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

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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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