

Keynote Speakers in HUPO2009 Plenary Sessions



P1 Lecture
On Monday AM By

Mathias Uhlen

Professor and Vice President
Microbiology
KTH, Sweden



P6 Lecture
On Tuesday AM By

Mark Baker

Professor and Chair
Proteomics and CEO of APAF
Macquarie University, Australia



P2 Lecture
On Monday AM By

Jan Schnitzer

Professor and Scientific Director
Vascular Biology Program
PRISM, US



P7 Lecture
On Tuesday PM By

Carol Robinson

Professor and Director
Churchill College
University of Cambridge, UK



P3 Lecture
On Monday PM By

Cathy Costello

Professor and Director
Mass Spectrometry Resource
Boston University, US



P8 Lecture
On Tuesday PM By

Christoph Borchers

Professor and Director
Genome BC Proteomics Centre
University of Victoria, Canada



P4 Lecture
On Monday PM By

John Yates

Professor and Director
Chemical Physiology
TSRI, US



P9 Lecture
On Wednesday AM By

Ruedi Aebersold

Professor and Director
Molecular Systems Biology
ETH-Zürich, Switzerland



P5 Lecture
On Tuesday AM By

Jack Greenblatt

University Professor
Medical Research
University of Toronto, Canada



P10 Lecture
On Wednesday AM By

Donald Hunt

Professor
Chemistry and Pathology
University of Virginia, US

A Human Protein Atlas for Profiling Cells, Tissues and Organs

Mathias Uhlen

School of Biotechnology, AlbaNova University Center, Royal Institute of Technology (KTH), Stockholm, Sweden

The new version 5.0 of the Human Protein Atlas (www.proteinatlas.org) contains 8,000 validated antibodies targeting 6,800 genes corresponding to approximately one third of the protein-encoded genes in humans. The portal contains more than 6 million high-resolution images generated by immunohistochemistry and confocal microscopy. The antibodies have been generated to regions of low homology (1) and the long-term objective is to generate paired antibodies towards the protein targets with separate and non-overlapping epitopes (2). Pilot projects have been initiated to also generate recombinant affinity reagents (3) and a community-based portal for sharing antibody and antigen validation data has recently been launched (4) as well as a pilot version of a Rodent Brain Protein Atlas (5). We have used the human protein atlas as a discovery tool to find potential biomarkers for cancer diagnostics.

1. Berglund et al (2008) Protein Science 17, 606-613.
2. Rockberg et al (2008 Nature Methods, 5(12):1039-45
3. Uhlen et al (2008) Nature Methods 5 (10): 854-855.
4. Björling and Uhlen (2008) Mol Cell Proteomics 7(10): 2028-37
5. Mulder et al (2009) Mol Cell Proteomics, in press.

Note: The full version of this abstract can be found at the section of *Human Proteome Projects* within this proceeding book (abstract #H121).

Proteomic Imaging of Endothelium and Caveolae for Targeted Penetration into Single Organs and Solid Tumors

Jan. E. Schnitzer

Proteogenomics Research Institute for Systems Medicine, San Diego, CA

Hundreds of disease biomarkers have been discovered. Accomplishing noninvasive targeted imaging and pharmacodelivery using biomarkers is challenged by *in vivo* barriers limiting access inside most tissues. For example, vascular endothelium prevents the penetration of intravenously injected biological agents into tissue where they can be effective. By integrating tissue subfractionation, subtractive proteomics, bioinformatic interrogation, antibody generation, expression profiling, and various imaging modalities, we can identify and actually validate the subset of biomarkers that are targetable *in vivo*. Tissue microenvironment extensively influences endothelial expression. Antibodies to select endothelial and caveolar proteins can not only very rapidly target a specific organ or tumor *in vivo* but also penetrate deep into the tissue. This greatly enhances the targeting of drugs, nanoparticles, gene vectors, and radioisotopes to facilitate disease imaging and treatment. This “organellar proteomic imaging” strategy creates a unique delivery system with many clinical opportunities to diagnose and treat a wide variety of diseases.

Note: The full version of this abstract can be found at the section of *Organelle Proteomics* within this proceeding book (abstract #O125).

Top-down Analysis of Native and Crosslinked Proteins to Locate Modifications and Investigate 3-Dimensional Structures

Catherine. E. Costello

Boston University School of Medicine, 670 Albany Street, Rm 511, Boston, MA 02118-2646 USA.

In biological systems, most proteins undergo one or more forms of post-translational modification; these changes strongly affect protein localization, interactions and turnover. In addition, multiple sequence variants can occur, and proteins associate into functional complexes. Proteolytic digestion of protein mixtures risks the loss of valuable information, since critical details regarding the extent and interrelationships of these important features is lost. We are pursuing top-down and crosslinking as methods to extract the maximum information from intact proteins that are identified as significant in proteomic investigations and are applying new strategies and software tools for this purpose. Top-down sequencing of native and crosslinked proteins has been carried out online and offline, on LTQ-Orbitrap, FT, Q-o-TOF and ion trap MS systems and multistage MSⁿ, including in-source fragmentation, CID, IRMPD, ECD and ETD. The spectra have been interpreted using the Boston University Protein Identifier – Top Down (BUPID-TD), and publicly available programs.

For the crosslinking experiments, we employed BS³, a homobifunctional crosslinker that contains an amine-reactive *N*-hydroxysulfosuccinimide (NHS) ester at each end of an 11.4-Å spacer arm. Conditions for the crosslinking reaction were carefully optimized to minimize the number of crosslinks, to focus on the most significant through-space interactions. Proteins of particular interest to our investigations of oxidative stress in cardiovascular disease, e.g., hemoglobin, and/or systemic amyloidoses, e.g., transthyretin, were chosen as the central objects of the research; other well-characterized proteins were used for method development and evaluation of results. Our results are consistent with published X-ray crystallographic data. When fully developed, the method should be applicable to novel proteins and sequence or post-translationally modified forms of known proteins, which occur in very small amounts and have no X-ray data.

Acknowledgements:

This project was funded NIH-NCRR grants P41 RR10888, S10 RR15942, S10 RR20946 and S10 RR25082 and by NIH-NHLBI contract N01 HV28178. We thank Bruker for loan of the amaZon and access to the solariX.

Note: The full version of this abstract can be found at the section of *Structural Proteomics* within this proceeding book (abstract #S213).

Driving Biological Discovery Using Quantitative Mass Spectrometry

John R. Yates

Department of Chemical Physiology, The Scripps Research Institute, LaJolla, CA, USA

A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale analysis of protein data from experiments. Advances in multi-dimensional separations as well as mass spectrometry have improved the scale of experiments for protein identification. This has improved the analysis of protein complexes, and more complicated protein mixtures. Quantitative mass spectrometry can be used to study biological processes such as protein-protein interactions, development or the effects of gene mutations on pathways. Recent studies on the interactions of the Cystic Fibrosis Transport Regulator as it progresses through the folding pathway will be presented. Metabolic labeling of whole organisms can now be readily accomplished using ¹⁵N labeled proteins as a food source for studies of *in vivo* biology. The use of this method in combination with Shotgun proteomics was used to measure protein and phosphoprotein expression in organelles over several developmental time points in the rat brain (P1 to P45).

Note: The full version of this abstract can be found at the section of *Quantification* within this proceeding book (abstract #Q155).

Protein Complexes and Functional Pathways in Yeast and Bacteria

Jack Greenblatt

Banting and Best Department of Medical Research, University of Toronto, Toronto, ON, M5S 3E1, Canada
Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

We used TAP-tagging, affinity-purification and mass spectrometry (APMS) to sort the *S. cerevisiae* and *E. coli* soluble proteins into complexes and recently extended the yeast PPI network to encompass membrane proteins. Yeast complexes have been assigned to functional pathways using synthetic genetic interaction analysis in an E-MAP version of the SGA approach for genes related to yeast nuclear processes, and we have developed an analogous method, eSGA, for *E. coli*. The co-functionality of the proteins in yeast membrane-associated protein complexes is being tested by comparing protein complexes with genetic interactions and assessing the effects of membrane proteins on the morphology of intracellular compartments. Our *E. coli* PPI network has also been integrated with systematic genomic context inferences to derive a probabilistic network of functional inferences and assign ~57% of the functional orphan proteins to discrete functional neighborhoods. Analogous APMS and RNAi approaches could be used to functionally classify human proteins.

Note: The full version of this abstract can be found at the section of *Assembling the Parts: Whole Cell Modeling* within this proceeding book (abstract #A205).



Membrane Proteomics: Putting the Peptide Jigsaw Back Together

Mark S Baker

APAF, Dept. Chemistry and Biomolecular Science, Macquarie University, NSW. Australia

Introduction Proteomics promises delivery of comprehensive coverage but in membrane proteomics problems of protein solubility and low copy number have made this difficult.

Our recent data on membrane protein:protein interactomics, membrane proteomics peptide IPG-IEF “shotgun” approaches with label-free quantitation and glycan analyses on cancer cell lines, human embryonic stem cells and from liver membranes from cancer-bearing animals are discussed in the context of APAF’s attempts to comprehensively increase membrane proteome coverage.

Methods & Results Using a benign tumour bearing mouse model and “shotgun” peptide IPG-IEF proteomics with label-free quantitation we have examined reproducible, significant changes in the liver proteome.

In control animal experiments, tryptic peptides isolated through methanol-assisted digestion showed optimal peptide coverage of ectodomain-containing membrane proteins, where ~95% were concentrated into 3 main pI range peaks and ~2,000 non-redundant proteins (513 predicted to have between 1 and 19 transmembrane domains) were identified (including most (42) rat CYP450 protein family members). Using pI as a filtering tool enhanced identification reliability.

In cancer-bearing mice, major changes in glycosylation pathways, drug metabolism and clearance (e.g., multi-transmembrane solute carrier family proteins), cell surface receptors, 11 electron transport chain complex I subunits and fatty acid catabolic enzymes were observed.

Subsequently, glycan analyses on Triton-X114 phase partitioned membranes from cancer-bearing mice closely paralleled changes anticipated from glycosylation enzyme changes.

We have deployed additional methods to map embryonic stem cell lines (with various monogenic diseases) membrane proteomes and to membrane changes when the malignant HCT116 colon cancer line is transformed to a benign counterpart (through antisense uPAR suppression).

We report how immunoprecipitation proteomics can identify true uPAR-interacting proteins involved in the “metastosome”. Subsequent detailed analyses of the exact sites of protein interaction between uPAR and the integrin $\alpha v \beta 6$ [using overlapping peptide array binding blots and ELISA-type peptide competition assays] suggest that some very interesting biology may be associated with these interactions. This may have implications for cancer sufferers as both cancer lynchpin proteins uPAR and $\alpha v \beta 6$ are known to be independent poor prognostic indicators of patient outcome, but little is known regarding outcome when they are co-expressed in cancers.

Innovative aspects

- Collectively, our data demonstrate that combination(s) of methods including “shotgun” peptide IPG-IEF, glycoprotein enrichment, glycan analyses and membrane protein complex immunoprecipitation can result in deep, comprehensive mammalian membrane proteome coverage.
- Studies like these are providing vital new information regarding complications involved in cancer treatment (e.g., cachexia), changes in glycoprotein biosignatures associated with disease and proteins that may drive the process of malignancy as well as potentially being novel therapeutic targets.

References

1. Saldanha RG, et al., Proteomic Identification of Lynchpin Urokinase Plasminogen Activator Receptor Protein Interactions Associated with Epithelial Cancer Malignancy. *J Proteome Res*, 2007 6, 1016-28.
2. Lee, A, et al., Rat liver membrane glycoproteome: enrichment by phase partitioning and glycoprotein capture. *J. Proteome Res*, 2009; 8:770-81.
3. Chick JM, et al., Liver Membrane Proteome Dynamics in Cancer Bearing Mice, *Mol. Cell Proteomics*, submitted July 2009.

Note: The full version of this abstract can be found at the section of *Assembling the Parts: Whole Cell Modeling* within this proceeding book (abstract #A204).

Mass Spectrometry and its Role in Structural Biology

Carol V. Robinson

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK

Standard proteomics techniques are unable to describe the stoichiometry, subunit interactions and organisation of assemblies since many are heterogeneous, present at low cellular abundance and frequently difficult to isolate. Using methods designed to maintain non-covalent complexes within the mass spectrometer we show how we can provide definitive evidence of interacting subunits based on the masses of complexes and subcomplexes generated by perturbation both in solution and gas phases. Structural models will be presented for a number of oligomeric protein complexes including the human U1snRNP and eIF3 complexes.

Developments in mass spectrometry have added a further dimension to the study of protein complexes. Using ion mobility mass spectrometry we have been able to add spatial restraints to our models with measurements of collision cross-sections.

Very recently we have had a considerable breakthrough which has enabled us to preserve intact membrane complexes in the gas phase. This enables us to establish lipid and nucleotide binding and to define the stoichiometry and post translational modifications within the intact transmembrane regions of a number of complexes.

Note: The full version of this abstract can be found at the section of *Structural Proteomics* within this proceeding book (abstract #S214).

Dynamic Processes in Proteins and Protein Interactions Studied by Mass Spectrometry Combined with Protein Chemistry

Christoph H. Borchers

University of Victoria/Genome BC Proteomics Centre, 3101-4464 Markham Street, Vancouver Island Technology Park, Victoria, BC V8Z 7X8, Canada

The combination of tertiary structure selective chemical modification in proteins (like acetylation of lysine residues), mass spectrometric approaches, and molecular modeling methods has been used to determine structure-function relationships in proteins. One main focus of our research is to combine photochemical reactions, H/D exchange and chemical crosslinking of proteins with mass spectrometric methods. This concept has proven useful for identification of target proteins and components of protein complexes, structural characterization of protein-ligand and protein-biomolecule interactions, and elucidation of dynamic processes in proteins. The photochemical reactions of proteins used for this purpose can be classified as photoaffinity labeling, photo-crosslinking, or time-resolved photochemical reactions. The purpose of the research is to develop and apply these approaches to the study of proteins involved in a number of disease states and biochemical and pharmaceutical processes. Photoaffinity labeling, combined with mass spectrometric approaches and molecular modeling, is being used to determine the drug binding structures of HIV integrase, an important drug target in AIDS research. Determination of drug binding structures is essential for understanding the mechanism of binding to the inhibitor, and to provide information useful for generating more efficient anti-HIV drugs.

For the concept of crosslinking combined with MS to structurally elucidate protein-protein interaction, novel crosslinker and crosslinking strategies are being developed. In particular, crosslinkers and strategies are developed towards the enrichment, detection and identification of inter-peptide crosslinks. Their feasibilities have been shown with numerous model complexes and first applications to address biological and biomedical questions including the elucidation of interaction sites of membrane/cellular adaptor protein complexes. Within this research area, our special interest is to study the topology of the anaphase-promoting complex (APC), a multi-protein ubiquitin ligase involved in cell cycle regulation that is essential for the metaphase to anaphase transition and exit from mitosis using chemical crosslinking and mass spectrometry.

Note: The full version of this abstract can be found at the section of *Proteomics of Macromolecular Complexes* within this proceeding book (abstract #P224).

Mapping and Measuring Proteomes

Ruedi Aebersold

Institute of Molecular Systems Biology, ETH Zurich, and faculty of Science, University of Zurich, Zurich, Switzerland

The human genome project has taught us that a complete map -in the case of the genome project the complete genomic sequence - along with computational tools to navigate the map, represent invaluable resources for experimental and theoretical biologists. A main consequence of such a complete map is that all the biological processes have to be explainable with the components that constitute the map.

For technical and conceptual reasons the generation of complete maps has been more challenging in the field of proteomics. One of the main difficulties has been the definition of a suitable end point, e.g. definitive proof that all the proteins in a sample have been identified. To overcome this limitation, we have chemically synthesized multiple proteotypic peptides for each ORF in the genome of the yeast *S. cerevisiae* and generated reference fragment ion spectra for these peptides, thus generating for the first time a complete map of a mass spectrometry observable proteome.

In this presentation we will discuss experimental and computational challenges related to the generation of complete proteomic maps and demonstrate the value of the generated reference spectra libraries for shotgun as well as selected reaction monitoring (SRM) based targeted proteomics strategies that will transform proteomics from perpetual proteome re discovery to accurate proteome measurement.

Note: The full version of this abstract can be found at the section of *Human Proteome Projects* within this proceeding book (abstract #H122).

Innovative Mass Spectrometry Technology for the identification of Cancer Immunotherapeutics

Donald F. Hunt

Departments of Chemistry and Pathology, University of Virginia, Charlottesville, VA 22904

At present, the most effective treatment for metastatic melanoma is adoptive T-cell therapy (ACT). In this approach, tumor-infiltrating lymphocytes (TIL) are isolated from resected tumor and expanded ex vivo. After the patient's immune system is ablated by a combination of chemotherapy and total body irradiation, the TIL plus cytokine (IL-2) are re-infused. Objective (tumor shrinkage) and complete responses for this therapy in a recent clinical trial of 25 late stage patients with metastatic melanoma were 72% and 16%, respectively. Tumors the size of grapefruit disappear in a matter of months. Reported here are efforts to identify class I and class II MHC phosphopeptide antigens that are presented (1-10 copies/cell) as a result of dysregulated signal transduction pathways in cancer cells and that will make it possible to employ adoptive T-cell therapy for many additional human cancers.

Note: The full version of this abstract can be found at the section of *Mass Spectrometry Innovations* within this proceeding book (abstract #M123).