Protein Adsorption Simulation – Calculation of Free Energy Using Biased Sampling

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Statement of Purpose: The study of protein adsorption to surfaces is of critical importance in the field of biomaterials. At this time relatively little is understood regarding the molecular level events that control these interactions; however, with recent advances in the field of molecular dynamics (MD) simulation and exponentially increasing computational resources, the molecular mechanisms involved in protein-surface adsorption can be studied in unprecedented detail. MD studies employ a potential energy function (referred to as a *force field*) to describe the force vectors between atoms in a molecular system. These forces are then used in Newton's equations of motion to calculate atomic motions, which are then analyzed using statistic mechanics relationships to determine thermodynamic and kinetic properties of the system. The force fields currently used in protein adsorption simulations, such as *CHARMM* [1], have been primarily developed for predicting the behavior of proteins folding behavior in solution. The main objective of this study was to evaluate the validity of the *CHARMM* force field to simulate protein adsorption onto synthetic surfaces by calculating the free energy of adsorption (ΔG_{ads}) for peptide adsorption from MD simulations using *CHARMM* for comparison with experimental results determined by surface plasmon resonance (SPR) [2].

Methods: In our initial studies, three peptides on two different functional surfaces were simulated using a host-guest peptide model designed with the amino acid sequence of TGTG-X-GTGT, where T & G are threonine and glycine and X is either valine (nonpolar), aspartic acid (negatively charged), or threonine (polar). Methyl-terminated (hydrophobic) and hydroxyl-terminated (hydrophilic) self-assembled monolayers (SAMs) were used as surface models. All simulations were performed at 298 K in physiological saline (TIP3P water plus 150 mM Na^+/Cl^- ions). ΔG_{ads} was calculated using the probability ratio method by the following equation:

$$\frac{p_i}{p_\infty} = \exp\left(-\frac{\Delta G_i}{RT}\right), \text{ or } \Delta G_i = -RT \ln\left(\frac{p_i}{p_\infty}\right) \qquad (1)$$

where P_i is the probability of the peptide being at a given surface separation distance (SSD_i) over the SAM surface, P_∞ is the probability of the peptide being sufficiently far from the surface to represent bulk solution conditions, R is the ideal gas constant, T is absolute temperature, and ΔG_i is the free energy state of the peptide at position SSD_i. The values of P_i are determined from the MD simulation and then integrated over SSD to calculate ΔG_{ads} for comparison with experimental SPR measurements. The determination of ΔG_{ads} is problematic because peptides become trapped in low-energy states close to the surface in a normal MD simulation [3]. This causes two types of sampling problems; one due to the peptide being trapped at the surface and the other due to incomplete conformational sampling of the peptide during the adsorption process. To solve this, we are conducting umbrella sampling [4] of the peptide as a function of its SSD. A potential of mean force (PMF) *vs.* SSD profile is extracted using statistical methods [5], which represents the ΔG_i vs. SSD profile. This profile is fitted with a DLVO function [6], the inverse of which is then applied as a biasing energy in replica exchange MD (REMD) simulations. REMD is an advanced sampling method that uses temperature to facilitate the crossing of activation energy barriers within a molecular system to enable the conformational space of a system to be properly sampled within a given simulated time. The biased REMD simulation thus provides sufficient sampling of both SSD and peptide conformation for the calculation of ΔG_{ads}.

Results and Discussion: Fig. 1 shows an example of a PMF vs. SSD profile, which was used to generate the biasing energy function for the biased REMD simulations.

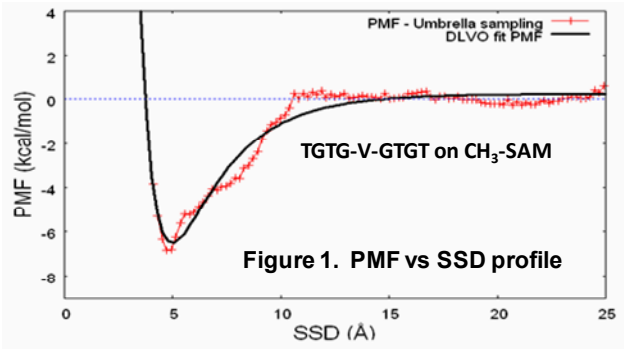

Figure 1. PMF vs SSD profile

Middle "X" residue	Free Energy (kcal/mol) ± 95% CI	
	Simulation	Experiment
Methyl SAM surface		
Valine	-4.33 ±0.74	-4.40 ±0.31
Threonine	-2.04 ±0.23	-2.76 ±0.28
Aspartic Acid	-0.62 ±0.34	-3.54 ±0.60
Hydroxyl SAM surface		
Valine	-0.24 ±0.10	-0.02 ±0.01
Threonine	-0.46 ±0.43	-0.00 ±0.01
Aspartic Acid	-0.23 ±0.05	-0.02 ±0.03

The above table summarizes the calculated and experimental ΔG_{ads} values from our simulations.

Conclusions: The force-field was not able to exactly match the experimental results thus indicating that parameter adjustment is needed before this force field can be used to accurately represent protein adsorption behavior. We are currently using these results as a basis for force field reparameterization to create a validated *CHARMM* force field for protein adsorption simulations.

References: [1]MacKerell et al., J.Phys.Chem B, 1998; [2] Yang et al., Langmuir 2008; [3] Raut et al., Langmuir 2005; [4] Torrie et al. J.Comp.Phys. 1977. [5] Kumar et al., J. Comput.. Chem 1992; [6] Wang et al., Biointerphases 2008.

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