using Far UV CD and FTIR revealed that the helix conformation induced at higher concentrations of TFE is non-specific. Additionally, the role of the charged amino and carboxyl groups at the ends of the peptides were studied to determine if these groups affect helix induction by TFE. 15-mers of poly-L-glutamic acid and poly-L-lysine were synthesized from amide resins and their C-terminals were acetylated. The protected poly-L-glutamic acid and poly-L-lysine were titrated with TFE at pH 2, 7 and 13, and the secondary structures were determined using CD: no significant difference was found when compared to the non-protected homopolypeptides. The results obtained in this study clearly question the validity of structures of short peptides characterized in high concentrations of organic solvents.

3004-Pos Board B51

Kinetics of Film Formation of Poly-L-Proline at High Temperatures

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At room temperature and in aqueous solution, poly-L-proline predominantly adopts a relatively open left-handed 3_1-helix termed polyproline II (PPII). The corresponding far UV electronic circular dichroism (ECD) spectrum exhibits a couplet with a pronounced minimum at ~ 204 nm, and a much less intense maximum at ~ 228 nm. When poly-L-proline is incubated in a quartz cell for two hours at temperatures above 60°C, a gradual decay of the PPII signal is observed. It is replaced by a spectrum which displays a strong negative signal at ~220 nm (?), thus being indicative of a right handed conformation. This PPII signal decay is thought to be caused by the formation of a film on the inner surface of the cell. After removal of the poly-L-proline solution from the cell, the ECD was measured on both the liquid and cell individually. The ECD of the removed liquid portion resembles a typical PPII spectrum, while that of the cell results in the aforementioned spectrum, with a significant minimum at ~220 nm, and a minor minimum at ~204 nm. The rate of film formation depends strongly on the incubation temperature. Bi-exponential rise and decay modeled the disappearance of the PPII signal in aqueous solution and growth of the film, respectively. The respective relaxation constants were determined to be 10^{-3} s^{-1} and 10^{-4} s^{-1} for the PPII signal disappearance and film formation, respectively, at T= 65C ? (Laura?). The analysis of the kinetics measured at different temperatures between 60° and 75° C reveals a non- Arrhenius behavior. A further structural characterization of the obtained poly-L-proline film and a thermodynamic analysis are currently being carried out in our laboratory.

3005-Pos Board B52

Thermophilic protein structure adaptation examined with Burial Depth and Travel Depth

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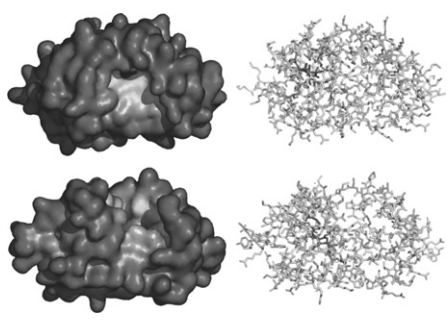
Organisms evolved at extreme temperatures (above 80° C) have constraints on their protein structures. These constraints result in differences in residue utilization and overall structure. By studying thermophile/mesophile pairs of homologous structures, we have examined these differences.

Geometric measures, specifically Burial Depth (distance from the molecular surface to each atom) and Travel Depth (distance from the convex hull to the molecular surface that avoids the protein interior), along with common metrics like packing are used to gain insight into the constraints experienced by thermophiles.

Our results show that extreme thermophiles show significant trends towards becoming more ''ball-like''. Their mean Travel Depth is less than their mesophilic counterparts, indicating smaller, less numerous and less deep pockets. Their mean Burial Depth is higher indicating that they bury more surface area and are more compact. This can be tracked on the individual residue level, for instance Alanine becomes more significantly buried under thermophilic conditions, and charged residues become less buried.

Shown is an example pair with the thermophile at top. At left are Travel Depth surfaces, at right Burial Depth. Note the fewer, smaller pockets and the deeper core in the thermophile.



3006-Pos Board B53

Small Conformational Changes Detected For Short Lived Transient Species During A Photoreaction

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he focus of the present study is firstly to investigate the origin of global conformational change during the photoreaction. Secondly, how such changes are transduced and spread to all over the protein molecule was investigated by studying the photoreaction of the site directed mutants. The diffusion of pG and pB of E46Q mutant showed the small difference, suggesting that the absence of negative charge at 46 position is responsible for the lack of partial unfolding in structure of PYP upon pB formation. The role of N-terminal region was further studied by replacing phenylalanine by alanine at position 6 (F6A). The diffusion constant of pB of F6A was not very different from that of pG, which is explained by a small conformational change in N-terminal tail upon pB formation. Previously, creation of a disulfide bond between position 6 (Phe6) and 121 (Phe121) showed the restricted movements of N-termius and less structural fluctuation upon pB formation. These results argued the more ordered and folded structure of pB of mutants lacking Phe6

3007-Pos Board B54

Probing the Dynamics and Conformations of Free and Ligand-bound Gamma-synuclein

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The synucleins are a family of natively unstructured, soluble proteins consisting of α-, β-, and γ -synuclein. They are primarily expressed in neurons, though they differ in their localization. Relatively little is known about the physiological roles of any of the synucleins, although the most studied of the group, α-synuclein, has been linked to Parkinson's disease. γ-synuclein was first identified in breast cancer cells, and was later found to be overly expressed in several other types of cancer, including ovarian cancer and retinoblastoma. Furthermore, subsequent studies found that γ-synuclein overexpression in breast carcinomas interferes with the activity of commonly used chemotherapeutic drugs, such as taxol and nocodazole. In the current study, we use a combination of single molecule Förster resonance energy transfer (FRET) and fluorescence correlation spectroscopy (FCS) to examine the conformations and dynamics of γ-synuclein alone and bound to potential ligands. Our findings will not only help understand the structural properties of a disordered protein, but will also lead to a better understanding of the mechanisms through which γ-synuclein alters the efficacy of antitumor drugs.

3008-Pos Board B55

Conformational Motion of Biological Macromolecules

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The beauty of biological macromolecules as a dancer in solution can be seen if we manage to understand graceful movements of the dancers. Biological macromolecules are dynamic machines so for proper functionality they need changing of shape, dancing through each other and non-contacting communicating to execute their ''understanding'' on distances. However there is no single theory to explain encountered experimental data and increase our understanding on the conformational motion. The problem is a suggestion of non-equilibrium nature of the macromolecules. Does it mean that non-equilibrium system like biological macromolecules can act as the dancer and can function properly? Does it mean that there is some rule that non-equilibrium macromolecules follow? Can the rule be generalized for any kind of non-equilibrium systems or not? My attempt to give a mathematical definition of the problems and point to their solution will be presented. Let consider bonded n point and correspond to the system some geometric figure with $V=n$ vertexes, virtual F,E faces and edges correspondingly. The conformational motion of the system is a changing the shape of the figure so we have two possibilities 1) the Euler Characteristic of the figure is changing in time as well as F,E changes; and 2) the Euler Characteristic of the figure is constant but F,E changes. Set of all possible {F,E} gives us the group with undefined operator f where f is operator which transfers elements of the set in each other $f:(F_i,E_j)\in\{F,E\}\rightarrow(F_g,E_k)$. The tusk could be solved if we manage to find the operator f. The value of the f can be immediately found by adding some physical conditions to the operator. The physical conditions and the equation of conformational motion will be presented at

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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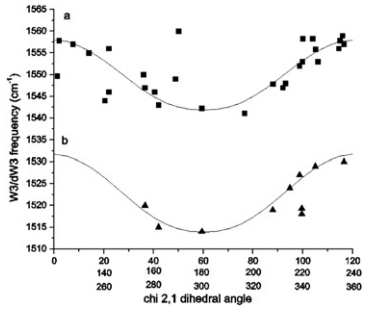
The correlation of the UVRR νW3 mode with the tryptophan $\chi^{2,1}$ dihedral angle (T. Miura et al. 1989, T. Maruyama & H. Takeuchi 1995, H. Takeuchi 2003) has been extended to a full, 360° rotation. The three-fold periodicity of the relationship (cos $3\chi^{2,1}$) over 360° results in up to six dihedral 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