differences in the conformational entropy of an IDP affect the binding affinity of a coupled folding reaction, and should give a better understanding of the mechanisms involved in this kind of binding. The importance of this research is highlighted by the high frequency in which folding is coupled to binding in protein-protein interactions.

318-Pos Board B104

Mechanism of Interaction Between Lung Surfactant Protein-D and Influenza A Virus Hemagglutinin

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Lung collectin surfactant proteins are pulmonary host defense proteins that contribute to innate, front-line defense against influenza A virus (IAV) and other inhaled pathogens. Collectins recognize viral glycans on the globular head of hemagglutinin (HA) on the IAV surface and initiate events leading to pathogen neutralization. Thus, effective pulmonary host defense requires fast recognition of IAV HA by collectins. In order to assist development of new approaches to collectin-based antiviral therapeutics, we investigated the mechanism underlying SP-D recognition of IAV HA using molecular dynamics simulations. Comparing the binding affinities of SP-Ds of human and swine on different IAV HA proteins, we showed that swine's SP-D has a higher binding affinity towards the glycans of HA proteins. In addition, starting from crystalized protein structures from X-ray diffraction experiments, our simulations identified the most stable docking configuration of the SP-D-HA complex, revealing how composition and location of glycans affect binding of SP-D to HA.

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Hybrid Molecular Mechanics/Coarse-Grained Calculations Applied to GPCR Receptors

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G-protein coupled receptors (GPCRs) comprise the largest membrane protein superfamily. They are involved in the majority of signaling process in humans and represent the target of a high percentage of the drugs used for clinic. In this context, elaborating a high performance computational strategy to guide drug design represents a highly relevant issue for this group of proteins. Among them, beta adrenergic receptors bind catecholamines, like adrenaline and noradrenaline, and are involved in heart muscle contraction and relaxation. GPCRs display a characteristic seven-transmembrane-domain architecture that is also present in prokaryotes. One example is the sensory rhodopsin II which functions as a light sensor.

Molecular mechanics/Coarse-grained (MM/CG) approaches aim to preserve the molecular detail in the active site, and accelerate the calculations by modeling the rest of the system using a simplified CG potential. in particular, in our approach the MM subsystem is represented with the united atom Gromos96 force field, with explicit solvation, while the CG subsystem is modeled with the Go potential. Additionally, we have included two wall potentials, that allow to represent the lipid bilayer and to avoid water to diffuse away from the active site.

In this work we performed hybrid MM/CG simulations on the B2-adrenergic receptor and sensory Rhodopsin II in presence of their agonist compounds. The simulations with the hybrid methodology were compared with long all atom molecular dynamics simulations and have shown to be able to capture the main structural and dynamical properties of the binding site. The results obtained in this work allow to validate the methodology and its applycability to the study of ligand biding in GPCRs receptors.

320-Pos Board B106

The Heterogeneity of DNA Unwinding by RecBCD Molecules Reflects Individual Conformations Stabilized by Ligand Binding: A Manifestation of the Ergodic Hypothesis

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Single-molecule studies can overcome the complications of asynchrony and ensemble-averaging in bulk-phase measurements, provide mechanistic insights into molecular properties and activities, and reveal interesting variations between individual molecules. The application of these techniques to the RecBCD helicase of *Escherichia coli* has put to rest some long-standing debates and given some mechanistic insights about its functions. Importantly, previous single-molecule analysis showed that the DNA unwinding rates of individual enzyme molecules vary significantly. However, the origin of this heterogeneity was unknown. Here we investigated the question by defining the components of the distribution, and the behavior of individual molecules in the distribution. The distribution of DNA unwinding rates is not unimodal: one third of the molecules unwound DNA significantly slower than the majority of the population. Although any individual RecBCD molecule unwound DNA at a constant rate, we discovered that transiently pausing a single enzyme-DNA complex, by depleting Mg^{2+}-ATP, changed the rates of some enzyme molecules. The proportion of molecules that changed rate increased exponentially with the duration of the pausing, with a half-time of 4 sec, suggesting a conformational change. We suggest that substrate binding locks the enzyme in one of a broad range of conformations that affect the rate-limiting translocation behavior of RecBCD, resulting the molecule-to-molecule variation in the helicase activity.

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Functional Conformational Changes in Lipoxygenase

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Structures of catalytic domains of lipoxygenases, including representatives from mammals, corals, plants and pathogenic bacteria, are similar. All reveal a bent cavity passing close to the non-heme iron involved in catalysis. However, specificity for oxidation at one of several sites on the unsaturated fatty acid substrates lacks an overarching rationale. We have positioned spin labels at selected sites on helices-2, −9, −13, −15 and −22 in soybean lipoxygenase-1 and examined whether the substrate analog lysooleoyllecithin induces conformational changes at any of these sites. Site-specific spin labeling of introduced cysteines was achieved on a cysteine-free background in which each natural cysteine was substituted by serine. EPR spectra revealed conformational changes in helix-2 with lysolecithin binding and with pH variation, while the other sites did not. Site-directed spin labeling of helix-2 includes a spin-label scan of single sites for responses to lyso lecithin and also changes of charged residues in helix-2 to alanines to characterize the pH dependent changes. The spin label sites on the other helices are conformationally invariant and serve as the basis for ongoing of a lipoxygenase structure by paramagnetic distance geometry. All spin labeled lipoxygenase sites examined have enzymatic properties similar to native enzyme.

322-Pos Board B108

Ligand Induced Dynamic Perturbations Highlight Important Regions Leading to Inhibition of Escherichia Coli Phosphofructokinase by Phosphoenolpyruvate

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Escherichia coli phosphofructokinase, a homotetrameric enzyme with a single tryptophan (W311) per subunit, experiences allosteric inhibition by phosphoenolpyruvate (PEP). The four active sites and four allosteric sites beget four unique heterotropic allosteric interactions, identified by the distance between the two sites; 23Å, 30Å, 33Å and 45Å, respectively. Each of the four interactions can be isolated through formation of hybrids containing three inactive tryptophan-minus subunits, and one active subunit retaining a single tryptophan that acts as a fluorescence reporter. Intrinsic fluorescence anisotropy and lifetime measurements are used to calculate the rotational correlation time of various tryptophan-shift mutants, which is used to evaluate how PEP inhibition perturbs the local dynamics around the tryptophan at specific locations. The purpose of this study is to compare the 23Å and 30Å interaction in terms of the ligand-induced perturbations of local dynamics throughout the enzyme. Rotational correlation times have been determined for W233 in unligated enzyme, enzyme with fructose 6-phosphate (F6P) or PEP bound, and enzyme with both F6P and PEP bound, in both the 23Å and 30Å interaction. In the 30Å interaction, perturbations to the rotational correlation time of W233 with both ligands bound (-8.2 ± 0.4ns) is not a sum of the effects from the individual binding of F6P (8.4 ± 0.4) and PEP (-2.4 ± 0.4) alone. This observation suggests that the region around W233 is involved in the allosteric coupling between F6P and PEP. The rotational correlation time of W233 is relatively undisturbed by F6P, PEP or both binding in the 23Å interaction (-1.3 ± 0.1, $0.5 \pm .2$, 0.6 ± 0.1ns respectively). These data suggest that the region around W233 is important for transmission of the allosteric signal in the 30Å interaction but not