Switch II has been identified as the main binding region between kinesin and the microtubule surface.

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Molecular Dynamics Simulations of Monomeric and Dimeric NAC in Alpha-Synuclein at Various Temperatures

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The aggregation of α-Synuclein (αS), a 140-residue presynaptic protein, is thought to be the primary step in the pathology of Parkinson's disease. Several studies have suggested that residues 61-95 of αS, known as the NAC region, may play a critical role in promoting the aggregation of the full-length αS. Furthermore, the first eighteen residues of NAC (i.e., residue 61-79) seem to be essential for the self-assembly of NAC. To better understand the dynamic structures of these proteins and mechanism by which they aggregate, molecular dynamics (MD) simulations have been performed on the NAC region of αS. In our MD simulations, the conformational changes of NAC are more sensitive to increased temperatures than the full-length αS. The initial monomeric NAC model, containing α-helix through the entire sequence, was obtained from the NMR minimized average structure of αS (PDB ID = 1XQ8). The dimeric peptide models were constructed through docking. Atomistic simulations for both monomeric and dimeric NAC in explicit water were conducted for 50 ns at 300 K and 372 K using the CHARMM22/CMAP force field. In simulations with monomeric NAC, the secondary structure of residues 74-84 and 87-92 were largely unchanged at both 300 K and 372 K, while residues 61-73 lost the initial helical structure much faster at 372 K than at 300 K. In simulations with dimeric NAC, a short region encompassing residues 64-71 appeared a higher propensity to form β structure than other regions. Inter-chain β-sheet structure was observed in this region at the beginning of the simulation at 372 K; however, this β-sheet was interrupted when the backbone of one peptide chain folded up, largely due to the electrostatic attraction between residue 61 (i.e., Glu) and 80 (i.e., Lys).

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How Biomolecules Influence Water Structure and Dynamics

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Interactions between solutes and water impact both water structure and structural dynamics as well as solute properties (e.g. conformational fluctuations of proteins). To understand these interactions we investigate water near disaccharides using classical atomistic molecular dynamics simulations. Disaccharides show topological and chemical complexity characteristic of larger biomolecules but are sufficiently small to permit detailed study. We observe that increases in hydrophobicity precisely map slow down in water translation and rotation of local water populations. In line with recent studies of proteins, we find that chemically similar functional groups may interact differently with water depending on neighboring functional groups.

To explain these observations we examine the mechanism of hydrogen bond exchange for waters hydrogen bonded to other waters but within the sugar first solvation shell, as well as waters hydrogen bonded to the sugar. Recent work showed that water in bulk rotates through large angular jumps that pass through bifurcated hydrogen bond intermediates and that rotation rates can be rationalized through transition state theory. Previous reports found that the rotational slow down of water near small solutes can be predicted from changes in the accessible transition state volume or the enthalpy of the hydrogen bonds. For our larger solutes we find that accounting for the transition state volume alone overestimates water rotational slow down. Differences in hydrogen bond enthalpy are also insufficient to predict rotational slowdown. Water slowdown can only be understood by additionally accounting for subtle changes in the free energy landscape associated with water rotation - reduction in the number of available reactant states and broadening of the transition state barriers. The presence of solutes of even moderate size thus affects water dynamics in ways difficult to predict using simple scaling considerations from bulk, making water response system dependent.

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Exploring Signal Transduction Mechanism of HAMP Domains via Molecular Dynamics and Metadynamics

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The HAMP domain is a linker region in prokaryotic sensor proteins which functions in two-component signal transduction pathways. HAMP exhibits a parallel coiled coil motif comprising four helices and transfers the signal from the sensor domain to the transmitter domain. We perfomed MD simulations of a number of HAMP structures in isolation. These structures include wild type and two mutants(A291V, A291F) of HAMP from A. fulgidus(PDB 2ASW) and three HAMPs in the triHAMP chain (PDB 3LNR) from the P. aeruginosa soluble receptor Aer2. Our simulations show that a) 2ASW retains its NMR structure in isolation; b) mutants of 2ASW exhibit slight higher flexibility but relax fast to NMR 2ASW if mutations are removed; c) while the N-terminal HAMP structure in the triHAMP chain is stable when isolated, the C-terminal domain and the inner HAMP show extensive rearrangements, indicating that the N-terminal constrains the flexibility of the triHAMP complex. Using MD in combination with adaptive biasing potentials, known as the metadynamics method, we explored different features of the signal transduction mechanism of HAMP. Our results indicate that HAMP can transfer signals by changing the tilt angle, the hydrophobic packing in the core of the complex and the rotation angle of the helices. The implications of these results for signal transduction proteins containing HAMP are discussed.

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Dominant Site Involved in Inhibition of Targets by Anesthetics Suggested Through Novel Free Energy Perturbation Calculations

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It is well-established that general anesthetics bind directly to many of their targets, but the location of binding sites in a well-investigated target family, the cys-loop receptors, remains unresolved. Although the structure of eukaryotic cys-loop receptors has proven challenging to determine crystographically, a recent prokaryotic anesthetic-sensitive member of the family (GLIC) has been crystallized in complex with both a volatile and injected anesthetic. Detergent molecules occupy the pore of both the apo and liganded GLIC receptor, preventing any direct observation of pore-block by anesthetics from the crystal structure. In recent microsecond atomistic molecular dynamics (MD) simulations of flooding by isoflurane of both the eukaryotic nicotinic acetylcholine receptor (nAChR) and GLIC, we observed two isoflurane molecules bind to the pore of each receptor. Circumventing the experimental obstacles, computational measurements of binding affinity to the pore site provide an alternate method for determining the relevance of pore-block to GLIC inhibition by anesthetics. In addition to a calculated binding affinity for isoflurane in the pore that corresponds well to the micromolar concentrations at which inhibition is observed, we also present novel measurements using the alchemical free energy perturbation method (FEP) to quantify negative cooperativity between the two isoflurane molecules in the pore. The low Hill coefficient (<0.5) calculated in this manner corresponds to that measured experimentally within 10%. The results suggest that the pore is probably occupied in GLIC exposed to experimental concentrations of isoflurane, and pore-block may provide the dominant source of inhibition.

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Computer Simulation Studies of an Antimicrobial Peptide and a Microbial Virulence Factor: Statistical Convergence of Equilibrium Properties from Massive Sampling

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Critical to the immune system of eukaryotes, antimicrobial peptides (AMPs) provide a line of defence against invading bacteria, often disrupting the integrity of the bacterial membrane. Concurrently, bacteria repair this damage by the action of their own integral membrane proteins. To gain insight into the molecular basis of AMP action and membrane repair, we used one third of the world's 28th fastest computer for one year to run massively repeated molecular dynamics simulations of one such antagonistic pair: the eukaryotic AMP indolicidin and the bacterial outer membrane enzyme and virulence factor PagP in explicit membranes. Attaining adequate statistical sampling of conformational space from molecular simulations of peptides and proteins in membranes is challenging because of the long correlation times of lipid bilayers. We analyze the convergence of equilibrium properties in these two systems. We first investigate the binding free energy of indolicidin to a lipid bilayer using umbrella sampling, simulating each window for 1.5 microseconds and repeating this simulation 30 times using different starting conformations. These simulations demonstrate the existence of long-lived metastable states, particularly when the peptide resides at or near the lipid-water interface. We show how many independent simulations reveal information that would likely be missed or misinterpreted if only a single simulation was performed. Second, we characterize the functional binding mode of PagP, a bacterial outer membrane acyltransferase that binds and catalyses its lipid solvent. 100 independent 5-microsecond simulations reveal the functional binding mode and associated gating mechanism. From these simulations, which total 2 milliseconds of simulation time, it is clear that the difficulty of reaching statistical convergence in atomistic