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Protein kinases regulate various important cellular signaling events by catalyzing phosphoryl transfer from ATP to the hydroxyl groups of their substrates. Aberrant protein phosphorylation is linked to fatal diseases including cancer and cardiac disease. However, due to high structural similarity between kinases, current kinase inhibitors that target the ATP-binding site are non-specific. Hence, to be able to control the activity of a kinase allosterically provides a promising alternative. An effective way to pursue this goal is by analyzing the motions of the inhibited state of a kinase. Here, we aim to characterize the dynamical behavior of the kinase prototype cAMP-dependent protein kinase A (PKA) when bound to standard potent inhibitors by NMR relaxation experiments. Preliminary results on both the amide backbone and methyl side chain conformational dynamics at multiple timescales of the complexes of PKA with inhibitors H89 and balanol will be presented. Our data showed that inhibitor binding shifted the timescales of the dynamics of the enzyme. Understanding the conformational dynamics of an inhibited enzyme may aid in the rational design of allosteric drugs that will fine-tune an enzyme's activity.

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Long-Range Protein Vibrations Dependence on Ligand Binding: Rate Promoting Motions

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It has been suggested that long-range intramolecular vibrations in enzymes may provide efficient access to intermediate state configurations, enhancing catalytic turnover rates. Recently we have successfully measured these long-range protein vibrations using an anisotropic THz near-field technique to measure protein crystals, called crystal anisotropy terahertz microscopy (CATM). The method isolates these motions from the isotropic librational background, revealing narrow band resonances. Recent measurements on free chicken-egg white lysozyme (CEWL) and tri-N-acetylglucosamine (3NAG) inhibitor bound CEWL reveal dramatic and reproducible changes in the intramolecular vibrational mode spectra with binding. A large resonance at ~70 cm^{-1} for free CEWL, is somewhat suppressed with binding, whereas a new resonance is observed at ~ 40 cm^{-1}, with binding. This dramatic change in the spectra with binding confirms that the observed resonances arise from intramolecular vibrations, and not crystal phonons. Using normal mode analysis, our calculated CATM spectra find a consistent increase in the low frequency signal with binding. While the density of states does not change appreciably with binding, the overall direction of motion shifts for vibrations in the frequency range where there is a large change in the optical signal. The change in the direction of the vibrational motion results in a change in the motion of the charge distribution and therefore, the dipole derivative, leading to the sensitivity to the trajectories in the terahertz optical absorbance. The remaining question is if these specific motions participate in the structural preorganization of the binding site, and thus promote catalytic activity. We will discuss measurements to answer this long standing question. This work was supported by NSF MRI^2 grant DBI295998.

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Rhodopsin Photoactivation Dynamics Revealed by Quasi-Elastic Neutron Scattering

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Rhodopsin is a G-protein-coupled receptor (GPCR) responsible for vision under dim light conditions. During rhodopsin photoactivation, the chromophore retinal undergoes cis-trans isomerization, and subsequently dissociates from the protein yielding the opsin apoprotein [1]. What are the changes in protein dynamics that occur during the rhodopsin photoactivation process? Here, we studied the microscopic dynamics of the dark-state rhodopsin and the ligand-free opsin using quasi-elastic neutron scattering (QENS). The QENS technique tracks the individual hydrogen atom motions in the protein molecules, because the neutron scattering cross-section of hydrogen is much higher than other atoms [2-4]. We used protein (rhodopsin/opsin) samples with CHAPS detergent hydrated with heavy water. The solvent signal is suppressed due to the heavy water, so that only the signals from proteins and detergents are detected. The activation of proteins is confirmed at low temperatures up to 300 K by the mean-square displacement (MSD) analysis. Our QENS experiments conducted at temperatures ranging from 220 K to 300 K clearly indicate that the protein dynamic behavior increases with temperature. The relaxation time for the ligand-bound protein rhodopsin was longer compared to opsin, which can be correlated with the photoactivation. Moreover, the protein dynamics are orders of magnitude slower than the accompanying CHAPS detergent, which forms a band around the protein molecule in the micelle. Unlike the protein, the CHAPS detergent manifests localized motions that are the same as in the bulk empty micelles. Thus QENS provides unique understanding of the key dynamics involved in the activation of the GPCR involved in the visual process.

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Role of Structural Flexibility of cpSRP43 in Binding Substrates during Post-Translational Targeting

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The ability of the chloroplast signal recognition particle (cpSRP) to post-translationally target light harvesting complex proteins (LHCs) to the thylakoid membrane relies on a chloroplast-specific subunit, cpSRP43. cpSRP43 is a multidomain protein that forms a heterodimer with the conserved GTPase, cpSRP54, and subsequently interacts with LHCP substrates to form a soluble targeting complex in the chloroplast stroma. Single-molecule Förster Resonance Energy Transfer (smFRET) and molecular dynamics simulations was employed to determine how the inter-domain structural dynamics of cpSRP43 is affected by binding to cpSRP54 to help address how the formation of the cpSRP heterodimer enables LHCPs to bind and adopt an insertion-competent transit complex. Our results reveal significant inter-domain dynamics (i.e. flexibility) in cpSRP43 across the whole protein with 3 major conformations being identified. Upon binding to cpSRP54, there is a reduction in the flexibility of cpSRP43 in certain regions of the protein. Using isothermal titration calorimetry, we found that the affinity of the L18 recognition site of LHCP to cpSRP43 increased when cpSRP43 was complexed with a cpSRP54 peptide, which corresponds to the cpSRP43 binding site on cpSRP54. These results support the model that cpSRP54 promotes transit complex formation by regulating the inter-domain structural dynamics of cpSRP43.

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Fluctuations within the Hydrogen Bond Network Modulate Cooperativity Across the Conformational Ensemble of Protein Structures

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Proteins are dynamic molecules that exhibit conformational changes limited by cross-linking H-bonds. Here we monitor how fluctuations within the hydrogen bond network (HBN) alter protein flexibility. Specifically, we characterize fluctuations in the HBN within two TEM β-lactamase enzymes by employing the Distance Constraint Model (DCM) on 100 ns explicit solvent molecular dynamics (MD) trajectories. A key feature of the DCM is that it employs network rigidity explicitly as a long-range mechanical interaction that accounts for nonadditivity in conformational entropy. Taking advantage of the fundamental link between mechanical and thermodynamic properties, we quantify protein flexibility using a variety of metrics that are calculated within the native state ensemble, and elucidate how fluctuations in the HBN modulate protein flexibility. We observe that the overall flexibility of the proteins is weakly anti-correlated to the overall strength of the HBN. Interestingly, equilibrium fluctuations lead to dramatic loss/gain in correlated rigidity throughout the structure. This translates to conformations that are more flexible, characterized by redundant cross-linking H-bonds break more readily upon slight temperature increases. These conformations also have tendency towards reduced cooperativity within unfolding transitions. The essential property leading to cooperative and non-cooperative structural networks is H-bond independence, which strongly depends on how the H-bonds are distributed. Dense regions of H-bonds that form rigid clusters do so with reduced conformational entropy reductions, thus increasing cooperativity within the folding transition and increasing the co-rigid nature of the native structure. A less dense HBN reduces molecular cooperativity because H-bonds are more likely to be independent. While cooperativity is a hallmark of protein stability, the extent of this cooperativity is modulated by fluctuations within the HBN. Consequently, native state HBN fluctuations have important ramifications to function, suggesting a more complete paradigm of structure/flexibility/function.