capable of probing changes in protein structure on time scales as short as ~100 ps. This infrastructure was first developed on the ID09B time-resolved beamline at the ESRF, and more recently at the ID14B BioCARS beamline at the APS. In these studies, a picosecond laser pulse first photoexcites a protein and triggers a structural change, then a suitably delayed picosecond X-ray pulse passes through the protein and the scattered X-rays are imaged on a 2D detector. When the sample is a protein crystal, this ''pump-probe'' approach recovers time-resolved diffraction ''snapshots'' whose corresponding electron density maps can be stitched together into movies that unveil the correlated motions that arise from the photoexcitation event. When the sample is a protein solution, we recover time-resolved small- and wide-angle X-ray scattering (SAXS/WAXS) patterns that are sensitive to changes in the size, shape, and structure of the protein. The time-resolved x-ray scattering diffractometer developed at the APS is capable of simultaneously probing both SAXS and WAXS regions with 100 ps time resolution. Importantly, scattering studies of proteins in solution unveil structural dynamics without the constraints imposed by crystal contacts; thus, these scattering ''fingerprints'' complement results obtained from diffraction studies. Together, these studies provide stringent constraints for putative models of conformational states and structural transitions between them. Time-resolved studies of heme proteins have unveiled at an unprecedented level of structural detail protein conformational dynamics as well as ligand migration and escape. This research was supported in part by the Intramural Research Program of the NIH, NIDDK. JSO is supported by NIH grants GM035469 and HL047020 and Grant C0612 from the Robert Welch A. Foundation.

Protein Dynamics

1217-Pos Board B127

Domain Motions in the Interpretation of Neutron Spin Echo Spectroscopy and Molecular Dynamic Simulation: A Case Study of Phosphoglycerate Kinase

Nikolay Smolin, Jeremy C. Smith.

The conformational dynamics of proteins are important for functions. Large-scale domain motions of enzymes are often essential for their biological function. Few techniques are available that can directly probe protein motions. Among the most direct is molecular dynamics simulation in which, using an empirical potential energy function, the equations of motion of a system of atoms are solved numerically. In this way detailed description of protein dynamics can be built up for timescales up to about 100 ns. Integration of molecular dynamics simulations and neutron scattering provide insights of process in the proteins in the atomic details. An exciting new development in the experimental detection of functionally-important domain motions in proteins is the application of neutron spin-echo spectroscopy (NSE). Spin echo directly probes coherent (i.e., pair correlated) scattering on the 10-100 ns timescale. Recent work has demonstrated that domain motions in the two domain protein phosphoglycerate kinase (PGK) can be positively identified and characterized with this technique. Inspired by these results, we performed molecular dynamics simulation of PGK. The measurable quantities, the intermediate scattering functions and small-angle neutron scattering profile were directly calculated from the MD trajectories. All results are in very good agreement with experimental data. Principal component analysis (PCA) was used to characterize the molecular dynamics trajectory. PCA extracts the essential motions sampled by MD simulation: the principal component modes. We have shown that combination of neutron scattering and molecular dynamics simulation is a powerful tool for characterization large scale motions in proteins.

1218-Pos Board B128

Dynamic Propagation of Long-Range Allosteric Signals by Nanoscale Protein Domain Motion Revealed by Neutron Spin Echo Spectroscopy

Zimei Bu, David J.E. Callaway.

NHERF1 is a multi-domain scaffolding protein that assembles signaling complexes, and regulates the cell surface expression and endocytic recycling of a variety of membrane proteins. The ability of the two PDZ domains in NHERF1 to assemble protein complexes is allosterically modulated by the membrane-cytoskeleton linker protein ezrin, whose binding site is located as far as 110 angstroms away from the PDZ domains. Here, using neutron spin echo (NSE) spectroscopy, selective deuterium labeling, and theoretical analyses, we reveal the activation of interdomain motion in NHERF1 on nanometer length scales and on sub-microsecond time scales upon forming a complex with ezrin. We show that a much simplified coarse-grained model suffices to describe inter-domain motion of a multi-domain protein or protein complex. We expect that future NSE experiments will benefit by exploiting our approach of selective deuteration to resolve the specific domain motions of interest from a plethora of global translational and rotational motions. Our results demonstrate that the dynamic propagation of allosteric signals to distal sites involves changes in long-range coupled domain motions on submicrosecond time scales, and that these coupled motions can be distinguished and characterized by NSE.

1219-Pos Board B129

Single Molecule FRET Study of Nucleotide Binding Effects on Muts Proteins

Ruoyi Qiu, Keith Weninger.

The DNA mismatch repair (MMR) system is an essential component of the cellular DNA replication process that ensures high overall fidelity. The protein MutS initiates MMR by binding specifically to base-base mismatches and insertion-deletion mismatches in double stranded DNA. The active MutS dimer includes two ATP binding sites that hydrolyze ATP in both DNA bound and free states. ATP is also hydrolyzed as part of the MMR cascade involving MutS. Biochemical characterizations have shown that distinct nucleotides produce different interactions between MutS and mismatched DNA substrates, but the details of the conformations of MutS-mismatched DNA complexes under exposure to different nucleotides are not well known. We used single molecule fluorescence resonance energy transfer (smFRET) to study MutS from Thermus aquaticus in isolation and in complex with mismatched DNA substrates when exposed to a variety of different nucleotide conditions. We report results using intramolecular FRET from MutS or the DNA substrate as well as intermolecular FRET between MutS and the DNA. Our results allow MutS-DNA conformations resulting from specific nucleotide bound states to be characterized. We suggest possible roles for ATP cycling that could regulate the function of MutS in DNA MMR.

1220-Pos Board B130

Is 10-100ps Spectral Relaxation of Trp An Indicator of Local Disorder in Proteins?

Arianna Biesso, Jianhua Xu, Olga Tcherkasskaya, Jay R. Knutson.

We have studied the time-resolved fluorescence of the Trp43 residue of the globular protein GB1 upon acid induced equilibrium unfolding.

NMR structural experiments have shown this protein is actually very acid stable above pH3.

Nanosecond time-resolved TCSPC data clearly suggest that, in the tryptophan environment, partial unfolding appears at surprisingly high pH values. In fact, GB1 exhibits signs of local lifetime changes for pH values as high as 6.9.

Further, femtosecond ultraviolet upconversion data reveal a ~30 ps component with a negative preexponential amplitude in the longer wavelength portion of the emission spectrum. Such a rise time on the red side of emission is a signature of generalized relaxation (solvent and/or protein) around the excited dipole of Trp. A similar term with a ~2ps exponential is always found for proteins in aqueous solution, representing bulk water motion. Slower terms (10-100ps) have previously been assigned to unusual water environments, protein dipolar relaxation, or the coupling between them.

Most intriguing is the fact that, for somewhat lower pH values where GB1 is locally (but not globally) unfolded to a larger degree, the amplitude of the observed 30ps term becomes larger (more negative).

Femtosecond Trp emission spectroscopy may thus provide new snapshots of proteins that are ''fully folded'' over longer time averaging but still have transiently unstructured regions.

1221-Pos Board B131

Anomalies in the Vibrational Dynamics of Proteins are a Consequence of Fractal-Like Stracture

Shlomi Reuveni, Yossi Klafter, Rony Granek.

Proteins have been shown to exhibit strange/anomalous dynamics displaying non-Debye density of vibrational states, anomalous spread of vibrational energy, large conformational changes, nonexponential decay of correlations, and nonexponential unfolding times. The anomalous behavior may, in principle, stem from various factors affecting the energy landscape under which a protein vibrates. Investigating the origins of such unconventional dynamics, we focus on the structure-dynamics interplay and introduce a stochastic approach to the vibrational dynamics of proteins. We use diffusion, a method sensitive to the structural features of the protein fold and them alone, in order to probe protein structure. Conducting a large-scale study of diffusion on over 500 Protein Data Bank structures we find it to be anomalous, an indication of a fractal-like structure. Taking advantage of known and newly derived relations between vibrational dynamics and diffusion, we demonstrate the equivalence of our findings to the existence of structurally originated anomalies in the vibrational dynamics of proteins. We conclude that these anomalies are a direct result of the fractal-like structure of proteins. The duality between diffusion and vibrational dynamics allows us to make, on a single-molecule level, experimentally