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

for non-equilibrium backbone dynamics; as the thiyl radicals separate under the influence of backbone motion, their recombination rate decreases, so that observation of the transient thiyl absorbance provides access to backbone dynamics. Unlike fluorescence or triplet quenching experiments, which often are used for the study of polypeptide dynamics, this method observes processes far from equilibrium, and has no intrinsic limitation of the accessible time scale.
The encounter probability of the thiyl radicals was found to decay with time following a power law $t^{-0.94}$ which is incompatible with simple diffusion. Thus, the relative motion of the radicals is affected by the dynamics of the connecting backbone, resulting in an unusual power law for the re-encounter probability which could be described as (fractal) diffusion in a reduced non-integer dimensional space. The scaling law was found to extend over the full experimental time window, covering nine orders of magnitude in time (1 ps to 1 ms), although very different processes govern backbone motion on these different time scales. Furthermore, the same scaling law was observed in a folding protein having secondary and tertiary structure, in simple model peptides forming only secondary structure, and in a protein under unfolding conditions, indicating an intrinsic behaviour of the polypeptide backbone itself.

1650-Pos Board B494
Mobility of a Loop of a B. subtilis Carboxylesterase and its Effect on Substrate Conversion
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Carboxylesterases (CEs) are ubiquitous enzymes responsible for the detoxification of xenobiotics. CEs can metabolize and hydrolyze a variety of esterified drugs, including the anticancer agent CPT-11. The specificity of CEs for a particular substrate or inhibitor depends on the enzyme's molecular structure and the dynamics of conformational substructures when a substrate is bound. We have used a series of biophysical techniques to understand differences in substrate selectivity of CEs. First, we used molecular dynamics simulations (MD) and normal mode analysis (NMA) to identify the loop region of high fluctuation in a CE from B. subtilis. Second, we calculated the root-mean-square deviation (RMSD) from both MD and NMA trajectory data. Then we used these RMSD data along with its secondary structure to make correlations with enzyme activity. Meanwhile, we generated a series of mutations at specific amino acid residues that are located near this flexible loop region in order to restrict its mobility. Then we measured enzyme activity of these mutant CEs and compared them with the wild type. Our hypothesis is that the molecular dynamics of this enzyme is correlated with substrate conversion efficiency for selected CEs. These experiments provide the first data toward testing this hypothesis.
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1651-Pos Board B495
Reality's A Drag: Accounting For Friction In Simple Protein Models
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Elastic network models (ENMs) are widely used for studying the global equilibrium dynamics of proteins because they predict motions on timescales that are generally inaccessible to molecular dynamics (MD) simulations. Although the slowest motions predicted by in vacuo ENMs have repeatedly shown to correlate well with experiment, the timescales of these motions do not. Here we develop a simple algorithm for scaling the characteristic timescales of slow motions predicted by an ENM to reflect the true timescales of the molecular motions. Using MD trajectories on the order of tens of nanoseconds, we calculate ideal friction constants for Langevin models of three proteins. We then demonstrate that the difference between the slowest vibrational frequencies predicted by the Langevin model and those predicted by an in vacuo ENM can be explained through simple physical arguments. We provide an expression for scaling the normal mode frequencies of an in vacuo ENM to realistic values and discuss the utility of our results in combining ENMs with MD simulations to predict large-scale protein dynamics.

1652-Pos Board B496
Coupling Of Solvent And Protein Dynamics: Mossbauer And Incoherent Neutron Scattering From Dielectric Relaxation Data
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A wide variety of protein dynamics are accounted for by two classes of solvent processes: the bulk-solvent viscosity and hydration-shell dynamics. In glass-forming solvents the bulk viscosity arises from the well-characterized microscopic alpha relaxation; in liquid solvents that freeze the alpha process is essentially molecular reorientation. In past work we showed that the solvent alpha relaxation determines the activation enthalpy of alpha-slaved protein motions.
We have now measured the dielectric spectrum of the hydration-shell dynamics in myoglobin solutions as a function of hydration, temperature and frequency. These hydration-shell data and a minimal model of protein-solvent coupling predict the temperature- and hydration-dependence of the Mossbauer effect. Furthermore, we show agreement between incoherent neutron scattering data and our measurements of hydration dynamics.
These improvements in understanding protein-solvent dynamical coupling will be discussed in terms of earlier work describing the slaving of many protein functional motions to the solvent alpha-process and the slaving of protein folding. We demonstrate that many enthalpy barriers to protein motion arise almost entirely from solvent dynamical processes.

1653-Pos Board B497
A Detailed Comparison Between The NMR Ensemble, Two X-ray Models And Computational Predictions Of Motions For A Designed Sugar Binding Protein
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Coarse-grained elastic network models with single point representation of amino acids are becoming increasingly popular for describing conformational flexibility and equilibrium dynamics of proteins. In particular, the Gaussian network model (GNM) predictions have been fairly successful in interpreting the residue-level root-mean-square variations in residue positions inferred from NMR ensembles of structural models for a given protein and the fluctuations in residue positions indicated by crystallographic B-factors. Here, we carried out a detailed analysis for a designed sugar binding protein whose structure was solved in two crystal forms by X-ray crystallography and by NMR. Comparison with experimental data and results from molecular dynamics simulations confirm that the GNM predicts well the equilibrium dynamics of this protein and correlates better with the NMR derived data than crystallographic B-factors. The results further stipulate the importance of examining multiple structures determined by different methods as well as performing both analytical and numerical studies, toward gaining an accurate understanding of the type and range of conformational motions accessible to a given protein under native state conditions.

1654-Pos Board B498
Molecular Dynamics Simulations of Phosphorylation-induced Conformational Transitions in the *Mycobacterium tuberculosis* Response Regulator PrrA
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Phosphorylation-mediated activation of response regulators (RRs) is predominantly used by microorganisms as a central strategy in the regulatory activities of their two-component systems, the underlying molecular mechanisms are however far from fully understood. In this work we have conducted molecular dynamics simulations of the phosphorylation-induced conformational transitions in the *Mycobacterium tuberculosis* RR, PrrA, to obtain the dynamical details that are relevant to the RR activation. From the full-length structure of unphosphorylated PrrA we generated a computational model for the phosphorylated PrrA state by changing the phospho-accepting aspartic acid Asp-58 in the regulatory domain to the phosphoaspartate phos-Asp-58. The resultant structural relaxations were simulated through a rapid sampling of protein motions using a conformation-biased all heavy-atom potential energy function without explicit solvent. Marked structural rearrangements have been observed across the interdomain interface of the phosphorylated PrrA, manifesting the global effect of the local phosphorylation upon a single residue of aspartate. Such changes have also been found to involve the domain-crossing motions that disrupt the hydrophilic and hydrophobic interactions within the interdomain space and thus transform PrrA from a compact structure to a more extended conformation featuring a wider domain-domain separation and a more exposed transactivation loop. These simulated motions reflect the essential early-stage activation dynamics for the relief of the inhibitory role of the regulatory domain in PrrA. In effect, each more extended PrrA becomes more suited to interact with DNA and RNA polymerase; the activation of many proteins also shifts the population-equilibrium of PrrA towards more active states, therefore leading to a phosphorylation-enhanced allosteric regulation for the control of transcription.