Estimation of Peptide-Surface Adsorption Free Energy for Material Surfaces Not Conducive to SPR and QCM using AFM

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Statement of Purpose: Understanding the fundamental factors controlling the interactions between peptides and proteins with material surfaces is of fundamental importance in many areas of biotechnology, including biosensors, enzyme-based technologies, regenerative medicine, implants, and biodefense. While surface plasmon resonance spectroscopy (SPR) and quartz crystal microbalance (QCM) methods have proven to be very useful for measuring the free energy of adsorption for peptide-surface interactions, these methods are largely restricted to use for materials that can readily form nanoscale-thickness films over the respective sensor surfaces. Many materials, however, including many types of polymers, ceramics, and inorganic glasses, are not readily suitable for use SPR and QCM methods. To address this limitation, we have recently conducted an atomic force microscopy (AFM) study[1] to show that desorption forces (F_{ads}) obtained from a standardized AFM method are linearly correlated to standard state adsorption free energy values (ΔG^{o}_{ads}) measured from SPR.[2] This provides a means to estimate ΔG^{o}_{ads} for peptide adsorption using AFM that can be applied to any flat materials surface. However, this initial AFM study was limited to a relatively small data set. The objective of the current study was therefore (*i*) to generate a more complete desorption force-free energy correlation for peptide adsorption on microscopically flat surfaces comparing AFM and SPR results, and (*ii*) to apply the developed correlation to predict ΔG^{o}_{ads} for a materials not conducive for use with SPR or QCM methods.

Materials and Methods

Host-Guest Peptide Model: A host-guest peptide was designed with an amino acid (AA) sequence of TGTG-X-GTCT with zwitterionic end groups. The types of AA residues used for X are shown in Table 1. Peptides were attached via the C residue to the AFM tip by a 3.4 kDa PEG tether as illustrated in Figure 1.

Table 1. Types of AAs Selected for the Guest X Residue

-X- residue	Side Chain	Property
Valine (V)	$-CH(CH_3)_2$	Non-polar
Glycine (G)	-H	Non-chiral
Phenylalanine (F)	$-CH_2-C_6H_5$	Aromatic
Tryptophan (W)	$-CH_2$-indole ring (C_8H_6N)	Aromatic
Threonine (T)	$-CH(CH_3)OH$	Neutral polar
Asparagine (N)	$-CH_2-CO-NH_2$	Neutral polar
Aspartic Acid (D)	$-CH_2COO^-$ (pK=3.97)	Negatively charged
Lysine (K)	$-(CH_2)_4-NH_3^+$ (pK=10.78)	Positively charged

Material Surfaces: Self-assembled monolayer (SAM) surface on gold were selected to provide a wide range of functionalities. These SAMs include hydrophobic surfaces (CH_3 and OC_6H_5), hydrophilic surfaces (OH, EG_3OH, $NHCOCH_3$, and $COOCH_3$), and partially charged surfaces ($COOH/COO^-$ and NH_2/NH_3^+). Poly(methyl-methacrylate) (PMMA), high density polyethylene (HDPE), quartz, and fused silica glass were then tested to estimate ΔG^{o}_{ads} from the developed force-energy correlation.

SPR Measurement of ΔG^{o}_{ads}: ΔG^{o}_{ads} was determined by SPR using methods developed by our group using a TGTG-X-GTGT peptide model.[2] This method was specifically designed for SPR to enable bulk-shift effects to be directly determined and to enable ΔG^{o}_{ads} to be calculated with minimal peptide-peptide influences.

AFM Measurement of Desorption Force: AFM was conducted as illustrated in Figures 1 and 2 to measure the force to displace the peptide from the surface.

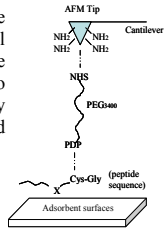
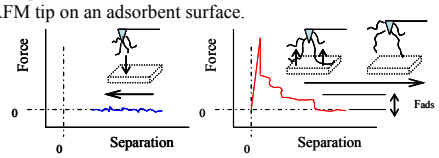


Fig. 1. AFM Tip linkage. Peptide sequences are coupled to AFM tips via a polyethylene glycol (PEG) crosslinker. The n-hydroxy-succinimide (NHS) end of the PEG is covalently bound to amines on the tip before the peptide is directly attached to the pyridyldithio-propionate (PDP) end via cysteine.

Fig. 2. Typical AFM force-separation curves recorded during adsorption-desorption of peptide sequences that are covalently attached to an AFM tip on an adsorbent surface.



Results and Discussion. Studies were first conducted using the SAM surfaces, which could be used with both SPR and AFM in order to evaluate the correlation between the pull-off force measured by AFM and ΔG^{o}_{ads} determined by SPR (Fig. 3). These combined results showed high correlation between F_{ads} and ΔG^{o}_{ads} (overall $R^2 = 0.96$). F_{ads} was then determined for the peptides interacting PMMA, HDPE, quartz, and glass surfaces by AFM, with ΔG^{o}_{ads} then estimated from the correlation shown in Figure 3.

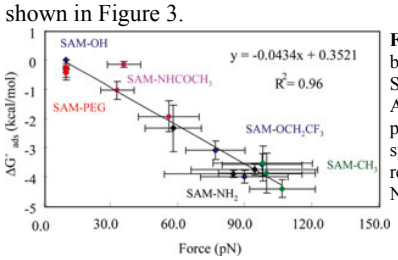


Fig 3. Correlation between $\Delta G°_{ads}$ by SPR and F_{ads} by AFM for a set of peptide-SAM systems. (Error bar represents 95% C.I.; N = 3.)

Table 2. F_{ads} measurements and $\Delta G°_{ads}$ estimation for TGTG-V-GTCT on selected surfaces in PBS; pH=7.4. * Mean (± 95% confidential interval), N = 3. $^{\#}\Delta G^{o}_{ads}$ estimated from the correlation derived from Fig 3.

Material	PMMA	HDPE	Glass	Quartz
* F_{ads} (pN)	35 (7)	65 (12)	20 (6)	10 (1)
$^{\#}\Delta G°_{ads}$ (kcal/mol)	- 1.2(0.8)	-2.5(0.8)	-0.5(0.8)	-0.1(0.8)

Concluding Remarks. These results show that high correlation exists between F_{ads} obtained from our standardized AFM method and ΔG^{o}_{ads} from SPR for a wide range of peptide-SAM surface systems. This correlation can then be used to estimate ΔG^{o}_{ads} for systems not conducive for use with SPR or QCM. These methods can provide important insights into the thermodynamics governing protein–surface interactions and useful data to validate parameters needed for molecular simulation.

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Refs: 1. Wei, Latour. Langmuir, in press. 2. Wei, Latour. Langmuir, 25: 5637-46 (2009).