representative conformations, and inter-residue interactions amongst these peptides are described here. These characterizations help illustrate the conformational landscapes of Aß monomers at atomic resolution and provide insight into the early stages of Aß aggregation pathways.

3206-Pos Board B67

Effect of a Single-Point Mutation on the Conformation and Dynamics of Islet Amyloid Polypeptide from Nanosecond-Resolved Intramolecular Contact Formation

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Islet amyloid polypeptide (IAPP) is an intrinsically disordered protein involved in regulating glucose metabolism and gastric emptying. It plays a crucial role in beta cell failure in diabetes type II, where it accumulates in the form of amyloid fibers. Human IAPP (hIAPP) is arguably the most amyloidogenic naturally occurring peptide known so far. Recent work by Raleigh and coworkers shows that a single-point mutation (I26P) converts it into an effective in vitro amyloid inhibitor[1,2]. The I26P mutation has been proposed to inhibit fibril formation by affecting the ability of the disordered C terminal tail to rearrange into the amyloid fibril structure, while maintaining intact the conformational properties of the N-terminal region, putatively responsible for the formation of aggregate intermediates[1,2]. Though such mechanism has been proposed, the conformational and dynamical properties of the I26P monomer have not been experimentally determined yet.

We use tryptophan triplet quenching by cystine to measure the rate of contact formation between the two ends of the I26P peptide. This technique has previously revealed conformational differences between rIAPP and hIAPP[3]. It allows detecting both changes in the equilibrium end-to-end distance distribution and in the diffusional dynamics of the end-to-end distance caused by fast reconfigurations of backbone dihedral angles. We compare results for I26P, hIAPP, rIAPP, and model worm like chain peptides of same length. We discuss the effect of proline substitutions on the conformation and dynamics of these intrinsically disordered proteins and their possible effect on amyloid aggregation.

Footnotes
[1] Abedini A. et al. JACS 129 2007
[2] Meng F. et al. JACS 132 2010
[3] Vaiana SM et al. BiophysJ 97 2009

3207-Pos Board B68

Intrinsically Disordered Regions as Affinity Tuners in Protein-DNA Interactions

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Intrinsically disordered regions, terminal tails, and flexible linkers, are abundant in DNA-binding proteins and play a crucial role by increasing the affinity and specificity of DNA binding. Disordered tails often undergo a disorder-to-order transition during interactions with DNA and improve both the kinetics and thermodynamics of specific DNA binding. The DNA search by proteins that interact nonspecifically with DNA can be supported by disordered tails as well. The disordered tail may increase the overall protein-DNA interface and thus increase the affinity of the protein to the DNA and its sliding propensity while slowing linear diffusion. The exact effect of the disordered tails on sliding rate depends on the degree of positive charge clustering, as has been shown for homeodomains and p53 transcription factors. The disordered tails, which may be viewed as DNA recognizing subdomains, can facilitate intersegment transfer events that occur via a ''monkey bar'' mechanism in which the domains bridge two different DNA fragments simultaneously. The monkey-bar mechanism can be facilitated by internal disordered linkers in multidomain proteins that mediate the cross-talks between the constituent domains and especially their brachiation dynamics and thus their overall capability to search DNA efficiently. The residue sequence of the disordered tails has unique characteristics that were evolutionarily selected to achieve the optimized function that is unique to each protein. Perturbation of the electrostatic characteristics of the disordered tails by post-translational modifications, such as acetylation and phosphorylation, may affect protein affinity to DNA and therefore can serve to regulate DNA recognition. Modifying the disordered protein tails or the flexibility of the inter-domain linkers of multidomain proteins may affect the cross-talk between the constituent domains so as to facilitate the search kinetics of non-specific DNA sequences and increase affinity to the specific sequences.

3208-Pos Board B69

Atomistic MD Simulations Reveal the Protective Role of Cholesterol in the Membrane Disruptive Effects of Dimeric Beta-Amyloid in Neuronal Membrane Mimics

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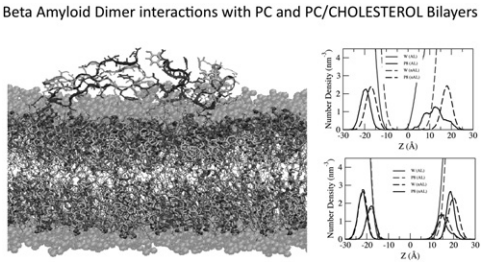
Detailed interactions of oligomeric beta-amyloid with neurons have been associated with the pathogenesis of Alzheimer's disease. The molecular interactions of different lipid components, particularly cholesterol (CHOL), of the membranes with the peptides are not clear. Using atomistic MD simulations, the water permeability barrier, surface area, density profile and order parameters of binary phosphatidylcholine (PC) and PC/CHOL lipid bilayers were examined from various 200 ns-simulation replicates. Our results suggest that the longer chain-length dimer (2 x 42 residues) perturbs the membrane more than the shorter one (2 x 40 residues). In addition, we discovered a significant protective role of cholesterol in the protein-induced disruptions of the membranes. The use of a new Monte-Carlo method in characterizing the structures of the conformal annular lipids in close proximity to the proteins will be presented. We propose that the neurotoxicity of beta-amyloid peptide may be associated with the raft-like nanodomains of the neuronal membranes during the early development of Alzheimer's.



3209-Pos Board B70

Conformational Sampling of FG-Nucleoporins using Extended Molecular Dynamics Simulations

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FG nucleoporins (FG-nups) are intrinsically-disordered proteins that fill the central channel of the Nuclear Pore Complex (NPC) and are believed to mediate the selective gating of cargo molecules through the NPC. Previous molecular dynamics (MD) simulations of FG-nup fragments approximately 100 amino acids (AAs) in length have shown that different FG-nups adopt different average shapes, ranging from compact premolten globules to extended coils, which are the basis for a new model for NPC gating. For MD studies of disordered proteins there are still open questions about the appropriate simulation times and protocols needed to sufficiently search conformation space, and how different force fields and ionic conditions affect the resulting structural ensembles. For this reason we have performed MD simulations on smaller, 25 and 50 AA fragments of key FG-nups for microsecond timescales, using both implicit and explicit solvent, as well as different ionic conditions. Our goal is to see if their dynamical properties exhibit previously-documented behavior for the larger fragments and to evaluate the thoroughness of conformational sampling for the FG-nups. We will represent the results of multiple replicate simulations on different FG- nups, analyzed using a variety of methods that range from conventional measures of protein size and structure, to new tools based on contact maps, clustering, and dimensionality- reduction techniques.

3210-Pos Board B71

Different Behaviors of the Structured and Unstructured Regions of Titin under Pressure

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Contrary to the classical view, according to which all proteins adopt a specific folded conformation necessary for their function, intrinsically unstructured proteins (IUPs) display random-coil-like conformation under physiological conditions, although they have specific biological function. Upon ligand binding, however, many of them may adopt a well-defined three-dimensional structure.

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)