Symposium: Electrostatics

1817-Symp
Charges in Hydrophobic Environments in Proteins
Bertrand Garcia-Moreno, Ph.D.
Biophysics, Johns Hopkins University, Baltimore, MD, USA.
Ionizable groups buried in the hydrophobic interior of proteins play central roles in a wide variety of essential biochemical processes. Owing to the inherent differences in the dielectric properties of water and of protein, buried ionizable groups usually titrate with anomalous pKa values shifted relative to normal pKa values in water in the direction that favors the neutral state. The determinants of these unusual pKa values are being examined systematically with a family of variants of staphylococcal nuclease with Lys, Arg, His, Asp and Glu in 25 internal positions. Some of these ionizable groups titrate with normal pKa values, but the majority of them have anomalous pKa values, some shifted by as many as 6 units relative to the normal values. Dozens of crystal structures confirm that the ionizable groups are buried, at least when they are in the neutral state, often in complex with water molecules never before observed at internal locations in SNase. The structures show that the microenvironments of the ionizable groups range between the fully hydrophobic to the highly polar, but they do not fully explain the unusual properties of these groups. NMR spectroscopy has been used to examine the range of structural reorganization that can be coupled to the ionization of the internal ionizable. While the ionization of some residues is not easily detected in HSQC spectra, in most cases there is clear evidence of modest charge-induced changes in local structure or increased conformational fluctuations. Interactions between internal groups and polar groups, surface charges, and other internal groups have also been measured. These data constitute an extensive, quantitative description of origins of the apparent dielectric properties of proteins. They also show that proteins tolerate internal charges without the need for any special structural adaptations.

1818-Symp
Manipulating the Protein Energy Landscape
Susan Marqusee.
University of California, Berkeley, Berkeley, CA, USA.
I will present new developments in our studies probing high-energy fluctuations on the energy landscape. using single molecule mechanical manipulation and thiol exchange techniques, we have been able to identify and monitor novel events on the conformational landscape. Our recent results on the differences in mechanical compliance between the molten globule and native states and our ability to monitor the conformational fluctuations leading to unfolding have important implications for mechanical processes in the cell.

1819-Symp
Connecting Sequence to Conformational Properties of Intrinsically Disordered Proteins: A Charged Relationship
Rohit V. Pappu.
Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, USA.
Intrinsically disordered proteins (IDPs) can be classified as coils or globules based on their net charge per residue (NCPR). Naïve annotation of a predictive phase diagram suggests that a majority of IDPs are likely to form disordered globules. Globule formers (as opposed to rigid, folded globules) are likely to have poor solubility profiles and it seems unlikely that the IDP proteome is enriched in globule formers. This raises the possibility that NCPR is an incomplete descriptor of IDP phase behavior. To address this issue, we carried out systematic computational studies on a set of synthetic and naturally occurring IDPs where NCPR is likely to yield questionable designations of IDP phase behavior. Our results show that the polyampholytic nature of IDPs provides a clear descriptor of sequence-ensemble relationships. Our results highlight the connection between linear patterning of oppositely charged residues in polyampholytic sequences and the phase behavior of IDPs / IDRs in sequences where more than 30% of the residues are charged. Analysis of sequence databases shows that ~ 70% IDPs/IDRs are sequence-patterned polyampholytes that are likely to form heterogeneous expanded ensembles. This has important implications for the accessibility of short linear interaction motifs that directly influence IDP function.

1820-Symp
Vibrational Stark Spectroscopy Directly Probes Electric Fields in Proteins
Steven Boxer[1], Sayan Bagchi[2], Stephen D. Fried[2], Nick Levinson[2], Miguel Saggu[2], Lin Xu[2].
[1]Department of Chemistry, Stanford University, Stanford, CA, USA, [2]Stanford University, Stanford, CA, USA.
We have developed vibrational Stark effect (VSE) spectroscopy to probe electrostatics and dynamics in organized systems, in particular in proteins where they can report on functionally important electric fields. The strategy involves deploying site-specific vibrational probes (-C≡N, -C-D, -C=O and -C-F) whose sensitivity to an electric field is measured in a calibrated external electric field by VSE spectroscopy. This gives the magnitude of the vibrational frequency shift associated with an electric field change in a protein, e.g. by making a mutation, changing pH, ligand binding, etc., projected along the bond axis, which is typically determined by x-ray crystallography. This concept can also be used to estimate the electrostatic contribution to non-covalent X-H••π interactions that stabilize protein structures. Recent results in which we attempt to calibrate the absolute magnitude of the field will be presented, along with complications that arise from non-electrostatic contributions to the frequency shifts. This requires a substantial development of simulations methods in parallel with experiments. By fully understanding the origins of these effects, they can be applied to obtain information on functionally-relevant fields at the active site of enzymes (see also presentation by Fried, Bagchi and SGB). This approach provides experimental benchmarks for high-level simulations which have suggested a critical role for electric fields and electric field gradients in biological function.

Symposium: Counting Atoms in Big Machines

1821-Symp
Of Membranes and Motors - New Insights from Mass Spectrometry
Carol Robinson.
University of Oxford, Oxford, United Kingdom.
Polydispersity, heterogeneity and insolubility are among the most challenging properties of protein complexes making them almost impossible to study by conventional structural biology approaches. Mass spectrometry is providing significant insight into this area uncovering the precise composition of intact complexes, the effects of phosphorylation and the dynamics of subunit exchange. Recent results will be presented from a range of both membrane and soluble complexes.

1822-Symp
Dynamics of the Ribosome Assembly Line in Living Cells
James R. Williamson.
The Scripps Research Institute, La Jolla, CA, USA.
The process of ribosome assembly is a major metabolic program that accounts for a third of the energy budget of a rapidly growing bacterium. using Stable Isotope Pulse Labeling and Quantitative Mass Spectrometry, we are investigating the dynamics of ribosome biogenesis in living cells. A distribution of ribosome assembly intermediates is observed that provides direct information on the order of ribosomal protein binding to the nascent ribosomal RNA transcript. Proteomics reveals the participation of many assembly cofactors at specific stages of assembly. using specific deletion strains and growth under perturbed conditions, we are developing a systems level approach to understanding the-critical assembly dynamics required to ensure the capacity for protein synthesis.

1823-Symp
Mass Spectrometric Characterization of Protein:Carbohydrate Non Covalent Complexes: What is Fact and What is Fiction
Julie A. Leary.
Department of Molecular and Cellular Biology, University of California, Davis, CA, USA.
This seminar will present Ion Mobility Mass Spectrometry data showing separation and detection of isomeric glycosaminoglycans (GAG) both with and without protein complexation. Chemokine proteins are involved in inflammation and as such complex with GAG during the activation step of chemotaxis. Comparison of different chemokine conformations suggests that the GAG will complex to different areas of the protein and that sulfation pattern may play a part in complexation. A relatively new modeling program, RAPTOR, is used for determining theoretical cross sections and overall conformation of proteins for which there is no crystal or NMR structure. Both RAPTOR and IM provide valuable information on isomeric separation, identification and conformation of both carbohydrate alone, and with its' protein partner.