the presentation. Conformational motion theory is believed to be found in group theory.

3009-Pos Board B56

Extension of the Tryptophan Dihedral Angle - W3 Band Frequency Relationship to a Full Rotation

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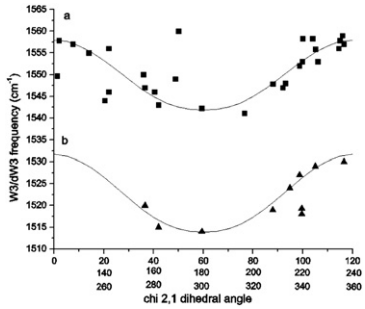
The correlation of the UVRR νW3 mode with the tryptophan $\chi^{2,1}$ dihedral angle (T. Miura et al. 1989, T. Maruyama & H. Takeuchi 1995, H. Takeuchi 2003) has been extended to a full, 360° rotation. The three-fold periodicity of the relationship (cos $3\chi^{2,1}$) over 360° results in up to six dihedral angles for a given νW3. Consideration of a circular plot of dihedral angles for proteinaceous tryptophans taken from the Protein Data Bank along with a Newman projection shows that steric hindrance limits the range of preferred dihedral angles, and reduces the possible $\chi^{2,1}$ to one or two, reasserting the general utility of the νW3 - $\chi^{2,1}$ relationship. However, not all proteinaceous tryptophans follow the relationship. DFT based calculations suggest that the discrepancies observed for the PGA-ligated mutant enzyme, *S. cerevisiae* TIM Trp90Tyr Trp157Phe, are due to electrostatic interaction between the indole ring of Trp-168 and the Glu-129 carboxyl.



3010-Pos Board B57

The Residue Network Architecture of a Protein-Protein Complex Reveals the Linkage between Dynamics and Energetics

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Among the toxins secreted by *Bacillus anthracis* the edema factor EF, an adenylate cyclase, provokes severe cellular dysfunction by accumulating cAMP from ATP. EF is activated by calmodulin (CaM), involved in many calcium signaling pathways. The stability of the EF-CaM complex depends on the level of calcium bound to CaM while the architecture of the complex loaded with 2, 3 or 4 Ca^{2+} ions remains practically unchanged. That is why modeling the electrostatic effect of Calcium through EF-CaM structure is challenging.

Here, we aim at describing the calcium-induced changes in EF-CaM dynamics and energetics through a consensual view of its residue network organization. The analysis of molecular dynamics (MD) simulations of EF-CaM with 0, 2 and 4 Ca^{2+} ions helped characterize CaM conformational plasticity and led to a model of the EF-CaM interaction, in which CaM acts as a spring that maintains EF in an open active conformation (Laine et al., 2008).

The computation of various dynamical covariances and energetic dependency maps from the MD trajectories further raised the concept of residue network connectedness. This connectedness quality provides a frame for unifying the dynamics and energetics of the complex and a criterion for assessing its stability (Laine et al., under revision).

Laine E., JD. Yoneda, A. Blondel and TE. Malliavin (2008). *Proteins*. 71: 1813-29.
Laine E., A. Blondel and TE. Malliavin. *Biophys. J. (under revision)*

3011-Pos Board B58

Picosecond Dynamics Evolution During Function For Photoactive Yellow Protein

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Picosecond protein dynamics refer to both diffusive motion at the protein surface and adjacent solvent as well as possible underdamped structural vibrational modes. Functional protein structure changes result in possible changes in both these diffusive and collective dynamics which can lead to either an increase or decrease in flexibility in the active state. In the case of photoactive yellow protein (PYP), a large conformational change occurs as one proceeds from the resting pG state to the active pB state with partial molten globule formation. Previous terahertz dielectric response has been used to monitor changes in picosecond dynamics for the photoactive protein PYP with opposing results [1, 2]. While in one set of measurements, hydrated films were used and the pG/pB relative state population was monitored, in another set of measurements low conc PYP solution was used without monitoring of the conversion. In this paper we present THz dielectric response as a function of photocycle state for fully solvated PYP with in situ monitoring of the conversion using UV/Vis absorbance, both at room temperature and below freezing. Freezing reduces the background relaxational absorption of bulk water, and increases conversion to pB by slowing the photocycling time.

3012-Pos Board B59

Native-Like Structure of Proteins at a Planar PAA Brush

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Applying ATR-FTIR (attenuated total reflection-Fourier transform infrared) and TIRF (total internal reflection fluorescence) spectroscopy, we have studied the secondary structure and aggregation properties of different proteins which are adsorbed at a poly-(acrylic acid) (PAA) brush that covers a macroscopically large, planar surface. The PAA brush has been prepared on the surface of an ATR silicon crystal or a quartz plate. The preparation includes the deposition of a thin poly-(styrene) film by spin-coating and the transfer of the diblock copolymer poly-(styrene)-poly-(acrylic acid) onto the hydrophobic film using the Langmuir-Schäfer technique. It has been found that the proteins hen egg white lysozyme, bovine serum albumin, bovine α-lactalbumin, and bovine insulin adsorb spontaneously at a PAA brush at neutral pD-values, albeit to different degrees. The secondary structure of the proteins was estimated from a decomposition of the amide I'-band in the observed ATR-FTIR spectra. Generally, the fractions of secondary structure elements recovered in this way were almost identical to those found when the proteins are native in solution. In addition, the tendency of insulin to form amyloid fibrils has also been tested when the protein is adsorbed at a planar PAA brush. Insulin is known to form amyloid fibrils in solution at low pH-values and elevated temperatures. The experiments performed in this study suggest that a PAA brush does not promote fibril formation of insulin. Rather, insulin that is adsorbed at a PAA brush seems to be excluded from fibril formation pathways even at pD = 2 and 60 °C, where fibril formation of insulin is triggered in solution. Overall, the results of this study demonstrate that a planar PAA brush may serve as a mild environment for immobilized proteins.

3013-Pos Board B60

Conformation of Beta-Lactoglobulin at an Oil/Water Interface as Determined From Single-Molecule Force Spectroscopy

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Understanding the structure, composition and mechanical properties of adsorbed protein layers is essential for controlling the physico-chemical stability properties of food colloids. We have used atomic force microscopy (AFM)-single molecule force spectroscopy to probe the conformational changes in β-lactoglobulin (β-LG) proteins adsorbed onto the interface of an oil droplet in water, with in situ changes in pH. Single oil droplets were mechanically trapped in the pores of a polycarbonate filter and the AFM tip was used to grab onto and unfold the β-LG molecules. The changes in the contour length upon each unfolding event were determined by fitting the wormlike chain (WLC) model of polymer elasticity to each of the β-LG peaks of the force-extension profiles. Our results show clearly that β-LG on the same oil droplet adopts different conformations for different pH values. At pH 2.5, the unfolded β-LG molecule has a contour length that is similar to the total length of a single monomer with two large unfolding barriers, whereas the molecule exists mainly as a dimer formed of several smaller domains at pH 6.8. Furthermore, at pH 9 the interactions between the AFM tip and the β-LG layer on the oil droplet surface are dominated by an important repulsion due to the highly negatively charged β-LG layer. This study demonstrates a novel application of single molecule force spectroscopy to investigate the underlying mechanisms by which proteins can be used to stabilize food products.

3014-Pos Board B61

Effect of Trifluoperazine on Ca^{2+} -Bound Calmodulin binding to Fas Death Domain for DISC Formation

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Fas death receptor-activated signaling pathway is one important regulating mechanism of apoptosis in a variety of cells. The formation of the death inducing signaling complex (DISC) is a critical step for Fas-mediated signaling of apoptosis. Recent experimental studies showed that calmodulin (CaM) binds to Fas and regulates Fas-mediated DISC formation and the binding of CaM to Fas is inhibited by CaM antagonist, trifluoperazine (TFP). However, the exact molecular mechanisms for the effect of TFP on Fas-mediated DISC formation are still unknown. Knowledge about these is important for identifying new drug candidate to regulate Fas-mediated signaling pathway for apoptosis. In

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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