Calculation of Free Energy of Peptide-Surface Interactions Using Biased-Sampling Molecular Dynamics

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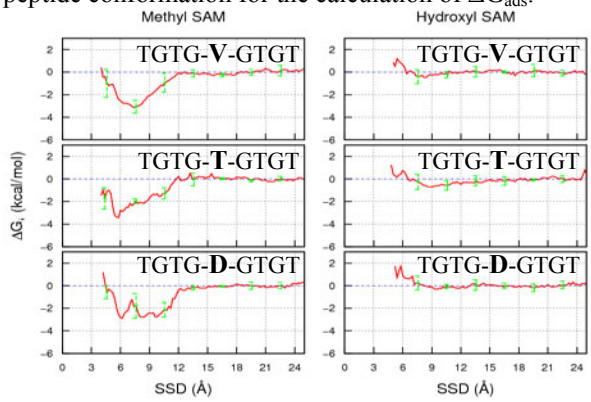
Statement of Purpose:
The study of protein adsorption to surfaces is of critical importance in the field of biomaterials. At this time relatively little is understood regarding the molecular level events that control these interactions; however, with recent advances in the field of molecular dynamics (MD) simulation and exponentially increasing computational resources, the molecular mechanisms involved in protein-surface adsorption can be studied in unprecedented detail. MD studies utilize a potential energy function (referred to as a *force field*) which is a collection of empirical equations and parameters that are used to calculate the energy of a molecular system as a function of atomic position. The force fields currently used in protein adsorption simulations, such as *CHARMM*,[1] have been primarily developed for predicting the behavior of protein folding in solution. The objective of this study was to evaluate the validity of using *CHARMM* to simulate protein adsorption on material surfaces by calculating the free energy of adsorption (ΔG_{ads}) for peptides from MD simulations using *CHARMM* for comparison with experimental results determined by surface plasmon resonance (SPR).[2,3]

Methods: 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, respectively, and X is either valine (V), threonine (T), or aspartic acid (D). 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 with 150 mM Na^+/Cl^- ions). ΔG_{ads} was calculated using the probability ratio method by equation (1), where P_i is the probability of the peptide being at

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

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 relative to bulk solution. The values of P_i are determined from the 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.[4] 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 performed umbrella sampling[5] of the peptide as a function of SSD. A potential of mean force (PMF) *vs.* SSD profile is extracted using statistical methods[6], which represents an initial estimate of the ΔG_i vs. SSD profile. This profile is fitted with a function, the inverse of which is then applied as a biasing energy in replica exchange MD (REMD) simulations[7]. 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 efficiently sampled. The biased-REMD simulation thus provides sufficient sampling of both SSD and peptide conformation for the calculation of ΔG_{ads}.



Results and Discussion: Each simulation was performed for 5 ns. The figure presented above shows the ΔG_i *vs.* SSD profiles from the biased-REMD simulations on methyl- and hydroxyl-SAM surfaces. All simulations were performed in triplets for calculating statistics.

Middle "X" residue	ΔG_{ads} (kcal/mol) ± 95% CI	
	Simulation	Experiment
Methyl-SAM surface		
Valine	-2.13 ± 0.62	-4.40 ± 0.31
Threonine	-2.30 ± 0.78	-2.76 ± 0.28
Aspartic Acid	-2.08 ± 0.27	-3.54 ± 0.60
Hydroxyl-SAM surface		
Valine	-0.03 ± 0.32	-0.02 ± 0.01
Threonine	-0.18 ± 0.34	-0.00 ± 0.01
Aspartic Acid	+0.11 ± 0.38	-0.02 ± 0.03

The above table summarizes the calculated ΔG_{ads} values from our simulations with comparison to experimental values[2,3].

Conclusions: The CHARMM force-field substantially underestimates the strength of adsorption on the hydrophobic surface while providing close agreement for adsorption on the hydrophilic surface. These results indicate that parameter adjustment is needed before this force field can be generally used to accurately represent protein adsorption behavior. We are currently evaluating 38 other peptide-SAM systems to compare with the SPR experimental results published by our group[3]. These results will be used as a basis for force field re-parameterization to create a validated *CHARMM* force field for protein adsorption simulations.

References: [1] MacKerell et al., J.Phys.Chem B, 1998; [2] Wei et al., Langmuir 2008; [3] Wei et al. Langmuir 2009. [4] Raut et al., Langmuir 2005; [5] Torrie et al. J.Comp.Phys. 1977. [6] Kumar et al., J. Comput.. Chem 1992; [7] O'Brien et al., Langmuir, 2008.

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