2]; Ronald Falk[2]; Dhavalkumar Patel[2]
[1]Invitrogen, Branford, CT; [2]University of North Carolina, Chapel Hill, NC

The presence of autoimmune disease-associated autoantibodies prior to the presentation of clinical symptoms has been well-documented, and can be exploited for the development of multiplex assays offering improved sensitivity, selectivity, and reproducibility over currently available immunodiagnostics. This study utilized high-content protein microarrays comprised of more than 5,000 human proteins, including 25 known autoantigens, to evaluate immunological profiles across panels of serum samples derived from healthy donors and Systemic Lupus Erythemasosus (SLE) patients. Candidate biomarkers were identified using the parallel application of three statistical approaches including M-statistics, volcano analysis, and fold change calculations. Validation experiments were subsequently carried out using both custom protein microarrays and Luminex bead sets. A validation rate of approximately 70% was observed across both technology platforms when the same set of disease and normal serum samples were used as probes. Improved discrimination between the two populations was observed when Principle Component Analysis was applied to data derived from novel, protein microarray-defined proteins relative to autoantigens with annotated associated with SLE. Leave-one-out cross-validation analysis using support vector machine learning calculated a classification error rate of 3.3% for the array-defined candidate biomarkers, relative to an error rate of 13.3% calculated for the annotated SLE biomarkers. Taken together, this study provides the experimental and statistical framework to support the adoption of protein microarray technology as a tool for immunological profiling for disease biomarker discovery.

Wednesday 1:15 – 2:45 pm
Bioinformatics for Systems Biology

Wed 1:15 - 1:45 pm: Understanding Protein Function on a Genome-scale using Networks

Mark Gerstein; *Yale University, New Haven, CT*

My talk will be concerned with topics in proteomics, in particular predicting protein function on a genomic scale. We approach this through the prediction and analysis of biological networks, focusing on protein-protein interaction and transcription-factor-target ones. I will describe how these networks can be determined through integration of many genomic features and how they can be analyzed in terms of various simple topological statistics. In particular, I will discuss a number of specific analyses: (1) Integrating gene expression data with the regulatory network illuminates transient hubs; (2) Integration of the protein interaction network with 3D molecular structures reveals different types of hubs, depending on the number of interfaces involved in interactions (one or many); (3) Analysis of betweenness in biological networks reveals that this quantity is more strongly correlated with essentially than degree; (4) Analysis of structure of the regulatory network shows that it has a hierarchiel layout with the "middle-managers" acting as information bottlenecks. (5) Development of a useful web-based tools for the analysis of networks, TopNet and tYNA.

http://bioinfo.mbb.yale.edu - http://topnet.gersteinlab.org

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Positive Selection at the Protein Network Periphery: Evaluation in Terms of Structural Constraints and Cellular Context. Philip M. Kim, Jan O. Korbel and Mark B. Gerstein PNAS 104: 20274-9

The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. H Yu, PM Kim, E Sprecher, V Trifonov, M Gerstein (2007) PLoS Comput Biol 3: e59.

Genomic analysis of the hierarchical structure of regulatory networks. H Yu, M Gerstein (2006) Proc Natl Acad Sci U S A 103: 14724-31.

Wed 1:45 – 2:15 pm: Protein Interaction Networks from Bacteria to Man

Peter Uetz; *J. Craig Venter Institute, Rockville, MD*

Protein-protein interaction networks have been pioneered in yeast, using both yeast two-hybrid (Y2H) screens and affinity purification combined with mass spectrometry (AP-MS). More recently other organisms have been studied more comprehensively, including several bacterial species and humans. While the coverage of Y2H screens is estimated to be on the order of 25%, the coverage of AP-MS screens remains unknown. However, it is clear that both methodologies recover different interactions.

Many interactions are probably not biologically relevant, given that in yeast only 20% of all genes are essential (under standard laboratory conditions). Besides mutating interaction epitopes (which would abolish specific interactions) another way to identify important interactions is to detect homologous interactions ("interologs") in multiple species. I will present some comparisons among bacterial interactomes and discuss their implications for other interaction data sets, including vertebrates.

Wed 2:15 - 2:30 pm: PeptideAtlas: Resource for Target Selection for Emerging Targeted Proteomics Workflows

Eric W Deutsch[1]; Henry Lam[1]; Ruedi Aebersold[2]
[1]Institute for Systems Biology, Seattle, WA; [2]Institute for Molecular Systems Biology,ETH Zurich, Zurich, Switzerland

A critical component of successful systems biology experiments is an assay that provides reliable quantitative measurements of each of the components of the system being studied. For proteomics to be a key part of systems biology experiments, it must be able to deliver accurate -- if possible, absolute -- quantification for all the components of the system for each perturbation of the system without gaps in the data. This will require a new approach to proteomics as opposed to incremental improvements of the shotgun method. The emerging targeted workflows provide the techniques needed to turn proteomics into a quantitative discipline. Two things are required to support targeted proteomics workflows successfully: more advanced instrumentation with appropriate control software, and a means to reliably select and validate the targets.

The PeptideAtlas Project is comprised of a growing, publicly accessible database of peptides identified in a large set of tandem mass spectrometry proteomics experiments, plus a set of software tools that enable the building of the PeptideAtlas as well as the use of the results by the community. PeptideAtlas is not a passive repository that contains static results, but rather an active repository in which new software tools and techniques are applied to all raw data to extract ever more information and present it to the community. We describe the PeptideAtlas project, its components, and related efforts, and show how, together, they provide the platform necessary to select and validate targets, thereby enabling the next revolution in proteomics: targeted quantitative workflows.

Wed 2:30 - 2:45 pm: Proteogenomic Mapping for the Human Genome: Technologies and Challenges for Identifying Protein-coding Sequences

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The ENCODE project, in which 1% of the human genome was studied extensively by multiple groups, revealed pervasive and complex transcription patterns that present a significant challenge to determining which parts of the genome are translated into proteins and when. Proteogenomic mapping uses mass spectrometry data from the analysis of expressed proteins to determine the genomic loci encoding them, providing direct information about which transcripts from the genome are translated. The approach has been applied successfully to smaller genomes, but there are significant technical challenges in applying it to large genomes with complex multi-exon genes. One of those is the computational cost of searching incomplete and noisy mass spectrometry data against a sequence the size of the human genome, with over 10^9 theoretical peptides, both rapidly and with useful accuracy. Despite the obstacles, this approach promises to reveal new information about the extent and nature of alternative splicing, and to provide definitive information about which transcripts encode proteins and in which biological circumstances.

We developed the Genome Fingerprint Scanning (GFS) software as a general platform for proteogenomic mapping. It matches tandem mass spectrometry (MS/MS) data from digested protein samples to a theoretical digest of an entire genome sequence, identifying the encoding DNA sequences. We are building a computational pipeline that uses GFS and large human proteomic data sets to reveal protein translation patterns in human cells. We recently developed several new computational approaches to improve the accuracy of the GFS search and to manage its results. First, we implemented a hidden Markov model based scoring system for matching MS/MS peptide spectra to genomic sequence, providing significantly enhanced accuracy for whole genome searches compared to commonly used MS/MS scoring methods. We also are developing a new, highly flexible heterogeneous data management system, that uses a relational database system to represents all information from the project as a set of rules within a small cluster of relational tables. New data types can be rapidly added to the system by modifying the meta-rules stored within the system, without changing the relational database structure. We have also made programmatic enhancements to GFS to provide for fine-grained cluster distribution and rapid genome-wide search by combining multiple stages of MS/MS search.

We will discuss the application of these new methodologies to a prototype project where we mapped a human blood plasma data set comprised of 1,200 MS/MS-analyzed peptides to their genomic sequence origins. We compare the results of whole-genome mapping to those of searching protein databases derived from existing gene annotations, highlighting both the promise of the approach in uncovering new information about which transcripts encode protein(s), and discussing the prospects for large-scale application of the method to enhance genome annotation.

Mon Poster 01: Analysis of Barrett's Esophageal Single Cells using Two-Dimensional Capillary Electrophoresis with Laser-Induced Fluorescence

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Analysis of cellular homogenate samples gives information only on the average expression within a cellular population, often masking expressions from cells of interest. Employing chemical cytometry to study protein expression within a single cell gives a more accurate description of varying expression within a heterogeneous cellular population. We have previously demonstrated the use of 2D-CE to monitor expression differences associated with tissue types and at different points in Barrett's Esophagus (BE) neoplastic progression. The grade of the dysplasia in BE is defined by the degree of the architectural atypia of the crypts. Variations in protein expression between cells within these crypts have enormous potential in giving prognostic information. Two-dimensional capillary electrophoresis (2D-CE) with laser-induced fluorescence (LIF) detection is used for high resolution separation of complex biological samples. In 2D-CE, two capillaries, with an i.d. of 30 µ, are aligned at a buffer filled interface. The proteins are separated by capillary sieving electrophoresis (CSE) in the first dimension. Fractions are electrokinetically transferred to the second capillary, and separated by micellar electrokinetic chromotagraphy (MEKC). A single cell is injected and lysed in the first capillary. Biomolecules are labeled by 3-(2-fluoryl)quinonoline-2-carboxaldehyde (FQ), a fluorogenic molecule that binds to primary amines to form highly fluorescent products. Fluorescent biomolecules are separated by 2D-CE and detected inside a sheath-flow cuvette with a 473 nm solid state laser and fiber-coupled avalanche photo diode (APD). 2D_CE-LIF is capable if detecting zeptomole (10^{-21}) quantities of FQ-labeled proteins. The 2D-CE system demonstrates high reproducibility of migration times. Same-day experiments have less than 1% variation in migration times and different-day experiments have less than 1.3% variation. The 2D-CE-LIF system routinely achieves spot capacities of hundreds to thousands. The sensitivity and resolution are ideal for single cell analysis.

Mon Poster 02: Isolation and Identification of Candidate Peptidomic Biomarker Proteins for Prostate Cancer Detection

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Prostate cancer is the most common non-cutaneous malignancy and the second-leading cause of cancer deaths among United States men. Measurment of serum prostate-specific antigen (PSA) levels has revolutionized the care of prostate cancer patients, although there are well-recognized limitations of the PSA use in clinical practice. Therefore, there is a need to identify new prostate cancer biomarkers for improving early diagnosis and prognosis. Human serum contains a large number and quantity of intact, modified or cleaved proteins. Some of these can originate in the cancer-tissue microenvironment and provide a record of the physiological status. In particular, the low molecular weight (LMW) protein fraction of the serum proteome, or peptidome, is thought to contain undiscovered, potentially useful disease biomarkers and as such constitutes a rich source of disease-specific information. The LMW protein fragments can be bound to highly abundant serum proteins, which preserve these molecules from renal clearance, but hinder identification and analysis. Liquid chromatography coupled on line to mass spectrometry (LC-MS) is a powerful technology that enables detection and identification of proteins and peptides in complex samples, such as serum. In this study we analyzed the serum LMW protein fraction from prostate cancer patients by liquid chromatography-tandem mass spectrometry (LC-MS/MS), to identify new biomarkers. For each patient, whole serum was collected immediately before the surgery and 6 weeks to 3 months following surgery. The LMW fraction was obtained by continuous elution electrophoresis, by which lower abundance protein fragments and peptides are recovered, and thereby isolated from highly abundant, higher MW serum proteins. The LMW protein fraction was subjected to in-gel trypsin digestion and then analyzed by LC-MS/MS (Thermo LTQ Orbitrap). The data were analyzed by database search (SEQUEST), manual identification confirmation, initial comparative analysis by MS-MS spectral counts (Scaffold) and then manual comparative analysis of peptide abundances. Several proteins/fragments were determined to be differentially abundant in pre-operative versus post-operative LMW fractions and therefore designated as candidate biomarkers to be evaluated further for their association with prostate cancer. Reverse phase protein microarrays and Western blot assays were used to validate these candidate biomarkers.

Mon Poster 03: Clinical Proteomics of Saliva of Squamous Cell Carcinoma Oropharynx and Leukoplakia

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Squamous cell carcinoma of oropharynx , the most common type of oral cancer ,accounts the sixth most common malignancy in US. The subtle clinical presentation of the disease and ambiguous clinico- histopathological picture of the disease has lead to delay in diagnosis and hence poor outcome of the medical and surgical treatments. Till date the sole criteria for diagnosis is scalpel based tissue biopsy , which often fails to correctly rule out or favour the diagnosis of the condtion. Our study of differential proteomic profile in saliva of patients of Squamous cell carcinoma oropharynx with that of leukoplakia(a premalignant condition) and normal saliva can lead to discovery of biomarker and hence a saliva based compliant , quick and accurate bed side diagnostic cum prognostic kit for the disease.

Mon Poster 04: Improving the Discovery Potential of 2D PAGE Proteomic Projects by Introducing a New Image Analysis System

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It is a well-established problem that the quality of two-dimensional (2D) gel electrophoresis results can suffer from personal bias and/or lab-to-lab variability during the image analysis stage. In addition, the Combined Correctness of 2D gel image analysis results has been shown to be the single most deciding factor in order to maximize discovery potential and minimize error rate. However, using conventional 2D gel image analysis software such as Progenesis Samespots, PDQuest, Decyder, ImageMaster etc, there is no way of monitoring and measuring this parameter in the 2D gel image analysis. Hence, using conventional software you are left to do 2D gel image analysis until you think it "looks good". As a control of the reliability of your results, you can calculate the mean-CV. But recently, mean-CV has been shown not to correlate very well to a high discovery potential and a low error rate in 2D gel image analysis.

Thus, a new way of performing 2D gel image analysis is needed that minimizes personal bias, eliminates lab-to-lab variability, optimizes discovery potential, and decreases error rate. We have devised a new system for analyzing 2D gel images that addresses all the abovementioned problems. The system comprises a professional image analysis center with standardized working procedures and well-defined quality metrics and uses proprietary software that allows optimizing Combined Correctness. Scientists are able to incorporate this system into their 2D gel proteomics workflow through a powerful, user-friendly, and workflow driven software that enables uploading images and the subsequent exploring of results.

In this study we show that this system minimizes personal bias, eliminates lab-to-lab variability, optimizes discovery potential, minimizes error-rate and decreases the overall turn-around time for 2D gel proteomics projects.

Mon Poster 05: Antibody Proteomics Mediated Biomarker Discovery

Laszlo Takacs

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Discovery and utilization of new, disease specific protein biomarkers will forward and accelerate the complex drug discovery and validation process. It also facilitates identification of the optimal patient population for important clinical trials and disease diagnosis. The two popular approaches of biomarker discovery are protein profiling by mass spectrometry and systems biology based exploration of multiple biomarkers. While these methods can generate some disease relevant candidates, the markers are generally based on abundant proteins and in most instances lack true disease specificity. Therefore, such activities are rarely translated into a clinical diagnostic assay. The bottleneck is in the validation/qualification process of the relevant candidates with sensitive, reproducible and easily applicable clinical assays.

Here we describe a novel biomarker discovery strategy that combines high throughput monoclonal antibody-based global disease specific analyte screening technology, with mass spectrometry-based identification of protein biomarkers of lung cancer. In a single step, profiling of the potential biomarker is accomplished in terms of the immunogenic space of a given complex protein sample (e.g. the human plasma proteome), in conjunction with the relevant protein IDs, and the first level of validation. Large-scale monoclonal antibody profiling also offers high sensitivity, good efficiency and automation of ELISA assays. The monoclonal antibodies derived by this novel platform can be readily incorporated into the further biomarker validation and clinical diagnostics development processes by the pharmaceutical and diagnostics industry to accelerate the product development cycle. Additionally, the libraries are amenable to global plasma proteome profiling by antibody microarrays.

Mon Poster 06: Microproteomic Analysis of FFPE Cancer Tissue: Spectral Count Quantitation and Differential Protein Expression

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Tissue microproteomics was employed to demonstrate large scale analysis of proteins in formalin fixed cancer tissue. Liquid Tissue® lysates were prepared from 15 different formalin fixed tissues spanning multiple tissue types and grades of cancer. Lysates were analyzed by LC-ESI-MS/MS and large numbers of peptides and proteins were identified in each sample ranging from 745 proteins (1048 peptides) to 2378 (3455 peptides). Spectral count analysis indicates hundreds of proteins expressed in common between all cancers while cluster analysis indicates differentially expressed proteins capable of segregating cancer types. The identified proteins include known cancer biomarkers, low-abundance proteins such as transcription factors and signal pathway proteins, and housekeeping proteins. Cluster analysis demonstrates the ability to segregate primary tumors that gave rise to metastatic disease from primary tumors that did not show signs of metastasis at time of collection. These results indicate the potential to determine the metastatic status of a primary tumor using mass spec-based proteomics.

Mon Poster 07: Protein Differential Expression Profiling of MCF7 Breast Cancer Cells

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The development of novel technologies for fast proteomic profiling of cancerous cells is essential for speeding up the discovery process of early disease biomarkers. Timely detection of abnormal physiological conditions will be highly beneficial for diagnosing various diseases and increasing survivability rates. We are reporting on the development of label-free and iTRAQ quantitation strategies for global differential expression analysis of MCF7 breast cancer cells cultured in the presence of estradiol and tamoxifen, and on the implementation of these technologies on high-throughput microfluidic analysis platforms. Disposability, cost-effective analysis with minimal carry-over, and capability to detect protein co-expression patterns, will launch such devices as viable bioanalytical platforms for large-scale population screening applications.

MCF7 cells were cultured to 70 % confluence in the presence of estradiol and tamoxifen. The cells were lysed, digested with trypsin and processed according to a shotgun 2D-SCX-LC-ESI-MS/MS protocol. A bench-top LC (Agilent, CA) interfaced to an LTQ mass spectrometer (Thermo Electron, CA), and in-house developed microfluidic chips, were used for completing the analysis. iTRAQ and label-free quantitation technologies were investigated for differential protein expression profiling of cells.

We have developed proteomic protocols that enabled the identification of >2,000 proteins in cancer cellular extracts. In the estrogen positive MCF7 breast cancer cell line, ~1,900 proteins were identified with p-values <0.001 (p being the probability of a random match for a given peptide amino acid sequence). The experimental distribution profile of protein numbers vs. molecular weight closely followed the theoretical distribution of the human proteome displayed by SwissProt. Over 200 proteins were categorized as being involved in cancer relevant cellular processes (i.e., cell differentiation, cell growth and proliferation, cell cycle regulation, cell adhesion, apoptosis and DNA repair), and a number of well-known putative cancer biomarkers such as PCNA, cathepsin D, E-cadherin, 14-3-3-sigma, Ki-67, TP53RK, calreticulin and keratins 8, 18 and 19, were identified. For quantitative experiments performed with iTRAQ reagents, typically, the experimental vs. true values were found to be within a range of true +/-50 % for ~80 % of all quantified proteins. Overall, quantitative profiling was estimated to generate reliable results for changes in expression levels ≥3X. Label free quantitation strategies that rely on spectral counting are under development.

Our long-term objective is focused on implementing this technology on disposable microfluidic platforms for reliable detection of protein co-expression patterns. The performance of such microfluidic chips, in terms of number of identified proteins, was comparable to bench-top instrumentation, if similar conditions were used. For the microfluidic experiment, and theoretical iTRAQ ratios of 0.2:1:1:5, the range of normalized ratios for individual standard proteins spiked into the MCF7 extract was (0.15-0.39):(0.52-1.16):1:(4:08-6.44). The larger the number of unique peptides that matched a given protein, the closer the normalized experimental to the true values were found to be. Typically, the injection of 0.1-1 ug cellular protein extract enabled the confident identification of 40-100 proteins and a panel of 5-6 putative biomarkers.

Mon Poster 08: Biomarker Discovery Based on MALDI MS Profiling in Combination with High Throughput Multiplexed, Electrophoretic Prefractionation

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In most proteomics studies enormous effort is directed towards identifying and cataloguing proteins in limited numbers of pooled samples. Proportionally little effort is directed towards quantifying protein changes across patient populations. Protein identification consumes enormous resources and distracts, in terms of time, sensitivity and precision, from efforts to discover new biomarkers. Our view is that the first priority in clinical proteomics is to establish reproducible quantitative differences between groups (e.g., cancer and no-cancer) that can guide clinical decision-making and once a biomarker candidate passes this criterion, targeted protein identification is performed.

Studies must incorporate representative samplings, but given the heterogeneity within the human population this can only be achieved by analyzing hundreds of samples. Approaches based on MALDI profiling are among the few options that provide the necessary throughput, but they have gained an execrable reputation due to their application in poorly designed studies. The reality, however, is that when employed in an appropriately designed study with the proper approach to sample preparation, data collection and interpretation, profiling can be a reproducible and precise discovery tool. To maximize the potential of these approaches we therefore investigated pre-fractionation strategies that can be combined with MALDI profiling with the objective of increasing the coverage of the proteome without significantly compromising throughput and reproducibility.

One approach, based on multiplexed electrophoretic pre-fractionation and concentration, showed considerable promise. This technique utilizes a multi-well electrophoretic device to deplete, fractionate, concentrate and desalt 96 samples in one hour. The precision of this method was assessed by replicate analysis (n=24) of a standard sample, ACTH [18-39] at 50 fmol/µL. The CV was 7.5% (cf. 5.2% for the same sample spotted directly onto the MALDI target). Replicate analysis (n=48) of a human serum sample gave reproducible, information-rich spectra. Analysis of serum from a single individual at pH 5.35 yielded ca. 200 anionic and another 200 cationic proteins. Reproducibility was computed for each ion found in all replicate samples. The median CV was less than 30%. Absolute sensitivity was determined by adding known amounts of ACTH (LOD, 1 fmol) and fibrinonectin binding protein, peptide D3 (LOD, 5 fmol) to 5 µL of human serum.

The combination of electrophoretic sample preparation and MALDI profiling was applied to the analysis of human plasma from breast and lung cancer patients. The approach provided a time and cost effective way to analyze multiple samples (in replicate) and gave a set of candidate biomarkers for further qualification and verification. When applied to the analysis of human serum from patients with breast cancer vs. matched controls, 37 differentially expressed proteins were observed. The ions corresponding to these differences were in the range m/z 1500-5000 and were distributed across all fractions. The 10 most statistically significant ions from each fraction classified patients with an accuracy of 95% based on hierarchical clustering. We detail these studies, our findings and evaluate the potential of this approach to accelerate and standardize MALDI MS for the discovery of protein biomarkers.

Mon Poster 09: Characterization of Stress Hormone-Mediated Drug Resistance in Breast Cancer Cells using SILAC Combined with High Resolution Mass Spectrometry

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Breast cancer is one of the most frequently diagnosed cancers among women and chemotherapy treatment has been a successful therapy, however, as many as 50% of patients will not benefit from this care because their breast cancer cells will become resistant to the chemotherapeutic reagents. Great effort is on going to understanding what causes drug-resistance in breast cancer and several different hypotheses have been formed. To gain a better picture of what might be happening at the proteomic level we used stable isotope labeling with amino acids in cell culture (SILAC) coupled with high resolution mass spectrometry to investigate drug-resistance in breast cancer cells. Initial results shown by traditional cell biology assay indicated that breast cancer cells treated with Paclitaxel demonstrated significant cell cytotoxicity. However, once cells were incubated with stress hormones the majority of cells survived the drug treatment. To gain an insight as to what is happening at the protein level, breast cancer cells were grown in light and heavy SILAC media both treated with Paclitaxel to induce cell death, and the heavy cells were also incubated with stress hormones. Cells were lysated, mixed together at a 1:1 ratio and protein separation was preformed using SDS-PAGE. Gel bands were digested and analyzed using a high resolution FTMS. Initial results show that stress hormones alter several proteins involved in drug resistance. . Taken together, We have shown that SILAC combined with nanoscale separations coupled online with high resolution mass spectrometry has been successfully used to study quantitative changes in protein synthesis in breast cancer cells and chemotherapy.

Mon Poster 10: Prevalence and Characteristics of Autoantibodies to Annexin A11 in Different Types of Human Cancer

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Annexin A11 is a member of the annexin superfamily of Ca2+ and phospholipids-binding, membrane-associated proteins implicated in Ca2+-signal transduction processes associated with cell growth and differentiation. Recently, we showed that annexin A11 is associated with cisplatin resistance and related to tumor recurrence in ovarian cancer patients. Previously, annexin A11 was identified as an autoantigen in 4.1~10.1% of patients with various systemic autoimmune diseases. The majority of these anti-annexin A11 autoantibodies belong to the IgG class, consistent with an antigen driven mechanism of autoantibody production, in contrast to that the autoantibodies to other annexins are primarily of the IgM isotype. Anti-annexin A11 autoantibodies do not cross-react with other annexin members, corroborating that they recognize the unique N-terminal domain of annexin A11. It is believed that autoantibodies can be viewed as reporters from the immune system revealing the identity of antigens, which might be playing roles in the pathophysiology of the disease process. How annexin A11 participates in the pathogenesis of human cancers and whether a similar mechanism in autoimmune diseases might be involved in human immune responses in cancer remains to be established. In this study, we develop a novel enzyme-linked immunosorbent assay (ELISA) to investigate the occurrence and features of autoantibodies against annexin A11 in sera from patients with different types of human cancer and diabetes as well as from healthy controls. Briefly, the recombinant protein of GST fused to the N-terminal domain (1-175 residues) of human annexin A11 (GST-Anx11N) was expressed in E. coli BL21 cells and purified by affinity chromatography using Glutathione-Sepharose 4B. The fusion protein was then used as antigen in ELISA and western blot for the detection of autoantibodies to annexin A11. A total of 246 serum specimens archived at the Johns Hopkins Hospital were analyzed, which includes sera from 77 healthy women; 72 patients with stage III/IV ovarian cancer (40 primary and 32 recurrent tumors); 18 patients with breast cancer; 19 patients with colon cancer; and groups of 20 patients each with pancreatic cancer, prostate cancer, or diabetes. The overall titer of autoantibodies to annexin A11 in ovarian cancer patients (or primary tumors only) was found much higher than that in healthy controls ($P<0.05$). At the cut-off value (mean OD + 2SD of healthy controls) designating positive reaction, autoantibodies to annexin A11 were detected in 12.5% (5/40) of primary ovarian cancer patients with a significant difference from 2.6% (2/77) of the healthy controls ($P<0.05$), but only in 6.25% (2/32) of recurrent tumors. ROC analysis demonstrated the potential diagnostic value of autoantibodies to annexin A11 in primary ovarian cancer patients with an AUC of 0.62 (0.52-0.73). Autoantibodies to annexin A11 were also detected in 5.26% (1/19) of colon cancer and 10% (2/20) of diabetes patients but without significant difference from the healthy controls. This study suggested that anti-annexin A11 autoantibodies frequently occur in primary ovarian cancer patients in contrast to healthy controls, and maybe involved in the pathogenesis of ovarian cancer.

Mon Poster 11: Discovery of Pancreatic Cancer Biomarker for Early Detection: Proteomic Analysis of Human Pancreatic Duct Fluid (Juice)

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Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer death in USA and has the lowest survival rate for any solid tumor. Most patients diagnosed with pancreatic cancer die within 12

months, and only about 4% survive 5 years after diagnosis due to the advanced stage of cancer at the time of diagnosis. Early diagnosis of pancreatic cancer may improve survival rate. Proteomic analysis of patient samples may provide a new diagnostic marker for early detection of pancreatic cancer. Pancreatic duct fluid (juice) is an ideal proteomic sample that is rich in cancer-specific proteins, given the proximity to the tumor. However, not many reports are available on global proteomic analysis of pancreatic juice. In this study, we analyzed twenty pancreatic duct fluid samples (tumor and non-tumor) using HPLC-Chip-MS technology in a high throughput manner using Gel-LC-MS/MS, MudPIT and peptide OFFGEL Electrophoresis (OGE) followed by MS/MS analysis. 1D-gel separated proteins were cut into 30 equal sized gel bands and in-gel trypsin digested. For OGE, proteins were trypsin digested and separated into 24 fractions between pH 3-10. For SCX, proteins were trypsin digested and fractionated into 15 salt fractions. Peptides were analyzed on Agilent's HPLC-Chip-XCT-Ultra ion-trap and/or q-TOF mass spectrometer and the CID data was analyzed with Spectrum Mill bioinformatics tool. From this analysis, over 1000 NR human proteins were identified in pancreatic juice with <1% false positive rate. Of these, ~100 proteins were found in common between multiple samples. More proteins were identified using OGE peptide fractionation than with 1D-Gel protein separation and SCX peptide separation method and all together yielded more number of proteins in pancreatic juice. Semi quantitative analysis based on spectrum intensity yielded ~60 proteins whose levels are changed >3 fold compared to benign tumor samples. Many of the identified proteins are pancreatic cancer related and therefore could be potential candidates for diagnostic markers leading to early detection. To date, this is the first report showing more than 200 proteins in a single analysis of pancreatic duct fluid. Some of the pancreatic cancer-related proteins found in this study will be further validated in plasma, tumor interstitial fluid (TIF) and tumor tissue samples of the same patient using QQQ mass spectrometer.

Mon Poster 12: Global Quantitative Analysis of the IR Response of $p53^{K317R}$ Knock-in Mouse Thymocytes

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p53 is a sequence-specific transcription factor that has crucial roles in apoptosis, cell cycle arrest, cellular senescence, and DNA repair. It is maintained at low levels in unstressed cells, but stabilized and activated following DNA damage through extensive post-translational modification. Among those modifications is acetylation of human p53 at Lys_{320} (Lys_{317} in mouse), the sole site to be modified by the acetylase PCAF. To elucidate functions of this specific post-translational modification, knock-in mice were generated in which Lys_{317} was mutated to Arg in both alleles of endogenous p53, maintaining a positive charge but blocking acetylation at this site. Using cleavable ICAT quantitative mass spectrometry, the effect of ionizing radiation (IR) on protein levels in either the wild type or $p53^{K317R}$ thymocytes was determined. 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. Many proteins were found to be significantly affected by IR in the wild type thymocytes, including several whose expression has been shown to be directly regulated by p53. When the effects of IR in the wild type and $p53^{K317R}$ samples were compared, 54 proteins were found to be affected by the mutation ($p<0.1$). The $p53^{K317R}$ mutation has widespread effects on specific protein levels following IR, including the levels of proteins involved in apoptosis, transcription, and translation. Pathway analysis of the affected proteins suggests an increase in p53 activity in the $p53^{K317R}$ thymocytes, as well as a decrease in TNFα signaling. These results suggest that acetylation of Lys_{317} modulates the functions of p53 and influences the cross-talk between the DNA damage response and other signaling pathways.

Mon Poster 13: The Application of Plasma Proteomic Patterns for Discrimination of Patients with Squamous Cell Carcinomas from Healthy Controls

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No satisfactory plasma biomarkers were discovered till now for the early detecting and monitoring of lung cancer, one of the most frequently encountered cancers. In the present research the new methodology for the search of tumor markers of lung cancer, involving profiling the low-molecular plasma proteomes (120 кDa) of blood, is developed, unified and approved. The given approach included three basic components: robotics pre-preparation of samples, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and bioinformatics software for mass spectral data processing. Opportunities and prospects of the developed approach for the detection of potential lung cancer markers were shown.The plasma samples have been prefractionated using magnetic beads kits functionalized with weak cation exchange coatings. We compiled MS protein profiles for 90 patients with squamous cell carcinomas (SCC) (stages I-II) and compared them with profiles from 187 healthy controls. The control group was subdi-vided into a preliminary set (123 controls) for model generation and a second validation set (64 controls). The SCC group was also subdivided into a training cohort with 59 patients and test cohort with 31 patients for validation. Peptide profiles were acquired with an Ultraflex II mass spectrometer in linear positive mode. The MALDI-TOF spectra were analyzed statistically using ClinProToolsTM bioinformatic software. We detected about 440 peaks/spectrum in a mass range of 1000 – 20000 Da. 33 of these proteins had statistically differential expression levels between SCC and control plasma ($P < 10\text{-}3$). Compared to controls, 3 peaks ($p<10\text{-}3$) are increased in SCC and 30 peaks are decreased. The series of the peaks were automatically chosen as potential biomarker patterns in the training set. They allowed to discriminate plasma samples from healthy control and samples from SCC patients (sensitivity – 90%, specificity – 95% for support vector machine) in external validation test. These results suggest that plasma MALDI-TOF MS protein profiling can distinguish SCC patients from normal subjects with relatively high sensitivity and specificity, and the MALDI-TOF MS is a potential tool for the screening of lung cancer.

Mon Poster 14: Proteomic Profiling of Biopsy Procured Tissues from Low and High Dicer Expressing Glioblastomas

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The ability to effectively profile human tissues is of fundamental importance since the proteomes isolated from cell lines may not accurately resemble those in situ. It has been shown that reduced expression of RNAse III endonuclease (Dicer) in tumorous tissue is associated with poor prognosis in patients diagnosed with certain type of cancer (e.g. lung cancer). Here, we present a simple method developed for comparative proteomic profiling of low Dicer vs. high Dicer expressing glioblastomas tissues procured by tumor biopsy. This approach relies on tissue homogenization coupled with two-stage solubilization and protein digestion in mixed aqueous-organic buffer. To characterize variation between glioblastoma proteomes exhibiting differential Dicer expression we analyzed four samples of low Dicer expressing glioblastomas and four samples of high Dicer expressing glioblastomas. Nanoflow LC-MS analysis resulted in the identification of 1,705 proteins from low Dicer expressing tissues and 1,747 in high Dicer expressing tissues. A total of 777 proteins were identified in both tissues. Based on the peptide count, high Dicer expressing glioblastomas showed an increased expression of epidermal growth factor receptor and LanC-like protein 2, which were previously found co-amplified and over expressed in ~20% of all glioblastomas.

Mon Poster 15: Characterization of the T-Cell Leukemia/Lymphoma (TCL1) Oncoprotein Interactome

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TCL1 is a proto-oncogene whose deregulation has been implicated in the pathogenesis of T- and B-cell lymphoproliferative disorders. Although recent studies indicate that dysregulated expression of TCL1 is important in mature B-cell transformation, very little is currently known about the function or interactions of TCL1, specifically in the B-cell context. Proteomic analysis provides an opportunity to carry out functional studies of protein-protein interactions and characterization of functional interactomes. Using a functional proteomic approach, we determined the identity of proteins that interact with TCL1 by co-immunoprecipitation with anti-TCL1 antibody followed by liquid chromatography (LC), electrospray ionization (ESI) and tandem mass spectrometry (MS/MS). Immunoprecipitates of the TCL1 expressing SUDHL-16 B-cell lymphoma derived cell line were compared to that of hyperimmune rabbit immunoglobulin and a cell line that does not express TCL1 (SUDHL-1) by 1-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Silver-stained protein bands from both immunocomplexes were excised and analyzed by ESI-LC-MS/MS. MS2 data was searched using k-score enabled X!Tandem against IPI Human (version 3.32). This search database included reverse protein sequences and common contaminants. Search results were subsequently processed through PeptideProphet and ProteinProphet keeping only those proteins identified with a probability of >0.8 (FDR <= 0.03). Proteins specific to the SUDHL16 pull down were then extracted from this list of proteins using in-house bioinformatics tools and subjected to pathway analysis by Ingenuity Pathway Analysis which reveal potential role of TCL1 in cellular pathways including AKT, SRC, MYC and RAS signaling. Our analysis provides unique insights into the potential role of TCL1, a protein whose deregulation is increasingly implicated in the pathogenesis of a variety of hematopoietic neoplasms, in various cellular processes such as protein synthesis, protein trafficking, cellular assembly and RNA metabolism.

Mon Poster 16: Analysis of MALDI-TOF Mass Spectrometry Data for Discovery of Peptide and Glycan Biomarkers of Hepatocellular Carcinoma

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We propose computational methods to analyze MALDI-TOF mass spectrometry data for quantitative comparison of peptides and glycans in serum. The methods are applied to identify candidate biomarkers in serum samples of 203 participants from Egypt; 73 hepatocellular carcinoma (HCC) cases, 52 patients with chronic liver disease (CLD) consisting of cirrhosis and fibrosis cases, and 78 population controls. Two complementary sample preparation methods were applied prior to generating mass spectra: (1) low molecular weight (LMW) enrichment of each serum sample was carried out for MALDI-TOF quantification of peptides, and (2) glycans were enzymatically released from proteins in each serum sample and permethylated for MALDI-TOF quantification of glycans. A peak selection algorithm was applied to identify the most useful peptide and glycan peaks for accurate detection of HCC cases from high-risk population of patients with CLD. In addition to global peaks selected by the whole population based approach, where identically labeled patients are treated as a single group, subgroup-specific peaks were identified by searching for peaks that are differentially abundant in a subgroup of patients only. The peak selection process was preceded by peak screening, where we eliminated peaks that have significant association with covariates such as age, gender, and viral infection based on the peptide and glycan spectra from population controls. The performance of the selected peptide and glycan peaks was evaluated in terms of their ability in detecting HCC cases from patients with CLD in a blinded validation set and through the cross-validation method. Finally, we investigated the possibility of using both peptides and glycans in a panel to enhance the diagnostic capability of these candidate markers. Further evaluation is needed to examine the potential clinical utility of the candidate peptide and glycan markers identified in this study.

Mon Poster 17: ProtExpress, a Web-Based Annotation Tool to Input and Format Experiment Informaion for Proteomics Research

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Increasing amount of proteomics research data, especially mass spectrometry data has been made available in the past few years. Several large scale repositories have been created to host proteomics experiment and protocol data. National Cancer Institute has implemented a public proteomics repository, based on Computational Portal and Analysis System (CPAS), developed at Fred Hutchinson Cancer Research Center. Currently, the repository provides public access to 6,176,247 peptide identifications from 244 ms-ms runs from Mouse Proteomics Technology initiative (MPTI). One problem for transporting the data from labs to public repositories is the lack of a standard format for experiment and protocol annotations. For example, CPAS uses eXperimental ARchive (XAR) format, while EBI pride database uses Pride XML format.

To deal with the issue, our team has developed a web based annotation tool for proteomics experiment, named protExpress. protExpress is an open source project utilizing industry standard best practices and is developed on the J2EE platform. protExpress provides an intuitive web-based interface to allow users to input and manage experiment and protocol information. It provides a framework for describing experimental procedures and steps. It also allows custom annotation with description of protocols, protocol input and protocol output. protExpress enables researchers to specify an experiment with a series of protocols, with specific inputs and specific outputs. The protocols can be shared and reused by authorized user groups. The software also format and export experiment information into the XAR format used by CPAS. The architecture of protExpress enables addition of other annotation formats for proteomics experiment.

Mon Poster 18: Identification of Sites of Phosphorylation from Tandem Spectrometry Data by Logical Analysis of Result Classes

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In tandem mass spectrometry, most peptide identification methods work by ruling against the null hypothesis that the number of matches between the masses of observed ion fragment masses and those of expected ones from a candidate peptide is due to chance. This method can be especially unsuitable for determining the correct phosphorylation site(s) of a phosphopeptide when there are multiple potential sites available. In addition, when these potential phosphorylation sites are close to each other, observed ion masses may be consistent with multiple isomers of the peptide and more than one may achieve a statistically high score. This problem of identification can be reframed into one of distinguishing among alternative hypotheses representing possible phosphorylation sites. We have implemented an algorithm based on the principle that observations that may be individually consistent with multiple isomers can be mutually inconsistent with each other for all but one of the structures, leading to the correct identification of the phosphate site(s). Our program converts the experimental data into logical constructs that lead to one or more independent conclusions regarding the phosphorylation site. The program automatically adjusts its sensitivity settings to select a set of observations that is logically consistent with a unique set of phosphorylation sites. The method works for peptides with one or more phosphates and if more than one set of phosphorylation sites

is consistent with the data, probabilities are calculated for the likelihood of correctness for each site. Examples from the analysis of actual data sets with this program are shown.

Mon Poster 19: RAId_DbS: Peptide Identification using Database Searches with Realistic Statistics

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Background: The key to mass-spectrometry-based proteomics is peptide identification. A major challenge in peptide identification is to obtain realistic E-values when assigning statistical significance to candidate peptides. Results: Using a simple scoring scheme, we propose a database search method with theoretically characterized statistics. Taking into account possible skewness in the random variable distribution and the effect of finite sampling, we provide a theoretical derivation for the tail of the score distribution. For every experimental spectrum examined, we collect the scores of peptides in the database, and find good agreement between the collected score statistics and our theoretical distribution. Using student's T-tests, we quantify the degree of agreement between the theoretical distribution and the score statistics collected. The T-tests may be used to measure the reliability of reported statistics. When combined with reported P-value for a peptide hit using a score distribution model, this new measure prevents exaggerated statistics. Another feature of RAId DbS is its capability of detecting multiple co-eluted peptides. The peptide identification performance and statistical accuracy of RAId DbS are assessed and compared with several other search tools.

Mon Poster 20: How to Predict Discovery Potential and False Positives in 2-Dimensional Electrophoresis Image Analyses

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In two-dimensional gel electrophoresis (2DGE) experiments, the achieved correctness in the image analysis plays a crucial part in maximizing the number of true protein changes found in the experiment as well as minimizing the error rate among the results. In this study, we measured the correctness of different aspects of the image analysis in order to investigate if there is a way to predict the reliability and completeness of the image analysis results. As relevant measures we used the present proportion of noise in the form of false positives as well as the number of verified, statistically significant changes in protein expression that are found, given that these are the most important factors in the desired outcome of most 2DGE experiments.

We had access to a large number of gel image analyses performed by the Ludesi Analysis Center as well as by a variety of organizations using analysis software such as PDQuest, DeCyder, Progenesis and Progenesis with SameSpots. A novel method of manually evaluating the correctness of different aspects of the analyses, such as spot detection, spot segmentation and spot matching, was devised. In addition, we calculated the mean coefficient of variation (mean CV) of the spot volumes in all the analyses. The resulting data show that a combined measurement of the spot detection correctness and spot segmentation and matching correctness display a great degree of correlation with the number of statistically significant protein regulations that are found, as well an inverse correlation with the fraction of errors among these regulations. In contrast, mean CV is much less useful when trying to predict the reliability and completeness of the analysis.

The results indicate that a measurement based on rigorous evaluation of the correctness of spot detection, spot segmentation and spot matching, which we have termed Combined Correctness (CC), provides a good indication of the reliability and completeness of the results from 2D gel image analysis.

Mon Poster 21: Absolute Protein Abundance Estimation Based on Spectrum Counting using Large Datasets in PeptideAtlas

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Modern tandem MS technologies enable high-throughput peptide identifications in many species across many labs. Number of unique peptides identified and the number of those spectra used to identify new and existing peptides have steadily increased for many species such as yeast, human and mouse over the last couple of years. PeptideAtlas was designed to capture all peptides confidently identified from tandem MS datasets collected from many labs for many species. Here we report that using the datasets currently stored in PeptideAtlas. we are able to estimate absolute protein abundances from yeast and human plasma fairly accurately. For yeast, 31 non_enrichment experiments totaling 3,457,179 spectra were searched using Sequest . Out of wihich, 2814 proteins were confidently IDed with a FDR of 0.7%. Spectra counts for each confidently IDed protein were used to estimate its absolute abundance in cell lysate or plasma. MRM experiments were conducted to measure absolute peptide concentrations based on the ratios to corresponding AQUA peptides from yeast and human plasma and the results of protein abundances inferred from measured peptides' abundances are in good agreement with our computational estimation.

Mon Poster 22: Statistical Analysis of Proteomics Datasets and Comparison to Reference Databases

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Dealing with large quantities of data generated in shotgun proteomics, is often followed by a further challenge: extraction of biologically meaningful information from the proteomics datasets. A number of questions often arise following dataset processing: how exhaustive was the analysis, how efficient was the purification, what was the proteome coverage and so on.

Here we show how ProteinCenter, a novel bioinformatics tool, provides a statistical overview of protein lists based on the gene ontology (GO) annotations and bioinformatical prediction tools. This tool enables straightforward analysis of proteomics datasets and their comparison to reference statistics derived from a number of protein sequence databases. Currently ProteinCenter supports reference statistics from the following species: Homo sapiens, Mus musculus,Rattus norvegicus, Drosophila melanogaster, Saccharomyces cerevisiae, Escherichia coli, Caenorhabditis elegans, Arabidopsis thaliana, Bos Taurus, Gallus gallus, Danio rerio, Xenopus tropicalis. ProteinCenter uses the Benjamini-Hochberg method for correction of multiple testing to determine which protein sets are selected with a particular false discovery rate (FDR) and statistically overrepresented in comparison to a reference dataset.

A number of examples of statistical analysis of proteomics dataset including purification of membrane mitochondrial proteins, pheromone response and others will be presented.

Mon Poster 23: A Novel Approach to Extract Biological Knowledge from Proteomics Data

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The success of LC-MS based proteomics has triggered the realization of a number of proteomics initiatives like the HUPO projects. At the same time a number of challenges remains in data interpretation, data validation and ability fast and in a correct way to decipher the biological relevant information from obtained data. As a result new software tools are needed to facilitate the processing of proteomics data.

Here we described our recent development in the ProteinCenter software package. The core of ProteinCenter is based on the master database that includes all major protein databases (automatically updated and keeping track of outdated accession codes) and currently contains more than 9.2 millions of proteins. That resolves a problem of the redundancy of protein databases and performs complex comparison between different datasets. It also includes instant BLAST analysis, prediction of

signal peptides and transmembrane regions as well as a gene ontology analysis
In the current study we present a new feature of ProteinCenter that enables significance statistical analysis of comparison data based on feature distributions that are significantly different between the selected data sets. The quantitative phosphoproteomics study applied to the yeast pheromone signaling pathway (Gruhler et.all, MCP 2005) was under investigation. The significance statistics were applied on the Gene ontology (GO) classification. The results of the comparison of up- and down regulated phosphorylation sites to the total pull of the detected phosphopeptides showed that the polarized growth and mating projection contribute most for the cellular components. It should be mentioned that mating projection is formed by unicellular fungi in response to mating pheromone. The detail analysis also shows that mating projection was caused by the tail projection but not the base projection. That example demonstrates the power of the software that allows extraction of biological important information in a matter of minutes.
Other examples of proteomics data processing will be included.

Mon Poster 24: Structure Based Discovery of Potential Inhibitor Leads for Sirtuin: A Novel Target of Entamoeba Histolytica

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Amebic dysentery and amebic liver abscess caused by anaerobic protozoan parasite Entamoeba histolytica, is a major causes of morbidity and mortality worldwide. The control of ameobic parasite infection relies primarily on chemotherapy which is itself limited by number of drugs available against this parasite. Therefore, there is a urgent need to identify new targets for development of novel antiamoebic drug candidates. Sirtuin protein was found to be essential for protozoal life and sirtuin inhibitors has shown a stage specific cell killing activity against Leishmanial parasites hence sirtuin could be a potential drug target in protozoa. SIRTUIN gene has been cloned into E. coli expression vector pQE31 and novel intron (54 nucleotide) was identified which was not revealed in genome sequencing. This gene sequence has been submitted in GenBank (GenBank accession no. EF202834). For functional characterization, intronless EHSIR2 gene was constructed using Site Directed Mutagenesis approach. EhSir2 protein has been expressed and purified by metal affinity matrix, subsequently characterized by using fluorometric activity assay. To find out novel broad spectrum less toxic inhibitors, homology model of E. histolytica, L. major and human sirtuin protein was constructed using MOE. The models were validated and the ligand selectivity for Entamoeba, Leishmania against human Sirtuin was evaluated using Molecular Electrostatic Potential (MEP) maps and cavity depth analysis, subsequently structure based virtual screening was performed to obtain a database of 1591 compounds, incorporating a molecular drawing interface from ZINC database and molecular docking was performed into the active site of EhSir2, LmSir2 and human sirtuin protein. Most of the molecules docked well, but few of the molecules demonstrated better affinity to EhSir2 and LmSir2 compared to human sirtuin.

Mon Poster 25: Retinal Pigment Epithelium Secretome and Age Related Macular Degeneration

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While the genetic factors responsible for age related macular degeneration (AMD) are becoming unravelled, the pathophysiology of the disease remains difficult to understand. AMD is characterized by an accumulation of drusen between the basal lamina of the retinal pigment epithelium (RPE) and Bruch's membrane resulting in progressive degeneration of RPE cells and photoreceptors. Understanding the cellular and molecular origins of drusen will help in finding new therapeutic targets to prevent or slow down the progression of the disease. The objective of this study is to determine the role of RPE cells in drusen formation. The underlying hypothesis is that RPE cells are involved in drusen formation and that an aberrant response of RPE from AMD donors to different environmental stressors is a cause of drusen accumulation and choroidal neo_vascularisation. Protein expression patterns and especially proteins secreted by RPE cells, detectable by proteomic techniques, are expected to be a direct reflection of the relationship between RPE cells and drusen formation. Using stable isotope labelling by amino acids in cell culture we show that RPE cells secrete a variety of proteins that have been reported to be constituent of drusen. Our data revealed that RPE cells from AMD donors (diagnosed by histological examinations of the macula and genotyped for the Y402H-CFH variant) secreted 2 to 3 fold more of these proteins than RPE from age matched healthy donors. Additionally, treatment of RPE cells with TNF-alpha resulted in an increased secretion of complement C3, a major component found in drusen.
We anticipate that the application of newly emerging proteomic techniques will result in an improved understanding of the pathways involved in AMD pathogenesis and will help in finding new therapeutic targets to delay or stop progression of the disease.

Mon Poster 26: Differential Proteomic Characterization of Multiple Sclerosis-Associated Proteins as Potential Biomarkers in Cerebrospinal Fluid (CSF)

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Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of unknown etiology that affects the central nervous system (CNS). Currently there is no specific diagnostic test for MS and the diagnostic process is often drawn out over months or years. 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 identify putative biomarkers, we performed an in-depth proteomic analysis of cerebrospinal fluid (CSF) derived from MS patients and control subjects to determine the differences in the expressed proteins. Previous studies suggested that the cleavage of the full-length protein Cystatin C may be an adaptive host response and may identify a subgroup of patients with Multiple sclerosis. Our study focused on other possible biomarkers, including cleavage products. A non-gel based multidimensional fractionation approach coupled with high resolution tandem mass spectrometry (LTQ-Orbitrap) was used to perform global differential proteomic analysis of the CSF samples. High abundance proteins including transthyretin and albumin in the CSF were first removed by adding different volume of organic solvent after overnight desalting. The remaining, lower abundance proteins were enriched into a pellet during fractionation. Reversed phase chromatography was used to further separate the proteins into sub-fractions, which were then digested with trypsin. The resulting peptide mixture was refractionated by reversed-phase liquid chromatography and analyzed using nanospray tandem mass spectrometry (LC-MS/MS).

In this study, assessment and optimization of this fractionation approach is presented. An inclusive CSF protein database containing over 200 non-redundant CSF proteins (minimum two peptide matches with acceptable score, excluding transthyretin and albumin) was established from a healthy individual under optimized methods. Among them, numerous secreted cellular and signal proteins were identified to be specifically associated with cerebrospinal fluid, such as N-terminal beta-trace protein (27 amino acid long) and Cystatin C, an important inhibitor of cysteine proteases including the cathepsins, showing unique cleavage sites in the samples. The detection of specific CSF proteins from healthy individuals reflects the physiological process that occurs in brain, which will have a significant impact on the direction to find the biomarkers by using generic proteins. Our preliminary analyses of diseased samples also revealed several proteins with significant differences between the diseased and the control groups of samples, which allow us to further pursue the significant of these findings.

Mon Poster 27: KATP Channel Knockout Causes Proteome Remodeling in Hypertensive Failing Hearts

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In systemic hypertension, knockout of the KCNJ11 gene, encoding the Kir6.2 pore of the sarcolemmal ATP-sensitive potassium (KATP) channel, predisposes to heart failure and death. To gain insight into the molecular mechanisms underlying poor outcome, the left ventricular cytosolic proteome of hypertensive Kir6.2 knockout (KO) and age- and sex-matched wild-type (WT) counterparts was profiled by comparative two-dimensional gel electrophoresis and linear ion trap quadrupole Orbitrap tandem mass spectrometry. Despite equivalent hypertension produced by chronic hyperaldosteronism, KO compared to WT ventricles displayed, out of >900 elucidated protein species, 82 altered protein spots corresponding to 115 unique identities. Bioinformatic analysis linked the primary biological function of the unmasked KATP channel-dependent protein cohort to energetic metabolism (62.6% of all proteins) followed by signaling infrastructure (37.4%) that included oxidoreductases, stress-related chaperones, and processes supporting protein degradation, transcription and translation, as well as cellular structure. Examination of protein-protein relationships authenticated the primary impact on metabolic pathways, delineating the KATP channel-dependent subproteome within a non-stochastic network. Iterative systems interrogation prioritized cardiac damage, enlargement and fibrosis pathways, exposing a maladaptive predilection in the hypertensive KO. Validating the proteomic forecast, structural and functional assessment of hypertensive KO hearts demonstrated left ventricular chamber dilation, increased cardiac and more specifically left ventricular size accompanied by interstitial fibrosis, and overt heart failure. Thus, ablation of the KCNJ11-encoded KATP channel engenders unfavorable proteomic remodeling in the hypertensive heart, diagnosing a composite molecular substrate for stress-associated channelopathy in cardiovascular disease.

Mon Poster 28: Identification of Novel Depression Biomarkers in Cerebrospinal Fluid using Antibody Microarrays

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Background: Many patients treated with interferon alpha (IFNα) become clinically depressed following treatment. The mechanism of this interferon-induced depression is not known. The biological fluids of patients with specific diseases often contain proteins that have leaked from the diseased cells. Thus, we anticipate that depression-specific protein biomarkers may be found in the cerebrospinal fluid (CSF) in this class of patients and may be indicative of biomarkers for depressed patients in general.

Hypothesis: We have hypothesized that diseased or distressed cells in the brain or the periphery of depressed subjects release low abundance cellular proteins into the circulation, which can be detected in the CSF by sensitive protein microarray platforms.

Methods: CSF was collected from Hepatitis C patients from three groups: no interferon treatment; IFNα-treated, but not depressed; and IFNα-treated and depressed. Protein biomarkers were identified using multiplex ELISAs (Pierce Searchlight Assays) and antibody microarrays. For antibody microarray analysis, CSF from each of the groups was pooled and CSF proteins were labelled using Cy5 and Cy3 fluorescent dyes. Proteins differentially expressed in depressed patients were identified using an antibody microarray that contains 507 antibodies printed in duplicate (Clontech Antibody Microarray 500). Fluorescent proteins were visualized using the Axon GenePix Microarray Scanner and differentially expressed proteins determined using GenePix software. Antibody microarray data was confirmed using reverse capture protein microarrays.

Results: We find elevated levels of TNFα, IL-6, sIL-6R and other mediators in the CSF of depressed patients. These high levels of different cytokines in the CSF are consistent with the cytokine-depression hypothesis posited by Miller and Manji. However, we have also identified approximately 40 novel, low abundance signaling proteins in the CSF of IFNα-treated, depressed patients that are statistically different from levels found in the CSF of IFNα-treated patients that did not develop depression. Most of these newly identified proteins are not commonly associated with neuropsychiatric diseases. Examples of more commonly known proteins that are decreased in depressed patients include the N-methyl-D-aspartate 2B receptor; potassium large conductance calcium-activated channel; β1-integrin; and Bcl-2-associated transcription factor (BTF). Examples of proteins that are increased in depressed patients are: nuclear transport protein 2 (NTF2); flotilin 2; heat shock transcription factor 4; TNF receptor-associated factor 4 (TRAF4) and protein kinase C-like protein 2. These data are quite unique because most of these low abundance proteins have not previously been identified in CSF.

Conclusions: The over 40 low abundance proteins that are differentially expressed in the CSF of depressed patients may be considered candidate biomarkers for depression. A CSF/serum protein signature consisting of multiple proteins is a considerably more powerful predictor of clinical state than considering individual proteins one at a time. Thus, these candidate biomarkers may constitute a depression-specific proteomic signature that can be used as a surrogate endpoint for the development of new therapeutic approaches for depression.

Acknowledgements: Support is gratefully acknowledged from NIMH.

Mon Poster 29: Chromatographic Techniques for Human Tissue and Cell Line Proteomic Profiling

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The tremendous complexity of the human proteome presents extreme analytical challenges in comprehensive analysis due to the wide dynamic range of protein concentrations. Therefore, robust sample preparation methods remain one of the most important steps in the proteome characterization workflow. Proteomic analysis of complex samples can be facilitated by protein fractionation prior to enzymatic or chemical fragmentation combined with MS-based identification of peptides.

It is important that fractionation steps minimize the number of sample manipulations to avoid inadvertent loss or modifications of low-abundant proteins. In addition, sample handling protocols have a direct effect on the accuracy of the final results, especially in validation experiments and during comparison of normal and diseased samples.

Here we present results on fractionation and identification of membrane proteins from human brain lipid rafts and HeLa cells, as well as the soluble proteins from a HeLa cell line. Human brain membrane raft proteins were fractionated by a high-recovery superficially macroporous reversed-phase (mRP) column and identified by SDS-PAGE in combination with in-gel digestion and LC/MS/MS analysis of the tryptic fragments.

Gel-free separation strategy was used for HeLa cell membrane and soluble fractions profiling. With the combination of the superficially macroporous mRP column and optimized chromatographic conditions determined by sample type, we have demonstrated enhanced peak resolution, enabled high recoveries >98%, obtained run-to-run reproducibility, and permitted higher column load tolerances. We also incorporated a novel microfluidic HPLC-Chip/MS system for tryptic peptide analysis. Integration of this technology provided ease of use and increased our productivity allowing unattended HPLC runs and MS analysis.

Through the use of optimized chromatography methods and efficient sample preparation, followed by protein identification by HPLC-chip based nano-LC/MS, we present a complete proteomic workflow for profiling human brain lipid rafts and a HeLa cell proteome.

Mon Poster 30: Proteomic Profile of Synaptosome-Associated Proteins in Spinal Dorsal Horn after Peripheral Nerve Injury

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Peripheral nerve injury leads to neuroadaptive changes of cellular signals in spinal cord that are thought to be one of central mechanisms underlying the development and maintenance of nerve injury-induced neuropathic pain. However, the global neuroadaptive changes of the complicated intracellular signals following peripheral nerve injury in the spinal cord are unclear. Using two-dimensional gel electrophoresis (2-DE) in combination with mass spectrometry, we, here, reported the global expressional changes of synaptosome-associated proteins in spinal dorsal horn after peripheral nerve injury. Neuropathic pain was induced by unilateral the fifth spinal nerve injury (SNI) in rats and evaluated by measurement of paw withdrawal threshed in response to mechanical stimuli on day 14 post-surgery. Sham surgery was performed as a control. Followed by behavioral examination, the fifth lumbar dorsal horns ipsilateral to spinal nerve injury or sham surgery were harvested and the total soluble fraction and synaptosomal fraction were isolated. The proteins derived from the synaptosomal fractions were resolved in 2-DE. We found that 30 proteins displayed different expression levels after SNI, including the proteins involved in transmission and modulation of central pain signaling, amino acid metabolism, oxidative stress, apoptosis, or energy metabolism. Among 30 identified differentially expressed proteins, 28 proteins were significantly up-regulated and 2 proteins down-regulated on day 14 post-nerve injury. Six proteins randomly chosen among these identified proteins were further validated by Western blot analysis in the synaptosomal fraction. Unexpectedly in the total soluble fraction, Western blot analysis showed that only one protein among these six proteins exhibited a significant expressional change after SNI. Our findings indicate that peripheral nerve injury not only leads to the expressional changes of dorsal horn proteins but also produces subcellular distribution changes of dorsal horn proteins in dorsal horn neurons. This suggests that peripheral nerve injury-induced expressional and/or trafficking changes of dorsal horn proteins might be involved in the central mechanism that underlies the maintenance of nerve injury-induced chronic neuropathic pain.

Mon Poster 31: Proteomic Profiling of Sera from Patients with HIV-1 Associated Dementia

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Advanced HIV-1 infection is associated with cognitive impairments (CI). However, the means to diagnose and track disease progression is often limited. The emergence of functional proteomics platforms including those recently developed in our laboratories could provide the means towards definitive diagnostic tests for HIV-1 associated cognitive impairments. 2-Dimensional Electrophoresis (2DE) with Difference Gel Electrophoresis (2-D DIGE) followed by DeCyder® 6.5 software analyses were employed to assess differentially expressed proteins in sera of HIV-1-infected people with or without CI. To facilitate analysis of low abundant proteins, we removed 12 of the most abundant proteins from sera samples. Proteins were identified by liquid chromatography mass spectrometry peptide sequencing of trypsin in-gel digested protein spots. Differential expression of identified proteins was validated by Western blot analysis. We used 14 sera samples obtained from National NeuroAIDS Tissue Consortium (NNTC). In each patient group a diagnosis was made for HIV-1 associated dementia (HAD) based on neuropsychological and clinical grounds.

In sera samples, 29 protein spots were identified by DeCyder® analysis as significantly different and proteins were positively identified in 17 of these spots. Ceruloplasmin, afamin and a broad range of components of complement system were identified. Differential expression of ceruloplasmin and afamin was validated by Western blot tests. Box plots of statistical analysis derived from Western blot images quantified in Image Quant software showed a normal distribution of these two proteins with statistical significance ($p<0.05$) of protein level differences between infected patients with and without HAD for respective proteins. In conclusion we posit that disease onset and progression could be made on joint sets of biomarkers (as were identified above) in which levels will be measured and combined with other neuropsychological and behavioral tests. These and other proteins are currently being developed using a larger patient cohort.

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Mon Poster 32: Signaling Pathways in Down Syndrome Platelets

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Down syndrome (DS) is a major genetic cause of mental retardation. The development of the segmental trisomy mouse model, Ts65Dn, allowed investigation of the molecular and developmental effects associated with DS. Abnormalities in signaling pathways and its impact on hippocampal plasticity have been reported in Ts65Dn mice; however, these results have never been verified in individuals with DS. Hematological abnormalities in DS and Ts65Dn mice have been found, further suggesting impairment of platelet metabolism and function. Investigations into signaling pathways of platelets in neurodegenerative disorders like Alzheimer disease have demonstrated that certain properties common to central nervous system (CNS) neurons also exist in blood platelets. Platelets, therefore, can provide a unique "window" into signaling pathway disturbances that impact CNS function. For this study, we examined the signaling proteome of platelets isolated from individuals with DS and Ts65Dn mouse which both exhibit deficits in hippocampal function.

Blood was drawn under IRB approved human use protocols (NNMC, CNMC and USUHS) from children with DS and age-matched controls and treated with prostacyclin to inhibit platelet activation. None of DS subjects has been diagnosed with acute megakaryocytic leukemia. Platelets were homogenized in the presence of protease and phosphatase inhibitors and either labeled with Cy3 and Cy5 for antibody microarrays or left unlabeled for Western blotting and reverse capture arrays. Reverse capture arrays were printed on a Genetix Mini array printer (Boston, MA). Comparative studies were conducted on the Ts65Dn mice.

Screens of the platelet proteome were conducted using commercial antibody arrays from Clontech (Mountain View, CA) and Labvision (Fremont, CA). Initial analysis showed that 29 proteins displayed decreased expression ranging from 38- to 5–fold and 77 proteins had increased expression ranging from 21- to 2.5-fold in the DS platelets when compared to the controls. Reverse capture arrays composed of platelet extracts from 3 DS patients (2 females and 1 males), and 6 controls (4 females and 2 males), were probed with antibodies against Ras, Akt, pAkt, AMPKα, JAK1, caspase3, IKKγ, PKCα and TNFR2. Preliminary results indicate hyperphosphorylation of Thr308 on Akt in DS platelets. Western blots were performed to substantiate the changes observed in the array assays. We found significant ($p =0.02$) reduction in bleeding time in Ts65Dn mice compared to diploid littermates (not reported in DS). We also found that Akt is hyperphosphorylated in Ts65Dn mouse platelets.

These results suggest that the PI3K pathway in DS and Ts65Dn platelets displays a deficit similar to the one detected in Ts65Dn hippocampus. A more detailed quantitative proteomic approach to investigate other signaling pathways is underway. Platelets express many of the molecular neuronal markers and exhibit some physiological and molecular properties that are characteristic for hippocampal neurons. Therefore, these results suggest that defective signaling pathways may underlie functional deficits in reported DS and Ts65Dn CNS.

Mon Poster 33: Identifying the Subproteome of Kinetically Stable Proteins via Diagonal 2D-SDS PAGE and Application 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 allow proteins to maintain activity under harsh conditions and to preserve the structure of proteins that are prone to misfolding. The biological and pathological significance of KS remains poorly understood due to the lack of simple experimental methods to identify this property, and its infrequent occurrence in proteins. 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 transthyretin (TTR) as a KSP. 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-PAGEs of samples containing WT TTR or FAP-related TTR mutants with compromised KS have shown clear differences in their migration pattern. Thus, our results suggest that D2D/SDS-PAGE can be used to screen human plasma for KSP, and may have diagnostic potential for diseases pathologically linked to the abnormal gain or lost of protein kinetic stability.

Mon Poster 34: Proteomic Analysis Identified Molecular Signatures for Diabetes Mellitus Associated Erectile Dysfunction

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Protein expression profiles in rat corpora smooth muscle were compared between animal models of STZ-induced diabetes mellitus (STZ-DM) and age matched controls (AMC) at one week and two months. At each time point, protein samples from four STZ-DM and four AMC rat corpora tissues were prepared independently and analyzed together across multiple DIGE gels using pooled internal standard sample to quantify expression changes with statistical confidence. A total of 170 spots were differential expressed among the four experimental groups. Unsupervised principal component analysis on expression data yielded four distinct expression patterns; which demonstrated high reproducibility between replicate samples but variability among the experimental groups. A subsequent mass spectrometry analysis of the 170 spots identified a total of 64 unique proteins. Of these proteins about 40 exhibited significant changes one week after confirmation of the STZ-induced hyperglycemia. A significant numbers of these proteins except about 12 proteins, that showed temporal changes during the initiation of the disease, and additional 22 proteins showed differential expression after two months. A network analysis of these proteins using Metacore ™ identified a hypothetical induction of transcriptional factors that are too low to be detected by 2D-DIGE. The proteins that are down regulated with diabetes include isoforms of collages, which are precursor to fibrils forming collagen type 1, hsp47 that assists and mediates the proper folding of procollagen, type I, alpha 1 and procollagen, type I, alpha 2 and proteins involved in muscle remodeling (eg. LIM protein). On the other hand, the proteins that are up regulated include proteins involved in oxidative stress (eg. Glutathione peroxidase 3), protein that neutralize the biological activity of nerve growth factor (eg. Anti-NGF 30), and proteins involved in inflammatory response (eg Fga, Fgb, Fgg, ApoA1, and ApoA4). Taken together, our result broadens the ranges of candidate proteins mediated by diabetes that may have accounted for the functional deficits known to occur in erectile dysfunction.

Mon Poster 35: Improved Fractionation and Distribution of Labeled Peptides in a Complex Sample

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The fractionation of complex proteomic samples is critical to successful protein characterization and quantitation using mass spectrometry. Such samples often consist of tens or hundreds of thousands of peptides over a very wide dynamic range. Currently, the most widely used method of peptide fractionation is strong cation exchange (SCX) chromatography followed by reverse phase separation of each SCX fraction prior to MS analysis. However, SCX exhibits less than optimal peptide resolution resulting in the same peptide appearing in many fractions. Additionally, SCX fractionation typically results in a very uneven (front loaded) distribution of peptides amongst the fractions. These issues reduce the achieved chromatographic separation, increasing the redundant analysis of the peptides and further complicating the mass spectrum. A different first dimension separation that does not exhibit such problems is therefore highly desirable.

We investigated the use of solution phase pI separation as the first dimension of a 2-D workflow and compared it with the standard SCX/reverse phase workflow for a complex cell lysate spiked with standard proteins at various concentrations. The comparison focused on the peptide distribution across fractions, number of proteins/peptides identified and improved quantitation. These results will be described in detail.

Mon Poster 36: Method Optimization for Label-Free Quantitative Proteomics of Complex Biological Samples Analyzed by Nanoflow LC-MS

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One of the goals of proteomics is to measure the up- or down-regulation of protein expression of the entire proteome as the cell system is perturbed. Label-free protein quantitation approaches facilitate this goal by determining relative protein abundances directly from datasets obtained using shot-gun proteomics without applying labeling techniques, thus circumventing the time-consuming labeling steps and selective peptide detection associated with labeling reaction schemes. In this study, a label free approach was tested using data acquired from a synthetic model in which the quantities of a standard protein were controlled. Specifically, a bovine serum albumin (BSA) digestion was added to the yeast digestates at nine different concentrations (0, 1, 2, 5, 10, 50, 100, 500, 1000 fmol/µL). These samples were then subjected to HPLC separation followed by LTQ-FT MS detection. BSA ratios within this complex protein mixture were measured using various label free software platforms including a commercially available software SIEVE (Statistical Iterative Exploratory Visualization Environment), a free-distributed software QUOIL (QUantification withOut Isotope Labeling), and an in-house developed software QGUI (QuantitationGUI). Label-free proteomics strategies were investigated in respect of reproducibility, sensitivity and sample throughput. Application of these software packages to data from BSA-spiked yeast study strongly indicate that the label-free method for quantitative expression profiling presented herein is an alternative to stable isotope based quantitation of peptide data sets generated by nanoflow LC-MS differentially labeled peptide mixtures.

Mon Poster 37: Cryogenic Fourier Transform Mass Spectrometry for Proteomic Applications

Peter B. OConnor

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The new Cryogenic MALDI-FTICR mass spectrometer involves a unique and practical design which yields large, combined advantages in instrument performance. Operating the ICR cell in the cold bore of the magnet allows 1) use of narrow bore magnets for increased field at decreased cost, 2) cryopumping operation at

the cell for minimum base pressure, and 3) low noise environment for a cryogenically cooled preamplifier for maximum signal/noise.

Designing, building, and testing such an instrument, understandably, involves new challenges. Specifically, existing FTICR instrument designs must be modified for cryogenic operation - taking into account a number of unusual problems such as the thermal contraction of ion optics, even down to the connections that are used to wire the cell. Many such new design issues arose during construction and had to be overcome.

Also, the construction of a preamplifier that can function well at 4K is a non-trivial problem because most silicon-based transistors and chips do not function below ~50K. A new, differential FET-based design using GaAs MESFETs was designed and constructed and tested. The performance of this circuit will be shown.

The first instrument of this type was recently constructed and has yielded first data. The instrument design, the performance advantages, and the up-to-date spectra from this instrument will be shown.

Mon Poster 38: Simplifying the Hunt for Optimal SRM Transitions: Utilizing Discovery Data to Expedite Targeted Peptide Quantitation Methods

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Greater 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. The typical workflow involves first performing discovery based experiments to identify protein expression levels that are confidently changing between a control and treated samples and generate product ion information used to sequence the precursor peptide. The difficulty arises in transferring discovery based methods directly over to validated quantitation methods since each is generally performed on separate mass spectral platforms. Low confidence has been placed on relating relative product ion abundance obtained from ion trap CID to that observed using a triple quadrupole mass spectrometer due to the difference in ion activation mechanisms and the timescale of the excitation. Thus, the only information transferred from one method to the other is protein id, peptide sequence, and the most abundant charge state resulting in further method development to complete the SRM assay. Common approaches to determine SRM transitions are based on a set of accepted rules to determine the best possible ion pair(s), which are then searched against the matrix database to determine the uniqueness of each mass pair. We contend that the relative abundance of product ions originating from ion trap CID can be a used to directly assign the most sensitive ion pairs for the targeted SRM methods.

We will present direct comparison of relative product ion abundance measurements for 100 plasma peptides between an ion trap and a triple quadrupole mass spectrometer. The selected peptides are broken down into sequence length ranging from 7 to 15 residues to determine consistency across the typical biomarker properties. Success rates for matching the most abundant product ions from each method to those predicted will be consolidated and reported.

Mon Poster 39: Identifying Peptides with Higher Order Charge States: Why Bigger is Better

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Analysis of peptide mixtures via protein digests using ion trap instrumentation has been a mainstay for shotgun proteomics. Probability based database searching algorithms from tandem mass spectrometry data acquired at unit mass resolution are effective for the identification of peptides possessing 1+, 2+ and 3+ charge-states. However, information from larger peptides with higher order charge states (*i.e.*, $\geq$ 4+) are typically missed due to the complexity of the tandem mass spectra (*e.g.*, poor identification of fragment ion charge state), incomplete fragmentation of molecular ions, and the "general pitfall" that many search engines simply fail to either adequately identify or score these types of peptides. For example, SEQUEST results scored from higher order charge state peptides generally lack the required distribution of discriminant scores in PeptideProphet to be statistically validated.

Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry results in accurate mass measurement where mass deviations of $\pm$2ppm are routine. With the advent of the linear ion trap hybrid instruments (*i.e.*, LTQ-FT, LTQ-OT), accurate mass measurement of both MS and tandem MS events are obtainable with duty-cycles within typical chromatographic time scales. Data dependent selection of peptide ions possessing higher order charge-states followed by tandem MS analysis at high resolution and mass accuracy represents a competitive and complementary strategy for peptide identification. Such an approach consists of charge-state driven data acquisition, accurate mass measurement of both precursor and fragment ions, a deconvolution processing step, and sequence database searching using Phenyx (GeneBio), a statistical-based search engine that utilizes high mass accuracy in peptide scoring.

Integration of this approach into a strategy for characterizing complex peptide mixtures, resulting in the identification of larger peptides, will be presented. Examples will include identification of atypical post-translational modifications (*i.e.*, protein-drug conjugates) and chemically cross-linked peptides (*i.e.*, to model protein-protein interactions). Data were acquired on LTQ-based Thermo-Fisher hybrid mass spectrometers.

Mon Poster 40: MALDI Quadrupole Orthogonal Acceleration-TOF Mass Spectrometry Enhanced with Ion Mobility Spectrometry for Tissue Imaging

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The application of MALDI mass spectrometers to determine the spatial distribution of endogenous and exogenous chemical species in tissue is a rapidly developing area of research. A promising branch of this technique is the study of the distribution of metabolites. MALDI imaging has great potential by offering complementary information to traditional techniques.
The two main instrumental challenges for the mass spectrometric analysis of these types of samples are sensitivity and specificity, i.e. how well the compound of interest can be distinguished from background ions.

The efficient detection of small molecules in a biological matrix is often difficult owing to competing background ions from the tissue. This effect is exacerbated in the case of MALDI imaging by the presence of the matrix. We introduce high definition mass spectrometry (HDMS) combined with MALDI imaging. HDMS offers a new dimension of separation, combining high efficiency ion mobility separation (IMS) with orthogonal TOF mass spectrometry. Separation of ions according to size, shape and charge state is possible. Using this technique it is possible to separate different compound classes.

The sample studied was a thin section of rat kidney. A 12µm section was produced using a cryotome and deposited on thick aluminium foil. An airbrush was used to apply several coats of α-cyano-4-hydroxycinnamic acid matrix evenly to the sample. The sample was mounted on a target plate. The area to be imaged was selected and data were acquired on a MALDI Synapt HDMS system operated in mobility Tof mode. After acquisition HDMS data evaluation was performed. Data were converted into Analyze file format and image analysis was performed using BioMap.

We present data demonstrating the separation of nominally isobaric species using IMS in an imaging experiment, producing distinct images for the separated compounds.

Mon Poster 41: Cell Surface Glycoproteomics: Finding New Biomarkers

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Cell surface glycoproteins are of particular interest as diagnostic markers due to their ability to sense and respond to their environment and their accessibility to antibodies. To enhance the discovery of specific protein markers of differentiation and potential therapeutic targets, a recently developed proteomic method is being employed to selectively identify cell surface N-linked glycoproteins.

Cell surface capturing (CSC) is a recently developed method that circumvents traditional challenges encountered in cell surface protein preparations by specifically labeling the extracellular glycan structure on intact cells, allowing for subsequent capture and identification of the glycopeptides with high specificity without contamination from intracellular membranes. Importantly, in addition to providing identification of the cell surface glycoprotein, the CSC technology also provides information about glycosylation site occupancy. Knowledge of site occupancy aids in determining the orientation of the protein in the plasma membrane and is useful to consider for downstream antigen selection and antibody design. Using custom analysis software to process the CSC data, the search for bona fide cell surface markers is remarkably fast.

We applied the CSC technology to study in vitro differentiation of stem cell-derived cardiac and skeletal mesodermal lineages. To date, the cell surface glycoproteins of undifferentiated mouse myoblasts have been cataloged, revealing more than 100 cell surface glycoproteins. Interestingly, for more than half of these proteins, the CSC method confirmed sites of glycosylation that have not been previously reported. Multiple sphingosine 1-phosphate (S1P) receptors have been identified using CSC. Thus, as S1P is an important signaling molecule in myoblast differentiation, the effect of S1P on the cell surface of undifferentiated myoblasts is currently being investigated using CSC in combination with quantitative iTRAQ measurements.

In a separate study, the CSC technology in conjunction with SILAC is being used to study the effects of hypoxia-induced mitogenic factor (HIMF) on pulmonary smooth muscle cells. HIMF is a cytokine for which the cell surface receptor and mechanism of action are presently unknown. To date, more than 80 cell surface glycoproteins have been identified in smooth muscle cells. Overall, typical experiments to date capture N-linked glycopeptides with greater than 80% specificity without contaminants from nuclear and mitochondrial membranes. The list of >200 cell surface glycoproteins identified in the myoblast and smooth muscle cells includes 113 CD molecules and is composed of proteins containing a range of 1-14 transmembrane domains.

Quantitative mass spectrometry of the cell surface glycoproteome using the CSC technology allows for the analysis of how the cell surface changes in response to environmental stimulation and differentiation, for example. Combining the CSC technology with downstream validation tools such as immunohistochemistry or FACS is anticipated to provide a fruitful method for defining cell surface markers that can be used in a wide range of biological applications, including stem cell research.

Mon Poster 42: Analysis of IgA1 *O*-glycosylation in IgA Nephrophathy

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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. To address this question we have obtained accurate mass profiles of IgA1 *O*-glycosylation isoforms from serum, serum isolated immune complexes, and IgAN patient derived B-cell lines by use of FT-ICR mass spectrometry. These profiles allow an assessment of the heterogeneity of IgA1 *O*-glycans isolated from different sources within a single patient as compared to healthy controls. We have identified IgA1 *O*-glycopeptides with as many as three *O*-glycan chains lacking Gal as well as other species with up to five *O*-glycan chains which do not lack Gal. Additionally, by use of electron capture dissociation (ECD) and electron transfer dissociation (ETD) tandem mass spectrometry (MS/MS) we can successfully map the sites of *O*-glycan attachment of individual IgA1 *O*-glycopeptides. The two fragmentation techniques produce similar if not identical *c* and *z* type fragmentation patterns that leave the *O*-glycan chains intact and allow for unambiguous assignment of sites of *O*-glycosylation. ETD fragmentation in a two dimensional linear quadrupole ion trap (LTQ XL) does allow for the further interrogation of individual charge-reduced species and fragment ions by MS^n techniques. This allows for confirmation of low abundance fragments as well as the ability to unmask other fragments which may be covered by more dominant charged-reduced species and signature ECD/ETD amino acid side-chain loss fragments. The combination of accurate mass profiles plus ECD/ETD fragmentation provides a comprehensive analysis of IgA1. *O*-glycosylation towards the goal of understanding the pathogenesis of the most predominant form of glomerulonephritis in the world.

Mon Poster 43: Express Bioinformatic Analysis of Sialic Acid-Containing Glycopeptides from Early-Onset Diabetes Type 1 Patients

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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 gene ontology 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 found in an N-linked glycan motif (NxS or NxT).

Statistical GO analysis with ProteinCenter™ showed a significant increase 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.

Mon Poster 44: Absolute Quantification of Plasma Glycoproteins by Multiple Reaction Monitoring Mass Spectrometry of Proteotypic Peptides in Hypotheis-Driven Biomarker Discovery

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Monitoring the plasma glycoproteome is a promising strategy for identifying biomarker candidates. With the wealth of available candidates in the literature, it becomes feasible to use a targeted approach to biomarker discovery and focus our effort on potentially interesting proteins. Multiple Reaction Monitoring (MRM) is a very powerful technique for chemical and biological analysis. The use of a tandem-in-space mass spectrometer offers two levels of mass selection and unparalleled sensitivity. The nature of MRM being hypothesis-driven requires that one knows the analytes, in order to interrogate the sample. Knowledge of the mass of the precursor and the most intense fragments is essential to select and optimize transitions that are the most sensitive and specific.

Glycoproteins known to be differentially expressed in a disease state were selected by mining of scientific literature and microarray data sources, namely Medgene and the Gene Expression Omnibus at NCBI. Proteotypic glycopeptides from those proteins were selected by searching the Unipep database, a large repository of glycopeptides identified by mass spectrometry. The proteotypic peptides selected must have been identified at least once in a tandem mass spectrometry experiment to be included. The candidate peptide list was then manually curated to select the best peptides, before being BLAST-searched to confirm the uniqueness and accuracy of the sequence. For absolute quantification, reference peptides with heavy lysine and arginine were purchased (Sigma, Saint Louis, MO) for all 70 peptides in the final list. MRM transistions were optimized using our own TIQAM software (Targeted Identification for Quantative Analysis by MRM) to find the 3 more sensitive ones for each peptide.

To validate this experimental approach, human plasma was obtained from breast cancer patients, as well as from healthy controls. Plasma-derived glycoprotiens were first captured to solid-phase hydrazide beads, and (deglycosylated) N-linked peptides recovered by sequential digestion in situ with trypsin and PNGase F. Recovered peptides were then separated on a Tempo (Applied Biosystems, Foster City, CA) nano HPLC before being analyzed by MRM mass spectrometry on a QTRAP 4000 mass spectrometer (Applied Biosystems). The ratios of the light vs. heavy peptides was calculated and averaged for each peptide pair using Multiquant software (Applied Biosystems), and absolute concentrations of endogenous peptides calculated for both normal and diseased plasma samples.

Mon Poster 45: Identifying Cell Surface Glycoproteins using Hydrazide Chemistry in Combination with 2D-LC/ESI-MS/MS

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Introduction

Cell surface proteins are important therapeutic targets and have been exploited for targeted treatment in several diseases including cancer. Thus, identification of cell surface proteins as therapeutic targets has been a prime area of interest in the proteomics field. However, technical difficulties have hampered efforts to effectively isolate and identify cell surface proteins. Many cell surface proteins are known to be glycosylated. Therefore, a strategy integrating periodate oxidation and hydrazide resin coupling into a proteomics approach for the identification of cell surface proteins would seem to have merit. In this study, we oxidized the carbohydrates on cell surface membrane glycoproteins with periodate, and enriched them via coupling to hydrazide resin, followed by identification of trypsin-released peptides and PNGaseF released, N-linked glycopeptides using 2D-LC/ESI-MS/MS (MudPIT).

Methods

HeLa cells were grown in culture, oxidized with periodate, lysed using a Tris buffered saline lysis solution containing a non-ionic detergent and protease inhibitors. The cells were scraped from the plates using a rubber policeman. The lysate was applied to the hydrazide column to isolate glycoproteins, and a series of washes were used to eliminate non-specifically bound proteins. Proteins retained on the hydrazide column were digested with trypsin and the tryptic peptides collected. The glycopeptides bound to the hydrazide column were released using PNGaseF and this fraction was collected. Fractions contained tryptic peptides and PNGaseF released glycopeptides were analyzed by MudPIT. The proteins were identified by Sequest and MASCOT programs.

Preliminary Results

In the development of this methodology, we observed that identification of both the tryptic digestion products and the PNGaseF released glycoproteins increased the number of glycoproteins detected by MS analysis. Our initial results demonstrate that the periodate/hydrazide column protocol efficiently enriches cell surface membrane proteins from cultured cells. The composition of the peptides released from the hydrazide column by trypsin demonstrated that the column washing steps effectively separated cell surface membrane glycoproteins from other cellular proteins present in the total cell lysate. Information on the sites of N-linked glycosylation was obtained from the peptides released from the hydrazide column by PNGaseF. Protein database searching programs incorporated into the proteomics approach provide information on cellular location of the identified proteins. The incorporation of a multidimensional liquid chromatography further improved the sensitivity and selectivity of the method. The application of the optimized protocol will be presented. In addition, the results obtained from the periodate/hydrazide column protocol will be compared to those obtained using lectin chromatography.

Mon Poster 46: The Discovery of Glyco-biomarkers in Breast Cancer Plasma Samples

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Glycosylation is one of the most structurally and functionally elaborate post-translational modification of proteins. Cancerous cells usually pesent a unique repertoire of glycans. Through fine-tuning and modulating protein structure and function, glycan motifs regulate various biological events. Thereby, a loss of this fine-tuning ability results in disease states. It has been recognized that glycan alteration is related to tumor cell proliferation, invasion, metastasis and angiogenesis. Although a number of glycoprotein and associated glycan changes have been identified in cancer, there has been little adoption of glycan biomarkers for cancer diagnosis due to the technical limitations. Because the large dynamic range and the extensive charge heterogeneity of glycoproteins in plasma, it is challenging to characterize glycan pattern of glycoproteins in a complex sample. A glycoproteomics study requires sample pre-fractionation steps and sensitive analytical techniques. However, performing too extensive pre-fractionation on a single plasma sample into multiple fractions is not feasible for a large clinical study. In the first stage of this research, we aim to detect glycoprotein differences in plasma samples between breast cancer and control subjects. The discovery of candidate glycoprotein biomarkers relies on an optimized multi-step protocol, including immunodepletion of abundant proteins, multi-lectin affinity chromatography (M-LAC), reversed phase chromatography, and mass spectrometry. In addition, a rapid method for monitoring glycosylation changes has been developed. This methodology is based on the comparison of plasma samples by SDS-PAGE, combined with a glycoprotein staining technique. The second stage is to investigate specific glycan changes associated with the candidate glycoproteins. Detail information about the glycosylation sites, glycan structure and micro heterogeneity of the potential glycoprotein biomarkers is being pursued using a high resolution MS technique, such as Fourier transform mass spectrometry (FT – MS) combined with collision induced dissociation (CID) and electron transfer dissociation (ETD). Additionally, glycan cleavage of the

candidate glycoproteins, followed by HPLC profiles and MALDI – TOF structural analysis will also be discussed.

Mon Poster 47: An IPG Mudpit Workflow for MS Analysis of Protein Complexes Purified by Blue Native Gels

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Many cellular processes - transcription, degradation, ligand-gated ion channel that regulates signaling across the neuronal synaptic junction such as acetyl choline receptors and vesicle budding for example, - are executed by protein aggregates such as the COPII transport vesicles. Shotgun proteomics offer significant advantages over gel-based techniques for hydrophobic protein complexes. However additional physical information, such as isoelectric point (pI) can enhance the confidence of assignments. It is also critical to optimize Native separations to study the activity of complexes with high fidelity. BlueNative PAGE permits efficient separation of protein complexes; often functionally bioactive. Recently, protocols were introduced to analyze the subunits of protein complexes directly from BlueNative gel. Here, we illustrate the preparation conditions for accurate and comprehensive identification complex subunits.

Nicotinic Acetylcholine Receptor complex from Torpedo californic (nAChR) was the generous gift of Dr. Michael Blanton and its isolation has been described elsewhere. The nAChR was precipitated using acetone and then reconstituted in Invitrosol LCMS followed by trypsin digestion. Bovine mitochondria and yeast Sec 23/24-Sar1 pre-budding complexes were isolated in TESS buffer were stored at –80°C in 250uL aliquots. Before loading onto a NativePAGE™ gel, NativePAGE™ 5% G-250 was added to a final concentration one-fourth to one-tenth that of the detergent. Bands were cut directly from Blue Native gels and were de-stained, dehydrated and digested directly in the gel. The digested peptides focused on commercial IPG gels. After cutting the IPG strip into 8 pieces, the focused peptides were extracted and concentrated. Extracted peptides were analyzed by LC-MS with and without prior focusing on Zoom™ Immobilized pH Gradient (IPG) strips. Data were processed with a combination of Mascot Server 2.1 and Scaffold 1.5.

The recovery of digested peptides following IPG focusing was exceptional and thus we were able to analyze the same samples in parallel using the Q-TOF LC-ESI/MS and 4700 MALDI-TOF/TOF.
On average, our sample preparation protocols yielded 1.5 times greater sequence coverage for nAChR than conventional “in-gel” or solution proteolysis protocols. In preliminary work, we also optimized direct in BlueNative digestion applied to bovine mitochondrial complexes IV and V. The peptide recovery from direct in BlueNative trypsin digestion was substantially higher than alternative second dimension SDS gel electrophoresis workflows. By coupling a second IEF dimension, we extended the peak capacity further during the analysis of Bovine Complex I. We identified 36 of 45 reported subunits in a single preparation. We applied the same paradigm to identify structural partners of the COPII coated transport vesicles extracted from Saccharomyces cerevisiae.

Mon Poster 48: A Collection of Novel Isotopically-Coded Crosslinkers for Structural Proteomics

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Crosslinking combined with MS is a promising approach for structural proteomics. However, identification of crosslinked peptides derived from proteolytically digested crosslinked protein complexes by MS is still challenging. The combinatorial nature of crosslinked peptides complicates their unambiguous assignment. Moreover, specific content of crosslinked peptides is usually low, which makes detection of the crosslinks somewhat problematic. Recently, we described a set of novel homo-bifunctional amine-reactive and isotopically-coded crosslinkers designed to address these issues. Here we present new additions to our collection and postulate the required features for a crosslinking reagent to be suitable for structural proteomics applications. Our collection consists of isotopically-coded BS3, DTSSP, EGSS, SDPS, TEABS, DNBDPS, BiPS and DTTDPS crosslinkers.

BS3-D12 is a classical, non-cleavable crosslinker reagent. DTSSP-D8 and EGSS-D12 are classical, chemically cleavable by DTT and bases, respectively. A new feature of DTSSP is CID cleavage of its linker region. Cleavage produces fragment ions of individual peptides, constituting interpeptide crosslinks, each uniquely labeled with four deuterium atoms. SDPS-D8 is a novel crosslinker containing a sulfonyl group in the linker region. CID cleavage of the bond adjacent to the sulfonyl group also produces the two halves of the crosslink, which are still uniquely labeled with four deuterium atoms. TEABS-D12 is a crosslinker chemically cleavable by bases and affinity purifyable on avidin beads. Cleavage with ammonia produces four pieces of the crosslink including two peptide-containing parts, which are isotopically labeled with four deuterium atoms of the succinic acid moiety of the crosslinker. DNBDPS-D8 can be affinity purified by anti-dinitrophenyl antibodies. A recently discovered feature of this crosslinker is a specific cleavage reaction of the linker region with DTT under 100oC. Cleaved halves of the crosslinker remain labeled with four deuterium atoms. BiPS-D8 is a fluorescent crosslinker that is photo-cleavable under MALDI conditions. Partial photo-cleavage results in mass spectrum containing peaks corresponding to an uncleaved crosslink labeled with eight deuterium atoms and individual peptides labeled with four deuterium atoms. DTTDPS-D8 is a novel crosslinker containing a diol group in the linker region. It is cleavable with periodate to produce individual peptides of the crosslink. Also the diol group constitutes a minimal affinity group, which can be employed for affinity enrichment of the crosslinks with immobilized borate chromatographic supports.

Thus, three key features of the crosslinker design allow us to address challenges related to the application of crosslinking in structural proteomics. Isotopic coding enables univocal detection of the crosslinker-containing peptides in a mass spectrum. Chemical, photo- or CID cleavage of the linker region produces specifically isotopically labeled halves of the crosslinks, allowing quick distinguishing of different types of crosslinks, such as dead-end, intra- and inter-peptide crosslinks and, most importantly, provides mass and sequencing information for the individual peptides, which constitute the crosslink. Affinity enrichment with immobilized anti-crosslinker antibodies, avidin or borate enriches specific content of the crosslinks, simplifies the MS spectrum and reduces suppression effects of interfering peptides. Combination of these features in individual crosslinking reagent structures makes them enviable for structural proteomics applications.

Mon Poster 49: Detection of Low Abundance Tissue Leakage Serum Proteins by ProteoMiner Technology

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The biggest challenge in human serum proteomics is to detect the low abundance proteins, especially the proteins from tissue leakage which might be the ideal candidates for disease biomarkers. ProteoMiner Technology is a protein separation tool that can remove most of the high abundance proteins in serum and allow the detection of low abundance proteins. To determine whether ProteoMiner can facilitate the detection of tissue leakage proteins in serum, we mixed 12 commercially available proteins at the concentrations reported in normal human serum. The proteins included albumin, immunoglobins, tissue leakage proteins such as myelin basic protein, and interleukins. The protein sample was fractionated by ProteoMiner technology and the proteins in the bound fraction were analyzed by 2-D gel electrophoresis. This study demonstrated the capability of ProteoMiner technology to enrich the low abundance tissue leakage proteins in serum for proteomics study.

Mon Poster 50: Real-Time Bimolecular Interactions Studies using Self-Assembled Protein Microarrays and Surface Plasmon Resonance Imaging

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In post-genome era having sequenced the human genome, one of the most important pursuits is to understand the function of gene-expressed proteins. Despite immense progress in molecular biology and genetics, only a small fraction of the proteome is understood at the biochemical level. Systems biology and proteomics strive to create detailed predictive models for molecular pathways based upon the quantitative behavior of proteins. Understanding these dynamic networks provides clues into the consequence of aberrant interactions and why they lead to disease like cancer. However, collecting biochemical data about protein behavior at scale has been daunting. Historically, methods capable of collecting quantitative data on biochemical interactions could only be used for one or a few proteins at the time. Here, we shoe the combination of two technologies that together could lead to the ability to measure binding events in real time for many protein interactions simultaneously using a label free technology. This could revolutionize the study of protein interactions networks by enabling quantitative comparisons of binding affinities across many molecular species, as well determining the kinetics rates of binding and release.

The first technology is the Protein microarrays have demonstrated its utility by characterizing biochemical process and providing information which is not obtainable by gene microarrays. Nucleic Acids Programmable Protein Microarrays (NAPPA) developed at Harvard Institute of Proteomics (HIP) revolution protein microarrays by replacing complex process of spotting purified proteins with simpler process of spotting plasmid DNA and simultaneous transcription/translation of all the genes in situ at the time of the assay. The second one is a Surface Plasmon Resonance device that has been adapted to multiplexed binding events from a planar surface that is compatible with the protein microarray. In addition this technique is sensitive, accurate and provides real-time date for both the equilibrium and the interaction kinetics. The project is focused at coupling NAPPA protein array technology to multiplexed real-time label-free SPR-based detection system (which allows thousands of binding events to be monitored in real-time without any loss in sensitivity). By SPRi we are able to detect binary interactions using NAPPA format.

Among gene collections generated in our lab, there are unique cDNA clone collections representing more than 500 human kinases and over 250 Human-Protein-coupled-Receptors (GPCRs) proteins. Protein kinases and GPCRs are know to play key processes including for example metabolism, transcription, cell cycle progression, cytoskeletal rearrangement, cell movement and apoptosis. An array displaying more than 700 proteins was probed with purified ABL1 kinase. Known interaction partners of Abl1, such as CDK5, ATM and ABL2 were readily detected. Recently, it was demonstrated the ability to detect phosphorylation events in a study of autophosphorylation using human kinases expressed with the NAPPA technology, for example: Abl1, FES, TNK1, Abl2. The combination of both technologies allow us to generate detailed kinetic data of interactions pathways.

Mon Poster 51: In-Depth Analysis of the *Arabidopsis thaliana* Proteome using Electron Transfer Dissociation

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A common technique to analyze proteins by mass spectrometry is to digest the proteins into peptides with an enzyme, separate the peptides by reversed phase chromatography, and introduce the peptides into a mass spectrometer by electrospray ionization (ESI) to obtain sequence information by tandem mass spectrometry (MS/MS). The enzyme most widely utilized for the digest is the serine protease, which cleaves C-terminal to Arg or Lys. The obtained peptides ionize via ESI to low charge states (generally +3 or less, with the +1 and +2 charge states most favored), which are optimal for collision-induced dissociation (CID) mass spectrometry. While CID MS/MS is a very powerful technique, it does impose some limitations on investigators in their efforts to identify proteins and understand their biological function.

Electron transfer dissociation (ETD) is a new method to fragment peptides that utilizes ion/ion chemistry [1,2,3]. ETD fragments peptides by transferring an electron from a radical anion to a protonated peptide. This induces fragmentation of the peptide backbone, causing cleavage of the Cα-N bond. This creates complementary c and z-type ions instead of the typical b and y-type ions observed in CID. Best ETD spectra are obtained from peptides detected at m/z 300-900 with a charge state of 3 or greater.

Here we present the utility of electron transfer dissociation (ETD) mass spectrometry for sequence analysis of the proteome of Arabidopsis thaliana. The availability of both a complete genome sequence and high quality genome annotation makes Arabidopsis an ideal system to develop new proteomics technologies. Furthermore, Arabidopsis proteomics research may serve as a paradigm for other plant proteomics research projects and the advancement of plant proteomics in general.

Due to the complexity of a dynamic proteome, different approaches have to be combined to measure protein expression and dynamics, stress- and developmental responses, posttranslational protein modifications and protein interaction. So far, about 700 proteins from isolated Arabidopsis thaliana chloroplasts have been identified by mass spectrometry whereas the predicted number of proteins targeted to the plastid is approximately 3000. Here we present a successful approach for increasing the number of identified proteins using Electron Transfer Dissociation experiments of a serine protease digested whole soluble fraction as well as gel-separated subfractions from isolated chloroplast stroma.

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Mon Poster 52: Accurate Mass LC/MS as a Tool for Label Free Quantitative Proteome Studies

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Recent improvements in ESI-QTOF instrumentation make a novel MS-based approach to label-free protein quantification feasible. This approach could be a significant complement to current quantification methods, like 2D gel, label, or MRM based methods.The scope of this presentation is an evaluation of accurate mass LC/MS for label free quantification involving a nano-HPLC (Ultimate 3000, Dionex) connected to an ESI-QTOF (micrOTOF-Q, Bruker Daltonics). Our approach enables hypothesis driven proteome analysis and discovery of potential markers resulting from supervised statistical analysis. While quantification is purely obtained from MS data, identification is done as subsequent step involving a targeted approach to obtain sequence information of relevant peptides and proteins from MS/MS data. Using newly developed software we analysed the reproducibility of peptide signals between different LC runs in respect of occurrence, retention time, chromatographic peak with, mass accuracy and peak intensity or area. As a suitable sample we chose human lung carcinoma cell line A549 spiked with a set of 12 standard proteins. For quantification we used two samples and for each standard protein the concentration ratio between the two samples was varied.

In a targeted approach, using the peptide masses from a theoretical digest of the known standard proteins, we extracted accurate mass compound data from the LC-MS datasets. Suitable bioinformatics tools enabled us to match the compounds within replicate analysis of the samples. Initial results show that statistically valid quantification results are obtained with our instrumentation when performing a number of replicate analyses. The study covers the current status, the limitations and chances of MS based label-free quantification and show that the used instrument will become an excellent tool for this application. The goals of the ongoing work are identification and validation of potential biomarkers from clinical studies.

Mon Poster 53: Core Shell Particles Perform Molecular Sieving, Concentration and Preservation of Biomarkers for Subsequent Quantitative Analysis

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Discovering novel biomarkers holds big promises for early detection of diseases. Two main problems have been associated with biomarker discovery: 1) disease-relevant biomarkers in blood or body fluids may exist in exceedingly low concentrations within a complex mixture of biomolecules and could be masked by high-abundance species such as albumin, and 2) degradation of protein biomarkers can occur immediately following the collection of blood or body fluid as a result of endogenous or exogenous proteinases. The goal of this study was to incorporate a bait in "smart" nano-particles in order to sequester and concentrate selected classes of proteins and peptides from complex mixtures of biomolecules such as serum, and protect them from degradation during subsequent sample handling. In order to prevent non specific association of protein, the bait containing core was surrounded by a sieving shell.

N-isopropylacrylamide/acrylic acid (NIPAm/AAc) core – NIPAm shell particles were synthesized by precipitation polymerization and characterized by means of light scattering and atomic force microscopy. The AAc component acted as bait for cationic proteins. By using a solution of protein molecular weight markers and SDS PAGE analysis, the molecular weight cut off (MWCO) of the proteins sequestered by the particles was found to be around 20,000 Da, which is significant since the vast majority of possible biomarkers are thought to reside in the MW range from 0 to 20,000 Da.

Particles were incubated with human serum, and the captured proteins were electroeluted from particles. Analysis of the electroeluted proteins with mass spectrometry indicated that the particles had sequestered rare and small-sized serum proteins and peptide fragments with total exclusion of albumin and other large and high abundant proteins.

In order to prove the important point of protein protection from enzymatic degradation, particles were incubated with a solution containing model proteins (e.g. lysozyme, even in its reduced and alkylated form) and proteases (e.g. trypsin) with different incubation times (1 hour, 3 hours and overnight). It was demonstrate that core shell particles completely blocked protease degradation of captured proteins even at 37 ºC.

Taken together, these results provide evidence that core shell particles are a very promising tool that could help the discovery of novel biomarkers for early stage diseases.

Mon Poster 54: Proteomic Determination of the Stoichiometry and Sequential Assembly of Membrane Protein Complexes using Inducible Dominant Negative Mutant Trapping

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The sequential assembly of multiple protein complexes during vesicular trafficking is a well-established physiological process involved in various cell signaling pathways. We are interested in the role of vacuolar protein sorting protein 4b (vps4b) in regulating the assembly of endosomal sorting complex required for transport III (ESCRT-III) and its interaction with various membrane receptors that are implicated in regulating ligand-receptor signaling.

Toward our goal of identifying the role of vps4b function in the development of endosomal/lysosomal pathologies, we have developed an approach that combines biochemistry, immunology, mass spectrometry and bioinformatics in order to objectively identify and statistically validate potential vps4b interactors in tetracycline-inducible HEK293 cells stably expressing c-myc tagged ATP hydrolysis-deficient vps4b(E235Q) without the use of interactor-specific antibodies. The introduction of the E235Q point mutation within the ATPase domain of vps4b is a well-characterized mutation known to abrogate ATP hydrolysis, thereby permitting this dominant negative vps4b mutant to bind tightly to and "trap" its normal substrates. Vps4b(E235Q)-myc expression was induced in HEK293 cells for either 4 or 9 hours followed by c-myc immunoprecipitation. LC-MS/MS analysis coupled with stable isotope labeling and spectral counting permitted the determination of the stoichiometry of the ESCRT-III complex in relation to the over-expression of vps4b(E235Q).

We determined that the binding of vps4b to ESCRT-III is saturable in that vps4b(E235Q) over-expression does not perturb the endogenous expression levels of its known binding partners: charged multivesicular body proteins (CHMPs) 2A, 2B, 4A, 4B, 4C, and 5 that comprise ESCRT-III. Also among the identified interactors of vps4b were proteins with roles in endosomal trafficking and multivesicular body (MVB) maturation, cytoskeletal organization, protein turnover, and protein folding. Specifically, our data demonstrate that increasing duration of expression of vps4b(E235Q) correlates with the association of vps4b with proteins with functions related to protein turnover, protein folding, and cell death - pathways that have been proposed to play a central role in neurodegeneration. We have therefore established a robust method to quantitatively establish the stoichiometry and sequential assembly of vps4b-associated protein complexes by using a dominant negative vps4b mutant that is inducibly expressed in HEK293 cells.

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Mon Poster 55: Altered Proteome Biology of Cardiac Mitochondria under Stress Conditions

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Myocardial ischemia-reperfusion (I/R) induces mitochondrial dysfunction and, depending upon the degree of mitochondrial injury, may lead to cardiac cell death. However, our ability to understand mitochondrial dysfunction has been hindered by an absence of molecular markers defining the various degrees of injury. To address this paucity of knowledge, we sought to characterize the impact of I/R damage on mitochondrial proteome biology. We hypothesized that I/R injury induces differential alterations in various mitochondrial sub-compartments, that these proteomic changes are specific to the severity of injury, and that they are important to subsequent cellular adaptations to myocardial ischemic injury. Accordingly, an in vitro model of cardiac mitochondria injury was established to examine two stress conditions: reversible injury (induced by mild calcium overload) and irreversible injury (induced by hypotonic stimuli). Both forms of injury had a drastic impact on the proteome biology of cardiac mitochondria. Altered mitochondria function was concomitant with significant protein loss/shedding from the injured organelles. In the setting of calcium overload, mitochondria retained functionality despite the release of numerous proteins, and the majority of mitochondria remained intact. In contrast, hypotonic stimuli caused severe damage to mitochondrial structure and function, induced increased oxidative modification of mitochondrial proteins, and brought about detrimental changes to

the sub proteomes of the inner mitochondrial membrane and matrix. Using an established in vivo model of regional myocardial I/R injury in mice, we validated key observations made by the in vitro model. This pre-clinical investigation provides function and sub-organelle compartment information on a repertoire of cardiac mitochondrial proteins sensitive to I/R stress and highlights protein clusters potentially involved in mitochondrial dysfunction in the setting of ischemic injury.

Mon Poster 56: Functional Heterogeneity and Organ Specific Assembly of 20S Proteasome Complexes

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The ubiquitin proteasome system represents the major components of the intracellular protein degradation machinery. A functional proteomic approach was utilized to comprehensively characterize highly purified cardiac and liver 20S proteasomes using a combination of blue-native gel electrophoresis and LC/MS/MS. Expression of the inducible subunits of the purified 20S complexes were found to be higher in the liver compared to the heart by both mass spectrometry of O16/O18 labeled samples and immunoblotting. Additional immunoblotting showed that the liver 20S proteasomes contained higher relative amounts of phosphorylated Serine, Threonine and Tyrosine residues than the heart 20S. Proteolytic activities of the cardiac and liver proteasomes also differed. The hydrophobic proteolytic activity of the cardiac 20S was significantly greater than that of liver 20S, whereas the acidic proteolytic activity of the liver 20S was greater than that of cardiac 20S (no detectable difference was observed in the 20S basic proteolytic activities). Two binding partners that are present in both liver and cardiac 20S proteasomes were further investigated: protein phosphatase 1 (PP1) and Casein kinase II (CKII). Addition of CKII to liver 20S significantly decreased all three proteolytic activities of the liver proteasome without affecting the cardiac proteasome. Inhibition of the endogenous CKII bound to 20S proteasomes resulted in an increase in all three proteolytic activities of the liver proteasome without affecting the cardiac proteasome. Addition of PP1 to cardiac 20S significantly enhanced the b5 activity (50% ± 2% increase when compared to heat-inactivated PP1), with no detectable effect on the b5 activity in liver 20S. Inhibition of the endogenous PP1 bound to 20S proteasomes reduced b5 activity of the heart proteasome with no effect on the liver proteasome. The ratios of total (cytosolic fraction) and assembled (purified cytosolic 20S) 20S subunits were determined by western blotting and found to drastically vary for each proteasome subunit investigated. This suggests that the ratios of free and assembled 20S subunits are different between subunits of the same tissue and different between tissues, all of which together, indicate organ specific heterogeneity of 20S proteasomes, with respect to their molecular composition, complex assembly, post-translational modification and proteolytic function.

Mon Poster 57: Regulation of Myocardial Contractile Function by O-Linked GlcNAc Modification of Myofilament Proteins

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O-linked phosphorylation of serines (Ser) and threonines (Thr) of myofilment proteins are known to regulate myofilament function. A number of recent studies suggest that *O*-linked modifications of Ser and Thr by N acetyl-D glucosamine (GlcNAc) may also regulate contractile function. To examine this we used a novel method to identify specific sites of modification of cardiac myofilament proteins. After first subjecting myofilament proteins to extensive dephosphorylation, an azido-modified galactosamine (GalNAz) was attached to the *O*-GlcNAc moiety, then a biotin tag was attached to the GalNAz and the modified peptides were enriched by avidin chromatography. To label the specific sites DTT was substituted for the GlcNAc-GalNAz-Biotin by β-elimination and Michael addition. The modified peptides were identified by LC-MS/MS. As a result, 32 of *O*-GlcNAc peptides from cardiac myofilaments were identified including cardiac myosin heavy chain, myosin light chain 1, myosin light chain 2, actin and troponin I. All these *O*-GlcNAc modification sites are novel. The *O*-GlcNAc sites found on MLC1 differed from phosphorylation sites previously reported during preconditioning (Thr69 and Ser200). Ser 150, a known phosphorylation site of TnI by *p*-21 activated kinase, has been identified as O-GlcNAc modified in our work. In order to assess the potential physiological role of the GlcNAc modification of myofilament proteins, tension-calcium relationships were determined in skinned rat cardiac trabeculae at baseline and then the trabeculae were exposed to GlcNAc or Glycerol (as a negative control). Trabeculae exposed to GlcNAc but not glycerol had a significant desensitization of the tension calcium relationship (pCa 50 1.75±0.04 vs 3.51±0.39, n=3, p< 0.05), whereas Fmax and Hill coefficient were not significantly modified. In conclusion, we have identified the specific sites of *O*-GlcNAc modification of cardiac myofilament proteins and demonstrated their potential role in regulating myocardial contractile function. Regulation of *O*-GlcNAc myofilament modification may represent a novel and useful therapeutic target in heart failure, especially in diabetic cardiomyopathy. (*contributed equally to this work).

Mon Poster 58: Development of a Novel Diffraction-Based Immunoassay for Characterizing the Primary and Ternary Structure of the Circulating Form of Cardiac Troponin

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The detection of cardiac muscle specific troponins in blood is the current gold standard for the diagnosis of patients with acute myocardial infarction (AMI). In cardiac muscle, the troponin (cTn) complex comprises 3 tightly interacting subunits cTnI, cTnT, and cTnC. With AMI, cardiac troponin is released from the heart into the circulation where it can be detected using a variety of immunoassays that independently quantify cTnI or cTnT. cTnI is a complex analyte. Its circulating form has the potential of disease-induced post-translational modifications such as the specific and selective degradation of the N- and/or C-terminus and the possibility of presenting different ternary structures. There is indirect evidence suggesting that the dominant circulating form of cTnI is the cTnI-cTnC complex and that other potential complexes with cTnT are rare or nonexistent. However, there has been no study that has directly characterized the circulating ternary form of these biomarkers, in part, due to a limitation in technology. Here we describe the development of a novel immunoassay to characterize the physical form of circulating cTnI using diffractive optics technology, or dot. Core to this technology is a diffraction grating formed from affinity reagents such as antibodies. This grating is comprised of a repetitive sequence of lines and generates a specific, reflected diffraction pattern when interrogated with a laser. As molecules exhibit affinity for the capture molecules that make up the grating, the diffraction efficiency is improved. In this investigation, cTnI was captured using a biotinylated anti-cTnI antibody to the constant region (a.a residues 137-148) that was immobilized on a pre-patterned avidin sensor and then probed i) with antibodies to either or both the N- and C-terminus to determine if cTnI was degraded or ii) sequentially with anti-cTnT and/or anti-cTnC antibodies to determine whether cTnI existed as a monomer, dimer or trimer. The binding of the immobilized antibody, protein analyte and other antibodies were observed in real time as an increase in diffraction signal intensity. By continuously monitoring the intensity we can characterize the differences in the circulating form of cTnI. Using this new method, we have now directly measured the circulating cTnI-cTnT complex in patients diagnosed with AMI.

Mon Poster 59: Quantitative Analysis of Mammalian 20S Proteasomes Heterogeneity using Proteomic Strategies

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Proteaomes play critical role in intracellular homeostasis. This mega protease complex has been found in archaea, yeast and mammalian. The inducible subunits (β1i, β2i and β5i), however, are only identified in mammalian species, indicating their important roles towards mammalian 20S proteasome regulation. The inducible subunits are expressed in most tissues with quantitative differences. To compare the molecular composition and assembly

of 20S proteasomes in the murine heart and liver, we have applied both label-free semi-quantification strategy and O18 labeling approach. Purified 20S proteasome complexes were resolved by blue-native gel electrophoresis, followed by in-gel digestion and LC-MS/MS analysis. Relative protein abundance was estimated based on peak area of the extracted precursor ion chromatogram detected by the mass spectrometer. For O18 labeling, the tryptic digests from murine liver and heart 20S proteasomes were further labeled by either O18-enriched or regular O16 water, the mixed sample were then subject to LC-MS/MS analysis using LTQ zoom scan mode. Both quantification methods showed that the murine liver proteasomes contain consistently higher amount of inducible subunits compared to that expressed by the murine heart proteasomes, especially for β1i and β5i. Using O18 labeling strategy, the relative amount ratio of β1i and β5i from liver and heart were 2.95 ± 0.03 and 2.87 ± 0.028, and the ratio calculated from the peak area were 3.06 ± 0.67 and 4.32 ± 0.73, respectively. The different amount of inducible subunits from mouse liver and heart were also validated by western blotting. Taken together, our studies have shown that both semi-quantification by peak area and O18 labeling methods are very effective ways for addressing relative protein abundance of 20S proteasomes in mammalian systems; murine liver and heart contains different proportions of inducible proteasome subunits and possess distinct-assembled 20S proteasome complexes. These inducible subunits are important for producing antigenic peptides and the differences in the amounts of these proteasome subunits may represent the adaptation of these tissues for this function.

Mon Poster 60: Proteomic and Network Mapping of the KATP Channel-Dependent Subproteome in Kir6.2-Knockout Hearts

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ATP-sensitive K+ (KATP) channels perform a vital rheostat-like function, adjusting membrane potential-dependent cellular functions to match energy demand. Genetic defects in channel subunits have been associated with susceptibility to life-threatening cardiomyopathy, yet the molecular substrate of KATP channel deficiency has not been dissected at the proteome level. Here, the cardiac proteomic response to targeted disruption of the KATP channel, through knockout of the KCNJ11-encoded Kir6.2 pore, was delineated by comparative expression profiling. Differential two-dimensional gel electrophoresis reproducibly resolved >800 protein species from hearts of wild-type (WT) and age- and sex-matched Kir6.2-knockout (KO) counterparts. KATP channel ablation significantly altered 71 protein spots, from which 102 unique identities were assigned following linear ion trap quadrupole Orbitrap tandem mass spectrometry. Bioinformatic annotation stratified this KATP channel-dependent protein cohort into a predominant bioenergetic module (63/102), along with focused sets of signaling components (39/102) comprising oxidoreductases, chaperones, proteasome subunits, and proteins involved in supporting cytostructure, transcription or translation. Protein-protein interaction mapping, in conjunction with respective expression level changes, localized the KATP channel-associated subproteome within a non-stochastic, scale-free network. Ontological assessment of the KATP channel deficient environment validated the primary impact on metabolic pathways, and revealed statistical overrepresentation of markers associated with cardiovascular disease and cardiovascular system development and function. This proteomic cartography establishes a critical role for the KATP channel in the homeostasis of the normal cardiac proteome, and provides a systems biology framework to predict outcome associated with deficit in this cardioprotective checkpoint.

Mon Poster 61: Characterization of Platelet Membranes using Peptide Centric Proteomics

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Precise knowledge of the composition of the platelet membrane proteome is an important prerequisite for the identification of new therapeutic target proteins regarding the treatment of cardiovascular diseases.

Several inherent limitations of gel-electrophoresis as the difficulty to detect low abundant and hydrophobic proteins led to the development of new gel-free techniques. In this study we use the Combined Fractional Diagonal Chromatography (COFRADIC) approach. The idea is to reduce sample complexity of peptide mixtures without changing the ratios of the different peptides in the samples. The approach provides a number of reverse-phase LC techniques that sort specific classes of peptides (e.g. aminoterminal, cystenyl and methionyl peptides).

Prior to the isolation of peptides by COFRADIC, membrane proteins were isolated by aqueous two phase partioning. Afterwards the protein mixture is digested with trypsin and separated by a primary run on a RP-HPLC column. The chromatographic separation and fractionation is performed with an Ultimate 3000 HPLC system in a fully automatic fashion. Different fractions are collected in relation to the gradient and a second derivatisation is performed. The intention of this modification step is to change column retention time of the peptide subset of interest in contrast to the unmodified remaining peptide mixture. During the second run modified peptides show a hydrophobic or hydrophilic shift resulting in earlier or later elution times, respectively. The extent of the peptide retention time shift depends on the character of the modification and on the frequency of the modified amino acid in a certain peptide. Thus, fractions containing peptide subsets of interest can be selectively selected and analysed nano-LC-MS/MS.

In total 173 proteins were identified by Mascot searches against the SwissProt database, whereas about 50% of all identified proteins were plasmamembrane or membrane associated proteins.

Mon Poster 62: Quantification and Normalization of Complex Label-Free Mass Spectrometry

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Comprehensive proteomic profiling of tissue, cells, organelles or even biological fluids can benefit greatly from replicate MS measurements and multiple analytical methods (see our accompanying paper). 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. Here, we develop and extensively validate a novel label-free quantitative method termed the spectral index, SI, which combines three prominent mass spectrum abundance features: peptide and spectral count with peak height ion intensity. Both relative abundances and estimated actual protein levels can be calculated. With proper normalisation, SI 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 validated beyond just statistical testing of different datasets from similar and distinct MS techniques to include both comparative immunoblotting and densitometry. This normalized spectral index method may advance further the utility of shotgun proteomic analysis in systems biology and biomarker/target discovery.

Mon Poster 63: Enhancing Identifications of Lipid-Embedded Proteins by Mass Spectrometry for Improved Mapping of Endothelial Plasma Membranes *in vivo*

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Lipid membranes structurally define the outer surface and internal organelles of the cell. Although clearly functionally important, the multitude of proteins embedded in the lipid bilayer of cell membranes represents a special challenge for current large-scale shotgun proteomic methods. Here, using endothelial cell plasma membranes isolated from lung tissue, we tested the effectiveness of four different mass spectrometry-based methods, each with multiple replicate measurements, to identify proteins, especially lipid-embedded proteins, and in doing so, expand greatly the endothelial cell plasma membranome to 1,834 proteins. Strategies combining SDS-PAGE prefractionation with 1D and 2D nano-HPLC peptide separation before mass spectrometry dramatically enhanced protein identifications including integral membrane proteins and endothelial cell marker proteins, profoundly improved the sensitivity and dynamic range of detection, and substantially reduced the amount of sample and the number of replicate measurements required for 95% analytical completeness. This prefractionation with 2DLC/MS/MS analysis enhanced the identification of single and multiple pass transmembrane proteins by 5-7-fold. Such expansion in comprehensiveness requires a trade-off in heavy instrument time but portends well for future advancement in truly defining the ever-important membranome with its potential in systems biology and the discovery of new disease biomarkers and therapeutic targets.

Mon Poster 64: Global Protein Analysis using SeraFILE™: A Novel Proteomic Profiling Tool

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SeraFILE™ is a novel proteomic profiling tool designed for rapid quantification and functional analysis of protein samples. SeraFILE™, a proprietary surface library and associated automated protocols, was developed to characterize changes in the global proteomic composition of prokaryotic and eukaryotic samples. SeraFILE™ enables researchers to 'shuffle the deck' and visualize all of the proteins in a given sample based on their interaction with the surface library. Each of the eleven SeraFILE™ matrices have different functionalities, facilitating protein separation based on several protein characteristics concurrently. Protein samples are applied and weakly bound to the 11 SeraFILE™ surfaces and subjected to a mild, consecutive, multiple elution scheme. When all matrices are used in combination, the resultant 77 biologically active sub-proteomes offer a comprehensive signature of the starting sample.

Serum studies confirm the value of SeraFILE™ in various types of profiling applications. SeraFILE™ does not require any depletion step to resolve low abundance proteins and nothing is removed from the primary sample. Some surfaces bind high abundance proteins while others leave them intact. For instance, albumin is removed in certain sub-proteomes, revealing low abundance serum proteins otherwise masked by albumin. In addition, the distribution of protein in sub-proteomes recovered from each matrix is reproducible independent of starting concentration. Functional profiles of the serum enzyme, alkaline phosphatase, verify that reproducible patterns of native activity are also generated.

SeraFILE™ offers a unique platform for Rational Proteome Prospecting™ and biomarker discovery. SeraFILE™ experiments were conducted with yeast lysates and patient matched tumor samples to study the Ubiquitin Proteasome Pathway. Preliminary data suggests that SeraFILE™ has the potential to shed light on regulatory factors and alternate conformers specific to certain cancers. Our multidimensional approach allows researchers to correlate changes in native enzyme function to corresponding simplified protein pools. These functional profiles may facilitate detection and identification of small molecule inhibitors and/or co-factors associated with the protein target of interest, thus offering a link to pre-clinical drug development.

Mon Poster 65: Building an SRM-Based, Targeted MS Quantitative Assay for the Anti-Aging Hormone Klotho

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Recently, the proteomics community has begun to complement unbiased, shotgun-based approaches to biomarker discovery with hypothesis-driven discovery utilizing targeted SRM-based analyses. Building a clinically useful SRM-based targeted assay requires careful choice of signature peptides and transitions, to achieve the best possible limit of quantitation and detection given the complexity of the biological matrix that biomarkers are expressed in.

The Klotho gene was originally discovered in mouse mutants that displayed accelerated loss of multiple functions resulting in a phenotype that resembled premature aging. In more recent studies, Klotho appears to act as a hormone that inhibits insulin signaling and acts as an aging supressor. Previously developed ELISA and Western blot assays for the Klotho protein in plasma are unsatisfactory due to the lack of specific antibodies and imprecise mass measurements for verification of analyte identity. The development of an accurate and robust assay for Klotho would facilitate accurate detection and quantification in human samples for further study.

We report here the development of a quantitative, selective reaction monitoring (SRM), SID-MS−based assay using triple quadrupole mass spectrometry and Aqua™ heavy isotope labeled peptides to detect and quantify Klotho protein in plasma from mice and human samples. These analyses are carried out using a novel software solution that dramatically strengthens method development by incorporating proteotypic peptide selection based on ranked spectral library matching and an analytical selectivity based score, automatically incorporating the most selective and sensitive product ions for SRM transitions. The automated data processing methodology utilizes alignment, probability scoring and ion ratio analysis for confirmation and quantitation of Klotho.

Mon Poster 66: Glycans and Peptides in Hepatocellular Carcinoma

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Early detection of cancer improves survival of patients. Our study of hepatocellular carcinoma in Egypt, a country with an epidemic of hepatitis C viral infection, identified a set of glycans as candidate biomarkers for early detection of the disease. Glycans enzymatically released from serum proteins were analyzed by MALDI-TOF/TOF following solid phase permethylation. Analysis of less than 0.05 ml of serum allowed relative quantification of 80 glycans. Approximately half of the glycans changed significantly at $p<0.01$ in HCC patients (n=73) compared to gender and age matched controls with liver cirrhosis (n=52). Individual glycans have prediction accuracy comparable or better than alpha-fetoprotein, the current clinical serologic marker. A set of 3 glycans is sufficient to classify HCC with 93 % prediction accuracy in a blinded validation set of samples (n=75). Other glycans change abundance at earlier stages of progression of HCV infection to HCC and could be used to track the natural progression of the disease. The structure of the glycans suggests which glycosylation processes are affected in the pathophysiology of liver disease. The glycans complement peptidic markers identified previously by MALDI-TOF/TOF analysis of enriched low mass fraction of serum. Sequencing of the candidates using nanoLC-MALDI-TOF/TOF detected many peptides related to hemostasis. Fragments of complement C3 and C4 were consistently observed and sufficient to separate HCC cases and cirrhotic controls with 85% prediction accuracy. In conclusion, we identified novel candidate markers for early detection of HCC among protein associated N-glycans and native peptides. The results provide leads for further improvement of analytical strategies in biomarker discovery.

Mon Poster 67: Towards the Quantitative Establishment of a Standard Operating Procedure for Reverse Phase Protein Array Experiments

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For the past decade, researchers have implemented Reverse Phase Protein Arrays (RPPAs) in the field of cancer cell signaling with great success and the literature continues to grow. However, published manuscripts using RPPAs employ a wide array of disparate analytical methods for calculating sample protein expression. Which analytical method delivers the most accurate and precise result for any given analyte remains an open question in the field. Additionally, researchers currently do not implement a quantitative method in order to determine the number of sample replicates to print on their arrays and as a result are unaware of the significance of detected protein concentration differences among samples. Furthermore, colorimetric detection is currently the most common method of expression detection for RPPA. We hypothesize that use of a fluorometric method rather than colorimetric will provide for an increase in analytical accuracy. Our group has set out to systematically assess the analytical issues surrounding the use of RPPAs and to determine the optimal methodology for accurate and precise determination of measurements. In the first experiment, we generate a method that will allow researchers to determine the number of replicates per sample to be printed on each array in order to detect particular protein concentration differences with a statistical significance of p=0.05 and a power of 0.80. Based on this replicate sample size calculation, we are able to compare the performance of RPPAs using colorimetric detection to those using fluorometric detection. We confirm that the use of fluorescence increases the dynamic range of detection and requires fewer replicates per sample to yield the same power achieved by colorimetric detection. This implies that by switching to a fluorometric method researchers may be able to save precious space on the arrays by decreasing both the number of replicates (due to increased statistical power) as well as the number of serial dilutions (due to increased dynamic range). The next set of experiments attempts to resolve which analytical method for RPPA analysis generates the most accurate and precise measurements. Clinical standards containing known concentrations of proteins are used to print samples of varying concentrations on RPPAs. Sample protein expression is then detected using both colorimetric and fluorometric methods. All analytical methods applied to these arrays can then be assessed based both on their precision and accuracy to the known sample protein concentrations. The methods that are evaluated include: three published methods that utilize entire sample dilution curves, a simplified method that uses a single dilution point (and could eliminate the need for printing dilution curves all together) and a new calibrated assay method that we have developed. Furthermore, this experimental setup provides the ability to confirm that we can detect effect sizes postulated using the replicate numbers calculated. These experiments will give us quantitative evidence to select the best detection method and the optimal analytical method for calculating expression values. The end result will be a quantitatively justified standard operating procedure for RPPA experiments that generates the highest degree of accuracy and precision.

Mon Poster 68: Developing Reliable MRM Assay for Protein Quantification Based on Parallel Multiplexing LC-MS/MS Analysis

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Because of its high sensitivity and specificity, the multiple reaction monitoring (MRM) assay has been adopted in quantitative proteomics for biomarker discovery and validation. One of the initial limitations of MRM-based proteomics was the lack of a rugged and reproducible nano-LC system that was capable to generate reproducible retention times for the same analyte (peptide) across a large number of samples. In addition, large scale MRM analysis of proteins is hindered by the time-consuming step of identifying of the best proteotypic peptides for each protein and the subsequent selection of the best MRM transitions of the chosen peptide.

Proteotypic peptides are those peptides which are most likely to be confidently observed by MS-based proteomics methods. Successful and accurate MRM measurements require the selection/inclusion of at least one unique proteotypic peptide for each protein isoform and, at least one of the most intense fragment ions from the collision-induced-dissociation (CID) process of this peptide, although two fragment ions are preferred in many cases. Although this information can be obtained during the protein identification process, the sorting process of precursor and fragment ions, according to the intensities of their response, is not a straightforward procedure. Furthermore, if the LC/MS/MS data is acquired using a data-dependent acquisition (DDA) method, the precursor selection during MS/MS data acquisition is often non-repeatable, especially in the case of the analysis of very complex samples (e.g. whole cell or serum digests). As a result, is difficult to find the proper MRM transitions.

We describe a method that circumvents the two obstacles discussed above. The method utilizes a non-split nanoflow chromatography system able to produce highly reproducible high resolution LC separations for complex digests. The selection of MRM transitions is based on the parallel multiplexing LC-MS/MS analysis (LC/MSE method). Unlike the traditional DDA method, the MSE method maximizes the duty cycle of quadrupole/time-of-flight (Q-TOF) mass spectrometry by admitting all the LC-eluted peptides into the collision cell with no precursor selection. Because the Q-TOF mass spectrometer is operated by alternating between a low-energy scanning mode (5 eV) (for accurate measurement of peptide precursors) and an-elevated energy mode (MSE) (collision energy ramping from 15 to 40 eV, for accurate mass measurement of peptide fragment ions), all the proteotypic peptides as well as the corresponding fragments are recorded simultaneously. As a result, the probability of missing some of the proteotypic peptides (which could occur in a DDA experiment), is eliminated.

We developed an automated process for the selection of peptide MRM transitions for large scale protein quantification. Our approach is demonstrated for the analysis of 40 serum proteins from a human serum digest, using two peptides/protein and two transitions/peptide for a total of 160 MRM transitions.

By using the MSE data to design the MRM experiment one can achieve very high success rates (approaching 100%) for the MRM analysis. Also, peak area reproducibility (CV's < 15% for 70 % of MRMs) indicates that the matrix interference due to ion suppression is not a significant problem when using 30 min LC gradients.

Mon Poster 69: Stabilization of Peptide Biomarkers in Human Blood Samples

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We have previously reported that intrinsic protease and peptidase activity in plasma and serum samples can cause ex vivo variability and instability of proteins and peptides (Yi et al, J. Proteome Res. (2007) 6: 1768-1781). In this study, we investigated the stability of peptide biomarkers in human plasma and serum by monitoring an isotopically labeled peptide (e.g., an AQUA peptide) added into a sample as a function of time. Several synthesized peptides of known biomarkers, including fibrinogen peptide A, bradykinin, GP120, BNP, and GLP-1, as well as peptides from complement components 3 and 4, were spiked into five blood samples: serum or plasma with either Citrate, Heparin, or EDTA as anticoagulant, or EDTA plus protease inhibitors. After incubation for a specified period of time ranging from 0 minute to 72 hours, the peptides were extracted and analyzed by MALDI-TOF MS and LC-ESI MS. The results indicate that all peptide biomarkers used in this study are decreasing in concentration over time, demonstrating their proteolytic instability in these different blood products. Within the same serum or plasma sample, each peptide biomarker shows a unique half life, suggesting that the peptidase specificity differs between peptides. Also, the same peptide shows a difference of half life among the five sample types, indicating that intrinsic peptidase activities vary among these five samples. However, the spiked peptides are the most stable in the protease-inhibited plasma by inhibition of intrinsic proteolysis. The stability of the

peptides in different collected samples is as following order: P100* plasma ≥ EDTA > Citrate & Heparin > Serum. Monitoring the fragments of the labeled peptides provides mechanistic insight into proteolytic activity. As isotopically labeled peptides have been widely used as controls for quantitative analysis, the applications of these observations to plasma proteome research will be discussed.
Footnote: * For research use only, not for diagnostic purposes.

Mon Poster 70: Application of Physicochemically Modified Silicon Substrates as Reverse Phase Protein Microarrays

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Reverse-phase protein microarrays enable high throughput screening of posttranslational modifications of important signaling proteins within diseased cells. One limitation of protein-based molecular profiling is the lack of a PCR-like intrinsic amplification system for proteins. For this reason, enhancement of protein microarray sensitivities is an important goal, especially because many molecular targets within patient tissues exist in low abundance. The ideal array substrate will have a high protein binding affinity and low intrinsic background signal. As fluorescent systems, such as quantum dots, are explored as potential reporter agents because of their multiplexing potential and potential for increased sensitivity, the intrinsic fluorescent properties of nitrocellulose-coated glass slides limit the ability to image microarrays for extended periods of time where increases in net sensitivity can be attained. In light of the emerging field of biomedical nanotechnology, silicon, which has low intrinsic autofluorescence, is being explored as a potential microarray surface. In a previous paper (Nijdam, A.J., Cheng, M.M.C., Fedele, R., Geho, D.H., Herrmann, P., Killian, K., Espina, V., Petricoin, E.F., Liotta, L.A., Ferrari, M. (2007) Physicochemically Modified Silicon As Substrate For Protein Microarrays Biomater, 28, 550-558), it was shown that physicochemical modification of silicon substrates increases the protein binding to silicon that was at a level comparable to that of nitrocellulose. Here, we apply such substrates in a reverse-phase protein microarray setting in two model systems. We demonstrate that physicochemically modified silicon substrates can provide for a high quality alternative for nitrocellulose-coated glass slides for use in reverse-phase protein microarrays.

Mon Poster 71: Further Development of IgY-Immunoaffinity Fractionation – IgY14, SuperMix, and SepproTip Technologies

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Avian polyclonal IgY (Immunoglobulin Yolk) antibodies have unique and advantageous features that allow for highly-specific and effective capture of protein targets. Previously, we have reported development and application of IgY microbeads for the one-step removal of top 12 highly-abundant proteins (HAP) from plasma using immunoaffinity columns (IgY12 columns). However, after removal of top HAP, the next level of moderately-abundant proteins (MAP) becomes an obstacle to access low-abundant proteins (LAP), where the majority of biologically interesting and clinically important biomarkers reside. To tackle this challenge, we further developed the IgY-microbead system in several directions: (1) Developing novel IgY14 system for removal of top 14 HAP. In addition to top 12 HAP it removes complement C3 and ApoB, which frequently interfere with the MS analysis; (2) Establishing a Plasma-SuperMix column system to separate MAP from LAP; (3) Completing novel unique immunodepletion system for separating the RuBisCo protein from the plant proteomic samples; (4) Innovating a Cell-SuperMix column system to enrich proteins from tissue leakage. These novel approaches enable deeper and more effective access into the population of LAP. By coupling HAP removal with SuperMix system, hundreds proteins less than 1ng/ml in plasma were detected by LC/MS/MS analysis. In addition to digging deeper, the SepproTip platform for automated, multiplex, and HTP sample preparation was developed. This system allows for processing 15 µl of each of 12 samples at a time with minimal hands-on manipulation. The turnaround time of 12 samples per 60 minutes allows large number of samples being processed without decrease in sample preparation quality. The SepproTip system makes “digging faster” possible for meeting the needs of HTP sample preparation. Specific data and case studies will be presented.

Mon Poster 72: Qualitative and Quantitative Proteomic Profiling of Cripto-/- Embryonic Stem Cells by means of LC-MS Analysis

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Embryonic stem (ES) cells derive from the inner cell mass of blastocyst, an early stage embryo. ES cells are pluripotent, being able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. They are therefore an outstanding model system for early development studies or for the comprehension of mechanisms of organ formation or organ regeneration studies in clinical research. Cripto is a growth factor involved in stem cells differentiation and mouse cripto-/- ES cells have been developed and utilized to investigate their fate under a variety of conditions.
Here, a 2D LC-MS/MS approach has been used to qualitatively profile the cripto-/- embryonic stem cell proteome. The method is based on the multidimensional separation of a complex tryptic digest mixture using a nanoscale LC system on-line connected to an instrument capable of data-directed switching between the MS and the product ion MS/MS modes. The research enabled the recording of a large data collection by loading low sample amounts. Protein identifications achieved via databank searching the ESI-MS/MS spectra, provide qualitative information on the proteins present in the complex mixture. Furthermore, the identified proteins have been classified in terms of subcellular localization, molecular function and biological process as defined by their associated Gene Ontology annotation. A quantitative profile of the cripto-/- embryonic stem cell proteome was obtained by performing a label-free quantitative LC-MS experiment, utilizing multiplexed (alternate scanning) LC-MS^E. The principle of this method is based on the intensity difference measurement of the peptides identified to their constituant proteins. Several differentially expressed proteins have been identified and quantified in cripto-deficient stem cells with respect to the control murine RI embryonic stem cells. Delineation of the relative amounts of these proteins, can provide an integral view of the alterations induced in stem cell functions by deleting the cripto gene.

Mon Poster 73: Synthesis of Full-Length Stable-Isotope Labeled Proteins for Use as Internal Standards in LC/MS-Based Clinical Protein Reference Methods

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The prevalence of clinical protein in vitro diagnostics, as well as the continuing search for novel protein biomarkers, has increased the demand for reference materials and methods for the quantitation of proteins in biological matrices. To accommodate such demands, the National Institute of Standards and Technology (NIST) requires reliable sources of suitable internal standards for the development of standard reference methods. Liquid chromatography/mass spectrometry (LC/MS) methods for measuring proteins often employ stable-isotope labeled peptides as internal standards to quantitate tryptic peptide levels in a protein digest mixture. However, peptide internal standards cannot account for: the loss or chemical modification of protein in processing steps prior to digestion and incomplete or non-specific digestion prior to analysis. Full-length, stable-isotope labeled proteins would function as more appropriate

internal standards for quantitative LC/MS-based methods since they closely mimic the extraction recovery, chromatographic retention time, and mass spectrometric ionization response of the endogenous analyte. In order to obtain custom, full-length stable-isotope labeled proteins, NIST is investigating multiple methods of protein expression to determine which are most appropriate for specific target proteins. These methods include more traditional in vivo expression in N15-labeling culture media and in vitro expression in commercial cell-free systems utilizing C13-labeled amino acids, which can be more cost-effective and efficient than cultured systems. The resulting intact, labeled proteins could then be utilized as internal standards for quantitative LC/MS methods. In preliminary work, E. coli lysate-based cell-free expression systems have been evaluated for their ability to produce an unlabeled and labeled control protein, with results demonstrating expression of green fluorescent protein (GFP). Standard molecular biology techniques have been utilized to engineer a DNA vector appropriate for expression of human serum albumin (HSA) in both in vivo and in vitro systems. Purification of the histidine-tagged GFP protein is accomplished by magnetic bead immobilized metal affinity chromatography (IMAC). HSA is purified by affinity methods. The level of isotopic incorporation is quantified by high-resolution mass spectrometry and comparison to computer simulations of isotopic distributions. Acquisition of full-length, stable-isotope labeled proteins for internal standards will allow for the development of quantitative LC/MS-based reference methods. With this scheme, we intend to expand the capabilities of NIST to develop reference materials and methods for the quantitative measurement of a multitude of clinical target proteins in various biological matrices, which will benefit the clinical chemistry and proteomics communities.

Mon Poster 74: Proteomics-Based Characterization of Influenza Virus Strains and Influenza Vaccine

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The single radial immunodiffusion (SRID) assay is currently used to identify the virus strain included in an influenza vaccine preparation, as well as to determine vaccine potency. In order to perform this essential test, hemagglutinin (HA), one of the antigenic proteins, must first be purified and then used to immunize sheep for the production of HA-specific antiserum. This poses a potential bottleneck for vaccine manufacture that is of particular concern for the generation of SRID reference reagents and antisera for pandemic H5N1 vaccines. Alternative proteomics-based assays that are independent of such reagents would prevent this potential bottleneck. This work reports on an ongoing effort to develop a mass-spectrometry (MS)-based technique that provides both qualitative and quantitative analysis of monovalent and trivalent influenza vaccine preparations. First, a peptide list was generated from in silico tryptic digest of HAs from influenza A subtypes H1N1 (A/New Caledonia/20/99), H3N2 (A/Wisconsin/67/2005), and influenza B (B/Ohio/01/2005), the strains that compose the 2006/07 Northern hemisphere vaccine, using protein sequences from the influenza virus database (http://influenza.genomics.org.cn/). Next, the list was matched with that of the mass spectra data obtained from MALDI MS analysis of the tryptic digest of purified recombinant HAs from each of the aforementioned virus types. In silico and tryptic peptides from HA from H3 drift variants, A/Wisconsin/67/2005, A/New York/55/2004, and A/Wyoming/3/03 were also compared. On the basis of this information and published data regarding antigenic sites of HA, peptides from each virus subtype were selected for qualitative characterization of vaccines. In addition, a peptide that is unique to each H3 variant was identified. The hypothesis that the selected peptides will identify a virus subtype as well as the specific strain contained in a trivalent influenza subunit vaccine was tested using a 2006/2007 formulation manufactured by Sanofi Pasteur Inc. (Swiftwater, PA). The vaccine was digested with trypsin and the resulting peptide mixture was analyzed using MALDI TOF/TOF instrument. The results showed the presence of subtype and strain specific peptides, confirming the presence of A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/2005 (H3N2) and B/Malaysia/2506/2004 in the vaccine preparation. Our current experiments aim to use the amount of peptide present to determine HA quantity, with the goal of establishing a method to measure the absolute amount of HA in a vaccine or reference antigen preparation. This is an essential first step in evaluation of vaccine potency.

"The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy."

Mon Poster 75: Metabolite and Protein Profiles of Cancerous and Infected Cells using LAESI Mass Spectrometry

Rachel Van Duyne[1]; Zachary Klase[1]; Peter Nemes[2]; Bindesh Shrestha[2]; Yue Li[2]; Akos Vertes[2]; Fatah Kashanchi[1]

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The in vivo characterization of cellular changes between a normal and disease state cell is critical to understanding virulence and tumor proliferation. A true global in vivo observation of a cell is nearly impossible with currently available proteomics techniques, however recent advances in mass spectrometry have started to make this a reality. Recently, we have developed a novel combination of infrared laser ablation with electrospray ionization (LAESI) which has been used to identify small biomolecules under ambient conditions. We have successfully applied this technique to the analysis of live cells in culture. Using this technique, we have identified the metabolite profile of HIV-1 infected cells as compared to control. This analysis will aid in future studies of viral pathogenesis.

Mon Poster 76: Respiratory Syncytial Virus (RSV)-Infection Downregulates the Expression of Antioxidant Enzymes in BALB/C Mice

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RSV is the one of the most important viral pathogen which cause upper and lower respiratory tract infections in infants and young children. Oxidative stress has been implicated in the pathogenesis of acute and chronic lung inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD). RSV infection induces lung inflammation and ROS production in vitro as well as in vivo as previously shown by our group. The mechanism of RSV-induced oxidative injury in the airways has not been investigated yet. Using a proteomics approach to detect changes in protein expression in the airways of infected mice, we identified a number of antioxidant enzymes (AOE) that were down regulated following RSV infection. To further investigate the mechanism of RSV-induced oxidative injury, we measured expression and activity of superoxide dismutase 1 (SOD 1), SOD 2, SOD 3, catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST) in mouse bronchoalveolar lavage (BAL). In mice, RSV infection resulted in a decrease of GSH/GSSG ratio in RSV infected mice compared to control animals. RSV infection induced a significant decrease in expression of SOD 1, SOD2, SOD 3, catalase and GPx. Enzymatic assay results showed that there was a significant decrease in the total SOD activity, and also catalase, GPx and GST activities. When we investigated AOE expression in cell extracts of conductive airways, there was a significant decrease in the expression of SOD1 and 3 where as SOD 2, catalase and GST remained unchanged. These results show that RSV-infection induces the down regulation of antioxidant system in mice suggesting that RSV-induced oxidative damage in mouse lung is the result of an imbalance between ROS production and airway antioxidant defenses. Modulation of oxidative stress, proper understanding of the molecular mechanisms that regulate expression of antioxidant enzymes/genes and associated signaling in response to RSV-infection may pave the way toward important advances in the therapeutic approach of RSV-induced acute lung disease.

Mon Poster 77: Proteomic Analysis of Altered Protein in Lymphoid Organs of Penaeus Monodon upon Yellow Head Virus Infection

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Yellow head virus (YHV) is a rod shaped and positive-stranded RNA virus . YHV is one of the major pathogens of the black tiger shrimp (Penaeus monodon). In YHV-infected shrimp, a deformation of lymphoid organ (LO) has been shown to be associated with the progression of yellow head disease (YHD). To better understand molecular responses of crustacean LO to YHV infection, a comparative proteomic analysis employing a gel-based approach was performed to identify a set of altered proteins in the LO that might be involved in the pathogenesis of YHD in P. monodon. At 24 hours after experimental challenge with YHV, the infected shrimps showed obvious signs of YHD, while the control (uninfected) shrimps remained healthy (n = 15 per group). Comparative 2-DE analysis of proteins extracted from the LO revealed significant alterations in abundance of several proteins in the infected LO. These altered proteins were then identified by MALDI-TOF MS and/or nanoLC-ESI-(Q-TOF)-MS/MS, including transglutaminase, protein disulfide isomerase, ATP synthase beta subunit, V-ATPase subunit A, and hemocyanin fragments, whose levels were significantly increased, and est_l_vannamei501, est_l_vannamei1751, Rab GDP-dissociation inhibitor, 6-phoshogluconate dehydrogenase, actin, fast tropomyosin isoform, hemolymph clottable protein, and cAMP-regulated protein, whose levels were significantly decreased in the infected LO. Some of these selected altered proteins were further investigated at the mRNA level using real-time RT-PCR, of which the results also confirmed the proteomic data. Identification of these altered LO proteins in the YHV-infected shrimps may provide some novel insights into the molecular responses of P. monodon to YHV infection.

Mon Poster 78: The Proteomics of HIV-1 Tat Transactivator: Implications for Tat PTMs

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Post translational modifications (PTMs) represent an additional layer of control in cellular pathways. The presence or absence of specific PTMs (such as acetylation, methylation and ubiquitinilation) can augment or block the specific activity of a protein or enzyme. The HIV-1 Tat protein is involved in the transactivation of the HIV-1 viral promoter and is regulated by PTMs. We have analyzed the association of various proteins with Tat and acetylated Tat and identified proteins with a preference for the acetylated state. Of particular interest is the recruitment of p32, a protein with implications for the regulation of splicing. Tat association with p32 influences the recruitment and function of CDK13 leading to changes in the ratio of splice to unspliced HIV-1 mRNA. We have also analyzed the methylation of Tat and identified methyl-transferases that bind to this protein. Modification of Tat also leads to changes in recruitment of various chromatin remodeling and transcription factor proteins to the viral promoter. Implications for Tat function, protein-protein interaction and activation of the HIV-1 LTR will be discussed.

Mon Poster 79: The Membrane Proteome of HIV-1 Infected Cells: Implications for Anti-Apoptosis

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Profiling integral plasma membrane proteins is of particular importance for the identification of new biomarkers for diagnosis and for drug development. We report in this study the identification of surface markers by performing comparative proteomics of established human immunodeficiency virus-1 (HIV-1) latent cell models and parental cell lines. To this end we isolated integral membrane proteins using a biotin-directed affinity purification method. Isolated proteins were separated by two-dimensional gel electrophoresis and identified by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) after in gel digestion. Seventeen different proteins were found to vary on the surface of T-cells due to HIV-1 infection. Of these proteins, 47% were integral membrane proteins, and 18% were membrane-associated. Through the use of complementary techniques such as Western blotting and fluorescent staining, we confirmed the differential expression of some of the proteins identified by MALDI-TOF including Bruton's tyrosine kinase and X-linked inhibitor of apoptosis. Finally, using phosphatidylinositol 3-kinase inhibitors and flavopiridol to inhibit Bruton's tyrosine kinase localization at the membrane and X-linked inhibitor of apoptosis protein expression, respectively, we showed that HIV-1 latently infected cells are more sensitive to these drugs than uninfected cells. Further studies track the change in expression of apoptosis related proteins using Q-RT-PCR.

Mon Poster 80: Protein Profile of HTLV-1 Tax-Proteome

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Infection with human T-cell leukemia virus type 1 (HTLV-1) results in adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis. Tax, a 40-kDa protein, regulates viral and cellular transcription, host signal transduction, the cell cycle and apoptosis. However, its role in binding to various host cellular proteins involved in tumorigenesis has not been fully described. In this study, we describe a proteomic method to identify Tax-associated proteins and their functions in cells. 32 proteins were identified to be associated with Tax by using size exclusion followed by Tax immunoprecipitation. The physical and functional association of Tax with the chromatin remodeling SWI/SNF complex was assessed using in vitro chromatin remodeling assays, chromatin remodeling factor BRG1 mutant cells, and RNA interference experiments. In conclusion, we described a systemic method in identifying Tax-associated proteins, which provides us comprehensive information about the Tax-proteome.

Mon Poster 81: Got Enolase? A Hit Parade of Notoriously Identified Differentially Expressed Proteins

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Conventional two-dimensional electrophoresis (2-DE) remains a fundamental tool in expression proteomics. However, after reading many 2-DE–based articles featuring lists of the identified differentially expressed proteins, one starts experiencing a disturbing sense of déjà vu. Heat-shock proteins again? Elongation factors, proteasome subunits or peroxiredoxins once more? The same proteins seem to predominate regardless of the experiment, tissue or species.

To explore this unsettling sensation and to quantify the occurrence of individual differentially expressed proteins in 2-DE experiment reports, we performed a simple proteomic meta-analysis. We compiled identities of differentially expressed proteins identified in human, mouse and rat tissues in 2-DE experiments published in 3 recent volumes (2004-2006) of PROTEOMICS. We than calculated the appearance of the most predominant proteins in the dataset and assembled the „TOP 15“ charts for individual human and rodent proteins and for protein families.

Results of our meta-analysis confirm that some proteins appear among the identified differentially expressed proteins very often, regardless of experiment, tissue or species. The “TOP 15” chart assembled for individual proteins includes mostly glycolytic enzymes, heat shock and stress proteins and cytoskeletal components. The most frequently identified differentially expressed protein in rodents is enolase 1. In humans, HSP27 is the most notoriously identified protein closely followed by enolase 1. This highly abundant glycolytic enzyme was identified as differentially expressed in about 30 percent of all published experiments in both

human and rodent tissues. Interestingly, nearly 25 percent of published articles showed differential expression of at least five proteins belonging among the “TOP 15” .

The “TOP 15” charts assembled for protein families demonstrate that keratins and peroxiredoxins are the most often differentially expressed protein families in human and rodent samples respectively. In 108 studies using human tissues keratins were differentially expressed 70 times, whereas peroxiredoxins were identified 34 times in 78 rodent experiments.

Our meta-analysis raises several thorny questions. Why should a conserved glycolytic enzyme such as enolase1 change its expression in response to so many different stimuli? Are we observing universal cellular sensors or rather technical artifacts and limitations of the technique? How reliable are GAPD, beta tubulin and actin as internal standards in expression stuides if they belong among the molecules that are often differentially expressed? Why are keratins identified as differentially expressed significantly more often in human samples than in rodent tissues?

We believe that our simple study demonstrates that such meta-analyses of proteomic data can provide invaluable information pertinent to various biological processes or methods involved. We suggest that our “TOP15” charts could be useful as blacklists that could prevent unjustified over-interpretation of 2-DE-based results in expression studies.

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Tues Poster 01: Calibrating E-values for MS/MS Database Search Methods

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Background: The key to mass-spectrometry-based proteomics is peptide identification, which relies on software analysis of tandem mass spectra. Although each search engine has its strength, combining the strengths of various search engines is not yet realizable largely due to the lack of a unified statistical framework that is applicable to any method.

Results: We have developed a universal scheme for statistical calibration of peptide identifications. The protocol can be used for both de novo approaches as well as database search methods. We demonstrate the protocol using only the database search methods. Among seven methods –SEQUEST (v27 rev12), ProbID (v1.0), InsPecT (v20060505), Mascot (v2.1), X!Tandem (v1.0), OMSSA (v2.0) and RAId_DbS– calibrated, except for X!Tandem and RAId DbS most methods require a rescaling according to the database size searched. We demonstrate that our calibration protocol indeed produces unified statistics both in terms of average number of false positives and in terms of the probability for a peptide hit to be a true positive. Although both the protocols for calibration and the statistics thus calibrated are universal, the calibration formulas obtained from one laboratory with data collected using either centroid or profile format may not be directly usable by the other laboratories. Thus each laboratory is encouraged to calibrate the search methods it intends to use. We also address the importance of using spectrum-specific statistics and possible improvement on the current calibration protocol.

Tues Poster 02: Protein-Centric Search and Analysis at the NIAID Biodefense Proteomics Resource Center

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The National Institute for Allergy and Infectious Diseases (NIAID) has created a biodefense proteomics program to identify targets for potential vaccines, therapeutics, and diagnostics, for agents of concern in bioterrorism. The program consists of seven Proteomics Research Centers (PRCs) and a Proteomics Resource Center (RC) charged with developing the bioinformatics infrastructure for public dissemination of the PRC data and reagents. The data includes transcriptional profiles, protein profiles, structures, and protein interactions, from pathogen and host proteins, in vivo and in vitro. For a summary of available data see http://www.proteomicsresource.org/Resources/Catalog.aspx.

Administering such a program presents challenges as large and heterogeneous data have to be integrated to allow for a systems approach to knowledge discovery. We adopted a protein-centric approach to organizing the information as proteins occupy a central functional role in most cell biological processes. All genetic, functional, structural, pathway and experimental results can be mapped and organized as a network of attributes of pathogen or host proteins. The entire sets of attributes are available for simple but powerful text searches or browsing starting from the RC home page http://www.proteomicsresource.org/.

Underlying this approach is a comprehensive set of protein/gene related ID mappings contained in the iProClass data warehouse and name mappings maintained in the Biothesaurus. All validated research results provided by the PRC's are mapped to UniProtKB accessions or if not possible (i.e. a pseudo gene or a sequence not in GenBank or another repository) to an iProClass record. Program and experiment specific attributes are created to summarize experimental conditions, results and publications. Select proteins are annotated with controlled comments to flag key experimental findings or additional validation.

The availability of proteomic and genomic data in an integrated database allows comparisons across different experiments and organisms. Here we show examples of analysis of proteins across different data sets. 1) Illustrates a query searching for proteins detected during infection of mouse macrophage cells by two different organisms (*B. anthracis* and *S. typimurium*) and two separate data types (microarray and mass spec.) led to a list of 17 mouse macrophage proteins found in three experiments. One protein, a 1173 aa mouse protein (K1033_MOUSE) of no known function is found in several eukaryotes and even in lower eukaryotes such as Entamoeba. Using protein family information we can broaden our search across additional organisms and datatypes and see the human ortholog of this protein was also detected in mass spec. data from Hela cells infected with orthopox viruses. The conservation of this protein in eukaryotes and the identification of this protein in both mass spectrometry and microarray based analysis in two host systems suggest that this protein might be prioritized for additional analysis and functional characterization. 2) Illustrates a simple keyword search from the home page for protein data on "*Francisella tularensis*" The results pull out 6 *F. tularensis* proteins and related human interactions provided by Myriad Genetics and 11 other mostly mouse host proteins associated with literature on *F. tularensis* infection.

Tues Poster 03: Connecting NCI Proteomics Repository to the Cancer Biomedical Informatics Grid (caBIG)

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National Cancer Institute has implemented public proteomics repository, based on Computational Portal and Analysis System (CPAS), developed at Fred Hutchinson Cancer Research Center. Currently, the repository provides public access to 6,176,247 peptide identifications from 244 ms-ms runs from Mouse Proteomics Technology initiative (MPTI). Making proteomics data from large initiatives accessible to public is important to allow further statistic analysis and validation in the research community. To achieve the goal, we decided to connect the NCI proteomics repository to the cancer Biomedical Informatics Grid (caBIG).

The caBIG is a virtual network for nation wide cancer research centers. It is developed by National Cancer Institute to connect interoperable databases and analytical tools for translational cancer research. Starting with CPAS caBIG client development kit developed by the Labkey group, necessary caGrid service components, such as schema mapping, caBIG client API, and server side module were generated. An open-source toolkit called Introduce was utilized to package these components and deploy the CPAS data service to the WS-Resource Framework (WSRF) compliant Grid service. The CPAS Grid data service enables the Grid access to the experiment and mass spectrometry data in CPAS proteomics repository. The Grid connection not only makes the data more visible through caBIG participated cancer centers, it also allows easier integration of proteomics data with other types of experiments.

Tues Poster 04: A Phylogenetic Paradigm for Dynamic Systems Biology: An Integrative Modeling of the Heterogeneities of the Omics Data and Disease

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The biological heterogeneity of diseases such as cancer, autoimmunity, and degenerative disorders, has not been qualitatively accounted for by dominant analytical paradigms and the modeling of their omics data need to accurately reflect their patterns of diversity. Proteomic, metabolomic, and genomic heterogeneity of diseased specimens points out the existence of several phenomena: complex models of systems biology; random pattern of mutation responsible for diversity and complexity; and probably multiple pathways of disease development.

The omics data are characterized as containing asynchronous, homoplastic, and heterotachous patterns of expression, therefore,

parsimony phylogenetic analysis seems to be the most suitable paradigm for the reconstruction of systems biology. Parsimony phylogenetics identifies natural classes of specimens, the clades, by their shared derived expressions--the synapomorphies. Synapomorphies are determined through polarity assessment (ancestral v. derived) of expression states or values followed by a maximum parsimony analysis. It offers a multidimensional systematic approach that is seamless, dynamic, and predictive; it reveals biological classes as natural phyletic units, disease ontogenies, and patterns of pathway relationships.

Additionally, a parsimony phylogenetic analysis reduces the provisionality of plausible biomarkers and redefines them as an integral component of systems biology. It accomplishes this by requiring each biomarker to be a synapomorphy, i.e., each synapomorphy is a shared proteomic/metabolomic/genomic expression by all the specimens of its clade. This characteristic is of major importance because it permits the use of biomarkers in interactome linkage within and between systems.

Since it is a major goal of systems biology to integrate data from several sources (genomic, metabolomic, and proteomic) in one analytical paradigm, parsimony phylogenetic analysis facilitates this process by permitting the pooling of polarized datasets together to carry out a super analysis. The parsimony algorithm models the data on a graphical tree with dichotomous branching termed cladogram. The branches of the cladogram represent the relationships among the specimens and each node of it is defined by one or more synapomorphies. Because of its hierarchical nature, the cladogram places the specimens with the highest number of expression changes at its upper part.

We have successfully applied our maximum parsimony analysis to the omics data and produced a phylogenetic model based on the following cancers: breast, gastric, leiomyosarcoma, lung, pancreas, pheochromocytoma, and prostate. The model revealed several shared observations that illustrated common patterns within the above cancers. Among those: (i) a transitional zone between cancerous and non-cancerous clades that encompasses the specimens with the least amount of altered expressions; (ii) a consistent major dichotomy in cancers that translates into two main developmental pathways; (iii) asynchronous protein and gene expression profiles that underscore the heterogeneity of omics can be used in modeling the disease; and (iv) the successful pooling and analysis of omics data from different datasets, platforms, cancers, and various high-throughput technologies.

Thus, our phylogenetic model of dynamic systems biology offers an optimal bioinformatic tool, which meaningfully integrates the heterogeneity of the omics expressions and contribute to the understanding and reconstruction of their systems biology.

Tues Poster 05: The Benefits of Measuring Spectral Pattern Reproducibility in Complex Spectra

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Introduction. Rather than calculating a mere average spectrum from replicate spectra, we present a method for validating the spectral pattern and distinguishing aberrant replicates. This is an important distinction because while spectral averaging and precision attenuation of the average allow for the incorporation of the most consistent features from spectra, pattern validation ensures that the replicates are indeed similar. Our data processing approach is based on the spectral dot product (DP) and introduces 95% confidence intervals (CI) on the spectral dot product to evaluate the strength of spectral correlation; it is the only calculation described to date which accounts for both the nonnormal sampling distribution of the dot product and the number of peaks the spectra have in common.

The data analysis scheme is applied to replicates of brain tubulin CNBr peptides to enable a robust comparison of tubulin isotype expression and posttranslational modification patterns in rat and cow brains. These complex spectra highlight the need for evaluating spectrum variability and comparing differing spectra because the number and variability of peaks confounds manual comparisons. As these methods are shown to work for tubulins, they represent a general methodology for evaluation of spectral reproducibility and similarity with the spectral dot product in complex spectra.

Methods. Microtubules are extracted from rat and cow brains and treated with CNBr by well-established methods. Each sample was spotted in triplicate on the MALDI target, and 30,000 shots – 10 spectra of 1000 shots per spot – were acquired in batch mode for each sample on an Applied Biosystems (ABI) 4700 operated in negative reflector mode. A consensus spectrum was generated from these 30 replicates, and the DP and 95% CI of each replicate against the consensus was calculated. Replicates with negative 95% CI were discarded, and the remaining replicates were used to assemble a new consensus spectrum. The consensus spectrum was then used to assign the isotypes and posttranslational modifications present in each tissue.

Preliminary data. The preliminary data demonstrate that use of a single spectrum introduces variance and ambiguity into the assignments while the most reliable information comes from consensus spectra that have been derived with mass and intensity precision and pattern validated using the spectral dot product.

Tues Poster 06: Protein-Centric Integration and Functional Analysis of Cancer Omics Data

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Analysis and interpretation of large-scale transcriptomic and proteomic data remain challenging and require effective use of public knowledge resources and advanced bioinformatics methods for data integration, mining, and comparative analyses. We previously developed a prototype system, iProXpress (Huang et. al., 2007), for large-scale expression data analysis, which had been applied to several gene expression and proteomic studies, including those of organellar proteomes. The system contains three major components: a data warehouse with information derived from over 90 databases, analytical tools for sequence analysis and functional annotation, and a graphical user interface for protein mapping, functional annotation, and function and pathway profiling. The system's unique features include its comprehensiveness of protein sequence coverage and annotation, high protein mapping rate of expression data including protein spliced forms, and its versatility of use on different types of omics data. The iProXpress system may serve as a model for protein-centric omics data integration and analysis, as illustrated below in two case studies, both aiming to elucidate molecular mechanisms underlying drug- or radiation-resistance in cancers.

The first study aims to identify early signaling pathways underlying the low-dose estrogen-induced apoptosis of a variant breast cancer cell line, which may have significant implications in designing novel therapies for anti-estrogen drug resistant breast cancers. The nuclear receptor co-activator AIB1 (or NCOA3) has been shown to play a critical role in estrogen-induced apoptosis of breast cancer cells. A high-throughput immunoprecipitation method using anti-AIB1 antibody was used to pull-down AIB1-interacting proteins in estrogen-treated vs. -untreated cells, followed by 1D-gel analysis, excision of bands of interest, followed by MS/MS. The iProXpress system facilitates protein mapping and functional annotation of the identified proteins, and integration and cross comparison of multiple datasets, including experimental repeats. AIB1 was found to interact with multiple proteins that were differentially pulled-down from different breast cancer cells, including an enriched group of proteins in estrogen-induced apoptotic cells that are involved in RNA metabolism and transcription, with functions including transcriptional regulation, chromatin interaction and regulation, mRNA splicing, and apoptosis.

The second study investigated pathways underlying cellular responses to ionizing radiation that lead to radiation resistance in cancer cells. ATM, or mutated in ataxia-telangiectasia, is a member of the serine-threonine protein kinase family and plays critical roles in radiation-induced responses. Human fibroblast cell lines expressing wild-type or mutated/truncated ATM proteins are used

as models for identifying important pathways or targets associated with ATM-mediated radiation responses. The two cell lines were subjected to global gene expression and proteomics experiments (2D-gel and MS/MS). The pathway analysis showed that purine metabolic pathways were significantly represented and differentially changed in the wild type- vs. mutated-ATM expressing cells. The system also allows direct association of gene expression data with proteomic data from the same cells, and facilitates the identification of truly changed proteins in the 2D-gel spots using differential expression pattern and common pathway knowledge gained through the functional profiling.

Reference: Huang H, Hu ZZ, Arighi CN, Wu CH. (2007) Integrated bioinformatics resources for large-scale expression data analysis. Frontiers in Bioscience, 12:5071-5088.

Tues Poster 07: A Pre-Clinical Data Resource for AIDS Research

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Structure-based drug discovery relies on efficient compilation and analysis of chemical, biological, pre-clinical and structural data. Factors such as inconsistent naming standards, publications and private archives with scattered and incomplete data, Web pages with little or no cross-talks act against this drug-design effort. Large scale effort on AIDS drug discovery has led to many issues ranging from archival and distribution of the information to annotation and naming standards for the data. To alleviate this situation we have been working on developing Web based public data resources (Fig) (HIV Structural Database (http://bioinfo.nist.gov/SemanticWeb_pr2d/chemblast.do and http://chemdb2.niaid.nih.gov). A significant part of the data found in these data resources is specific to the resources and they were obtained by examining literature or by direct request to individual researchers. To our knowledge, these Web resources have the largest collection of pre-clinical data and this effort may serve as a model for future efforts focused on other diseases.

Tues Poster 08: Isolation of Proteins from Subcutaneous Tumor and Adipose Tissues using a Pressure Cycling Technology

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Extremely small and rare tissue samples must be processed with high efficiency to enable reliable proteomic analysis. For small samples of murine tumor and adipose (10-100 mg) or rat liver (0.5-10 mg), Pressure Cycling Technology (PCT) yielded more protein in 30 minutes compared to pulverization of tissue frozen under liquid nitrogen (LNP) followed by buffer extraction of the triturate for 0.5-48 hours. For four subcutaneous tumor types, protein extraction efficiency was 4.4% ± 0.3% for PCT compared to 1.5% ± 0.6% for LNP. Further, a coefficient of variation (CV) of 7.8% was observed between the four tumor types for PCT compared to 37.7% for LNP. Also, PCT extracted as much as four times more protein from small adipose tissue samples than LNP. Finally, it is demonstrated that PCT derived sufficient protein from less than 1 mg of rat liver (0.7 ± 0.3 mg) by PCT to enable highly reproducible analyses by two-dimensional gel electrophoresis (2DGE).

Tues Poster 09: Analysis of the Effects of Ultrafiltration Conditions on Enrichment of Low Molecular Weight Serum Proteins

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Purpose: Emerging proteomic technologies facilitate the identification of cancer biomarkers. We report a study of marker discovery in hepatocellular carcinoma (HCC) in an Egyptian population with high incidence of HCC. The goal of our study is to identify serum peptides associated with the development of HCC. Methods and Results: We developed MALDI-TOF methods for high throughput analysis of low molecular weight serum proteins/peptides in order to decipher statistically significant differences in abundance. To identify the differentially abundant peptides, we have explored several parameters in the enrichment using centrifugal ultrafiltration. We studied the effects of membrane molecular weight cut-off, buffer components, temperature, and centrifugation speed on protein recovery and identification. We have used MALDI mass spectrometry, gel electrophoresis, and protein quantification to evaluate the filtration efficiency under various conditions. LC-MALDI-MS/MS was used for protein identification in the serum ultrafiltrates. The studies identified more than 50 native peptides under the selected conditions. Conclusion: A combination of centrifugal ultrafiltration and LC-MALDI MS/MS mass spectrometry allows identification of biomarker candidates in serum samples. Centrifugal ultrafiltration is a simple procedure for the elimination of high abundance proteins. Analysis of the enriched low molecular weight species led to the identification of several peptides that detect HCC with high prediction accuracy. These peptides may be useful in examining the progression of chronic hepatitis C viral infection to malignancy.

Tues Poster 10: Proteomic Identification of Therapeutic Response Biomarkers in A431 Tissue

Kian Kani[1]; Vitor Faca[2]; Babak Shahbaba[3]; Roland Leuthy[1]; Wenxuan Zhang[1]; Sharon Pitteri[2]; Qing Zhang[2]; Jonathan Erde[1]; Jonathan Katz[1]; Anjali Jain[1]; Parag Mallick[1]; Sam Hanash[2]; David Agus[1]

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Clinical oncology is challenged by the lack of viable biomarkers available for the accurate assessment of therapeutic response, in patient subpopulations, to different pathway-targeted therapies. Consequently, there is a profound need for the application of integrated technologies for the discovery, and translational validation, of biomarkers for individualized therapy. Here we present a study where a panel of Iressa sensitivity markers discovered by proteomic analysis of a cell-line model of cancer showed strong concordance with Iressa response in vivo and in vitro.

A431 tissue, an epidermoid cancer cell line that over-expresses the Epidermal Growth Factor Receptor (EGFR), is a model system for sensitivity to EGFR targeted therapies (e.g. IRESSA). Stable Isotope Labeling of Amino acids in Culture (SILAC) was used with MS Proteomics to quantitatively analyze protein changes in A431 in response to IRESSA treatment. Proteomic analysis was done separately in three sub-proteomes: a) shotgun MS/MS to assess the intracellular proteome; b) biotin-capture-based cell-surface profiling methods, consisting of a comprehensive analysis, that identifies and quantitates constituents of the cell-surface proteome; and c) solid- phase extraction of glycoprotein (SPEG) profiling for the enrichment and subsequent study of the secretome.

We identified 3,707 intracellular proteins, 1,276 cell surface proteins, and 879 secreted proteins (peptide/protein prophet >0.9). Three out of four proteins identified had quantitative information, a subset consisting of 400 proteins showed a statistically significant change in abundance following IRESSA treatment. Using a Bayesian approach we observe that proteins with SILAC ratios (± 1.5) are enriched in integrin signaling and leukocyte extravasion pathways. By coupling SILAC with SPEG enabled we were able to identify proteins whose N-linked glycosylation (e.g. CD44, EGFR, and DSDG1) state was altered in response to therapy. 15 potential biomarkers were discovered and validated using SDS/PAGE and immunoblot.

To translate our in vitro proteomics findings, we transplanted mice with A431 tissue and dosed them with Iressa. A431 xenograft mice demonstrated similar Iressa sensitivity as A431 tissue. The biological response to Iressa of our putative biomarkers was highly similar in vivo and in vitro suggesting our panel of putative biological response biomarkers may be useful for screening models that differ in terms of sensitivity to Iressa and therefore markers of therapeutic response.

Tues Poster 11: Biomarker Identification in Prostate Cancer Cell Lines using EGFR Targeted Treatment: A Proteomics Approach to Individualized Therapy

Kian Kani[1]; Vitor Faca[2]; Lindsey Hughes[1]; Babak Shahbaba[3]; Roland Leuthy[1]; Wenxuan Zhang[1]; Sharon Pitteri[2]; Qing Zhang[2]; Jonathan Erde[1]; Jonathan Katz[1]; Anjali Jain[1]; Parag Mallick[1]; Sam Hanash[2]; David Agus[1]

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Clinical oncology is challenged by the lack of viable biomarkers available for the accurate assessment of therapeutic response in patient subpopulations to different pathway-targeted therapies. Consequently, there is a profound need for the application of integrated technologies for the discovery and translational validation of biomarkers for individualized therapy. We utilize the proteomic profiling of prostate cancer cell lines and ex vivo tumor cells that differ with respect to their sensitivity to EGFR targeted therapies in order to facilitate the identification of biomarkers indicative of therapeutic response.

The use of Stable Isotope Labeling of Amino acids in Culture (SILAC) enabled quantitative analysis of protein changes in response to Tyrosine Kinase Inhibitors (Iressa) and antibody based therapeutics (2C4) in three sub-proteomes: a) shotgun MS/MS to assess the intracellular proteome; b) biotin-capture-based cell-surface profiling methods, consisting of a comprehensive analysis, that identifies and quantitates constituents of the cell-surface proteome; and c) solid-phase extraction of glycoprotein (SPEG) profiling for the enrichment and subsequent study of the secretome.

A total of 7,489 proteins were identified in the three sub-proteomes of the prostate tissues. Cell surface profiling identified 1413,1262 and 1094 proteins in Du145, 22Rv1 and LACP9-AI, respectively, with less than 1% false discovery rate. Protein quantitation was obtained from SILAC ratios or Acrylamide labeling. Preliminary biomarker validation of numerous androgen response genes (e.g. XAR2) and EGFR pathway members was accomplished by immunoblot. Cross validation of biomarkers between cell lines with differential sensitivity to therapeutics will identify markers of clinical response.

Tues Poster 12: A Label-Free Quantitative Proteomics Study to Identify Candidate Breast Cancer Biomarkers in Response to Hypoxia Treatment

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Breast cancer cells cultured at low oxygen levels (hypoxia) undergo a variety of biological responses, including the activation of signaling pathways that regulate proliferation, angiogenesis, cell migration, and apoptosis. Cancer cells have adapted to these growth conditions, allowing tumors to grow and survive. These processes thus contribute to the malignant phenotype and to aggressive tumor behavior. Since many of the oncogenic pathways overlap with hypoxia-induced pathways, we were interested in analyzing and comparing the N-glycoprotein profiles of different cell lines, treated under hypoxic conditions, and thus allow us to discover new, cell-type specific breast cancer biomarkers.

We chose several breast cancer cell lines that reflected the heterogeneity of human breast cancer, as well as different stages of invasiveness. Among them was MCF7, representative of a low-invasive cell line, and Hs578T for a high invasive cell line. We also included, as a control, an immortalized benign cell line derived from primary breast epithelial cells. The breast cancer cell lines were subjected to normoxic (21% O2) and hypoxic (1% O2) conditions for 6 and 24 hours. N-glycopeptides were enriched from these different treated cell lines, and were analyzed using an Orbitrap mass spectrometer.

We used a label-free approach to quantify glycoproteins that were up-regulated upon hypoxia treatment. The Corra pipeline, used for alignment and statistical analyses, generates MS1-based target peptide features, by detecting features that display statistically significant differences upon hypoxia treatment, and among the cell lines. Preliminary data showed a clear clustering of glycopeptide features between the control, the MCF7 and the Hs578T cell lines under normoxic, as well as hypoxic conditions. Moreover, we observed a difference in the hypoxic response between the low and the high invasive breast cancer cell lines. After 6 hours treatment, normoxic and hypoxic glycopeptide features cluster distinctly for the Hs578T breast cancer cell line but not for the MCF7 cell line. After 24 hours of treatment, normoxic and hypoxic glycopeptide features cluster distinctly for the MCF7 cell line but not anymore for the Hs578T cell line. These results indicate that the regulation of glycoproteins upon hypoxia treatment might reflect a cell-specific response, related to invasiveness and cancer cell survival, which could potentially be used to identify new markers for breast cancer. The differentially abundant MS1 features will next be targeted for MS/MS identification.

Tues Poster 13: Regulation of Mucin Expression and Glycosylation by Pro-Inflammatory Stimuli in Pancreatic Cancer Cells

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Mucins are high-molecular-weight glycoproteins that maintain the integrity, lubricate and protect the epithelial surfaces within the human body. Among the mucin members, MUC1, MUC5ac, MUC16 and their glycoforms have been implicated in diagnosis, prognosis, and as therapeutic targets due to their aberrant expression in various malignancies including pancreatic cancer. A high level of tumor-associated inflammation is another indicator of poor prognosis of cancer, although the associated mechanisms are not well understood. In this study, we investigate the hypotheses 1) that inflammatory stimuli (oxidative stress and certain cytokines) may account for the deregulated expression of mucins and their glycoforms in tumor cells; and 2) that the response to the inflammatory stimuli depends on the differentiation status of the pancreatic cancer cells, as defined by the surface expression of three pancreatic cancer stem cell (CSC) markers (ESA, CD24, and CD44). A novel antibody array method developed in our lab was used for the multiplexed detection of mucin core peptides and the glycans on individual mucins. We characterized the levels of the pancreatic CSC-defining markers on fourteen different pancreatic cancer cell lines using flow cytometry, and then measured MUC1, MUC5AC, MUC16 and their associated glycan structures in the secretions from six of the cell lines under different types of inflammatory stimuli, including H2O2, IFNgamma, IL1alpha, and TNFalpha. TNFalpha and IFNgamma induced significant changes in mucin levels and their associated glycan structures in certain cell lines. The two cell lines bearing all three CSC markers, BxPC3 and MPanc96, showed up-regulation of MUC5AC, MUC16 and their CA 19-9 glycan structures, whereas the two cell lines with only one CSC marker, AsPC2 and MIAPaca, showed up-regulation of MUC1, but unaffected or down-regulated levels of CA19-9 on the three mucins. In addition, only the BxPC3 and MPanc96 cell lines showed an up-regulation of galactose and lactosamine glycan structures, as measured by the lectins BPL and RCA120, on the mucins MUC16 and MUC5ac. The increased galactose and lactosamine on mucins is also observed in blood specimens taken from pancreatic cancer patients. Our study demonstrates for the first time that not only the expression of mucin core peptides but also the glycosylation status of mucins can be regulated by a variety of pro-inflammatory stimuli in pancreatic cancer cells. Since these mucins and glycan structures have been functionally linked to tumor-promotion, this response of the CSC-like cells could point to a mechanism by which a pro-inflammatory tumor environment promotes tumor progression.

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Tues Poster 14: Proteomic Strategies for the Identification of Potential Drug Targets within the T cell mTOR-Raptor Mediated Signal Transduction Pathway

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The mammalian Target of the immunosuppressive drug Rapamycin (mTOR) and its binding partner Raptor comprise the central components of an environ-specific signaling pathway essential to cell survival. This pathway is activated by formation of a stoichiometric complex between the protein kinase mTOR and its binding partner Raptor. The newly formed complex then samples the surrounding environment for specific stress indicators (in terms of available nutrients, energy, cytokines, and growth factors). Integration of specific cues from the surrounding intercellular milieu serves as the axis of control, determining the specific nature of the downstream pathway propagated by the mTOR-Raptor complex. The resultant kinase-dependant signal transduction pathway functions to regulate cellular growth, size, and proliferation in a contextually specific way, ensuing that cellular growth or proliferation does not exceed available intercellular resources.

In T cells we hypothesize that this Raptor-dependent mTOR-mediated signaling pathway integrates environmental cues in the presence or absence of cellular stress and thereby dictates the ultimate outcome of antigen recognition (tolerance or activation) by the T cell receptor complex (TCR). Thus, the mTOR-Raptor signal transduction pathway plays a central role in determining the outcome of antigen recognition and is of critical importance to the regulated functioning of our immunodefense system.

The ability to selectively target and employ the host's immune system against self-cancerous cells has been the topic of intensive investigation for many cancer researchers. Given the significance of the mTOR-Raptor signaling pathway in either activating or anergizing the host's cell-mediated (T-cell dependant) immune response against a specific antigen, the success of an immuno-specific cancer defense may be dependant upon one or more of mTOR or Raptor's protein signal partners. However, a comprehensive elucidation of the pathway is lacking from the current literature.

The current work proposes to identify the major proteins implicated in both the T cell activation mTOR pathway and the T cell tolerance mTOR pathway using a gel-independent proteomics approach. Immune response to antigen recognition will be mimicked in vitro by addition of external signal factors with conditions replicating both anergistic and activation signaling. Co-immunoprecipitation with antibodies specific to either Raptor or mTOR will be preformed on differentially stimulated cellular lysates to isolate proteins associated with the mTOR-Raptor signaling pathway. IP elutant will be subject to digestion with proteomics grade trypsin. Digestion will be coupled directly to LC/multistage tandem MS (MSn) analysis performed using a Thermo Scientific LTQ-Orbitrap (Sunnyvale, CA). Analysis will incorporate a full mass scan, in-source collision-induced dissociation (CID), and data-dependent MSn. Identification of resultant peptides will be based upon data dependent CID analysis. In parallel to the global proteometric identification of Raptor and mTOR signalling partners, we hope to map the differential phosphorylation sites of mTOR itself. Previous work by our group suggests that the site-specific phosphorylation pattern of mTOR is dependant upon specific signal transduction during antigen recognition. By using MS/MS spectra obtained using the electron transfer dissociation (ETD) capability of the Orbitrap mass spectrometer, we hope to confirm our previous phosphorylation map and elucidate previously undetected phosphorylation sites.

Tues Poster 15: Differential Protein Expression Analysis of the MCF7 Breast Cancer Cell Line using iTRAQ-RP-LC/MS/MS

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Breast cancer is one of the most frequent forms of cancer affecting women in United States and across the world. Although the pathway that leads to breast cancer development remains to be completely elucidated, certain hormones such as estradiol are known to play a major role in breast cancer development. Innovative treatments are constantly being implemented to fight this form of cancer; among which tamoxifen has proven effective in improving survival rates. To gain a better insight into the proteome of cancerous breast cells, and to understand the effect that estradiol and tamoxifen have on breast cancer development and treatment, a quantitative proteome study is proposed using the MCF7 breast cancer cell line as a study model.

Here, we report an iTRAQ-LC-MS/MS strategy for the quantitative proteomic analysis of MCF7 breast cancer cell lines using a linear ion trap LTQ instrument, operated under the PQD mode. The developed iTRAQ-LC-MS/MS approach was implemented for protein differential expression analysis in MCF7 cells grown in the presence of estradiol and tamoxifen. MCF7 breast cancer cells were divided in four batches, cultured, and harvested with estradiol and/or tamoxifen. Following lysis, the protein extract was spiked with 10 standard proteins, treated with trypsin, and cleaned up with C18 SPE cartridges. The resulting peptide mixtures were separately labeled with the iTRAQ reagents 114, 115, 116 and 117. These four fractions were then combined and cleaned up with SCX SPE cartridges; after which, RP-LC/MS/MS analysis were conducted. Data processing was performed with the BioWorks 3.3 software. In an extended application, the implementation of this strategy to microfluidic chips was explored for the first time.

Experiments conducted with standard proteins revealed a labeling efficiency reaction of ~ 100 %. Proteins were reproducibly identified across replicate LC-MS/MS runs, with ~ 65-70 % overlap of identified proteins with $p < 0.001$ between duplicate runs, ~ 50 % overlap among triplicate runs. The accuracy of the method was determined to be influenced by protein concentration, sample complexity, and increased with the number of peptides used for protein identification. For example, > 85 % of the proteins quantified in MCF7 extracts by 5 unique peptides had % variations of iTRAQ ratios within 0-30%. 407 proteins with $p < 0.001$ were identified in MCF7 cell treated with estradiol and/or tamoxifen from a multiconsensus file containing data from five consecutive LC/MS/MS analysis; a list of up and down regulated proteins was generated from these results. The standard deviation of the experimental average iTRAQ ratios calculated for five LC/MS/MS runs was within the range of 0.08-0.09, demonstrating the reproducibility of the analysis. These results provide a valuable insight into the performance of the iTRAQ/PQD technology for protein quantitation and protein differential expression analysis.

Tues Poster 16: Combination of Affinity Depletion of Abundant Proteins and Reversed Phase Fractionation in Proteomic Analysis of Human Plasma/Serum

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Serum and plasma represent the most complex sample of the human proteome, composed of the homeostatic blood proteins as well as tissue leakage proteins. The tremendous complexity of this biofluid proteome presents extreme analytical challenges in comprehensive analysis due to the wide dynamic range of protein concentrations (spanning over 10 orders of magnitude). Consequently, robust sample preparation methods remain one of the important steps in the proteome characterization workflow.

Depletion of high-abundant proteins in serum and plasma has become an essential, routine and accepted technique. These high-abundant protein components interfere with identification and characterization of important low-abundant proteins by limiting the dynamic range for mass spectral and electrophoretic analyses. We are presenting the results on a new column for the specific depletion of 14 high-abundant proteins from serum and plasma. Through depletion of the 14 high-abundant proteins we are removing ~94% of the total protein mass. The depletion process is robust, easily automated and highly efficient. The column depletes the 14 targeted proteins reproducibly during 200 runs and has excellent depletion efficiency as determined by ELISA. Results on

the identification of the bound proteins indicate specific removal of the targeted proteins.

We have depleted plasma of 14 high-abundant proteins and performed a subsequent fractionation using a high-recovery macroporous reversed-phase column (mRP-C18). The chromatographic conditions and methods enabled high protein recovery while permitting robust and reproducible fractionation. The collected column fractions were trypsin-digested and analyzed on a microfluidic HPLC-Chip/MS system, providing a reliable and fast peptide separation combined with ease of use, robust ionization and fast data acquisition. High-abundant protein depletion and RP fractionation of plasma showed an improved dynamic range for proteomic analysis resulting in reduced ion suppression in electrospray MS. The multi-dimensional workflow approach presented here allowed the identification of low-abundant plasma proteins.

Tues Poster 17: Discovery of O-Linked Glycoprotein Cancer Biomarker with Multi-Lectin Enrichment and Lectin Microarray Binding Pattern in Human Sera with MALDI-QIT

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Introduction: Pancreatic cancer is one of the most dangerous cancers with a 5-year survival of less than 4%. Discovering new biomarkers in serum with high sensitivity and specificity would increase the chance of early prognosis of the cancer. Glycosylation is the most common modification in serum and both N- and O-linked glycoprotein have been found responded to different kinds of cancers. Compared to N-linked glycosylation, the mapping of O-linked glycosylation is still under development. Due to lack of a general core structure in O-linked glycans, O-linked glycoproteins are more difficult to selectively enrich from serum. And coexistence of N-linked and O-linked glycan on single protein furthermore complicates this task.
Method: We here present a new method of biomarker discovery by mapping O-linked glycoproteins with multi-lectin enrichment and validating the potential biomarkers with lectin microarray binding patterns. The serum sample is first depleted by IgY antibody column. The flow-through fractions are incubated with PNGase F for 24 hours to release the N-linked glycans as much as possible. Then affinity columns packed with Jacalin and HPA respectively are used sequentially to capture the O-linked glycoproteins. Elutions from the lectin affinity columns are combined and separated by RP-HPLC to find the differences between normal and cancer sample in the UV chromatogram. Interesting RP-HPLC fractions are further digested and analyzed by ESI-MS/MS for protein IDs. O-linked glycans are released by beta-elimination and analyzed by MALDI-QIT.

When O-linked glycoproteins are found have changed either their expression level or glycosylation structure in cancer serum, they are selected as potential biomarkers and their lectin binding patterns are studied by antibody microarray with lection detection. After the antibodies are printed, the slides for the microarray study are chemically blocked following a protocol developed by Brian Haab to prevent nonspecific lectin binding to glycans on the antibodies. The slides are incubated with normal and cancer sera and the captured proteins are detected by a number of lectins targeting different glycan structures.
Result: Sera sample were tested for this procedure and result showed simplified chromatogram and a lot of new peaks coming out in hydrophobic fractions. ESI-MS/MS identified more than 20 glycoproteins with O-linked glycosylation, 10 of which have both N- and O-linked glycosylation and were not identified before when the sera were first cleaned with ConA to get rid of N-linked glycoproteins.

Tues Poster 18: Proteome Characterization of Prostate Proximal Fluids as a Source of Cancer Biomarkers

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The current strengths and limitations of the prostate specific antigen (PSA) serum test for early detection of prostate cancers, as well as its limitations in prognosis and treatment planning, are well documented.
The search for other serum biomarkers of prostate cancer in serum or plasma remains a challenge due to low concentrations of prostate specific proteins in these fluids. Prostate tissues as a biomarker source are generally available in limiting amounts and have many issue related to their acquisition. We have begun collection and evaluation of a new fluid for prostate cancer biomarker discovery, expressed prostate secretions (EPS). EPS represent proximal fluids secreted by the prostate gland which can be collected in urine, or prior to surgery. Based on the collection of EPS from men with benign disease and prostate cancers, comprehensive characterization of the proteome of these fluids has been initiated.

The EPS urines were obtained following gentle prostate massage during digital rectal examination prior to scheduled prostate biopsy procedures. For comparison, a subset of individuals provided urines for baseline comparison prior to EPS urine collection. Undiluted EPS was collected pre-operatively from individuals with prostate cancer prior to prostatectomy. A combinatorial proteomic approach involving sample fractionation prior to mass spectrometry identification was used. This included lectin capture of glycoproteins, two-dimensional gel and liquid chromatography separations combined with quantitative isotope tagging methods. Following tandem mass spectrometry sequencing of proteins within these fractions, over 500 individual proteins have been cumulatively identified. The predominant molecular function of these proteins were proteases, signaling and growth regulation, cell adhesion and receptors. Prostate specific antigen and prostatic acid phosphatase were two of the most abundant proteins identified in EPS and are present at microgram per milliliter concentrations as compared to low nanogram per milliliter levels in blood. Analysis of the protein constituents in the pre-prostate massage urines and undiluted EPS samples were also done to confirm which proteins were of prostate origin.

EPS represents a rich reservoir of potential prostate cancer biomarkers, and its collection can be easily incorporated into routine prostate examinations for benign and cancerous conditions. The high concentration of secreted glycoproteins and regulatory molecules will facilitate characterization of expression differences in disease states like cancer, and for characterization of potential post-translational differences like glycosylation.

Tues Poster 19: Identification of Liver Cancer Biomarkers through Metabolic Oligosaccharide Engineering

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Biomarkers are indicators of an organism's normal metabolism. Those that are uniquely or aberrantly expressed in cancer can be used to identify cancerous tissue and thus represent potential diagnostic targets. To date, few protein biomarkers are used for reliable cancer detection and diagnosis. Discovery of new cancer-specific biomarkers would alleviate the heavy demand for new cancer diagnostics. A number of changes in glycosylation have been associated with malignant transformation and may contribute to the proliferation and metastatic potential of cancer. Given the diversity of oligosaccharides on cell surfaces, novel cancer-associated glycoproteins represent an untapped source of new biomarkers. Previous research in our laboratory has established that metabolic oligosaccharide engineering (MOE) using azide-modified monosaccharides reveals patterns of glycosylation in disease in immortalized human cell lines and in healthy mice when probed with appropriate secondary chemical labeling agents. This form of labeling gives a specific metabolic readout of glycoprotein biosynthesis in cells and tissues and reveals changes in protein expression as a function of disease state, drug treatment, or other biological stimuli, providing more valuable metabolic information

than traditional, non-specific labeling methods. MOE has now been applied to the identification of novel cancer biomarkers in a conditional transgenic mouse model of liver cancer, in which the human oncogene, c-myc, is constitutively expressed. Analysis of tumor compared to healthy tissue using MOE reveals metabolic differences that may represent potential biomarkers of disease.

Tues Poster 20: The Breast Cancer Salivary Fragmentome

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Serum and plasma peptides and proteins have been associated with many disease states such as cancers, diabetes, neurological and cardiovascular diseases [1]. Despite the limited success of a handful of biomarkers, most diseases lack sensitive and specific serum biomarkers. The only current minimally-invasive clinical biomarker tests in regular use for cancer are the prostate-specific antigen (PSA) test for prostate cancer, CA-125 for ovarian cancer, and NMP22 for bladder cancer. Salivary diagnostics offers a new opportunity for less-invasive tests and potential diagnostic tools for disease detection and surveillance [2]. High-resolution mass spectrometry is herein applied to breast cancer saliva samples to explore the low molecular weight saliva fragmentome. Samples were fractionated using affinity capture and hydrophobic enrichment. Analyses by MALDI Orthogonal-TOF MS and LC-MS/MS identified over 400 interesting protein fragments and several potential salivary biomarkers for early stage breast cancer, including an over-expression of a fragment of Proline-Rich Haelli Subfamily 2 (PRH2) protein at 1731.9 Da. This finding is further supported by quantitative gene expression profiling experiments of cancer tissues (GEPIS database). Other potential biomarkers, including markers correlating to HER2 positivity, will also be presented. This data demonstrates the viability of utilizing salivary fragmentomics for biomarker discovery and provides a powerful new diagnostic opportunity.

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Tues Poster 21: Improving the Discovery Potential of 2D PAGE Proteomic Projects by Introducing a New Image Analysis System

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It is a well-established problem that the quality of two-dimensional (2D) gel electrophoresis results can suffer from personal bias and/or lab-to-lab variability during the image analysis stage. In addition, the Combined Correctness of 2D gel image analysis results has been shown to be the single most deciding factor in order to maximize discovery potential and minimize error rate. However, using conventional 2D gel image analysis software such as Progenesis Samespots, PDQuest, Decyder, ImageMaster etc, there is no way of monitoring and measuring this parameter in the 2D gel image analysis. Hence, using conventional software you are left to do 2D gel image analysis until you think it “looks good”. As a control of the reliability of your results, you can calculate the mean-CV. But recently, mean-CV has been shown not to correlate very well to a high discovery potential and a low error rate in 2D gel image analysis. Thus, a new way of performing 2D gel image analysis is needed that minimizes personal bias, eliminates lab-to-lab variability, optimizes discovery potential, and decreases error rate. We have devised a new system for analyzing 2D gel images that addresses all the abovementioned problems.

The system comprises a professional image analysis center with standardized working procedures and well-defined quality metrics and uses proprietary software that allows optimizing Combined Correctness. Scientists are able to incorporate this system into their 2D gel proteomics workflow through a powerful, user-friendly, and workflow driven software that enables uploading images and the subsequent exploring of results. In this study we show that this system minimizes personal bias, eliminates lab-to-lab variability, optimizes discovery potential, minimizes error-rate and decreases the overall turn-around time for 2D gel proteomics projects.

Tues Poster 22: Accurate Re-estimation of Precursor-Ion Mass Improves Peptide Identification

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Mass spectrometry-based proteomics experiments have become an important tool for studying biological systems. Identifying the proteins in complex mixtures by assigning tandem mass spectra to peptide sequences is an important step in the proteomics process.
Hybrid instruments, like the LTQ-FT, have two mass spectrometers that operate mostly in parallel: a slower Fourier transform-ICR (FT) and a faster ion trap (IT). A typical cycle consists of an IT MS scan followed by an FT MS scan in parallel with several IT MS/MS scans whose precursor ions are selected in a data dependent manner. The determination of which ions are to be selected can use either the information from the preceding IT spectrum or from a medium resolution preview scan derived from the first quarter of the FT scan as the data are being acquired. The triggering-m/z is an estimate of the intact parent m/z of the peptide selected for fragmentation and is used by the identification program to aid in the inference of the sequence.
In both the IT and FT scenarios the reported precursor m/z values do not take advantage of the high resolution of the full FT spectrum. Consequently, the difference between the inferred parent mass and the actual peptide mass can be significant. For example in datasets with identified peptides from 18 known proteins the mean difference between the estimate of the precursor mass value and the calculated mass of the identified peptides is 171 ppm for ion trap data and 56 ppm for FT-triggered MS/MS. These deviations are significantly larger than the expected 2 ppm mass accuracy of the hybrid instruments. To improve these deviations we introduce msPrefix, a program that intercedes between data collection and computational identification to improve the precision of the precursor mass by inspection of the preceding full-resolution FTMS survey-scan. This re-estimation significantly improves delta mass: For the datasets mentioned above, the IT triggered data now has a mean of 0.7 ppm and for FT triggered data the mean delta mass is now 1 ppm. These deviations are more consistent with the expected mass accuracy of the instrument.
To determine the impact msPrefix has on peptide identifications we next performed our TANDEM searches using a variety of precursor tolerance cutoffs both with and without msPrefix. By using msPrefix, the majority (98 percent) of correct identifications are covered with a threshold below 4 ppm for both IT and FT-triggered experiments. To achieve a similar number of true positives without msPrefix a threshold of 1000 ppm was required.
In order to test the performance of msPrefix for complex samples we applied it to blood plasma samples and RV1 whole cell lysates. In the case of the whole cell lysates, at its optimal precursor tolerance value of 200 ppm msPrefix confidently identifies about 200% more peptides than using native data with the same tolerance, and 20% more peptides than searching native data at its best tolerance of 1000 ppm.
These results suggest that integration of msPrefix into shotgun proteomics workflows can significantly improve identification results.

Tues Poster 23: The ProteoWizard Library: An Open Source Software Library for Rapid Proteomics Software Development

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We introduce the ProteoWizard Library (PWL), a software library providing a basic proteomics analysis framework, including data file access, chemistry computations, and LCMS dataset analysis. Although a common set of computations are widely used throughout the proteomics community, there is no single standard, cross-

platform, modular and extensible software package. Here we present a draft library to fill this need.

PWL has been designed with three primary layers: utility, data, and analysis. The **utility layer** contains common computations and operations like binary-to-text data encoding (including floating point precision conversion, endianization, and compression), XML parsing and writing, file hashing, COM object communication for RAW file access, and miscellaneous mathematical functions. The **data layer** provides access to data objects, like spectra, peaks and features, and supports reading and writing of RAW, mzXML, mzML, MIDAS, FASTA, and emerging peaks and feature file formats. PWL provides an LCMS data file abstraction interface that allows file format-independent data access. This data file abstraction is built using a newly-designed C++ model of the HUPO-PSI mzML standard, with typesafe access to the PSI-MS controlled vocabulary. A convenient and flexible iterator interface handles data file traversal, freeing the programmer to focus on analysis of the data. The **analysis layer** contains common proteomics computations, including calculations involving proteins, peptides, and chemical formulas for determining ion m/z's and theoretical isotope envelopes. Also included are modules for computing LCMS run statistics, selected ion counts, extracting data slices, psuedo-2D gel plotting, and peak detection and estimation.

Applications (e.g. the recently released raw2xml converter, msPrefix and msPicturePlus) are easily constructed using these three interfaces. PWL is cross-platform using native compilers (MSVC on Windows, gcc on Linux/Cygwin/OSX), and was built from the ground up using modern C++ techniques. PWL source code is available from http://www.sfcap.cshs.org/download. PWL is actively maintained with community feedback welcomed.

Tues Poster 24: Simplified Extensive Peptide Identification Using Sequence Temperature Values and Feature Probabilities

Ignat V. Shilov; Alpesh A. Patel; Wilfred H. Tang; Alex Loboda; Christie L. Hunter; Lydia M. Nuwaysir; Daniel A. Schaeffer; Sean L. Seymour

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Informatics methodologies to more deeply characterize the peptides and proteins analyzed in bottom-up proteomics by mass spectrometry beyond what was possible with early search engines are still evolving. One approach to identifying peptides with less common features like non-tryptic termini, infrequent modifications, and amino acid mutations is the 'multi-pass' approach where search space is expanded stepwise in a series of searches. Although some search engines now enable this approach, optimal search parameterization is only possible for researchers with substantial informatics expertise. Thus, a tool that could achieve at least the same depth of analysis as multi-pass approaches without a complicated search workflow would enable a broader range of researchers to conduct quality proteomics experiments. Although some would argue 'the world does not need another search engine', it is with this motivation that we have developed the Paragon™ Algorithm for peptide identification.

Our recent report [Shilov, I. et al, Mol. Cell. Proteomics, 6:1638-1655, (2007)] presents this algorithm and a benchmarking comparison to Mascot. The first of the two key technical innovations of this tool is the use of Sequence Temperature Values (STV), which measure the extent to which each 7-residue segment of a protein database is implicated by the collective evidence of many small sequence 'taglets' of various certainties from a single MS/MS spectrum. The size of search space is modulated on a continuum proportional to the STV of each segment, making this the first algorithm we are aware of that applies different search space to different regions of a database for the search of a single spectrum. Not only does this continuous modulation of search space obviate the need for segmentation strategies in multi-pass approaches, but it may also yield fundamentally better results. The second technical innovation provides the quantitative approach to determine which features are allowed at any point in the spectrum of small to large search space. A translation layer allows the user to set up a search by simply describing what was done in the lab, and the implications of these actions are automatically translated into a probability-based description of features like modifications and digestion cleavage events. For example, one enters the name of the reagent used for cysteine alkylation such as 'Iodoacetamide', rather than the modifications that would result from it.

We will present the results of our initial report as well as subsequent advances in several areas, including confidence model and error rate assessment improvements, the applicability of the Paragon Algorithm to all mass spectral platforms, and implications for protein inference.

Tues Poster 25: Advances in the Assembly and Use of Annotated Reference Libraries of Peptide MS/MS Spectra

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Searching libraries of consensus mass spectra has been shown to be a more sensitive method for the identification of peptides by tandem mass spectrometry than sequence searching [1, 2, 3]. The primary reason for this is that the relative abundances of all consensus peaks (e.g. sequence, neutral loss, etc.), derived from actual spectra, are captured and annotated in the library record. This information can be directly used by modified dot product or similar correlation-based scoring algorithms to more robustly score experimental spectra. The major limitation of this approach has been the availability of comprehensive, curated reference libraries. In this talk we will discuss the latest releases of the NIST libraries of peptide fragmentation spectra. The current human library has been compiled from >16 million peptide identifications, extracted from >50,000 data files. It contains 187,790 consensus spectra, identifying 116,839 distinct peptide sequences. Each of these peptides maps to one or more proteins in the Human IPI database (3.10) covering a maximum of ~12% of all protein sequences. Each identification has been made by up to 4 separate search algorithms and re-scored based on a marginal false positive rate calculated from a decoy database search. Identifications passing a threshold of 5% false positive were entered as candidate replicate spectra. Protein matches represented by only a single peptide ion were required to pass more stringent filters. Clusters of 100 or less best-scoring replicate spectra from different sources were used to construct a single consensus spectrum for each ion. The library contains roughly 90% tryptic peptides and 10% semi-tryptics. Modifications include C carbamidomethylation (25,229), M oxidation (22,933), C ICAT (10,585), n-term pyro-glu (5,093), n-term acetylation (2,401); unmodified peptides (125,909) make up the majority of the consensus spectra. This represents a ~4-fold increase since the initial June 2006 release due to the inclusion of many new public and private data donations. The majority of the candidate spectra were acquired on Thermo LCQ, LTQ and hybrid-LTQ mass spectrometers. We will outline the construction of these new libraries, discuss their composition in detail, and address current methods to search them individually or in combination with sequence searching (OMSSA). Relative performance compared to 'current practice' and to searching theoretical mass spectra [4, 5] will also be discussed.

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Tues Poster 26: A Graph-Based Approach for Protein Identification

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Tandem mass spectrometry has been a very important technique used in protein identification. However, it is challenging to determine the amino acid sequence from a tandem mass spectrum due to noisy and missing peaks. Moreover, there are generally two different ion types (b-ions and y-ions) in a spectrum and they cannot be included at the same time to generate a protein sequence. We introduce a new linear-time graph-based approach that can identify noisy peaks and the ion types of the other peaks in a spectrum. Each peak in a spectrum is a vertex and we connect pairs of vertices with different edges depending on the differences of their mass values. We then cut the graph into three sets: a set of b-ion peaks, a set of y-ion peaks and a set of noisy peaks. Our experiments show 93% to 100% for our 16 experimental spectra and 96% to 100% accuracy for 2000 simulated spectra.

Tues Poster 27: Peak Detection and Quantitative Analysis of Isotope Labeled Samples

C. Nicole Rosenzweig; Hui Zhang; Yuan Tian; Zhen Zhang; Daniel W. Chan
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The simultaneous comparison of proteins derived from cancer and noncancer individuals is the hallmark of cancer proteomics research. Identifying biomarkers with the potential for discrimination relies on a quantitative comparison of profiles. Isotopic labeling provides a relative measurement of the amount of protein in multiple samples. In order to accurately identify the isotope-labeled protein species, reliable monoisotopic peak detection is required. In cases where the labeled peptides are co-eluted, additional contextual information is available within the spectra which can be used to improve peak detection.

Two separate 2DNanoLC-MALDI evaluations were analyzed by the method described here. In the first experiment, sera from healthy mice, sera from cancer-laden mice, and mouse skin cancer tissue were evaluated simultaneously by labeling with D0 (light), D4 (+4) and D4-13C4(+8) succinic anhydride respectively. The labeled mouse cancer tissue was evaluated again in a second independent experiment. The samples were separated via 2DNanoLC and analyzed on the Applied Biosystems 4800 TOF/TOF mass spectrometer. A novel analysis method was developed to identify monoisotopic peaks as well as matching the isotopic cluster pattern in the tissue-only experiment. Raw spectra files collected from the mass spectrometer are used for analysis. In this experiment, proteins with an isotopic cluster from both tissue and sera from cancer-laden mice were of particular interest. Because an isotopic cluster from at least two of the protein sources was required to be observed in the combined experiment, heuristics could be added to the peak detection algorithm evaluating contextual information present in the spectra.

The algorithm uses a rigid description of a singly charged isotopic cluster to aid in the detection of the monoisotopic peaks without using isotope distribution calculations. Each LC-MALDI experiment is evaluated independently in the first phase. Once one isotopic cluster was identified, the additional isotopic clusters were searched for at the user defined mass differences with similar contextual patterns. The same numbers of isotope labels are not required to be used in all the experiments. Once all peaks were selected, peaks were compared between the experiments. A comparison of monoisotopic peak detection completed by the ABI 4800 demonstrated that a clear improvement in accuracy was made by this algorithm. Further, a number of peptides were identified which were differentially expressed in the normal and cancer sera samples. This method improved peak detection and identified isotope-labeled peptide species both within and between LC-MALDI experiments.

Tues Poster 28: Performance Comparison of *de novo* Peptide Sequencing Algorithms and Identification of a Novel Zinc-Dependent Membrane Protein of Amycolatopsis Japonicum

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Protein identification is most often performed using tandem mass spectrometry, whereby tryptic digests of proteins are fragmented to reveal their amino acid sequence. For proteins of known sequence several computer programs exist that can match peptide tandem mass spectrometry data to their exactly corresponding database sequences. However, we frequently obtain high-quality tandem mass spectrometry spectra of tryptic peptides from proteins that have not previously been sequenced and therefore no obvious database match can be made. Since de novo sequencing of peptides manually is time-consuming, we evaluated the performance of four different sequencing algorithms that automatically identify sequence tags for each peptide. For this study we measured a tryptic BSA digest using a quadrupole TOF (QSTAR Pulsar, ABI) and a triple quadrupole linear ion-trap mass spectrometer (QTRAP 4000, ABI). The resulting spectra were analysed by de novo peptide sequencing with BioAnalyst (ABI), MASCOT Distiller (Matrixscience), PEAKS Online (Bioinformatics Solution Inc.) and PepNovo (http://proteomics.bioprojects.org/MassSpec/). In general, all four algorithms performed better for QSTAR data than for QTRAP data. The performance order of the algorithms is PEAKS online > MASCOT distiller > BioAnalyst > PepNovo for data generated using both QSTAR and QTRAP. Compared with the other three algorithms, PEAKS online out-performed the tested programs by a factor of 2 or greater as it identified at least 9 sequence tags of at least 10 amino acids in length. For ion trap data, 81% of the acquired peptides were sequenced correctly and 5 peptides of 41 (12%) were sequenced completely correct by PEAKS online. For quadrupole TOF data, 88% of the acquired peptides were sequenced correctly and 14 peptides of 34 (4%) were sequenced completely correct by PEAKS online.

In this study, PEAKS Online provided the best algorithm for automated de novo sequencing and this algorithm was therefore used to identify novel proteins from Amycolatopsis japonicum, an organism not fully genome sequenced to date. A. japonicum synthesises EDDS (ethylene-diamine-disuccinic acid), a structural isomer of the synthetic chelator agent EDTA (ethylene-diamine-tetraacetic acid). Unlike EDTA, EDDS is fully biologically degradable and is therefore a potential substitute for EDTA in detergents and cosmetics. For A. japonicum EDDS serves as a zinc siderophore, hence EDDS is exclusively produced under depleted zinc conditions. Zinc concentrations above 3 µM completely suppress EDDS production.

To find differentially expressed proteins we compared A. japonicum cultivated on zinc medium with A. japonicum grown in the absence of zinc. From a 2D gel one protein spot was extracted, digested with trypsin and, by using the QSTAR data and PEAKS online algorithm for de novo sequencing, we obtained high quality spectra and sequenced a total of 6 peptides. For evaluation and identification of the respective protein at the DNA level, we selected 2 of these 6 peptides and accordingly synthesised 2 degenerate DNA primers. That way we identified the zinc-dependent membrane protein/gene of A. japonicum with homology to a YibE/F-like protein from Saccharopolyspora erythrea. Note: Claudia Fladerer and Stephan Jung contributed equally to this work.

Tues Poster 29: Respiratory Syncytial Virus (RSV) Induces Cellular Oxidative Stress by Down Regulating the Expression of Antioxidant Enzymes

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Respiratory syncytial virus (RSV) is one of the most important cause of lower respiratory tract infection in infants and young children. Oxidative stress has been shown to play an important role in the pathogenesis of acute and chronic lung inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD). As a protective mechanism from oxidative injury, various antioxidants such as superoxide dismutases (SOD 1, cytosolic copper zinc SOD; SOD 2, mitochondrial manganese Mn SOD and SOD 3, extra cellular EC SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase-mu (GST-mu) are produced in the system. We have previously shown that RSV infection induces reactive oxygen species (ROS) in vitro and oxidative stress in lungs in vivo. Using a proteomics approach to detect changes in protein expression in the airways of infected mice, we identified a number of antioxidant enzymes that were down regulated following RSV infection. To further investigate the mechanism of RSV-induced oxidative injury, we measured expression and activity of SOD1, SOD2, SOD3, catalase, GPx and GST-mu in A549 cells, a type II-like alveolar epithelial cell line, and in small airway epithelial cells (SAE), normal airway epithelial cells derived from terminal bronchioli. RSV infection induced a significant increase of lipid peroxidation products such as malondialdehyde (MDA), 4-hydroxylnonenal (HNE) and F2-8 isoprostanes in infected cells compared to control cells. There was a significant decrease in the levels of GSH/GSSG ratio in RSV compared to control cells. There was also a significant decrease in expression of SOD 1, SOD 3, catalase, GPx and GST with a concomitant increase in the concentrations of SOD 2 in infected A549 cells compared to control cells. Enzymatic assay results showed that total SOD activity was increased but catalase, GPX and GST activities were decreased. Similar results were also found in SAE cells. These findings suggest that RSV-induced cellular oxidative damage is the result of an imbalance between ROS production and antioxidant cellular defenses. Oxidative stress and chronic lung inflammation are the important features in the pathophysiology/pathogenesis of RSV. From our study since it is evident that a variety of antioxidants are implicated in the pathogenesis of RSV it is possible that therapeutic administration of multiple antioxidants will be effective in the treatment of RSV and other airway inflammatory diseases. Alterations in the oxidants and antioxidants in RSV infected cells revealed that diminished antioxidants serves as a biomarker of the inflammation induced by RSV and can be used in drug development for RSV infections.

Tues Poster 30: Time-Dependent Changes in Plasma Proteome Caused by Interferon/Acetaminophen Co-Therapy Induced Hepatotoxicity

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Interferons (IFNs) are biologics most commonly prescribed as therapy against various forms of hepatitis, tumors, leukemia, multiple sclerosis, AIDS-related Kaposi's sarcoma and human papilloma virus. Interferon is implicated in a significant number of adverse drug reactions, the most common being flu-like symptoms. Acetaminophen (APAP) is commonly used in combination with IFNs to help with the relief of these symptoms. There is a potential for adverse reactions resulting from co-therapy in addition to adverse reactions seen in treatment with IFN or APAP-only. It is of interest to investigate how the adverse drug reactions correlate to the blood plasma proteome changes when co-therapy or mono-therapy is applied in order to better understand the biologic basis of these effects. We found previously that the degree of up or down-regulation of expression of plasma proteins in co-therapy in comparison to the IFN-only therapy depends on the dose of interferon. Here, we present a detailed time-dependent study of the influence of IFN/APAP co-therapy on protein expression in mouse plasma using a proteomics approach. Experiments were performed in vivo on CD-1 mice, by injection with high doses of: IFN only, APAP only and IFN/APAP in combination. The doses were determined by applying findings of a previous study. The mice were sacrificed via cardiac puncture under anaesthesia at different time intervals after dosing: 1.5 h, 4 h, 8 h and 24 h. The blood was collected and plasma separated from the cells using the polymeric erythrocyte aggregating agent Ficoll. Blood plasma was depleted using Proteome Lab IgY-R7 spin columns that separate seven most abundant plasma proteins from the remainder. Proteomes of high-abundant and low-abundant fractions were then analyzed. To identify the whole blood plasma protein content, in-solution and in-gel tryptic digests were applied, followed by analyses of resulting mixtures of peptides by liquid chromatography and tandem mass spectrometry using a Waters UPLC-Q-TOF MS Global Ultima system. Proteins were identified using a Mascot search engine. To quantify protein changes between mice injected with different therapeutics and sacrificed at different times, iTRAQ quantitation and comparison of search scores were utilized. Results show the differences between the proteins identified in plasma of mice injected with a co-therapy compared to mono-therapy as well as both compared to the control. The quantitative differences in the protein expression among differently treated mice will be discussed in detail with emphasis on the change of protein expression with time.

Tues Poster 31: Proteomic Analysis of Gill of Penaeus Monodon Identifies Ribosomal Protein SA/Laminin Receptor Homologue as the Yellow Head Virus Binding Protein

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Yellow head virus is an RNA virus that causes the yellow head disease in Penaeid shrimp. To understand the response of shrimp during viral infection, a 2-DE technique was used to analyze the differential expression of shrimp proteins. Penaeus monodon shrimp were intramuscularly infected with YHV or mock-infected. At 6, 18 and 36 hour post infection (hpi), the protein lysates from gill tissues were subjected to 2-DE analysis. With respect to the mock-infected shrimps, nineteen protein spots were differentially expressed with at least a 1.5-fold increase or a 0.65-fold decrease following viral infection. One of these differentially expressed proteins, the ribosomal protein SA (RpSA/ laminin receptor), was up-regulated and modified at 6 hpi. A virus overlay protein binding assay revealed that YHV was capable of binding to recombinant RpSA in situ. Far-Western analysis showed that the recombinant RpSA bound to the YHV gp116 structural protein. An in vitro GST-pull down assay and co-immunoprecipitation of YHV with RpSA using anti-serum against YHV also confirmed the interaction of both proteins. Immunofluorescent microscopic analysis of YHV-infected hemocytes showed that the YHV gp116 envelope protein was co-localized with shrimp RpSA.

Tues Poster 32: A Targeted Comparative Proteomic Approach for Identification of Phosphorylation Dependent Protein-Protein Interactions of α-Synuclein

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Mutations in the gene coding for the neuronal protein a-synuclein (aS) have been linked to familial Parkinson disease (PD). S129 phosphorylated aS has been shown to be highly enriched in Lewy bodies, a pathological hallmark of PD. Our lab has also shown that the C-terminus tail of aS can be phosphorylated on Y125. However, little is known of the normal function of aS phosphorylation. In this work we use a mass spectrometric based targeted functional proteomic approach to identify qualitative and relative quantitative differences in protein-protein interactions of the phosphorylated versus non-phophorylated C-terminus of a-synuclein.

Biotinylated peptides of the aS C-terminus tail have been synthesized as non-phosphorylated, S129 phosphorylated, Y125 phosphorylated, and a scrambled sequence as a control for non-specific binding. Protein pull-down assays are performed on streptavidin coated magnetic beads, where the bound peptide is incubated with mouse brain synaptic lysate. Eluted proteins are separated by SDS-PAGE. Each lane is cut into 40 slices, in-gel digested, and analyzed by LC-MS/MS followed by protein sequence library searches for peptide assignments. All pull-down assays were performed in triplicate with different pooled lysates. To facilitate parsimonious assignment, comparison of datasets, and relative quantification of peptide and putative protein assignments across these large datasets, both for a given gel and across gels, in-house software MassSieve was developed.

This data provides evidence that nonphosphorylated aS preferentially interacts with mitochondrial electron transport chain proteins, while phosphorylated aS interacts with cytoskeletal proteins, which suggests a change in function of aS upon phosphorylation.

Tues Poster 33: Proteomic Profiling of Activated Macrophages by Isotope Coded Affinity Tagging and Capillary Electrophoresis with Laser-Induced Fluorescence Detection

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The three species of the genus Yersinia that are responsible for plague, gastroenteritis, and lymphadenitis inhibit phagocytosis and inflammatory response via a type III secretion system and the effector proteins translocated by this system. Host macrophages infected by Yersinia are highly susceptible to YopJ-dependent apoptosis. Macrophages that have been activated by lipopolysaccharide (LPS) previous to infection by Yersinia instead undergo caspase-1 mediated proinflammatory cell death caused by production of inflammatory cytokines IL-18 and IL-1β , a process termed pyroptosis. Better understanding of the differing responses of naïve and activated macrophages to infection will provide insight into the immunopathogenesis involved in establishing an ongoing infection, as well as in generating protective host immune responses.

In this work, we explore this difference at the level of the whole proteome of cultured mouse macrophages. Isotope coded affinity tagging (ICAT) is used to identify changes in protein expression between naïve and LPS-activated RAW cells. ICAT results are compared with protein fingerprints obtained by two-dimensional capillary electrophoresis with laser-induced fluorescence detection.

Tues Poster 34: The New Phase of the HUPO Plasma Proteome Project

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The longterm scientific goals of the HUPO Plasma Proteome Project (PPP) are to: (1) comprehensively analyze human plasma and serum proteins; (2) identify and quantitate physiological, pathological, and pharmacological sources of variation in plasma proteins within individuals over time; (3) determine the variation across and within populations; and (4) establish plasma as the common pathway to biomarkers from organ and disease proteome studies. The Pilot Phase of the PPP yielded a special issue of Proteomics in Aug 2005 and a Wiley book in 2006 entitled Exploring the Human Plasma Proteome. The datasets are publicly-accessible at www.ebi.ac.uk/pride [EBI]; www.peptideatlas.org/repository [ISB]; and http://www.bioinformatics.med.umich.edu/hupo/ppp [UM]. A core dataset of 3020 proteins based on two or more peptides is a starting point for highly flexible analyses.

The New Phase of the PPP emphasizes (1) quantitation and subproteome (PTMs) analyses with new methods and advanced instruments; (2) robust bioinformatics analyses, using repositories at ISB and EBI and the Tranche distributed file-sharing system for large plasma proteome datasets; and (3) close coordination with organ and disease proteome studies. The PPP invites each HUPO initiative to analyze EDTA-plasma alongside the tissue or biofluid analyzed (liver, brain, kidney/urine, saliva) or disease studied (CV, cancers). The PPP Database and Bioinformatics unit will provide collaborative cross-analyses of these and other available datasets. Co-Chairs Aebersold, Omenn, and Paik are eager to encourage analyses of the PPP primary data, especially with new tools of analysis. [Supported by MEDC GR687, SAIC/NCI 23X110A, and NIH U54DA021519]

Tues Poster 35: Neurovascular Proteomics of Thrombolytic Therapy at the Bedside

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Introduction: Neurovascular injuries, such as those resulting from acute ischemic stroke, have few therapeutic options. The only FDA-approved medical therapy for acute ischemic stroke, tissue plasminogen activator (tPA), a serine protease, while highly efficacious, can have lethal side effects in tPA-related hemorrhage. tPA has also been implicated in the intricate cross-talk of the protease-fibrinolytic cascade, in particular with respect to matrix metalloproteinases (MMPs). It has been shown in animal models to have pleiotropic effects regulating the expression of MMPs and other proteases involved in cell-cell signaling both in the blood and in the brain. In humans, at least one MMP (MMP9) is thought to play a role in tPA-related hemorrhage, and has been shown to be up-regulated by tPA. Proteomic study of both focused simultaneous screening of multiple MMPs in relevant biochemical pathways, and broad analysis of the protease substrate profile of tPA directly at the bedside, may reveal more about tPA's therapeutic efficacy.

We hypothesize that in patients given tPA, there will be peripheral perturbation of not just a single MMP, but of a panel of MMPs, and there will be unique protease substrate profiles which may correlate to therapeutic efficacy.

Methods: Plasma from tPA-treated acute stroke patients, untreated stroke patients and healthy controls were matched with respect to age, gender, and stroke severity. Patient blood was drawn at three time-points post stroke, in the hyperacute (pre-tPA), acute (post-tPA), and subacute stages. We perform quantitative proteomic profiling of an array of 8 MMPs and their inhibitors, which may be implicated in plasminogen activation in human plasma, in conjunction with protease substrate profiling of corresponding subjects.

Plasma samples were also fractionated by size exclusion chromatography (SEC) and analyzed on LTQ-FT. We postulate that the later fractions will contain in part the protease substrates of interest. The same plasma was analyzed using protein microarrays to quantify expression of low-abundance MMPs and inhibitors of interest.

Results: tPA-treated patients demonstrate differentially expressed MMPs and TIMPs compared to non-tPA patients and controls. Three of the 8 MMPs measured showed trends toward statistical significance (see Figure 1). Figure 1 illustrates the ranked MMP and TIMP levels in representative control (dotted), tPA-treated (solid) and untreated (dashed) samples at 8hrs post stroke-symptom onset. Corresponding to these individual MMP profiles, tPA treatment demonstrated differential expression patterns of small peptides in treated acute stroke patients in comparison with non-tPA treated patients.

Conclusion: Our results suggest that in addition to the previous finding of a tPA-related upregulation of MMP-9, thrombolysis treatment affects the global profile of a panel of MMPs and their inhibitors in the plasma of acute stroke patients. The mechanisms of therapeutic efficacy in thrombolysis may be better understood by a system-oriented study of the MMP-fibrinolysis pathway through complimentary proteomic protease substrate profiling and microarray quantitation, and thus bring us a step closer toward individualizing thrombolytic treatment at the bedside. Comparisons with other thrombolytic agents and clinical outcomes are underway.

Tues Poster 36: Antibody Microarray Analysis of Neurological Disorders

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Antibody microarray technology has been increasingly utilized in biomedical research. The antibody microarray technique is attracting a great deal of interest because it can simultaneously examine a large number of proteins that are involved in a specific signal transduction pathway. We have been optimizing and refining conditions and parameters for microarray analysis, and have developed a novel method for assessing the outcome accuracy of microarray experiments. We also have been applying this cutting-edge technique in studies of neurological disorders such as Alzheimer's disease, epilepsy, and chronic alcoholics. Microarray data generated from human autopsy samples and animal models revealed differential protein levels between diseased and control brain tissues. Several identified proteins were further assessed and validated by means of Western blot analyses. These differentially expressed proteins may play an important role in pathophysiological processes of neurological disorders. Our results suggest that the protein microarray technique has the potential to lead to a better understanding of molecular mechanisms underlying neurological disorders, and to discoveries of candidate proteins as potential drug targets for the treatment of neurological disorders. It is anticipated that the antibody microarray technique will eventually become a routine laboratory tool for studies of differential protein expressions, as well as a clinical diagnostic tool for identification and validation of surrogate markers in body-fluids or tissues that correlate with different diseases.

Tues Poster 37: Effects of a 1,25-Dihydroxyvitamin D3 Analogue, TX527, on Human Dendritic Cells: A 2D-DIGE Approach

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The use of vitamin D and its derivatives is being considered for immune intervention in type 1 diabetes. The active form of vitamin D, 1,25-dihydroxyvitaminD3, (1,25(OH)2D3) and its immunopotent analogues (eg TX527), have important immune effects, mainly mediated by their actions on dendritic cells. Previous studies have shown that 1,25(OH)2D3 induces a more tolerogenic DC phenotype, with a reduced capacity to process and present antigens and to fully activate T-cells. The aim of this study was to investigate global protein changes in this tolerogenic DC phenotype induced by TX527, using bidimensional difference gel electrophoresis (2D-DIGE). Human CD14+ monocytes, isolated from peripheral blood mononuclear cells, were differentiated towards immature DCs (IM-DC) (6 days in IL-4/GM-CSF) or to mature DCs (M-DC) (2 additional days in IFNγ/LPS/GM-CSF), with/without TX527 (10-8M) (n=4). Protein profiles were analyzed by 2D-DIGE, separating protein samples in 2 different pH ranges (pH4-7 and 6-9). Differentially expressed spots were identified by MALDI-TOF/TOF. Approximately 3500 spots were detected in all groups of comparison, of which 97 (IM-DC versus M-DC), 119 (IM-DC with/without TX527) and 143 (M-DC with/without TX527) were significantly changed ($p<0.01$). Of these, 193 spots have been identified by now, revealing many proteins that were previously unknown to play a role in DC maturation or to be altered by TX527. The results indicate that major changes are taking place in antigen presenting capacity and in actin cytoskeleton rearrangement of DCs. In addition to data brought by FACS and ELISA analysis, these findings reinforce the capacity of TX527, to silence some important features of DCs that make them potent antigen presenting cells, inducing a more tolerogenic state.

Tues Poster 38: Mass Spectrometry-Compatible Surfactant for Protein Sample Preparation

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Protein solubilization, protease digestion efficiency, and peptide recovery after digestion are all significant factors in the identification of proteins by mass spectrometry. Approaches to singularly enhance any one of these factors can adversely affect other factors. For example, solubilizers such as urea or sodium dodecylsulfate (SDS) can increase protein solubilization and denaturation, but concomitantly can destabilize trypsin thereby reducing proteolytic activity or can interfere with attempts to fractionate a peptide mixture by liquid chromatography (LC). Here we present a surfactant for augmenting effectiveness of sample preparations to yield substantially improved data quality by mass spectrometry. This surfactant enhances the solubility of protein and peptide, and accelerates proteolytic activity, while avoiding unwanted side effects observed with common denaturants such as urea, guanidine or SDS. To prevent interference with downstream analytical methods such as solid-phase sample extraction, liquid chromatography, mass spectrometry analysis, the surfactant was designed to degrade into innocuous components by the end of a typical digestion reaction. In this presentation, we will present studies showing enhanced efficacy of protein solubilization relative to denaturants like urea, enhanced efficacy of trypsin proteolysis on model proteins, and enhanced peptide recovery from gel slices after proteolytic digestion. Data will be presented demonstrating that improved in-gel protein digestion and peptide recovery, including longer and more hydrophobic peptides, give increased sequence coverage and higher Mascot scores. We will also show, using a complex membrane fraction as a model, that enhanced protein solubilization and digestion can improve proteome coverage for shotgun proteomic analysis approaches by 1D and/or 2D LC-MS/MS.

Tues Poster 39: Rapid Nanoflow LC/MS Analysis of 1D and 2D Gels

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A major challenge in the field of proteomics is the identification of low-abundance proteins from complex protein mixtures. A common approach used in addressing the issue has been to run the protein mixture on 1 D or 2-D gels. The resolving power of the gel and subsequent in-gel digestions followed by mass spectrometry analysis has facilitated the identification of these low-abundance proteins from their more abundant counterparts. Nanoflow LC/MS is among the most sensitive techniques for the identification of proteins and is well suited for the identification of proteins from in-gel digests. Typically, electrospray ionization results in better sequence coverage compared to MALDI, especially from gel spots containing multiple proteins, but MALDI-based analysis can be faster. Using a microfluidic-based nanoflow LC/Q-TOF system allows for rapid analysis of gel spots and bands.

An E. coli lysate was separated by both 1D SDS-PAGE and 2D-gel electrophoresis. Gels were Coomassie-stained, and then spots or bands were excised. Each gel spot or band, which represents a protein mixture with low-to-medium complexity, was reduced, alkylated and digested with trypsin using a standard protocol. The in-gel digests were analyzed by reversed-phase microfluidic-based nanoLC on a Q-TOF mass spectrometer using rapid gradients. Protein database searching was used to assess protein sequence coverage and determine the minimal time required for optimal results.

Tues Poster 40: Using Multiple-Reaction Monitoring for the Confirmation of Putative Biomarkers

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Biomarker discovery using mass spectrometry is now well established. As a consequence of this, it is logical that mass spectrometry should also be used for confirmation of candidate biomarkers. Multiple-reaction monitoring (MRM) on a triple quadruple (QQQ) mass spectrometer provides superior sensitivity and selectivity for targeted compounds in a complex sample. MRM also offers high precision in quantitation and fast scan speed, which

makes it an ideal technology for validating biomarkers in a high-throughput fashion. In this study, we tested the confirmation methodology using peptides from proteins present in immunodepleted human serum.

The selected proteins were digested in silico using Spectrum Mill Peptide Selector to predict the peptides and their optimum MS/MS product ions. These predicted results were then compared to experimental results from digests of the standard proteins and the lists of MRM transitions were then created. The plasma samples were analyzed by robust and reproducible nanoflow LC/MS using the HPLC-Chip/MS interfaced to a high performance QQQ mass spectrometer. Multiple peptides from each protein were used for the MRM acquisition parameters. The accuracy and precision for the quantitation will be reported to demonstrate the utility of this workflow for validation of putative biomarkers.

Tues Poster 41: Proteomic Quantitation for Reference Databases - Relative Expression or Absolute Expression?

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While a diverse array of proteomic technologies have been developed to provide relative quantitation between test and control samples, their biological impact has been limited due to the fact that each experiment provides information relevant only to the specific test and controls samples analyzed in each experiment. This has been a major impediment to the creation of databases of protein expression which can be used not simply within a given experiment, but across experiments and across laboratories. This limitation has been an integral part of essentially all proteomics experiments, from the initial 2D gels to modern LC/MS methods (isotope labeled and label free). The utility of any proteomics method will be greatly enhanced if it could provide not only relative quantitation, but also absolute quantitation information regarding all of the proteins identified. This provides a more complete understanding of the biology by providing stoichiometric information, moreover, providing absolute quantitation information allows the results of any specific proteomic experiment to be translated to other experiments, performed under different biological conditions at different times and in different laboratories.

Absolute quantitation on a targeted scale by LC/MS/MS with multiple reaction monitoring has been performed for over twenty years. However, it was not possible on an 'omic scale until 2005 when Silva, et al. reported a method for absolute quantitation based on the empirically observed fact that the average LC/MS response for the three most intense tryptic peptides per mole of protein is constant (+/- 10 %) across a widely diverse array of proteins. Spiking a known amount of an internal standard protein into the sample permits the calculation of a universal response factor (counts/fmol), which can be used along with tandem MS information to identify a peptide and convert the peptide intensities to protein quantity, so long as the LC/MS data acquisition method records accurate abundance information for all peptides at all times.

This work describes our initial efforts towards the creation of a proteomics database of absolute quantitation information for nuclear and cytoplasmic compartments of cancer cell lines. This database addresses breast cancer and non-small cell lung cancer cell lines, specifically including those whose genotypic differences lead to refractory responses to drug therapies or for which genomic response to certain external stimuli has already been measured. The LC/MS analyses are accomplished using alternating scans of hi-lo collision energies, providing quantitative and qualitative information in a single LC/MS run.

Tues Poster 42: Rapid, Comprehensive and High-Resolution Intact Protein Separation for Proteomics

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Sample complexity is one of the key challenges facing contemporary proteomic analysis. New developments in column technology have allowed us to perform rapid improved-resolution MS based identification of intact proteins from complex samples. Sample types investigated to establish the utility of the methodology include bacterial lysates (Bordetella parapertusis, and Escherichia coli), a eukaryotic parasite (Leishmania donovani), and transformed human cell lines.

Here we report the separation of complex protein mixtures using online 2D liquid chromatography on derivitized polystyrene-divinylbenzene (PSDVB) pellicular ion-exchange resins and PSDVB monolithic reversed-phase columns. Proteolytic digestion of the fractions followed by rapid LC-MSMS was used to complete the analysis. An alternative methodology, relying on direct analysis of the second dimension eluents by top-down methodology, using the Apex IV 12T FTICR-MS has allowed identification from intact Leishmania proteins and PTM mapping of histone H4.

Separation of a typical amount of lysate (200ug) was performed using anion exchange columns, followed by reversed phase separation using rapid gradients on a 500 um PS-DVB monolith. Fractions (20uL) from the second dimension were collected in 384 well microtitre plates and subjected to trypsin digestion.

The use of parallel 200um monoliths for tryptic peptide separations ensured maximum capacity, minimum sample loss and high sample throughput, with no loss of sensitivity. For simple mixtures, reversed-phase separation times could be reduced to a few minutes without significantly affecting data content, although rapid scanning capability was essential due to the very narrow peak widths.

Analysis of the digested fractions gave good coverage of the proteome. Proteins representing low (8kDa) and high (500kDa) molecular mass and extremes of predicted pI were identified, as well as a number of membrane proteins. Resolution of the intact protein separation was such that single protein species often occurred in one or two fractions for both the ion-exchange and reversed phase separations, with the fractions varying in complexity. Separation of modified proteins in the ion-exchange dimension demonstrated separation of isoforms.

Quantitation is of paramount importance to any proteomic technique, and liquid chromatographic separation of intact proteins provides unparallelled flexibility for differential analysis of complex samples. UV absorbance maps were generated and could be used for differential analysis of samples. In addition, isotopic labelling techniques have been employed for more in-depth analysis of quantitative differences between samples. Additionally, label-free techniques have been employed for protein quantitation by LC/FT-ICR-MS.

Tues Poster 43: Antibody-Mediated Biomarker Discovery and Validation (AMBIODV)

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One of currently-unmet biomedical R&D demands is to annotate gene expression profiling at protein level. Using antibody to delineate the relationship between gene expression and biomarker characteristics is one of proven effective approaches. The antibodies isolated from egg yolk are called IgY (Immunoglobulin Yolk). We have developed a proprietary technology that can generate libraries of gene-specific and domain-targeted polyclonal antibodies in chickens based upon bioinformatic analysis of gene-expression data and preparation of gene-specific antigens.

The antibodies thus produced carry specific gene identity or gene domain information. With these antibodies, GenWay can localize and identify unknown proteins by screening against arrays of human tissues and cells. This approach is called "Antibody-Mediated Biomarker Discovery and Validation (AMBIODV)", which is the methodology of using antibodies to bridge the gap between gene expression and protein profiling. This process has been further developed by applying multiplex technology (antibody chips, antibody beads or tissue arrays) to identify unknown proteins in different tissues under various physiological or pathological conditions (e.g., protein expression profiling). The IgY-mediated protein separation for sample preparation of proteomic studies can also greatly enhance this process. Novel proteins or biomarkers can be further analyzed and validated via 2DE and mass

spectrometry. Large quantities of data can be generated and organized into an integrated and relational database. More specifically, the AMBIODV consists of the following steps and processes:
1. Human Genes - Groups of genes with unknown function can be selected based upon gene family sequence alignments or other bioinformatics criteria such as expression profiling.
2. Antigen Preparation -The selected genes are subcloned into gene-expression vectors to generate antigens. Other forms of antigens such as peptides may also be used.
3. Immunization - Animals, especially chickens, are injected with antigens. The animals' immune systems will produce antibodies against the antigens.
4. Gene-Specific Antibodies - The antibodies produced carry specific gene product information and can be characterized and tested for their specificities of binding proteins via standard quality control assays.
5. Protein Target Screening - The antibodies are used as probes to detect protein targets in human tissue or cell samples. The screening methods use multiplex formats such as antibody arrays or tissue arrays, suitable for protein identification and understanding of protein expression.
6. Proteomic Profiling - Expression of the identified new proteins can be further quantified via Western blotting or other immuno-based assays. Semi-quantitative profile of target proteins can be obtained.

Tues Poster 44: Patterned Porous Gold as a Platform for Improved Interrogation of Protein Arrays with Mass Spectrometry

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Protein microarrays, typically printed on functionalized glass slides, have become an integral technique for high-throughput studies of systems proteomics. Two recent advancements in this technique are the use of surfaces with hydrophobic/hydrophilic patterns and the use of mass spectrometry for detection of binding to the arrays. Patterned surfaces with hydrophilic spots against a hydrophobic background contain spots in a precisely defined area, improving spot uniformity and reproducibility and eliminating cross-talk between spots. Using mass spectrometry to interrogate arrays provides molecular information for bound species, eliminating the need for tags while detecting and potentially characterizing (via MS/MS) unanticipated or unknown binding partners from complex mixtures. We have engineered a surface using porous gold and alkanethiol self-assembled monolayers (SAMs) that can be modified with custom hydrophobic/hydrophilic patterns and has dramatically enhanced surface area to facilitate more sensitive detection with mass spectrometry.

Porous gold is produced by electrochemical deposition of gold salt on a flat gold surface, such as a gold-coated microscope slide. The large surface area of porous gold allows more protein to be loaded onto arrayed spots, increasing the amount of analyte that can bind at the spots and thereby amplifying the signal obtained when the array is interrogated. Additionally, enhanced surface area amplifies the observable surface chemistry effects of surface modifications. Methyl and carboxy-terminated self-assembled monolayers (SAMs) were custom-patterned on porous gold substrates with equipment commonly used to print protein arrays, without complex surface chemistry protocols. Proteins were covalently immobilized on printed hydrophilic carboxy-terminated SAM spots, while the remainder of the surface was coated with a methyl-terminated SAM to produce the hydrophobic/hydrophilic pattern on the surface. With previous protein arrays using hydrophobic/hydrophilic patterns, exposure of the surface to protein solutions had to be carefully controlled to avoid non-specific adsorption on the hydrophobic regions, which ruins the pattern. With our surface, the enhanced surface area of the porous gold amplifies the hydrophobicity imparted by functionalizing the background of the slide with a hydrophobic SAM. The background is rendered so hydrophobic that solution contact with these superhydrophobic regions is minimal and non-specific protein adsorption is avoided.

An antibody-antigen model system was used to demonstrate the utility of patterned porous gold substrates as array platforms. Antibodies were immobilized in the hydrophilic regions of the patterned porous gold and probed with bovine plasma spiked with a mixture of the peptide antigens. Mass spectra, MS/MS sequence data, and MS images were obtained for the array which reflected the effective binding of each antigen at its corresponding arrayed antibody spot. Porous gold yielded increased signal relative to flat gold surfaces and the hydrophobic/hydrophilic pattern was maintained on the porous gold even when the slide was immersed in plasma. Currently, we are studying the binding activity for mixtures of peptide-based kinase inhibitors using arrays on porous gold with mass spectrometry detection. Using mass spectrometry for detection, an array can be probed with a full library of kinase inhibitors in one experiment.

Tues Poster 45: Mapping Dynamic Changes in Phosphorylation of the Vasopressin-Sensitive Water Channel Aquaporin-2 using Targeted MRM Methods (MIDAS™) and the QTRAP™ Instrument

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Due to the variable quality and overall unavailability phospho-specific antibodies, quantification of protein phosphorylation in cell signaling pathways remains quite challenging. Studies aimed at elucidating cell signaling networks that respond to short-lived biochemical stimuli via rapid phosphorylation and dephosphorylation events at multiple sites can only be addressed by employing very selective and targeted approaches, such as multiple-reaction monitoring initiated sequencing and detection (MIDAS™). It has been demonstrated that the hormone vasopressin mediates osmotic water absorption by the inner medullary collecting duct (IMCD) cell and that this regulation occurs through regulated trafficking of water channel AQP2 to and from the plasma membrane, a process which has been postulated to be regulated by phosphorylation of AQP2. MS experiments profiling phosphoproteins of the rat kidney inner medullary collecting duct (IMCD) cell has revealed the presence of four phosphorylation sites, all serines, within the last 16 amino acids of the C-terminal tail of the water channel AQP2. Using phosphospecific antibodies to each of the four sites, we have demonstrate that phosphorylation at all 4 sites is modulated through short-term vasopressin exposure. Using MIDAS™ Workflow and the 4000 QTRAP™ instrument, carefully designed MRM transitions distinguishing each phosphorylated forms of the C-terminal tail of AQP2 were used to identify and confirm the presence of pS256, pS261, pS264, and pS269 sites in samples incubated in the presence of vasopressin in a 30 min time course experiment. Relative quantitation between control and vasopressin treated IMCD suspensions revealed a dynamic stiochiometry between phospho-forms of the AQP2 C-terminal tail demonstrating an increase of phosphorylation at S256 after 5 min and S269 after 30 min in response to vasopressin. A static phosphorylation at S261 was observed through the vasopressin time course, as well as in control samples, indicating a conserved phosphorylated form of AQP2 in the absence of vasopressin. Furthermore, using synthetic AQP2 phosphopeptides, a near absolute amount of phosphorylation per serine residue can also be determined using MRM-based quantitation.

Tues Poster 46: 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 Initiative providing support for seven years to ten interactive Proteomic Centers operating within US research institutes and universities. The aim is to develop and enhance innovative proteomic technologies and apply them to substantive 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 developed within the Initiative for open access and dissemination to the scientific community.

The participating centers are: the Cardiovascular Proteomics Center at Boston University School of Medicine, 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 School of Medicine, 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. As part of the official program of the US HUPO 2008 Annual Meeting, members of the NHLBI Proteomics Initiative will present an evening session highlighting technological developments and biological applications.

Tues Poster 47: Solid Phase Extraction - Liquid Chromatography (SPE-LC) Interface for Automated Peptide Separation and Identification by Tandem Mass Spectrometry

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Solid phase extraction (SPE) is a simple and widely used technique for desalting and concentrating peptide and protein samples prior to mass spectrometry analysis. Often, SPE sample preparation is done manually and the samples eluted, dried and reconstituted into 96 well titer plates for subsequent LC MS/MS analysis. To reduce the number of sample handling stages and increase throughput, we developed a robotic system to interface off-line SPE to LC-ESI-MS/MS.

Samples were manually loaded onto disposable SPE tips that subsequently were connected in-line with a capillary reverse phase (RP) column. Peptides were recovered from the SPE precolumn and separated on the RP column using isocratic elution conditions and analyzed by electrospray tandem mass spectrometry. Flow was delivered by two nanoflow piston pumps operated with Advanced Flow Control (AFC) (Proxeon, Odense, Denmark). Using a modified autosampler for mounting and disposal of the SPE tips, the SPE-LC-MS/MS system could analyze 8 samples per hour, and up to 96 SPE tips in one batch.

The chromatographic performance of the SPE-LC system was evaluated in terms of peptide ion peak widths, column peak capacity and retention time reproducibility based upon the analysis of tryptic BSA and a 12 protein component mixture. Peptide mixtures eluted within approximately 5 minutes, with individual peptide peak width of ~5 seconds (FWHM), making the SPE-LC suited for high throughput analysis.

The relatively high sample throughput, sufficient separation power and high sensitivity makes the automated SPE-LC MS/MS setup attractive for proteomics experiments as demonstrated by the identification of the components of simple protein mixtures and of proteins recovered from SDS-PAGE and 2DE gels.

Tues Poster 48: The Development of a Targeted MRM Assay for the Quantitation of Low Abundance Proteins

Amy Bartlett; Christopher Hughes; Johannas P C Vissers; Scott Geromanos; Catalin Donneanu; Therese McKenna; James Langridge

Waters Corporation, Manchester, United Kingdom

Proteomics profiling of complex biological mixtures using a non biased LC MS/MS strategy, Identity^E and MS^E on a Q-Tof mass spectrometer, produces a list of candidate proteins whose differential expression ratio changes between control and disease state. Specific peptides from these proteins can be targeted as a marker for that protein in a screening assay using a triple quadrupole mass spectrometer operating in the multiple reaction monitoring (MRM) mode. The MRM method which is robust and reliable is used to detect specific ions from target molecules and has recently received considerable attention in this area where the simultaneous quantitation of large numbers of low abundance proteins needs to be performed. In this mode of analysis the sensitivity and dynamic range are improved and providing sufficient data points across a chromatographic peak are recorded then quantitation is accurate. This high sensitivity coupled with the specificity/selectivity afforded by MRM transitions allows extensive panels of peptide biomarkers to be monitored in a single experiment from complex mixtures.

The sensitivity of MRM analysis is further improved by coupling nanoscale UPLC to the mass spectrometer, which delivers separated components at high peak concentrations to the nano-electrospray ionization source with reproducible retention times. Appropriate MRM transitions and acquisition parameters, including, accurate retention time windows, cone voltage and collision energies were determined from the Identity^E experiments.

In this study we have obtained a proteomic profile of protein expression changes using label-free LC-MS^E and Identity^E processing of rat microsomal cells following perturbation with a range of chemical inducers and from there determined a panel of target peptides for MRM quantitation studies.

Tues Poster 49: Quantitative Phosphoproteome Analysis via Label-free Proteomics: Applications to Hypoxia Stress in Breast Cancer Cells

Yong Zhou[1]; Mi-Youn Brusniak [1]; Safia Thaminy [1]; James Eddes [1]; Bernd Bodenmiller [2]; Lukas N. Mueller [2]; Julian D. Watts [1]; Ruedi Aebersold [1]

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Low oxygenation (hypoxia) triggers a multifaceted cellular response that has important roles in normal physiology and in many human diseases. Although it is well-known that tumors are hypoxic and that there is a correlation between the level of hypoxia and prognosis, little work has been done to investigate the relationship between hypoxia and cancer. Recent studies suggest that activation of the hypoxia-inducible transcription factor (HIF) plays a central role in the hypoxia response. It could be interesting to know about the variation in the protein phosphorylation cascades response to hypoxia before the transcriptional level responses in normal and cancer cells.

In this study, by using the Label-free Quantitative Proteomics techniques, we profiled protein phosphorylation in response to hypoxia in the breast cancer cell line MCF7. Phosphopeptides enriched from MCF7 cells exposed to 1% O2 at 6 different time points were analyzed using a LTQ-Orbitrap Hybrid mass spectrometer. MS1 features from 14 runs were detected and aligned using the new developed SuperHirn and Corra tools. Among 49824 aligned features, 605 were statistically significant in time trend.

The results show a strong acute response to hypoxia treatment at the protein phosphorylation level in MCF7 cells. The average level of protein phosphorylation significantly decreases in MCF7 cells from 0.5h to 3h of hypoxia treatment, and comes back to normal level at 6h while the hypoxia induced transcription factor-1a (HIF-1a) reaches its maximum expression level. Interestingly, 58% of annotated features, which are mostly high abundant ones, decrease

more than 2-fold versus normoxia condition. We observed that, other than oxidative phosphorylation associated kinases and subtracts (e.g. PFKP), some important cell survival associated kinases (e.g. PIK4CB), were also down regulated during hypoxia.

Further analyses, including applying different distribution models in Corra and targeted analysis on features unannotated by tandem MS/MS, will give us more information to establish the understanding of cell level response to hypoxia in cancer diseases.

Tues Poster 50: Quantitative Profiling of DNA Damage Response Proteins Using iTRAQ Labeling and LTQ Orbitrap XL

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The DNA damage response pathway is critical in maintaining genome stability, and proteins within this pathway are commonly mis-regulated in cancer cells. Camptothecin is an anti-cancer drug that inhibits topoisomerase I DNA unwinding and leads to DNA damage in cells undergoing DNA replication. It has been shown that protein concentrations and post-translational modifications, particularly phosphorylation, change in response to DNA damage and other cellular stresses. Quantitation of the responses of different proteins or phosphorylation sites to camptothecin or other agents will help in the elucidation of the signaling pathways and development of new anti-cancer drugs.

Here, we employed a mass spectrometry (MS)-based proteomics approach to identify and characterize proteins responding to campothecin-induced DNA damage in A549 cells. A549 cells were treated with 5 µM camptothecin and harvested at 0, 2, 8 and 24 hours post treatment. The new Thermo Scientific Pierce Phosphoprotein Enrichment kit was used for the enrichment of phosphorylated proteins (PE). Multiplexed iTRAQ labeling was used to determine relative changes in overall protein abundance and phosphorylation state of proteins. The labeled samples were analyzed using nanoLC-ESI-MS/MS with LTQ Orbitrap XL. Selected parent peptide ions were fragmented in the HCD (High Energy Collision Dissociation) collision cell. The HCD MS/MS spectra have high mass accuracy and resolution since they are acquired in the orbitrap and display a similar fragmentation pattern to those from a quadrupole collision cell. More than 500 proteins were quantified by iTRAQ (CV< 5-15% for peptides) using the HCD method from A549 whole cell and PE lysates. Analysis of the phosphoprotein-enriched fraction yielded an additional 145 protein identifications. To specifically quantify the phospho sites, we further purified phosphopeptides from the iTRAQ PE fraction by IMAC (gallium-IDA) spin columns. After IMAC enrichment, we were able to identify and quantify an additional 30 phosphorylation sites from a very limited amount of sample (15 µg of PE lysate digest).

Tues Poster 51: Strong Anion Exchange and MS/MS of Membrane Proteins Reveals Novel Phosphorylation Sites of AQP-1 in Cardiac Ischemia Reperfusion Model

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Global analysis of protein phosphorylation of cardiac sarcoplasmic reticulum (SR) and membrane proteins presents a technical challenge. We applied a recently reported pH step elution based strong-anion exchange (SAX) to enrich the phosphopeptides from cardiac membrane enriched proteins. Rat hearts were subjected to ischemia reperfusion (I/R) in Krebs-Henseleit (K-H) solution equilibrated with 95% O_2 and 5% CO_2 at 37°C. The I/R protocol consisted of 15 min perfusion, 20 min non flow ischemia followed by 25 min of reperfusion in a Langendorff apparatus. Control rat hearts were perfused for 60 min. As a result, 83 phosphopeptides and 640 non redundant proteins were identified by mass spectra (more than 400 proteins were identified by more than one peptide). SR proteins such as SERCA, RyR2, PLB and calsequestrin were detected by MS and confirmed by western blotting. Semi-quantitative data on phosphopeptides between control and I/R samples were obtained by comparing the number of tandem MS hits for specific peptides. Furthermore phosphopeptides from I/R hearts were observed to have different phosphorylation sites compared to control hearts for specific proteins. For example aquaporin-1 (AQP-1), a water channel membrane protein previously co-localized with T-tubules and caveolar proteins, was detected with two novel phosphorylation sites at S247 and S262. Interestingly, S247 was found to be phosphorylated in control only. In conclusion, application of this novel method to membrane enriched proteins from control and I/R rat hearts identified multiple phosphopeptides including novel and differentially phosphorylated sites in I/R.

Tues Poster 52: Improved Coverage and PTM Characterization of Complex Samples through the Use of Complementary Proteases and Fragmentation Methods

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The analysis of complex protein samples by mass spectrometry is complicated by the occurrence of proteins expressed at low copy levels, proteins resistant to trypsin proteolysis, and peptides that don't fragment efficiently using traditional collision induced dissociation (CID) approaches. These result in missed protein identifications, low sequence coverage, and missed protein modifications. Efforts to identify disease markers and to fully characterize protein modifications using mass spectrometry would be enhanced if these challenges could be addressed. Sequence coverage can be improved through the parallel use of multiple proteases to generate overlapping complementary peptides, and Electron Transfer Dissociation (ETD) to fragment larger peptides without disrupting labile post-translational modifications (PTMs). In order to evaluate the relative benefits of multiple proteases and multiple MS/MS fragmentation methods, we combined these approaches and assessed the sequence coverage and PTM mapping of several purified, modified proteins, as well as whole cell lysate samples separated by 1D-PAGE and analyzed by GeLC-MS/MS. Our analysis workflow included parallel digestion of samples with multiple proteases, MS analysis using both CID and ETD, and MultiConsensus reports in a beta version of Thermo Proteome Discoverer to analyze and combine the data. The combination of multiple digestion and fragmentation approaches improved the sequence coverage and PTM identification when compared to analyzing the same samples using trypsin and CID alone. This analysis was used to identify the most complementary proteases and fragmentation methods for the in-solution and in-gel analysis of complex protein samples.

Tues Poster 53: Multidimensional Protein Chromatography and Mass Imaging for Targeted PTM Characterization

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Abstract: Identification, quantitation and characterization of protein post-translational modifications (PTMs) represents perhaps the most challenging aspect of mass spectrometry-based proteomics. Multiple forms, low % site occupancy, temporal flux and varied isoforms of PTMs require enhanced separation and detection in MS and MS/MS and robust and sensitive approaches for data analyses. Here we describe the use of 1D- and 2D-protein chromatography coupled with an advanced proteomics workflow for enhanced characterization of protein PTMs. Protein profiling is performed via 1D- and 2D-protein HPLC with a Beckman Coulter PF2D: 1D; Chromatofocusing or SEC, 2D; rp-C4 or C8, with detection via UV at 214 nm. Fractions were analyzed directly by MALDI TOF MS for protein profiling or by trypsin digestion and PMF MS for tentative identification and peptide profiling. Select fractions were further characterized by LC-MS/MS. Protein standards were used to develop methodology and evaluate system performance. 1D- and 2D-protein HPLC offered improved automation, reproducibility and sensitivity compared with 1D- and 2D SDS-PAGE analyses when using Coomassie stained gels. Improved S/N for intact protein MS, PMF and MS/MS were observed. Differential UV detection enabled facile comparison of proteomics datasets and targeted MS analyses of select fractions. Differential and cluster analysis of protein MALDI-TOF MS data and PMF data allowed for fraction-location

and preliminary identification of differentially expressed proteins and proteins which were post-translationally modified and led to further, more complete characterization by LC-MS/MS. Enhanced PTM search strategies were implemented. Improved database search results were obtained from the LC-MS/MS data sets obtained from the protein fractions as similar information space approaches could be applied in comparison to MUDPIT where data is spread across an elongated time space. This approach facilitated location and assignment of PTMs, by integration of the intact molecular weight information, with PMF MS and MS/MS database searches.

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Tues Poster 54: Methionine Oxidation in Calmodulin Binding Domains

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Calmodulin is a calcium-binding protein that binds to and activates over 40 target proteins. The affinity of targets for calmodulin is modulatable by posttranslational modifications such as phosphorylation or Tyr nitration in or near the target protein calmodulin-binding domain. In addition, calmodulin itself can be phosphorylated on Tyr or oxidized at Met residues and those modifications inhibit calmodulin activation of its targets. The hypothesis for this project is that Met oxidation in calmodulin-binding domains will result in loss of calmodulin affinity for those targets.

Purified calcineurin, a prominent calmodulin target in the brain was subjected to oxidation with hydrogen peroxide. The oxidation rate of Met406, in the calmodulin-binding domain, was measured by LC/MS and determined to be 4.4x10-3 M-1 s-1, similar to the oxidation rate for Met in a free peptide analog (4.9x10-3 M-1 s-1). Oxidation of Met406 in calcineurin caused a 3.3 fold decrease in affinity for calmodulin and disrupted the calmodulin activation profile.

In preparation for global assessment of the susceptibility of calmodulin-binding domains to oxidation, calmodulin-binding proteins were isolated from rat liver and brain by affinity purification on calmodulin-sepharose. Separation by 1D SDS-PAGE was followed by automated excision of overlapping protein spots from the entire lane of the gel for each sample using ProPicII. In-gel digestion and LC/MS/MS resulted in identification of 161 proteins in at least 2 of 3 replicate liver preparations. Digestion followed by MuDPIT style 2D-LC/MS/MS resulted in the identification of 255 proteins in at least 2 of 3 liver preparations. A total of 289 proteins isolated from liver were identified in 2 out of 3 preparations by one of the two methods, with 127 identified by both. Less than 20% of all proteins isolated by calmodulin affinity chromatography and identified in this study are known calmodulin-binding proteins. It is likely that many of the proteins identified resine in multi-protein complexes with one protein binding to calmodulin. The susceptibility of these proteins to oxidation at Met residues and effects of Met oxidation on calmodulin binding to these proteins is predicted to modulate Ca2+ dependent signaling. Supported by NIEHS grant P30 ES06639.

Tues Poster 55: High Throughput Screen to Identify Novel Substrates for E3 Ubiquitin and Ubiquitin-like (Ubl) Ligases Using Human Protein Microarray Technology

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Protein modification by ubiquitin or the ubiquitin-like proteins (UBLs) has been implicated in a variety of cellular processes including proteosomal degradation, cell signaling, gene transcription, DNA repair, and protein localization. The nature, location, and extent of these post translational modifications appear to influence their cellular fate. In all cases, modifications are conferred through an enzymatic cascade involving an E1 activating enzyme, an E2 conjugating enzyme and an E3 ligase, with the E3 enzyme being primarily responsible for conferring substrate specificity. These enzymes are known to play a role in a variety of human diseases including cancer, and thus represent attractive therapeutic targets. Despite efforts employing cell-based assays and proteomic technologies such as mass spectrometry, the substrates of most E3 enzymes remain unknown. Our study utilized functional protein microarrays comprised of approximately 8,000 human proteins deposited in an addressable format on nitrocellulose-coated glass slides to identify novel substrates for E3 ligases. The arrays were utilized to evaluate modification by recombinant SUMOylation enzymes in the presence of three different SUMO paralogues (SUMO-1, SUMO-2, and SUMO-3). Candidate substrates for the E3 ligase were cross-checked against SUMOylation targets previously defined by mass spectrometry, and were subsequently validated using both homogenous assays and more classical immunoblotting approaches. Our results highlight protein microarrays as a technology platform suitable for the global discovery of E3 substrates, enabling a more detailed understanding of their cellular function and opening new avenues of investigation for therapeutics modulating their activity.

Tues Poster 56: Integrating Two Affinity Pull-down Strategies with Mass Spectrometry to Characterize a Novel PTM in the Escherichia coli ribosomal protein S12

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The ribosome is the universal macromolecular machine that translates the mRNA transcript into polypeptides. Analytical techniques have facilitated characterization of this complex and the identification of various ribosomal protein isoforms that result from post-translational modifications (PTMs). Beta -methylthioaspartic acid was previously identified as a novel PTM at position 88 in the Escherichia coli ribosomal protein S12. D88 is universal among all S12 bacterial orthologs and mutations at this position are lethal. This unusual PTM has also been identified in the equivalent position of ribosomal protein S12 from three phylogenetically distinct bacteria suggesting, at the bacterial level, conservation of the modification. Our goal is to elucidate the enzymology of modification and biological function of this novel PTM by identifying specific binding partners. Toward this goal we are currently employing two affinity pull-down strategies. The first involves the utilization of an endogenously expressed recombinant E. coli S12 protein (created by homologous recombination of the chromosomal S12 gene) that has two C-terminal affinity tags to pull-down proteins that form interactions. This allows the entire protein to serve as bait for the identification of proteins that form a stable protein complex and for proteins that form non-stoichiometric transient interactions. The second involves creation of synthetic biotinylated peptides that mimic the recognition site on S12. In brief peptides containing the modified (in progress) and unmodified Asp 88 serves as baits. The peptide pull-down has the advantage of identifying proteins that directly bind or interact with the S12 recognition site. A correlation between the two approaches improves the confidence of the identification. To detect binding proteins from both approaches we have utilized 1D PAGE and LC/MS/MS. Several interacting proteins, including putative methyl transferases, will be discussed.

Tues Poster 57: Extension of Microwave-Accelerated Residue-Specific Acid Cleavage to Glycoproteins

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Recently the Fenselau group has reported that microwave-assisted acetic acid incubation cleaves proteins at aspartate residues, with high specificity in seconds or minutes (1) and that tandem mass spectra of the peptide products of this proteolytic reaction provide suitable data for bioinformatic searching in support of high throughput proteomic strategies (2). In the present study we evaluate the suitability of this chemical proteolysis for analysis of glycoproteins.

Ribonuclease A and ribonuclease B protein have the same sequence except that the former does not contain carbohydrate while the latter one carries a single glycan at Asn-34. These two proteins both contain 4 disulfide bonds, which were reduced by adding dithiothreitol to the acetic acid solution used to cleave the polyamide backbone. MALDI-MS analysis of the products revealed full coverage of the ribonuclease A sequence, and 80% coverage of ribonuclease B. The glycopeptide [15-38] which accounts for the other 20% of the ribonuclease B sequence, could be detected when the product mixture was fractionated by reverse phase HPLC interfaced to an electrospray mass spectrometer. Carbohydrate heterogeneity in the acid cleavage product was characterized by mass spectrometry. This carbohydrate composition was compared to that reported in the literature (3), (4) and to that characterized by MS analysis of the products of a tryptic digestion. Based on the behavior of this first model system, we propose that microwave accelerated acetic acid treatment provides residue specific cleavage of the polyamide backbone in glycoproteins, without hydrolyzing the carbohydrate side-chain.

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Tues Poster 58: Tristetraprolin Phosphorylation Site Analysis

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Tristetraprolin (TTP) is a member of the CCCH zinc finger proteins and is an anti-inflammatory protein. Mice deficient in TTP develop a profound inflammatory syndrome with erosive arthritis, autoimmunity and myeloid hyperplasia. TTP expression is reduced in fats of obese people with metabolic syndrome and brains of suicide victims.

TTP gene expression is induced by insulin and cinnamon polyphenol extract in adipocytes, by lipopolysaccharide in macrophages, and by green tea polyphenol extract in rats. TTP binds to mRNA AU-rich elements with high affinity for UUAUUUAUU nucleotides and causes destabilization of those mRNA molecules. TTP is phosphorylated extensively in vivo and is a substrate for multiple protein kinases in vitro. A number of approaches have been used to identify its phosphorylation sites.

This presentation highlights the recent progress and different approaches utilized for the identification of phosphorylation sites in mammalian TTP. Important but limited results are obtained using traditional methods including in vivo labeling, site-directed mutagenesis, phosphopeptide mapping and protein sequencing. Mass spectrometry including MALDI/MS, MALDI/MS/MS, LC/MS/MS, IMAC/MALDI/MS/MS and multidimensional protein identification technology (MudPIT) has led the way in identifying TTP phosphorylation sites. The combination of these approaches has identified multiple phosphorylation sites in mammalian TTP, some of which are predicted by motif scanning to be phosphorylated by several protein kinases. This information should provide the molecular basis for future investigation of TTP's regulatory functions in controlling pro-inflammatory cytokines.

Tues Poster 59: Separation and Detection of Protein Post-Translational Modifications using a Mass Spectrometer Equipped with High Efficiency Ion Mobility Separation

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Post-translational modification (PTM) of proteins plays a fundamental role in cellular processes and their determination is one of the main goals of modern proteomic research. Phosphorylation, glycosylation and acylation are the best characterized, however, the variety, diversity and heterogeneity of these modifications requires novel analytical tools for their qualitative and quantitative assessment. We have investigated the potential of a novel travelling wave ion mobility spectrometer equipped with both MALDI and atmospheric ionization sources for the separation, detection and mass determination of post-translationally modified proteins. Species were ionised and the resulting ions separated based upon their ion mobility, or collision cross section, through the IMS device and subsequently mass analysed using the oa-TOF analyser.

Peptides and phosphopeptides originating from proteolytic digestion of the protein mixture were analysed directly or purified using TiO2 columns as previously described. The LC- IMS MS separations were performed using a nanoscale UPLC system in trapping mode described previously, in combination with a Synapt HDMS instrument. In these experiments the m/z, drift time and UPLC retention time of phosphorylated and non-phosphorylated species was determined. We will show the potential of IMS in combination with mass spectrometry to discriminate between these species and show that by synchronizing the output from the IMS device with the pusher pulse of the oa-TOF mass analyser we improve sensitivity between 3 to 10 fold compared to normal TOF MS mode of acquisition.
In addition by raising the collision energy on the travelling wave collision cell fragmentation of the peptide or modified peptides can be induced allowing structural information to be obtained. We will present data obtained from glycopeptides analysed by MALDI IMS MS. The released glycans were initially analysed using IMS MS then further analysis was performed on the individual glycans using Time aligned parallel (TAP) fragmentation.

Tues Poster 60: Normalization Methods for LC-MS Spectral Peaks Based on a Large-Scale Calibration Experiment

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Spectral peaks from liquid chromatography mass spectrometry (LC-MS) can be used to identify and quantitate proteins in complex biological samples. However, LC-MS experiments are susceptible to many sources of systematic bias, including imperfect sample preparation, sample run order, or even day-to-day variation in the instrument. Here, we characterize typical biases in LC-MS spectral peaks using a large-scale calibration experiment. We compare several existing normalization methods and propose a method based on smoothed peptide-specific bias estimates that is demonstrated to capture biases with great accuracy without overfitting the data.

To determine the factors that contribute most to observed technical variability, we analyze a large-scale calibration dataset. Samples of Salmonella (S. typhi) under two biologically distinct conditions were mixed together in five different concentrations, with equal concentrations of a mixture of quality-control (QC) proteins added to each. Five replicates of each of the five concentration groups were obtained in five batches using a randomized block design. With this design, we are able to definitively separate signal from bias due to systematic differences between batches. Furthermore, by comparing observed and expected patterns in the QC peptides, we can evaluate and compare normalization methods on their ability to remove biases.

We examine several standard normalization methods for LC-MS peak intensity normalization, including global scaling, peptide-specific ANOVA models, and scatterplot smoothing techniques like lowess and quantile normalization. We propose a functional regression model, where biases are modeled as functions across peptides. By allowing for a functional relationship in bias effects across peptides, we are able to capture systematic biases with great accuracy without overfitting the data. Using the calibration data, we compare the various normalization methods and demonstrate that the proposed functional regression model performs very well.

Tues Poster 61: Shotgun Sequencing by MS/MS to Discover Candidate Biomarkers from Spectral Counts using Non-Parametric Techniques

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Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) continues to be a leading method for the identification of the protein composition of complex biological samples. While stable-isotope labeling (ICAT, ITRAQ, and SILAC) are the most widely used methods to measure relative protein abundance, they have inherent practical difficulties, the most important of which is that they reduce the sensitivity of protein and peptide detections. Several label-free methods have recently been shown to correlate highly with protein expression, removing the problems found in stable-isotope labeling. Specifically, spectral counts—the total number of MS/MS spectra matched to a given protein—have been shown to increase linearly with increasing protein abundance levels. Spectral counts are easier to manage in that they are provided directly by database searching algorithms such as SEQUEST, Mascot, and XTandem! and in that they do not require analysis of ion current peak areas. However, their very nature makes them difficult to use for statistical analysis: the data is discrete, and the underlying distribution is not normal. We attempted to normalize a data set consisting of 20 MS/MS samples (10 disease, 10 control) and found no method to achieve satisfactory normality. In addition, proteins found in only one run of a multi-run experiment will result in zero entries, which greatly skew the data. As a result, the usual normalization procedures and statistical tests (t-test, local pooled error [LPE]) are not applicable on this type of data. Therefore, we adapted non-parametric tests and permutation-based procedures to probe spectral count data from patients with abdominal aortic aneurysm (AAA). AAA is the fifteenth leading cause of death in the United States, largely due to the low level of detection: there are very few risk factors aside from age and gender, and diagnosis requires expensive imaging. To discover candidate biomarkers for AAA, samples were taken from patients prior to undergoing surgical AAA repair. Matched controls were taken from patients with similar age, gender and co-morbidity distribution prior to undergoing exploratory balloon angiography. After microparticle preparation, the samples were analyzed via LC-MS/MS and spectral counts were determined by SEQUEST. Of the 760 proteins identified, over 20 were differentially expressed by Wilcoxon rank-sum test with a full correction for multiple comparisons. Using supervised learning techniques, including principle component analysis, clustering, linear discriminant analysis, and misclassification penalized posterior (MiPP), we were able to construct a minimal classifier consisting of only five proteins. Applying this classifier on a second AAA dataset successfully separated the AAA patients from the controls with a confidence level greater than 90%.

Tues Poster 62: Peptide Identification by Spectral Matching of Tandem Mass Spectra using Hidden Markov Models

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The peptide fragmentation spectra generated by tandem mass spectrometry workflows exhibit characteristic fragmentation patterns that can be used to identify the fragmented peptide. In other fields, where the compounds of interest do not have the convenient linear structure of peptides, fragmentation spectra are identified by comparing new spectra with libraries of previously identified spectra, using an approach called spectral matching. In contrast to sequence based tandem mass spectrometry search engines used for peptides, spectral matching uses the intensities of fragment peaks in library spectra to assess the quality of a match.

We demonstrate a completely new approach to spectral library matching for peptide identification. Using hidden Markov models to summarize many examples of a peptide's fragmentation spectrum in a generative probabilistic model, we are able to capture both the consensus and variation in each peak's intensity. We show that this approach, called HMMatch, has superior sensitivity and specificity compared to sequence database search engines and dot-product based spectral comparison tools. HMMatch is able to confidently assign peptide identifications (p-value < 1e-5) to thousands of publicly available tandem mass spectra that neither Mascot nor X!Tandem was able to identify with E-value less than 0.05. For comparison, on the same spectral dataset, NIST's MS Search software and consensus spectra were only able to assign a few hundred identifications with match factor 0.9 or better. HMMatch achieves good results from relatively few training spectra, is fast to train, and can evaluate many spectra per second. A statistical significance model permits HMMatch scores to be compared with each other, and with other peptide identification tools, on a unified scale. Finally, we show that it is possible to extrapolate HMMatch models beyond a single peptide's training spectra to the spectra of related peptides, expanding the application of spectral matching techniques beyond the set of previously observed peptides.

We describe our growing library of HMMatch peptide fragmentation spectra models, open-source tools for building and searching HMMatch models, and a public web-site for searching tandem mass spectra against the HMMatch models for peptide identification. We also show how our library of HMMatch models can be used to guide the selection of peptides and their fragment ions for protein quantitation by multiple reaction monitoring.

Tues Poster 63: Global Bioinformatical Analysis of Human Liver Proteome

Ole Vorm; Mortem Bern; Christian Ingrell; Alexandre Podtelejnikov

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To resolve a complete proteome of a particular species, organelle or tissue is not visible by performing experiments in one particular laboratory. Such a task requires multiple diverse experiments performed in different laboratories using different enrichment methods, diverse purification protocols and methods of data processing. It also requires sophisticated bioinformatics tools to analyze such diverse datasets. Here we present the global bioinformatics analysis of human liver proteome using ProteinCenter – a universal tool that can process proteomics data independently of the source of information. It is based on a consolidated biological annotated protein sequence database derived from all major protein databases and it allows filtering, clustering and statistical bioinformatical analysis from single, combined or comparison datasets using a broad range of criteria, including gene ontology, signal peptide and transmembrane predictions, biochemical pathways, disease association, etc.

All currently available HUPO liver proteome projects (in total of 202) 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. ProteinCenter collapsed 22636 protein identifications down to 5378 protein IDs. Among which 4087 proteins contained PFAM domains, 999 proteins had a predicted signal peptide and 2417 had predicted transmembrane regions. A significantly high number of 2227 proteins had disease annotation. Statistical Gene ontology analysis of liver proteome shows high content of membrane protein (38%), involvement in metabolism (57%) and catalytic activity (45%) A detailed distribution of liver proteome accordingly to the gene ontology categorization including cellular component, biological processes and molecular function will be presented. A comprehensive comparison analysis based on the peptide or homology clustering on selected liver proteome projects as well as examples on cross-species comparison of liver proteomes will be demonstrated.

Tues Poster 64: Using False Discovery Rates in Protein Identification

Tom Blackwell

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The False Discovery Rate (FDR) in any subset of protein identifications from a MudPIT mass spectrometry experiment is defined as the expected number of false positive identifications in the subset, divided by the size of the subset. The expected number of false positives can be estimated in several ways: by searching a decoy database, from the posterior probabilities provided by peptide

prophet / protein prophet, or from the peptide coverage of identified proteins. The yield curve for an experiment is a graph showing how the net number of proteins correctly identified increases as more false positives are included. This graph provides a useful way to compare different experimental or analytical strategies. It is more informative than simply stating the total number of identifications above a fixed threshold (a single point on the graph). The shape of the yield curve may suggest useful groupings of protein results. The local or "instantaneous" false discovery rate for protein identifications close to a threshold can be much worse than the average rate in the subset as a whole.

Tues Poster 65: A Predictive Model for Identifying Proteins by a Single Peptide Match

Roger Higdon; Eugene Kolker
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The standard for identifying peptides through tandem mass-spectrometry of trypsin digests still hovers at the recommendation for at least 2 unique peptides within a single protein. Yet identification need not be limited by this convention. We will present a method for identifying true and false identifications among the hundreds of proteins identified by only a single peptide in a typical high-throughput experiment. This method uses randomized database searching and logistic regression models, along with cross-validation.

We discuss three bacterial sample case studies, where our method enabled recovery of 68–98% of the correct single-hit proteins with an error rate of <2%. This results in a 22–65% increase in number of identified proteins. Identifying true single-hit proteins will lead to discovering many crucial regulators, biomarkers and other low abundance proteins.

Tues Poster 66: New Functionality for the Trans-Proteomic Pipeline: Improving the PeptideProphet Classifier

Eric Deutsch[1]; Hyungwon Choi[3]; James Eddes[4]; Jimmy Eng[2]; Johan Malmstroem[4]; Luis Mendoza[1]; Alexey Nesvizhskii[3]; David Shteynberg[1]; Joshua Tasman[1]; Ruedi Aebersold[4]
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The Trans-Proteomic Pipeline (TPP) is a freely available, open-source software suite for the analysis of shotgun mass-spectrometry data. The suite includes tools for conversion of raw instrument data to our open mzXML format; import of spectral search engine (Sequest, Mascot, X!Tandem, Phenyx, OMSSA, and Comet) results to our open pepXML format; peptide-level statistical validation of search engine results with PeptideProphet, protein-level statistical validation with ProteinProphet and export to our open protXML format; and quantitation for many differential labeling techniques including SILAC, ICAT, and iTRAQ. We also provide tools for visualizing and interacting with the data as it is processed through the pipeline. The software is available for Windows and Linux systems. The Windows distribution includes an easy-to-use installer, which installs and configures a webserver for a graphical user interface to the tools.

The PeptideProphet classifier is a statistical tool that is an integral part of the TPP. This tool uses the expectation maximization (EM) algorithm to model the most likely distributions of spectral matches, returned by database search algorithms, among correctly and incorrectly assigned peptide ion matches. Here we present recently added features for improving the PeptideProphet classifier. We extended PeptideProphet to consider retention time, as calculated by the SSRCalc algorithm, in the model. This allows PeptideProphet to gain some additional power for discriminating between correct and incorrect peptide IDs. Also, PeptideProphet was extended to use searches where some of the peptides in the database correspond to known decoys. PeptideProphet will now use these decoy hits to help guide the model of incorrect peptide matches to a more likely solution. Finally, PeptideProphet was enhanced so that the distributions it uses to model the database search scores no longer have to rely on any particular shape defined by a parametric distribution. The novel PeptideProphet semi-parametric model uses the decoy hits and kernel density estimation to learn the shape of the distribution of the incorrect assignments. It then applies the EM algorithm to find the most likely apportionment of the non-decoy matches among the incorrect and correct distributions. The semi-parametric model opens the door for PeptideProphet to model the results of practically any search engine that are in pepXML format, for which accurate and robust parametric distributions could not be found (e.g. OMSSA).

The TPP was developed by the Seattle Proteomic Center (the NHLBI Proteomics Center at the Institute for Systems Biology) through the Aebersold Lab.

Tues Poster 67: Unified Sample Preparation Approach using Hydrostatic Pressure Cycling: Simultaneous Isolation of Proteins, Nucleic Acids and Lipids from a Single Sample

Vera S. Gross; Greta Carlson; Gary B. Smejkal; Ada T. Kwan; Timothy Straub; Alexander V. Lazarev
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Systems biology studies require the incorporation of methods used in the fields of genomics, transcription profiling and proteomics, as well as the rapidly emerging area of metabolomics. While powerful and sensitive techniques are available for the individual isolation and analysis of nucleic acids, proteins, lipids and small molecules, major bottlenecks arise because multiple sample replicates are usually required if all of these cellular components are to be analyzed in the same sample. Current sample preparation techniques rely upon mutually incompatible sample preparation methods and solvents to isolate nucleic acids, proteins, lipids and small molecules from cells and tissues. Moreover, the strong detergents and chaotropic agents often used to solubilize samples tend to interfere with subsequent separation and downstream analyses. We have developed a novel detergent-free sample preparation technique which allows concurrent isolation and fractionation of protein, nucleic acids and lipids from samples as diverse as cell cultures, brain tissue, adipose tissue and liver. This novel method relies on a synergistic combination of cell disruption by alternating hydrostatic pressure (Pressure Cycling Technology, or PCT) and optimized reagents that dissolve and partition distinct classes of molecules into separable fractions. We report rapid simultaneous isolation of nucleic acids (DNA and/or RNA), lipids, proteins and small molecules from samples of cultured human fibroblasts, PC-12 cells and several types of mammalian tissues. Gel electrophoresis and real-time PCR confirm that high recovery of intact genomic DNA and good yields of intact RNA are obtained using this novel technique. Small molecule recovery has been confirmed by HPLC. Total protein fractions have been analyzed by SDS-PAGE and 2D-PAGE, confirming excellent reproducibility of the new method and high recovery of total protein. Several proteins extracted with significantly higher yields by the novel method versus conventional detergent-based techniques have been identified by MALDI-TOF mass spectrometry.

Tues Poster 68: Identification of Novel Interacting Partners of Protein Kinase CK2: Disclosing Unanticipated Links Between CK2 and a Variety of Biochemical Events

Giorgio Arrigoni[1]; Mario Pagano[2]; Stefania Sarno[2]; Luca Cesaro[2]; Peter James[1]; Lorenzo Pinna[2]
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Protein phosphorylation is probably the most important post translational modification and the most common way the cell uses to regulate all its fundamental functions. Over the past few years protein kinases and phosphatases have become promising targets of the pharmaceutical research since it has been demonstrated that a deregulation of a phosphorylation mechanism is probably responsible for the pathogenesis and development of many diseases. Cancer, neurodegenerative and autoimmune diseases, diabetes, hypertension and many other disorders have all been associated to an altered activity of these enzymes. Among all the protein kinases, CK2 is one of the most studied because of its involvement in many pathological disorders, particularly in cancer. In order to better understand the complex role of this highly pleiotropic kinase and to screen for potential therapeutic targets, we

have applied a combination of affinity chromatography and mass spectrometry to identify novel interacting partners of CK2. 144 proteins were identified with high confidence, many of which appear to be new and unexpected partners of this kinase. More than 60% of the proteins we identified are known to be phopshorylated in vivo and of those, over 40% are known substrates of CK2. Moreover, we identified many proteins that are strongly involved in the pathogenesis of several tumors (glioma, neuroblastoma, prostate, breast and ovarian cancer) and that were never reported before to be associated with CK2. Finally several other proteins were identified as involved in the pathogenesis of neurodegenerative diseases (Parkinson, Alzheimer, Lesch-Nyhan disease and Zellweger syndrome). Our findings support the view that CK2 is strongly involved in the development of cancer and other diseases and the new partner proteins identified disclose unanticipated links between CK2 and a variety of biochemical events.

Tues Poster 69: Development of BIATECH-54 Standard Mixtures for Assessment of Protein Identification and Relative Expression

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Mixtures of known proteins have greatly advanced the assessment and validation of methods in proteomics. But these mixtures are simple, with a few proteins at near equal concentration or a single protein at varied concentrations. To overcome this limitation, this presentation presents ways of simulating studies of complex biological samples. Only with such complex mixtures can we start to realistically assess and evaluate the long-term potential of different approaches to protein identification and label-free differential expression.

Using a complex but standard mixture of 54 proteins in variable concentrations, we will present the advantages and strengths of using complex but standard mixtures. We will also discuss the method for creating our 54-protein complex mixture, which comprised 16 off-the-shelf Sigma–Aldrich proteins and 38 S. oneidensis proteins produced in-house. The standard proteins were systematically distributed into three main concentration groups (high, medium, and low) and then the concentrations were varied differently for each mixture within the groups to generate different expression ratios. The mixtures were analyzed with both low mass accuracy LCQ and high mass accuracy FT-LTQ instruments. Our 54-protein mixture closely follows the molecular weight distributions of both bacterial and human proteomes. Methodology and experimental design developed in this work can be readily applied in future to development of more complex standard mixtures for HTP proteomics studies.

Tues Poster 70: Protein Families at the Postsynaptic Density

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The postsynaptic density (PSD) is a ~one million KDa protein complex lining the postsynaptic membrane at the active zone of the synapse. The complex contains receptors and signal transduction molecules that are tightly organized through association with specialized scaffolding molecules. The identities and arrangement of the molecules making up the PSD are expected to be important determinants of synaptic transmission. We used a highly purified PSD fraction, obtained by conventional subcellular fractionation followed by affinity purification with an antibody against PSD-95, to identify PSD constituents (Dosemeci et al. MCP 2007). Many of identified PSD proteins appear to belong to families of proteins with substantial sequence similarity. Moreover, western immunoblots with antibodies directed to identified components of the PSD suggest co-existence within the PSD of more than one member of the same family and/or more than one form of the same protein. Proteins in an affinity-purified PSD preparation were fractionated by SDS-PAGE, and tryptic fragments from individual gel slices were analyzed by LC/MS/MS, extending our previous study by using an LTQ/Orbitrap. Data has been evaluated separately for each gel slice using MassSieve with the aim of identifying proteins with shared sequences but different electrophoretic mobilities. We report the protein profile of the purified PSDs with proteins identified in individual electrophoretic bands, with particular attention to distinguish isoforms from families of proteins previously reported. For example, Shank1, Shank2, and Shank3 are readily distinguished on gel, identified by distinct peptides, and provide molecular markers along with other characteristic PSD constituents for isoform discrimination. BRAG1 and BRAG2b have been similarly electrophoretically localized and characterized. Variations on a protein (sequence and/or post-translational modification) may serve for the regulation of specific functions.

Tues Poster 71: Comparative Modeling of Human Metallothionein Isoform-3 (MT-3)

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Metallothioneins are low molecular weight cystein rich proteins expressed in response to heavy metals. Out of the four classes, Metallothionein isoform 3 (MT-3) which is known as a growth inhibitor factor, has deviated characteristics. Thus most of its functions and interactions are yet to be discovered which include its neuronel growth inhibitory property at the N terminus and negatively charged hexapeptide (EAAEAE) at the 55th position. The Protein Bata Bank (PDB) structures 2fj4 and 2fj5 are corresponding to human MT-3 alpha domain, where as the structure of beta domain and entire structure is not available. We there fore submitted the Uniprot sequence corresponding to human MT-3, having the primary accession number P25713 to the Swiss model work place template search. The PDB structure 2mhu, having 70% similarity with the beta domain of human MT-3 was selected for modeling the beta domain using the program modeller. Out of 30 comparatively modeled structures, the most suitable structure was selected based on the DOPE score and selected model of beta and the alpha domains were matched with rat Metallothionein-2 using UCSF Chimera with a BLOSUM-65 matrix, gap opening penalties as 18 for intra-helix and intra-strand, and 6 for any other. Secondary structure score was 30 %. Removal of 31st and 32nd residue from rat MT-2 allowed adhering the terminals of alpha and beta domains. Full model of the human MT-3 was generated by the geometry optimization of the peptide bond connecting the N and C terminals of alpha and beta domains using Arguslab docking and molecular editing program. Currently we are studying MT-3 interactions with cell cycle proteins using molecular dynamics simulation and docking.

Tues Poster 72: Absolute Quantification using Fluorescent and Isotope Labeled Concatenated Peptide Standards: Human Serum Albumin

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We reported recently a general approach for the determination of absolute amounts and the relative stoichiometry of proteins in mixtures using fluorescence and mass spectrometry (Nanavati et al. MCP 2007, in press). This method uses concatenated labeled signature peptides fused in tandem to green fluorescent protein. The fluorescence signal from GFP provides a unique measure of the absolute concentration of recombinant protein in a solution, and complements the information derived from the signature peptide peaks about relative amounts of proteins present in a protein mixture. Our design of signature peptides includes upstream and downstream regions to mimic the native protein with respect to the tryptic digestion site properties and emulating the digestion characteristic of full length protein. This approach has the advantage that it can be scaled to analyze complex mixtures and provides the means to generate clinically relevant and regenerable analytical standards to profile and quantify key protein blood biomarkers. In this work, we demonstrate a GFP fused 15N, 13C labeled standard protein for human serum albumin synthesized using in vitro translation methods. Our goal is to develop a protein specific protocol for the absolute quantification of targeted proteins that is broadly applicable, and to expand this strategy to other commonly measured human serum proteins (insulin, hemoglobin, plasminogen, etc) and biomarkers for clinical diagnostics.

Tues Poster 73: Integrating Peptide Identification Data with Clinical and Biological Annotation Data to Support Query, Reporting, and Data Visualization

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We report a software solution that integrates the generated mass spectrometry-based peptide and protein identification data with the biological annotation information available from public repositories, such as SwissProt and Trembl, and with clinical data stored in spreadsheets. The solution calculates various proteomics and statistical metrics, such as spectral counts, spectral count ratios, standard deviation of spectral counts, coefficient of variations, number of signal proteins identified, number of missed cleavages, number of phosphorylated proteins identified, and number of mitochondrial proteins. The solution comes prepackaged with a large set of highly annotated reports (e.g. biomarker reports, statistical analysis reports, timeseries reports, gene ontology reports, missed cleavages reports, sample summary reports, protein biological features reports). End-users can easily and quickly modify these pre-built reports or construct a new query/report from scratch. These newly modified or constructed reports can then be saved in the solution's central repository so that others can access them in the future.

Our solution uses the commercial Oracle database to capture data. The data model follows star schema modeling which permits very fast reporting on very large data sets. The front-end user interface is implemented through Microsoft Excel where we have found a way to sidestep the typical challenges faced with the program, such as poor performance, row limits, and security issues.

We currently have 25 pre-built reports that contain over 100 fields. Now that the underlying infrastructure has been developed, we are projecting that approximately 15 new reports will be developed every month in the next year. Our solution's strengths lie in the pre-built set of reports that are highly annotated, the flexibility and ease with which these reports can be modified, and the integration of all the biology information found in SwissProt and Trembl with peptide and protein identification data generated from experiments and with the clinical data stored in spreadsheets.

Tues Poster 74: Early Markers of Kidney Transplant Rejection: Quantitative Proteomic Workflows for Discovery and the Development of Non-Invasive, Targeted Assays

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The accurate diagnosis of renal allograft rejection currently depends on a biopsy. Transplant medicine would benefit greatly from the availability of non-invasive tests for early detection of rejection as well as immunosuppressive drug therapeutic monitoring. Only a limited number of studies have been published to date on specific proteins associated with allograft rejection. In this study, we describe the development of a workflow utilizing on-line liquid chromatography coupled with high resolution tandem MS on an LTQ-FT hybrid mass spectrometer specifically geared toward the identification of differentially expressed proteins and protein fragments in urine. Quantitative differential analysis of tryptic peptides was carried out using label-free analysis with SIEVE software algorithm. A selection of protein or peptide biomarkers identified in the high-resolution discovery workflow were subsequently quantified in targeted selective reaction monitoring (SRM) assays on a Quantum triple quadrupole mass spectrometer. Proteotypic peptides and transitions for the targeted SRM assays were selected using a novel software algorithm automatically incorporating the most selective and sensitive predictions for SRM transitions. The automated data processing methodology utilizes alignment, probability scoring and ion ratio analysis for confirmation and quantitation. The described quantitative workflows were used to analyze urine samples from normal, early rejection and acute humoral rejection transplant patients.

Tues Poster 75: Expression Profiling and Identification of Serum and Plasma Proteins using Immobilized Trypsin Beads with LIFT-MALDI-TOF/TOF

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Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry offers the capability of higher throughput protein profiling analysis of large numbers of clinical samples like serum. Many profiling strategies typically employ single chemical affinity beads or surfaces to decrease the sample complexity and dynamic concentration range of clinical fluids. However, a majority of the intact proteins fractionated in this manner are not detected in the lower mass ranges where MALDI-TOF mass spectrometers are most effective.

We describe an expression profiling workflow that incorporates immobilized trypsin paramagnetic bead digestions following front-end affinity bead capture. This approach allows analysis of any protein bound to the capture resin to be reduced to peptides that fall into the ideal range for profiling by MALDI-TOF mass spectrometry and direct LIFT-MALDI-TOF/TOF sequence determinations. Workflows using weak cationic, weak anionic or lectin bead surfaces in tandem combinations with the trypsin bead digestions will be described. Resulting peptides were captured on C18 beads, eluted and spotted directly with matrix on MALDI plates. We found that the bead-based trypsinization method was highly reproducible and efficient in digesting large serum protein fractions at short incubation times, and that the resulting peptides were readily able to be identified by LIFT-MALDI-TOF/TOF. Representative lists of proteins present in a pooled healthy serum sample will be presented.

As a proof-of-concept for clinical applications, the method was used in a plasma profiling study that had the goal of predicting individuals that would respond most effectively to influenza vaccines. Using both the weak anionic and cationic magnetic beads in tandem with the bound trypsin beads, we were able to identify four plasma proteins that are elevated prior to vaccination in eventual vaccine responders relative to non-responders. The level of each peptide can be monitored in plasma obtained across multiple time points.

Overall this scheme has shown to be reproducible, and because the fractionation and trypsinization steps are performed via paramagnetic particles, this method can be automated using a robotic liquid handling platform. Similarly, the workflow is amenable to further fractionation and analysis schemes like LC-MALDI and quantitative isotope tagging approaches. In addition, the influenza study presented here has demonstrated that we may effectively profile samples and identify clinically relevant, differentially expressed peptides using weak cationic and weak anionic magnetic beads in tandem with the immobilized trypsin.

Tues Poster 76: iTRAQ and SELDI-TOF Analysis of Plasma Processed With and Without Protease Iinhibitors: Pilot Study

Adriana Aguilar-Mahecha[1]; Marguerite Buchanan[1]; Sylvain Tessier[2]; Martin Latterich[2]; Mark Basik[1]
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Biomarker studies on plasma and serum samples require highly controlled protocols for blood processing and storage in order to minimize preanalytical variability. Ideally, blood samples should be processed and frozen immediately after collection to minimize protein degradation. However, in the clinical setting, it often takes several hours before the blood sample gets processed and stored. The BD P100 blood collection tube contains a cocktail of protease inhibitors and has been shown to provide some benefit to stabilize blood proteins during blood collection and processing. Appropriate use of these tubes requires that samples be processed within half hour of blood collection. The objective of the present pilot study is to investigate the effect of protease inhibitors on plasma stability in blood samples processed 6 hours after blood collection. Blood from the same individual was collected in EDTA and P100 tubes and processed immediately (time 0) or after 6 hours on the bench at room temperature. Samples were delipidated and immunodepleted

prior to iTRAQ analysis and fractionated prior to SELDI-TOF analysis. For each experimental method, the results from different tubes and processing times were compared among each other. We identified a total of 97 proteins in all samples analysed by iTRAQ. Analysis of P100 tubes at t=0 and t= 6hrs showed a slight decrease in relative protein abundance for 6% of the proteins detected in samples processed after 6 hours of blood collection. In contrast, 6% of detected proteins slightly increased in abundance in EDTA samples at t=6 when compared to t=0. SELDI analysis revealed more significant changes in peak detection and intensity in the 2500-25000 Da window compared to the higher molecular weight proteome. The only significant change in P100 tubes was a decrease in intensity for 7.5 % of detected peaks in blood samples processed after 6hrs on the bench compared to time 0. For samples collected in EDTA tubes, 9% of peaks detected were differentially expressed between both time points. Peak intensity decreased in 4.5% of peaks detected after 6 hours of collection. The results from this pilot study suggest that, given the proteome coverage of iTRAQ and SELDI-TOF technologies, the delay in processing of blood collected in both EDTA and P100 tubes does not result in very significant alterations in plasma protein and peptide levels.

Tues Poster 77: Absolute, Multiplexed, Mass Spectrometry Based Quantitation of the 47 most Abundant Proteins in Human Plasma

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Conventional approaches to protein quantitation using mass spectrometry (MS) are plagued by difficulties largely attributable to variable signal intensities between analyses and signal suppression from background sample complexity. Recent quantitative approaches have incorporated stable isotope labels (both chemical and metabolic) and have improved the reproducibility of relative quantitation between samples within a limited dynamic range of quantitation. But, these approaches are both laborious and confined to comparing small sample sets. Additionally, these analyses are incapable of absolute analyte concentration determination, which must be overcome to enable translation of these analyses into assays for clinical applications.

Triple quadrupole tandem mass spectrometers are capable of sensitively monitoring generation of a fragment ion by collision induced dissociation (CID) from an isolated precursor ion using multiple reaction monitoring (MRM) a tandem MS (MS/MS) scan mode. MRM scans have been routinely used in pharmaceutical, clinical and environmental applications for absolute quantitation of low molecular weight organic analytes (<1,000 amu). The combination of triple quadrupole MS instrumentation with nanolitre flow rate high performance liquid chromatography (HPLC) and nano-electrospray ionization has provided the necessary gains in sensitivity required to enable detection and analysis of biological macromolecules (peptides) in complex samples using LC-MS/MS. When combined with the use of synthetic, isotopically labeled peptide internal standards, MRM analysis is capable of sensitive and absolute determination of peptide concentrations across a wide linear dynamic range (10^3-10^4 orders of magnitude).

We have synthesized isotopically labeled peptide standards (Lys[$13C_6$] or Arg[$13C_6$]) for 47 high abundance human plasma proteins. These peptides have been purified by reversed phased HPLC and their concentrations determined by amino acid analysis. Pure peptide solutions were analyzed by electrospray ionization using direct infusion to optimize precursor and fragment ion selection and instrument specific parameters (declustering potential and collision energy). These peptides represent unique, tryptic peptides containing no missed cleavages and are reproducibly detectable by LC-MRM analysis of 1 µg of trypsin digested plasma. The abundance ratios of the internal standard mixture have been balanced to ensure that the molar ratio of natural peptide to the internal standard is within ±50:1 which is well within recognized ±100:1 linear dynamic range limit of the method. Our MRM methodology, using normalization of endogenous peak areas to internal standard peak areas, has excellent analytical (< ±1% RSD) and technical (< ±5% RSD) reproducibility. Data presented will demonstrate simultaneous quantitation of a subset of the top 47 plasma protein targets in a single 60 min LC-MRM analysis of trypsin digested human plasma.

Tues Poster 78: A New Strategy for Affinity Removal of Global Abundance Proteins

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The dynamic ranges of protein concentrations in biological fluids or tissues/organs can be from mg/ml to pg/ml or from mg/g to pg/g levels. Existing technologies tend to be robust for the separation and characterization of the major proteins of biological mixtures. However, low abundance proteins are often lost in the process and/or cannot be analyzed. Numerous findings indicate that low abundance proteins in complex mixtures can provide important information about the status of a cell and a disease. In order to enrich for low abundance, disease-associated proteins, a new strategy for depleting commonly shared, highly and moderately abundant (or so-called abundant) proteins was developed using reciprocal affinity depletion (RAD). In this experiment, chicken polyclonal antibodies against normal and asthmatic lung total proteins were used to establish global abundance protein affinity removal columns (GAPAR) for reciprocal depletion of abundance proteins. Our data show that GAPAR are effective against diseased and normal rat tissue total proteins and can deplete up to 95% of the total proteins using the RAD strategy. Many unique protein spots could be identified on 2-DE gels after RAD, whereas these protein spots could not be identified in the original samples on 2-DE gels. Thus technology allows detection of differentially expressed, as well as those with low abundance and non-antigenic proteins. Unique protein spots from asthmatic lung and normal lung RAD were picked up for MALDI-TOF MS analysis to identify the protein species. Several of these protein spots were identified as asthma-associated proteins which are consistent with the published data. These results suggest that disease-relevant proteins can be identified using the RAD method along with other proteomic technologies such as 2-DE and MALDI-TOF MS.

Tues Poster 79: Statistical Demonstration of the Utility of Edge™ Technology in the Evaluation of Biomarkers

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The identification of biomarkers is an essential element for early prediction of diseases and, in the future, personalized medicine. Biomarker discovery, evaluation and validation are the key steps in biomarker development processes. The recent development of high-throughput proteomics, including high sensitivity mass spectrometry and automation of protein identification, significantly increases the data base of potential biomarkers. However, the evaluation and validation steps of the biomarker development process remain a bottleneck. Edge (Enhanced Density Gradient Extraction) technology, a density based extraction method, provides 1) a simple and reproducible technique for fractionation of tissue homogenates/cell lysates, 2) a unique statistical method for evaluation of potential biomarkers, and 3) the opportunity for generating data for further clinical validation. To demonstrate the utility of Edge technology in biomarker evaluation, rat models on high fat diet were used in this study, since high fat diet has a profound impact on brain function and cardiovascular 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 heart oxidative stress, rat brains and hearts tissues were homogenized then fractionated using the Edge 200 separation system. Fractions were subjected to western blot analysis using synaptic function marker p-synapsin I and oxidative stress marker GRP75. Results were analyzed statistically giving clustered data for each of the markers. The results show that Edge technology represents a unique method for easy and rapid evaluation of any potential biomarker.

Tues Poster 80: Immuno Tandem Mass Spectrometry (iMALDI) Assay for Clinical Proteomics: Development and Application of iMALDI for EGFR Diagnosis

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The epidermal growth factor receptor (EGFR) is highly expressed in a variety of cancer tumours, and is therefore an important biomarker and target for cancer therapy. We have developed a novel peptide-based immuno-tandem mass spectrometry (iMALDI) diagnostic assay for highly sensitive, specific and absolute quantitative analysis of EGFR. This peptide-based assay offers several advantages including the elimination of extensive protein separation steps, ability to multiplex to detect a panel of proteins, and the ability to capture low abundance peptides in complex samples, thus providing a wide dynamic range of detection and better molecular characterization. iMALDI offers an improved approach to protein chip technology that should prove beneficial in diagnostics and render it useful for a wide variety of applications. Not only will it lead to a better molecular characterization of cancer, it will provide us with valuable information such as the extent of contribution of the proteins in the EGFR pathway, the modification levels of the protein of interest, and efficacy of current drugs in targeting the pathway which, in turn, will indicate potential targets for further drug development. In the iMALDI approach, customized anti-peptide antibodies are immobilized to CNBr-activated sepharose beads according to the manufacturer's protocol. The sample to be analyzed is digested with trypsin; subsequently, the digest is incubated with the immobilized anti-peptide antibody beads. Stable-isotope labeled derivatives of the epitope-containing peptide, synthesized at the UVic-Genome BC Proteomics Centre, are added as internal standards for absolute quantitation. Finally, the beads are directly placed on the MALDI target (to limit sample loss) with matrix solution in a microarray array format for MS and MS/MS analysis. In our experiments, we chose the peptide MHLPSPTDSNFYR (aa 963-975) from the EGFR protein after digestion and MALDI-MS/MS, as it had the best flight when compared to the other peptides within the protein. iMALDI was capable of detection of the EGFR peptide at attomole levels, whereas EGFR in solution could only be detected at femtomol levels. Both tumour tissue and cancer cell lines showed attomole sensitivity as well, and very few individual cells were required for sample preparation. We confirmed the identity of the immuno-precipitated peptide through MALDI-MS/MS. Additionally, through a standard curve generated from varying ratios of labeled and unlabeled EGFR peptide, we were able to accurately measure the absolute amount of EGFR protein in the biopsy samples. /Moving forward from this approach, we are in the process of multiplexing iMALDI. Since EGFR is part of a complex signaling network, and its cellular expression is a combinatorial effort between EGFR and other closely linked receptors, a diagnostic test must include as many downstream products as possible for greater accuracy. As a result, the following proteins in the EGFR pathway will be targeted for characterization in addition to EGFR: Src, Ras, PKC, Akt, MEK, and RAF. These particular proteins are readily available, along with antibodies, from Invitrogen. As part of the development of this assay, absolute expression and modification levels of the above mentioned proteins will be determined in cancer cells and tumour tissue.

Tues Poster 81: Using Ettan™ DIGE System for Analysis of Proteome Changes in Transformed E. coli

Maria Winkvist; Åsa Hagner-McWhirter; Bengt Westerlund; Gunilla Jacobson; John Flensburg; Gunnar Malmquist; Lennart Björkesten

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The 2D Fluorescence Difference Gel Electrophoresis (2D DIGE) method allows for accurate differential quantitation of individual proteins through the use of a pooled internal standard, which removes technical variation otherwise common when using 2D electrophoresis. The use of an internal standard eliminates the need for technical replication and thereby saves time and cost. The use of biological replication allows for the identification of statistically significant biological variation.

2D DIGE was used to assess the proteomic changes as a result of time and temperature in transformed E. coli. E. coli was transformed with a target protein regulated by an IPTG sensitive promoter and grown at 20° C or 37°C. Samples were collected at different time points after induction with IPTG and the proteome analysed with 2D DIGE. Also, the levels of target protein were monitored in all samples. Analyses using DeCyder™ software revealed differences in protein expression as a result of temperature as well as differences over time. Proteins with significant variation in abundance over time and temperature were identified and related to levels of target protein.

Preparative gels were post-stained with Deep Purple™ total protein stain and protein spots were identified by mass spectrometry using MALDI.

Tues Poster 82: Automated Off-line Capillary 2D-LC for Separation of Complex Peptide Samples

Robert van Ling; Bas Dolman; Evert-Jan Sneekes; Remco Swart

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Multidimensional liquid chromatography (MDLC) is a valuable technique for bottom-up and top-down workflows in proteomics. Various approaches of MDLC, e.g. MudPit, have been described for the separation of peptides. Off-line MDLC techniques have several advantages over on-line approaches: i) higher flexibility with respect to column dimensions and mobile phase selection, ii) easier method development and iii) the ability to perform re-analysis of the fractionated effluent. On the other hand off-line LC methods are labour intensive and time consuming.

Here we discuss a novel off-line 2D-LC method for the separation of tryptic peptides. The peptides are separated on a 300 µm i.d. packed SCX column. Micro fraction collection and re-injection of the peptide samples onto a reversed phase monolithic trap column has been automated. The second dimension separation is achieved on a 200 µm i.d. monolithic column which is connected to an ion-trap mass spectrometer.

The method was evaluated with a complex protein tryptic digest sample. Peptide identification by tandem MS allowed the assessment of the quality of the 2D-LC separation. It was found that most of the peptides, around 85%, were present in single or adjacent SCX fractions. The method repeatability was studied by performing consecutive 2D-LC experiments, demonstrating excellent retention time precision data for both the first and second dimension separation.

Tues Poster 83: Proteomic Analysis of Softwood Degrading Fungi towards Biomimetic Enzyme Applications

Sonam Mahajan; Emma R Master

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Biocatalysts are important tools for harnessing the potential of wood fibres since they can perform highly specific reactions with low environmental impact. Challenges to bioconversion technologies as applied to wood fibres include accessibility of plant cell wall polymers and stability of the biocatalysts used. Moreover, variability in the molecular structure and distribution of cell wall polymers in different plant species and tissues impacts bioprocess efficiency. This project describes proteomic analysis of enzymes secreted by the white-rot basidiomycete Phanerochaete carnosa, and the brown-rot basidiomycete Postia placenta, while transforming lignocellulosic substrates. It is anticipated that this project will identify profiles of expressed microbial enzymes that are specific to transformation of particular lignocellulosic fibres. This will enable tailored applications of enzymes for processing wood fibres in the future. Notably, white-rot fungi secrete oxidative enzymes that depolymerize lignin and enable growth on hemicellulose and cellulose, while brown-rot fungi degrade polysaccharides in wood, leaving lignin essentially intact. Thus, the comparison of proteins secreted by P carnosa and P placenta will also provide further insight into the unique biotransformation strategies that have evolved in brown-rot and white-rot fungi.

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