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 α2β2 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