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

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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

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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

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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

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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

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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

folding/unfolding region by obtaining atomic scale detail of core hydrophobic interactions.

1644-Pos Board B488
Functional Dynamics in Chlorella virus DNA Ligase
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DNA ligases specifically recognize and seal double stranded nicked DNA by catalyzing the formation of a phospho-diester bond between the 3′OH and 5′ phosphate termini. In physiological conditions these ubiquitous enzymes are essential for DNA replication, repair and recombination, while in tumor cells they can play a critical role in apoptosis resistance.
Chlorella virus ligase is a pluripotent ATP-dependent ligase composed by two domains, a N-terminal nucleotidyltransferase domain, hosting the catalytic site, and a C-terminal OB-domain, both of which participate to DNA binding. A number of crystallographic studies have elucidated important structural details of the nick-sealing process. DNA binding, in particular, appears to require a large reorientation of the two domains, as well as relevant structural rearrangements localized mainly in the N-terminal region (1). Indeed this protein appears to be a highly dynamic system whose internal motions are closely linked to both the DNA recognition and to the catalytic process. So far, however, the actual nature of these motions is still largely unknown, not only for Chlorella virus ligase, but also for the entire protein family.
We therefore tried to close this gap by undertaking the analysis of the dynamic properties of Chlorella virus liagase by solution NMR spectroscopy.
Reference:
(1) Nair P.A., Nandakumar J., Smith P., Odell M., Lima C.D., Shuman S. (2008) Nat Struct Mol Biol. 14, 770-8.

1645-Pos Board B489
Investigating Protein Dynamics Via A Multivariate Frequency Domain Analysis
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A novel method, which is based on a multivariate frequency domain analysis (MFDA), is proposed to extract collective vibrational modes of protein. The idea of the MFDA is to perform band-pass filtering of multivariate time-series using the multitaper Fourier transformation technique before multivariate analyses (e.g., singular-value decomposition) are carried out. The MFDA is compared with the standard multivariate analysis, principal component analysis (PCA), which solely utilizes the information of the equilibrium distribution of protein dynamics. It is found that, compared with the PCA, the MFDA well represents the vibrational behavior of protein and gives us an insight into the high-dimensional vibrational motion of protein. In our poster, we will show the recent extension of the MFDA on the time-frequency domain. Using the time-frequency domain extension of the MFDA, the anharmonic aspects of vibrational motion of protein will be discussed.

1646-Pos Board B490
Role of Cationic Residues in Fine Tuning the Flexibility of Charged Single α-helices
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A few highly charged natural peptide sequences have recently been suggested to form stable α-helical structures in aqueous solution. Here we show that these sequences represent a more widespread structural motif called 'charged single α-helix' (CSAH). We have developed two conceptually different computational methods capable of scanning large databases: SCAN4CSAH is based on sequence features characteristic for salt bridge stabilized single α-helices, while FT_CHARGE applies Fourier transformation to charges along sequences. Using the consensus of the two approaches, a remarkable number of proteins were found to contain putative CSAH domains. Recombinant fragments of 50-60 residues in length corresponding to selected hits (from myosin 6, Golgi complex associated protein-60, and mitogen-activated protein kinase M4K4) were found to adopt a highly stable α-helical structure in water. Molecular dynamic simulations of five CSAH peptides (the above three plus peptides from caldesmon and myosin 10) showed that the formation of dynamic ion-pair clusters significantly contribute to the stability of the helices. Furthermore, cationic residues were found to play a differential role in tuning the local flexibility of the CSAH domains. We conclude that sequence specific tuning of flexibility of CSAH peptides could have important role in the mechanical performance of CSAH-containing myosin motors, such as myosin 6 and 10, or in other protein functions.
This work was supported by the Hungarian National Science Fund (OTKA).

1647-Pos Board B491
Multidimensional IR Study Of The Structure And Dynamics Of Elastin Protein
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Elastin protein is responsible for the elasticity of organs including the skin, lungs, and arterial tissues. Upon heating to physiological temperature the elastic region undergoes an inverse temperature transition (ITT) from an extended to a folded state. It is hypothesized that the ITT results from changes in water structure around the elastic regions hydrophobic side chains. To investigate this hypothesis we have performed temperature dependent FTIR and 2D IR experiments on the amide I, amide A, and water OH stretching vibrations of bovine neck elastin and synthetic peptide mimics. FTIR spectra of hydrated films in the OH stretch region show spectral signatures which are indicative of water molecules interacting with the protein. This spectral signature displays a large absorption band centered at 3450cm-1, extending as far as 3600cm-1. This band is suggestive of weakly hydrogen bound water and may be an indicator of hydrophobic hydration. In 2D IR experiments we have observed a vibrational coupling between the high frequency water band and absorption bands at lower frequency that correspond to the amide A vibration of the protein backbone. Vibrational coupling between these modes may be an indicator of water molecules located at the protein water interface. Finally, 2D IR spectra on the amide 1 vibration of the protein backbone display a cross peak between a well resolved proline vibration centered at 1610cm-1 that originates from the elastic region of the protein, and a vibration centered at 1675cm-1 whose associated secondary structure is currently undetermined. From this data we hope to determine water's role in the ITT and propose a secondary structure for the elastic region of the protein.

1648-Pos Board B492
Dynamical Studies Of A Temperature-Sensitive Mutant Of The Tryptophan Repressor Protein, L75F-TrpR
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The overall research goal of these NMR dynamic studies is to enhance our knowledge of the dynamical properties of the tryptophan repressor (TrpR) protein and to establish the origin of flexibility changes that take place in TrpR mutants which exhibit conservative single point amino acid replacements that lead to altered L-trp and/or DNA binding properties.
A second objective is to understand how differential flexibility modulates L-tryptophan (L-trp) co-repressor binding to TrpR, and may be at the origin of the non-local long-range effects observed in the temperature-sensitive (ts) mutant of the tryptophan repressor protein, L75F-TrpR, which cannot simply be rationalized by small structural changes in the 3D fold of L75F-TrpR when compared to the 3D structure of wild-type (WT) TrpR.
We have undertaken 15N NMR relaxation studies to investigate the motional properties of backbone amides in the apo and L-trp bound (holo) forms of L75F-TrpR in solution and to compare the dynamical properties of mutant TrpR to that of wild type repressor. We have identified interesting differences between the flexibility profile of WT-TrpR vs. L75F-TrpR. The overall picture that emerges is that although both proteins exhibit similar ps-ns motion patterns for many residues in the core helices (i.e. helices A, B, C, and F) differences are detected in the DNA binding region (i.e. helix D-turn-helix E motif).

1649-Pos Board B493
Universal Scaling Law for Polypeptide Backbone Dynamics on the Pico- to Millisecond Time Scale
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UV-photolysis of an aromatic disulfide bond which holds a protein or peptide in a non-native conformation has been used to trigger polypeptide backbone relaxation. Geminate recombination of the disulfide bond was used as probe