October 5, 2009

Dear Participant:

On behalf of the National Cancer Institute’s (NCI) Clinical Proteomic Technologies for Cancer (CPTC) Initiative, we welcome you to the CPTC Third Annual Meeting, October 5-7, 2009, in Bethesda, Maryland. The NCI has a long history of supporting scientific programs to provide both discovery and development of foundational data and knowledge to accelerate progress in the field of cancer biomarkers. Although proteomics offers great promise to enable nearly all aspects of the discovery and development of diagnostics and therapeutics, many problems have hindered the advancement of the field for several years. To turn this challenge into progress, the NCI launched the CPTC in late 2006.

The NCI’s CPTC is a nationwide, interdisciplinary group of partnerships among a multitude of centers and investigators working to establish and advance a more reliable and efficient biomarker development pipeline. CPTC investigators are achieving this goal by addressing the barriers and issues related to applying proteomic insight to clinical practice (e.g., proteomics technologies, biospecimen collection, reagents).

In just 3 years, the CPTC has made great strides in the development of a robust proteomics discovery pipeline that includes community reference data, critical reagents, and standard protocols. Importantly, this program is bridging the gap between laboratory advances and the clinical utility of proteins by developing a pipeline with greater reliability/accuracy through the introduction of metrics at the discovery stage and efficiency by the introduction of a preclinical stage called “verification.”

As a result, the CPTC is the leader in defining proteomics platform performance parameters and standards to define proteomics states that are anticipated to produce better credentialed candidate leads that will ultimately accelerate the discovery of new cancer biomarkers. The CPTC’s work will have far-reaching impacts, including the ultimate goal of reducing the burden of suffering and death due to cancer. The CPTC has achieved a tremendous amount of progress, which is a reflection of deep commitment to teamwork and collaborative science focused on achieving common goals.

This year’s meeting has been planned along major scientific themes that are being conducted in the program, and each session will showcase a panel of presentations reflecting a major proteomics discipline. In addition to presenting a significant amount of work produced by our teams and individual investigators who are helping advance our understanding of protein biology in cancer and accelerate discovery research and clinical applications, this year’s meeting will highlight keynote talks, special plenary lectures from national and international experts in the field of proteomics research, public-private partnerships, and community-based resources such as the NCI’s Antibody Characterization Program, a component of the CPTC.

The CPTC meeting will begin on Monday morning, October 5, 2009, and close by Wednesday noon, October 7, 2009. This year’s event will be held in conjunction with the NCI’s Innovative Molecular Analysis Technologies (IMAT) program to foster greater collaboration among its investigators. The IMAT meeting will kick off with a joint CPTC and IMAT session on the morning of Wednesday, October 7, 2009. The official IMAT meeting will begin on Wednesday afternoon.

We look forward to your participation!

Clinical Proteomic Technologies for Cancer Program Coordinating Committee:

Steven A. Carr, Ph.D.          Amanda G. Paulovich, M.D., Ph.D.
Susan J. Fisher, Ph.D.          David F. Ransohoff, M.D.
Bradford W. Gibson, Ph.D.      Fred E. Regnier, Ph.D.
Joe W. Gray, Ph.D.             Henry Rodriguez, Ph.D., M.B.A.
Leland H. Hartwell, Ph.D.      Steven J. Skates, Ph.D.
Daniel C. Liebler, Ph.D.       Paul Tempst, Ph.D.
Gordon B. Mills, M.D., Ph.D.
MEETING PLANNING COMMITTEE

Emily Boja, Ph.D.
National Cancer Institute, NIH

Steven A. Carr, Ph.D.
Broad Institute of MIT and Harvard

Susan J. Fisher, Ph.D.
University of California, San Francisco

Bradford W. Gibson, Ph.D.
Buck Institute for Age Research
University of California, Los Angeles

Joe W. Gray, Ph.D.
Lawrence Berkeley National Laboratory

Leland H. Hartwell, Ph.D.
Fred Hutchinson Cancer Research Center

Tara Hiltke, Ph.D.
National Cancer Institute, NIH

Christopher Kinsinger, Ph.D.
National Cancer Institute, NIH

Daniel C. Liebler, Ph.D.
Vanderbilt University Medical Center

Mark David Lim, Ph.D.
National Cancer Institute, NIH

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National Cancer Institute, NIH

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The University of Texas M.D. Anderson Cancer Center

Amanda G. Paulovich, M.D., Ph.D.
Fred Hutchinson Cancer Research Center

Amir Rahbar, Ph.D., M.B.A.
National Cancer Institute, NIH

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The University of North Carolina at Chapel Hill

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Purdue University

Robert Rivers, Ph.D.
National Cancer Institute, NIH

Henry Rodriguez, Ph.D., M.B.A.
National Cancer Institute, NIH

Steven J. Skates, Ph.D.
Massachusetts General Hospital

Paul Tempst, Ph.D.
Memorial Sloan-Kettering Cancer Center
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AGENDA

Day 1 – Monday, October 5, 2009

7:30 a.m. - 8:00 a.m.  Registration and Morning Break

8:00 a.m. - 8:10 a.m.  Welcome and Introduction
   Henry Rodriguez, Ph.D., M.B.A.
   National Cancer Institute, National Institutes of Health

8:10 a.m. - 8:20 a.m.  Role of Patient Advocates in Clinical Proteomics
   Elda Railey
   Research Advocacy Network

   James Heath, Ph.D.
   California Institute of Technology

9:00 a.m. - 10:40 a.m.  Theme 1: Biomarker Discovery Platforms
   Chair: Steven A. Carr, Ph.D.
   Broad Institute of MIT and Harvard

   9:00 a.m. - 9:25 a.m.  Discovery and Verification of Protein Biomarkers in Cancer, Precancer and Normal Tissues
      Daniel C. Liebler, Ph.D.
      Vanderbilt University Medical Center

   9:25 a.m. - 9:50 a.m.  Posttranslational Modifications of Secreted Proteins as Breast Cancer Biomarkers: Splice Variants
      Susan J. Fisher, Ph.D.
      University of California, San Francisco

   9:50 a.m. - 10:15 a.m.  Transformation of Anecdotal Clinical Peptidomics Into Activity-Based Biomarker Screens and Verification
      Paul Tempst, Ph.D.
      Memorial Sloan-Kettering Cancer Center

   10:15 a.m. - 10:40 a.m.  A HUPO Equimolar Test Sample for Proteomics
      John Bergeron, D.Phil.
      McGill University

10:40 a.m. - 10:50 a.m.  Break
Day 1 – Monday, October 5, 2009 (continued)

10:50 a.m. - 12:30 p.m.  Theme 2: Platforms and Tools for PTM Studies
Chair: Bradford W. Gibson, Ph.D.
Buck Institute for Age Research, University of California, San Francisco

10:50 a.m. - 11:15 a.m.  
**Affinity-Based Biomarker Discovery Workflows Targeting Cancer-Specific Posttranslationally Modified Proteins in Human Plasma**
Bradford W. Gibson, Ph.D.
Buck Institute for Age Research, University of California, San Francisco

11:15 a.m. - 11:40 a.m.  
**Using SILAC To Study Tyrosine Kinase Signaling in Neurons**
Thomas Neubert, Ph.D.
New York University School of Medicine

11:40 a.m. - 12:05 p.m.  
**Modeling Phosphopeptide Gas Phase Fragmentation**
William Old, Ph.D.
University of Colorado at Boulder

12:05 p.m. - 12:30 p.m.  
**Proteomic Phosphopeptide Chip Technology for Protein Profiling**
Xiaolian Gao, Ph.D.
University of Houston

12:30 p.m. - 2:00 p.m.  
**Lunch and Poster Session**

2:00 p.m. - 3:40 p.m.  Theme 3: Bioinformatics Resources and Tools for Proteomic Community
Chair: Christopher Kinsinger, Ph.D.
National Cancer Institute, National Institutes of Health

2:00 p.m. - 2:25 p.m.  
**caTranche: A CPTAC Community Resource for Sharing Proteomic Data**
Phil Andrews, Ph.D.
University of Michigan

2:25 p.m. - 2:50 p.m.  
**TagRecon Identifies Protein Mutations and Modifications**
David Tabb, Ph.D.
Vanderbilt University Medical Center

2:50 p.m. - 3:15 p.m.  
**Analysis and Statistical Validation of Proteomic Datasets**
Alexey Nesvizhskii, Ph.D.
University of Michigan

3:15 p.m. - 3:40 p.m.  
**Improving the Sensitivity of Peptide Identification From Tandem Mass Spectra Using Meta-Search, Grid-Computing, and Machine-Learning**
Nathan Edwards, Ph.D.
Georgetown University

3:40 p.m. - 4:40 p.m.  
**Poster Session/Break**
Day 1 – Monday, October 5, 2009 (continued)

4:40 p.m. - 5:55 p.m. Theme 4: Advances in Proteomic Technologies
Chair: Paul Tempst, Ph.D.
Memorial Sloan-Kettering Cancer Center

High-Throughput, High-Sensitivity Proteomics Platform for Improved Biomarker Discovery and Verification
Richard D. Smith, Ph.D.
Pacific Northwest National Laboratory

5:05 p.m. - 5:30 p.m.
Immunosignaturing as Biomarkers of Early Chronic Disease, Including Cancer
Stephen Albert Johnston, Ph.D.
The Biodesign Institute, Arizona State University

5:30 p.m. - 5:55 p.m.
Group Quantification of Mass-Tagged Peptides Using Spectrum Clustering With PICquant and MAZIE
Dennis J. Templeton, M.D., Ph.D.
University of Virginia Health System

5:55 p.m. - 6:00 p.m.
Wrapup and Adjournment
Henry Rodriguez, Ph.D., M.B.A.
National Cancer Institute, National Institutes of Health

Day 2 – Tuesday, October 6, 2009

7:30 a.m. - 8:00 a.m. Registration and Morning Break

8:00 a.m. - 8:05 a.m.
Welcome
Henry Rodriguez, Ph.D., M.B.A.
National Cancer Institute, National Institutes of Health

8:05 a.m. - 8:45 a.m.
Keynote Lecture: Restructuring Biomarker Proteomics: Using Mass Spectrometry To Bridge Toward the Clinical Laboratory
Leigh Anderson, Ph.D.
Plasma Proteome Institute

8:45 a.m. - 9:25 a.m.
Special Plenary Lectures: International Perspectives in Clinical Proteomics
Chair: Daniel C. Liebler, Ph.D.
Vanderbilt University Medical Center

8:45 a.m. - 9:05 a.m.
Focused Integration and Valued Output: Korean Proteomics Efforts To Discover Biomarkers
Myeong-Hee Yu, Ph.D.
Korea Institute of Science and Technology
Day 2 – Tuesday, October 6, 2009 (continued)

9:05 a.m. - 9:25 a.m.  
**Drug Rescue: The Application of Quantitative Mass Spectrometric Methods to Personalized Medicine Development**  
K.W. Michael Siu, Ph.D.  
Ontario Cancer Biomarker Network

9:25 a.m. - 11:30 a.m.  
**Theme 5: MS Platform Benchmarking: CPTAC Multisite Assessment Studies**  
Chair: Fred E. Regnier, Ph.D.  
Purdue University

9:25 a.m. - 9:50 a.m.  
**A Multisite Assessment of Multiple Reaction Monitoring MS for Protein Quantitation in Human Plasma: Toward a Robust Tool for Verification of Biomarker Candidates**  
Steven Hall, Ph.D.  
University of California, San Francisco

9:50 a.m. - 10:15 a.m.  
**Break**

10:15 a.m. - 10:40 a.m.  
**Applications of the CPTAC Yeast Reference Proteome Standard**  
Daniel C. Liebler, Ph.D.  
Vanderbilt University Medical Center

10:40 a.m. - 11:05 a.m.  
**Metrics for LC-MS/MS Performance in Proteomics**  
Stephen Stein, Ph.D.  
National Institute of Standards and Technology

11:05 a.m. - 11:30 a.m.  
**Repeatability and Reproducibility in Proteomic Analyses by Liquid Chromatography-Tandem Mass Spectrometry**  
David Tabb, Ph.D.  
Vanderbilt University Medical Center

11:30 a.m. - 12:20 p.m.  
**Theme 6: Quantitative Proteomics**  
Chair: N. Leigh Anderson, Ph.D.  
Plasma Proteome Institute

11:30 a.m. - 11:55 a.m.  
**Measuring Cancer Biomarker Candidates by Targeted MS and Ab Enrichment**  
Steven A. Carr, Ph.D.  
Broad Institute of MIT and Harvard

11:55 a.m. - 12:20 p.m.  
**What I've Learned From Being a Member of the Proteomics Research Group**  
Michael MacCoss, Ph.D.  
University of Washington

12:20 p.m. - 1:30 p.m.  
**Lunch and Poster Session**
Day 2 – Tuesday, October 6, 2009 (continued)

1:30 p.m. - 2:30 p.m.  Theme 7: Special Panel on Biospecimen and Experimental Design/Statistical Considerations
Moderator: Helen Moore, Ph.D.
National Cancer Institute, National Institutes of Health

1:30 p.m. - 1:45 p.m.  Design and Statistics of Marker Studies: What Can We Learn From Netflix?
David F. Ransohoff, M.D.
The University of North Carolina at Chapel Hill

1:45 p.m. - 2:00 p.m.  The Love/Avon Army of Women: A New Model for Partnering Women and Scientists To Find the Cause and Prevention of Breast Cancer
Dixie Mills, M.D.
Dr. Susan Love Research Foundation

2:00 p.m. - 2:15 p.m.  Statistical Models for Longitudinal Biomarker Behavior
Steven J. Skates, Ph.D.
Harvard Medical School/Massachusetts General Hospital

2:15 p.m. - 2:30 p.m.  Using Data To Ensure Good Statistical Design
Nell Sedransk, Ph.D.
National Institute of Statistical Sciences

2:30 p.m. - 3:45 p.m.  Poster Session/Break

3:45 p.m. - 4:05 p.m.  Role of Clinical Chemists in Translating Novel Biomarkers Into Clinical Practice: AACC Perspective on Clinical Proteomics
Saeed Jortani, Ph.D.
University of Louisville

4:05 p.m. - 4:15 p.m.  Update on National Cancer Institute-FDA Interagency Oncology Task Force on Molecular Diagnostics
Zivana Tezak, Ph.D.
U.S. Food and Drug Administration

4:15 p.m. - 5:55 p.m.  Theme 8: Reagents Development
Chair: Mehdi Mesri, Ph.D.
National Cancer Institute, National Institutes of Health

4:15 p.m. - 4:40 p.m.  CPTC’s Antibody Characterization Pipeline Update
Tara Hiltke, Ph.D.
Clinical Proteomic Technologies for Cancer
National Cancer Institute, National Institutes of Health
Day 2 – Tuesday, October 6, 2009 (continued)

4:40 p.m. - 5:05 p.m. Antibody Validation by Means of Protein Array and Immunohistochemistry
Stephen Hewitt, M.D., Ph.D.
National Cancer Institute, NIH

5:05 p.m. - 5:30 p.m. Development of Reference Materials for Proteomics
David Bunk, Ph.D.
National Institute of Standards and Technology

5:30 p.m. - 5:55 p.m. Synbodies: An Alternative Protein Affinity Reagent
John Chaput, Ph.D.
Arizona State University

5:55 p.m. - 6:00 p.m. Wrapup and Adjournment
Henry Rodriguez, Ph.D., M.B.A.
National Cancer Institute, NIH

Day 3 – Wednesday, October 7, 2009

Joint CPTC/IMAT Session

7:30 a.m. - 8:00 a.m. Registration and Morning Break

8:00 a.m. - 8:05 a.m. Welcome and Introduction
Mark David Lim, Ph.D.
National Cancer Institute, National Institutes of Health

Henry Rodriguez, Ph.D., M.B.A.
National Cancer Institute, National Institutes of Health

8:05 a.m. - 8:45 a.m. Keynote Lecture: Elucidation of Tumor Biology and Assessment of Cancer Patients Using Quantitative Mass Spectrometry
John Koomen, Ph.D.
H. Lee Moffitt Cancer Center and Research Institute

8:45 a.m. - 11:30 a.m. CPTC and IMAT Joint Theme Session: Immunoaffinity Platforms and Protein Arrays
Cochairs: CPTC Representative (Susan J. Fisher, Ph.D.)
IMAT Representative (TBD)

8:45 a.m. - 9:10 a.m. Oxidized Proteins in Breast Cancer Patient Plasma
Fred E. Regnier, Ph.D.
Purdue University
Day 3 – Wednesday, October 7, 2009 (continued)

9:10 a.m. - 9:35 a.m.  The Prevalence and Nature of Glycan Alterations on Specific Proteins in Pancreatic Cancer Patients Revealed Using Antibody-Lectin Sandwich Arrays
Brian Haab, Ph.D.
Van Andel Research Institute

9:35 a.m. - 10:00 a.m.  Clinically Quantifying Low-Abundance Serum Proteins by LC-MS/MS: Potential Benefits and a Case Study
Andy Hoofnagle, M.D., Ph.D.
University of Washington

10:00 a.m. - 10:15 a.m.  Break

10:15 a.m. - 10:40 a.m.  Bead Array-Mass Spectrometer: Equipment and Method Development
Scott Tanner, Ph.D.
University of Toronto

10:40 a.m. - 11:05 a.m.  Multiplex Mass Spectrometric Immunoassays
Dobrin Nedelkov, Ph.D.
Intrinsic Bioprobes, Inc.

11:05 a.m. - 11:30 a.m.  FDA Regulation of Protein-Based Diagnostic Assays
Jeffrey N. Gibbs, J.D.
Hyman, Phelps and McNamara, P.C.

11:30 a.m. - 11:45 a.m.  Diversity Training Opportunities
Mark David Lim, Ph.D. (introduction)
National Cancer Institute, National Institutes of Health
Kristina Pohaku Mitchell (M.S., Ph.D. candidate)
University of California, San Diego

11:45 a.m. - 1:00 p.m.  Lunch and Poster Session

1:00 p.m.  CPTC Meeting Adjournment
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**Indicates that the abstract has a corresponding poster.**
caTranche: A CPTAC Community Resource for Sharing Proteomic Data

Phil Andrews

University of Michigan, Ann Arbor, MI

The Tranche data repository houses the CPTC data sets and is integrated with the refactored proteomecommons.org Web site that provides project management and annotation support. The main dissemination site for CPTC data also is located on proteomecommons.org, and additional Web pages are generated as these data sets are made public, which then are indexed by the major search engines. This allows researchers to locate the data sets through the proteomecommons.org search tools or via commercial search engines. Public data sets in the Tranche repository also are mined by several central proteomics databases (e.g., Peptide Atlas, GPMdb, PRIDE), providing wider dissemination of the data sets and potentially increased citations of CPTC research. The proteomecommons.org Tranche Annotation Data Resource (caTranche) passed all requirements for the caBIG Silver Compliance at the beginning of September 2009. This first stage required building an external data model to allow interfacing the dynamic data model to the caBIG static data model and exposed about 25% of the annotation terms to caBIG. This includes information allowing users to find and access the target data sets in Tranche. The complete data model will be exposed to the caBIG in future development efforts. The caTranche resource allows the caBIG resources to develop query and access tools for CPTC data sets in the Tranche repository.

Protein Quantification by Targeted Mass Spectrometry: The Way Out of Biomarker Purgatory

Steven A. Carr¹, Amanda Paulovich², Leigh Anderson³, Terry Pearson⁴, Susan E. Abbatiello¹, Terri Addona¹, Michael Burgess¹, Angela Jackson⁶, Eric Kuhn¹, Hasmik Keshishian¹, DR Mani¹, Matt Pope⁴ Morteza Razavi⁴, Derek Smith⁵, Steven Skates⁵, Janice Williamson¹, Jeffrey Whiteaker²

¹Broad Institute of MIT and Harvard; ²Fred Hutchinson Cancer Research Center; ³Plasma Proteome Institute; ⁴University of Victoria, ⁵UVic/Genome BC Proteomics Centre, University of Victoria, ⁶Massachusetts General Hospital

Lack of robust quantitative methods with sufficient sensitivity, reproducibility, and throughput has significantly hampered our ability to credential candidates coming from unbiased proteomic discovery efforts, since useful antibody reagents for the vast majority do not yet exist. The National Cancer Institute’s Clinical Proteomics Assessment for Cancer (CPTAC) program has focused on addressing this serious barrier by developing robust targeted assay methods employing mass spectrometry to screen and quantify low abundance proteins in plasma. We recently have demonstrated that multiplexed assays for proteins at the low ng/mL level in plasma using 50-100 uL of plasma can be configured using Stable Isotope Dilution (SID)-Multiple Reaction Monitoring (MRM) Mass Spectrometry. Large-scale interlaboratory studies conducted under the auspices of the CPTAC program have demonstrated that these assays can be reproducibly configured, deployed, and run in multiple laboratories with assay CVs approaching clinical performance. Further improvements will come from the use of protein and peptide enrichment technologies coupled with quantification using mass spectrometry. One method employing immunoaffinity enrichment of peptides, Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA) holds particular promise for simplifying sample preparation and increasing both throughput and sensitivity of MRM-based assays. Using SISCAPA, assays can be readily configured that enable quantification of proteins present at low ng/mL levels directly from 10-50 uL of digested plasma, and sensitivity in the 10 pg/mL range has recently been achieved by capture from 1 mL of plasma. This presentation will focus on the further development and application of MRM-MS and SISCAPA technologies by our group in the context of cancer and other diseases.
Synbodies: An Alternative Protein Affinity Reagent

John C. Chaput

The Biodesign Institute and Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ

Developing high-quality affinity reagents to the human proteome represents a grand challenge in basic and applied biomedicine. Reaching this goal will require transformative ideas that shift the current paradigm away from methodologies that are costly and time consuming and that focus on novel solutions capable of revolutionizing the process by which protein capture reagents are created. In response to this challenge, we are working to develop a new class of synthetic antibodies that recognize protein targets with high affinity and specificity but that do not require animal immunization or recursive selection steps for their discovery. Instead, our approach relies on a versatile two-step strategy in which noncompeting ligands are rapidly discovered and assembled into a series of multivalent protein capture reagents. This strategy, which is general and amenable to high throughput, has the potential to become an enabling technology by providing a simple method for creating high-quality synthetic antibodies. We anticipate that these affinity reagents could be used in multiple formats to interrogate the nature of the human proteome and facilitate deeper insights into the molecular basis of human health and disease.


Nathan Edwards

Department of Biochemistry and Molecular and Cellular Biology, Georgetown University, Washington, DC

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 continues 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. Although high-confidence assignments can easily be achieved by setting an arbitrarily stringent E-value cutoff, the resulting loss of peptide and protein identification sensitivity is unacceptable. We demonstrate that multiple search engines, heterogeneous grid-computing, and unsupervised machine-learning result reconciliation can substantially improve the number of high-confidence peptide identifications from tandem mass spectrum data sets.

The PepArML MS/MS meta-search engine computes peptide identifications using the Mascot, X!Tandem, X!Tandem with KScore scoring plug-in, OMSSA, and MyriMatch search engines, automatically reformatting spectral data and constructing search configurations for each search engine from a simple, unified search specification. Spectrum identifier tracking, decoy searches, C13 precursor correction, and, if necessary, charge enumeration are carried out by the meta-search engine, eliminating spurious disagreement due to individual search engine quirks. The configured searches are automatically scheduled on a heterogeneous mix of local and remote compute nodes, including the Edwards Lab cluster at Georgetown University and National Science Foundation TeraGrid compute resources at Purdue University, utilizing secure communication protocols for spectra and results. Results from target and decoy searches are reconciled using the PepArML machine-learning-based result combiner.

The PepArML meta-search engine current integrates five search engines, automatically reformatting spectrum data files and sequence databases and building configuration files. The grid-computing scheduler manages the queue of waiting search jobs and provides compute nodes with spectra and search configurations as needed. The commercial
Posttranslational Modifications of Secreted Proteins as Breast Cancer Biomarkers: Splice Variants

Eric Johansen1, Demetris Iacovides2, Akrporn Prakobphol1, Penelope Drake1, Simon Allen1, Richard Niles1, Lakshmi Jakkula2, Anna Lapuk2, Heidi Feiler2, Henry Marr2, John Conboy2, Miles Braten1, Maria Hassis1, Joe Gray2, Susan Fisher1

1University of California, San Francisco, San Francisco, CA; 2Lawrence Berkeley National Laboratory, Berkeley, CA

Breast cancer is a heterogeneous disease that includes a number of distinct biological entities that are associated with specific morphological/immunohistochemical features and clinical outcomes. For many decades, these cancers have been classified by a combination of factors, including patient age, tumor size, pathological grade, and the degree of lymph node involvement. Recent genome-wide microarray analyses of primary tumors enabled further their classification into major subtypes. The luminal cell-like breast cancers are generally ER positive, and the two basal cell-like subtypes (A and B), which are ER negative, share mRNA expression patterns with normal basal/myoepithelial cells of the breast. Patients with luminal subtype tumors generally have higher survival and lower recurrence rates than those with cancers that have a basal phenotype.

Microarray analyses of a collection of breast cancer cell lines that were derived from primary tumors showed that they maintained the defects in gene copy number and mRNA expression patterns that were observed in the primary tumors (Neve et al. 2006). Accordingly, these breast cancer cell lines also clustered into basal- and luminal-like subsets. Very recently, we used exon microarray techniques for high-resolution transcriptome analyses of the same panel of breast cancer cell lines and demonstrated that a portion of the alternative splicing events appeared to be subtype specific. As such, these molecules are candidate biomarkers, which could be used to diagnose the basal-type tumors that have the highest morbidity and mortality.

As a proof of principle, we are constructing mass spectrometry (MS)-based assays for detecting these splice variants in samples of conditioned medium from a subset (44) of the breast cancer cell lines (8 basal A, 14 basal B, 18 luminal, 4 unclassified) that were used for the microarray analyses. To increase the probability that a splice variant circulates in blood, we focused on proteins that are secreted and/or subject to proteinase cleavage and release from the cell surface. Briefly, we translated the RNAs that encode these alternatively spliced molecules into protein sequences and predicted (in silico) the peptide products of trypsin digestion. Then we selected candidate peptides for
constructing multiple reaction monitoring (MRM) assays, which enable MS-based quantitative measurements (Addona et al. 2009). Each peptide included a novel junction or exon and was in the molecular weight region that is amenable to analysis on an Applied Biosystems 4000 QTRAP mass spectrometer. Interestingly, a number of the candidate biomarkers had the expected subtype specificity. Thus, we developed a set of MRM assays that, in future experiments, can be used to analyze plasma samples collected from breast cancer patients and control individuals with the goal of identifying a subset of peptides that cross novel splice junctions and track with the basal-type tumors.


Proteomic Phosphopeptide Chip Technology for Protein Profiling

Xiaolian Gao¹, Tongbin Li², Xiaochuan Zhou³

¹University of Houston, Houston, TX; ²University of Minnesota, Minneapolis, MN; ³LC Sciences, Houston, TX

Cancers are malignant growths caused by misregulated and uncontrolled cell division; these abnormal cellular activities are typically accompanied by unusual protein expression profiles. Signaling and network proteins are groups of proteins of direct link associated with such protein expression variations. Our project is based on the idea to use the interactions between phosphopeptides and phosphoprotein binding domains (PPEPs-PPBDs) as a means to look at the proteins through target binding. Specifically, more than 10 families of PPBDs are known to be present in several hundred proteins (the binders). These PPBDs are associated with thousands of PPEPs from at least hundreds of host proteins. Our research has focused on technology advancement (integration high-density peptide microarrays [peparrays], computational and Web tools) and development of peparrays for cancer pathway-related proteins. We have prototyped 30,000 high-density peparrays, identified signature phosphopeptides for important network proteins containing SH2 domains, gained capability to measure multiple SH2-containing proteins qualitatively and quantitatively, established a Web tool for peparray design (http://pepcyber.umn.edu/uPepArray/) which can also use Web-accessible PPEP databases (PepCyber and Phospho.ELM), and refined the iterative processes of statistical analysis of peparray experimental results ↔ new generation of peparray design and assays of proteins. These results will be presented.

FDA Regulation of Protein-Based Diagnostic Assays

Jeffrey N. Gibbs

Hyman, Phelps & McNamara, Washington, DC

The U.S. Food and Drug Administration (FDA) regulates the commercialization of new in vitro diagnostic (IVD) assays in the United States. Before a new IVD assay can be introduced into interstate commerce, it must be the subject of an FDA approval or clearance. Thus, the FDA serves as the gatekeeper for the sale of new protein-based IVDs in the United States.

There are two primary routes by which new IVD kits can enter the market in the United States. The first is through the submission of a 510(k) premarket notification; this method is available to Class I and Class II devices. A 510(k) can be
cleared by the FDA if the agency determines that the new IVD is “substantially equivalent” to a “predicate device.” A predicate device is either another assay that was on the market prior to May 1976 or one that previously has been cleared by the FDA. Companies submitting 510(k)s for protein-based assays will need to rely on the latter category. In addition to identifying a predicate device, the applicant must show that the product (1) has the same intended use as the predicate device and the same technological characteristics or (2) the same intended use, is as safe and effective as the predicate device, and does not raise different questions of safety and effectiveness. In applying and interpreting this requirement, the FDA has substantial latitude. The FDA has cleared 510(k)s in which the new product uses a fundamentally different technology and the intended use is different from the predicate device’s intended use. 510(k) applications for protein-based IVDs must be supported by clinical data.

The second primary route is the premarket approval application (PMA). New Class III devices must obtain PMA approval, which requires showing that the product is safe and effective for its intended use. Obtaining PMA approval in general, requires more data from clinical studies than a 510(k). Due to various regulatory requirements, the PMA submission is lengthier and more costly. PMAs are also subject to additional postmarket controls that do not apply to devices that enter the market through the 510(k) process.

An IVD may be placed in Class III due to its riskiness. Cancer screening assays generally are placed in Class III. An IVD also can be placed in Class III due to the lack of a predicate device. If a company submits a 510(k) for a novel protein-based assay and fails to identify an acceptable predicate device, the product is automatically placed in Class III. In that situation, the FDA has the flexibility to “de novo” reclassify the device to Class II and, in essence, treat the submission as a 510(k). This mechanism, which can greatly shorten the time to market, has been used by the FDA for a number of novel IVDs.

Not all new assays enter the market through the FDA process. Many new tests have been developed and offered by laboratories. Historically, the FDA has not regulated laboratory-developed tests (LDTs). Although the FDA has asserted that it has the authority to regulate LDTs, generally it has not exercised that authority. Instead, these tests have been regulated under the Clinical Laboratory Improvement Amendments, by the States, and by professional organizations.

Obtaining FDA clearance or approval for most novel protein-based IVDs is not simple or routine. Companies that wish to pursue this process will need to engage in both good science and thorough planning of its regulatory strategy, including maintaining appropriate communications with the FDA.

**Affinity-Based Biomarker Discovery Workflows Targeting Cancer-Specific Posttranslationally Modified Proteins in Human Plasma**

Penelope M. Drake¹, Eric B. Johansen¹, Richard K. Niles¹, Michael Lerch¹, Haichuan Liu¹, Bensheng Li², Anna Zawadzka², Simon Allen¹, Kwanyoung Jung¹, Steven C. Hall¹, Birgit Schilling², H. Ewa Witkowska², Lee Makowski², Fred Regnier⁴, Susan J. Fisher¹, Bradford W. Gibson¹,²

¹UCSF Sandler-Moore Mass Spectrometry Core Facility, University of California, San Francisco, San Francisco, CA; ²Buck Institute for Age Research, Novato, CA; ³Argonne National Laboratory; ⁴Purdue University, West Lafayette, IN

Most commonly, the design of discovery-stage biomarker experiments is based on the principle that oncogenic processes effect changes in protein expression. It is less well appreciated that posttranslational modifications (PTMs) undergo significant disease-related alterations, even when the levels of the mRNAs that encode the peptide backbones do not change. Alterations in phosphorylation and glycosylation, along with the accumulation of protein
oxidative damage, are common features of many cancers, and as such, they are interesting targets that can be exploited for biomarker discovery.

Accordingly, as part of the NCI CPTAC Network, we are developing mass spectrometry-based strategies for plasma analyses that incorporate one or more affinity-selectors, thus enabling enrichment of proteins that carry cancer-related PTMs. All parts of the workflow, from sample collection to data analysis, have been tailored for this purpose. For example, blood collection protocols were modified to include the immediate addition of phosphatase inhibitors or hydrazide regents to freshly isolated platelet-depleted plasma to protect phosphorylated and oxidized sites, respectively. With regard to separation strategies, the samples were chromatographed on a MARS column, which removed the 14 most abundant plasma proteins, before they were subjected to trypsin digestion. Then they were fractionated using an affinity reagent that bound the PTM of interest. In each case, we developed peptide and/or protein standards containing (or lacking) the relevant PTMs to serve as controls, which is allowing us to optimize each workflow. We are testing these platforms in multiple CPTAC laboratories to assess selectivity and robustness for isolating glycopeptides, phosphopeptides and/or oxidized carbonyl-containing (carbonylated) plasma proteins. Future directions include applying these workflows, in a consortium-wide CPTAC study of plasma samples from breast cancer patients and control individuals, with the goal of identifying proteins that carry cancer-specific PTMs. We anticipate that future validation experiments could employ alternative strategies such as peptide-based multiple reaction monitoring (MRM) assays.

A Multisite Assessment of Multiple Reaction Monitoring MS for Protein Quantitation in Human Plasma: Toward a Robust Tool for Verification of Biomarker Candidates


1University of California, San Francisco, San Francisco, CA; 2Broad Institute of MIT and Harvard, Cambridge, MA; 3Buck Institute for Age Research, Novato, CA; 4Massachusetts General Hospital, Boston, MA; 5Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD; 6Texas A&M University, College Station, TX; 7Vanderbilt University, Nashville, TN; 8University of Victoria-Genome BC Proteomics Centre, Victoria, BC, Canada; 9Purdue University, West Lafayette, IN; 10Kimmel Center for Biology and Medicine at the Skirball Institute and Department of Pharmacology, New York University School of Medicine, New York, NY; 11National Cancer Institute, Bethesda, MD; 12The University of North Carolina at Chapel Hill, Chapel Hill, NC; 13Monarch Life Sciences, Indianapolis, IN; 14Indiana University School of Medicine, Indianapolis, IN; 15Fred Hutchinson Cancer Research Center, Seattle, WA; 16Plasma Proteome Institute, Washington DC; 17Memorial Sloan-Kettering Cancer Center, New York, NY. 18Present address: Synta Pharm, Lexington, MA.

Immunoasays such as ELISA have long been the primary technology for verification of candidate protein biomarkers and for their preclinical validation. However, when confronted with the hundreds of candidate protein biomarkers coming out of proteomic-based discovery analyses, all of which are in need of verification, immunoassays present significant problems, including lack of availability of suitable antibodies and difficulties in multiplexing the assays. Multiple reaction monitoring mass spectrometry (MRM-MS) with quantification using stable isotope-labeled versions...
of the analyte peptides (a technique referred to as “stable isotope dilution” [SID]), is poised to provide a much-needed alternative to immunoassay. This technology is already widely used to measure small molecules and drugs in plasma and has shown early promise for assaying proteins in blood. However, before SID-MRM-MS can be accepted as a viable component of the biomarker pipeline, this technique must be shown to be transferable and reproducible between laboratories. We have carried out a multilaboratory study to assess the reproducibility, recovery, linear dynamic range, and limits of detection for SID-MRM-MS assays of seven target proteins in plasma. Using common reagents and standardized operating procedures we demonstrated that these MS-based assays for proteins can be highly reproducible within and across laboratories and instrument platforms in unfractionated plasma, with performance approaching those of clinical assays. Sensitivity is currently limited to low ug/mL protein concentrations but can be improved using protein and/or peptide enrichment prior to measurement by SID-MRM-MS. We also describe typical problems that are encountered in developing and applying MRM-SID-MS assays to quantify proteins in human plasma and demonstrate how to detect and overcome them.


Clinically Quantifying Low-Abundance Serum Proteins by LC-MS/MS: Potential Benefits and a Case Study

Andy Hoofnagle

Department of Laboratory Medicine, University of Washington, Seattle, WA

The quest for novel biomarkers may one day result in new assays for the clinical laboratory. Currently, protein biomarkers are measured by immunoassays, which have fundamental flaws that can lead to patient harm in a surprisingly large number of patients. We set out to solve this important clinical problem by using immunoaffinity enrichment of target peptides from tryptic digests of whole serum and measurement of the target peptides by liquid chromatography-tandem mass spectrometry. With thyroglobulin as the canonical troubled tumor marker and an assay employing stable isotope-labeled internal standard peptide and external calibrators, we were able to achieve good agreement with a relevant immunoassay and a 4 pM limit of detection in normal human serum specimens. The translation to the clinical laboratory of research proteomic methods that use mass spectrometry could lower the risk of misdiagnosis and lead to better patient care. This study puts us one step closer to a new paradigm.

Role of Clinical Chemists in Translating Novel Biomarkers Into Clinical Practice

Saeed A. Jortani

University of Louisville School of Medicine, Louisville, KY

Clinical laboratorians and in particular clinical chemists are routinely involved with the development, management, and interpretation of in vitro diagnostic procedures routinely used in medicine. Their typical day involves interactions with the in vitro diagnostic companies that produce the clinical assays, technologists who perform the testing, and clinicians who order and use the results for patient care. In fact, novel tests have to be put through a rigorous analytical validation process and an operating procedure must be developed for each assay’s implementation into clinical practice. These processes are handled and directed by clinical chemists. Many individuals in this profession work in industry (i.e., diagnostics companies) setting up the new tests onto automated instruments, seeking regulatory approval, and overseeing their evaluation in diagnostic clinical trials. From “bench to bedside” or from “discovery to clinical use,” several steps must be considered that may not be clear to the majority of investigators. It is therefore imperative that clinical chemists be included in the teams charged with discovery and validation of new
cancer biomarker candidates for patient care. In this session, we will outline the interactions between the CPCT and the proteomics division of the American Association for Clinical Chemistry in an effort to involve the professionals in the clinical biomarker implementation business with the new cancer biomarker discovery efforts by the National Cancer Institute.

**Elucidation of Tumor Biology and Assessment of Cancer Patients Using Quantitative Mass Spectrometry**

*John Koomen*

*H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL*

Applications for quantification of protein expression and posttranslational modification using liquid chromatography coupled to multiple reaction monitoring (LC-MRM) are currently being explored to determine their impact on critical issues in cancer biology research and patient assessment and treatment.

Quantitative mass spectrometry enables multiplex analysis of signaling networks and components of biological processes; assay development is under way for proteins relevant to tumorigenesis, progression, metastasis, and drug resistance. After development and implementation in preclinical models such as cell lines, these assays can be rapidly adapted to clinical samples, effectively translating knowledge gained from basic science into a clinical context. Quantification of expression of selected hypothesis-driven and investigator-driven protein targets has been the focus for assay development; the methods and reagents will be made available online in the Quantitative Assay Database at proteome.moffitt.org/QuAD.

LC-MRM has the potential to quantitatively evaluate substantial numbers of cellular functions and response mechanisms in parallel. As proof of concept, we have been quantifying expression of direct drug targets and apoptosis-related proteins in multiple myeloma models of drug resistance using SDS-PAGE fractionation of whole-cell lysate coupled with LC-MRM analysis. The BH3 domain containing proteins provide unique challenges for LC-MRM; because of their small size, few tryptic peptides can be detected from each protein. Furthermore, truncation and other posttranslational modification events are also critical to the full assessment of their function. Currently, assays for proteins from the NFκB and DNA damage response pathways are being developed and integrated in parallel. Combined with microenvironmental models and established drug-resistant cell lines, these quantitative mass spectrometry assays prove to have high utility in developing preclinical models for minimal residual disease and in implicating potential combination therapies.

LC-MRM also is being used for assessment of multiple myeloma patients to illustrate the potential advantages and limitations of the technology as part of a patient care strategy. Quantification of monoclonal antibodies in serum collected from multiple myeloma patients is being explored in an effort to detect patient relapse earlier than the current clinical method, serum protein electrophoresis (SPEP). With detection limits around 1 mg/mL for the monoclonal antibody, SPEP sensitivity could be significantly improved. LC-MRM can quantify each of the antibodies and their isoforms, providing additional information over SPEP and immunofixation, while lowering the limits of detection required to observe disease recurrence.

Finally, the integration of LC-MRM into the institutional initiative for personalized medicine, Total Cancer Care, will be discussed. Each tumor type could be evaluated with quantitative proteomics to develop signatures of protein expression and posttranslational modification that would complement current efforts in recording detailed medical histories and gene expression profiles.
Applications of the CPTAC Yeast Reference Proteome Standard

Daniel C. Liebler

CPTAC Discovery Working Group; Jim Ayers Institute for Precancer Detection and Diagnosis, Vanderbilt-Ingram Cancer Center, Nashville, TN

The CPTAC Discovery Working group has performed interlaboratory studies to evaluate the performance of LC-MS/MS proteomics platforms and to develop and employ standard materials and performance metrics to evaluate system performance. Optimal performance of LC-MS/MS platforms is critical to generating high-quality proteomic data. Although individual laboratories have developed quality control samples, there is no widely available performance standard of biological complexity (and associated reference data sets) for benchmarking of platform performance for analysis of complex biological proteomes across different laboratories in the community. Individual preparations of the yeast \textit{Saccharomyces cerevisiae} proteome have been used extensively by laboratories in the proteomic community to characterize LC-MS platform performance. The yeast proteome is uniquely attractive as a performance standard since it is the most extensively characterized complex biological proteome and the only one associated with several large-scale studies estimating the abundance of all detectable proteins. In this study, we describe a standard operating protocol for large-scale production of the yeast performance standard and offer aliquots to the community through the National Institute of Standards and Technology, where the yeast proteome is under development as a certified reference material to meet the long-term needs of the community. Using a series of metrics that characterize LC-MS performance, we provide a reference data set demonstrating the typical performance of commonly used ion trap instrument platforms in expert laboratories; the results provide a basis for laboratories to benchmark their own performance, improve on current methods, and evaluate new technologies. Additionally, we demonstrate how the yeast reference, spiked with human proteins, can be used to benchmark the power of proteomic platforms for detection of differentially expressed proteins at different levels of concentration in a complex matrix, thereby providing a metric to evaluate and minimize preanalytical and analytical variation in comparative proteomics experiments. Additional studies to be described have applied the yeast reference to evaluate affinity capture methods, enhance multidimensional LC-MS/MS platforms, and generate data sets for development and testing of statistical and computational tools for proteomics.

Discovery and Verification of Protein Biomarkers in Cancer, Precancer, and Normal Tissues

Daniel C. Liebler

CPTAC Discovery Working Group; Jim Ayers Institute for Precancer Detection and Diagnosis, Vanderbilt-Ingram Cancer Center, Nashville, TN

The Vanderbilt CPTAC program evaluates technology platforms for unbiased biomarker discovery and biomarker candidate verification by targeted protein quantitation. Discovery platforms employ shotgun proteomics by multidimensional LC-MS-MS, whereas targeted quantitation employs liquid chromatography-multiple reaction monitoring-mass spectrometry (LC-MRM-MS). Recent work compared IEF fractionation platforms (GE IGPbhor 24 cm strips, Invitrogen 10 cm Zoom-IPG Runner strips, and Agilent 24 cm Off-Gel IPG strips) with different protein load numbers of fractions. This work provides objective criteria for matching peptide separations to available tissue protein input, MS instrument time, and overall sample numbers. We have developed a statistical model for distinguishing proteotypes corresponding to phenotype differences from shotgun proteomics data sets. The model identified proteomic differences between significantly different tissue phenotypes (e.g., colon carcinoma vs. normal colon) typically corresponding to approximately 200 differentially expressed proteins. This approach also was used to compare proteomes from colon cancer cell lines that differ in mismatch repair (MMR) capacity due to mutations in
MSH and MLH genes. Shotgun proteome inventories of MMR+ and MMR- cell lines yielded ~6,000 confident protein identifications with variations of less than 15% in total numbers of spectra mapped to peptides and proteins. Spectral count data verified the predicted absence of protein products of the MSH and MLH genes. The data illustrate the ability of discovery proteomics analysis platforms to detect discrete, cancer-relevant changes in proteomes that correspond to cancer-specific mutations and phenotypes. In related work, we have evaluated methods for preparation of tryptic digests from proteins captured by laser capture microdissection of breast tumor tissues. A parallel project developed a novel, biotinylating tag that is cleaved in mild base and can be used to enrich cysteinyl peptides from tryptic digests. Cysteinyl peptide enrichment enhances detection of low-abundance proteins in human cell proteome digests and with the CPTAC yeast proteome reference material. Other work has demonstrated the equivalency of formalin-fixed paraffin-embedded (FFPE) and frozen tissue specimens in shotgun proteomic analyses. Analysis of archival colon adenoma FFPE specimens indicated equivalent numbers of MS/MS spectral counts and protein group identifications from specimens stored for up to 10 years. Additional work is focused on assessment of plasma proteome integrity under conditions of collection and storage. Global shotgun proteome analysis indicates broad stability of plasma proteins under conditions of extended room temperature blood storage in EDTA-plasma tubes and after multiple freeze-thaw cycles for EDTA-plasma. We have evaluated stable isotope dilution, “single peptide standard,” and “label-free” methods for LC-MRM-MS analysis of protein biomarker candidates in cell and tissue specimens. This work provides a basis for selecting and optimizing cost-effective strategies for preliminary verification studies of candidate markers in tissues and biofluids. Finally, we have developed a collaboration with the MacCoss laboratory at the University of Washington to develop and employ the “Skyline” software to the design and analysis of LC-MRM-MS analyses of biomarker candidate proteins. (Supported by NIH cooperative agreement award U24CA126479)

Love/Avon Army of Women: A New Model for Partnering Women and Scientists To Find the Cause and Prevention of Breast Cancer

Susan Love1, Dixie Mills1, Marc Hurlbert2, Effie Eraklis1

1Dr. Susan Love Research Foundation, Santa Monica, CA; 2Avon Foundation for Women, New York, NY

One of the barriers to elucidating the etiology of breast cancer is the difficulty basic scientists have in involving healthy women and in obtaining human tissues, fluids, and information processed according to their needs. However, the Dr. Susan Love Research Foundation has had success in recruiting healthy community women for studies. In 2006 we partnered with a Texas Tech University scientist who had planned to do his research in an animal model because he felt it would be impossible to obtain blood and core needle biopsies from the women fitting his strict criteria. However, by accessing our registry of potential volunteers, we were able to collect all the specimens. A grant from the Avon Foundation for Women allowed us to develop the Love/Avon Army of Women (AOW).

The AOW was launched in October 2008 by Dr. Love on national media with the goal of recruiting one million women who are willing to participate in clinical research into the cause and prevention of breast cancer. Researchers contact the AOW and sign in with their studies. Projects are reviewed by the AOW Scientific Advisory Committee, which includes members nominated by the AACR and the NBCC. If a researcher cannot obtain his or her own specimens, there are regional AOW Centers where the appropriate data and samples can be collected according to the specifications of the scientist. After a study has been completed, the researcher reports back to the participating women the overall findings of the study via webcast. This ensures a partnership between the women and the researchers in moving toward the goal of eradicating breast cancer.

To date, over 300,000 women have signed up at www.armyofwomen.org. Of these, 85% are Caucasian, with age ranges from 18 to 89 years with a mean of 56 years. There are members in all 50 States. An eblast announcing the
The first AOW study was sent out in mid-October 2008, describing the study, the criteria for participation, and what would be involved. Women then self-selected and RSVPed to the AOW. In 48 hours, 1,600 women had responded and qualified, and in 2 weeks, 2,300 were enrolled. A second study was sent out a few weeks later, again accruing more women than the researchers had anticipated. As of August 1, 2009, 11 studies had been sent out via email; 6 are now closed and 5 are still open. Over 11,000 women are now participating in clinical research. Efforts are under way to launch a large cohort study of AOW members along with caBIG and the City of Hope. We are also currently looking for more scientists who have projects needing human breast tissue, ductal fluid, urine, saliva, or blood as well as data for epidemiology.

The Love/Avon Army of Women is a novel resource for breast cancer scientists to obtain exactly the type of specimens they need when they need it. This new partnership between women and scientists can revolutionize research and accelerate efforts to eradicate breast cancer.

**What I’ve Learned From Being a Member of the Proteomics Research Group**

*Michael MacCoss*

*Department of Genome Sciences, University of Washington, Seattle, WA*

The Proteomics Research Group (PRG) is a volunteer organization that is dedicated to assisting scientists and facilities in the analysis of proteins. The PRG sponsors annual research studies that examine current techniques and approaches used by research laboratories for the analysis of protein mixtures. I will present the studies developed by the PRG during my 3-year involvement and the rationale behind their designs. I will also present the results from these studies. Using these results, I will illustrate the importance that these studies play in evaluating the performance of the community as a whole and in the self-evaluation of individual laboratories to benchmark themselves within the community.

**Degradable, Iron(III)-Doped, Hollow Silica Nanoparticles**

*Kristina K. Pohaku Mitchell*

*Trogler Laboratory, University of California, San Diego, San Diego, CA*

Nanoparticles are being investigated for their potential use in targeted drug delivery. Ideally, nanoparticles should possess the following properties for use in drug delivery: a long shelf life, synthesis that can be done on a commercial scale, and no accumulation of particles in the body. Silica nanoparticles can be synthesized on a commercial scale and, once calcined, can have a long shelf life. Although silica is FDA approved for consumption, silica nanoparticles are questioned for use in intravenous applications because of its potential to accumulate in the body. We are incorporating iron(III) into the silica matrix of the nanoparticles in an attempt to make the silica degradable. A degradable property would prevent accumulation of nanoparticles in the body, thus making them useful as delivery agents for chemotherapeutic drugs.
Multiplex Mass Spectrometric Immunoassays

Dobrin Nedelkov

Intrinsic Bioprobes Inc., Tempe, AZ

Targeted proteomics approaches can facilitate the full assessment of the human protein variations and their association with cancer. Typically, affinity interactions are utilized to selectively retrieve specific proteins from complex biological fluids and are followed by mass spectrometric analysis of the targeted proteins. Mass spectrometric immunoassays are similar to traditional enzyme immunoassays in that they use antibodies as reagents for affinity retrieval of specific proteins. However, instead of enzymatic reaction for indirect protein detection, mass spectrometric analysis is used for direct identification of the proteins and their modifications. This rather straightforward concept is realized through affinity pipette devices that enable high-throughput integration and assaying of thousands of samples per day. Described in this presentation will be the development of several single and multiplex mass spectrometric immunoassays toward cancer-related proteins. The assays’ characteristics and their performance will be compared with existing approaches, and the application of these assays in clinical setting will be discussed.

Using SILAC To Study Tyrosine Kinase Signaling in Neurons

Thomas A. Neubert, Daniel S. Spellman, Katrin Deinhardt, Moses V. Chao, Guoan Zhang

Kimmel Center for Biology and Medicine, Skirball Institute, New York University School of Medicine, New York, NY

Communication between neurons often relies on the stimulation of receptor tyrosine kinases with ligands for these receptors. Much can be learned about how this signaling works by using stable isotope labeling methods such as stable isotope labeling by amino acids in cell culture (SILAC) and mass spectrometry to compare the proteins in signal transduction complexes from stimulated and nonstimulated cells. Cultured primary neurons are a well-established model for the study of neuronal function in vitro. We have demonstrated that SILAC can be applied to a differentiated, nondividing cell types such as primary neurons as long as corrections can be made for each protein in cases of incomplete labeling with heavy amino acids. More recently, we have overcome this problem by using only heavy isotope-labeled cell populations rather than labeled and unlabeled cells.

We applied this technique to assess changes in the neuronal phosphotyrosine proteome and associated proteins in response to stimulation by three ligands that are important for the development and regulation of neuronal connections: brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4. Our results provided clues as to how these ligands can produce different signaling outcomes, even though all three ligands can activate the same receptor tyrosine kinase TrkB receptor. These results demonstrate that the combination of primary neuronal cell culture and SILAC can be a powerful tool for the proteomic study of neuronal molecular and cellular dynamics.
The success of proteomic projects critically depends on the availability of robust and accurate methods of data analysis. One of the key areas in the field of proteomics is statistical validation, interpretation, and mining of the lists of protein identifications obtained using tandem mass spectrometry (MS/MS). Due to the improvements in mass spectrometry instrumentation, the size of MS/MS data sets generated in typical proteomic experiments keeps increasing. Often, multiple replicates of the same or similar samples are acquired and processed simultaneously (e.g., in applications utilizing label-free quantification based on spectral counts for differential protein analysis). Furthermore, multiple diverse data sets can be merged to create large repositories such as the PeptideAtlas. At the same time, many currently used statistical methods for estimating protein identification error rates were initially developed using much smaller data sets. In this work, we investigate the performance of the commonly used protein inference tool ProteinProphet using a large publicly available data set generated on human T leukemic cells using an LTQ linear ion trap instrument (14 technical replicates, 246 mass spectrometry runs, 2.6 million MS/MS spectra). Based on this analysis, we suggest an improved strategy for filtering and error-rate estimation. We also describe a new computational approach that further improves on the standard PeptideProphet/ProteinProphet by incorporating additional information in the model, such as matches made by multiple database search engines and repeated MS/MS sequencing of the same peptide ion in one or across multiple LC-MS/MS runs, in different charge states, and in different modification states. The performance of the new computational tool, iProphet, is tested using several large collections of data taken from the Human PeptideAtlas data repository.

Modeling Phosphopeptide Gas Phase Fragmentation

William M. Old

Department of Chemistry and Biochemistry, University of Colorado at Boulder, Boulder, CO

Conventional peptide search algorithms are limited by their inability to evaluate fragment ion intensity information in MS/MS spectra, using scoring systems that lack specificity when evaluating thousands of candidate sequences. As a consequence, high score thresholds are required to control false discovery rates, resulting in large numbers of false negatives, a problem compounded by the large search spaces required for identification of posttranslational modifications. We have developed a method for postsearch validation to address this issue, termed “Manual Analysis Emulator (MAE),” which improves accuracy by using expert rules to assign fragment ions and assesses chemical plausibility of fragmentation by scoring for similarity between observed MS/MS spectra and theoretical spectra calculated from a kinetic model of gas phase fragmentation (MassAnalyzer, developed by Zhong-qi Zhang). These features of MAE greatly improve discrimination between correct and incorrect sequence assignments. In order to add new peptide fragmentation mechanisms and extend it to phosphopeptides, we created “S3,” our own version of MassAnalyzer. Model parameters are simultaneously optimized using the Levenberg-Marquardt algorithm, bypassing constraints of MassAnalyzer to fit only one parameter at a time. Overall model prediction accuracy was significantly increased using our fitted parameter set. We are currently extending S3 to phosphopeptide fragmentation. To provide data for model fitting, 13 combinatorial phosphopeptide libraries were synthesized with degenerate amino acids incorporated at different positions, spanning thousands of sequences with diverse chemistries. Dephosphorylated forms of the libraries provide MS/MS spectra for both the unphosphorylated and phosphorylated forms of each peptide. Data mining of these libraries have provided insights into the factors that affect neutral loss of phosphate and MS/MS and MS3 cleavage around phosphorylated residues. The goal will be to
improve the accuracy of predicting phosphopeptide fragmentation spectra, which will enable the development of more sophisticated tools for large-scale phosphopeptide identification.

Oxidized Proteins in Breast Cancer Patient Plasma

Ashraf Madian, Fred Regnier

Department of Chemistry, Purdue University, West Lafayette, IN

Oxidative stress (OS) plays an important role in aging, neurological diseases, inflammatory diseases, and some types of cancer. Irreversible oxidative damage from chronically high OS appears to be cell type and organ specific in many cases, leading to unique oxidative fingerprints in particular proteins and at specific sites. This in turn leads to partial or total loss of function with concomitant weakening cells and often apoptosis. Carboxylation is among the more prominent forms of oxidation. Proteins can be carboxylated in multiple ways including oxidation of arginine, threonine, lysine or proline, Michael addition of carboxyl containing fragments of fatty acids, and by oxidation of advance glycation end products. All of these types of oxidation are distinguishable by mass spectrometry, making it possible to identify the mechanism of oxidative damage.

We report here the first proteomic based identification and characterization of oxidized proteins in human plasma. Biotin hydrazide was added to normal human plasma samples and the resulting Schiff’s bases were reduced by sodium caynoborohydride. Proteins thus biotinylated were affinity enriched by avidin affinity chromatography. Affinity selected proteins were digested with trypsin, the peptide fragments separated by RPC, and identified by ESI-MS/MS and MALDI-MS/MS. Approximately 0.2% of the total protein content of plasma was affinity selected in a small group of 30 year old male subjects from which 65 proteins were identified. Some proteins and sites within these proteins were more prone to oxidation than others. Twenty four oxidative modifications (e.g., kynurenin, pyroglutamic and aminoadipic semialdehyde) were identified in fourteen proteins. Oxidative damage was found to be substantially higher in 60 year old breast cancer patients, from which 1% of the total protein was avidin selected after biotinylation.

High-Throughput, High-Sensitivity Proteomics Platform for Improved Biomarker Discovery and Verification


Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA

With the ability to quantify peptides and proteins in organisms, tissues, cells, and biological fluids, mass spectrometry (MS)-based technologies are playing a growing role in the discovery of new candidate biomarkers. In spite of significant advances, MS technologies remain challenged by biofluid samples such as blood plasma, where protein concentrations span a dynamic range greater than 10 orders of magnitude and inadequate measurement throughput hinders the ability to effectively account for biological diversity. Achieving higher analysis throughput with concurrent increase in sensitivity and dynamic range of measurements to allow detection and quantitation of lower abundance peptides and proteins from biological fluids is a major analytical challenge. In response to this shortcoming, we are developing a new platform with greatly improved measurement throughput, sensitivity, robustness, and quantitative capability for cancer biomarker discovery, verification, and preclinical validation.
Our proteomics platform encompasses fast capillary LC separations coupled via a greatly improved electrospray ionization interface to an ion mobility spectrometer (IMS) that is interfaced to a time-of-flight mass spectrometer (TOF-MS). The fully automated capillary LC system incorporates high-pressure LC pumps, an autosampler, and a four-column fluidics system. Electrospray ionization (ESI)-generated ions are accumulated in an electrodynamic ion funnel trap before being injected into an 84-cm-long IMS drift tube. To increase IMS-TOF-MS sensitivity, we have developed a novel multiplexing approach that increases the number of ion pulses that can be separated in a given time by >50-fold. Downstream of the IMS drift tube, spatially dispersed ion packets are recollimated by an electrodynamic ion funnel into a commercial orthogonal acceleration TOF-MS. A high-performance data acquisition system based on an 8-bit 1 GS/s analog-to-digital converter was developed for high mass accuracy and dynamic range measurements. The total duration of LC-ESI-IMS-TOF-MS analysis (i.e., for each column) is 15 minutes.

Initial evaluation of the LC-IMS-TOF-MS system has been performed using a number of different approaches. In general, we have used blood plasma sample in which 12 of the most abundant proteins were depleted. The evaluation efforts have consistently demonstrated significant gains in both the throughput and coverage provided by other proteomics platforms available at our laboratory. In the past year, a growing portion of our efforts has involved advancing the informatics data analysis tools since the LC-IMS-TOF-MS platform evaluates samples in four different dimensions (elution time, drift time, m/z, intensity). Recent informatics improvements include incorporation of the ion mobility information into the AMT tag database, instituting multiple “deisotoping” algorithms, and developments in multidimensional peptide feature finding. All of these informatic enhancements are expected to greatly increase data quality and accelerate biomarker discovery efforts. The poster will summarize overall platform performance.

Metrics for LC-MS/MS Performance in Proteomics

Qian Dong, Bhaskar Godugu, Lisa Kilpatrick, Yuri Mirokhin, Pedatsur Neta, Paul Rudnick, Dmitrii Tchekhovskoi, Eric Yan, Stephen Stein

National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD

LC-MS/MS is now a routine and widely used method in proteomics, where peptides from tryptic digests are identified and linked to their parent proteins using known protein sequences. Instruments have become highly sophisticated, and data are analyzed by many accepted methods. However, the variability of results from this platform is only poorly understood and rarely monitored, though it can seriously influence the reproducibility and quality of results. Part of the CPTAC Program has been devoted to dealing with these issues, and the latest results of these studies will be reported here. Analysis makes use of results of interlaboratory studies on two selected “standard” mixtures—a simple protein mix and a yeast lysate, spike-ins as well as other simple tryptic digests studied to elucidate specific aspects of this variation. This has led to the development of a large number of performance metrics, each of which reports a value linked to a different aspect of the experiment and each derived from the original data in a straightforward manner. Two varieties of metrics have been developed—one based on the analysis of a single data file, the other expressing differences between the peptides in common between pairs of runs. One use is the monitor changes in results for a standard mix, where any significant variation in metrics indicates that a problem has arisen and corrective action is needed. Another application is to objectively examine differences between results between of different labs and to assist in pinpointing the origin of significant deviations. These metrics also provide a means of monitoring changes in this complex experiment as changes are made to the analysis method. A data analysis pipeline that produces these metrics is freely available from http://peptide.nist.gov/.
Repeatability and Reproducibility in Proteomic Analyses by Liquid Chromatography-Tandem Mass Spectrometry

David Tabb

Vanderbilt University, Nashville, TN

The CPTAC Unbiased Discovery Working Group generated an interlaboratory LC-MS/MS data set featuring multiple sample types, technical replicates, and instrument platforms throughout the consortium. We examined these data to measure the variability among technical replicates (repeatability) and in the face of methodology changes (reproducibility). Peptide-level repeatability was relatively robust against changes in sample type and concentration but was improved for Orbitraps versus LTQ instruments. The most consistently repeated peptides were those that conformed to two standard trypsin cutting sites, produced intense MS signals, and originated in the best-evidenced proteins of the sample. Statistical evaluation of spectral counts revealed that more replicates are necessary in LTQs to achieve the same level of discrimination between samples as in Orbitraps. Reproducibility was also enhanced for Orbitrap instruments.

TagRecon Identifies Protein Mutations and Modifications

David Tabb

Vanderbilt University, Nashville, TN

The Jim Ayers Institute for Precancer Detection and Diagnosis, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center has produced large amounts of MS/MS data from clinical samples and from cancer-related cell lines. Our sequence tag-based analysis of Ayers data sets reveals that these data sets contain evidence of mutations and modifications that correlate with disease state. TagRecon has enabled the recognition of proteomic sequence variability in response to mutation mismatch repair deficiency. The software also revealed the differentiation of tumor and adjacent samples by hydroxyprolination.

Group Quantification of Mass-Tagged Peptides Using Spectrum Clustering With PICquant and MAZIE

Kenneth G. Victor, Charles E. Lyons, Sergey Moshnikov, Dennis J. Templeton

Department of Pathology, University of Virginia, Charlottesville, VA

PICquant is a software platform that aims to perform mass spectrometric quantification of anonymous peptides labeled with $^{13}\text{C}(6)$ isotopic versions of phenylisocyanate (PIC). M+0 and M+6 Dalton mass pairs are quantified in high-resolution MS1 spectra. Replicate quantitative determinations result from ion sampling of both heavy and light labeled ions and from data-dependent resampling. Early versions of PICquant sought to average these replicate determinations by grouping repeated determinations of homologous ions identified by similarities in MS-MS spectra. This approach has proven suboptimal for several reasons, including inefficiency in spectrum clustering and mass inaccuracy owing to precursor mass imprecision and sampling of isotopic variants with M+1 to M+3 mass variation.

We have recently described MAZIE, a Mass and Charge (Z) Inference Engine, that calculates monoisotopic mass from ion envelopes that result from isotopic mass variation and also corrects precursor mass imprecision. MAZIE also determines accurate ion charge. MAZIE is useful for enhancing spectrum data files and results in increased high-
confidence sequence identifications, principally because the enhanced mass precision requires far fewer candidate ions to be searched and, therefore, far fewer false-positive identifications.

The enhanced mass and charge precision resulting from MAZIE also presents an alternative means to group ion quantifications using PICTquant. The resulting monoisotopic mass precision is in the range of 0.1 Da. This is sufficient to cluster individual ion families resulting from heavy and light PIC labeling, plus M+0 to M+4 isotopic mass variants, using monoisotopic mass alone, when analyzing complex tryptic samples producing 8-10,000 MS-MS spectra. Examples of clustering results from urine peptides samples fractionated by isoelectric focusing will be described quantitatively.

**Transformation of Anecdotal Clinical Peptidomics Into Activity-Based Biomarker Screens and Verification**

Paul Tempst

Memorial Sloan-Kettering Cancer Center, New York, NY

It has often been stated in editorials and conference roundtables that the prevailing, less-than-optimistic view of proteomics as a way to discovering novel biomarkers is primarily related to the overhyped and then maligned cancer serum peptidomic efforts of the first half of this decade (and beyond). The repeated criticisms over the years include (1) substandard analytical technology; not reproducible; not quantitative; peptides not identified; underlying biology not understood; (2) fragments derive from abundant blood proteins; interference from acute phase response, malignant coagulopathy, and other epiphenomena; (3) bias in sample collection; no thorough SOP in clinical environment; and (4) bias in human subject demographics (e.g., gender, age) and genetic variation of the population. Sadly, these criticisms continue to be reiterated in recent reviews; presumably through copy-and-paste and not based on an appraisal of the recent literature describing optimized and technically adequate approaches that have evolved, and continue to evolve rapidly, from the SELDI-based peptidomics of old.

Over the course of the past 6 years, our lab and others have tried to answer these criticisms and, in the process, have transformed shaky diagnostic peptidomics into better controlled and reproducible plasma proteolytic assays. Among the sequential improvements, rational adaptations, and innovations are (1) use of high-end MALDI-TOF; automation of peptide processing; strict sample collection SOP; (2) the biology is now fairly well understood and a model of “exopeptidase-activities-as biomarkers” has been proposed, which also explained the sample variability problems and convinced us that a different approach was needed in the future; (3) a wide-ranging healthy human subject study indicated no appreciable gender and age differences; (4) a new test utilizing externally added, labeled peptide substrates avoids reproducibility problems related to sample collection, storage and handling and copes with variability in endogenous peptide precursor levels owing to hemostatic alterations in cancer patients; (5) spiked, nondegradable reference peptides allow for relative quantitation of all individual degradation products; (6) development of second-generation substrates and choice of class-specific and enzyme-specific inhibitors restrict multiplexed assays to one or a few candidate exopeptidases each; and (7) mouse models of prostate cancer resulting from two different genetic lesions and WT controls have been assayed in recent studies and confirm our earlier working model. A synopsis of how we have restructured approaches and measurements will be given, illustrated with recent applications.
Focused Integration and Valued Output: Korean Proteomics Efforts To Discover Biomarkers

Myeong-Hee Yu

Functional Proteomics Center, The 21C Frontier R&D Initiatives, Korea Institute of Science and Technology, Seoul, Korea

Among various technological approaches to finding disease-related proteins, proteomics has been considered a revolutionary technology for discovering phenotype-based biomarker candidates. Innovation in biomarker discovery is needed not only in developing sensitive high-throughput detection tools but also in coordinating various disciplines toward value creation. For efficient use of our limited resources, we have formed a national network of proteomics core facilities and have focused on establishing high-throughput platforms for biomarker discovery. This strategy of networking allows us to share individual expertise in specific areas and to become a competitive team as a whole. During this collaborative effort, we developed various in-house softwares for interpreting mass spectra data, such as MODi, RAPID, and MpM, through which data interpretation and processing are more informative and faster. To validate clinical relevance of the discovered candidates, collaborative clinical research networks were also set up through which a systematic collection of clinical samples with detailed patient records is provided. Key factors of resource allocation in biomarker discovery will be highlighted, and bottlenecks in translation into value chain will be addressed.
# POSTER ABSTRACTS

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*Author could not attend meeting*
A Lectin Affinity-Based Biomarker Discovery Protocol Targeting Cancer-Specific Glycopeptides in Human Plasma

Penelope M. Drake1, Michael Lerch1, Eric Johansen1, Richard K. Niles1, Birgit Schilling1, Haichuan Liu1, Bensheng Li2, Simon Allen1, Steven C. Hall1, H. Ewa Witkowska1, Fred E. Regnier3, Bradford W. Gibson2, Susan J. Fisher1

1University of California, San Francisco, San Francisco, CA; 2Buck Institute for Age Research, Novato, CA; 3Purdue University, West Lafayette, IN

Glycans are an important class of posttranslational modifications that decorate secreted and extracellular molecules. As products of extensive biosynthetic pathways, glycan structures reflect cell status. Thus, they radically change under pathogenic conditions such as cancer. Increases in sialylation and fucosylation are prominent among malignancy-associated modifications. We (among others) propose that these cancer-related carbohydrate variants be exploited for biomarker discovery aimed at diagnosing early-stage disease. Accordingly, we developed a mass spectrometry-based workflow that incorporates chromatography on affinity matrices formed from lectins, proteins that bind specific glycan epitopes. Here we focused on Sambucus nigra agglutinin (SNA) and Aleuria aurantia lectin (AAL), which bind sialic acid and fucose, respectively. The lectins were covalently coupled to POROS beads (Applied Biosystems) and packed into PEEK columns for high-pressure liquid chromatography (HPLC). Plasma was depleted of the 14 most abundant proteins using a multiple affinity removal system (MARS-14; Agilent). Depleted plasma was trypsin digested and spiked with predigested glycoprotein standards—fucosylated and sialylated human lactoferrin glycopeptides (as positive controls), and high mannose glycopeptides from Saccharomyces cerevisiae (as negative controls). Samples were loaded onto the lectin POROS columns and separated into flow-through and bound fractions, which were treated with PNGaseF to remove N-linked glycans. Deglycosylation conditions were optimized by using LC/MS/MS to monitor reaction efficiency. The deglycosylated peptide fractions were interrogated by LC/MS/MS on a QStar Elite. Data were analyzed using Mascot and in-house software programs. Key elements of this workflow included (1) optimizing the ratio of starting material to column capacity based on the relative abundance of potential ligands in the sample, (2) spiking in standards to monitor the specificity of lectin capture, (3) increasing reproducibility by employing an HPLC format, and (4) including a PNGaseF digestion step, which allowed glycopeptide identification based on the appearance of deamidated Asn-Xxx-Ser/Thr motifs in peptide sequences. With this optimized protocol, following lectin chromatography of depleted plasma, we typically recovered 1,000-1,300 peptides in the flow-through fraction, 2-4% of which were glycopeptides. By contrast, the bound fraction contained 200-400 peptides, 30-50% of which were glycopeptides. Next, we applied this methodology to the analysis of conditioned medium samples from breast cancer cell lines and identified many known cancer-related glycoproteins in the bound fractions. Now, we are poised to compare families of SNA- and AAL-binding plasma glycoproteins isolated from breast cancer patients and control individuals.
Developing a Mass Spectrometry-Based Workflow Targeting the Plasma Phosphoproteome for Cancer Biomarker Discovery

Anna M. Zawadzka¹, Bensheng Li¹, Penelope M. Drake², Miles Brater², Birgit Schilling¹, Lee Makowski², Lynda Dieckman³, Susan J. Fisher², Bradford W. Gibson¹,²

¹The Buck Institute for Age Research, Novato, CA; ²University of California San Francisco, San Francisco, CA; ³Argonne National Laboratory, U.S. Department of Energy, Argonne, IL

In many tumors, malignant tissues, and cancer cell lines, significant changes occur in phosphorylation and the downstream signaling pathways this process governs. However, it remains unclear whether these cancer-related phosphoproteins can be observed in peripheral blood, where they might serve as potential early-stage disease biomarkers. To address this question, we optimized our plasma isolation protocol by adding phosphatase inhibitors to platelet-depleted plasma in an effort to preserve the phosphorylation status of the proteome. Then the samples were subjected to several separation and processing steps. Specifically, the 14 most abundant proteins were removed using a multiple affinity removal system (MARS-14). Depleted plasma was trypsin-digested, subjected to phosphopeptide-affinity selection, and analyzed by mass spectrometry. A variety of phosphopeptide enrichment strategies were evaluated to optimize recovery from plasma, including immobilized metal affinity (IMAC, both iron and gallium based) and titanium dioxide (TiO₂) chromatography. A set of commercially available phosphopeptides served as a positive control to evaluate the efficiency of these workflows. In collaboration with Argonne National Laboratory, we are also generating N-15 labeled phosphoproteins for this purpose. Mass spectrometric analyses of recovered plasma phosphopeptides were performed on multiple platforms, including a QqTOF (QSTAR-Elite) and a vMALDI-LTQ linear ion trap; analysis soon will be extended to an LTQ Velos Dual-Pressure Linear Ion Trap in both CID and ETD modes. To date, starting with 20 μL of plasma, over 26 phosphopeptides (62 unique phosphopeptides) have been confidently identified following TiO₂-enrichment, and 11 phosphopeptides (29 unique phosphopeptides) have been distinguished following IMAC enrichment. The list of observed phosphoproteins includes kininogen-1, vitronectin, selenoprotein P, antithrombin-III, alpha-2-HS-glycoprotein, kinesin-like protein KIF2B, and zinc finger protein 469. A significant and novel aspect of our study is the demonstration that addition of phosphatase inhibitors to freshly isolated plasma substantially increases the number of phosphopeptide identifications. Future directions include using this optimized workflow in mass spectrometry-based discovery experiments on plasma from breast cancer patients and control individuals.

Identifying Metrics for Digestion Effectiveness

Amy-Joan L. Ham, Corbin W. Whitwell, Lisa E. Kilpatrick, Lisa J. Zimmerman, Dmitrii Tchekhovskoi, Xinjian Yan, Pedatsur Neta, Bhaskar Godugu, Daniel C. Liebler, Stephen E. Stein

Vanderbilt University, Nashville, TN; Chemical and Biochemical Reference Data Division, National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg MD; Hollings Marine Laboratory, National Institute of Standards and Technology, U.S. Department of Commerce, Charleston, SC

One of the least examined sources of variability in mass spectrometry-based proteomics is the efficiency of digestion. The concentration of peptides in the digest may not match concentrations of their precursor proteins, and this mismatch can depend on details of the digestion process and the starting protein mixture. We report early results of studies that aim to find metrics for assessing the variability of digestion using mixtures of well-defined proteins. These studies employ a mixture of 9 proteins of variable size, structural and molecular properties, and “digestibility” and include beta-galactosidase (E. coli), serum albumin (human), alkaline phosphatase (E. coli), carbonic anhydrase (bovine), Invertase (S. cerevisiae), alpha-1-acid glycoprotein (human), apomyoglobin (equine), C-reactive protein
Peptides are analyzed using both traditional search methods and spectral library searching, with an MS1 intensity derived for each identified peptide. Peptides are classified by whether they contain missed cleavages, semitryptic, nonspecific cleavages and variable modifications. In our initial studies, we performed triplicate trypsin digestions using a urea digestion protocol and performed a time-course study of digestion at 1, 2, 6, 24, and 48 hours of digestion. Following digestion, equivalent amounts of peptides (based on total protein concentration) were resolved using a reversed phase precolumn (100 μm x 6 cm) and packed tip (100 μm x 11 cm) packed with C18 resin (Jupiter C18, 5 micron, 300 angstrom, Phenomenex, Torrance, CA) and separated using a 95-minute 0.1% formic acid/acetonitrile gradient on an LTQ-Orbitrap mass spectrometer (Thermo Scientific). The samples were analyzed with replicate injections to distinguish digestion variability from variability due to instrumental analysis. In additional experiments, several peptides were also spiked into the samples to monitor run-to-run instrument variations. The data were searched and filtered using either the Myrimatch and IDPicker algorithms or using spectral library searching. The data were further analyzed using a newly developed program, NISTMSQC, to identify and monitor changes in proteolytic products. In general, the number of tryptic peptides did not significantly increase after 6 hours of digestion. In addition, the total number of missed cleavages decreased with time, and the number of semitryptic cleavages increased over time, including some cases where the numbers of semitryptic peptides identified were as much as 50% of the number of identified peptides after 48 hours of digestion. Analysis of individual tryptic peptides demonstrated that some peptides were more stable over time than others, possibly a consequence of differences in stability of different peptides in the digestion mix. Additional studies investigating different digestion protocols with human serum albumin were performed and will be reported. (Supported by NIH cooperative agreement award U24CA126479)

**Discovery of Cancer Biomarkers Based on Tyrosine Phosphor-Proteomics**

*Srinivasan Krishnamoorthy*

*Physical Sciences Inc., Andover, MA*

Protein phosphorylation mediates many critical cellular responses and is essential for many biological functions during development. About one-third of cellular proteins are phosphorylated, representing the phosphor-proteome, and phosphorylation can alter a protein’s function, activity, localization, and stability. Tyrosine phosphorylation events mediated by aberrant activation of receptor tyrosine kinase (RTK) pathways have been proven to be involved in the development of several diseases, including cancer. To understand the systems biology of RTK activation, we have developed a phosphor-proteome focused on tyrosine phosphorylation events under insulin and EGF signaling pathways using the PhosphoScan® technique coupled with high-throughput mass spectrometry analysis. Comparative proteomic analyses of all these tyrosine phosphorylation events revealed that around 70% of these pY events are conserved in human orthologs and paralogs. A careful analysis of published in vivo tyrosine phosphorylation events from literature and patents revealed that around 26% of pY events from *Drosophila* proteins conserved on 185 human proteins are confirmed in vivo tyrosine phosphorylation events. Hence, the data are validated partially based on available reports, and the credibility of the remaining 43% of novel conserved sites on more than 300 human proteins that are unpublished so far is very high but requires further followup studies. The novel pY events found in this study that are conserved on human proteins could potentially lead to the discovery of drug targets and biomarkers for the detection of various cancers and neurodegenerative diseases.

The novel tyrosine phosphorylation events from the *Drosophila* proteome conserved in 100 human orthologs and paralogs constitute a valuable resource to translate the missing signaling connections and nodal points in the human proteome from the perspective of disease development. The data warrant further validation in human tumor cell lines and tissue samples to determine whether these pY events are upregulated or downregulated in GF signaling with...
respect to human disease phenotypes. Many of these novel pY events on proteins involved in various cancers and neurodegenerative diseases could potentially become biomarkers or drug targets for the respective diseases.

Recent reports indicate the importance of genes in glycolytic pathways in cancer progression. Mechanisms of tumor growth based on the selective switching of cellular processes toward anabolic pathways rather than oxidative phosphorylation also stress the importance of glycolytic proteins in cancer development. Our study in the fly proteome reveals that several proteins involved in glycolytic pathways are tyrosine phosphorylated and that these candidate human proteins with conserved tyrosine residues merit further study. Upregulation of monocarboxylic transporters (MCA) in type 1 diabetic patients indicate the possibility of increased capacity of the brain to use nonglucose substrates to meet energy requirements during hypoglycemia. Our study reveals that several MCA transporters are tyrosine phosphorylated upon RTK activation. It will be interesting to see whether the respective human orthologs are involved in similar mechanisms in brain-cell energetics.

Based on the available protein expression data, interesting candidate proteins could be selected for analysis of the dynamics of tyrosine phosphorylation with respect to disease development. Reverse-phase protein microarrays could be a very useful tool in this direction. Peptide arrays containing novel conserved pY modifications could be used to probe SH2 domain containing protein arrays to expand the signaling network with respect to a particular pathway.

**Novel Cleavable Probe for Cysteinyl-Peptide Enrichment and LC-MS/MS Analysis in Complex Proteome Mixtures**

De Lin, Ned A. Porter, Daniel C. Liebler

Departments of Biochemistry and Chemistry, Vanderbilt University, Nashville, TN

The complexity of cell and tissue proteomes presents one of the most significant technical challenges in proteomic biomarker discovery. Multidimensional LC-MS/MS-based shotgun proteomics is frequently coupled with selective enrichment of modified peptides or proteins containing phosphorylated, glycosylated, and cysteine-containing residues to reduce sample complexity. Here we report cysteinyl-peptide enrichment (CPE) with a new cleavable cysteine-reactive probe N-(2-(2-(2-(2-(3-(1-hydroxy-2-oxo-2-phenylethyl)phenoxy)acetamido)ethoxy)-ethoxy)ethyl)-5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (IBB). This probe contains three functional elements, a thiol-reactive iodoacetyl group, a mild base-cleavable benzoin linker, and a biotin affinity tag. We evaluated the efficiency of CPE using the IBB reagent followed by analyses using isoelectric focusing (IEF) of tryptic peptides coupled to LC-MS/MS. IBB was synthesized starting from 3-hydroxylbenzaldehyde by a seven-step route. Initial studies examined capture of the peptide Ac-AVAGCAGAR (Ac-TpepC), which was efficiently modified by IBB in PBS/TFE to form an S-alkyl-(IBB)-Ac-TpepC conjugate that was stable at room temperature in 50 mM acetate, pH 5.0 for up to 24 hours. However, in mild base buffer (pH 8.0, 50 mM ammonium bicarbonate), this conjugate hydrolyzed completely to form S-carboxymethyl-Ac-TpepC at room temperature in 5 hours. To evaluate the CPE technique, samples of IBB-alkylated tryptic peptides were prepared either by streptavidin capture followed by hydrolysis (CPE) or by hydrolysis followed by streptavidin capture (global peptide sample). Both global and CPE and samples from *Saccharomyces cerevisiae* (yeast) or RKO colon carcinoma cells were analyzed by reverse phase LC-MS/MS analysis after with or without IEF fractionation. We demonstrate that this approach is capable of not only enriching Cys-containing peptides but also enhancing low-abundance protein detection in both yeast and RKO samples. (Supported by NIH cooperative agreement award U24CA126479)
A Pipeline for Production of Protein Standards

Lee Makowski, Amina Aziz, Lynda Dieckman, Suneeta Mandava, Diane Rodi, Wen Zhang

Biosciences Division, Argonne National Laboratory, U.S. Department of Energy, Argonne, IL

Providing protein standards for MS experiments and antigens for production and evaluation of antibodies is a critical component of the CPTC Program. Argonne National Laboratory has taken on the role of providing these reagents. Proteins are selected for production on the basis of their relevance to cancer and, in particular, their potential as cancer biomarkers. Additional proteins relevant to proteomic studies also are being expressed, for instance, high-abundance plasma proteins. Human proteins are produced in *E. coli* hosts using a variety of vectors designed to provide uniform, high-quality reagents using a medium- to high-throughput pipeline. Production includes unlabeled proteins and N15 labeled proteins. To date, approximately 115 clones have been constructed, leading to the expression of 537 proteins, of which 262 have expressed in a soluble form. All clones leading to expressed proteins are deposited in the Arizona State University proteomics repository (formerly HIP). Current efforts include production of phosphorylated protein standards using in vitro phosphorylation of expressed targets. Protein standards have been designed and produced for a variety of proteomics experiments integral to the CPTC Program. Such high-quality, well-characterized, and readily available proteins and peptides will be a key resource in advancing proteomic technologies and capabilities and will be essential for developing highly specific antibodies to proteins associated with cancer.

Enhancement of MS Signal Processing Toward Improved Cancer Biomarker Discovery

Dariya Malyarenko

Department of Applied Science, College of William and Mary, Williamsburg, VA

In the third year of the project, we continued testing and integrating our signal processing and statistical pattern recognition tools into an R-package for automated selection of diagnostic proteins from mass spectra. The R-based flat-file parser was developed to import raw Ultraflex TOF spectra and instrumental meta-data so that large data sets could be automatically loaded into analysis pipelines. An analysis of amplitude and correlation statistics was performed for multiple replicates of a protein standard mixture and pooled serum samples to identify and characterize the origin of strong, nonrandom, between-peak amplitude correlations. Different normalization methods were explored to set the thresholds for automated detection of ionization states, ionization adducts, and protein modifications, which were identified as a major source of the strong amplitude correlations. This identification also provides for an intelligent, automated reduction of variables. Automated Bayesian inductive methods, combined with nonmodel-dependent information theory scoring, were developed to find diagnostic feature sets. This probabilistic methodology was capable of naturally accounting for the uncertainties in measurements and provided automatic detection of correlated features and diagnostic groups of features, leading to improved performance over standard statistical significance tests. The feature sets obtained appear to show sufficient stability under cross-validation to provide not only biomarker candidates but also families of features for further biochemical analysis. The integrated tools were successfully applied to an analysis of broad-range MALDI-TOF spectra for prostate cancer and for adult T-cell leukemia blood serum. As a result of improved signal processing, a more than tenfold enhancement of sensitivity and selectivity was achieved for detection of molecular species from serum spectra. This enabled a significant extension of the useful m/z-range for detection of potential biomarkers. Progress was also made in designing and testing sample preparation and data acquisition protocols for the identification and verification of diagnostic molecular markers from magnetic bead-purified serum.
Robust and Automated Detection of Interferences in Peptide Quantitation by MRM Mass Spectrometry

D.R. Mani, Susan E. Abbatiello, Steven A. Carr

The Broad Institute of MIT and Harvard, Cambridge, MA

Multiple reaction monitoring (MRM) mass spectrometry (MS) is increasingly being used for the quantitation of peptide targets in complex samples. Although the method is proven to be reproducible and sensitive, interferences in the MRM signals arising from sources other than the peptide target can drastically undermine and invalidate results from these quantitative experiments. Interferences usually are detected by painstaking and subjective manual examination of raw data. Here we demonstrate an efficient, automated, and objective method to detect interferences in MRM signals generated for quantitative peptide analyses in a variety of background matrices.

The interference detection algorithm is based on the observed constancy of the ratio of peak areas for any two transitions (fragments) of a peptide. Unexpected deviations of these ratios, indicative of interferences, are objectively detected using a statistical test. The algorithm has been validated using 28 isotope dilution MRM-MS data sets composed of a 9-point calibration curve for each of 10 peptides (with internal standards). Compared to expert manual inspection, the algorithm achieves an accuracy of 93-96%, with a sensitivity of 99% and specificity ranging from 45-71%. Use of the automated procedure drastically reduces manual inspection of transitions from 60,480 to 4,288. The method can pinpoint transitions that are affected by interference arising from a range of sources, including co-eluting nontarget peptides, improper or inconsistent peak shapes, and incorrect peak integration. The algorithm is also robust in the face of multiple transitions for a peptide incurring interference.

Quantitative Methods for Multiplexed Proteomic Assays

Tim Randolph

Fred Hutchinson Cancer Research Center, Seattle, WA

An update on progress and plans regarding the extraction and quantification of protein profiles in three platforms: (1) LC-MS/MS, (2) immunohistochemical tissue microarrays, and (3) Raman spectroscopy. After briefly reviewing progress on the first two types of data, we focus on a newly developed method aimed at the analysis of advanced Raman spectroscopy being used in immunoassays and molecular imaging. Our methods provide rigorous statistical estimation of high-dimensional parameters (i.e., the estimation of a coefficient function) in functional linear models. Here, the predictors are spectral curves that comprise the output from a vibrational spectroscopy assay. Applications include the detection of posttranslational modifications of proteins in single cells and of antibody-conjugated nanoparticles used in multiplexed assays.

Use of Glycan-Targeting Antibodies To Identify Cancer-Associated Glycoproteins in Human Plasma

Fred E. Regnier, Wonryeon Cho, Kwanyoung Jung, Qiang Gao

Department of Chemistry, Purdue University, West Lafayette, IN

Aberrations in the glycosylation of surface glycoproteins play a prominent role in the loss of cellular adhesion during cancer progression, metastasis, tumor-cell binding at remote sites, and secondary tumor colonization. This paper focuses on the issues of how to (1) recognize and identify cancer-associated glycoproteins shed into blood and (2) use them as a molecular signature for various types of cancer. Plasma of disease-free and breast cancer patients was
applied directly to immunoaffinity chromatography (IAC) and lectin affinity chromatography (LAC) columns targeting deviant glycoproteins. IAC selected 25-30 proteins and provided at least 2,000-fold purification in a single step. The number of proteins captured by LAC depended on lectin selectivity. Glycoproteins captured by affinity chromatography were eluted with an acidic mobile phase and identified both by (1) tryptic digestion, reversed phase chromatographic fractionation of the digest, and identification of peptides in collected RPC fractions by MALDI-MS/MS and (2) by further RPC fractionation before proteolysis of individual chromatographic fractions and identification by MALDI-MS/MS and ESI-MS/MS. Quantification was achieved with global internal standard technology. Of the proteins selected and identified, 15 were breast cancer marker candidates based on their elevation in breast cancer patients. The potential of these candidates as cancer markers is being validated in larger, more diverse populations of breast cancer patients. Plasma samples from ovarian, prostate, and colon cancer patients were examined in a similar fashion. Some of the marker glycoproteins, whereas common to all the types of cancer examined while others were type specific.

**NIST Peptide MS/MS Spectral Libraries: Reference Data for Proteomics**

*Paul Rudnick*, Qian Dong, Lisa Kilpatrick, Yuri Mirokhin, Pedatsur Neta, Jeri Roth, Dmitrii Tchekhovskoi, Stephen Stein

**National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD**

The National Institute of Standards and Technology (NIST) has been compiling and maintaining reference mass spectral libraries for the past 20 years. The primary use of these electron impact (EI) spectra has been for the identification of small molecules during GS-MS analysis. The widespread use of this library (NIST SRD1A) and the recent availability of MS/MS spectra from proteomics experiments initiated the development of peptide fragmentation libraries for use by the proteomics community. To build these libraries, we take advantage of the power of multiple search algorithms (OMSSA, X!Tandem, Protein Prospector, Inspect, etc.) and strict quality control filters (e.g., fraction of unexplained abundance) to compile both consensus and “best-replicate” libraries. The human library now has grown in excess of 250,000 MS/MS spectra, covering roughly 16% of all human protein sequences (IPI 3.10) since this project began in 2005. Some 90% of these represent fully tryptic peptides. Several recent publications have shown that searching these libraries has distinct advantages over traditional sequence-based searches alone (Lam et al. 2007). Most notably, search algorithms designed to use these libraries can take advantage of actual fragment peak intensities and noncanonical peaks present in the empirical spectra (H. Lam, ASMS 2009), a difference that better distinguishes correct from incorrect matches. The Chemical Reference Data Group at NIST develops libraries for both ion trap and QTOF instruments. As well as being reference data for routine MS/MS peptide searches, these spectra are also a valuable resource for SRM assay developers using applications like Skyline (MacCoss Lab) where a priori knowledge of a peptide fragmentation mass spectrum is important for selecting transitions. Some of the data for this project are generated in-house, but most come from public donations and from data acquired during CPTAC interlaboratory studies. The human library also has benefitted from large contributions of cancer-related spectra made by the CPTAC team at Vanderbilt University Medical Center. NIST offers these libraries in ASCII text and formats suitable for use with our own MS Search 2.0 and MS PepSearch software, or SpectraST, a part of ISB’s Trans-Proteomic-Pipeline. NIST libraries and search tools can be downloaded free of charge at http://peptide.nist.gov.
Comparison of Stable Isotope Dilution and Label-Free Quantitation of Proteins by Liquid Chromatography-Multiple Reaction Monitoring Mass Spectrometry

Haixia Zhang, Qinfeng Liu, Robbert J.C. Slebos, Daniel C. Liebler

Jim Ayers Institute for Precancer Detection and Diagnosis, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN

Liquid chromatography-multiple reaction monitoring mass spectrometry (LC-MRM-MS) has become an important method for targeted protein quantitation. LC-MRM-MS is most commonly done with isotope-labeled peptide internal standards (stable isotope dilution [SID]). However, the need to implement targeted quantitation for many proteins and peptides, as in biomarker verification studies, is limited by the expense of labeled peptide standards. Here we compared SID with a similar method using a single isotope-labeled peptide internal standard (human β-actin GYSFTTTAER) and with a label free method based on peak area extracted from LC-MRM-MS product ion chromatograms.

First, six peptides from *Escherichia coli* proteins, alkaline phosphatase (AP), and β-galactosidase (BG) were synthesized in both unlabeled and isotope-labeled forms; different concentrations of peptide solutions were prepared; and the three approaches were used to construct the calibration curves for the unlabeled peptides. Second, AP and BG proteins were spiked into a lysate from RKO human colon carcinoma cells at different concentrations, and the six synthetic isotope labeled peptides from both proteins were spiked in as internal standards. The peak area linearity of six unlabeled peptides from AP and BG tryptic digest was evaluated using conventional SID, the single-peptide standard, and label-free quantitation approaches. Under all conditions studied, good linearity between signal response and peptide amount was observed ($r^2>0.95$ for most peptides), which indicated that normalization using one isotope-labeled peptide internal standard or based on un-normalized peptide peak area is acceptable for a wide concentration range of peptide quantitation. Of the three approaches, SID gave the best linearity ($r^2\geq0.99$) and exhibited the lowest variation.

We also evaluated the methods in five pairs of normal lung and lung adenocarcinoma and in five pairs of normal lung and squamous cell carcinoma tissues. One isotope-labeled peptide each from the proteins AGER, β-actin, and annexin A1 were spiked in as internal standards, and the performance of SID was compared with that of the single-peptide standard and label-free methods. Compared with SID, the single-peptide standard method led to constant measurement deviation for all samples yet was suitable for relative quantitation of target peptides among different samples. The label-free method exhibited higher measurement variation than SID, but this deviation was also relatively constant, and two out of three peptides had low measurement variations (CV<10%). Finally, quantitation of 10 biomarker candidates in these 10 pairs of lung tumor and normal samples was demonstrated using these three approaches. The methods yielded similar results for peptides, with significant expression differences between samples. Inconsistency between methods was observed only in comparisons involving weak peptide MRM signals or similar absolute expression levels. The results suggest that the single-peptide standard and label-free methods are acceptable, cost-effective alternatives to SID for preliminary LC-MRM-MS screens of biomarker candidate proteins prior to the development of SID assays. (Supported by NIH cooperative agreement award U24CA126479)
Assessment of Plasma Proteome Stability

Lisa J. Zimmerman, Robbert Slebos, Julie A. Coleman, Constantin Aliferis, Douglas Hardin, Alexander Statnikov, Daniel C. Liebler

Departments of Biochemistry and Biomedical Informatics and Vanderbilt Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN

Although many of the studies examining the stability of plasma and serum have focused solely on the analysis of the low-molecular-weight proteome of serum using MALDI-TOF mass spectrometry, information regarding the overall global integrity of proteins, particularly in plasma, has not been previously described. Here we report our ongoing efforts to characterize changes in the plasma proteome using shotgun proteomic analysis for the development of quality control metrics used to evaluate sample viability for biomarker discovery and verification assays.

Experiments analyzed blood collected in EDTA collection tubes in which plasma was isolated at 4 °C or room temperature after a period of 0, 4, 24, or 72 hours or 1 week. Aliquots of each plasma sample were analyzed using MALDI mass spectrometry, and the acquired spectra were analyzed using univariate statistical tests and building multivariate predictors. Additional plasma samples were collected and subjected to 0, 1, 2, 3, 5, 10, or 25 freeze-thaw cycles. All plasma samples were digested using trypsin and analyzed using LC/MS/MS. Tandem spectra were identified using a semitryptic database search, and results were filtered using IDPicker with a 5% FDR. Shotgun data sets were analyzed using MonsterMod to identify modifications and cleavage products resulting from such processing and handling conditions. In addition, statistical analysis of LC-MS/MS data sets was carried out using an in-house software tool, Quasi-Tel. LC-MRM analysis was performed on all plasma digests to measure levels of truncated peptides derived from fibrinogen and complement C3f in addition to those peptides found to be significantly different, based on spectral count information, between each of the handling conditions.

Time and temperature have a very significant effect on the form of the produced MALDI spectra; however, the most notable observations were visual trends in a change of intensity values of several peaks with changing temperature and/or time. Analysis of plasma samples subjected to various collection conditions and freeze-thaw cycles followed by tryptic digestion and reverse-phase liquid chromatography/mass spectrometry revealed minimal changes in proteins of plasma collected in EDTA tubes, with only modest degradation of those proteins previously shown to be highly susceptible to cleavage (i.e., fibrinogen, complement C3). Initial shotgun proteomic analysis indicates that overall numbers of peptide and protein identifications are similar between each time and temperature point and between freeze-thaw cycles. Further analysis of the shotgun data using MonsterMod software identified no significant truncations resulting from cleavage of fibrinogen, complement C3f, complement C4 precursor, or interalpha trypsin inhibition. Changes in the global distribution at the peptide level, both tryptic and semitryptic, in stored plasma are not detected upon digestion and LC-MS/MS analysis. Preliminary MRM-based studies, targeting previously reported truncated peptides originating from fibrinogen and complement C3, revealed only minimal cleavage products even at the most extreme of the handling conditions. MRM studies have been expanded to include those peptides identified by statistical analysis that appear to change over time and freeze-thaw cycles. Our results indicate that degradation occurring during storage is randomly distributed, and although some proteins may display higher instability, most proteins are relatively unaffected under such conditions. (Supported by NIH cooperative agreement award U24CA126479)
Comparison of Isoelectric Focusing Platforms for Complex Peptide Mixtures

Lisa J. Zimmerman, Sarah R. Stuart, Robbert J. Slebos, Corbin A. Whitwell, Misti A. Martinez, Amy-Joan L. Ham, Daniel C. Liebler

Department of Biochemistry, Mass Spectrometry Research Center, and Jim Ayers Institute of Precancer Detection and Diagnosis, Vanderbilt University, Nashville, TN

The use of isoelectric focusing (IEF) for the fractionation of peptides derived from complex mixtures has gained considerable attention as an alternative to the traditional approach of using strong cation exchange chromatography (SCX) as the first dimension of separation in shotgun proteomics. As the standard method for the fractionation of peptides in our laboratory, we were interested in comparing the performance of various IEF-based platforms that include the GE Healthcare Ettan IPGphor, Invitrogen ZOOM IPGRunner, and the Agilent OFFGEL system and their ability to fractionate a complex mixture generated from a human colon adenocarcinoma cell line (RKO).

For initial experiments, a standard protein load of 200 μg of a RKO cell lysate digest was fractionated by each IEF platform using either pH ranges 3.5-4.7 and/or 4-7. Peptide fractions were analyzed using reverse-phase liquid chromatography mass spectrometry (LC/MS/MS). Based on the initial results, the ZOOM system using immobilized pH gradient (IPG) strips in the 3.5-4.7 pH range afforded the highest number of peptide and protein identifications (an average of 6,687 and 1,752, respectively), outperforming each of the other platforms. Due to the overall poor performance of the Agilent OFFGEL system, subsequent comparisons were carried out using only the Ettan IPGphor and ZOOM systems. A series of followup experiments were performed using decreasing proteins loads (200 μg, 100 μg, 75 μg, and 50 μg) per IPG strip while keeping the number of fractions constant at 20. In addition, the effect of cutting the IPG strips into 10, 15, or 20 gel pieces using two protein loads, 50 μg and 200 μg, also was evaluated.

The current comparison of several IEF platforms offers insight into the overall advantages and limitations for each method. We have demonstrated that the ZOOM system offers superior reproducibility and efficient resolution for peptide fractionation relative to comparable IEF systems. Advantages such as significant decreases in focusing times and fractions collected allow for an increase in sample throughput critical for biomarker discovery pipelines. (Supported by NIH cooperative agreement award U24CA126479)
KEYNOTE SPEAKER BIOSKETCHES

N. Leigh Anderson, Ph.D.

Dr. Anderson is Founder and Chief Executive Officer of the Plasma Proteome Institute (PPI), Washington, DC (www.plasmaproteome.org). The Institute aims to foster a comprehensive exploration of the plasma proteome and the rapid application of novel protein measurements in clinical diagnostics and clinical trials. Prior to founding PPI, he was Chief Scientific Officer at Large Scale Biology Corporation, which he cofounded in 1985. Dr. Anderson obtained his B.A. degree in physics with honors from Yale and a Ph.D. degree in molecular biology from Cambridge University (England). He has served on the boards of directors of three public technology companies and consults through the Anderson Forschung Group.

James R. Heath, Ph.D.

Dr. Heath is the Elizabeth W. Gilloon Professor and Professor of Chemistry at the California Institute of Technology (Caltech), Professor of Molecular and Medical Pharmacology at the University of California, Los Angeles (UCLA), and Director of the National Cancer Institute’s (NCI) NSB Cancer Center. He received a B.Sc. degree in 1984 (Baylor University) and a Ph.D. degree in chemistry (Rice University) in 1988, where he was the principal student involved in the Nobel Prize-winning discovery of C60 and the fullerenes. Dr. Heath was a Miller Fellow at University of California, Berkeley, from 1988-1991 and was on the technical staff at IBM Watson Labs from 1991-1993. In 1994 he joined the faculty at UCLA. Dr. Health founded the California NanoSystems Institute in 2000 and served as its Director until moving to Caltech. He has investigated quantum phase transitions and has developed architectures, devices, and circuits for molecular electronics. Dr. Heath’s group recently has been applying its advances on nanoelectronics circuitry toward addressing problems in cancer. He has received a number of awards, including a Public Service Commendation from Governor Grey Davis, the Sackler Prize, the Spiers Medal, the Feynman Prize, the Jules Springer Prize, and the Arthur K. Doolittle Award. Dr. Heath has founded or cofounded several companies, including NanoSys, MTI, MoB, and Homestead Clinical and serves on the boards of a number of organizations, including the Board of Scientific Advisors of the NCI.

John M. Koomen, Ph.D.

Dr. Koomen received a Ph.D. degree in chemistry from Texas A&M University in 2002. From 2002-2005, he worked as a junior faculty member at The University of Texas M.D. Anderson Cancer Center, where he was involved in basic science and clinical proteomics projects. Since 2005 he has been Assistant Professor and Scientific Director of Proteomics at the H. Lee Moffitt Cancer Center and Research Institute. Research in the Koomen laboratory focuses on analytical chemistry development, elucidation of biological processes, and implementation of clinical assays using proteomics and quantitative mass spectrometry. Much of this work is performed using multiple reaction monitoring (MRM); three examples are detailed below. In colon cancer, broad-scale proteomics employing relative quantification techniques and gene expression profiles have determined candidate biomarkers related to the adenomatous polyposis coli protein. Targeted analysis of this panel of proteins, coupled with the interrogation of protein expression in relevant signaling pathways (including Wnt, TGF/SMAD, and Notch) in tumors and adjacent normal tissue, can be used to select and evaluate candidate biomarkers for predicting patient outcomes. In multiple myeloma, the same techniques and technologies are being applied in cell lines to develop preclinical models that will provide a molecular basis for rational chemotherapy. In these experiments, direct drug targets and apoptosis-related proteins are being monitored in parallel with specific signaling pathways, including NFkB. These data will elucidate mechanisms of drug resistance and provide methods to evaluate combination therapy. Finally, direct patient assessment is being performed in multiple myeloma using MRM. Current clinical assays use gel or capillary electrophoresis to measure the antibody secreted by the myeloma, which is used to stage the patients and estimate tumor burden. Quantitative
mass spectrometry should be significantly more sensitive at detecting and quantifying these antibodies. Improvements in the analytical technique would lead to earlier detection of disease relapse, more rapid intervention with second-line therapies, and potentially improved patient outcomes. These projects share the common goal of changing the standard of patient assessment and care using mass spectrometry.
ABOUT THE CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Overview

Researchers have long sought biomarkers that could detect cancer at an early stage or predict the optimal cancer therapy for specific patients. Recent technological advances in genomics, proteomics, and metabolomics have enabled researchers to identify molecular fingerprints of specific cancers. The translation of these research findings could potentially improve cancer screening, diagnosis, and treatment, which in turn could profoundly alter the economic burden of cancer. Despite a great deal of work, this has proven to be an extremely difficult undertaking. There are well over 1,000 cancer protein biomarker candidates that have been described in the scientific literature over the past decade, and this list continues to grow. So why is the clinical pipeline so sparse when we have all of these candidates at our fingertips? Why are there not a greater number of protein biomarkers already in the clinic for the early detection and treatment of cancer? The proteomics community is struggling with this discrepancy. The NCI and experts in the field believe that part of the problem lies in technological and methodological variability, the extents of which we still do not understand entirely.

Recognizing the challenges facing the proteomics community, the NCI launched the Clinical Proteomic Technologies for Cancer (CPTC) Initiative to address and reduce the layers of variability at every step of the biomarker discovery pipeline (experimental design, sample collection and preparation, protein/peptide fractionation and detection, and protein/peptide identification and quantification—data analysis). Addressing these challenges is to be achieved through the optimization of platforms, development of appropriate standards with performance metrics, development of new technologies, development of standard operating procedures, and development and availability of high-quality reagents.

Improvements in DNA sequencing launched the genomics revolution, and the same will hold true for proteomics. The NCI, recognizing the promise of clinical proteomics for the early detection and treatment of cancer, has taken on this challenge.

Through the CPTC Initiative, the NCI is providing critical resources to the entire cancer research community to build the foundation for the next generation of molecular diagnostics. Recognizing that launching a proteomics “revolution” will take a community, the CPTC has brought together the best minds in proteomics by partnering with scientists from nearly 50 Federal, academic, and private sector organizations.

CPTC Mission and Goals

The overall objective of this 5-year, $104 million undertaking is to build the foundation of technologies, data, reagents and reference materials, analysis systems, and infrastructure needed to systematically advance our understanding of protein biology in cancer and accelerate discovery research and its clinical applications.

CPTC goals include:

- Enhancing and optimizing technical abilities to identify and measure proteins accurately and reproducibly in biological systems
- Advancing proteomics as a reliable, quantitative field that can accelerate discovery and translational research
Components of the Initiative

- **Clinical Proteomic Technology Assessment for Cancer (CPTAC)** comprises a multidisciplinary team network of leading proteomics centers that is conducting rigorous technology assessment and optimization, developing standard protocols and technology performance mixtures, and evaluating methods to ensure data reproducibility.

- **Advanced Proteomic Platforms and Computational Sciences** supports investigator-initiated development of highly innovative new tools for quantitative protein/peptide measurement. It also supports enhanced algorithm development and computational methods to interrogate emerging preprocessed data sets.

- **Proteomic Reagents and Resources Core** serves the investigator community as a central public source for reagents, data, standards of practice, and other information developed in the course of CPTC work.

The National Cancer Institute’s (NCI) Clinical Proteomic Technologies for Cancer (CPTC) initiative is composed of three major, integrated components, all working in tandem to translate protein biomarker discoveries into clinical utility: the Clinical Proteomic Technology Assessment for Cancer (CPTAC), the Advanced Proteomic Platforms and Computational Sciences, and the Proteomic Reagents and Resources Core. Collaboration is an integral part of the CPTC initiative, and CPTC also encourages industry partnerships through the NCI’s Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) Programs.
PARTICIPANT LIST
(as of September 27, 2009)

Susan E. Abbatiello, Ph.D.
The Broad Institute of MIT and Harvard
(617) 714-7653
susana@broadinstitute.org

Michail A. Alterman, Ph.D.
U.S. Food and Drug Administration
(301) 827-1753
michail.alterman@fda.hhs.gov

N. Leigh Anderson, Ph.D.
Plasma Proteome Institute
(301) 728-1451
leighanderson@plasmaproteome.org

Oleg A. Andreev, Ph.D.
University of Rhode Island
(401) 874-2054
andreev@mail.uri.edu

Philip Andrews, Ph.D.
University of Michigan
(734) 763-3130
andrewsp@umich.edu

Oceguera Villanueva Antonio, M.D.
Instituto Jaliscience de Cancerologia
33 361 59164
ocegueraantonio@hotmail.com

Don W. Arnold, Ph.D.
Eksigent Technologies, LLC
(925) 560-2602
dwarnold@eksigent.com

Suresh K. Arya, Ph.D.
National Cancer Institute, NIH
(301) 496-8783
aryas@mail.nih.gov

LeeAnn Bailey, Ph.D.
Center to Reduce Cancer Health Disparities, NIH
(301) 496-8589
baileyj@mail.nih.gov

Erin Baker, Ph.D.
Pacific Northwest National Laboratory
(509) 371-6219
erin.baker@pnl.gov

Peter E. Barker, Ph.D.
National Institute of Standards and Technology
(301) 975-5402
peter.barker@nist.gov

Maureen Beanan, Ph.D.
National Institute of Allergy and Infectious Diseases, NIH
(301) 451-3247
beananm@mail.nih.gov

Ronald Beavis, Ph.D.
(204) 290-9105
beavis.ron@gmail.com

John J.M. Bergeron, D.Phil.
McGill University
(514) 398-1259
john.bergeron@mcgill.ca

Josip Blonder, M.D.
SAIC-Frederick Inc.
(301) 846-7211
blonderj@mail.nih.gov

Emily S. Boja, Ph.D.
National Cancer Institute, NIH
(301) 451-1079
bojae@mail.nih.gov

Christoph Borchers, Ph.D.
University of Victoria/Genome British Columbia
Proteomics Centre
(250) 483-3221
christoph@proteincentre.com
David M. Bunk, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 951-9047
david.bunk@nist.gov

Steven A. Carr, Ph.D.
The Broad Institute of MIT and Harvard
(617) 714-7630
scarr@broad.mit.edu

Alessandra Cesano, Ph.D.
Nodality
(650) 827-8017
alessandra.cesano@nodality.com

Arnon Chait, Ph.D.
AnalizaDx, LLC
(216) 432-9050
chait@analiza.com

Maria M. Chan, Ph.D.
U.S. Food and Drug Administration
(301) 796-5482
maria.chan@fda.hhs.gov

John Chaput, Ph.D.
Arizona State University
(480) 727-0392
john.chaput@asu.edu

Amrita K. Cheema, Ph.D., M.S.
Georgetown University
(202) 687-2756
akc27@georgetown.edu

Joon-Yong Chung, Ph.D.
National Cancer Institute, NIH
(301) 443-4969
chungjo@mail.nih.gov

Patrick L. Coleman, Ph.D.
U.S. Department Health and Human Services
(202) 260-0448
patrick.coleman@hhs.gov

Deborah Collyar
PAIR: Patient Advocates In Research
(925) 260-1006
collyar@att.net

Rina Das, Ph.D.
National Cancer Institute, NIH
(301) 496-8589
dasr2@mail.nih.gov

Gene Davis
Asterand, Inc.
(301) 526-5700
gene.davis@asterand.com

Maryellen de Mars, Ph.D.
The Critical Path Institute
(301) 762-7650
mdemars@c-path.org

Moyez Dharsee
Ontario Cancer Biomarker Network
(416) 673-8427
mdharsee@ocbn.ca

Michael J. Difilippantonio, Ph.D.
National Cancer Institute, NIH
(301) 496-6278
difilipm@mail.nih.gov

Ivan Ding, M.D.
National Cancer Institute, NIH
(301) 451-3864
dingi@mail.nih.gov

Gareth Steven Dobson, Ph.D., M.S.
SRI International
(650) 859-3917
gareth.dobson@sri.com

Nathan Dodder, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 975-3389
nathan.dodder@nist.gov
Qian Dong, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 975-2569
qian.dong@nist.gov

Nathan Edwards, Ph.D.
Georgetown University Medical Center
(202) 687-7042
nje5@georgetown.edu

Virginia Espina, M.S.
George Mason University
(703) 993-8062
vespina@gmu.edu

Luis A. Espinoza, Ph.D., M.S.
National Cancer Institute, NIH
(301) 435-3098
espinozala@mail.nih.gov

Greg Evans, Ph.D.
National Cancer Institute, NIH
(301) 594-8807
evansgl@mail.nih.gov

Paul Ko Ferrigno, Ph.D.
University of Leeds
113 343 8644
p.koferrigno@leeds.ac.uk

Giovanna Fantoni, Ph.D.
Clinical Center, NIH
(301) 435-2301
gfantoni@cc.nih.gov

Dorothy Farrell, Ph.D.
National Institutes of Health
(301) 496-5652
farrellld@mail.nih.gov

Susan J. Fisher, Ph.D.
University of California, San Francisco
(415) 476-5297
sfisher@cgl.ucsf.edu

Tony Fong
ProteoMonitor
(212) 651-5633
tfong@genomeweb.com

Keolu O. Fox
National Human Genome Research Institute, NIH
(301) 346-1307
foxko@mail.nih.gov

Yali Fu, Ph.D.
National Cancer Institute, NIH
(301) 496-8787
fuyali@mail.nih.gov

Dan Gallahan, Ph.D.
National Cancer Institute, NIH
(301) 496-8636
dg13w@nih.gov

Xiaolian Gao, Ph.D.
University of Houston
(713) 743-2805
xgao@uh.edu

Timothy Geddes
William Beaumont Hospital
(248) 551-0520
timothy.geddes@beaumont.edu

Ning Geng, D.D.S., Ph.D.
National Institute of Dental and Craniofacial Research,
NIH
(301) 402-7537
gengn@mail.nih.gov

Gradimir Georgevich, Ph.D.
IONICS Mass Spectrometry Group, Inc.
(301) 461-0259
gg@ionicsmse.com

Melkamu Getie-Kebtie, Ph.D.
U.S. Food and Drug Administration
(301) 827-1742
melkamu.getiekebtie@fda.hhs.gov
<table>
<thead>
<tr>
<th>Name</th>
<th>Organization</th>
<th>Phone Number</th>
<th>Email Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mousumi Ghosh, Ph.D.</td>
<td>Memorial Sloan-Kettering Cancer Center</td>
<td>(212) 639-6012</td>
<td><a href="mailto:ghoshm@mskcc.org">ghoshm@mskcc.org</a></td>
</tr>
<tr>
<td>Jeffrey Gibbs, J.D.</td>
<td>Hyman Phelps &amp; McNamara, PC</td>
<td>(202) 737-4288</td>
<td><a href="mailto:jng@hpm.com">jng@hpm.com</a></td>
</tr>
<tr>
<td>Bradford W. Gibson, Ph.D.</td>
<td>University of California, San Francisco</td>
<td>(415) 209-2032</td>
<td><a href="mailto:bgibson@buckinstitute.org">bgibson@buckinstitute.org</a></td>
</tr>
<tr>
<td>Jeff Gildersleeve, Ph.D.</td>
<td>National Cancer Institute, NIH</td>
<td>(301) 846-5699</td>
<td><a href="mailto:gildersj@mail.nih.gov">gildersj@mail.nih.gov</a></td>
</tr>
<tr>
<td>Bhaskar Godugu, Ph.D.</td>
<td>National Institute of Standards and Technology, U.S. Department of Commerce</td>
<td>(301) 975-2721</td>
<td><a href="mailto:bhaskar.godugu@nist.gov">bhaskar.godugu@nist.gov</a></td>
</tr>
<tr>
<td>Radoslav Goldman, Ph.D.</td>
<td>Georgetown University</td>
<td>(202) 687-9868</td>
<td><a href="mailto:rg26@georgetown.edu">rg26@georgetown.edu</a></td>
</tr>
<tr>
<td>Ira Gordon, D.V.M.</td>
<td>National Cancer Institute, NIH</td>
<td>(301) 451-3701</td>
<td><a href="mailto:gordoni2@mail.nih.gov">gordoni2@mail.nih.gov</a></td>
</tr>
<tr>
<td>Maryam Goudarzi, M.S.</td>
<td>George Mason University</td>
<td>(703) 505-1901</td>
<td><a href="mailto:mgoudarzi@gmu.edu">mgoudarzi@gmu.edu</a></td>
</tr>
<tr>
<td>Christian Graves</td>
<td>National Cancer Institute, NIH</td>
<td>(301) 443-4798</td>
<td><a href="mailto:gravesc2@mail.nih.gov">gravesc2@mail.nih.gov</a></td>
</tr>
<tr>
<td>Marjan Gucek, Sc.D.</td>
<td>National Institutes of Health</td>
<td>(301) 594-1060</td>
<td><a href="mailto:marjan.gucek@nih.gov">marjan.gucek@nih.gov</a></td>
</tr>
<tr>
<td>Brian Haab, Ph.D.</td>
<td>Van Andel Research Institute</td>
<td></td>
<td><a href="mailto:brian.haab@vai.org">brian.haab@vai.org</a></td>
</tr>
<tr>
<td>Steven C. Hall, Ph.D.</td>
<td>University of California, San Francisco</td>
<td>(415) 502-8423</td>
<td><a href="mailto:steven@cgl.ucsf.edu">steven@cgl.ucsf.edu</a></td>
</tr>
<tr>
<td>Amy Ham, Ph.D.</td>
<td>Vanderbilt University</td>
<td>(615) 322-2948</td>
<td><a href="mailto:amy.ham@vanderbilt.edu">amy.ham@vanderbilt.edu</a></td>
</tr>
<tr>
<td>Maria Hammond, M.S.</td>
<td>Uppsala University</td>
<td>46 18 471 4072</td>
<td><a href="mailto:maria.hammond@genpat.uu.se">maria.hammond@genpat.uu.se</a></td>
</tr>
<tr>
<td>Brian Hampton</td>
<td>University of Maryland</td>
<td>(410) 706-8207</td>
<td><a href="mailto:bhampton@som.umaryland.edu">bhampton@som.umaryland.edu</a></td>
</tr>
<tr>
<td>Bingnan Han</td>
<td>SAIC-Frederick, Inc.</td>
<td>(301) 846-6805</td>
<td><a href="mailto:hanb3@mail.nih.gov">hanb3@mail.nih.gov</a></td>
</tr>
<tr>
<td>Tamara Harris, M.D., M.S.</td>
<td>National Institute on Aging, NIH</td>
<td>(301) 496-1178</td>
<td><a href="mailto:harris99@mail.nih.gov">harris99@mail.nih.gov</a></td>
</tr>
<tr>
<td>James R. Heath, Ph.D.</td>
<td>California Institute of Technology</td>
<td>(626) 395-8920</td>
<td><a href="mailto:heath@caltech.edu">heath@caltech.edu</a></td>
</tr>
<tr>
<td>Stephen M. Hewitt, M.D., Ph.D.</td>
<td>National Cancer Institute, NIH</td>
<td>(301) 496-0040</td>
<td><a href="mailto:genejock@helix.nih.gov">genejock@helix.nih.gov</a></td>
</tr>
</tbody>
</table>
Tara Hiltke, Ph.D.
National Cancer Institute, NIH
(301) 451-8511
hiltket@mail.nih.gov

Andy Hoffnagle, M.D., Ph.D.
University of Washington
(206) 598-6131
ahoof@u.washington.edu

Howard M. Jacobson, M.B.A.
Protagen, Inc.
(908) 879-1445
howard.jacobson@protagen.com

J. Milburn Jessup, M.D.
National Cancer Institute, NIH
(301) 435-9010
jessupj@mail.nih.gov

Libin Jia, M.D.
National Cancer Institute, NIH
(301) 496-5808
libinj@mail.nih.gov

Donald Johann, M.D.
National Cancer Institute, NIH
(301) 402-6298
johanned@mail.nih.gov

Stephen Albert Johnston, Ph.D.
Arizona State University
(480) 727-0792
stephen.johnston@asu.edu

Saeed Jortani, Ph.D.
University of Louisville
(502) 852-8835
sjortani@louisville.edu

Jacob Kagan, Ph.D., M.S.
National Cancer Institute, NIH
(301) 496-9541
kaganj@mail.nih.gov

Marcia Kean, M.B.A.
Feinstein Kean Healthcare
(617) 413-1295
marcia.kean@fkhealth.com

John Kenten, Ph.D.
Meso Scale Diagnostics
(240) 631-2522
jkenten@mesoscale.com

Shahid Khan, Ph.D.
(214) 282-9431
shahidkhan786@gmail.com

Kelly Kim
National Cancer Institute, NIH
(301) 496-8639
kimke@mail.nih.gov

Youngsoo Kim, Ph.D.
Seoul National University College of Medicine
biolab@snu.ac.kr

Christopher R. Kinsinger, Ph.D.
National Cancer Institute, NIH
(301) 496-1550
kinsingc@mail.nih.gov

John Matthew Koomen, Ph.D.
H. Lee Moffitt Cancer Center
(813) 745-8624
john.koomen@moffitt.org

Srinivasan Krishnamoorthy, Ph.D.
Physical Sciences, Inc.
(978) 738-8136
sk@psicorp.com

Srilatha Kuntumalla, Ph.D.
(301) 795-7000
ksril@hotmail.com

Wilhelm Lachnit
(415) 309-6882
wlachnit@cellbiosciences.com
Cheolju Lee, Ph.D.
Korea Institute of Science and Technology
82 2 958 6788
clee270@kist.re.kr

Joshua D. Levin, Ph.D., M.A.
U.S. Food and Drug Administration
(301) 796-6695
joshua.levin@fda.hhs.gov

Dai Li, M.D., Ph.D., M.Sc.
U.S. Food and Drug Administration
(301) 796-6174
dai.li@fda.hhs.gov

Hong Li, Ph.D.
New Jersey Medical School Cancer Center
(973) 972-8396
liho2@umdnj.edu

Yuxue Liang, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 975-4417
yuxue.liang@nist.gov

Daniel C. Liebler, Ph.D.
Vanderbilt University Medical Center
(615) 322-3063
daniel.liebler@vanderbilt.edu

De Lin, Ph.D.
Vanderbilt University Medical Center
(615) 936-7020
de.lin@vanderbilt.edu

Jeff R. Livingstone, Ph.D.
Monarch LifeSciences
(508) 838-9663
jrl@monarchlifesciences.com

Christian M. Loch, Ph.D., M.P.H.
LifeSensors, Inc.
(610) 644-8845
loch@lifesensors.com

Michael MacCoss, Ph.D.
University of Washington
(206) 616-7451
maccoss@u.washington.edu

Lee Makowski, Ph.D.
Argonne National Laboratory
(630) 252-3917
lmakowski@anl.gov

Parag Mallick, Ph.D.
University of Southern California
(323) 442-2839
mallick@usc.edu

Dariya Malyarenko, Ph.D., M.S.
College of William and Mary
(757) 221-4735
dimaly@wm.edu

D.R. Mani, Ph.D.
The Broad Institute of MIT and Harvard
(617) 714-7483
manidr@broad.mit.edu

Elizabeth Mansfield, Ph.D.
U.S. Food and Drug Administration
(301) 796-4664
elizabeth.mansfield@fda.hhs.gov

Sanford Markey, Ph.D.
National Institute of Mental Health, NIH
(301) 496-4022
markeys@mail.nih.gov

Francisco Martinez
U.S. Food and Drug Administration
(301) 796-6201
francisco.martinez@fda.hhs.gov

Mehdi Mesri, Ph.D.
National Cancer Institute, NIH
(301) 451-2149
mesrim@mail.nih.gov
Myrtle Davis Millin, D.V.M., Ph.D.
National Cancer Institute, NIH
(301) 443-3404
myrtledavis@mail.nih.gov

Dixie Mills, M.D.
Dr. Susan Love Research Foundation
(310) 828-0060
dixie.mills@dslfr.org

Nrusingha Mishra, Ph.D.
U.S. Army Medical Research and Material Command
(301) 619-7782
nrusingha.mishra@amedd.army.mil

Kristina Mitchell, M.S.
University of California, San Diego
(619) 916-7692
kpohaku@ucsd.edu

Suresh Mohla, Ph.D.
National Cancer Institute, NIH
(301) 435-1878
mohlas@mail.nih.gov

Helen M. Moore, Ph.D.
National Cancer Institute, NIH
(301) 496-0206
moorehe@mail.nih.gov

Bethanie Morrison, Ph.D.
SAIC-Frederick, Inc.
(301) 846-6810
blm0331@gmail.com

Laurie Nadler, Ph.D.
National Institute of Mental Health, NIH
(301) 443-5288
lnadler@mail.nih.gov

Dobrin Nedelkov, Ph.D.
Intrinsic Bioprobes Inc.
(480) 804-1778
dnedelkov@intrinsicbio.com

Alexey I. Nesvizhskii, Ph.D.
University of Michigan, Ann Arbor
(734) 764-3516
nesvi@umich.edu

Thomas A. Neubert, Ph.D.
New York University School of Medicine
(212) 263-7265
neubert@saturn.med.nyu.edu

Dianne Newton, Ph.D.
SAIC-Frederick, Inc.
(301) 846-6809
newtondianne@mail.nih.gov

Rachel Yuan Nong, M.S.
Uppsala University
46 18 471 4907
rachel.nong@genpat.uu.se

William Old, Ph.D.
(303) 735-4019
william.old@colorado.edu

Isabel Victoria Otero, M.P.H.
National Cancer Institute, NIH
(301) 435-4434
isotero@mail.nih.gov

Vyomesh Patel, Ph.D.
National Institute of Dental and Craniofacial Research, NIH
(310) 402-7456
vpatel@mail.nih.gov

John Phipps
New England Peptide, LLC
(978) 630-0020
john.phipps@newenglandpeptide.com

Ian Pike, Ph.D.
Proteome Sciences
44 771 475 1764
ian.pike@proteomics.com
Ronald M. Przygodzki, M.D.
U.S. Department of Veterans Affairs
(202) 461-1665
gizbab@verizon.net

Menon Radha, Ph.D.
U.S. Food and Drug Administration
(301) 796-6180
exr@cdrh.fda.gov

Amir Rahbar, Ph.D., M.B.A.
National Cancer Institute, NIH
(301) 496-1550
rahbaram@mail.nih.gov

Elda Railey
Research Advocacy Network
(877) 276-2187
erailey@researchadvocacy.org

Timothy Randolph, Ph.D.
Fred Hutchinson Cancer Research Center
(206) 667-1079
trandolp@fhcrc.org

Robert Clay Rivers, Ph.D.
National Cancer Institute, NIH
(301) 451-1083
riversrc@mail.nih.gov

Karin Rodland, Ph.D.
Pacific Northwest National Laboratory
(509) 371-6935
karin.rodland@pnl.gov

Henry Rodriguez, Ph.D., M.B.A.
National Cancer Institute, NIH
(301) 496-1550
rodriguezh@mail.nih.gov

Donna Roscoe, Ph.D.
U.S. Food and Drug Administration
(301) 796-6183
donna.roscoe@fda.hhs.gov

Paul Rudnick, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 975-5828
paul.rudnick@nist.gov

Paul Russo, Ph.D.
George Mason University
(703) 993-4824
prusso@gmu.edu

Rick Saul, Ph.D.
SAIC-Frederick, Inc.
(301) 325-6669
rgsaul@comcast.net

Birgit Schilling, Ph.D.
Buck Institute for Age Research
(415) 235-7812
bschilling@buckinstitute.org

Nell Sedransk, Ph.D.
National Institute of Statistical Sciences
(919) 685-9300
sedransk@niss.org

David Ransohoff, M.D.
The University of North Carolina at Chapel Hill
(919) 966-1256
ransohof@med.unc.edu

Fred E. Regnier, Ph.D.
Purdue University
(765) 494-3878
fregnier@purdue.edu

Habtom W. Ressom
Georgetown University
(202) 687-2283
hwr@georgetown.edu

Jens Rittscher, D.Eng.
General Electric
(518) 387-4410
jens.rittscher@research.ge.com
Kenna Shaw, Ph.D.
National Cancer Institute, NIH
(301) 435-3864
shawk@mail.nih.gov

Douglas Sheeley, Sc.D.
National Center for Research Resources, NIH
(301) 594-9762
dms.nih.gov

Rong-Fong Shen, Ph.D.
National Institute on Aging, NIH
(410) 558-8275
shenr2@mail.nih.gov

Yi Shi, M.D., Ph.D.
Innovative Drug Discovery
(610) 358-1356
yishi2007@gmail.com

K.W. Michael Siu, Ph.D., M.S.
Ontario Cancer Biomarker Network
(416) 673-8425
kwmsiu@yorku.ca

Steven Skates, Ph.D.
Massachusetts General Hospital
(617) 726-4309
ssskates@partners.org

Richard Smith, Ph.D.
Pacific Northwest National Laboratory
(509) 371-6576
rds@pnl.gov

Bruce A. Stanley, Ph.D., M.S.
Pennsylvania State University College of Medicine
(717) 531-5329
bstanley@psu.edu

James Stave, Ph.D.
Strategic Diagnostics Inc.
(302) 456-6785
cshockley@sdix.com

Stephen E. Stein, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 975-2505
steve.stein@nist.gov

Martin Stengelin, Ph.D., M.B.A.
Meso Scale Diagnostics
(240) 631-2522
mstengelin@mesoscale.com

Luke Hunter Stockwin, Ph.D.
SAIC-Frederick, Inc.
(301) 846-5431
stockwin@ncifcrf.gov

David Tabb, Ph.D.
Vanderbilt University Medical Center
(615) 936-0380
david.l.tabb@vanderbilt.edu

Anita Tandle, Ph.D.
National Cancer Institute, NIH
(301) 443-4402
tandlea@mail.nih.gov

Zhiqun Tang, Ph.D.
(202) 687-7369
zt26@georgetown.edu

Michael Tangrea, Ph.D.
National Cancer Institute, NIH
(301) 594-7580
tangream@mail.nih.gov

Scott Tanner, Ph.D.
University of Toronto
sd.tanner@utoronto.ca

Feng Tao, Ph.D.
Armed Forces Institute of Pathology
(202) 782-2815
feng.tao@afip.osd.mil

Dennis J. Templeton, M.D., Ph.D.
University of Virginia Health System
(434) 924-1946
dt9b@virginia.edu
Paul Tempst, Ph.D.
Memorial Sloan-Kettering Cancer Center
(212) 639-8923
p-tempst@mskcc.org

Zivana Tezak, Ph.D.
U.S. Food and Drug Administration
(301) 796-6206
zivana.tezak@fda.hhs.gov

Jeffrey Thomas, Ph.D.
National Cancer Institute, NIH
(301) 846-5465
jeffreyt@mail.nih.gov

Magdalena Thurin, Ph.D.
National Cancer Institute, NIH
(301) 496-1591
thurinm@mail.nih.gov

Yelizaveta Torosyan, M.D., Ph.D.
Uniformed Services University of the Health Sciences
(301) 295-9379
ytorosyan@usuhs.mil

Lois Ann Tully, Ph.D., M.S.
National Institutes of Health
(301) 594-5968
tullyla@mail.nih.gov

Abraham Tzou, M.D.
U.S. Food and Drug Administration
(301) 796-6221
abraham.tzou@fda.hhs.gov

Peter Ujhazy, M.D., Ph.D.
National Cancer Institute, NIH
(301) 439-8528
pu5s@nih.gov

Arti Patel Varanasi, Ph.D., M.P.H.
Westat, Inc.
(301) 294-3952
artivaranasi@westat.com

Mukesh Verma, Ph.D.
National Cancer Institute, NIH
(301) 594-7344
vermam@mail.nih.gov

Bhadrasain Vikram, M.D.
National Cancer Institute, NIH
(301) 496-6111
vikramb@mail.nih.gov

Paul Wagner, Ph.D.
National Cancer Institute, NIH
(301) 496-9424
wagnerp@mail.nih.gov

Anil Wali, Ph.D.
National Institutes of Health
(301) 594-1628
walia@mail.nih.gov

Jason Wan, Ph.D.
National Institute of Dental and Craniofacial Research,
NIH
(301) 594-9898
jasonwan@mail.nih.gov

Guanghui Wang, Ph.D.
National Heart, Lung, and Blood Institute, NIH
(301) 402-2299
guanghui.wang@nih.gov

Honghui Wang, Ph.D.
Clinical Center, NIH
(301) 496-8445
hwang2@cc.nih.gov

Mu Wang, Ph.D.
Indiana University
(317) 278-0296
muwang@indiana.edu

Songfeng Wu, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 975-2529
songfeng.wu@nist.gov
Dan Xi, Ph.D.
National Cancer Institute, NIH
(301) 594-9983
xida@mail.nih.gov

Gary Guishan Xiao, Ph.D.
Creighton University
(402) 280-5911
gxiao@creighton.edu

Xinjian Yan, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 975-3758
xinjian.yan@nist.gov

Liming Yang, Ph.D.
National Center for Research Resources, NIH
(301) 402-0304
lyang@mail.nih.gov

Li-Rong Yu, Ph.D.
U.S. Food and Drug Administration
(870) 543-7052
lirong.yu@fda.hhs.gov

Myeong-Hee Yu, Ph.D., M.B.A.
Korea Institute of Science and Technology
mhyu@kist.re.kr

Elzbieta Zakrzewska, Ph.D.
University of Vermont
(802) 656-4558
ezakrzew@uvm.edu

Boris Y. Zaslavsky, Ph.D.
AnalizaDx
(216) 432-9050
bz@analiza.com

Haixia Zhang, Ph.D.
Vanderbilt University Medical Center
(615) 936-7020
haixia.zhang@vanderbilt.edu

Hui Zhang, Ph.D.
Johns Hopkins University
(410) 502-8149
hzhang32@jhmi.edu

Yufang Zheng, Ph.D.
National Institute of Standards and Technology,
U.S. Department of Commerce
(301) 975-3798
yufang.zheng@nist.gov

Ming Zhou, Ph.D.
National Cancer Institute, NIH
(301) 946-7199
zhouming2@mail.nih.gov

Lisa Zimmerman, Ph.D.
Vanderbilt University
(615) 936-7020
lisa.zimmerman@vanderbilt.edu
MEETING INFORMATION

Hyatt Regency Bethesda
The Hyatt’s main number is (301) 657-1234; the fax number is (301) 657-6453.

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Additional Materials
- CPTC Factsheet
- CPTC Annual Report 2007
- CPTC Patient Brochure
- eProtein Winter 2007
- eProtein Spring 2009
- eProtein Summer 2009

**These materials are available at the registration desk, and an electronic copy has been loaded on the memory stick provided at registration.**
http://proteomics.cancer.gov