26-Subg
Insights into the Binding Mechanism of IDPS from Molecular Simulation
Robert Best.
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No abstract.

27-Subg
Accessible Conformations of N-Terminal Acetylated Alpha-Synuclein: Implications for Fibril Formation
Jean Baum.
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No abstract.

28-Subg
Diverse Transient Structures in Small Oligomers of α-Synuclein Probed by Single-Molecule Force Spectroscopy
Michael Woodside[1,2].
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Intrinsically-disordered proteins such as α-synuclein display a wide range of behavior, from transient, fluctuating structures to stable oligomers and fibrillar aggregates. We characterized the structures formed in individual α-synuclein molecules and small oligomers by using single-molecule force spectroscopy to determine the size of the structures, their stability, and their rates of formation. Applying force to single protein molecules with optical tweezers and measuring the molecular extension as the force was ramped up to unfold any structure that might have formed, we found that most often, no discrete structural transitions could be observed, as might be expected for a disordered protein. Some of the time, however, discrete transitions representing the unfolding of a stable or metastable structure were observed. Even small oligomers formed numerous metastable structures, with a surprisingly broad range of sizes. Comparing the structures formed in monomers, dimers and tetramers, we found that average stability increased with oligomer size. Most structures formed within a minute, with size-dependent rates. Intriguingly, the pulling curves that showed no evidence of discrete transitions nevertheless revealed the presence of rapid fluctuations at low force, arising from marginally-stable interactions. These results provide a new window onto the complex landscape for α-synuclein aggregation.

29-Subg
Control of Disorder and order in Signaling by Proteins
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Proteins serve as switches in signaling networks and their switch-like behavior is often controlled by inputs such as ligand binding, post-translational modification, changes in micro-environment (pH, oxidation state), mechanical force, and light irradiation. Disordered proteins and domains, which are prevalent in eukaryotic proteomes, often serve as hubs within signaling networks. Motifs within disordered protein regions mediate their multifarious interactions, and these interactions are often regulated by post-translational modifications. Of course, folded proteins also participate in signaling networks and also exhibit switch-like behavior. Interestingly, we have observed that some folded proteins function as signaling switches through transitions to disordered states due to ligand binding or post-translational modification_a phenomenon we term ''regulated unfolding''. It is well appreciated that many proteins that are disordered in isolation play their regulatory roles by folding upon binding their biological targets. We have observed that these proteins, in their functional, largely ordered bound states, also experience regulated unfolding as a mechanism of signaling. We will discuss several examples of regulated unfolding from the realms of both folded and disordered proteins. Observations from us and others suggest that the biological palette of protein disorder is more diverse than currently appreciated. The emerging view is that both regulated order-to-disorder and disorder-to-order transitions contribute to and enhance the functional complexity of proteins.

30-Subg
NMR Studies of the free Energy Landscape of Intrinsically Disordered Proteins in their free and Bound Forms
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No abstract.

31-Subg
Linking Intrinsic Disorder to Allosteric Regulation in the NMDA Receptor
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Ionotropic glutamate receptors mediate excitatory synaptic transmission in the central nervous system. Ligand binding to the extracellular domain triggers opening of a transmembrane ion channel domain allowing sodium influx. The NMDA-sensitive receptor subtype also permits calcium influx, which plays important roles in regulating synaptic strength. With the evolution of vertebrates, the NMDA receptor isoform GluN2B acquired a large cytoplasmic domain (CTD) that enables allosteric modulation by Src kinase. GluN2B-containing receptors are inhibited by extracellular zinc but the block can be reversed by phosphorylation of two tyrosine residues in the distal CTD. The mechanism whereby the CTD affects gating of the ion channel is unknown. The CTD is predicted to contain large stretches of intrinsic disorder. A central palmitoylation motif splits the CTD into two domains. Using ensemble biophysical and biochemical methods, we confirmed that the distal cytoplasmic domain (CTD2) is a disordered globule. Single molecule FRET revealed stochastic transitions that appear to arise from slow conformational dynamics on the second timescale. We found that Src phosphorylation caused a uniform expansion of the polypeptide. We were able to induce compaction in CTD2 by removing proline residues, which retarded the Src-induced expansion. Scanning mutagenesis also revealed a role for proline in aggregation as well as CTD2 binding to small molecules, like the fluorophore ANS, and a PDZ domain involved in synaptic targeting of the receptor. Thus, proline appears to modulate the attractive potential of short linear motifs within regions of intrinsic disorder. In the context of the native NMDA receptor, proline depletion near the Src phosphorylation sites uncoupled allosteric regulation of the channel by zinc and eliminated the zinc block. This suggests that allosteric modulation is linked to the conformational dynamics within the disordered cytoplasmic domain.

32-Subg
Decoding Sequence-Ensemble Relationships of IDPS
Rohit Pappu.
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Synthesis of various independent observations suggests that IDPs can be classified as globule vs. coil formers based on their net charge per residue. Closer scrutiny of the resultant classifications reveals considerable complexity and conformational richness that is masked by considerations of amino acid composition alone. I will present results from our recent work on polyampholytic IDPs, which show that conformational properties of at least a third of naturally occurring IDPs are governed by the linear sequence distribution of oppositely charged residues. This has implications for de novo design of sequence-ensemble relationships as a tool for modulating functions of IDPs. Preliminary results highlighting the utility of sequence design to afford tunability of IDP functions will be discussed. Finally, I will close with a discussion of recent advances that are allowing us to probe how being in cis to ordered domains modulates the sequence-encoded conformational properties of intrinsically disordered regions.

Subgroup: Nanoscale Biophysics

33-Subg
Nanoscale Mechanisms Underlying HIV-1 Viral Particle Assembly and Release
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The Human Immunodeficiency virus (HIV-1) life cycle involves several highly choreographed steps during which the virus assembles at the plasma membrane (PM) of an infected cell and buds off the membrane as a viral particle. We have used conventional and superresolution imaging approaches to investigate the cell biological mechanisms underpinning three key steps in the viral assembly/budding pathway: clustering of the viral coat in the plasma membrane; recruitment of proteins into the viral bud; and virus budding off the membrane. In the first step involving viral Gag coat assembly, we show it is critically dependent on viral and/or host mRNA, which drives Gag clustering through RNA-Gag electrostatic interactions. In the second step involving protein recruitment into the viral bud, we demonstrate that Env proteins incorporate into viral buds through dynamic partitioning into a specialized microenvironment created by multimerization of Gag at the PM. In the final step involving viral abscission from the PM, we examine the 3D molecular organization of