1343-Pos
Reverse Regulation: Controlling Intrinsically Disordered Domains with Structured Elements
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Intrinsic disorder in proteins correlates with alternatively spliced motifs, protein interaction domains, and post-translational modification sites. Consequently, there are many examples of intrinsically disordered regions sensing multiple cellular signals and responding by modulating the activity of a structured functional domain. Conversely, we have discovered two examples of the reverse process - regulation of an intrinsically disordered domain by a structured protein element - using the *Drosophila* Hox transcription factor Ultrabithorax (Ubx) as a model system. Both *in silico* and *in vitro* approaches identified a large (~150 a.a.) intrinsically disordered domain within the Ubx transcription activation domain, which is bounded on its C-terminus by an alpha helix. In cell culture promoter-reporter assays, point mutations that enhance helix stability increase transcription activation, whereas mutations that destroy helix structure abrogate transcription activation, leaving repression and DNA binding intact. Indeed, two amino acid changes are sufficient to disable a 150 a.a. intrinsically disordered domain. These mutations alter Ubx function in a tissue-dependent manner in *Drosophila*, emphasizing the fact that *in silico* prediction and *in vitro* characterization of intrinsically disordered domains is relevant to the function of the protein in a live animal. In the second example, we monitored DNA binding as a function of osmotic stress to discover DNA binding triggers a conformational change that exposes significant additional surface area in N-terminal half of Ubx, including the intrinsically disordered domain. This conformational change provides an opportunity for DNA, via the structured DNA-binding homeodomain, to impact both transcription regulation and protein interactions by the intrinsically disordered domain. This regulatory mode could potentially select the mode (activation vs. repression) of transcription regulation by Ubx in response to DNA sequence.

1344-Pos
Single-Molecule FRET Reveals Altered Binding-Induced Folding Landscape of PD-Related Mutant Protein Alpha-Synuclein
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The abundantly-expressed and intrinsically disordered protein (IDP) α-synuclein represents an interesting target for biophysical studies of both protein folding and the molecular mechanism(s) of neurodegenerative diseases in humans. Filamentous intracellular inclusion bodies, composed primarily of this protein, are the defining hallmark of Parkinson's disease (PD, the second most common neurodegenerative disorder after Alzheimer's disease) and other related synucleinopathies. Furthermore, three single-amino acid substitutions in α-synuclein have been linked to dominantly inherited familial forms of the disease. Despite much effort and growing interest, much remains to be understood about its normal physiological and disease-related function and the structures associated with them. Like many other IDPs, α-synuclein lacks a well-defined *in vitro* structure, and appears to rely on molecular binding partners to modulate its structure and induce potentially functional folds. Due to the protein's characteristic structural plasticity, structural features that do exist are difficult to observe by conventional techniques that rely on ensemble conformation averaging. To overcome this limitation and begin to understand the complicated folding behavior of this and similar proteins, we employed single-molecule Förster resonance energy transfer (smFRET), which avoids conformation averaging, to probe conformation distributions of a PD-related mutant of α-synuclein as a function of binding-induced folding. These experiments resulted in a detailed binding-induced folding landscape for this α-synuclein mutant. A comparison of this folding landscape with that of the wild-type protein revealed clear conformational consequences of the disease-related mutation and may represent not only a potential clue to the molecular mechanism(s) of PD, but also to the fundamental protein-folding phenomenon of aggregation and amyloid formation.

1345-Pos
α-Synuclein N-Terminus Elicits Vesicle Binding and Folding Nucleation
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α-Synuclein (αS) is a natively unfolded protein predominantly found in pre-synaptic terminals of the central nervous system and implicated in several neurodegenerative disorders, such as Parkinson's disease. The αS monomer may undergo a transition from its disordered solution state into an amphipathic helical conformation upon membrane interaction. Its folding may regulate the fusion of synaptic vesicles with the pre-synaptic nerve terminal. Moreover, the structured monomer may be a necessary intermediate to forming high molecular weight species characteristic of the disease state (1). Here we show the affinity of the αS N-terminus to bind to and fold on highly curved lipid bilayers. Using CD spectroscopy and isothermal titration calorimetry, we investigated lipid-protein interactions of αS N-terminal synthetic peptides and truncated mutants with small unilamellar vesicles having a phase transition near physiological temperature. Moreover, lipid mixtures that undergo phase separation were studied (2). We found the membrane-induced binding and helical folding of the first 25 residues to be highly cooperative. Folding occurs electrostatically or as a result of a change in lipid ordering across the lipid phase transition. Stepwise removal of the first five amino acids of αS by site-specific truncation resulted in a substantial decrease in mean residue ellipiticity. This specifically indicates which N-terminal residues are critical for lipid binding and folding nucleation in the full-length protein. A chemically truncated mutant lacking portions of the N- as well as the C-terminus yielded only a small decrease in binding affinity in comparison to wild-type αS, but led to significant helical destabilization. Our findings highlight the importance of the αS N-terminus in folding nucleation and provide a framework for elucidating lipid-induced conformational transitions in the full-length protein. [1] K. Beyer (2007) Cell Biochem. Biophys. 47, 285-299. [2] T. Bartels et al. (2008) JACS 130, 14521-14532.

1346-Pos
Effects of Vesicle Diameter and Lipid Composition on α-Synuclein Binding
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Parkinson's Disease is characterized by the presence of fibrillar deposits of alpha-Synuclein (αS) in the *substantia nigra*. αS is an intrinsically unstructured protein that becomes α-helical upon binding lipid membranes. Many studies indicate that the toxic form of αS may be pre-fibrillar oligomers formed in solution or upon binding to cell membranes or synaptic vesicles. The effects of curvature and lipid composition on αS binding were studied by using Fluorescence Correlation Spectroscopy (FCS) to quantitatively measure the binding affinity of αS for synthetic lipid vesicles. Vesicles were prepared with different diameters, and lipid compositions included several anionic lipids, fluid phase, and gel phase vesicles. Binding of the wild-type protein was compared to the three pathological mutants: A30P, A53T, and E46K. Our findings indicate that bilayer curvature does affect the affinity of αS for net negatively charged vesicles, while affinity is mostly invariant to the anionic lipid used.

1347-Pos
Vesicle-Bound Alpha-Synuclein: Are the Helices Anti-Parallel or Extended?
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The Parkinson's disease-related protein α-Synuclein (αS) is a 140 residue protein that is natively unfolded in solution. The membrane-binding properties of αS are implied in its physiological or pathologic activity. The protein was investigated by spin-label EPR using the electron-electron-double resonance (DEER) method to measure the distance between pairs of spin labels. For four double mutants of αS, distances were determined in the vesicle-bound and free form. An antiparallel arrangement of the helices 1 and 2 was found, revealing a horseshoe conformation (Drescher et al., JACS 2008). Applying the same method to single mutants, aggregates with an antiparallel arrangement of helix 2 of the partners are found. Mobility analysis of five singly spin-labeled mutants showed that the membrane affinity of helix 2, comprising residues 45 - 90, decreases with decreasing negative charge of the membrane surface, suggesting differential binding of αS to membranes (Drescher et al., CBC 2008). Recently, there is substantial debate about the actual conformation of αS on different membranes (Jao et al., PNAS 2008; Georgieva et al., JACS 2008; Bortuolus et al., JACS 2008). We will discuss this and show further aspects of the interaction of αS with small unilamellar POPG vesicles, highlighting the structure of the bound form of αS under these conditions.

1348-Pos
A Single Molecule Fluorescence Study on the Consequences of Alpha-Synuclein Oxidation
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Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the deposition of fibrillar amyloid inclusions in the substantia nigra region of the brain. Aggregation of alpha-synuclein (aS), an abundant presynaptic protein, is thought to play an essential role in the pathogenesis of PD. Oligomeric intermediates of the aggregation process have been implicated in neuronal cell death, possibly by compromising cell membrane integrity. Oxidative stress appears to