k-Hefutoxin1 were lysed by sonication. Obtained proteins were finally purified and structurally analyzed by CD spectroscopy and NMR. In this study, we were able to ascertain the effect of absence of one or both of the disulfide bonds on the structure of k-Hefutoxin1.

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Characterization of HIV-1 Protease-Inhibitor Interaction by Interflap Distance Measurement, NMR Spectroscopy, and Solution Kinetics

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HIV-1 protease (HIV-1 PR) is an important drug target for the treatment of HIV/AIDS. Currently, there are several commercially available protease inhibitors (PIs) that improve the lives of patients. However, viral mutation often renders the PIs less effective after continuous use. In this study, we compare the effects of several PIs such as Indinavir, Atazanavir, Lopinavir, Saquinavir and Nelfinavir on the activity of the wild-type (PMPR), clinical isolate V6 and MDR769 HIV-1 proteases. We also use 2D HSQC NMR of uniformly 15N labeled samples and DEER spectroscopy with K55MTSL as the reporter site to study the conformational change in HIV-1 PR as a function of various inhibitors. Preliminary solution kinetics data show strong inhibition of PMPR by various inhibitors, but not for V6 and MDR769. The NMR spectra of unbound PMPR, V6, and MDR769 differ markedly from one another, and significant changes in the protein chemical shifts of PMPR and V6 are seen in the presence of inhibitors. The DEER distance distribution profiles reveal altered flap conformations in the uninhibited state of V6 and MDR769 compared to PMPR. Finally, in the presence of inhibitors such as Indinavir, the flap conformations in V6 and MDR769 show a minor change, whereas data for PMPR reflects a closing of the flaps to a conformation consistent with X-ray crystallographic structures.

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Picosecond Dynamics Of Surface Water As A Function Of Hydrophobicity

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Previously we and others have shown terahertz sensitivity to protein-ligand binding, possibly arising from the change in low frequency structural modes [1]. Another possibility is that the water adjacent to the protein, which strongly contributes to the THz response, may be affected by the change in the protein surface with binding. Pollack and coworkers have shown an ordered water film (from nm up to hundreds of um thickness) is formed on a smooth hydrophilic surface [2, 3]. To study how picosecond dynamics of water are affected by hydrophilicity of a surface, we performed a series of terahertz dielectric measurements as a function of water film thickness and hydrophicility of the surface. Measurements were made on solution cells with windows made of polyethylene or quartz. The hydrophilicity of the surfaces was modified by air plasma treatments, and characterized with contact angle measurements. Terahertz time domain spectroscopy measurements were made as a function of thickness and the absorption coefficient and index were extracted. The results were analyzed at selected frequencies to study the absorption trend with respect to the change of thickness. These measurements suggest a smaller THz response for water adjacent to hydrophilic surfaces. This lower response may possibly come from an overall decrease in water density at the surface or a stronger ordering inhibiting rotational motions contributing to the picosecond response.

1. Chen, J.-Y., et al., *Terahertz Dielectric Assay of Solution Phase Protein Binding.* Appl. Phys. Lett., 2007. **90**: p. 243901.
2. Zheng, J., et al., *Surfaces and interfacial water: Evidence that hydrophilic surfaces have long-range impact.* Advances In Colloid and Interface science, 2006. **127**: p. 19.
3. Rand, R.P. and V.A. Parsegian, *Hydration forces between phospholipid bilayers.* Biochimica et Biophsica Acta, 1989. **988**: p. 351.

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The Scaffolding Subunit of PP2A is a Coherent Linear Elastic Object That Can Transmit Mechanical Information Along Its Length

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HEAT repeat protein PR65 is the scaffolding component of protein phosphatase PP2A, which has been implicated in tension sensing during chromosome segregation and in diverse other chromosomal processes. PR65 is composed exclusively of 15 HEAT repeats, i.e. pairs of anti-parallel alpha helices connected by short 1-3 residue turns, that stack in parallel to form a solenoid structure in which the packed helices form one continuous hydrophobic core. Molecular dynamics analysis reveals that tensile or compressive forces applied at the protein termini produce evenly-distributed shape changes (straightening/bending) via longitudinal redistribution of stress, with elastic coherence resulting from the continuous meshwork of van der Waals interactions created by the aligned helix/helix interfaces. At higher forces, fracturing occurs via loss of a specific helix/helix contact between adjacent repeats, accompanied by relaxation that spreads outward from the fracture site through the adjacent regions. Fracturing is nucleated by "flaws" resulting from atypical residues in inter-helix turns along the edges of the structure. Such flaw sites exhibit competition, such that only one of them fractures, as well as cooperation to create bounded regions of increased strain. Thus, PR65 is a coherent linear elastic object, capable of transducing mechanical information from one position along its length to another. We propose that HEAT repeat scaffolds, including PR65, exist to place bound components in mechanical linkage so that their promoted molecular reactions are sensitive to externally-imposed mechanical forces. More generally, since analogous elastic coherence should be present in many types of helical repeat proteins, cells may be filled with mechanically-tunable molecules, and mechanical stress may be a common currency for subcellular information transfer.

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The Closure Mechanism Of M. Tuberculosis Guanylate Kinase Relates Structural Fluctuations To Enzymatic Function

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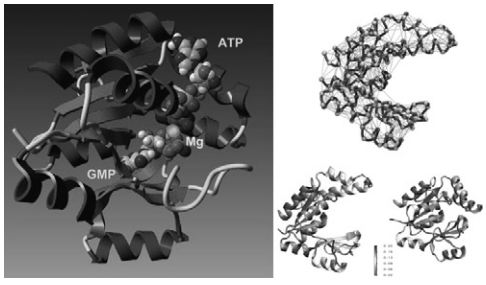
The Allosteric Spring Probe (ASP) technique allowed Zocchi et al. to act on the enzymatic activity of guanylate kinase (GK) by applying tension upon the molecular structure of this enzyme [Choi et al., Biophys. J., 2007]. These experiments raise the question about the underlying conformational modifications leading to such an observation.

In order to elucidate the results from these ASP studies, we investigate the conformational dynamics of GK and its mechanical properties. We use both high resolution atomistic molecular dynamics and low resolution Brownian Dynamics simulations.

The enzyme is subject to large conformational changes, leading from an open to a closed form, and further influenced by substrate or co-factor docking. A reduction or perturbation of the conformational space available to GK can be related to the activity loss encountered in the ASP experiments.

We describe a detailed picture of GK's closure mechanism characterizing the hierarchy and chronology of structural events essential for the enzymatic reaction. Rigidity profiles obtained from simulations of distinct states hint at important differences. We have investigated open vs. closed, apo vs. holo or substrate vs. product-loaded forms of the enzyme.



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The Closed <-> Open Transition of Adenylate Kinase From Crystal Structures and Computer Simulations

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Many proteins function as dynamic molecular machines that cycle between well-defined states. A mechanistic and atomic-scale understanding starts with crystal, NMR or electron microscopy structures in these states. Typically, none or only very limited structural information is available for the intermediates along the transition. Computational methods can simulate transitions between states but due to the absence of intermediate structures it is hard to verify that the simulated transition path is correct. One exception is the enzyme adenylate kinase. It is well studied and a large number of crystal structures are available. Vonrhein et al [1] suggested early on that some of these structures would be transition intermediates due to stabilization by crystal contacts and created a 'movie' from nine structures. We took this idea one step further and compare 45 experimental structures to hundreds of transitions of E. coli AdK simulated with the dynamic importance sampling method (DIMS). We find that DIMS trajectories, which only require a crystal structure for the starting and the end point of the transitions, contain all intermediate crystal structures (RMSD for matches: <4 Å with median 1.2 Å). The crystal structures

can be time-ordered according to the DIMS trajectory and this ordering is essentially the same for forward and backward transitions. These results suggest that DIMS is capable of simulating realistic macromolecular transitions. From the simulated trajectories we can present a molecular detailed picture of a macromolecular transition. We discuss the conformational change of AdK with respect to the presence or absence of ligands, the relevance of salt bridges, and the motions of rigid domains.

[1] C. Vonrhein, G. J. Schlauderer, and G. E. Schulz. Movie of the structural changes during a catalytic cycle of nucleoside monophosphate kinases. Structure 3 (1995),483–490.

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Conserved Protein Flexibility And Pathways Of Energy Flow In Enzyme Catalysis

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Conformational fluctuations in enzymes have significant affect on catalysis. Several enzymes show the presence of a network of coupled motions associated with the catalytic step. Here, we describe our recent studies to identify and characterize coupled motions in members of a diverse family of enzymes namely the dinucleotide binding Rossmann fold proteins (DBRP), sharing a common sub-step of hydride transfer from the dinucleotide cofactor to the substrate.

Results show that in spite of low sequence/structural homology, the overall intrinsic dynamical flexibility during the course of the enzyme reaction is conserved. These dynamical fluctuations span from the exterior surface regions to the active site of the protein and form pathways. These pathways are connected via hydrogen bonds/hydrophobic interactions, which are conserved across prokaryotes and eukaryotes alike.

In order to characterize the energy flow within these pathways, we use an integrated information theoretic and biophysical approach to study how energy may propagate within the DBRP super-family. The studies reveal for the first time how energy is propagated from the exterior flexible surface regions of the protein to the active site of the protein.

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Accelerated Target Selection By Repair Enzymes Through Charge Transport

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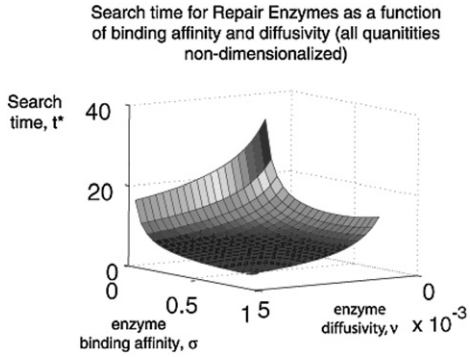
A Charge Transport (CT) mechanism has been proposed in several papers (for example see Yavin *et al.* PNAS **102**, 3546 (2005)) to explain the co-localization of Base Excision Repair (BER) enzymes to lesions (damaged bases) on DNA. The CT mechanism relies on the presence of iron-sulfur clusters on the enzymes; these clusters can undergo redox reactions to modify the enzymes' binding affinity. The redox reactions are mediated by the DNA strand and involve the exchange of electrons between individual BER enzymes. This process effectively increases the desorption rate of enzymes to promote their redistribution and co-localization to lesions.

We study the search times of BER enzymes to lesions by using a mass action model of enzyme dynamics and electron transport. We show that when the enzyme copy number is small, the CT mechanism reduces the search time of otherwise ''passive'' enzymes that simply attach to the DNA without desorbing. Other physical effects in our enzyme model include an explicit treatment of their dynamics in solution, diffusion along the DNA and facilitated adsorption by guanine radicals.



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Reference-Free Identification of Dynamic Structural Domains in Proteins: Comparison of Numeric Predictions with NMR Measurements

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Understanding proteins' functionalities, which are intimately related with their structural conformations, require a robust characterization of conformational changes which occur in proteins in response to external impacts, as well as spontaneously. This presentation introduces a novel numeric methodology to identify dynamic structural domains in proteins, which is based on the recent theoretic invention [M. Stepanova, Phys. Rev. E76 (2007) 051918]. The methodology employs a fundamental, reference-free approach including identification of essential collective coordinates by the covariance analysis of molecular dynamics trajectories, construction of the Mori projection operator with these collective coordinates, and analysis of the corresponding generalized Langevin equations (GLE). The dynamic domains are identified as groups of atoms that show a dynamic coupling in the GLE. Since the methodology is based on a rigorous theory, the outcomes are physically transparent: the dynamic domains are associated with regions of relative rigidity, whereas off-domain regions are relatively soft. In the presentation, applications of the new structural analysis are demonstrated for the examples of protein G and prion proteins. Experimental NMR-based model-free S2 profiles, random coil indexes, and amplitude correlation data are compared with the numeric analysis, which includes (i) robust systems of dynamic structural domains and (ii) dynamically consistent local flexibility descriptors. It is shown that these numerical results agree well with the available NMR experiments. It is also demonstrated that the dynamic domains and the corresponding flexibility descriptors represent highly sensitive scores for characterization and comparison of proteins' conformations. Even very subtle changes in collective behaviors in macromolecules can be easily detected, visualized, and interpreted. The introduced methodology provides the community with a novel powerful tool for interpretation of NMR experiments, as well as for characterization, comparison, and dynamic analysis of proteins' conformational behaviors.

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Spontaneous Substrate Binding and Formation of the Bound State in Glycerol-3-Phosphate Transporter (GlpT)

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GlpT is an antiporter mediating the uptake of glycerol-3-phosphate (G3P) across the membrane using preexisting gradient of inorganic phosphate (Pi). GlpT is believed to function through an alternating access mechanism, in which the two functional states inter-convert through rocker-switch type of conformational changes. However, the crystal structure of GlpT is only available in its cytosol-open state. Furthermore, the location of the binding site and residues involved in substrate binding are largely unknown. We have carried out an exhaustive set of long (50 ns or longer) molecular dynamics simulations of GlpT in the presence of all physiologically relevant substrates, i.e., monovalent and divalent Pi and G3P, as well as in the apo state as control. The substrate is placed at the opening of the lumen in the beginning of each simulation. In all of the simulations, we observe rapid, spontaneous binding of the substrate in less than 10 ns. All trajectories consistently yield a common binding pathway, composed of several conserved residues: K80 acting as a ''fishing hook'', one of the symmetrically positioned arginines (R45), and H165. The phosphate moiety of the substrate first binds to K80, which brings the substrate to a close contact with R45 and H165. Despite its symmetrical position to R45, no direct contact with conserved R269 is observed in any of the simulations. Neutralizing any one of the above residues impairs binding as revealed by additional simulations. Moreover, substrate binding results in appreciable closure of the helices in the cytoplasmic side illuminating initial steps of the rocker-switch mechanism. Our MD simulations reveal a common pathway involved in binding of the substrate, a detailed view of the binding site, and initial protein conformational changes induced by substrate binding.

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Single Molecule Fret Reveals Novel Dynamic Structure And Stoichiometry Of L27 Domain-mediated Polarity Complexes Formed By Drosophila Sdt/DPatj/DLin-7

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Cellular differentiation is frequently regulated by multi-protein complexes where the spatial proximity of the components facilitates biological function. There is immense interest in isolating the individual components involved as