PRESENTER ABSTRACTS

How High Mass Accuracy Measurements Will Transform Targeted Proteomics

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A weakness of the shotgun method is the stochastic nature of sampling, which often yields spotty coverage and reduced sensitivity. This limitation has propelled recent fervor in targeted MS methods (SRM) in which prior system knowledge pre-selects peptide targets. SRM offers high sensitivity and reproducibility; however, the inability to simultaneously monitor multiple transitions and the inherent low resolution of QqQ systems limit SRM throughput. Drawing on the high resolution and accuracy achievable via FT-MS, we describe a targeted method that allows for the simultaneous monitoring of all precursor-to-product transitions (parallel reaction monitoring, PRM) and dynamic, real-time adjustment of target candidates. The method does not require prior knowledge of preferred transitions and offers ~20-50X higher throughput as compared to SRM.

Electron-Based Dissociation Methods for Glycans, Glycopeptides and Glycoproteins

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We are applying Electron Transfer Dissociation (ETD) and Electron Capture Dissociation (ECD), with and without prior or post-activation, for detailed structural analysis of oligosaccharides, glycopeptides and glycoproteins. We have investigated adduction with different metals, the use of alternative ETD reagent anions, and varied ECD parameters and are exploring the theoretical basis for the observed fragmentation.

We reported recently the generation of a variety of fragmentation types for milk sugars that were initiated by ETD on an ion trap using fluoranthene as the chemical reagent [Han and Costello, *J Am Soc Mass Spectrom*, **2011**, 22, 997]. Cross-ring cleavages helped to clarify the different linkage types and branching patterns of the glycans. Stable isotopic labeling verified the fragment ion assignments. Now we have extended our prior results for to include a wider variety of cation-adducted and permethylated oligosaccharides, biantennary and disialylated biantennary *N*-linked glycans and native glycopeptides and the performance of ETD MS/MS with different chemical reagents. We will summarize further glycan analyses using ETD on ion trap, FT-ICR and Orbitrap instruments and the top-down analyses of glycoproteins with a 12-T hybrid QhFTICR MS equipped for CID, ECD, ETD and IRMPD.

ETD spectra with other chemical reagents now reveals complementary information. In ECD, both the nature of the metal adduct and the electron energy strongly influence the ECD fragmentation of glycans; these results shed light on the dissociation mechanisms. In top-down analyses of glycoproteins that used ETD or ECD, loss of any portion or the full glycan was minimal (<2%), protein sequence coverage is high and glycosylation sites can be clearly defined. The methods are being applied to the analysis of free sugars, glycans released from infectious microorganisms, and to intact glycoproteins, *e.g.*, Ribonuclease B, human serum amyloid P.

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Top-Down Disease Proteomics of Myofilaments for Understanding Heart Failure

Ying Ge, Ph.D.

Heart failure is the leading cause of mortality and morbidity in the United States. The underlying molecular and cellular mechanisms of heart failure are very complex and poorly understood. Myofilament proteins in the sarcomeres not only play essential roles in cardiac contractility but also are critical elements in signal reception and transduction during the onset and progression to heart failure. The hypothesis is that both extrinsic and intrinsic stresses trigger the molecular signaling processes that result in altered modifications to myofilaments leading to contractile dysfunction and eventually heart failure. Herein, we aim to establish top-down mass spectrometry (MS)-based disease proteomics platform to examine intact myofilament proteins extracted from both normal and diseased tissues to establish a correlation between altered modifications of myofilaments and cardiac dysfunction. We have

shown that top-down with electron capture dissociation has unique advantages in unraveling the molecular complexity, quantifying multiple modified protein forms, complete mapping of modifications with full sequence coverage, discovering unexpected modifications, and identifying and quantifying positional isomers and determining the order of multiple modifications. We have established a simple and robust top-down quantitative proteomics methodology featuring affinity chromatography and high resolution MS for biomarker discovery from tissues. We have systematically analyzed thirty-six clinical human heart tissue samples and identified phosphorylation of cardiac troponin I (cTnI) as a candidate biomarker for chronic heart failure. Moreover, we found phosphorylation of cTnI is up-regulated in spontaneously hypertensive rats (SHR) in comparison to age-matched Wistar-Kyoto rats (WKY) and identified upregulated PKC phosphorylation sites of cTnI in SHR. Recently, we have developed a protocol which effectively extracted myofilament proteins from less than 1 mg of myocardial tissue and separated/analyzed intact myofilament proteins via on-line two dimensional liquid chromatography (LC)/MS within 4-5 hours. In summary, we have demonstrated the unique power of top-down disease proteomics methodology for deciphering protein modifications toward a better understanding and diagnosis of heart failure.

Precursor Acquisition Independent from Ion Count: Extending Proteomic Dynamic Range without Prior Fractionation
David R. Goodlett, Ph.D.

Data-dependent precursor ion selection is widely used in shotgun proteomics to profile the protein components of complex samples. Although very popular, this bottom-up method presents major drawbacks in terms of detectable dynamic range. In Panchaud et al (Anal Chem. 2009) we demonstrated the superior performance of a data-independent method we term precursor acquisition independent from ion count (PAcIFIC). Our results showed that almost the entire, predicted, soluble bacterial proteome could be thoroughly analyzed by PAcIFIC without the need for any sample fractionation other than the C18-based liquid chromatograph used to introduce the peptide mixture into the mass spectrometer. Importantly, we also showed that PAcIFIC provides unique performance for analysis of human plasma in terms of the number of proteins identified (746 at FDR < or = 0.5%) and achieved dynamic range (8 orders of magnitude at FDR < or = 0.5%), without any fractionation other than immuno-depletion of the seven most abundant proteins. Since this publication we have gone on to show in Panchaud et al (Anal Chem. 2011) that PAcIFIC can 1) be used with spectral counting for quantitation, 2) may be combined with tandem mass tags to provide multiplexed quantitation and 3) detect the full dynamic range of yeast without prior fractionation. We'll review these data and discuss extensions that reduce the number of analyses required for a full PAcIFIC experiment.

Can Mass Spectrometry and Protein Footprinting Play a Role in Structural Biology?
Michael L. Gross, Ph.D.

MS offers means of determining protein interactions, folding, and unfolding by using chemical footprinting. Driving this approach is the wide availability of mass spectrometers for analytical proteomics; these should also be applicable to protein footprinting. To this end, we are developing fast photochemical oxidation of proteins (FPOP) and implementing HD exchange, and other covalent modification approaches to interrogate protein interactions, interfaces, and dynamics of folding/unfolding. A significant advantage of FPOP is its speed, owing to the use of free radicals as footprinting reagents. Radical generation occurs in low nanosec, and the radical reactions are complete in 1 microsec. Only a single conformation of the protein exists during the footprinting because the distribution of oxidation products follows a Poisson distribution. Besides OH radicals, other radicals are possible including the sulfate and carbonate radical anions and the iodide radical. FPOP can also be the probe in a classic, two-laser "pump-probe" experiment whereby a temperature-jump perturbation is produced as a pump by one laser and a second laser initiates FPOP as the probe. Subsequent analysis is by MS. This experiment is capable of probing protein structural dynamics at the sub millisecond level with improved sensitivity and more detailed structural resolution than any physical chemical method. Better understanding of folding will emerge from this approach, which is more informative than ultrafast mixing, temperature-jump or pressure-jump coupled with time-resolved fluorescence, UV-Raman, IR, nuclear magnetic resonance and circular dichroism.

Electron Irradiation of Cat- and Anions: A Potential Analytical Tool in both Proteomics and Glycomics
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Electron capture dissociation (ECD) and electron transfer dissociation (ETD) are well established as valuable complementary activation techniques in peptide tandem mass spectrometry (MS/MS) analysis. Our research has shown that gas-phase ion-electron reactions (ECD and electron detachment dissociation (EDD)) are also valuable for generating extensive dissociation of oligosaccharides, including cross-ring fragmentation that is crucial for assigning carbohydrate linkages. We have extended this work to fluorescently labeled glycans. Such labels, which allow UV/fluorescence detection and reverse-phase chromatography, show altered fragmentation behavior in EDD with reduced structural information in some cases. We hypothesize that this reduction in cross-ring fragmentation is due to altered charge location in gas-phase glycan anions. By contrast, labeling improves fragmentation in both positive and negative ion mode electron induced dissociation (EID), presumably due to facilitated electronic excitation from the introduction of aromatic electrons. For doubly charged glycan cations, ECD appears to generate more structural information than ECD. Recently, we have discovered a novel MS/MS technique: negative ion

electron capture dissociation (niECD), which appears particularly promising for sulfo- and phosphopeptides that show improved ionization efficiency in negative ion mode. niECD provides ECD/ETD-like fragmentation for peptide anions with complete retention of posttranslational modifications.

Application of Proteomics to Cancer and Personalized Medicine

Sam Hanash M.D., Ph.D.

Strategies to achieve personalized medicine and improve public health encompass assessment of an individual's risk for disease, early detection and molecular classification of disease resulting in an informed choice of the most appropriate treatment instituted at an early stage of disease development. A major contribution of proteomics in this field is the development of blood based tests to achieve the goals of personalized medicine. An integrated cooperative effort is currently under way for the identification of biomarkers of cancer risk, early detection of cancer and identification of altered signaling pathways based on serum and plasma analysis. This is illustrated in proteomic studies of epithelial cancer that encompass analysis of specimens collected before onset of symptoms for the identification of risk and early detection markers and elucidation of signatures in plasma for altered signaling pathways in tumors. This overarching effort also benefits from the availability of subject cohorts and from the availability of engineered mouse models and cell lines that inform with respect to proteins involved in altered signaling pathways. Such an effort requires and benefits from the availability of in-depth quantitative proteomics methods, bioinformatics resources and integration with other broad based molecular profiling technologies.

Developing Integrated Proteomic Approaches for Comprehensive Profiling of Protein Complexes

Lan Huang, Ph.D.

Proteins form stable and/or dynamic multi-subunit protein complexes under different physiological conditions to control various biological processes and thus maintain normal cell homeostasis. Protein-protein interactions are vital for modulating macromolecular protein complex composition, assembly, structure, stability and function. Aberrant protein-protein interactions can have severe biological consequences and often result in human disease or cancer. In order to understand molecular mechanisms underlying how protein complexes function in response to various excellular cellular cues, it is essential to characterize protein interaction networks of protein complexes and determine their structural dynamics. In recent years, we have developed several novel mass spectrometry-based proteomic strategies by integrating new sample preparation techniques, affinity purification methods, quantitative mass spectrometry with bioinformatics tools to obtain a comprehensive proteomic profile of protein complexes. In addition, we have developed new cross-linking strategies to identify protein interaction interfaces for structural elucidation of large protein complexes. To evaluate these strategies, we have characterized the proteome dynamics of protein complexes involved in regulating intracellular protein degradation in the ubiquitin-proteasome system (UPS). The results have provided new mechanistic insights on the regulation of the UPS at different physiological states. The methodology presented here can be applied for the study of other protein complexes.

A Top Down Approach to the Human Proteome

Neil Kelleher, Ph.D.

Although the more widely used proteomics strategy has been peptide-based, unambiguous characterization of entire protein sequences and their post-translational modifications (PTMs) are often best achieved through the analysis of intact proteins. Previously, the field of Top Down proteomics has lagged behind Bottom Up in terms of high-throughput and comprehensive analysis. This was attributed to underdevelopment of separations, MS instrumentation and bioinformatics. Recently, our lab has developed a platform for the large scale use of multiplexed 2D separations coupled to capillary LC-LTQ-FTMS to achieve unprecedented proteome coverage. Given the complexity of the human proteome, our separation platform involves sIEF (solution IEF), GELFrEE, and capillary-RPLC; which fractionate according to charge, mass and hydrophobicity, respectively. An analysis of HeLa S3 cells (+/- etoposide) using this 3D platform results in approximately 50 fractions covering 4 < pI < 10 and MW up to 100 kDa in a total separation time of 3.5 hours. These fractions were further separated using capillary-RPLC coupled online to LTQ-FTMS for direct analysis of intact proteins. Online detection and identification resulted from data dependent scan events that included both MS1 scans using FTICR or ion trap mode and fragmentation scans using both CID and nozzle skimmer dissociation. Data were analyzed using ProSightPC software on a 48-core computer cluster. Proteins identified with different accession numbers with E-Value < 10^-2 were considered unique hits. Proteins identified with termini sequence homology were filtered for redundancy. With six days of automated instrument time, 580 unique accession numbers were identified. Compilation of identifications between untreated and DNA damaged samples led to an unprecedented total of unique Top Down identifications. This study shows that we achieved the highest levels of throughput with record-breaking proteome coverage across a range of protein physiochemical properties, with detection of post-translational dynamics of HeLa S3 cells in response to intrinsic DNA damage.

Comprehensive Glycomics for Biomarker Discovery and Nutrition

Carlito B. Lebrilla, Ph.D.

Proteins are often modified by the attachment of sugar units called glycans during the normal course of protein production. It is estimated that over 70% of all human proteins are modified in this way, making glycosylation one of the most common form of post-translational modification. It is also the only PTM with significant structure. The development of new analytical methods, specifically mass spectrometry and separation science, has significantly

increased the progress in understanding the role of the glycome in many biological areas. Research in our group has focused on glycans as disease markers and their role in nutrition. For disease markers, a new paradigm for cancer biomarker discovery is proposed. The detection and analysis of the glycans that decorate the underlying

polypeptide in glycoproteins may provide more specific detection of cancer rather than examining the proteins themselves. There are numerous studies that demonstrate glycans produced in cancer cells are different from those in normal cells. The aberration in glycosylation is observed with many types of diseases as well. In the studies in our laboratory, we harvest glycoproteins and extract the glycans in patient serum to determine whether the glycosylation has changed in cancer patients compared to healthy patients. This new glycans assay is used to discover biomarkers for the diagnosis of breast, prostate, ovarian, and gastric cancer. For nutrition, glycans and glycoproteins make up a large component of human milk. As human milk is the model for the perfect nutrition, we are investigating the role of glycans and glycoproteins in the infants' development. We find that the free milk oligosaccharides, which have no nutritional value to the infant, are food for the intestinal bacteria and are key to establishing the gut flora. In addition, glycosylation on milk proteins work to mediate bacterial binding to epithelial cells.

Developing Mass Spectrometry-based Tools for Biomarker Discovery in Neurological Disorders
Lingjun Li, Ph.D.

Mass spectrometry (MS) – based proteomic approaches have become increasingly popular tools for biomarker discovery. However, their applications to neurological disorders remain to be challenging due to sample complexity and limited dynamic range for detection. Here, I will present our efforts on the search and discovery of biomarkers in several neurological diseases, including the prion disease and the Alexander disease (AxD). For prion disease there is a lack of sensitive and reliable pre-mortem diagnostic test. By applying glycoprotein enrichment via lectin affinity chromatography and 2D RP-RPLC prior to tandem MS experiments we have improved separation of peptides, yielding identifications of more than 700 proteins with three different time points of prion disease infection and progression. Relative quantitation of a panel of proteins was obtained by a combination of isotopic labeling and validated by spectral counting. For cerebrospinal fluid (CSF) samples obtained from control and glial fibrillary acidic protein transgenic mice as a mouse model for AxD, a shotgun proteomics approach incorporated with immunodepletion by a modified IgY-14 protocol was employed. This approach resulted in the identification of 266 proteins with relative quantitation performed using distributive normalized spectral abundance factor (dNSAF) spectral counting analysis. A panel of biomarker proteins with significant changes in the CSF of transgenic GFAP overexpressor mice has been identified, demonstrating the utility of our methodology and providing interesting targets for future investigations on the molecular and pathological aspects of AxD. Finally, imaging mass spectrometry (IMS) technology has been utilized to reveal spatial distributions of several putative protein and peptide biomarkers in different brain regions of a rodent model of the autism spectrum disorders (ASD), following brief exposures to compounds that alter excitatory/inhibitory neurotransmission in the brain during critical periods of development. Collectively, these examples highlight the unique advantages of MS-based proteomic tools for biomarker discovery in neurological diseases.

From Innovative Technologies to Model Organisms: The Search for Biomarkers for the Detection of Early Stage Epithelial Ovarian Cancer
David Muddiman, Ph.D.

Mass-spectrometry has the ability to elucidate new diagnostic, prognostic, and therapeutic biomarkers and translate them to the clinic. However, there are numerous challenges that exist that must be overcome. This presentation will discuss the challenges specific to the study of epithelial ovarian cancer (EOC) in humans and how these challenges have directed our thinking in terms of the development of mass spectrometry-based bioanalytical strategies. First, to augment the human model, we have developed a chicken model of spontaneous EOC which allows us to control the environment and genetic background, a model which is characterized by rapid onset and progression of disease, and allows us to conduct longitudinal sampling. Second, we have developed hydrophobic tagging reagents to increase the electrospray response of *N*-linked glycans which has the major added benefit of being able to incorporate a stable-isotope label for use in relative quantification experiments. Finally, we have developed new ionization methods that can be used for direct analysis and tissue imaging; the latter being a fruitful arena for biomarker discovery.

COPa Library: A Protein Knowledgebase for Cardiovascular Biology and Medicine
Peipei Ping, Ph.D.

We designed, organized, and constructed a Cardiac Organellar Protein Atlas Library (COPa library) as a targeted and interactive protein resource for cardiovascular biology and medicine. Three types of protein data are collected, protein image data by antibodies or affinity tag labeling, protein pathway data by protein arrays; and protein peptide spectra by mass spectrometry. This study focuses on the database construction and the peptide spectral library. Specifically, annotated spectra are hosted using a relational database (MySQL) in an organelle- and species-specific modular fashion. Thus far, a total of 100,988 spectra have been disseminated. A web portal was established to navigate the library via parallel set of identifiers, e.g. protein name, gene symbol. In parallel, a web-service (.NET framework) was engineered to annotate the identities of query spectra. This workflow dissects native spectral files into small data packages, surpassing network limitations and enabling parallel data submission and search. At the COPa server, an optimized dot product algorithm was coded to weigh the correlations between query

spectra and spectra in the library. Additionally, an innovative decoy mechanism minimized rate of false discovery, and overcame the propagation of spectra with inaccurate annotations. A benchmark test showed that a library search covered 93.4% of the proteins identified via a database search, as well as an additional 23.9% at the same

statistical cutoff. To foster synergy among proteomic researchers, each user is invited to participate the restructuring of the core datasets with individually approved consent. A secured wiki-like web interface was incorporated to develop consensus knowledge based on these core datasets. Overall, the COPa library search supports targeted proteomic characterization, which complements database searching for exploratory survey. The implementation of the COPa library-based protein knowledgebase leverages state-of-the-art technology and annotated datasets among the research community at large. Its application bridges discovery-driven and hypothesis-driven research while fostering translational medicine.

Proteomic Technologies for the Genome Age
Lloyd M. Smith, Ph.D.

The successful sequencing of the human genome has provided us with the blueprints of life.... but now the likely greater challenge of understanding those blueprints lies squarely before us. Again, information is needed. The proteins that are encoded by the genome come in many different types and forms. We need to know what proteins are where, when, and in what form, how they change with time and in response to internal and external signals, and how they interact with one another. This information is a first step towards developing an understanding of the complex web of networks and pathways that comprise functioning biological systems. As was true for the Genome Project, new technologies are needed to provide this information. This talk will present challenges, opportunities, and progress in the development of such new technologies, with a particular emphasis on surface science, biological mass spectrometry, and the conjunction of the two.

Advances in LC-MS Based Proteomics
Richard D. Smith, Ph.D. (Keynote)

Advances in the quality, resolution, and the speed of separations for proteomics samples combined with the sensitivity and performance of mass spectrometry continue to extend applications in proteomics, including the coverage of proteomics measurements and the quality of quantification for both broad discovery and targeted measurements. However, significant challenges remain related to: the coverage of very low abundance proteins, sample size limitations, the need for sufficient throughput to address large numbers of samples, and the ability to identify and distinguish protein modifications. This presentation will discuss the role of advanced approaches and instrumentation for addressing these challenges, illustrate their applications in the context of both broad plasma and CSF biomarker discovery and highly sensitive targeted measurements, and end with a discussion of the potential for further significant advances.

Regulation of SMN and Identification of its Downstream Targets
Judith A. J. Steen, Ph.D.

Spinal Muscular Atrophy (SMA), a disease caused by the mutations of *Survival Motor Neuron 1 (SMN1)* gene, is the most common genetic cause of infant mortality. Humans have two copies of the *SMN* gene, the telomeric *SMN1*, which encodes for a full-length form (FL-SMN), and the centromeric *SMN2*, which encodes primarily for a rapidly-degraded truncated form (SMNΔ7) as well as the full-length form. In the most severe form, Type 1 SMA, there are 1 or 2 copies of the *SMN2* gene, and patients die within 2 years of age due to respiratory failure. Patients with more copies of the *SMN2* gene, however, manifest a less severe form of SMA (Type III SMA). SMA occurs due to decreased amount of FL-SMN protein in spinal motor neurons. Therefore, much of the effort for therapeutic interventions in SMA has focused on increasing the level of FL-SMN protein products. Proteomic, immunofluorescence microscopy and biochemical results suggest that an E3 ubiquitin ligase, the APC (Anaphase Promoting Complex) is involved in regulation of SMN stability and function by targeting SMN to the proteasome for degradation. Since there is a tight correlation between the amount of FL-SMN and the severity of disease, studies to understand the interaction between APC and SMN as well as elucidate the downstream RNA targets of SMN were performed. These results provide important insights into the biology of SMA and have the potential to generate new treatment options for this disease.

Mass Spectrometric Analysis of Neuropeptides from Single *Ascaris* Neurons
Antony O.W. Stretton, Ph.D.

Numerically, nematodes have very simple nervous systems. The female parasitic nematode *Ascaris suum* has only 298 neurons, and the hermaphroditic free-living *Caenorhabditis elegans* has 302. *A. suum* is large (ca 35 cm), and has large neurons suitable for electrophysiological recording. We assembled a functional circuit from the morphological synapses, scored by electron microscopy, and the physiological properties of the neurons and their synapses. The predicted activity of this circuit matched that actually recorded from neurons in dissected preparations that were opened to allow microelectrode penetration. However, it differed dramatically from the activity recorded from these same neurons in semi-intact behaving preparations. Something was missing from the circuit description. We have now shown that there are numerous neuropeptides (at least 250) present in *A. suum*, and the ones we have sequenced have potent activity on individual neurons. We think that they were washed out of the dissected preparations, thus losing their modulatory activity on individual neurons. For peptide identification,

initially peptides were purified by HPLC and sequenced by Edman degradation. Now we are using mass spectrometry, which has speeded up the discovery process more than one hundred-fold. In particular, we are now dissecting single identified neurons and subjecting them to MALDI-TOF MS and tandem MS for sequence

determination. All neurons examined so far contain peptides. Most contain previously unknown peptides, and the unknown peptides often outnumber the known peptides. This is a powerful method of peptide discovery. It has the distinct advantage that it simultaneously solves the identity and the cellular expression of the peptide. It also has the advantage that it identifies the peptide actually expressed by a particular neuron, rather than relying on predictions from cDNA or genomic DNA sequences, and on reporter constructs for expression patterns. Neuropeptides are processed from precursor proteins, and the rules of the proteolytic cleavage are not yet robust enough for accurate prediction of processing.

The Cell-Environment Interface – Applications to Heart Disease and Biomarkers
Jennifer Van Eyk, Ph.D.

The hearts' response to injury is synchronous involving the interaction between different cell types; cardiac fibroblasts, embryonic or cardiac derived stem cells and myocytes. This coordination is due, in part, to the reciprocal interplay between cells and their response to changes in their local environment. The cell-environment interface (CEI) comprises both the secretome and the cell surface and is the main site of this response. Sensing of local changes in the ECM microenvironment occurs on the cell surface, which acts as a conduit, translating the extracellular signal into a cellular response. We have been using proteomic based methods to investigate the protein composition of the cell surface and determine how they sense and respond to changes in their environment. Using cell surface capturing technology we have specifically targeted the cell surface N-glycoproteome of myoblasts (cardiac and skeletal) and embryonic stem cells and have identified the differences in the protein composition of these cell types. This has allowed us to identify a number of potential markers of cell differentiation. In addition, we have optimized the proteomic analysis of secreted proteins from embryonic and cardiac derived stem cells, cardiac myocytes and cardiac fibroblasts. As with the cell surface subproteome, there is a high degree of cell specificity with groups of secreted proteins observed only in one cell type. Several of the cell specific proteins are also found to be elevated in the plasma of individuals who undergo myocardial ischemia and or necrosis. These findings suggest that relative concentration of each protein may be crucial for cellular intercellular communication allowing the various cells types to act synergistically and that several maybe useful as cell –specific biomarkers.

Unraveling Novel Molecular Mechanisms of Action of Thiopurine Drugs
Yinsheng Wang, Ph.D.

Acute lymphoblastic leukemia (ALL) remains the leading cause of cancer-related deaths in children. There is a pressing need for developing novel and effective *therapeutic* avenues for ALL treatment, thereby improving the cure rate and quality of life of ALL patients. Thiopurine drugs, including 6-mercaptopurine and 6-thioguanine, are among the most widely prescribed drugs for ALL treatments. The molecular mechanisms underlying the therapeutic activity of these drugs, however, remain elusive. We employed LC-MS/MS and assessed three potential mechanisms, i.e., 6-thioguanine induces cell death by triggering mismatch DNA repair, by perturbing epigenetic pathway through reactivating epigenetically silenced genes, and by altering cell cycle progression in ALL cells. Our metabolite quantification and SILAC-based quantitative proteomic profiling demonstrated that thiopurine drugs may exert their antineoplastic effect through the perturbation of multiple cellular pathways, which may involve the participation of both thioguanine nucleotides and DNA 6-thioguanine.

Proteomic Technologies Towards the Membrane Proteome: Applications in Neuroscience
Christine Wu, Ph.D.

[TBA]

Discovery and Characterization of Novel Lysine Modifications
Yingming Zhao, Ph.D.

Of the 20 ribosomally coded amino acid residues, lysine is the most frequently post-translationally modified, which has important functional and regulatory consequences. In this presentation, we will report the identification and verification of previously unreported forms of protein post-translational modifications (PTMs) – lysine malonylation and lysine succinylation. The modified residues were initially identified by mass spectrometry (MS) and protein sequence alignment. The modified peptides derived from *in vivo* proteins were verified by Western blot analysis, *in vivo* labeling with isotopic succinate, MS/MS and HPLC coelution of their synthetic counterparts. We also show that these two modifications are evolutionarily conserved and dynamically respond to different physiological conditions. Furthermore, we identified the first regulatory enzyme that can modulate status of these PTMs. Given the apparent high abundance and the significant structural changes induced by the PTMs, it is expected that the two PTM pathways will have important cellular functions.