Thermo Fisher Scientific Life Sciences Mass Spectrometry Symposium

Wednesday, November 7, 2018

Institution: North Carolina State University
Location: NCSU Hunt Library
Duke Energy Hall
Second Floor
Rooms: 2110A, 2110B
Address: 1070 Partners Way, Raleigh, NC 27606
Time: 8:00 a.m. - 5:00 p.m.

Agenda:

8:00 - 8:45 a.m.	Registration & Continental Breakfast
8:45 a.m.	Introduction to NC State and METRIC Partnership by Jonathan Horowitz, Assistant Vice Chancellor for Research
9:00 a.m.	Trixie Ueberheide
10:00 a.m.	John Rogers
11:00 a.m.	Tim Stratton
12:00 - 1:00 p.m.	Lunch
1:00 p.m.	Lingjun Li
2:00 p.m.	John Rogers
3:00 p.m.	Scott Peterman
4:00 p.m.	Ken Hsu
5:00 p.m.	Final Comments

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This symposium is sponsored by the Kenan Institute for Engineering, Technology & Science and Thermo Fisher Scientific to celebrate the formation of The Molecular Education, Technology and Research Innovation Center (METRIC). METRIC is focused on advanced molecular characterization and is one of five NC State Shared Core Research Facilities administered by the Office of Research and Innovation. We offer technical expertise and state-of-the-art instrumentation encompassing magnetic resonance spectroscopy, mass spectrometry, and X-ray crystallography.

Application of Mass Spectrometry: From Virulence Factor Discovery to Epitope Mapping

Beatrix Ueberheide, Ph.D, Assistant Professor – Department of Biochemistry and Molecular Pharmacology, NYU School of Medicine

Staphylococcus aureus related illnesses result in greater than 300,000 hospitalizations and more than 40,000 deaths in the United States annually. The majority of these infections are caused by highly virulent, drug resistant strains, but the difference in virulence among strains is not well understood. We will present strategies to characterize the virulence of different clinical strains using mass spectrometry and hot spot analysis. A better understanding of the virulence will aid in vaccine development against this pathogen. Once a suitable target is identified, the epitope of that antigen against neutralizing antibodies is one of the next steps of vaccine development. This presentation will describe an easily implemented bench-top method for oxidative footprinting to probe the solvent accessible surface of proteins and protein-protein interaction surfaces in a dose-dependent manner. Mass spectrometry is then used to quantitatively evaluate binding interfaces between antibody and antigen in the case for epitope mapping and generally by comparing the accessibility of surface residues of proteins in complex relative to their unbound forms. Benchmarking studies as well as application of epitope mapping and complex analysis will be discussed.

Novel reagents, acquisition, and analysis strategies for high confidence protein cross-link identification with mass spectrometry

John Rogers – Senior R&D Manager, MS Reagents

Chemical crosslinking in combination with mass spectrometry is a powerful method to determine protein-protein interactions. This method has been applied to recombinant and native protein complexes, and more recently to whole cell lysates or intact unicellular organisms in efforts to identify protein-protein interactions on a global scale. With the combination of MS cleavable crosslinkers and isobaric mass tags, Orbitrap technology for high resolution, accurate mass detection capabilities, and novel analysis algorithms, we provide scientists with the best tools for new applications into cellular dynamics and structural proteomics. This presentation will highlight reagents and sample preparation workflows in combination with multi-dimensional data acquisition and analysis approaches for fast and accurate identification of cross-links using Orbitrap Fusion Lumos and Q-Exactive HF mass spectrometers together with a new XlinkX node in Proteome Discoverer 2.3.

Evolution of Identification – Software tools for resolving unknowns

Tim Stratton, Ph.D., Manager – Library Technologies, Thermo Fisher

Mass spectrometry provides an excellent tool for analysis of complex samples to discover valuable biological insight. While the MS data helps to identify the wealth of compounds, individual chemical entities, in the sample and can provide information on which ones are important, it does not mean we know what each of those compounds are. Identification of many of the detected components remains a particular challenge.

Spectral library searching is one of the primary means by which an identification of an unknown is performed. While spectral libraries continue to grow, they still remain extremely small by comparison to the total 'known' chemical space of potential compounds we may observe, so how do we measure our confidence in a spectral library match? We will discuss work on creating a measure of confidence in a single spectral library match through the Confidence score algorithm for library searching and how this may be applied to understand the relative value of a spectral library match. In addition we will discuss tools beyond a simple 'identity' spectral library match to leverage spectral library to provide useful information on the majority of compounds that simply do not exist in spectral libraries.

Expanding the Chemical Toolbox for High-Throughput Quantitative Proteomics and PTM analysis

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

Recent advances in mass spectrometry (MS) have made MS-based omics a central technology for biomedical research. Quantification of proteins, peptides and metabolites present in complex biological systems is often key to understanding dynamic changes of many essential physiological and pathological processes. Chemical labeling with multiplex isobaric tags offers an effective strategy for parallel comparative analyses of many samples during liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Here, I will present our recent progress on the design and development of several novel chemical tags, including dimethylated leucine (DiLeu) isobaric tagging reagents, which offer cost-effective implementations that enable higher orders of multiplexing. The utilities of these novel chemical tags will be demonstrated through several biomedical applications.

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Targeted Mass Spectrometry Assay Kits for Absolute Quantitation of Signaling Pathway Proteins

John Rogers – Senior R&D Manager, MS Reagents

Many genetic mutations in cancer cells alter expression of proteins in the AKT, RAS and TP53 pathways. Quantitative measurements of alterations in the expression of pathway proteins and post-translational modifications (PTM) are necessary for understanding disease biology and drug actions, classifying disease states, monitoring cancer progression, and determining treatment response. Major bottlenecks for quantitation of these proteins are a lack of rigorously verified methods and reagents, and reliance on Western blotting. To address these bottlenecks, we have optimized a multiplex immunoprecipitation (IP) to targeted mass spectrometry (MS) workflow to develop pathway panel kits, thereby achieving simultaneous enrichment and absolute quantitation of multiple total and phospho proteins from the AKT pathway, RAS, and TP53.

Novel Approaches for Small Molecule Identification and Characterization Using Orbitrap ID-X Tribrid Mass Spectrometer

Scott Peterman, Ph.D., Sr. Global Marketing Manager, Thermo Fisher Scientific

Small molecule analysis is an integral component for many areas of academic and industrial research, ranging from metabolomics and metabolite identification to extractables and leachables and natural products. One of the primary goals of small molecule studies is to exhaustively characterize samples to identify compounds of interest and perform structural elucidation. The primary challenge is attributed to determining what are the compounds of interest relative to the background or matrix. Both are primarily singly charged and distributed across similar molecular weight ranges. In addition to sampling compounds of interest, comprehensive data processing is required for identification and structural elucidation in which the majority of compounds do not have validated spectral library entries.

Recently, Thermo Fisher Scientific introduced a novel workflow dedicated for small molecule analyses. The workflow is centered around the Orbitrap ID-X Tribrid mass spectrometer that can acquire HRAM MS and ion tree product ion spectra and a unique automated acquisition scheme performing exhaustive feature sampling. Lastly, updated software routines were created to leverage the unique, high-quality MS and MSn ion tree data to dramatically increase compound identification and structural elucidation for knowns, known unknowns, and unknown unknowns.

The seminar will provide insights into the workflow as it applies to the different small molecule research areas. A general overview of the instrument, acquisition schemes and software tools will be presented, and then how the workflow supports unknown metabolomics, lipidomics metabolite ID, flavonoids, and extractables & leachables.

Exploring ligand binding space to discover new lipid biology

Ken Hsu, Ph.D., Assistant Professor – Chemistry, University of Virginia, School of Medicine

Lipid phosphorylation is a key regulatory mechanism for cell metabolism and signaling. Specifically, diacylglycerol kinases(DGKs) modulate intracellular levels of the secondary messengers diacylglycerol and phosphatidic acid, which mediatecell biology through specific lipid-protein interactions. Development of isoform-selective DGK inhibitors is challenging but needed to understand specificity of DGK biology invivo. Towards this goal, we use ATP acylphosphate activity-based probes and quantitative mass spectrometry to define,for the first time, the DGKATP-binding site (DAGKc/DAGKa subdomains) and propose a potential role for C1 domains in DGK catalytic function. We discovered fragments from old drugs (ritanserin) that reveal pharmacophores required for selective binding to DGK active sites that are not in the canonical ATP binding pocket. We provide evidence of a single contiguou inhibitor binding site composed of DAGKc, DAGKa, and C1 sites by demonstrating equipotent inhibition using a fragment inhibitor. Our current working model is that C1 domains of DGKs are involved in substrate and inhibitor recognition of type1, 4, and 5 DGKs and is a site for pursuing development of isoform-selective inhibitors. We describe new lead compounds that will optimized into the first selective and invivo-active DGK-alpha inhibitor.

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