this study, we investigated the effect of TFP on CaM/Fas binding with molecular dynamics simulations. Conformation and binding free energy analyses were performed to examine the connections between the conformational changes of CaM by TFP and CaM/Fas binding affinity. Conformational characteristics of Fas by TFP were also examined for the further determining TFP effects on Fas recruiting FADD to form DISC. Binding free energy analyses showed that CaM antagonist, TFP inhibited CaM binding to Fas. The results are consistent with experimental results. The further conformational analyses showed that TFP significantly changed the CaM conformation, resulted in the increased Fas conformational fluctuations and the degree of correlation between motions of the residues in Fas, which provides structural insight for Fas further binding to FADD for DISC formation. Understanding the molecular mechanisms of CaM antagonist TFP in CaM/Fas binding for Fas-mediated DISC formation should provide important insight into the function of CaM antagonists in regulating Fas-mediated apoptosis.

Keywords: CaM antagonist TFP; CaM/Fas binding; DISC; binding free energy, conformational analysis

3015-Pos Board B62

Investigation Of A 6-fluorotryptophan Substituted scFv

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For many years our laboratory has pursued an understanding of the protein characteristics which confer specificity and affinity to the antibody for its antigen using a family of monoclonal antibodies to hen egg white lysozyme (HyHEL26, 10, 8 and 63, primarily.) We find that the binding is best characterized by a two-step model representing an association complex becoming a docked complex, evidencing a conformational change.

In a recently produced scFv variant of HyHEL10 in which all the tryptophans were substituted with the 6-fluoro form we studied kinetic behavior by Biacore SPR, using our usual protocol to obtain kinetic characterization. We observed that the affinity to lysozyme was concentration dependant, though it did not reflect oligomerization; it changes gradually, allowing investigation, decreasing by an order of magnitude over a period of 3 hours and that most of the change is due to the decrease in the docking step. This repeatable behavior is reversed upon sample reconcentration and delayed by cold. To explore the possible role of folding or water movement we investigated the impact of TMAO, glycerol and some detergents. We also did further exploration by SPR, fluorescence spectroscopy, and other biophysical characterizations in order to better understand the molecular events responsible for this dramatic affinity change.

3016-Pos Board B63

Unique Assembly Structure Of Human Haptoglobin Phenotypes 1-1, 2-1, And 2-2 And A Predominant *Hp1* Allele Hypothesis

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Human plasma haptoglobin (Hp) is classified as three phenotypes, Hp 1-1, 2-1 and 2-2. They are attributed by *Hp 1* and *Hp 2* alleles with each producing a $\alpha 1\beta$ and $\alpha 2\beta$ polypeptide chain, respectively. Owing to the various content of -SH groups in each peptide, the heterogeneous and polymeric structural arrangement via the disulfide linkages is totally different among the phenotypes. The resulting molecular size of Hp 2-2 $[(\alpha 2\beta)_n]$ and 2-1 $[(\alpha 1\beta)_2\ (\alpha 2\beta)_n]$ is dramatically larger than that of 1-1 $[(\alpha 1\beta)_2]$. In the present study, we observed that there were as many as 20 repeated units in Hp 2-2 as compared that only 10 repeats in Hp 2-1. We had reported that the concentration of Hp 1-1 is significantly and differentially higher than that of Hp 2-1 and 2-2 in normal human subjects. Based on our experimental and theoretical data, we hypothesized that the gene activity of *Hp 1* is much more predominant than *Hp 2* that is responsible for these differential concentrations as well as the unique assembly of Hp 2-1. Understanding the molecular arrangement in Hp polymers may provide insight into the underlying mechanism by which Hp phenotype is correlated with the development of inflammation-related diseases.

3017-Pos Board B64

Monitoring and Discerning the Conformational Change of the Most Common Peptide Related to Neuritic Plaques in Alzheimer's Disease

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Alzheimer's disease (AD) is a serious degenerative disease affecting millions of elderly individuals worldwide. Some of the most common symptoms include: loss of memory, cognitive function, and motile coordination, as well as social behavior alteration. The onset causes are not yet clear, however, three important hallmarks of this disease are known: amyloid beta peptide plaques formation (Aß, primarily Aß1-40 and Aß1-42), presence of neurofibrillary tangles, and finally neuronal death. Our work is oriented towards understanding the mechanism of plaque formation and more recently the clearance of these plaques. In this work, we studied and monitored the different aggregation pathways followed by Aß1-40, Aß1-42, and their mixture (1:1). Atomic force microscopy is used as the main analytical tool, served to monitor and study the topological changes suffered by each case studied. It was observed that the mixture of these peptides aggregated at a faster rate forming dense plaques, this observation was confirmed with Transmission Electron Microscope (TEM). The understanding of the trend in aggregation patterns is an important contribution to the comprehension of our ongoing project: targeting Amyloid beta plaques using an immunotherapeutic approach for the prevention and treatment of Aß plaques in the brain.

3018-Pos Board B65

Structural Studies Of Recombinant And Natural Spider Silk Proteins Studied By Nuclear Magnetic Resonance; Insights For The Spinning Process

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Spider silk is a biomaterial with astonishing properties that compete with the best synthetic man made materials such as Kevlar. For example, the dragline fiber is as strong as steel and the total energy to break is 6 times higher than Kevlar. These mechanical properties confer to the spider silk several potential medical and military applications such as bullet-proof vests, stitches, ligaments and tendons from tissue engineering. Nexia biotechnologies Inc. were able to make fibers from recombinant proteins but without achieving the same mechanical properties as the natural spider dragline.

The secondary structure that the two proteins adopt is known to be very important for the mechanical properties of silk. So our work is to study the structure-function relationship of the proteins by solution and solid-state nuclear magnetic resonance (NMR) spectroscopy and dynamic light scattering (DLS). One of the goals of our research project is therefore to study the proteins in solution, at the beginning of the spinning process and at the fiber state and to understand the conditions in which the structural transition is done. More specifically, we are investigating the structure of the two proteins, the aggregation processes and the level of compaction as a function of temperature, pH and salt concentration by solution NMR spectroscopy and DLS. In the solid-state, we are investigating the gland content *in situ* under MAS to compare between the recombinant, the natural and the *in situ* behavior. The comparison of the results gives insights on the role of the physicochemical modifications in the spiders' natural spinning process and supports the idea of using recombinant spider silk proteins as the source of raw material for industrial production of spider silk.

3019-Pos Board B66

Segmented Transition Pathway Of The Receiver Domain Of Nitrogen Regulatory Protein C

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The receiver domain of nitrogen regulatory protein C (NtrC) has two distinct conformations. The largest differences between the two conformations occur in the alpha4-helix. In addition to rigid body translocation and rotation, the alpha4-helix gains half a turn at one end and loses half a turn at the other end when the protein transforms from one conformation to another. The transition pathway between the two conformations is explored by the targeted molecular dynamics (TMD) algorithm in explicit solvent. It is segmented with four consecutive and distinct transition stages. Each transition stage has its own characteristic motion. We propose the reaction coordinates for each transition stage. By projecting the quasi-harmonic principal components along the first stage of the transition, we show that the dynamics of the nano-second time scale overlaps well with the beginning segment of the whole transition. The TMD pathway suggests that several transient hydrogen bonds help stabilize the intermediate structure and facilitate the transition.

3020-Pos Board B67

Activation Of Interferon Regulatory Factors Revealed By The Crystal Structure Of Dimeric IRF-5

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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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