Subgroup: Molecular Biophysics

1-Subg
Fast Photochemical Oxidation of Proteins (FPOP) for the Characterization of Macromolecules
Lisa M. Jones.
Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA.
Structural analysis of macromolecular complexes (e.g., antibody-antigen complexes and viruses) is hampered owing to their size and complexity. High resolution biophysical methods for structural analysis are not applicable to proteins that have a high molecular mass. In order to analyze these large structures, methods based on mass spectrometry (MS) can be employed. MS-based methods are advantageous because they are not limited by size and require only microgram quantities of protein. Footprinting methods coupled with MS are a powerful tool for studying higher order structure of proteins. These methods can be used to moniter protein conformational changes and identify protein-protein and protein-ligand interaction sites. The protein footprinting method fast photochemical oxidation of proteins (FPOP), a radical footprinting approach, utilizes hydroxyl radicals to oxidize solvent-exposed residues on a short time scale. The irreversible label of FPOP has several advantages including the ability to purify proteins after labelling. Here we report the efficacy of FPOP for studying protein conformational differences and identifying protein-interactions in large complexes.

2-Subg
Protein Hydrogen Exchange Measured by Mass Spectrometry
S. Walter Englander.
Biochem/Biophys, University of Pennsylvania, Philadelphia, PA, USA.
Newly advanced hydrogen exchange - mass spectrometry technology provides a uniquely powerful method for the study of protein properties - structure, change, interactions, dynamics, and energetics, at amino acid resolution, and how this limited biophysical repertoire is used to produce myriad protein functions. This talk will present an introduction to the method with examples of recent applications.

3-Subg
Protein Folding and Binding Characterized by Mass Spectrometry
Lars Konermann, Siavash Vahidi, Modupeola A. Sowole.
University of Western Ontario, London, ON, Canada.
This presentation will illustrate how the combination of solution phase labeling with mass spectrometry (MS) can elucidate mechanistic aspects of protein behavior. We will focus on two ongoing projects in our laboratory. (A) The initial (submillisecond) stages of protein folding represent a formidable experimental challenge. We have begun to address this issue by using submillisecond mixing with laser-induced oxidative labeling. Apomyoglobin (aMb) serves as a model system for these measurements. Exposure of the protein to a brief pulse of hydroxyl radical (•OH) at different time points during folding introduces covalent modifications at solvent accessible side chains. The extent of labeling is monitored using MS-based peptide mapping, providing spatially-resolved measurements of changes in solvent accessibility. The technique introduced here is capable of providing in-depth structural information on time scales that have thus far been dominated by low resolution spectroscopic probes. (B) The bacterial protease ClpP is a multi-subunit complex with a central degradation chamber that can be accessed via axial pores. In free ClpP these pores are obstructed. Acyldepsipeptides (ADEPs) are antibacterial compounds that bind ClpP and cause the pores to open up. The ensuing uncontrolled degradation of intracellular proteins is responsible for the antibiotic activity of ADEPs. We use hydrogen/deuterium exchange MS to obtain insights into the ClpP behavior with and without ADEP1. Our data point to a mechanism where the pore opening mechanism is mediated primarily by changes in the packing of N-terminal nonpolar side chains. We propose that a ''hydrophobic plug'' causes pore blockage in ligand-free ClpP. ADEP1 binding provides new hydrophobic anchor points that nonpolar N-terminal residues can interact with. In this way ADEP1 triggers the transition to an open conformation, where nonpolar moieties are clustered around the rim of the pore.

4-Subg
Native Mass Spectrometry for Structural Biophysics
Justin L.P. Benesch.
University of Oxford, Oxford, United Kingdom.
We use a combination of mass spectrometry (MS) based approaches to interrogate directly the quaternary structure and dynamics of proteins in the 100 kDa to 1 MDa range, intact in vacuum [1]. Of particular interest to us are the small heat-shock protein molecular chaperones, which are responsible for ensuring proteins reach and maintain their native fold in the cell [2]. Their study however is often hampered however due to their frequent heterogeneity and motions at equilibrium.
MS and tandem MS enable us to identify and quantify the relative abundances of different protein stoichiometries present in solution, while real-time experiments allow the extraction of quaternary fluctuations. This leads us to obtain equilibrium and rate constants for the underlying protein-protein and protein-ligand interactions. Concurrently, ion mobility (IM) MS measurements provide information as to the physical size of the proteins. Together with information from other sources, these experiments therefore provide powerful restraints in modeling the structures of protein assemblies that are difficult and time-consuming to study by means of conventional structural biology approaches.
[1] Benesch, J.L.P., Ruotolo, B.T. ''Mass spectrometry: come of age for structural and dynamical biology'' Current Opinion in Structural Biology (2011), 21, 641-9
[2] Hilton, G., Lioe, H., Stengel, F., Baldwin, A.J., Benesch, J.L.P. ''Small heat-shock proteins: paramedics of the cell'' Topics in Current Chemistry, (2012), 328, 69-98

5-Subg
A Novel Soft Ionization Process and Applications in Imaging Mass Spectrometry
Lorelie Imperial[1], Sashiprabha M. Vithanarachchi[1], James Wager-Miller[2], Ken Mackie[2], Matthew J. Allen[1], **Sarah Trimpin**[1].
[1]Wayne State University, n/a, MI, USA, [2]Indiana University, n/a, IN, USA.
A motivation of our research is to make inroads in the daunting task of chemical analysis of the brain's composition and temporal changes. We recently introduced a new soft ionization process for use in mass spectrometry (**MS**). This new technique called laserspray ionization (**LSI**) has advantages of speed of analysis, high spatial resolution for imaging, mass range extension, and improved fragmentation common with multiply charged ions. We have interfaced LSI with ion mobility spectrometry (**IMS**) for separation of gas phase ions from mixtures by charge and cross-section (size/shape) and, in a second dimension, with high resolving power by mass-to-charge permitting powerful deconvolution of sample complexity, even with identical masses (isomers), directly from surfaces. LSI interfaced with mass spectrometers having electron transfer dissociation (**ETD**) capabilities produces similar backbone fragmentation as ESI as demonstrated for the protein ubiquitin as well as for peptides directly from tissue. We have extended LSI to vacuum mass spectrometers and determined that ionization occurs with vacuum or thermal assistance without the need of voltage or lasers. LSI imaging of mouse brain tissue sections is demonstrated to determine the location of gadolinium-based complexes synthesized for use as magnetic resonance imaging (MRI) agents. Conventional MALDI failed. The LSI images are complemented by MRI and microscopy results of the same mouse brain and, additionally, provide a molecular view of the endogenous chemical composition. MS images of mouse brain tissue from a clozapine treated mouse are compared with results obtained for endogenous lipids, peptides and small proteins in the same tissue. Protocols are being developed for high spatial resolution LSI imaging.

6-Subg
Tissue Shotgun Proteomics: Applications to the Clinical Laboratory
Surendra Dasari[1], Jason D. Theis[2], Julie A. Vrana[2], Ahmet Dogan[3], Paul J. Kurtin[2].
[1]Health Sciences Research, Mayo Clinic, Rochester, MN, USA, [2]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA, [3]Pathology and Laboratory Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
Shotgun proteomics method starts by digesting the proteins into peptides. The peptide mixture is subjected to tandem mass spectrometry. Peptides and proteins are identified from the resulting tandem mass spectra using database search software. This technology has matured in the research laboratories and is poised to enter clinical laboratories. However, the road to this transition is sprinkled with major technical unknowns like long-term stability of the platform, reproducibility of the technology and clinical utility over traditional antibody-based platforms. Further, regulatory bodies that oversee the clinical laboratory operations are unfamiliar with this new technology. As a result, diagnostic laboratories have avoided using shotgun proteomics for routine diagnostics.
In this presentation, we describe the clinical implementation of a shotgun proteomics assay for amyloid subtyping, with a special emphasis on standardizing the platform for better quality control and earning clinical acceptance. This assay is the first shotgun proteomics assay to receive regulatory approval for patient care. Implementation of this assay in a CAP/CLIA clinical laboratory has transformed the amyloidosis standard of care at the Mayo Clinic and across