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Members of the Interferon Regulatory Factor (IRF) family of proteins play important roles in development of the immune system, host defense, inflammation and apoptosis. Activation of these proteins in the cytoplasm is triggered by phosphorylation of Ser/Thr residues in a C-terminal autoinhibitory region. Phosphylation stimulates dimerization, transport into the nucleus and assembly with the coactivator CBP/p300 to activate transcription of type I interferons and other target genes. We have determined the 2.0Å resolution crystal structure of a dimeric form of the IRF-5 transactivation domain (residues 222-467) in which Ser 430 has been mutated to the phosphomimetic Asp. The structure reveals a striking mechanism of dimerization in which the C-terminal autoinhibitory domain attains a highly extended conformation permitting extensive contacts to a second subunit. Mutational analysis of dimeric interface residues strongly supports the observed dimer as representing the activated states of IRF-5 and IRF-3. Based on comparison with crystal structures of IRF-3, these results provide a structural basis for the coupling between dimerization and CBP/p300 binding in IRF family members, in which the C-terminal autoinhibitory domain plays a dual role. In the unphosphorylated form, the C-terminal autoinhibitory domain binds to and masks the hydrophobic CBP/p300 binding surface. Phosphorylation stimulates the unfolding of the C-terminal autoinhibitory domain which then forms extensive contacts with a second IRF-5 subunit, leaving a hydrophobic surface free for binding CBP/p300.

3021-Pos Board B68
Altering the Process of Aß-Plaque Formation: Effect of Monoclonal Antibodies
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Amyloid diseases are a steadily expanding group of debilitating human disorders, including Alzheimer's disease and Parkinson's disease, which are characterized by deposits of insoluble protein fibrils in various tissues. High-visibility studies have found that amyloid mature fibrils are one of the main pathogenic agents in Alzheimer's, resulting in the deposition of extracellular Amyloid beta (Aß) plaques. Hence, determining the kinetics of amyloid fibril formation, characterizing the morphology of intermediate aggregates and relating them to underlying changes in protein structure are essential for their prevention and removal. Equally important, experimental techniques to provide in-situ characterization of amyloid-ß aggregation, aggregate structures and associated changes in protein structure are critical for testing drug targets for their ability to disrupt fibril formation. We have used atomic force microscopy (AFM), transmission electron microscopy (TEM), and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy to monitor the time course of changes in topology and conformation of the peptide aggregates. These measurements allow us to detect changes in intra and intermolecular beta sheets, beta turns, and alpha helix conformational rearrangements and relate them to topography conformations. We have tested the effects of different monoclonal antibodies (anti-Aß mAbs), both N-terminus and C-terminus, on preformed fibrils. We found that for molar ratios of 10:1 to 50:1 (amyloid:antibody), the dissolution process proceeds to completion within 144 hours. We determined that lower stoichiometric molar ratios of antibodies (1000:1) in preincubated solutions of Aß peptides also promoted defibrillization, but the time to achieve complete removal is more than 6 days. The outcomes of this study provide an in-vitro quantitative model to understand the potentially catalytic capacity of anti-Aß mAbs to monomerize assemblies of Aß and instruct the design and interpretation of ongoing clinical trials of these therapeutics in Alzheimer' disease patients.

Protein Folding & Stability III

3022-Pos Board B69
Equilibrium Thermodynamics of Urea Denaturation of Trp-cage Miniprotein
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Urea is a denaturant commonly used in protein folding studies. Simulation studies of the effect of urea on protein stability have concentrated on how urea unfolds proteins - not on how urea affects the folding/unfolding equilibrium. Here we report the first simulation studies of the reversible folding and unfolding equilibrium of a protein - the Trp-Cage miniprotein. Replica exchange MD was performed in all atomic detail, starting from an unfolded (extended) configuration in three different solvent conditions viz. 2M, 4M and 6M in Urea. The Kirkwood-Buff model for Urea was employed. Fifty replicas of the system at each concentration were simulated for 150 ns per replica per urea concentration (22.5 microseconds total simulation time), enabling us to obtain folding-unfolding equilibrium data in the temperature range of 283 K to 579 K. In addition, we have performed REMD simulations in 0 M urea i.e pure water (4 microseconds total simulation time). During these simulations we observe all replicas to fold and unfold multiple times. The equilibrium properties, as a function of T and [Urea], show a clear shift in equilibrium towards the unfolded state with increasing urea concentration. Details of the solvent structure around the protein backbone and side chains will be presented.
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3023-Pos Board B70
Structural Consequences of the Ionization of Internal Lys Residues in a Protein
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Internal ionizable residues in proteins play important functional roles in a variety of biological processes. The molecular determinants of their pKa values are poorly understood. Previously we measured the pKa of Lys, Asp, Glu and Arg at 25 internal groups in staphylococcal nuclease. 98 of these 100 variants are fully folded and native-like at pH 7. The pKa values of the majority of these groups are perturbed, some by as much as 5 pKa units, all in the direction that favors the neutral state. NMR spectroscopy was used to examine the structural and dynamic consequences of ionization of the internal lysine residues. In 9 of 10 crystal structures of Lys-containing variants the Lys side chain is completely buried, some in entirely hydrophobic microenvironments. In some cases the buried amino group makes contact with polar residues. The NMR experiments showed that in two variants the ionization of an internal Lys causes global unfolding. In five variants the ionization of the internal Lys triggers local structural changes and increased dynamics. The presence of conformational exchange in response to ionization appears to be correlated with the global stability of the variant proteins. Surprisingly, in the majority of cases, the changes in structure coupled to the ionization of the internal Lys residues are modest. These data demonstrate that proteins can tolerate internal ionizable residues, even those that exhibit large shifts in pKa values, and even in their charged states. The internal charged groups somehow manage to become solvated without disrupting the overall fold of the protein.

3024-Pos Board B71
Investigating The Mechanical Stability Of Sap-1 Transcription Factor By Single Molecule Force Spectroscopy
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Transcription factors play an essential role in biological systems, binding specifically to their target DNA sequences to regulate gene expression. Such a binding process must occur on a fast timescale while ensuring a high level of specificity. In order to meet such stringent requirements, DNA-binding proteins search for their specific DNA sequences by first diffusing to nonspecific DNA sites and then sliding to their target sites, thereby speeding up the overall exploration process. In order to facilitate the diffusive process, the ''fly-casting mechanism'' proposes that DNA-binding proteins such as SAP-1 are partially unstructured in the unbound state, while exhibiting a correct fold when bound to DNA. To learn more about the structural architecture of SAP-1 and to test if it is mechanically stable in the absence of DNA, we engineered polyproteins which combine the I27 module with the ETS-domain of the SAP-1 in a four tandem repeat, $(I27SAP\text{-}1)_4$. Since the mechanical properties of I27 are well-characterized, we can unambiguously fingerprint the mechanical stability of SAP-1. Here we show that pulling the engineered polyproteins at constant speed by atomic force microscope (AFM) results in saw-tooth unfolding patterns. We observe that SAP-1 unfolds at a force of 50 ± 26 pN, indicating that SAP-1 is mechanically stable even in its unbound state. The unfolding of each individual SAP-1 module increases the protein length by $\Delta L_C = 26 \pm 3$ nm, releasing ~65 amino acids hidden behind the unfolding transition state. We suggest that mechanical unfolding occurs upon shearing hydrogen bonds involving the β2-sheet. Remarkably, the distribution of contour length increments is broader than that found for other mechanically stable proteins, demonstrating the folded state of SAP-1 is surprisingly flexible in the absence of DNA.

3025-Pos Board B72
Identification Of Amino Acids That Are Critical For Structural Stability And Functionality Within The Negative Regulatory Region (NRR) Of Notch Proteins
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