In the present work we investigated the structural dynamics of PEVK, using FRET spectroscopy, on synthetic peptides of different contour length (11 and 21 residues) containing donor and acceptor fluorophores Trp and IAEDANS on the N- and C-termini, respectively. Because in this molecular arrangement FRET efficiency allows the calculation of the equilibrium mean end-to-end distance of the peptides, predictions based on statistical polymer models may be tested, and the effect of solution variables on global configuration may be measured. We find that the scaling of end-to-end distance with contour length deviates from the square-root law predicted for a purely statistical polymer chain, suggesting that the PEVK fragments studied acquire non-random conformation. To explore structural dynamics further, we measured the effect of temperature, chemical denaturation, pH and ionic strength on FRET efficiency. Increasing temperature, pH or ionic strength increased FRET efficiency. By contrast, denaturation with guanidine-HCl resulted in decreasing FRET efficiency. We hypothesize that PEVK may acquire a non-random structure in which electrostatic interactions play an important role. Whether local flexibility of the domain may be tuned by electrostatic mechanisms under physiological conditions, remains to be explored further.

1634-Pos Board B478

Cyanylated Cysteine Is a Site-Specific Vibrational Probe of Disorder-to-Order Transitions In Helical Protein Domains

Lena Edelstein, Katherine A. Clark, Casey H. Londergan.
Haverford College, Haverford, PA, USA.

The time scale of molecular vibrations allows infrared spectroscopy to be a picosecond probe of fluctuations in the local solvent and the structural environment of distinct vibrations. Solvent-exposed, free cysteine side chains are easily modified to thiocyanate through established reaction chemistry. Following site-directed mutagenesis to introduce cysteine, this modification allows the site-specific placement of thiocyanate in disordered domains implicated in binding or other structure-inducing events. Using a natural system (the Ntail protein from measles virus) and model helical peptides, we demonstrate that the frequency and line shape of the CN stretching band of cyanylated cysteine are sensitive to formation of both secondary structure and tertiary/quaternary or lipid contacts. The CN line shape indicates significant attenuation of the dynamics of water surrounding well-formed secondary structures.

1635-Pos Board B479

Effects of Phosphorylation on the unbound states of an intrinsically disordered protein: A Computational Approach

Debabani Ganguly, Jianhan Chen.
Kansas State University, Manhattan, KS, USA.

Intrinsically disordered proteins (IDP) can exist as ensembles of disordered conformations under physiological conditions, and such intrinsic disorder often plays important roles in their functions. The kinase-inducible transactivation domain (KID) from cAMP-response element-binding protein (CREB) has distinct ordered structure with its binding partner KIX, but is mostly unstructured in unbound state. The phosphorylation on Ser133 residue of KID increases the binding potency of the peptide toward KIX, but its impacts the disordered states remain unclear. We have carried out atomistic simulations in an implicit solvent to study effects of above-mentioned phosphorylation on the structure of unbound KID peptide. The results reveal that while the phosphorylation does not affect the average residue helicities, but has importance consequences on the flexibility of the peptide as well as the length and population of the transient helical segments. In particular, phosphorylation appears to restrict the accessible conformational space of the loop connecting two helices, and reduces the entropic penalty of folding upon binding. This entropic contribution, estimated to be ~1.5R from 4D joint backbone torsion distributions of Arg130 and Arg131 residues of KID, supplements the salt-bridges between pSer133 of KID and Lys662 and Tyr658 residues of KIX. This effect was not previously recognized due to inaccessibility of the structural details of the disordered ensembles from experiments. Success of these simulations is very encouraging, and demonstrates the feasibility of an implicit solvent-based computational framework for accurate atomistic simulation of IDPs.

Protein Dynamics II

1636-Pos Board B480

Computational Study of Signal Propagation in The Complex of *Thermus Thermophilus* Leucyl-tRNA Synthetase (leuRS) and Its Cognate tRNA

Yohsuke Hagiwara[1,2], Osamu Nureki[3], Masaru Tateno[1,2].
[1]Center for Computational Sciences, University of Tsukuba, Tsukuba, Japan, [2]Graduate School of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Japan, [3]Institute of Medical Sciences, University of Tokyo, Tokyo, Japan.

Aminoacyl-tRNA synthetases (aaRS's) play a critical role in decoding genetic information located on genome DNA, through catalyzing attachment of their cognate amino acid to 3′-end of the specific tRNA. The fidelity of translation is assured by their strict discrimination of the specific amino acids from non-cognate ones. For Ile, Val, and Leu, which are similar in the sizes and hydrophobicity, their specific aaRS's generate mis-aminoacylated tRNA, such as Val-$tRNA^{Leu}$; those enzymes accomplish ''editing'' through which mis-products are hydrolyzed. However, reaction mechanisms have not yet been clarified; the reasons are as follows: (i) No crystal structures of the enzymes in complex with the mis-aminoacylated tRNA have not yet been determined. (ii) Nucleophile for the reaction has not been identified.

In this study, to perform molecular docking of LeuRS, $tRNA^{Leu}$, and a nonspecific amino acid such as Val, we adopted a novel molecular docking algorithm developed by our group; characteristic features of our scheme are to enable us to predict conformational changes of protein induced by interaction with substrate and waters. Accordingly, this scheme is referred to as the Fully Solvated Dynamical Docking (FSDD). Thereby, we have successfully identified structural water molecules forming stable hydrogen bond networks in the active site of the enzyme. It has been found that one of such waters is located at the appropriate position as nucleophile in the modeled structure. Furthermore, using MD simulations of the LeuRS●Val-$tRNA^{Leu}$ complex, we have identified dynamical motions correlated between two distinct tRNA-binding domains of the enzyme, which are apart by ~100 Å. We have further found that those dynamical properties are induced by the interdomainal communication, for which the signal is propagated through the $tRNA^{Leu}$ molecule connecting the two domains in the complex.

1637-Pos Board B481

Transient Nonlinear Infrared Spectroscopy of Ubiquitin Unfolding Dynamics

Hoi Sung Chung, Ziad Ganim, **Kevin C. Jones**, Andrei Tokmakoff.
Massachusetts Institute of Technology, Cambridge, MA, USA.

We have structurally resolved the nanosecond to millisecond unfolding of ubiquitin with transient amide I two dimensional infrared (2D IR) and dispersed vibrational echo (DVE) spectroscopy following variable temperature jumps. 2D IR and DVE, a measurement related to the 2D IR spectrum projected onto the ω_3 axis, are nonlinear techniques capable of measuring secondary structure content with picosecond time resolution. The equilibrium 2D IR spectrum reveals features resulting from delocalized β-sheet vibrations with dipoles oriented parallel ($\nu_{\parallel}$) and perpendicular ($\nu_{\perp}$) to the strands. Transient 2D IR spectra show a blue shift of the $\nu_{\perp}$ vibration and disappearance of a cross peak between $\nu_{\perp}$ and $\nu_{\parallel}$ over μs to ms time scales. Diagonal peak intensities and homogeneous linewidths also indicate the melting away of sheet structure and the concomitant increased mobility of β-strand amide groups. These changes reflect the sequential unfolding of the β-sheet beginning with the labile strands III-V and followed by strands I-II. This pathway is confirmed through transient DVE of ubiquitin mutants, in which local mutations affect the timescales assigned to specific structures. The free energy landscape is evaluated through comparison of experiment and 2D IR spectra calculated from molecular dynamics simulations of ubiquitin unfolding using a structure-based model. The separation of timescales, stretched exponential relaxation, and probe-dependent response are consistent with the observation of μs downhill unfolding of a sub-ensemble that is prepared at the transition state followed by ms activated unfolding kinetics. The downhill unfolding is characterized through temperature jumps initiated and ending at variable temperatures. The increased downhill unfolding amplitudes and slowing timescales that accompany increases in temperature indicate that multiple unfolding pathways become accessible at higher temperatures.

1638-Pos Board B482

Class A β-lactamase backbone dynamics - At the crossroads of molecular dynamics and NMR spectroscopy

Olivier Fisette, Patrick Lague, Stephane M. Gagne.
Laval University, Quebec, QC, Canada.

Protein dynamics reveal crucial information about structure-function relationships. We complement the information obtained through NMR spectroscopy relaxation experiments and model-free analysis for class A beta-lactamases TEM-1 and PSE-4 with results from bioinformatics techniques, chiefly molecular dynamics (MD). Molecular dynamics allows the simulation of a protein's dynamics. The timescales probed using this technique differ from those accessible by NMR spectroscopy, giving a more complete picture of the backbone dynamics. Moreover, comparison of order parameters where the timescales of motions are accessible to both methods serves to validate our in silico approach. Also, MD hints at the atomic details associated with a residue's

particular motions. NMR spectroscopy data were re-analyzed under the light of this complementary information, yielding refined model selection and dynamics parameters. Our results suggest that highly structured backbone is a common characteristic of class A beta-lactamases. Nanosecond timescale motions taking place in the omega loop bordering the active site are observed with both techniques.

1639-Pos Board B483

Protein Engineering as a tool For Probing Potential Protein Dynamics in HIV-1 Protease

Seema Mittal, Celia A. Schiffer.
University of Massachusetts medical School, Worcester, MA, USA.

Human immunodeficiency virus 1 (HIV-1) protease is a symmetric, homodimeric aspartyl protease, crucial for viral maturation. From analysis of molecular dynamics simulations, 19 core hydrophobic residues appear to facilitate the conformational changes that occur in HIV-1 protease. This region has been suggested to undergo sliding motions facilitated by the exchange of hydrophobic van der Waals contacts between the core residues. Many of these residues are away from the substrate-binding site, yet have been implicated in conferring drug resistance, the mechanism of which still remains elusive.

We believe that this hydrophobic core dynamics governs protease activity and mutations within this region that alter this sliding motion, will potentially change the interactions between hydrophobic residues and consequently impact the catalytic activity of the protease. To determine whether locking the hydrophobic core using covalent chemistry compromises protease activity, we have engineered protease variants with novel disulfide bridges within the hydrophobic core region. Activity assays and crystal structures of the wildtype and mutant protease in the presence of the substrate will help elucidate effects of loss of core flexibility on protease function.

1640-Pos Board B484

Molecular Dynamics Simulation Of A Fatty Acid β-Oxidation Multienzyme

Tadaomi Furuta[1], Akinori Kidera[2].
[1]RIKEN, Wako, Japan, [2]Yokohama City University, Yokohama, Japan.

Recent biochemical studies suggest that many enzymes are organized into multifunctional enzyme complexes in the cytoplasm or subcellular organelles. Despite importances in cellular mechanisms, those structural bases to account for efficient enzymatic mechanisms have not been established yet. Among them, a fatty acid β-oxidation multienzyme complex (FOM) is the subject of intense investigation, because its function is an important catabolic process by which most organisms use fatty acids as energy and carbon sources (HUB in metabolic network). Also defects of FOM lead to several well-known metabolic disorders including metabolic syndrome which is popular recently. So the purpose of this investigation is to understand multi-enzymatic mechanism of FOM at atomic level. FOM structure was determined in several forms by Morikawa group in 2004, which were $\alpha 2\beta 2$ hetero complexes, had three kinds of ligands (Ac-CoA, NAD, C_8E_5), and had missing residue regions. FOM multi-functions are the last three of four β-oxidation enzymatic activity, i.e., 2-enoyl-CoA hydratase (ECH), L-3-hydroxyacyl-CoA dehydrogenase (HACD), and 3-ketoacyl-CoA thiolase (KACT). In preparatory investigation, we conducted structural modeling for those missing residue regions and determined force field parameters of ligands using RESP charges by quantum chemical calculation. In this presentation, we will show the results of molecular dynamics simulations of FOM with/without ligands and discuss the structural stability and multi-enzymatic mechanism of FOM at atomic level.

1641-Pos Board B485

Phosphorescence Probes of Molecular Mobility, Oxygen Permeability, and Dynamic Site Heterogeneity in Amorphous Soybean Glycinin

Andrew R. Draganski, **Richard D. Ludescher**.
Rutgers University, New Brunswick, NJ, USA.

The physical properties of amorphous biomolecules are important to the stability of low-moisture foods and pharmaceuticals. In the amorphous solid state, slow molecular motions are suitable for study by phosphorescent techniques. We use phosphorescence of erythrosin B dispersed in soy glycinin films to characterize the molecular mobility, oxygen permeability, and dynamic heterogeneity of the protein matrix. Films are spread from concentrated solutions of probe/protein at mole ratios of 0.045/1. Measurements as a function of temperature are made of phosphorescence intensity decays, emission spectra, and time-resolved emission spectra. Decays are fit with a stretched exponential function and both lifetimes and stretching exponents decrease with temperature. Arrhenius analysis of non-radiative quenching constants suggests that the protein matrix undergoes a broad softening transition between 70 and 120C during which additional modes of molecular motion are activated. The stretching exponent, a measure of the breadth of distribution of lifetimes and hence probe site heterogeneity, decreases gradually with temperature up to 70C and more steeply at higher temperatures, providing evidence of the onset of softening at 70C. Oxygen quenching rates, calculated from a comparison between emission lifetimes in the presence and absence of oxygen, vary roughly linearly with collisional quenching rates, which suggests that the local molecular mobility responsible for collisional quenching also modulates oxygen permeability. Delayed emission spectra are fit to a double lognormal function that provides peak emission energy and bandwidth. Bandwidth increases dramatically above 70C, which reflects an increase in the width of the distribution of energetically distinct matrix environments and provides further evidence of softening. The emission spectra blue shift with increasing delay time providing strong evidence that probes reside in distinct sites that vary in molecular mobility. Research supported by CSREES.

1642-Pos Board B486

A New Bend-Twist-Stretch Model Enables Coarse Graining of Elastic Network Models and of Any 3D Graph Irrespective of Atom Connectedness

Joseph N. Stember, Harel A. Weinstein, Willy Wriggers.
Weill Cornell Medical College, New York, NY, USA.

A stable parameterization of biomolecular elastic network models (ENMs) is proposed to enable coarse graining of the representation and to model any 3D graph irrespective of the atom connectedness of a system. Traditional ENMs rely on a distance cutoff which is unforgiving in the presence of false negatives in the connectivity, giving rise to unbounded zero-frequency motions when atoms are connected to fewer than three neighbors. A large cutoff is therefore chosen in an ENM, resulting in many false positives in the connectivity that reduce the spatial detail that can be resolved. The required connectivity also has the undesired effect of limiting the coarse-graining, i.e. the network must be dense even in the case of low-resolution structures that exhibit few spatial features. To facilitate such a coarse graining, the newly proposed potential includes 3- and 4-atom interactions (bending and twisting, respectively), in addition to the traditional 2-atom stretching. Thus, in our new Bend-Twist-Stretch (BTS) model the complexity of the parameterization is shifted from the spatial level of detail to the potential function. The additional potential terms were parameterized using continuum elastic theory, and the distance cutoff was replaced by a parameter free competitive Hebb connection rule. We validate the approach on a carbon-alpha representation of adenylate kinase, and illustrate its use with electron microscopy maps of RNA polymerase, ribosome and CCT chaperonin, which were difficult to model with traditional ENMs. For adenylate kinase, we find excellent reproduction (>95% overlap) of the ENM modes and B-factors when BTS is applied to the carbon-alpha representation as well as to coarser descriptions. For the volumetric maps, coarse BTS yields similar motions (75-90% overlap) to those obtained from denser representations with ENM.

1643-Pos Board B487

Conformational pathways of Adenylate Kinase characterized by computations, pressure and experiments

Dimitar Pachov, Scott J. Kerns, Dorothee Kern.
Brandeis University, Waltham, MA, USA.

Why and how do enzymes undergo conformational changes in order to perform their function?

The protein Adenylate Kinase (ADK) has two major conformations, the open and closed states. The conformational transition is important for the biological function of the protein in that, 1) the protein has to transform between the two conformations for catalytic function, and 2) the conformational transition is the rate limiting step during the catalytic cycle as shown by NMR experiments. The goal of our computational studies is to answer the questions about ''why and how'' these conformational transitions happen. We approach this problem indirectly by analyzing how different external pressure conditions affect the dynamics and functions of both P. profundum ADK (Padk), which lives at 700 atm pressure in the deep sea, and its homologue E. coli ADK (Eadk) living at ambient pressures.

Using NMR, we showed the rate of opening/closing transition in Padk increases with increasing pressures indicating that the protein possesses smaller partial molar volume in the transition state compared to its open and closed conformational states. MD simulations under pressure and TMD simulations we used to evaluate pathways of transitions in atomistic detail. Volume and surface accessible solvent area calculations per residue basis revealed physical principles underlying the different adaptations under pressure. Solvent exposure of charged residues combined with formation of ionic bridges was found to be the mechanism of the transition. The predicted pathways were verified by testing how mutations of key residues affected the enzyme conformational dynamics. The initially found steep pressure dependence of Padk in contrast to Eadk was mimicked by both the experiments with mutations and high-pressure simulations, the latter extending the conformational energy landscape to the