Published in final edited form as:

Langmuir. 2012 April 3; 28(13): 5687–5694. doi:10.1021/la300315r.

Determination of Peptide–Surface Adsorption Free Energy for Material Surfaces Not Conducive to SPR or QCM using AFM

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Abstract

The interactions between peptides and proteins with material surfaces are of primary importance in many areas of biotechnology. While surface plasmon resonance spectroscopy (SPR) and quartz crystal microbalance (QCM) methods have proven to be very useful in measuring fundamental properties characterizing adsorption behavior, such as the free energy of adsorption for peptide–surface interactions, these methods are largely restricted to use for materials that can readily form nanoscale–thick films over the respective sensor surfaces. Many materials including most polymers, ceramics, and inorganic glasses, however, are not readily suitable for use with SPR or QCM methods. To overcome these limitations, we recently showed that desorption forces (F_{des}) obtained using a standardized AFM method linearly correlate to standard state adsorption free energy values (ΔG^o_{ads}) measured from SPR in phosphate buffered saline (PBS: phosphate buffered 140 mM NaCl, pH 7.4). This approach thus provides a means to determine ΔG^o_{ads} for peptide adsorption using AFM that can be applied to any flat material surface. In this present study, we investigated the F_{des} : ΔG^o_{ads} correlation between AFM and SPR data in PBS for a much broader range of systems including eight different types of peptides on a set of eight different alkanethiol self-assembled monolayer (SAM) surfaces. The resulting correlation was then used to estimate ΔG^o_{ads} from F_{des} determined by AFM for selected bulk polymer and glass/ceramic materials such as the poly(methyl-methacrylate) (PMMA), high-density polyethylene (HDPE), fused silica glass (SiO₂), and a quartz (100) surface. The results of these studies support our previous findings regarding the strong correlation between F_{des} measured by AFM and ΔG^o_{ads} determined by SPR, and provides a means to estimate ΔG^o_{ads} for peptide adsorption on macroscopically thick samples of materials that are not conducive for use with SPR or QCM.

I. INTRODUCTION

The interactions between peptides and proteins with material surfaces are of primary importance in many areas of biotechnology, including biosensors,^{1, 2} enzyme-based technologies,^{3, 4} regenerative medicine,^{5, 6} implants,^{7–9} and biodefense.^{10, 11} SPR^{12–14} and QCM^{15, 16} are two powerful label-free detection techniques that have been applied for many applications to characterize protein-surface or peptide-surface interactions due to high sensitivity and the capability to quantify peptide or protein adsorption/desorption on surfaces in real time. One such application using SPR that was developed by our lab^{17, 18} involved the estimation of adsorption free energy (ΔG^o_{ads}) for a host–guest peptide (TGTG-X-GTGT, where G and T are glycine and threonine, and X represents a “guest” amino acid residue) on

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Supporting Information Available This information is available free of charge via the Internet at <http://pubs.acs.org>. This supporting information contains (i) the brief descriptions about the experimental procedures for the SPR studies and how to calculate ΔG^o_{ads} from corresponding SPR signals and (ii) detailed coupling chemistry for the AFM tip assembly and the resulting values from force–separation curves by AFM for desorption force corresponding to peptide on SAM surfaces in PBS solution.

alkanethiol self-assembled monolayer (SAM) surfaces. We developed this method to not only provide a quantitative characterization of peptide–surface interactions but also, very importantly, to provide the kind of experimental data that are sorely needed (and largely lacking) to be able to assess, modify, and validate empirical force fields that are utilized in molecular modeling to enable peptide and protein adsorption behavior to be reliably represented in a molecular simulation.^{19,20}

However, one of the limitations in using SPR or QCM for generating thermodynamic dataset of peptide–surface interactions is that these methods require nanoscale–thick surfaces to be coated on a sensor chip (e.g., nanoscale layer of coatings on a gold sensor–chip for SPR or on a quartz sensor–chip for QCM), thereby limiting the materials that could be tested.^{21–23} Hence, while the use of methods such as SPR and QCM are readily amenable for use with SAMs on gold–coated sensor chips,^{17, 24, 25} their use becomes problematic for a broad range of other common material surfaces that may need to be evaluated, therefore necessitating the development of alternative methods to quantitatively evaluate the adsorption behavior of peptides on surfaces of bulk material samples.

To address this limitation, we recently developed an AFM technique that was shown to provide strong linear correlation with ΔG^o_{ads} values obtained by SPR.²⁶ This AFM protocol²⁶ involved tethering a host–guest peptide (TGTG–X–GTCT with X = valine (V), aspartic acid (D), or leucine (L)) to a standardized AFM tip with a 3.5 kDa polyethylene glycol (PEG) spacer via the thiol side–group from cysteine (C) to enable the peptide to interact with the surface in a nearly unhindered manner. This method was then used to measure the force required to desorb the peptide (F_{des}) from a set of SAM surfaces that could also be used with SPR. Comparisons between F_{des} from AFM and ΔG^o_{ads} obtained by SPR showed that these values were strongly correlated. We then proposed that the generated correlation provides a means to estimate the values of ΔG^o_{ads} for peptide–surface systems that cannot be readily tested by SPR or QCM, thus providing the capability of quantitatively characterizing peptide adsorption behavior to any material with a microscopically flat surface irrespective of the thickness of the sample.^{27–29}

The correlation between F_{des} from AFM and ΔG^o_{ads} by SPR that we obtained from our previous study,²⁶ however, was done using a relatively small data set of only 18 different peptide–SAM systems (i.e., three host–guest peptides on six SAM surfaces). Therefore, the objective of this current research was to conduct experiments using our standardized AFM protocol for a much broader set of peptide–SAM systems to further investigate the correlation of F_{des} with the ΔG^o_{ads} values that we had obtained from our previous large set of SPR studies.¹⁸ If the same linear correlation could be found, the subsequent objective of this work was then to apply this AFM method and the resulting F_{des} : ΔG^o_{ads} correlation to determine effective values of ΔG^o_{ads} for the interaction of peptides with material samples that are not conducive for use with SPR or QCM, thus demonstrating how this method may be applied to expand capabilities to study the thermodynamics of peptide–surface interactions for a much broader range of materials.

II. EXPERIMENTAL METHODS

II.a. Surface Preparation and Characterization

The surfaces used in this study include alkanethiol self-assembled monolayers (SAMs) on gold, fused silica glass, quartz (100) surface plane, high density polyethylene (HDPE), and poly(methyl–methacrylate) (PMMA).

II.a.1. Preparation of Alkanethiol SAM Surfaces—The alkanethiol SAM monolayers had a general structure of $\text{HS}(\text{CH}_2)_{11}\text{--Y}$, with Y representing a variable surface functional

group: Y = OH, CH₃, OC₆H₅, NH₂, COOH, NHCOCH₃, COOCH₃, and EG₃OH (EG: ethylene glycol segment, $-(O-CH_2-CH_2)-$) (alkanethiols were purchased from Aldrich, Milwaukee, WI, USA; Prochimia, Sopot, Poland; or Assemblon, Redmond, WA, USA). The bare gold surfaces were purchased from Biacore (SIA Au kit, BR-1004-05, Biacore, Inc., Uppsala, Sweden). Prior to coating, the gold substrates were cleaned by sonicating (Branson Ultrasonic Corporation, Danbury, CT) in (a) "piranha" (7:3 (v/v) H₂SO₄ (Fisher Scientific, Fair Lawn, NJ)/H₂O₂ (Fisher Scientific, Fair Lawn, NJ, USA), and (b) basic solution (1:1:3 (v/v/v) NH₄OH (Fisher Scientific, Fair Lawn, NJ, USA)/H₂O₂/H₂O) at 50°C for 1 minute. After each stage of the washing process, the gold slides were rinsed in nano-pure water and dried under a steady stream of nitrogen gas (National Welders Supply Co., Charlotte, NC, USA). The cleaned slides were then rinsed with 100% (absolute) ethanol and incubated in the appropriate alkanethiol solution (1.0 mM in absolute ethanol (Pharmco-Aaper, Shelbyville, KY)). Because amine-terminated alkanethiols have the general tendency to form an upside-down layer and/or multilayers when adsorbed in their protonated state³⁰ the solution pH for this surface was shifted to pH $\approx$ 12 by adding a few drops of triethylamine solution (Sigma Chemical Co., St. Louis, MO) to the alkanethiol solution to minimize this occurrence. All slides were stored in their respective alkanethiol solution for at least 16 hours in the dark. Prior to use, all slides were sonicated in absolute ethanol and then in nano-pure water, and dried under a steady stream of nitrogen gas.

II.a.2. Preparation of Material Surfaces—Fused silica glass from Chemglass Life Sciences (Vineland, NJ) and quartz plates with (100) surfaces from MTI Corporation (Richmond, CA) were used as the silica substrates. HDPE and PMMA surfaces were spin-coated from dodecalin (0.5% (w/w) at 1500 rpm for 60s) and chloroform solutions (1.5% (w/w) at 1000 rpm for 60s), respectively, onto the fused silica glass slides. All chemicals such as the monomers of HDPE (Mw=125,000, Sigma Chemical Co., St. Louis, MO) and PMMA (Mw=350,000, Sigma Chemical Co., St. Louis, MO) as well as the solvents such as dodecalin (Sigma Chemical Co., St. Louis, MO) and chloroform (EMD Chemicals, Gibbstown, NJ) were used as supplied by the manufacturer. Prior to being used for desorption force measurement by AFM, all material surfaces were sonicated (Branson Ultrasonic Corporation, Danbury, CT) at room temperature for 30 min in 0.3 vol. % Triton X-100 (Sigma Chemical Co., St. Louis, MO).

II.a.3. Characterization of Material Surfaces—Surface characterization was performed to determine the static air–water contact angle, atomic composition, film thickness, and surface roughness of the substrates used. For all the surfaces, the static air–water contact angle values were analyzed using a CAM 200 optical contact–angle goniometer (KSV Instruments Inc., Monroe, CT). Similarly, for all the surfaces the atomic compositions were verified via X-ray photoelectron spectroscopy (NESCA/BIO, University of Washington, Seattle, WA) and the averaged surface roughness was analyzed using MFP-3D AFM (Asylum Research, Santa Barbara, CA) over an area of 5 μ m $\times$ 5 μ m. The film thickness of the SAMs and polymer films were characterized using a GES-5 variable angle spectroscopic ellipsometer (Sopra Inc., Palo Alto, CA).

II.b.1. Host–Guest Peptide Model—For these studies, we used a unique custom–designed peptide model, which was introduced in our previous papers.^{18, 26} The host–guest model peptides, which were synthesized by Biomatik (Wilmington, DA) and characterized by analytical HPLC and mass spectral analysis with at least 98% purity, were designed with the amino acid sequence of TGTG–X–GTGT or TGTG–X–GTCT with zwitterionic end groups, where G, T, and C are glycine (–H side–chain), threonine (–CH(CH₃)OH side–chain), and cysteine (–CH₂SH side chain), respectively. The X represents a "guest" amino acid residue, which was positioned in the middle of the peptide to best represent the

characteristics of a mid-chain amino acid in a protein by positioning it relatively far from the zwitterionic end groups. The threonine residues and the zwitterionic end groups were selected to enhance aqueous solubility and provide additional molecular weight for SPR detection while the nonchiral glycine residues were selected to inhibit the formation of secondary structure, which, if present, would complicate the adsorption process.

TGTG-X-GTGT was initially used in our previous SPR studies^{17, 18} while the TGTG-X-GTCT was used in place of TGTG-X-GTGT and specially designed for AFM force measurements²⁶. The cysteine (C) residue was required for the AFM studies as the linker to connect our host-guest peptide sequences to the AFM tip via a poly(ethylene-glycol) (PEG) cross-linker (3.4-kDA pyridyldithio poly(ethylene-glycol) succinimidylpropionate (PDP-PEG-NHS), Creative PEGWorks, Winston Salem, NC; polydispersity index = 1.08)²⁶ (Fig. 1). To address concerns of the effect of the swapping of 'G' in TGTG-X-GTGT for 'C' in TGTG-X-GTCT on the adsorption behavior, we previously conducted studies²⁶ to confirm that cysteine could be incorporated into this host-guest peptide model without significantly changing the free energy of adsorption of the peptide. By confirming this equivalence, our previously determined ΔG_{ads}^o values¹⁸ measured by TGTG-X-GTGT could then be directly correlated with the AFM results using the modified peptide model, TGTG-X-GTCT. Host guest peptides with eight different "guest" amino acid residues (-X-) were used in these studies, which as presented in Table 1 along with their side-chain chemical structure and their characteristic property.

II.c. Solvent Environment

All peptide-surface interactions were investigated in phosphate buffered saline (PBS, Fisher Scientific, Fair Lawn, NJ) at a bulk solution pH of 7.4. PBS is a complex mixture of salts in aqueous solution (140 mM sodium chloride, 10 mM sodium phosphate, 2.7 mM potassium chloride, and 1.8 mM potassium phosphate). The buffer solutions were filtered and degassed prior to use.

II.d. Determination of ΔG_{ads}^o by SPR

The adsorption experiments from SPR in this current study were conducted using a Biacore-X SPR spectrometer (Biacore, Inc., Piscataway, NJ) in PBS. The detailed methods have been previously described.^{17, 18} Briefly, the SPR sensorgrams were recorded in the form of resonance units (RU; 1 RU = 1.0 pg/mm²)³² as a function of time for six independent runs of peptide concentrations over each SAM surface at 25°C. The data obtained were then used to generate isotherm curves by plotting the raw SPR signal as a function of peptide solution concentration. The equations that were used for the determination of ΔG_{ads}^o from the adsorption isotherms were then derived based on the chemical potential of the peptide in its adsorbed and bulk solution state.¹⁸ Examples of adsorption isotherms and the equations to calculate adsorption free energy are presented in the Supporting Information (Fig. S.1 & S.2 and Eqns. (S.1) & (S.2)).

II.e. AFM Studies

High-resolution force spectroscopy measurements were conducted using AFM (MFP-3D instrument, Asylum Research, Santa Barbara, CA) with DNP-10 silicon nitride cantilevers (Veeco Nanofabrication Center, Camarillo, CA). As indicated in Fig. 1, the host-guest peptide sequences were tethered to AFM tips via a PEG cross-linker, PDP-PEG-NHS. Detailed coupling chemistry of AFM tip assembly is listed in the Supporting Information (Section S.II.a.).

II.e.1. Surface Density of Peptides Tethered to AFM Tip—Imaging of the AFM tips was performed with an Hitachi 4800-field-emission gun-scanning electron microscope

(FEG–SEM). Tip radii, which were about 32 nm, were measured by drawing a circle on the captured images such that the arc of the circle coincided with the curvature on the end of the tip.

The effectiveness of our tip coupling chemistry was developed and demonstrated by Hinterdorfer, et al. and Ebner, et al., by showing that it could be used to detect individual antibody–antigen recognition events by AFM.^{33, 34} From their studies with similar tip chemistry (coupling chemistry shown in Fig. 1), the surface density of protein attached to the probe tip was determined and calibrated by a sensitive high–resolution fluorescence imaging method and enzyme chip assay, which showed about 1,500–2,000 molecules per square micron. This molecular surface density translates to about ten peptide molecules attached to the AFM tip, which has an estimated surface area from a Hertzian model (contact–mechanics model used in AFM–based techniques)³⁵ of $\sim 6,000 \text{ nm}^2$ for the AFM tips used in our studies (surface area of hemi–spherical AFM tip end = $2\pi R^2$; $R \sim 32 \text{ nm}$). Although there is uncertainty in the probe density of tethered peptides, as long as the AFM force measurements for peptide–surface interactions are obtained using a standardized methodology, similar probe tip densities (although unknown) could be expected and therefore could be correlated to the free energy of adsorption for a common set of peptide–surface systems determined by SPR.

II.e.2. Determination of F_{des} by AFM—Force measurements were performed using our standardized AFM technique, which has been previously described.²⁶ Briefly, all force spectroscopy experiments were performed at room temperature in a fluid cell filled with droplets of PBS (pH=7.4). The functionalized tip with the peptide was then brought in contact with various substrate surfaces for one second of surface delay and then retracted at a constant vertical scanning speed of $0.1 \mu\text{m/s}$. Tips with PEG–OH (i.e., without peptide) were used as controls. The deflection signals (volts) were converted to force (Newton) using the settings of: (a) deflection sensitivity in the range of 40–100 nm/volts, (b) spring constant of tips of 0.058–0.065 N/m (from the thermal–tune method³⁶) and (c) applying correction for offset deflection. The thermal noise associated with the experimental set–up involving the cantilever (DNP-10), instrumentation (MFP-3D-Bio) and the data acquisition mode (*Force scan rate*: 0.99 Hz, *Approach scan rate*: 1 Hz) used in our current study with PBS, were kept minimal ($< 2 \text{ pN}$) and was verified with the “*amplitude spectral density*” function provided with the instrumentation.

The peptide–surface interaction force, F_{des} , was then recorded for tip–sample surface separation distance on approach (i.e., tip–probe moving towards the sample surface at a constant rate) and retraction (i.e., tip–probe moving away from the sample surface at a constant rate). To minimize the offset error resulting from potential damage to the SAM surface at the initial probe contact point, following initial contact, the tips were carefully moved upward just above the surface and then laterally to different locations on the SAMs before the force–separation curves were obtained.

From the retraction–force versus separation–distance data (Fig. 2), the unbinding force that was measured during the plateau region ending right at the separation distance (max sep) and corresponding to the contour length of PEG spacer and the peptide sequence (around 31 nm for our system)²⁶ was taken as the force that characterizes the desorption of the peptide from the surface (F_{des}),^{37, 38} while the larger force peak registered prior to this plateau region (usually $< 10 \text{ nm}$ separation distance) is believed to be caused by the interaction between the AFM tip material and the SAM surface.^{38, 39}

For each of the peptide–surface systems for which the force–separation curves were recorded, two different substrate samples from the same material were used, with at least

three distinct sites on each substrate being chosen for force measurements. A minimum of ten force–separation curves were recorded at each site. In total, more than 60 force–separation curves were used to generate a histogram (Fig. 3), from which one averaged ' F_{des} ' and the maximum separation distance were obtained for each of the peptide–surface systems.

III. Results and Discussion

III.a. Surface Characterization

Table 2 presents the results analyzed by the surface characterization techniques applied to the surfaces used in these studies. All of the measured values reported in Table 2 fall within the expected range for these surfaces.^{24, 40–45}

III.b. $F_{des} : \Delta G^o_{ads}$ Correlation between AFM and SPR data for 64 Peptide–SAM Interactions in PBS (pH=7.4)

Both of our current and previous AFM studies²⁶ showed that it is possible to directly correlate the data between AFM and SPR based on the following observations: (i) The comparison of F_{des} values for TGTG–V–GTCT on a hydrophilic SAM–OH surface vs. a hydrophobic SAM–CH₃ surface resulted in $F_{des} = 0.004 \pm 0.002$ nN and $F_{des} = 0.095 \pm 0.015$ nN (Supporting Information, Table S.3, mean $\pm$ 95% confidence interval and in Ref. 26), respectively, which showed the F_{des} value on SAM–CH₃ surface is significantly higher than that on the SAM–OH surface ($p < 0.001$) and demonstrated that the proposed AFM method is sufficiently sensitive to determine F_{des} with significant differences for peptides adsorbed on our SAM surfaces with different functionalities.²⁶ (ii) Our previous SPR studies comparing the adsorption experiment of TGTG–X–GTCT vs. TGTG–X–GTGT peptides on SAM surfaces did not show significant differences in ΔG^o_{ads} for a broad range of surface chemistries²⁶ (data shown in Supporting Information (Fig. S.3) and in Ref. 26). This comparison thus shows that the TGTG–X–GTCT peptide can be used in AFM studies as an equivalent model for the TGTG–X–GTGT peptide. (iii) Our previous comparison between F_{des} from AFM and ΔG^o_{ads} from SPR data showed a strong, linear correlation. However, this correlation was constructed using a relatively small data set, thereby necessitating further validation with a broader range of peptide–surface systems.

In order to expand the data sets applied to re–evaluate the $F_{des} : \Delta G^o_{ads}$ correlation based on the same protocol mentioned above, we probed the interactions from the host–guest peptide sequences (TGTG–X–GTCT for AFM and TGTG–X–GTGT for SPR) with eight different –X–residues (Table 1) on eight different SAM surfaces (presented in Section II.a.1) in PBS (pH=7.4). The resulting $F_{des} : \Delta G^o_{ads}$ correlation is shown in Fig. 4 (individual data points for ΔG^o_{ads} and F_{des} are presented in Table S.2 and S.3, respectively, of the Supporting Information, with the ΔG^o_{ads} value being obtained from our previous SPR studies¹⁸). As indicated by Fig 4, a strong linear correlation ($R^2 = 0.89$) is shown for this large set of peptides–SAM systems, which is in agreement with the linear relationship also found in our prior, but much more limited, studies.²⁶ However, it must be noted that comparison between the regression line for this new data set with that determined from our previous studies²⁶ reveals that the slopes are significantly different ($p=0.0003$ and $p < 0.0001$ with and without outliers included when determining the regression line for current new data, respectively; statistic analysis was done by a Student's t–test at the 5% level of significance ($\alpha=0.05$); all regression curves were shown in Supporting Information (Fig. S.4)). This difference is attributed to instrumentation differences, with intervening instrumentation problems having caused us to use two different AFM instruments for the two separate studies (i.e., Dimension TM 3100 AFM, Veeco Metrology Group, Santa Barbara, CA used for the previous data set; MFP–3D AFM instrument, Asylum Research, Santa Barbara, CA used for the present data

set), with all other experimental procedures being identical. While the apparent instrumentation differences found are certainly undesirable, the important relationship for this method is the remaining strong linear correlation between ΔG^o_{ads} from F_{des} , which can then be used to estimate effective ΔG^o_{ads} values from F_{des} measured by AFM for our host–guest peptide models on surfaces not conducive to SPR, with the constraining condition applied that the same AFM instrument that was used to generate the correlation plot must be used for subsequent F_{des} measurements on all other surfaces as well.

III.c. Estimation of ΔG^o_{ads} for Peptides on Surfaces that are not Conducive for Use with SPR in PBS (pH=7.4)

Since the comparison shown in Fig. 4 indicates a strong linear force–energy correlation, we investigated the reasonableness of applying this newly developed $F_{des} : \Delta G^o_{ads}$ correlation to estimate the ΔG^o_{ads} for peptide–surface systems that are not conducive for use with SPR. The regression equation from Fig. 4 was used to estimate ΔG^o_{ads} for each individual peptide on flat bulk–material substrates such as HDPE, PMMA, fused silica glass, and quartz (100) using the F_{des} determined from AFM (Table 3). All measurements were done using the host–guest peptide model, TGTG–X–GTCT, in PBS solution.

Unfortunately, attempts to directly validate the estimated ΔG^o_{ads} values for these selected surfaces by existing techniques such as isothermal titration calorimetry (ITC)⁵⁰ or by the area under the AFM force plateau⁵¹ were hampered by the numerous assumptions and the practical difficulties involved.^{26, 52} For example, although the free energies of adsorption could be estimated directly from the area under the velocity-independent force plateau, this method is only valid if the probe tip density is known, which, however, cannot be accurately determined for our technique and therefore was not applied.

Despite these limitations, the reasonableness of these data can also be generally assessed by plotting the estimated F_{des} values to the cosine of the static water contact angle of these surfaces along with the SAM surfaces as an additional check on the validity of these results. The results of these comparisons are presented in Fig. 5.

As shown in Fig. 5, the relationship between the cosine of the static water contact angle and F_{des} for this set of additional material surfaces (HDPE, PMMA, and quartz) agrees extremely well with the data for the non–charged SAM surfaces. The highly correlated trend between the F_{des} values measured by our standard AFM method and the cosine of the static water contact angle of the surface for the non–charged surfaces are very similar to our previous findings from SPR.¹⁸ As shown, a strong correlation exists between F_{des} and the water contact angle for each of the neutrally charged SAM surfaces, with peptide adsorption affinity increasing in a linear manner with the hydrophobicity of the SAM surfaces over the full range of contact angles. For the charged SAM surfaces (e.g., SAM–NH₂ and –COOH) and the silica glass, the strength of adsorption is substantially higher than the correlation line for the non–charged surfaces, reflecting the additional attraction considered to be provided by electrostatic interactions between the peptides and the surfaces, similar to what we have observed from our previous SPR¹⁸ and AFM²⁶ results.

IV. Conclusions

In this research, the correlation between F_{des} measured by AFM and ΔG^o_{ads} measured by SPR was investigated for an expanded data set using our standardized SPR¹⁸ and AFM methods.²⁶ The ΔG^o_{ads} values measured from SPR were found to strongly correlate in a linear manner with the desorption forces obtained from AFM studies for set of 64 peptide–SAM surface systems in PBS. The developed $F_{des} : \Delta G^o_{ads}$ correlation was then applied to estimate ΔG^o_{ads} for peptide–surface interactions for a set of four material surfaces that were

not amenable for analysis using SPR in PBS. The peptide desorption forces measured by AFM for both the SAM surfaces and the new set of material surfaces were also shown to correlate strongly with the cosine of the static water contact angle for both the uncharged SAM surfaces and the materials surfaces studied in PBS, with surfaces presenting charged functional groups resulting in additional contributions to the desorption force that are not reflected in the cosine of the water contact angle. The correlation between the peptide desorption force and the cosine of the static water contact angle provides an additional means of qualitatively assessing the reasonableness of the AFM desorption force results prior to using the data to estimate ΔG_{ads}^o for a given peptide–surface system. Besides, demonstrating how this method could be applied to expand capabilities to study the thermodynamics of peptide–surface interactions for a much broader range of materials, the variation in slopes when drawing the $F_{des} : \Delta G_{ads}^o$ correlation from different AFM instrumentation also suggest that, to put this method into practice, correlation plots need to be first generated using a common set of peptide–surface systems that can be tested by SPR (or QCD) vs. AFM and developed from the specific methods and instruments used.

These combined methods thus provide the ability to make thermodynamic measurements of peptide–surface interactions for any microscopically flat surface, including surfaces that are not amenable for use with SPR and QCM methods. In addition to providing the ability to experimentally characterize fundamental interactions that are important to help understand protein–surface adsorption behavior, these methods also provide a means to obtain experimental data that is essential for the evaluation, modification, and validation of empirical force field parameters that are needed to enable peptide and protein adsorption behavior to be accurately represented by molecular simulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge DTRA for funding support for this research (DTRA grant # HDTRA1). We also would like to thank Dr. James E. Harriss of Clemson University for assistance with the various aspects of SPR biosensor chip fabrication for these studies, Dr. Delphine Dean of Clemson University for assistance with the various aspect of AFM force measurement, and Ms. Megan Grobman, Dr. Lara Gamble, and Dr. David Castner of NESAC/BIO at the University of Washington for assistance with surface characterization with XPS under the funding support by NIBIB (grant # EB002027).

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Figure 1.

Linkage of peptide to the AFM tip via a PEG tether. Tethering of the peptides involved a three-step process of (i) amino-functionalization of the AFM cantilever tips, (ii) linking (PDP-PEG-NHS to the amino-functionalized tips, and (iii) coupling the peptide to the PEG tethered cantilever tip via cysteine.

Figure 2.

Measurement of desorption force (F_{des}) of TGTG-T-GTCT on four material surfaces that are used in the current study (a) HDPE, (b) Glass, (c) PMMA and (d) Quartz. The approach force traces are shown in red while retract force traces is shown in blue. The black dotted box for each of the raw force traces represents the separation distance (*max sep*) for which the F_{des} have been recorded (shown in the respective inset images). For a given material surface, the individual inset image represents one of the sixty raw force traces that were used for determining the F_{des} for an individual peptide-surface system. For an individual raw force trace, the baseline is defined as the region beyond the '*max sep*' where retract force, plateaus without any further step increase and is therefore defined as the *upper plateau region* (upper black dotted line). The force corresponding to *upper plateau region* is defined as '0 pN' and is obtained by averaging the individual data points within the single raw force trace for over twenty nm separation distance. The force plateau immediately preceding the baseline represents the force associated with the peptide adsorption on individual surfaces and is therefore defined as the *lower plateau region* (lower black dotted line). The averaged force plateau over the *lower plateau region* is obtained by averaging the individual data points within the raw force trace over the separation distance spanned by this plateau. The difference between the *upper and lower plateau regions* then represents F_{des} (shown with a solid black arrow) associated with each individual force trace.

Figure 3.

Histogram for (a) $F_{des} = 65 \pm 12$ pN and (b) maximum separation distance = 31 ± 5 nm for TGTG-V-GTCT on HDPE in PBS. (Mean $\pm$ 95% confidence interval (C.I.), N = 6.)

Figure 4. Correlation between ΔG°_{ads} by SPR and desorption force by AFM for an equivalent set of 64 peptide–SAM systems in PBS; pH=7.4. Outlier data points (analyzed by the Studentized–Residual method⁴⁷) are shown in red and were not considered when determining the trend line.

Figure 5.

Desorption force vs. cosine of the static water contact angle for F_{des} averaged from AFM pull-off force for TGTG-X-GTCT with eight different -X- (Table 1) on each of the SAM functionalized surfaces with specific functionality and the selected material surfaces in PBS, pH=7.4. The trend line shows the linear regression for the non-charged surfaces (i.e., excluding the SAM and material surfaces expected to show surface charged density: SAM- NH_2 ($\text{pK} \sim 6.5$)⁵³ and - COOH ($\text{pK} \sim 5.5$)⁵⁴ surfaces, which present positively and negatively charged surface at pH=7.4, respectively, and the glass which is expected to be negatively charged at pH=7.4.⁵⁵) The error bars represent the 95% C.I. with $N = 48$ for desorption force and $N = 3$ for contact angles.

Table 1

List of the eight designated amino acids used for the X residue in TGTG–X–GTCT or TGTG–X–GTGT. Each amino acid has the general structure of (–NH–CHR–CO–) with **R** presenting the side chain structure as presented here.

–X– residue	Side Chain (R)	Property
Phenylalanine (F)	–CH ₂ –C ₆ H ₅	Aromatic
Valine (V)	–CH(CH ₃) ₂	Non-polar
Tryptophan (W)	–CH ₂ –indole ring (C ₈ H ₆ N)	Aromatic
Threonine (T)	–CH(CH ₃)OH	Neutral polar
Glycine (G)	–H	Non-chiral
Asparagine (N)	–CH ₂ –CO–NH ₂	Neutral polar
Lysine (K)	–(CH ₂) ₄ –NH ₃ ⁺ (pK=10.78) ³¹	Positively charged
Aspartic Acid (D)	–CH ₂ COO [–] (pK=3.97) ³¹	Negatively charged

Table 2

Surface characterization: Atomic composition, static contact angle, film thickness, and surface roughness analyses for each surface. An asterisk (*) indicates negligible value for atomic composition data. (Mean $\pm$ 95% C.I., N = 3.)

Surface Moiety	C (%)	S (%)	N (%)	O (%)	Roughness (nm)	Contact Angle (°)	Thickness (Å)
-OH	56.7 (0.8)	2.8 (0.6)	*	7.5 (0.2)	< 0.5	16 (2)	13.0 (1.0)
-CH ₃	64.9 (0.7)	2.8 (0.2)	*	*	< 0.5	110 (3)	11.0 (1.0)
-(EG) ₃ OH	54.8 (0.3)	2.3 (0.1)	*	13.2(0.6)	< 0.5	32 (3)	19.0 (3.0)
-NH ₂	54.0 (0.9)	2.0 (0.2)	4.0 (0.3)	3.3 (0.3)	< 0.5	48 (2)	14.7 (2.5)
-COOH	47.6(1.8)	1.6(0.1)	*	7.6(0.3)	< 0.5	18 (1)	15.8 (2.0)
-OC ₆ H ₅	56.2(0.9)	2.4(0.2)	*	5.3(0.9)	< 0.5	80 (4)	14.4 (4.0)
-NHCOCH ₃	48.6 (0.6)	1.7 (0.1)	4.0 (0.1)	6.0 (0.7)	< 0.5	48 (2)	17.0 (2.0)
-COOCH ₃	45.4(4.3)	2.5(0.2)	*	10.8 (0.6)	< 0.5	63 (2)	11.0 (4.8)
Fused Glass**	25.0 (2.0)	*	< 1.0	49.0 (2.0)	< 10.0	23 (4)	—
Quartz (100)**	15.0 (2.0)	< 1.0	< 2.0	53 (1.0)	< 1.5	13 (3)	—
PMMA	76.0 (1.0)	*	*	24.0 (1.0)	< 1.5	63 (3)	90 (10) in nm
HDPE	96.0 (3.0)	*	*	3.0 (3.0)	< 8.0	97 (5)	100 (10) in nm

* indicates a negligible value;

** Fused glass slide also contains Zn (<1%), Al (<1%) and Si (22.0 $\pm$ 1.0%) while the quartz surface of specific orientation (100) contains Si (30.0 $\pm$ 3.0%) in atomic composition by XPS (not shown). The presence of extra carbon composition is believed to be originating from surface contamination due to the exposure of samples to air after the cleaning procedure. These are the typical adventitious and unavoidable hydrocarbon impurities that adsorb spontaneously from ambient air onto the glass and quartz surfaces.⁴⁶

Table 3

F_{des} and corresponding ΔG°_{ads} (estimated from F_{des} and correlation curve in Fig. 3) for TGTG-X-GTCT on selected surfaces in PBS, pH=7.4. Mean ($\pm$ 95% C.I. for F_{des} and $\pm$ 95% prediction interval (P.I.)^{48, 49} for effective ΔG°_{ads} values, N = 6.)

-X-	F_{des} (pN)				ΔG°_{ads} (kcal/mol)			
	Quartz	Glass	PMMA	HDPE	Quartz	Glass	PMMA	HDPE
-F-	18 (2)	39 (2)	39 (2)	64 (10)	-0.8(0.2)	-2.2(0.1)	-2.2(2.0)	-3.9(0.3)
-V-	6 (1)	20 (6)	35 (7)	65 (12)	0.0(0.2)	-0.9(0.1)	-2.0(0.8)	-4.0(0.3)
-W-	6 (2)	10 (2)	20 (2)	37 (2)	0.0(0.2)	-0.3(0.2)	-0.9(0.7)	-2.1(0.1)
-T-	6 (2)	21 (6)	11 (2)	32 (6)	0.0(0.2)	-1.0(0.1)	-0.3(0.3)	-1.8(0.1)
-G-	20 (9)	34 (8)	37 (2)	59 (5)	-0.9(0.1)	-1.9(0.1)	-2.1(0.3)	-3.6(0.2)
-N-	24 (3)	38 (6)	47 (5)	58 (7)	-1.2(0.1)	-2.2(0.1)	-2.8(0.4)	-3.5(0.2)
-K-	9 (3)	20 (2)	24 (6)	64 (7)	-0.2(0.2)	-0.9(0.1)	-1.2(0.4)	-3.9(0.3)
-D-	8 (2)	9 (2)	43 (13)	48 (11)	-0.1(0.2)	-0.2(0.2)	-2.5(0.4)	-2.8(0.2)