located in the intrinsically disordered regions. We studied the effect of phosphorylation on protein binding for different types of complexes from human proteome. We estimated the effect of phosphorylation on complex stability and found that the majority of complexes do not show significant stability differences upon phosphorylation or de-phosphorylation. However, we also found that phosphorylation sites tend to be located on binding interfaces, may orthosterically modulate the strength of interactions and for about one-third of all complexes cause relatively large changes in binding energy. All this suggests that processes of phosphorylation, binding and disorder-to-order transitions might be tightly coupled with each other. Indeed we showed that there is significant association between phosphorylation, disorder and binding for serine and threonine residues in human. However, tyrosine phosphorylation might not be necessarily associated with binding through disorder-to-order transitions and is often observed in ordered regions which are not disordered in unbound state. We suggest possible mechanisms of how phosphorylation might regulate protein-protein binding via intrinsic disorder, for example, phosphorylation in disordered regions may prevent disorder-to-order transition and subsequent protein-protein binding.

975-Plat
Single-Molecule and Ensemble Fluorescence Study of Cryptic Disorder and Oligomerization in Nucleophosmin
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Nucleophosmin (Npm) takes part in several important cellular processes including ribosome biogenesis and centrosome duplication. Past studies have shown that the Arf tumor suppressor protein interacts with the N-terminal region of Npm to form MDa sized complexes in vivo (1, 2). Npm shuttles between the nucleolus and cytoplasm and mediates assembly and maturation of ribosomal particles. We hypothesize that this shuttling activity is influenced by Npm's oligomeric state. Recently, the N-terminal region of Npm (residues 1-130, Npm-N) containing the oligomerization domain and a conserved disordered acidic stretch, was shown to interconvert between a monomeric disordered state and a folded, pentameric state, in response to changes in its ionic environment (3). However, the structural biophysics of the Npm/Arf system is not well understood, in part due to its complexity and high aggregation propensity. Here, we use the strengths of single-molecule (SM) and ensemble fluorescence in combination to study the folding and structural interconversion of Npm-N. In addition, we studied the interaction of Npm-N with various Arf fragments. These experiments shed light on the kinetics and mechanism of salt- and Arf-induced coupled folding and oligomerization of Npm-N, revealing multiple steps and the associated timescales. Moreover, our results suggest that Arf might arrest Npm-N in the oligomeric form, thus modulating the structural equilibrium of Npm-N, which we speculate is the underlying mechanism of inhibition of Npm's role in ribosome biogenesis by Arf. Thus, our high-resolution fluorescence studies of folding and oligomerization of Npm-N provide key biophysical insights into how Npm's shuttling and ribosome biogenesis functions are controlled.
(1) Ithana, K. et al. (2003), Mol. Cell., 12, 1151-1164
(2) Bertwistle, D. et al. (2004), Mol. Cell. Biol., 24, 985-996
(3) Mitrea, DM. et al, manuscript in preparation

976-Plat
Cooperatively Folded Conformational Sub-States in Intrinsically Disordered Proteins as Revealed by EPR Spectroscopy
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The discovery of intrinsically disordered proteins (IDPs) has started revolutionizing structural biology in recent years. Despite the, from a traditional point of view, lack of well-folded structures IDPs fulfill many vital functions and hence seem to contradict or at least amend the classic structure = function paradigm. The structural and functional characterization of IDPs remains challenging; only a few experimental techniques are suitable and appropriate theoretical concepts are rare. Here, we show that nanoscale distance measurements (double electron-electron resonance, DEER) based on electron paramagnetic resonance (EPR) spectroscopy can provide unique insights into the physical character of IDPs. EPR spectroscopy is used to provide quantitative information about the thermodynamic behavior and populations of cooperatively folded sub-states. The methodology was applied to the IDP Osteopontin (OPN), a cytokine involved in metastasis and tumor progression. We demonstrate that the solution structure of OPN is best described as a heterogeneous structural ensemble in which the individual, transiently formed conformational sub-states are largely different, ranging from extended coils devoid of stabilizing interactions to cooperatively folded compact structures with accentuated side-chain interactions and binding sites preformed to accommodate authentic binding partners. Thus, OPN is best understood as a polymer, whose large conformational space is governed by a subtle interplay of electrostatic and hydrophobic forces and significantly enriched with robust tertiary conformational sub-states. The unprecedented finding that IDPs are able to sample conformations reminiscent of globular stably folded proteins under native conditions, goes far beyond the classical view of intrinsically unstructured proteins and calls for a reassessment of the binary description scheme (ordered vs. disordered) proposed for this enormously important protein class.

977-Plat
Breaking Bad - Phospholipase Cβ Disrupts Alpha-Synuclein Aggregation in Neuronal Cells
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Alpha-synuclein (AS) is the main component of neurodegenerative plaques seen in Alzheimers and other diseases. There is evidence that oxidative stress promotes AS aggregation and plaque formation. Phospholipase Cβ1 (PLCβ1) transmits G proteins signals from hormones and neurotransmitters to induce the release of calcium from intracellular stores and subsequent activation of a host of calcium-sensitive enzymes. We have recently found that in neuronal cell lines, AS binds to PLCβ1, protecting it from calcium-regulated proteases that result in down-regulation of PLCβ1. In this study, we subjected neuronal cells to oxidative stress. This condition results in the down-regulation of many proteins, including PLCβ1. However, the levels of AS were unchanged under oxidative conditions. We postulated that the loss of PLCβ1 could promote aggregation of AS. In support of this idea, we found evidence that PLCβ1 prevents AS aggregation in vitro, and over-expression of PLCβ1 inhibits AS aggregation in live cells as indicated by live cell fluorescence imaging methods. Similarly, down-regulation of PLCβ1 promoted AS aggregation and preliminary studies suggest similar result for PLCβ1 down-regulation due to oxidation. We identified the interaction site between PLCβ1 and AS and are currently testing peptide inhibitors of AS aggregation based on their interaction. Our studies indicate that PLCβ1, or possibly other AS binding partners, might reduce damage by AS aggregation under basal or oxidative stress conditions. This work was supported by NIH GM053132.

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The Intrinsically Unstable SH3-DRKN Protein: Compactness, Conformations and Speed
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SH3 is a common binding domain that mediates protein-protein interactions. The N-terminal SH3 domain of the Drosophila adaptor protein Drk is marginally stable under physiological conditions, showing nearly 50/50 equilibrium between the folded and unfolded states. Due to the dynamic ensemble nature of this unfolded state under non-denaturing conditions, the structural characterization of SH3 is difficult using ensemble measurements. Experimental data have been used to define sets of heterogeneous conformations but ensemble averaging minimizes the information content [1]. Importantly, little is known about interconversion rates within the unfolded ensemble.
We performed fluorescence correlation spectroscopy (FCS) experiments on the wild-type and on a single-site mutant stabilizing the folded state. Based on this data, we estimated the size (hydrodynamic radii), of the folded (Rf), unfolded (Ru) and denatured (Rd) states. The size of unfolded state measured by FCS is in excellent agreement with the NMR-measured value, Ru/Rf = 1.30 ± 0.01 [1]. Quite surprisingly, our results indicate that the unfolded state under non-denaturing conditions is less compact than the chemically-denatured state, as Rd/Rf = 1.21 ± 0.03. This agrees with previously reported NMR and SAXS data when adequate shape factors for different conformations are considered. Using an environment sensitive dye and fitting the full correlation curve reveals a fast (~200 ns) process within the unfolded ensemble that is absent in the folded state.
Single-molecule FRET experiments were performed to quantify end-to-end distance distributions. We probed directly the conformational heterogeneity of the wild-type protein as well as the response to denaturants and osmolytes.