

Overview. The Human Proteomics Program at the University of Wisconsin–Madison and the BioPharmaceutical Technology Center Institute are pleased to offer this excellent symposium. Nationally recognized leaders in targeted proteomics and systems biology will discuss topics in cutting-edge proteomics technology development, as well as applications to human disease—including cancer, cardiovascular disease, neurodegenerative disease and virology. Researchers will also present their recent work in scientific poster sessions.

Schedule

7:30am–8:30 am	Registration, Continental Breakfast & Exhibits
8:30am–9:30 am	Welcome – Richard Moss, Ph.D. <i>Honorary Symposium Chair</i> Keynote Address: Tools for Defining the Molecular Choreography of Virus–host Interactions – Brian T. Chait, D. Phil.
9:30am–10:10 am	Emerging Roles for Acetylation in Regulating Host Defense Mechanisms Against Viral Infection – Ileana M. Cristea, Ph.D.
10:10am–10:25 am	Break
10:25am–11:05 am	Systems Proteomics of Chromatin Structure: Insights into Heart Disease Pathogenesis – Tom Vondriska, Ph.D.
11:05am–11:45 am	Top-down Targeted Proteomics for Cardiac Systems Biology – Ying Ge, Ph.D. <i>Lloyd M. Smith, Ph.D. (Moderator – Morning Session)</i>
11:45am–1:00 pm	Lunch, Poster Sessions & Exhibits
1:00pm–1:45 pm	Keynote Address: Imaging Mass Spectrometry: Molecular Mapping Beyond the Microscope – Richard M. Caprioli, Ph. D.
1:45pm–2:25 pm	Developing Mass Spectrometry-based Molecular Imaging and Proteomics Strategies for the Studies of Neurological Diseases – Lingjun Li, Ph.D.
2:25pm–3:05 pm	Quantitative Proteomics to Ubiquitin Signaling and Human Diseases – Junming Peng, Ph.D
3:05 pm–3:20 pm	Break
3:20pm–4:00 pm	Kinase Assay Linked Phosphoproteomics to Identify Direct Kinase Substrates – W. Andy Tao, Ph.D
4:00pm–4:40 pm	Regulation of Mitochondrial Metabolism by Post-translational Modifications – David Pagliarini, Ph.D. <i>Martha M. Vestling, Ph.D. (Moderator – Afternoon Session)</i>
4:40pm–4:45 pm	Closing Remarks
4:45pm–5:45 pm	Reception <i>(Sponsored by Michael, Best & Friedrich LLC)</i>

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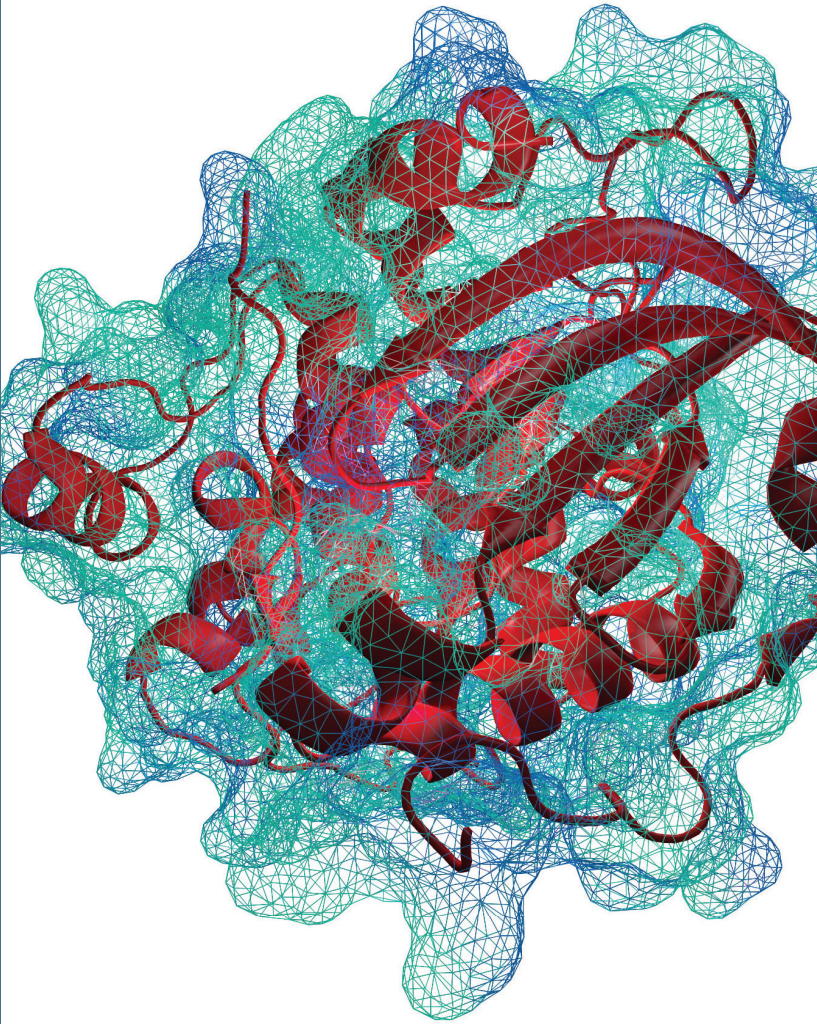
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Targeted Proteomics and Systems Biology

IN HEALTH AND DISEASE

Wisconsin Human Proteomics Symposium
August 1, 2013 | Madison, WI

Speakers

Keynote Speakers

Richard M. Caprioli, Ph. D.
Stanford Moore Chair in Biochemistry Professor, Departments of Biochemistry, Chemistry, Pharmacology and Medicine
Director, Mass Spectrometry Research Center
Vanderbilt University School of Medicine | Nashville, TN

Brian T. Chait, D.Phil.
Camille and Henry Dreyfus Professor, Laboratory of Mass Spectrometry and Gaseous Ion Chemistry
The Rockefeller University | New York, NY

Speakers

Ileana M. Cristea, Ph.D.
Assistant Professor of Molecular Biology
Princeton University | Princeton, NJ

Lingjun Li, Ph.D.
Professor, School of Pharmacy and Department of Chemistry
University of Wisconsin–Madison | Madison, WI

David Pagliarini, Ph.D.
Assistant Professor, Department of Biochemistry
University of Wisconsin–Madison | Madison, WI

Junming Peng, Ph.D.
Associate Member, St. Jude Faculty
Director, St. Jude Proteomics Facility
St. Jude’s Children’s Research Hospital | Memphis, TN

W. Andy Tao, Ph.D.
Associate Professor – Analytical Chemistry and Chemical Biology
Department of Chemistry
Purdue University | West Lafayette, IN

Tom Vondriska, Ph.D.
Associate Professor of Anesthesiology, Medicine and Physiology
David Geffen School of Medicine
University of California–Los Angeles | Los Angeles, CA

Ying Ge, Ph.D.
Assistant Professor of Cell and Regenerative Biology and Chemistry
Director of Mass Spectrometry, Human Proteomics Program
School of Medicine and Public Health
University of Wisconsin–Madison | Madison, WI

Moderators

Lloyd M. Smith, Ph.D.
Professor, Department of Chemistry
University of Wisconsin–Madison | Madison, WI

Martha M. Vestling, Ph.D.
Director of the Mass Spectrometry Facility
Department of Chemistry
University of Wisconsin–Madison | Madison, WI

Honorary Symposium Chair

Richard Moss, Ph.D.
Professor, Department of Cell and Regenerative Biology
Senior Associate Dean for Basic Research, Biotechnology and Graduate Studies
Executive Director, Master of Science in Biotechnology
Director, UW Cardiovascular Research Center
School of Medicine and Public Health,
University of Wisconsin–Madison | Madison, WI

Abstracts

(in order of presentation)

Tools for Defining the Molecular Choreography of Virus–host Interactions

Brian Chait, D.Phil.

Viruses efficiently utilize their host's machinery for their own growth and replication. This feat is usually achieved using just a small number of virally-derived macromolecules. In this respect, viruses master and commandeer for their own purposes key aspects of the biology of their hosts. Understanding how viruses do this can provide insights both into the detailed mechanisms of viral infections as well as into fundamental cellular processes, including for example responses to stress and the control of gene expression.

Towards this goal, I plan to discuss methods that we have developed for elucidating viral–host interactomes as a function of time and space. I will also discuss a new approach for defining antibodies that specifically neutralize infection by viruses, and speculate on generalizing the approach in order to elucidate extensive repertoires of tightly binding antibodies against virtually any chosen antigen.

Emerging Roles for Acetylation in Regulating Host Defense Mechanisms against Viral Infection

Ileana M. Cristea, Ph.D.

Recent years have established mass spectrometry as an essential component of biological discovery. In infectious disease research, mass spectrometry is becoming a fundamental addition to virology techniques, as changes in the proteome, metabolome and lipidome of infected cells are being recognized as key markers of the infection status. Following the infection of a host, viruses utilize finely-tuned mechanisms for hijacking host cellular processes. The outcome of a viral infection is influenced by the balance between host defense mechanisms and virus modulation of cellular pathways. Investigating these temporally-regulated virus–host interactions is essential to an understanding of viral pathogenesis and our ultimate ability to control infections and develop new therapies. We have integrated proteomics-based approaches with techniques from virology, genomics, biochemistry and bioinformatics to generate a systems biology view of infection, as well as gain insights into specific pathways subverted by viral proteins during infection. This presentation will focus on our identification of host defense mechanisms targeted during infections with herpes simplex virus 1 (HSV-1) and human cytomegalovirus (HCMV). For example, one of our recent finding is that viral DNA can be detected in host nuclei by specialized host proteins that act as nuclear DNA sensors to trigger the expression of antiviral cytokines. Within this biological context, strategies for identifying and quantifying protein interactions, building functional interaction networks, and determining the roles of posttranslational modifications during infection will be discussed.

Systems Proteomics of Chromatin Structure: Insights into Heart Disease Pathogenesis

Tom Vondriska, Ph.D.

The endogenous structure of the genome is unknown. Using a systems biology approach, the Vondriska lab is examining the protein constituents of the nucleus that endow the genome with its structure in non-mitototic, full differentiated cells like cardiac myocytes. Because the major causes of death in the developed world (cardiovascular disease and cancer) involve dedifferentiation and re-expression of primitive gene programs, understanding the global mechanisms of chromatin structure and its remodeling during disease has major implications for revealing new diagnostic, prognostic and therapeutic strategies.

Top-down Targeted Proteomics for Cardiac Systems Biology

Ying Ge, Ph.D.

Heart disease is the leading cause of morbidity and mortality in developed countries and is reaching epidemic proportions in aging populations worldwide. Transformative insights at the systems level have great potential to elucidate disease mechanisms and to develop new therapeutic treatments. In the post genomic era, proteomics is essential for deciphering how molecules interact as a system and for understanding the functions of cellular systems in health and disease. However, the unique characteristics of the proteome including the extreme complexity and heterogeneity due to the various post-translational modifications (PTMs), presents tremendous challenges.

To address such challenges, we aim to develop an integrated top-down mass spectrometry (MS)-based targeted proteomics platform for cardiac systems biology. We separate, identify, characterize, and quantify intact proteins extracted from cardiac tissues and reveal changes in the proteome in response to extrinsic and intrinsic stresses. We have shown that top-down targeted proteomics with ultra high-resolution MS has unique advantages for unraveling the molecular complexity, deep sequencing of intact proteins, quantifying multiple modified protein forms, complete mapping of modification sites with full sequence coverage, discovering unexpected modifications, and identifying/quantifying PTM changes during the disease progression. Success of our research will provide analytical tools to advance our understanding of the molecular basis of diseases and foster the development of new strategies for early diagnosis, prevention and better treatment of heart diseases.

Imaging Mass Spectrometry: Molecular Mapping Beyond the Microscope

Richard M. Caprioli, Ph.D.

MALDI Imaging MS produces molecular maps of peptides, proteins, lipids and metabolites present in intact tissue sections. It employs desorption of molecules by direct laser irradiation to map the location of specific molecules from fresh frozen and formalin fixed tissue sections without the need of target specific reagents such as antibodies. Molecular images of this nature are produced in specific m/z (mass-to-charge) values, or ranges of values, typically covering the MW range 200–100,000. We have also developed a similar approach for the analysis of targeted areas of tissues by integrating mass spectrometry and microscopy, termed histology-directed molecular analysis, whereby only selected areas of cells in the tissue are ablated and analyzed.

We have employed Imaging MS in studies of a variety of biologically and medically relevant research projects. One area of special interest is the molecular mapping of changes observed in diabetes in both a mouse model and in the human disease. Major molecular alterations have been recorded in advanced diabetic nephropathy. Other applications include developmental studies of embryo implantation in mouse, renal cancers as well as that in other organs, and neurodegenerative disease. Molecular signatures have been identified that are differentially expressed in diseased tissue compared to normal tissue and also in differentiating different stages of disease. These signatures typically consist of 10–20 or more different proteins and peptides, each identified using classical proteomics methods. In addition, Imaging MS has been applied to drug targeting and metabolic studies both in specific organs and also in intact whole animal sections following drug administration.

This presentation will focus on recent technological advances both in sample preparation and instrumental performance to achieve images at high spatial resolution (1–10 microns) and at high speeds so that a typical sample tissue once prepared can be imaged in just a few minutes. Finally, new biocomputational approaches will be discussed that deal with the high data dimensionality of Imaging MS and our implementation of 'image fusion' in terms of predictive integration of MS images with microscopy and other imaging modalities.

Developing Mass Spectrometry-based Molecular Imaging and Proteomics Strategies for the Studies of Neurological Diseases

Lingjun Li, Ph.D.

Advances in mass spectrometry (MS) have made MS-based proteomics a promising tool for protein profiling and biomarker discovery in various types of biological samples including tissues and biofluids. This presentation will focus on our recent progress in the development and application of MS tools for biomarker discovery in several neurological diseases, such as neurodevelopmental disorders, Alzheimer's disease (AD) and Alexander disease (AxD). The unique challenges associated with the study of nervous systems make it beneficial to focus on neural tissues or proximity fluids.

In an effort to gain insights into the impact of pharmacologically induced excitatory/inhibitory imbalance in infancy on the brain proteome, we employed mass spectrometric imaging (MSI) technology to study changes in protein expression in postnatal day 10 (P10) rat brains following neonatal exposure to the NMDA receptor antagonist dizocilpine (MK801). Analysis of rat brains exposed to vehicle or MK801 and comparison of their MALDI MS images revealed differential abundances of several proteins. Our findings indicate that a brief neonatal exposure to a compound that alters excitatory/inhibitory balance in the brain has a long term effect on protein expression patterns during subsequent development, highlighting the utility of MALDI-MSI as a discovery tool for potential biomarkers.

In addition to brain tissues, cerebrospinal fluid (CSF) provides another excellent source for putative biomarkers indicative of neurological diseases. Using a label-free spectral counting approach, we investigated CSF samples obtained from a mouse model for AxD. In a more recent study of biomarker discovery in AD patients, we conducted a large-scale comparative glycoproteomic analysis via lectin affinity chromatography to enrich glycoproteins from CSF samples collected from control, mild cognitive impairment (MCI) and AD groups. Preliminary results from these on-going studies will be presented. Collectively, these examples highlight the unique advantages of MS-based proteomic tools for biomarker discovery in neurological diseases.

Quantitative Proteomics to Ubiquitin Signaling and Human Diseases

Junmin Peng, Ph.D.

Proteomics is the systematic, large-scale study of protein identity, quantity, structure and function and mass spectrometry (MS) is the mainstream technology in proteomics. We have developed MS technologies to improve automation, sensitivity, throughput, and data processing using modern instruments (e.g. Orbitrap Elite and Q Exactive), with a focus on studying protein posttranslational modifications (e.g. protein ubiquitination) and neurodegenerative diseases.

Ubiquitin (Ub) plays an essential role in regulating almost all cellular events, by modifying substrates in forms of monomer and polyUb chains. The scope of ubiquitination is vast in eukaryotic cells, evidenced by >700 Ub enzymes in human proteome. During the last decade, we have developed a series of methods to enrich, identify, and quantify thousands of ubiquitinated proteins (ubiquitinome). Using both bottom-up and middle-down MS strategies, we determine Ub-modified amino acid residues and analyze the structure of polyUb chains. More interestingly, we have used the novel tools to reveal unexpected complex structures of polyUb chains in cells. Further functional analysis raises a central concept that diverse polyUb chains provide a structural basis for downstream signaling specificity in Ub pathways (Xu P *et al. Cell* 2009; 137:133–45).

In addition, we utilize proteomics approaches to profile neurodegenerative tissues from animal models and postmortem human brains. Combining laser capture microdissection and mass spectrometry, we analyzed localized proteome in tissues and identified dynein dysregulation in Alzheimer's disease. More recently, we used biochemical fractionation to concentrate protein inclusions in clinical samples, and then by large-scale profiling of ~10,000 proteins, identified and validated a number of novel disease-related proteins in neurodegenerative disorders. The pathological role of these novel proteins are further investigated in cellular and animal models.

Kinase Assay Linked Phosphoproteomics to Identify Direct Kinase Substrates

W. Andy Tao, Ph.D.

Protein kinases and their substrates represent the largest signaling network that regulates protein–protein interactions, subcellular localization, and ultimately cellular functions. While many kinases are excellent therapeutic targets, the precise connection between protein kinases and their direct substrates has not been fully elucidated for a majority of protein kinases. We introduce here an integrated proteomic strategy, termed Kinase Assay Linked Phosphoproteomics (KALIP), which combines a sensitive kinase reaction with kinase-dependent phosphoproteomics to identify direct substrates of protein kinases. The novel in vitro kinase reaction is carried out in a highly efficient manner using a pool of proteins derived directly from cellular components and labeled 18O-ATP. The resulting newly phosphorylated proteins are then isolated and identified by mass spectrometry. A further comparison of these in vitro phosphorylated proteins with those derived from endogenous proteins isolated from cells in which the kinase is either active or inhibited reveals new candidate protein substrates.

The KALIP strategy was applied to identify novel direct substrates of multiple kinases, including Syk, a protein tyrosine kinase with dual properties of an oncogene and a tumor suppressor in distinctive cell types, PINK1, a key player in Parkinson's disease, and Erk1/MAPK3, a serine/threonine kinase involved in both physiological and pathological cell proliferation. A number of known and novel substrates were identified to shed lights on new functions of the kinases. The proteomics-based KALIP strategy can be a powerful tool to decipher complex signaling cascades one kinase and one cell type at a time.

Regulation of Mitochondrial Metabolism by Post-translational Modifications

David Pagliarini, Ph.D.

Mitochondria are complex and dynamic organelles that are essential to the survival of nearly every eukaryotic cell. To generate a foundation for systematic investigations of mitochondrial function and adaptation, we recently established a protein compendium of these organelles across a wide range of tissues from healthy mice. This resource, termed MitoCarta, provides a robust, yet static view of the mitochondrial proteome. We are now applying MitoCarta as a framework for quantifying how mitochondrial proteins and post-translational modifications (PTMs, e.g., phosphorylation and acetylation) change during acute and chronic metabolic perturbations, and to elucidate the role of these changes in regulating mitochondrial activity. To do so, we blend state-of-the art multi-plexed mass spectrometry-based proteomics with focused biochemistry and molecular biology approaches.

In particular, we have recently taken this approach to capture the mitochondrial proteome dynamics during fasting, the onset of obesity, aging, caloric restriction and acute iron deprivation. Our analyses have revealed hundreds of dynamic phosphorylation and acetylation events and have produced quantitative, searchable maps of mitochondrial alterations across a spectrum of metabolic states. We have leveraged these data to demonstrate that key steps in ketogenesis, the TCA cycle, branched-chain amino acid degradation and fatty acid oxidation are regulated by reversible PTMs, and that the mitochondrial oxidative phosphorylation machinery is highly calibrated to cellular iron content. Moving forward, we plan to further elucidate the mitochondrial signaling network by identifying the regulatory enzymes (e.g., kinases, acetyltransferases, etc.) responsible for managing mitochondrial PTMs, and to define the functions of uncharacterized mitochondrial proteins mutated in human disease.

