Titin is a giant protein responsible for striated-muscle elasticity. It contains a series of ordered domains and a large disordered segment called the PEVK domain. It acts as an entropic spring and is thought to be responsible for the generation of passive contractile force in muscle. The ordered domains belong to the immunoglobulin (Ig) type C2 and fibronectin (FN) type III superfamilies. We expressed a 171-residue-long fragment of the PEVK domain (polyE) and an Ig domain (I27) in BL21 derivative E.coli Rosetta competent strains. FTIR spectroscopy combined with a diamond anvil cell was used as a non-perturbing method for investigating the secondary structures of these recombinant proteins. Fluorescence spectra of I27 were also recorded.
PolyE preserves its disordered characteristics across a wide range of pressure (0-16 kbar), temperature (0-100 °C), pD (3-10.5) and in presence of several cosolvents. Upon pressure treatment, titin I27 unfolds at 10.7 kbar at 30 °C. As the function of temperature we observed two transitions. At 50 °C the secondary structure is loosened, and the protein transforms into a molten-globule state. At 70 °C the protein completely unfolds. Unfolding is followed by aggregation at ambient pressure. Moderate pressures (>2 kbar), however, can prevent the protein from aggregation. We determined the detailed temperature-pressure phase diagram of titin I27, which contains metastable regions as well.

3211-Pos Board B72
Kinetic Enhancement of NF-KB/DNA Dissociation by IkBalpha
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The nuclear factor kappa B (NF-kB) family of transcription factors is involved in inter- and intracellular signaling, cellular stress response, growth, survival, and apoptosis. Specific inhibitors of NF-kB transcription including IkBα, IkBβ, and IkBε, block the transcriptional activity of p65 and c-Rel-containing NF-kB dimers. DNA binding by NF-kB is inhibited by the ankyrin repeat protein kappa B (IkBα), which sequesters NF-kB to the cytosol. The mechanism and kinetics of DNA binding inhibition by IkBα are still unknown, but we recently demonstrated that IkBα enhances the dissociation of NF-kB from DNA transcription sites. We are investigating the effect of IkBα on the association and dissociation rates of the NF-kB/DNA complex using titration measurements, stopped- flow fluorescence and Isothermal Titration Calorimetry (ITC). We are using pyrene labeled DNA, and IkBα Tryptophan fluorescence to study the fluorescence changes occurring during the enhanced dissociation process. Our results show that IkBα increases the dissociation rate of the DNA from the NF-kB complex in a concentration-dependent manner and with high efficiency. We repeated the experiments using a different mutant of IkBα, C186P/A220P (CPAP). We studied also the formation and dissociation of a forward- and a backwards-ternary complex between, IkBα-NFkB-DNA using pyrene labeled DNA, and IkBα Tryptophan fluorescence. The rates of association and dissociation of DNA, IkBα and CPAP to form the ternary complexes were also compared to interpret the kinetics of the enhanced dissociation process.

3212-Pos Board B73
Functional Regulation of the Anti-Apoptotic Protein BCL-xL through Post-Translational Modification of its Intrinsically Disordered Loop
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Several studies reported functional down-regulation of the anti-apoptotic protein BCL-xL as a consequence of phosphorylation or deamidation of amino acids within its large intrinsically disordered loop. We meant to elucidate these poorly understood mechanisms of apoptotic regulation, and at the same time develop a case study of functional interplay between folded and disordered segments within the same protein. We present here preliminary results towards a structural and mechanistic understanding of these phenomena. Our studies suggest that the post translational modification of its intrinsically disordered loop may trigger conformational rearrangements in the folded core of BCL-xL that decrease its affinity for BH3-only protein partners.

3213-Pos Board B74
Ultrabithorax, an Intrinsically Disordered Protein, Selects Protein Interactions by Topology
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Interaction between two structured proteins requires both complementary topologies to generate a sufficient interface and surface groups capable of forming bonds within this interface to stabilize the complex. When one (or both) partners is intrinsically disordered as a monomer, but folds upon interaction, the same rules for partner selection - complementary topology and surface chemistry - are expected to apply. However, many proteins do not fold even when forming stable protein interactions, creating ''fuzzy'' protein complexes. In these cases, the extreme instability of one partner may preclude forming a well-defined interface. Despite the apparent lack of constraints, these proteins specifically and reliably select the correct protein partners *in vivo*. To understand the rules that determine partner selection when forming fuzzy complexes, we have evaluated protein interactions formed by the *Drosophila melanogaster* Hox transcription factor Ultrabithorax (Ubx). Ubx interacts *in vitro* with 29 other proteins. All of these interactions require the intrinsically disordered regions within Ubx. Surprisingly, despite the extreme lack of structure within these regions, Ubx appears to select protein interactions by topology: 22 of the 29 partners include one of five protein folds out of the nearly 1200 folds listed in SCOP. These data suggest that topology remains a constraint even in fuzzy complexes. Although some Ubx partners bind equally well to both large intrinsically disordered regions within Ubx, many partners clearly prefer binding to the disordered alternatively spliced microexons. Partners preferring the microexon region bind various Ubx splicing isoforms differentially. Consequently, surface chemistry is likely important for these interactions. Together, our data suggests that both topology and surface chemistry are key criteria for partner selection, even in fuzzy complexes.

3214-Pos Board B75
The Nanomechanics of Neurotoxic Proteins Reveals Common Features at the Start of the Neurodegeneration Cascade
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Amyloidogenic neurodegenerative diseases are incurable conditions caused by specific largely disordered proteins. However, the underlying molecular mechanism remains elusive. A favored hypothesis postulates that a critical conformational change in the monomer (an ideal therapeutic target) in these ''neurotoxic proteins'' triggers the pathogenic cascade. Using force spectroscopy with unequivocal single-molecule identification we demonstrate a rich conformational polymorphism at their monomer level. This polymorphism strongly correlates with amyloidogenesis and neurotoxicity: it is absent in a fibrillization-incompetent mutant, favored by familial-disease mutations and diminished by a surprisingly promiscuous inhibitor of the monomeric β-conformational change and neurodegeneration. The demonstrated ability to inhibit the conformational heterogeneity of these proteins by a single pharmacological agent reveals common features in the monomer and suggests a common pathway to diagnose, prevent, halt or reverse multiple neurodegenerative diseases.

3215-Pos Board B76
High Resolution Characterization of Tertiary Contacts in Intrinsically Disordered Amyloidogenic States of α-Synuclein Provides New Scaffolds for Structure-Aided Drug Discovery
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The realization that transient population of partially unfolded conformations precedes the toxic aggregation of several amyloidogenic proteins has raised major interest in the design of compounds that could prevent protein misfolding. Long-range tertiary contacts offer a unique opportunity for the implementation of structure-based drug discovery strategies to find inhibitors of pathological protein aggregation. Representation of such transient contacts has, however, traditionally invoked the generation of low resolution and highly heterogeneous ensembles of structures that are impractical for in silico use. Here we show that it is possible to determine a single structural fold that describes at high resolution all tertiary contacts transiently established by the intrinsically disordered protein (IDP) α-synuclein under low and high amyloidogenic conditions. To generate the models we use paramagnetic relaxation enhancement (PRE) data as it directly probes transiently formed tertiary contacts, while being insensitive to other ensemble descriptors, such as size distribution, which are of little interest in docking studies. In our calculation strategy we refuse to comprehensively describe the conformational ensemble of the IDP (i.e. fulfilling average size and size distributions)