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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.

This work was supported by the NSF MCB-0543769.

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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The integral role that Notch proteins play in the normal development and tissue homeostasis of metazoan animals is orchestrated through the tightly regulated proteolytic processing of its Negative Regulatory Region (NRR). The NRR is composed of three linear notch repeats (LNR) followed by a heterodimerization (HD) domain that is N-terminal to the transmembrane region. Proteolytic Notch processing begins during transport to the cell surface with the cleavage of the HD domain at site S1 by a furin-like protease so as to produce a non-covalent heterodimer of one extracellular (NEC) and one transmembrane (NTM) subunit. Ligand binding at NEC initiates Notch activation by facilitating ADAM-type-metalloprotease-dependent extracellular cleavage at an NTM site (S2) that enables a subsequent intramembrane γ-secretase-mediated cleavage at site S3. These cleavages permit the translocation of the intracellular domain of Notch from the cell membrane to the nucleus to activate transcription. In this work we present the algorithms we have developed to evaluate the relative importance of specific amino acids for the structural stability and functionality of the NRR. These include predictive models derived from bioinformatic analysis such as phylogenetic inference and computational protein modeling as well as experimental data from multiple biophysical and biochemical techniques such as differential scanning calorimetry (DSC), circular dichroism (CD), and pull-down assays. Our results provide valuable insight to the mechanism of action of Notch.

3026-Pos Board B73

Macromolecular Crowding Affects Stability Properties: A Comparison Study For Titin And Ubiquitin

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Macromolecules can occupy a large fraction of the volume of the cell and this affects many properties of the proteins inside the cell, such as thermal stability and rates of folding. We present a comparison of the effects of molecular crowding in ubiquitin and titin. We have used an atomic force microscope based single molecule method to measure the effects of macromolecular crowding on the mechanical stability of these proteins. We used dextran as the crowding agent with two different molecular weights, with concentrations varying from zero to 300 grams per liter in the buffer solution. The results show that the forces that are required to unfold molecules are enhanced when high concentration of dextran molecules is added to the buffer solution.

3027-Pos Board B74

Properties Of Contact Matrices Induced By Pairwise Interactions In Proteins

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The total conformational energy is assumed to consist of pairwise interaction energies between atoms or residues, each of which is expressed as a product of a conformation-dependent function (an element of a contact matrix, C matrix) and a sequence-dependent energy parameter (an element of a contact energy matrix, E matrix). Such pairwise interactions in proteins force native C matrices to be in a relationship as if the interactions are a Go-like potential [N. Go, Annu. Rev. Biophys. Bioeng. **12**:183, 1983] for the native C matrix, because the lowest bound of the total energy function is equal to the total energy of the native conformation interacting in a Go-like pairwise potential. This relationship between C and E matrices corresponds to (a) a parallel relationship between the eigenvectors of the C and E matrices and a linear relationship between their eigenvalues, and (b) a parallel relationship between a contact number vector and the principal eigenvectors of the C and E matrices; the E matrix is expanded in a series of eigenspaces with an additional constant term, which corresponds to a threshold of contact energy that approximately separates native contacts from non-native ones. These relationships are confirmed in 182 representatives from each family of the SCOP database by examining inner products between the principal eigenvector of the C matrix, that of the E matrix evaluated with a statistical contact potential, and a contact number vector. In addition, the spectral representation of C and E matrices reveals that pairwise residue-residue interactions, which depends only on the types of interacting amino acids but not on other residues in a protein, are insufficient and other interactions including residue connectivities and steric hindrance are needed to make native structures unique lowest-energy conformations. Reference: Phys.Rev.E, **77:**051910, 2008.

3028-Pos Board B75

The Charge on Beta-Lactoglobulin A and B under Self-associating Conditions

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From electrophoretic and hydrodynamic measurements, the charge on monomeric proteins may be calculated accurately. It is not clear, though, how to analyze data from a self associating system. β-Lactoglobulin (β-LG) is a major whey protein abundant in cow milk. It has several genetic variants, is stable and self-associates under well characterized conditions. These factors make β-LG a good model to be used in the study of net charge and self-association. Both β-LG A and β-LG B were analyzed at pH 3, in a range of salt concentrations and protein concentrations. Under these conditions, self association is favored by higher salt concentrations. The mobility of β-LG was determined using capillary electrophoresis, with the Stokes radius determined using analytical ultracentrifugation. Self-association of β-LG A was characterized using sedimentation equilibrium. For both β-LG A and β-LG B, as ionic strength increased the mobility of the faster-moving peak decreased. With increased concentrations of β-LG A and β-LG B, the apparent mobility of this peak stayed the same. However, the boundary shapes observed in capillary electrophoresis were consistent with β-LG undergoing a mass-action association. It is difficult to interpret the capillary electrophoresis data quantitatively. Accordingly, membrane confined electrophoresis experiments are being used to determine the charge change on association.

3029-Pos Board B76

Volume changes in protein folding: A Modular Approach

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Pressure effects of protein conformational transitions, which are manifest via the associated changes in molar volume, remain rather poorly understood, and regrettably so given that they are clearly linked to changes in the magnitude and type of hydration that accompany such transitions. Given that the role of solvent in protein energetics and structural dynamics remains one of the key questions in the field of protein folding, a better understanding of the physical basis for the volume changes that accompany folding reactions could provide direct means to quantify this differential solvation. We have recently begun high pressure studies on the folding of the ankyrin repeat domain of the protein Notch bearing 7 ankyrin repeats (Nank7) and a series of smaller constructs differing is the number of repeats, and/or their sequences. We reasoned that such a system would provide a means of incrementally testing the role of the size of the hydrophobic core, and the importance of the specific amino acid sequence in determining the volume change upon unfolding, ΔVu. We present here a complete P-T equilibrium and p-jump kinetic study of the full-length Nank7 construct, as well as on a number of the smaller constructs. We find that the volume change of the full-length construct depends strongly upon temperature, and that the expansivity of the transition state ensemble is similar to that of the unfolded state, while its molar volume is closer to that of the folded state. Preliminary results on two very small constructs indicate that the total volume change is a linear combination of the volume change associated with the unfolding of each individual repeat.

3030-Pos Board B77

Effect of Macromolecular Crowding on Protein Folding Stability

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The interior of a cell contains ~300 g/l macromolecules. In order to understand the biophysical properties of proteins under in vivo conditions, it is necessary to consider the crowding effects of bystander macromolecules [1]. Statistical-thermodynamic models show that crowding increases the chemical potentials of both the folded and the unfolded states of a protein and predict a modest increase in folding stability [2], which has been experimentally confirmed in our group [3]. Here we report additional experimental results for crowding effects on FKBP12 mutant. At a fixed concentration measured in weight per volume, we observed an optimal dextran size at which stability increase is maximal, just as predicted previously [4]. In addition, we found that the stabilizing effect of a mixture of dextran 6K and Ficoll 70 is greater than the sum of the two individual crowding agents. These findings will have profound implications for understanding macromolecular crowding inside cells.

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