Correlation Between Desorption Force Measured by Atomic Force Microscopy (AFM) and Adsorption Free Energy Measured by Surface Plasmon Resonance Spectroscopy (SPR) for Peptide-Surface Interactions

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Statement of Purpose. SPR is a useful technique for measuring peptide-surface interactions; however, its usefulness is limited to materials that can form nanoscale-thick films on a metallic surface. AFM, on the other hand, can be used with any flat surface, thus making it more versatile for studying peptide-surface interactions. AFM, however, has the drawback of data interpretation due to questions regarding peptide probe-tip density. This problem could be overcome if results from AFM could be correlated with SPR results for a similar set of peptide-surface interactions so that AFM studies using a standardized method could be extended to characterize peptide-surface interactions for surfaces that are not amenable for characterization by SPR. The aims of this research were to conduct AFM studies to measure peptide-surface adhesion forces for a similar set of peptide-surface systems for which adsorption free energy (ΔG^{o}_{ads}) values are available from SPR measurements,[1,2] to determine if a linear correlation exists between the AFM and SPR data, and to then apply these methods to estimate the values of ΔG^{o}_{ads} for peptide-surface systems that cannot be tested by SPR.

Materials and Methods

Host-Guest Peptide Model A host-guest peptide was designed with an amino acid sequence of TGTG-X-GTCT with zwitterionic end groups. The peptides were attached via the C amino acid to the AFM tip by a 3.4 kDa PEG tether as illustrated in Figure 1.

Adsorbent Surfaces Self-assembled monolayer (SAM) surfaces on gold were selected to provide a wide range of functionality, including SAM-CH_3, -OCH_2CF_3, -OH, -EG_3OH, -$NHCOCH_3$ and -NH_2/NH_3^+. Teflon®, quartz and 304-stainless steel surfaces were also tested to extend the AFM technique to surfaces not readily suited for SPR.

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.[1,2] 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 influence from peptide-peptide interactions.

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

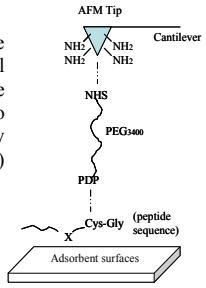
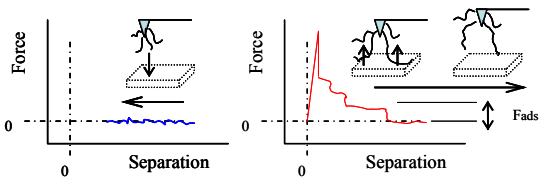


Fig. 1. AFM Tip linkage. Peptide sequences are coupled to AFM tips via a polyethylene glycol (PEG) crosslinker. The n-hydroxy-succinimide (NHS) end of the PEG is covalently bound to amines on the tip before the peptide is directly attached to the pyridyldithio-propionate (PDP) end via cysteine.

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. Fig. 3 presents ΔG^{o}_{ads} data from SPR to compare the effect of substituting C for G for the 8th amino acid residue in the peptide to provide a site to tether the peptide to the tip for the AFM studies. As shown, this substitution had minimal influence on the adsorption behavior of this peptide. Once this was confirmed, a set of AFM studies were conducted for a series of peptide-SAM surface systems with the measured AFM pull-off force plotted against the corresponding benchmark values of ΔG^{o}_{ads} determined by SPR (Fig. 4). As shown from this plot, the AFM force results are linearly related to the ΔG^{o}_{ads} results by SPR, with a correlation coefficient of $R^2 = 0.93$. AFM testing was then applied to three different material surfaces that are not readily amenable for thin-film formation as needed for ΔG^{o}_{ads} determination by SPR, with the linear relationship from Fig.4 used to estimate the ΔG^{o}_{ads} values for each of these peptide-surface systems (Table 1).

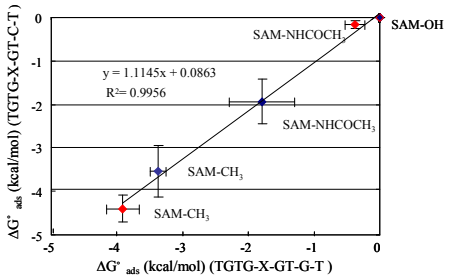


Fig 3. Comparison of experimentally measured $\Delta G°_{ads}$ by SPR between TGTG-X-GT**C**T and the sequence TGTG-X-GT**G**T.[2] X= valine (red) and aspartic acid (blue) on different SAMs. (Error bar: 95% C.I., N ≥ 3).

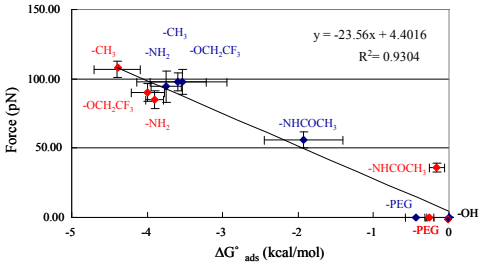


Fig 4. Correlation between $\Delta G°_{ads}$ by SPR and force by AFM for an equivalent set of peptide-SAM systems. Midchain amino acid (X) = valine (red) and aspartic acid (blue) on different SAMs in PBS; pH=7.4. (Error bar represents 95% C.I.; N = 60 for AFM, N ≥ 3 for SPR).

Table 1. Force measurements and $\Delta G°_{ads}$ estimation for TGTG-D-GTCT on selected surfaces in PBS; pH=7.4.

Material	304-S.S.	Teflon	Quartz
* Force $_{adsorption}$ (pN)	111 (9)	91 (10)	48 (13)
#$\Delta G°_{ads}$ (kcal/mol)	− 4.5	− 3.7	− 1.9

* Mean (± 95% confidence interval), N = 30.
#$\Delta G°_{ads}$ estimated from the correlation derived from data in Fig 4.

Concluding Remarks

These results show that the desorption force obtained from a standardized AFM test method to characterize peptide-surface interaction is linearly correlated with ΔG^{o}_{ads} measured from SPR, thus providing a means to estimate ΔG^{o}_{ads} for peptide-surface systems that are not amenable for evaluation by SPR.

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Refs: [1] Wei and Latour, Langmuir, 24: 6721-29 (2008).
[2] Wei and Latour, Langmuir, 25: 5637-46 (2009).