Thermodynamic analysis of marine bacterial attachment to oligo(ethylene glycol)-terminated self-assembled monolayers

Linnea K Ista¹,²* and Gabriel P López³

Abstract
Colloidal models are frequently used to model the thermodynamics of bacterial attachment to surfaces. The most commonly used of such models is that proposed by van Oss, Chaudhury and Good, which includes both non-polar and polar (including hydrogen bonding) interactions between the attaching bacterium, the attachment substratum and the aqueous environment. We use this model to calculate the free energy of adhesion, \( \Delta G_{adh} \), for attachment of the marine bacterium Cobetia marina to well defined attachment substrata that systematically vary in their chemistry and their ability to attach bacteria, namely a series of oligo(ethylene glycol) (OEG) terminated self-assembled monolayers that vary in the number of OEG moieties. For this system, the values of \( \Delta G_{adh} \) calculated using VCG do not correlate with observed attachment profiles. We examine the validity of a number of assumptions inherent in VCG and other colloidal models of adhesion, with special attention paid to those regarding bacterial surfaces.

Keywords: Fouling resistance; Thermodynamics; Oligo(ethylene glycol); Cobetia marina; Surface tension; Interfacial tension; Bacterial attachment

Background
Attachment of microorganisms to a submerged solid support is the first step in development of a biofilm [1-3]. Interactions of attaching bacteria with the substratum may strongly influence the final properties of the subsequent biofilm including structure [4], adhesion strength [5] and global developmental processes, such as quorum sensing [6] and exopolysaccharide production [7]. As such, attachment is the most logical place to prevent, as in the case of biofouling, or engineer, as in the case of microbial biofuel cells, biofilm formation. Accurately modeling initial attachment events is, therefore, critical not only to understanding a fundamental biological process, but also to optimizing the formation of biofilms for a variety of applications.

Colloidal models remain a popular approach to predicting attachment for bacteria that do not have specific attachment peptide or sugar binding proteins commonly found on pathogenic or commensal organisms [8-11], or for which such specific attachment mechanisms are unknown [12,13]. Bacteria exhibit some colloid-like properties: their sizes fall within the upper limits of colloid particles as generally defined (~1 μm) and they, like colloidal particles, tend to collect at interfaces. If and until selection pressure requires specific attachment mechanisms, bacteria may be best served by exploiting colloidal interactions at surfaces rather than expending metabolic energy to encode and express specific attachment molecules, thus allowing them a greater range of likely supports for biofilm formation.

A number of colloidal models have been proposed to explain biomolecular attachment in general, and bacterial attachment in particular [12,14-17]. Of these the van der Waals-Lewis-Acid–base model proposed by van Oss, Chaudhury and Good (VCG) in the late 1980s [18] 0020 seems to most accurately reflect the interfacial processes most likely to be important in biological attachment: nominally apolar Lifshitz-van der Waals interactions and polar Lewis acid–base interactions, including the special

* Correspondence: lkista@unm.edu
¹ Center for Biomedical Engineering, Department of Chemical and Nuclear Engineering, Albuquerque, NM, USA
² Department of Biology, The University of New Mexico, Albuquerque, NM 87131, USA
Full list of author information is available at the end of the article

© 2013 Ista and López; licensee Springer. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
cases of hydrogen-bonding [13,19,20] and electrostatic interactions [13]. In addition to the VCG model being used itself to study microbial interactions at the interface, it further informs the extended Derjaguin-Landau-Verwey-Overbeek model, currently in widespread use [21-23] and also the recently developed Chen/Qi ratio [24].

We recently used VCG to examine the role of the substratum-water interfacial tension (γSL) in elucidating differences in fouling resistance between oligo(ethylene glycol) (OEG)-terminated self-assembled monolayers (SAMs), correlating γSL, and its components, with increased degrees of hydrogen bonding between the SAM and water [25]. In that study, the increase in hydrogen bonding between OEG-SAMs and water correlated with increased bacterial attachment. In this manuscript, we expand these studies to calculations of free energy of adhesion (ΔGadh), based on the VCG model, for attachment of the marine bacterium, C. marina, to OEG-SAMs.

SAMs of alkanethiolates on gold terminated with varying lengths of OEG [26] are a particularly attractive model system for studying the relationship between bacterial attachment and an estimated ΔGadh. The number of ethylene glycol (EG) units (n = 1-6) in a SAM determines its resistance to protein adsorption [27], as well as attachment of mammalian cells [28], algal zoospores [29] and marine bacteria [30,31]. The difference between OEG-SAMs that resist and permit cellular adsorption is one EG moiety [32]; for example, for the marine organism, Cobetia marina, an OEG-SAM with three OEG moieties (EG n = 3) permits bacterial attachment, whereas one in which EG n = 4 resists bacterial attachment [25]. The OEG system is also unique in that the relatively low attachment observed on these surfaces means that over the course of our experiments (2 hr) it is extremely unlikely that an attachment maximum will be encountered, resulting in consistent attachment kinetics throughout the course of the study. Previous studies [33] suggest ΔGadh for non-specific attachment to SAMs that attach bacteria will be negative. Because the difference between OEG-SAMs that attach and those that do not attach microbes is one residue, the VCG model predicts that ΔGadh be negative for those SAMs attaching C. marina and positive for those not attaching the organism, with the transition from negative to positive being associated with an increase of one EG residue. We observed no correlation between number of attaching bacteria and ΔGadh of OEG-SAMs calculated using the VCG model of microbial attachment. In the process, we examined several parameters that might lead to this non-correlation and conclude that both errors endemic to the laboratory use of VCG and assumptions, common to all colloidal models of attachment, about the bacterial surface are the main source of the observed nonconcordance.

**Methods**

**Preparation of self-assembled monolayers**

SAMs were prepared as described previously [31]. Briefly, glass coverslips (Fisher, Fairlawn, NJ) were treated with 70:30 H2SO4/H2O2 for at least 1 hour, rinsed in copious amounts of deionized water, and dried under dry nitrogen. The samples were then loaded into a thermal evaporator. After evacuating the chamber to 1 millitorr, 15 Å Cr was deposited followed by 300 Å Au. For Wilhelmy plate contact angle evaluation, metal was deposited on both sides of the sample.

After metal deposition was complete, samples were immersed in 1 mM ethanolic solutions of OEG-terminated alkanethiols (EG n = 1-6; all from Prochimia, Poland; a kind gift from the lab of M. Grunze), 1-mercaptopoundecanol (OH; Aldrich, St. Louis MO), undecanethiol (CH3; Aldrich, St. Louis, MO), 1-mercaptopoundecyl trimethylamine (NMe3+; Prochimia, Poland) or poly(ethylene glycol) substituted undecanethiol (EG500; MW 2000, Rapp Polymere, Tübingen) and incubated for at least 2 hours. Prior to use, samples were sonicated in fresh ethanol for 5 minutes and dried under a stream of dry nitrogen immediately before analysis. Structures of thiols used for this study are shown in Table 1.

**Bacterial strains and culture conditions**

All media and buffers were prepared with de-ionized water generated by a system using tap water processed sequentially through water softening, reverse osmosis and
ion exchange (Barnstead-Thermolyne RoPure/Nanopure system). The final resistivity of the processed water was greater than 18 MΩ cm⁻¹. Marine Broth 2216 (MB, Difco, Franklin Lakes, NJ) was prepared according to manufacturer’s instructions. Marine Agar (MA) was prepared by the addition of 1.5% Bacto agar (Difco) to MB. Artificial sea water (ASW) contained 400 mM NaCl, 100 mM MgSO₄·7H₂O, 20 mM KCl, 10 mM CaCl₂ [34]. Modified basic marine medium plus glycerol (MBMMG) contained 0.5× ASW plus 19 mM NH₄Cl, 0.33 mM K₂HPO₄, 0.1 mM FeSO₄·7H₂O, 5 mM Trishydroxymethane hydrochloride pH 7, and 2 mM glycerol [34,35]. *Cobetia marina* (basonym, *Halomonas marina*) ATCC 25374, is stored in a frozen (−70°C) stock aliquots, made from first generation cultures of the original ATCC lyophilate, in MB containing 20% glycerol. Experimental stock preparations were maintained on MA slants and were stored at 4°C for up to 2 weeks. Prior to inoculation into a chemostat, a single colony from a MA slant was inoculated into 50 mL of MB and grown overnight with shaking at 25°C. A chemostat culture was established by inoculating 3 mL of the overnight culture into MBMMG. The chemostat was maintained at a flow rate of 1 mL min⁻¹ (dilution rate, 40–70 minutes after drying commenced [33]. Contact angles were determined, using the vane Oss-Chaudhury-Good (VCG) equation [18]:

\[ 1 + \cos \theta = \frac{2(\sqrt{\gamma_{LV} + \gamma_{LV}^{*}} + \sqrt{\gamma_{SV} + \gamma_{SV}^{*}})}{\gamma_{LV}} \]

where: \( \gamma_{SV}^{LW} \) and \( \gamma_{LV}^{LW} \) are the Lifshitz-van-Der-Waals components of the surface tensions of the substratum and the probe liquid, respectively, \( \gamma_{SV} \) and \( \gamma_{LV} \) are the Lewis basic (electron donating, hydrogen bond accepting) components, and \( \gamma_{SV}^{*} \) and \( \gamma_{LV}^{*} \) are the Lewis acidic (electron accepting, hydrogen bond donating) components. \( \gamma_{LV} \) is the total surface tension of the probe liquid. Because there are 3 unknowns, contact angles were taken with three different probe liquids and the unknowns \( \gamma_{SV}^{LW}, \gamma_{SV} \) and \( \gamma_{SV}^{*} \) calculated by simultaneously solving the three equations using MATLAB software (Mathworks, Natick). Values for the surface tension of bacteria (\( \gamma_{SV} \)) were obtained by substituting the contact angle of the probe liquids on bacterial mats into Equation (1).

Interfacial tensions between the bacterium and the substratum (\( \gamma_{BS} \)), the bacterium and water (\( \gamma_{BL} \)) or the substratum and water (\( \gamma_{SL} \)) were calculated by inserting measured on the sample six times per mm (a total of 60 data points per sample). For each SAM formulation, a minimum of three samples was measured per probe liquid (deionized water, diiodomethane, formamide, glycerol or hexadecane).

Contact angles of bacteria were taken on mats of bacteria supported on cellulose acetate filters (0.2 μm pores; Millipore, Billerica) [33,36]. Approximately 120 mL of chemostat culture were filtered, followed by an equal volume of deionized water to remove residual salt. The filtered bacteria were then allowed to air dry before contact angle analysis. To ensure that the surface of the mat was dry without being totally dehydrated, water contact angles were initially taken every 10 minutes during the drying cycle until they became stable; contact angles for analysis were taken during the time period in which it was determined that the water contact angle did not change, in our case, 40–70 minutes after drying commenced [33]. Contact angles were determined, using the angle analysis tool on ImageJ image processing software (NIH; [37]), from photographs taken with DROPImage software (Ramé-Hart, Succasunna, NJ) linked to a Ramé-Hart contact angle goniometer. Bacterial contact angles were measured with the same liquids as used for SAMs (deionized water, diiodomethane, formamide, glycerol and hexadecane).

### Calculation of surface and interfacial tensions using VCG

Surface tension and components were calculated using the van Oss-Chaudhury-Good (VCG) equation [18]:

\[ 1 + \cos \theta = \frac{2(\sqrt{\gamma_{LV} + \gamma_{LV}^{*}} + \sqrt{\gamma_{SV} + \gamma_{SV}^{*}})}{\gamma_{LV}} \]

Contact angles of bacteria were taken on mats of bacteria supported on cellulose acetate filters (0.2 μm pores; Millipore, Billerica) [33,36]. Approximately 120 mL of chemostat culture were filtered, followed by an equal volume of deionized water to remove residual salt. The filtered bacteria were then allowed to air dry before contact angle analysis. To ensure that the surface of the mat was dry without being totally dehydrated, water contact angles were initially taken every 10 minutes during the drying cycle until they became stable; contact angles for analysis were taken during the time period in which it was determined that the water contact angle did not change, in our case, 40–70 minutes after drying commenced [33]. Contact angles were determined, using the angle analysis tool on ImageJ image processing software (NIH; [37]), from photographs taken with DROPImage software (Ramé-Hart, Succasunna, NJ) linked to a Ramé-Hart contact angle goniometer. Bacterial contact angles were measured with the same liquids as used for SAMs (deionized water, diiodomethane, formamide, glycerol and hexadecane).
the relevant components of $\gamma_{SV}$, $\gamma_{BV}$ and $\gamma_{LV}$ into the following equation (shown here for $\gamma_{SL}$) [13]:

$$\gamma_{SL} = (\sqrt{\gamma_{SV}^L} - \sqrt{\gamma_{LV}^S})^2 + 2(\sqrt{\gamma_{SV}^L} - \sqrt{\gamma_{LV}^S})(\sqrt{\gamma_{SV}^L} - \sqrt{\gamma_{LV}^S})$$

(2)

$\Delta G_{adh}$ calculations. $\Delta G_{adh}$ was calculated by two different methods in order to insure fidelity of sometimes-complicated equations and for further analysis of components of $\Delta G_{adh}$. The basic equation for calculating $\Delta G_{adh}$ using colloidal models is a special case of the Dupré equation [14,18,38]:

$$\Delta G_{adh} = \gamma_{BS} - \gamma_{BL} - \gamma_{SL}$$

(3)

and is quickly calculated using the values for $\gamma_{BS}$, $\gamma_{BL}$, and $\gamma_{SL}$ obtained from Equation (2).

The VCG model, however, specifies that $\Delta G_{adh}$, like surface and interfacial tension, can be divided into two components, one apolar ($\Delta G_{adh}^{LW}$) and one polar ($\Delta G_{adh}^{AB}$) [13,18]:

$$\Delta G_{adh} = \Delta G_{adh}^{LW} + \Delta G_{adh}^{AB}$$

(4)

Both components of $\Delta G_{adh}$ can be derived according to Equation (3), with the interfacial tensions being calculated using iterations of Equation (2). The resulting components of $\Delta G_{adh}$ are [13,18]:

$$\Delta G_{adh}^{LW} = \left(\sqrt{\gamma_{BV}^{LW} - \gamma_{LV}^{SV}}\right)^2 - \left(\sqrt{\gamma_{BV}^{LW} - \gamma_{LV}^{SV}}\right)^2$$

(5)

$$-\left(\sqrt{\gamma_{LV}^{SV} - \gamma_{LV}^{LV}}\right)^2$$

And

$$\Delta G_{adh}^{AB} = 2\left[\sqrt{\gamma_{LV}^{SV}}(\sqrt{\gamma_{BV}^{BV} + \sqrt{\gamma_{SV}^{SV} - \gamma_{SV}^{SV}})}\right]$$

$$+ \sqrt{\gamma_{LV}^{SV}}(\sqrt{\gamma_{BV}^{BV} + \sqrt{\gamma_{SV}^{SV} - \gamma_{SV}^{SV}}})$$

$$-\sqrt{\gamma_{BV}^{BV}\gamma_{SV}^{SV} - \gamma_{BV}^{BV}\gamma_{SV}^{SV}}$$

(6)

Results and discussion

Attachment of C. marina to SAMs

The number of cells attached to OEG-SAMs after 2 hrs exposure to C. marina ($7.5 \times 10^7$ cells/mL) is shown in Figure 1. Included for comparison is the number of cells attached to a methyl terminated SAM (CH$_3$-SAM), which we have previously shown to attach C. marina in relatively large numbers [31,39]. As previously reported [25], the number of C. marina attached to OEG-SAMs over a 2 hour period decreases with increasing length of EG, with no attachment occurring when EG$_n \geq 4$. These data are consistent with those reported for zoospores of the macroalga Ulva linza, on a similar series of OEG-SAMs [32].

Contact angles

Advancing contact angles ($\theta_{AX}$ where “X” is the liquid) of SAMs are summarized in Table 2. $\theta_{AX}$ for OEG-SAMs with EG $n = 2$-6 were statistically identical (~34°; $p = 0.33$), but were different from EG $n = 1$ and OH (EG$_n = 0$) ($p \leq 0.01$); the latter two SAMs had statistically similar ($p = 0.14$) contact angles (average = 26°). There was no significant difference for advancing contact angles of formamide ($\theta_{AX}$) or dioodomethane ($\theta_{AD}$). Although differences were observed between advancing

Figure 1 Two hour attachment of Cobetia marina to EG-SAMs and a methyl-terminated positive control. Attachment was considered to be 0 when the number of cells mm$^{-2}$ was less than one (EG$_n \geq 1$). Error bars represent 95% confidence levels.
contact angles of hexadecane (θ_{AH}), all were less than 20°, and thus, few differences were observed when \(\cos \theta_{AH}\), the input into all subsequent calculations, was compared. Glycerol contact angles (θ_{AG}) were statistically different between all EG-SAMs. As previously observed [25], θ_{AG} was able to differentiate between fouling (OH\(_{-}\), EG\(_{-(n=1-3)}\)) and nonfouling (EG\(_{-}\) n = 4-6, PEG) SAMs.

Contact angles of *C. marina* bacterial mats supported on cellulose acetate filters using the five probe liquids from this study are presented in Table 3. Uncoated cellulose acetate filters were permeable to all liquids, rendering them effectively completely wettable (θ\(_{AX}\) = 0°).

### ΔG\(_{adh}\) calculations

ΔG\(_{adh}\) was calculated using both the Dupré Equation (3) and by the method proposed by VCG, as outlined above and in Equations (4), (5) and (6). To calculate ΔG\(_{adh}\) according to the Dupré equation, the interfacial tensions \(\gamma_{BS}\), \(\gamma_{SL}\) and \(\gamma_{SL}\) were first calculated according to Equation (2). \(\gamma_{BS}\) on all surfaces was statistically zero; \(\gamma_{SL}\) was \(-6.2 \pm 3\) mJ m\(^{-2}\). \(\gamma_{SL}\) for this series of OEG-SAMs has been reported previously [25] and is large and negative, driving ΔG\(_{adh}\) in a positive direction. Both methods of calculation yielded identical results (p = 1.0) for all SAMs; results are summarized in Figure 2.

As demonstrated in Figure 2, ΔG\(_{adh}\) as calculated from contact angles of water, diiodomethane and glycerol on bacteria and OEG-SAMs did not reflect the resistance to attachment of bacteria to OEG-SAMs with OEG > 3, nor was attachment correlated in a systematic way to ΔG\(_{adh}\). Based on previous applications of the VCG model, [35,40] one would predict that ΔG\(_{adh}\) for SAMs with EG ≤ 3 would be negative; they are not. Clearly ΔG\(_{adh}\) as calculated from contact angles and the VCG model is either not physically meaningful or is not capturing all the relevant information in the system. We now consider how the inputs into the equations for ΔG\(_{adh}\), ((3) and (4)) influence this value and from where the discrepancy between attachment of *C. marina* and estimates of ΔG\(_{adh}\) may stem.

We have previously demonstrated [31,39] that *C. marina* attaches in far greater numbers to SAMs terminated with a methyl group (CH\(_{3}\)-SAM) when compared to OH-SAMs. When we calculated ΔG\(_{adh}\) for *C. marina* attaching to a CH\(_{3}\)-SAM using liquid combination water, diiodomethane and glycerol (WDG), a value of \(-32.7 \pm 5\) mJ m\(^{-2}\) was obtained; the value is negative, as would be expected for a SAM attaching large numbers of bacteria (average coverage 1,121 ± 192 cells mm\(^{-2}\) under the same experimental conditions as for OEG-SAMs). When we calculated ΔG\(_{adh}\) for attachment of *C. marina* to a trimethylamine-terminated SAM (NMe\(_{3}\)-SAM; average coverage 662 ± 44 cells cm\(^{-2}\) under the same experimental conditions as for OEG-SAMs) using inputs from WDG, however, ΔG\(_{adh}\) = 24 ± 4 mJ m\(^{-2}\) is positive and statistically similar to OH-SAMs (ΔG\(_{adh}\) = 21 ± 4 mJ m\(^{-2}\)) that attached one fifth of the cells attached to NMe\(_{3}\)-SAMs (Figure 1). Because the ΔG\(_{adh}\) calculated for CH\(_{3}\)-SAMs is due only to Lifshitz-van-der-Waals interactions, with no polar components, and it had at least the expected negative sign for ΔG\(_{adh}\), we explored the possibility that the VCG method of calculating ΔG\(_{adh}\) misses information about the polar components.

Our first examination was whether the polar liquids used to measure contact angles on SAMs and bacteria affected the resulting values of ΔG\(_{adh}\). The three contact liquids used for VCG type analysis usually include one

### Table 2 Contact angles of SAMs with different contact angle liquids

| SAM         | Formamide | Glycerol | Hexadecane | Water | Diiodomethane |
|-------------|-----------|----------|------------|-------|---------------|
| CH\(_{3}\)  | 35 ± 1°   | 24 ± 1°  | 39 ± 2°    | 5 ± 3° | 24 ± 2°       |
| OEG\(_{1}\) | 25 ± 1°   | 29 ± 2°  | 35 ± 2°    | 7 ± 2° | 28 ± 2°       |
| OEG\(_{2}\) | 25 ± 2°   | 29 ± 1°  | 39 ± 1°    | 15 ± 1° | 32 ± 1°       |
| OEG\(_{3}\) | 27 ± 2°   | 23 ± 2°  | 38 ± 3°    | 18 ± 1° | 33 ± 1°       |
| OEG\(_{4}\) | 24 ± 3°   | 27 ± 2°  | 42 ± 1°    | 1 ± 1°  | 33 ± 1°       |
| OEG\(_{5}\) | 32 ± 2°   | 26 ± 2°  | 44 ± 3°    | 11 ± 1° | 34 ± 1°       |
| OEG\(_{6}\) | 27 ± 2°   | 24 ± 3°  | 47 ± 2°    | 11 ± 1° | 33 ± 2°       |
| PEG         | 29 ± 1°   | 21 ± 1°  | 60 ± 1°    | 3 ± 2°  | 37 ± 1°       |
| CH\(_{3}\)  | 66 ± 1°   | 90 ± 1°  | 96 ± 0°    | 36 ± 1° | 108 ± 2°      |
| NMe\(_{3}\) | 26 ± 2°   | 25 ± 1°  | 39 ± 2°    | 9 ± 3°  | 18 ± 2°       |

### Table 3 Contact angles (θ) of mats of logarithmic phase *C. marina* supported on cellulose acetate filters

| Liquid          | θ     |
|-----------------|-------|
| Diiodomethane   | 34 ± 2° |
| Formamide       | 53 ± 2° |
| Glycerol        | 64 ± 2° |
| Hexadecane      | 2 ± 1°  |
| Diiodomethane   | 34 ± 2° |
apolar and two polar liquids, although, if the model is robust, any combination of contact angle liquids should result in the same value for $\Delta G_{adh}$. As can been seen in Figure 3, this was clearly not the case; $\Delta G_{adh}$ values calculated from $\theta_{AX}$ taken three different sets of liquid: (i) water, diiodomethane and formamide (WDF), (ii) diiodomethane and glycerol (WDG) and (iii) water, glycerol and formamide (WGF) revealed different trends for $\Delta G_{adh}$.

One criticism of the VCG method is that it tends to underestimate $\gamma_{SV}^+$ on surfaces [12], which could significantly alter values calculated relying on this component of surface tension. For the OEG series, there was no statistical difference between different OEG surfaces using either WDG or WDF inputs, although the values obtained were less than one [25] which is much lower than the literature values for ethylene glycol ($\gamma_{LV}^+ = 1.6$ m J m$^{-2}$) [41]. To see how well this method captures $\gamma_{SV}^+$ on a surface for which $\gamma_{SV}$ is expected to be dominated by Lewis acidic interactions, we tested all liquids on a SAM terminated with trimethylamine (NMe$_3^+$), which, as an onium, should be Lewis acidic. Figure 4 shows $\gamma_{SV}$ and its components for NMe$_3^+$ SAMs for all liquid groups. That NMe$_3^+$-terminated SAMs appear to be hydrogen bond accepting is highly unexpected since the lone pair of electrons that normally mediate hydrogen bond formation with nitrogen are fully occupied. Surface analysis including X-ray photoelectron spectroscopy (data not shown) confirms that
no adducts are found on this surface, so this observation is not a result of surface contamination. We suspect that this underestimation of $\gamma_{SV}^+$ is due to the fact that, although water and glycerol have significant Lewis acidic components, they are not sufficiently large or dominant for their contact angles to result in accurate estimation of $\gamma_{SV}^+$. The lack of polar organic liquids with a significant Lewis acidic monopole was noted by van Oss, Chaudhury and Good in their original proposal of their model [18,20] as a possible shortcoming and the situation has not noticeably improved in the last two decades. Only bromoform ($\gamma_{LV}^+ = 1.72 \text{ mJ m}^{-2}$) has a $\gamma_{LV}^+$ value $>1 \text{ mJ m}^{-2}$, the minimum value considered by VCG to be useful in calculations, but it cannot be used in a general laboratory setting as its safe handling requires a respirator.

Most applications of the VCG model, and indeed the original model itself, assume that for water $\gamma_{LV}^+ = \gamma_{LV}^- = 25.5 \text{ mJ m}^{-2}$ [13,19,35,40], whereas others, most notably Lee [42], have shown that above 0°C, water is, in fact, much more likely to donate hydrogen bonds (be more Lewis acidic) than accept them and that, at room temperature, $\gamma_{SV}^+ = 1.8\gamma_{SV}^-$. Because the acid and base components for all other liquids were calculated based on the VCG assumption, surface tensions for other liquids, and the resulting surface tensions for measured solid surfaces, overestimated the Lewis-basic component [12,42]. When we used WDF contact angle values to calculate $\Delta G_{adh}$ the use of Lee values resulted in lower values for $\Delta G_{adh}$ whereas there was no statistical difference ($p \geq 0.05$) for $\Delta G_{adh}$ when calculated using contact angles from the WDG liquid set. Use of the Lee values in calculations did not, however, shift $\Delta G_{adh}$ such that it was negative for SAMs attaching cells, nor did it sufficiently raise the values of $\gamma_{SV}^+$ for NMe$_3^+$-SAMs such that they now were predominately Lewis acidic. We must, therefore, conclude, in agreement with others [35], that using the modified values of the components of $\gamma_{LV}$ does not have a substantial effect on VCG calculations.

An assumption that, as far as we know, remains unchallenged with regard to the VCG model and, more accurately, its application to analysis of surface tension has to do with the way the apolar components of sub-stratum and bacterial surface tension, $\gamma_{SV}^{LW}$ and $\gamma_{BV}^{LW}$, are calculated. VCG includes in these values not only attractive London dispersion interactions resulting from fluctuating dipoles and resulting induced dipoles, but also possible repulsive interactions between fixed dipoles (Keesom interactions) and fixed dipole (Deybe) interactions [13]. Although the latter are considered to be insignificant [13], we maintain that they are neglected, particularly given that the apolar liquid of choice for most VCG analysis [13,19,35,40,43], diiodomethane, although considered strictly apolar [13,19,33,40,42,43], has a small, but possibly significant, acid monopole (0.72 mJ m$^{-2}$) [44]. To test the significance of this monopole, we examined the effect of including this monopole on calculations of surface tension components, $\Delta G_{adh}^{LW}$ and $\Delta G_{adh}^{AB}$. As an added test, we compared values obtained with diiodomethane, both including and ignoring the acid monopole, with those obtained with hexadecane, which is known to be completely apolar.

The differences in $\gamma_{SV}^{LW}$ of polar SAMs as measured with hexadecane and diiodomethane is substantial, whether or not the acidic monopole of the latter is considered; $\gamma_{SV}^{LW}$ calculated from diiodomethane is $\sim 45 \text{ mJ m}^{-2}$, if the acid monopole is included that value drops to $\sim 35 \text{ mJ m}^{-2}$.
whereas that using hexadecane is ~27 mJ m⁻², similar to that for the CH₃-SAM. The inclusion of the acid monopole calculation of γSV LW, thus, has a profound effect on γSV LW. These results indicate that either the supposition that either Keesom or Debye interactions are insignificant may be false (as they seem to account for nearly 10 mJ m⁻² when the acid monopole included in the calculation) or the assumption that acid or base components <1.0 mJ m⁻² are insignificant [13,18] is unwarranted; in either case, further re-examination of these assumptions is suggested by these results.

The value of γSV LW for organic polymers and biopolymers is noted by van Oss to be universally ~45 mJ m⁻² [13,18], but we propose that this may be an artifact based on the use of diiodomethane as an apolar liquid that seems to always result in this value (see also the value of γBV LW in Figure 4). Examination of the γSV LW of SAMs as calculated with diiodomethane and hexadecane seems to indicate that this value may be an artifact. γSV LW of an OH-SAM, is, for example, about 20 mJ m⁻² higher than γSV LW of an CH₃-SAM using contact angles of diiodomethane and ignoring the acid component. If we compare γLV LW for n-decane (23.8 mJ m⁻²) and 1-decanol (22 mJ m⁻²) we see no such increase [41]. More to the point, the total surface tension for dodecane (similar to CH₃-SAM) is 25.6 mJ m⁻², whereas that for dodecanol is 28.6 mJ m⁻². Taking into account that γLV AB for most alcohols [41] is 3–6 mJ m⁻², it seems very unlikely to us that a similar change on a SAM surface would nearly double the value of γSV LW. On the other hand, a SAM surface is well ordered, and the main surface exposed would be OH (or NMe₃⁺) so a slightly higher value for OEG-SAMs might be expected. If we take into account, however, the published values of γLV LW for (21.8 mJ m⁻²), glycerol (34 mJ m⁻²) and ethylene glycol (29 mJ m⁻²) [41], are still much lower than those proposed for most polymer surfaces calculated using diiodomethane. We propose, therefore, that hexadecane or some other completely apolar liquid is the most relevant when analyzing SAMs. We should note, however, that using hexadecane alone is insufficient to bring about a correlation between ΔGadh and attachment.

The second part of the system that must be considered in reconciling attachment to ΔGadh is the surface energetic components of the attaching bacteria, presented in Figure 5. Based on preliminary data from microbial adhesion to solvents (MATS; data not shown), a qualitative method of assessing general surface tension of bacteria [19] and also its affinity for CH₃-terminated SAMs, we expected C. marina to be quite hydrophobic, rather than only moderately hydrophobic (θAW = 52°) as results from contact angles on bacterial mats suggest. In this analysis, C. marina was also Lewis basic, which would explain its low attachment to Lewis basic surfaces (OH, COO⁻, short chain OEG-SAMs [25,39]) and its propensity to attach to amine-containing surfaces. The value of γBS with regard to the polar SAMs was statistically zero, regardless of which polar liquids were used for contact angle analysis. For comparison, when γBS is calculated for the interaction between C. marina and CH₃-SAMs, which attach C. marina in relatively large numbers (in this study 1,215 bacteria mm⁻² compared to 135 ± 19 bacteria mm⁻²)

---

**Figure 5** The surface tension and components of logarithmic phase Cobetia marina calculated from contact angles of water, diiodomethane and formamide and water, diiodomethane, and glycerol (circles) using the VCG model. Error bars represent 95% confidence levels.
for OH-SAMs), it is \(-6 \text{ mJ m}^{-2}\); unfortunately, this value is also positive, which, according to the Dupré equation [3] would tend to disfavor attachment. \(\gamma_{\text{BL}}\) between \(C. \text{ marina}\) and water was \(-6.7 \text{ mJ m}^{-2}\), which would tend to favor attachment (\(\gamma_{\text{BL}}\) is subtracted from \(\gamma_{\text{BS}}\) in equation [3]). Clearly, then \(\Delta G_{\text{adh}}\) is dominated by \(\gamma_{\text{SL}}\), which we have shown previously to be negative, and increasing in magnitude with longer length OEG-SAMs, while highly positive for CH\(_3\)-SAMs.

The role of the organization of water around the OEG-SAMs as it relates to thermodynamics has been extensively discussed both in our previous work on the relationship between \(\gamma_{\text{SL}}\) and attachment of \(C. \text{ marina}\) [25] and its included references, with the conclusion that \(\gamma_{\text{SL}}\) calculated using VCG supports current mathematical theories that suggest hydrogen bonding between OEG moieties and water renders bacterial attachment to these surfaces entropically disfavored. The observation that bacterial attachment to a CH\(_3\)-SAM is increased in this system is energetically favored might lead one to make similar arguments that attachment of bacteria is entropically favored near a hydrophobic surface; in other bacterial systems [31,39,43], however, attachment to CH\(_3\)-SAMs is lower than to other SAMs, suggesting that the influence of the increased entropy upon bacterial attachment is not significant.

We suspect, however, that calculations of \(\gamma_{\text{DV}}\) and the resultant interfacial tensions \(\gamma_{\text{BS}}\) and \(\gamma_{\text{BL}}\) do not capture information most relevant to attachment. An assumption common to even the most carefully considered models of non-specific bacterial attachment is that surface tension is uniform across the bacterial cell (and, indeed, across a monoculture population). This assumption is simply invalid. The role of extracellular appendages such as flagella and pili in attachment are well known [45,46], and even though we deliberately chose \(C. \text{ marina}\) as a model organism partly due to the lack of observable extracellular structures [47], years of observation have led us to conclude that the surface is very likely not uniform as we have observed the cells orient themselves differently on different SAMs during attachment. We and others have also proposed that bacteria have different attachment mechanisms on different surfaces [4,35,48]. Furthermore an emerging field of study, based on observations of attachment of \(C. \text{ crescentus}\), has made a strong case that during cell division the biochemistry of the cell envelope of the two daughter cells may be very different, and that genetically programmed biochemical heterogeneity on individual cells is present [49]. The relevant \(\gamma_{\text{DV}}\) and components to include in a free-energy calculation are, therefore, less likely to be those of the whole bacterium, but rather that of the part of the cell which is interacting with the SAM. We are currently developing a method by which the areas of individual cells involved in attachment to each SAM may be accurately assayed; preliminary results indicate that different regions of the \(C. \text{ marina}\) cell surface do, indeed, interact differently with when SAM surface chemistry is altered.

We also initially assumed that attachment of \(C. \text{ marina}\) to SAMs is nonspecific, i.e., unmediated by receptor-ligand interactions. The lack of correlation \(\Delta G_{\text{adh}}\) and attachment called this assumption into question. It has been demonstrated previously that \(\Delta G_{\text{adh}}\) calculated using VCG does not correlate with attachment when the interaction between the bacteria and the surface is ligand mediated, i.e., specific [33,40]. It is possible that \(C. \text{ marina}\) possesses receptors that interact specifically with components of SAMs. We also considered the possibility that exopolymeric substances (EPS) secreted by planktonic \(C. \text{ marina}\) might form conditioning films that would present specific ligands for attachment. Marine bacteria are known to produce exopolymeric substances while growing planktonically [50] and to attach to conditioning films of EPS formed on surfaces [51]. It was not hard, therefore to envision a scenario in which \(C. \text{ marina}\) could produce EPS, even while in carbon-limited chemostat conditions, that could form conditioning films on SAMs, to which \(C. \text{ marina}\) could bind specifically.

We tested for the presence of EPS deposited onto SAMs from filtered (0.45 \(\mu\)m nylon) chemostat and also tested the filtered effluent directly for carbohydrate, DNA and protein. The lectin concavalin A (ConA) binds specifically to \(\alpha\)-D-mannosyl and \(\alpha\)-D-glucosyl groups in carbohydrates and glycoproteins. These residues are frequently found in the EPS of \(Pseudomonas \text{ aeruginosa}\) [52] and that of marine pseudomonads [50]. Alexa-dye-conjugated ConA staining of CH\(_3\)-SAMs exposed for two hours under flow to filtered chemostat effluent showed no discernable deposits, whereas the same SAMs exposed to 1 \(\mu\)g mL\(^{-1}\) dextran stained easily. The amount of dissolved carbohydrate in filtered chemostat effluent was estimated using the phenol-sulfuric acid method as modified by Jain for use in salt water [53] and no detectable (i.e. < 1 \(\mu\)g/mL) carbohydrate was found when compared with a glucose standard. Bradford assays for protein of the filtered effluent were similarly negative and no absorption peak was detected at 260 nm indicating the absence of nucleic acid. We thus conclude that, at least under our experimental conditions, EPS-derived conditioning films do not play a role in attachment of \(C. \text{ marina}\) to SAMs.

A final criticism of VCG may lie in the ability of calculations based on so many contact angles and their attendant errors may make error propagation an issue, particularly for low contact angles (as in OH-SAMs in this study) where accuracy and precision during contact angle measurement is difficult. A model developed for much simpler systems may not be applicable to more
complex systems involving heterogeneous cells and populations. We believe that the measurements required to accurately model this system are becoming increasing possible, however, and that more comprehensive explanations will be rapidly forthcoming.

Conclusions
We have undertaken a systematic study of VCG model as it pertains to bacterial attachment and $\Delta G_{adh}$. The key to this investigation is a series of SAMs differing only in the length of ethylene glycol chains presented at the surface, resulting in profoundly different attachment profiles. $\Delta G_{adh}$ as calculated using the VCG model, however, did not reveal even a qualitative correlation between this value and attachment. We conclude that the VCG model as currently utilized is insufficient to describe the relevant interaction occurring between the bacteria, attachment substratum and water. These results call into question the generalized use of contact angles and colloidal models based on them, such as VCG, as a substitute for surface energy in thermodynamic analyses of bacterial attachment. Because VCG is used not only in its own right, but also informs other models of attachment, most notably, the highly popular extended DLVO model, caution should be taken when drawing conclusions regarding the effect of substratum surface energy on attachment of microbes, at least until such time as contact angle probe liquids are identified which can accurately account for all components of surface tension.

In addition to the limitations VCG as applied to calculating $\gamma_{SV}$, we suggest a second important factor preventing accurate modeling of microbial attachment is inherent assumptions made about the surface energy of bacteria themselves. To date, all surface energy analyses of microbial cells assume that the population is uniform and that the surfaces of cells themselves are homogenous [12]; the average surface energy of both the population and individual cells is, thus the relevant information needed for input into VCG or any colloidal model. We and others, however, have demonstrated that different part of the cell are relevant for attachment to different substrata [34,48,54]. We are currently developing a method to probe which parts of the bacterial surface are relevant to attachment to different substrata is currently underway that might eventually lead to a more nuanced view of the bacterial cell surface and its relevance in attachment.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
LKI designed and carried out all experimental protocols and drafted the manuscript. GPL conceived the idea of using OEG SAMs as a systematic tool for these studies and participated in the drafting of the manuscript. Both authors read and approved the final manuscript.

Acknowledgements
This work was supported by grants N00014-08-1-0741 and N00014-10-1-09007 from the Office of Naval Research and HDTRA-1-11-1-0004 from the Defense Threat Reduction Agency. We thank M. Grunze and A. Rosenhahn (University of Heidelberg and Karlsruhe Institute of Technology) for thoughtful discussion and for providing some of the OEG thiolics used in this study. We also thank M. Werner-Washburne, D. Northup, and C. Takacs-Vesbach for helpful discussions. Technical assistance from Ms. Maria Pillar Arpa-Sancet (KIT/UH), Mr. Phanindhar Shivapooja (UNM/Duke) and Mr. José Cornejo (UNM) is greatly appreciated. We also thank Mr. Shivapooja and Dr. Kristin Wilde for careful reading of this manuscript.

Author details
1 Center for Biomedical Engineering, Department of Chemical and Nuclear Engineering, Albuquerque, NM, USA. 2 Department of Biology, The University of New Mexico, Albuquerque, NM 87131, USA. 3 Department of Biomedical Engineering, Duke University, Durham, NC 27708, USA.

Received: 11 July 2013 Accepted: 23 August 2013
Published: 3 September 2013

References
1. Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nature Rev 2:95–108
2. O’Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Annu Rev Microbio 54:49–79
3. Stoodley P, Sauer K, Davies DG, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbio 56:187–209
4. Dalton H, Stein J, March P (2000) A biological assay for detection of heterogeneities in the surface hydrophobicity of polymer coatings exposed to the marine environment. Biofouling 15:83–94
5. Gadh A (2007) Interfacial Forces in Aqueous Media. Taylor and Francis, London.
6. Labatte M, Zhu H, Thung L, Bandara R, Larsen MR, Wilcox MDP, Givskov M, Rice SA, Kjelleberg S (2007) Quorum-sensing regulation of adhesion in Serratia marcescens MG1 is surface dependent. J Bacteriol 189:2708–2711
7. Becker K (1996) Exopolysaccharide production and attachment strength of bacteria and diatoms on substrates with different surface tensions. Micro Ecol 32:23–33
8. Downie JA (2010) The roles of extracellular proteins, polysaccharides and signals in the interactions of hizobia with legume roots. FEMS Microb Rev 34:150–170
9. Klemm P, Hancock V, Schembri MA (2010) Fimbrial adhesins from extraintestinal Echerichia coli. Environ Microbio Rep 2:628–640
10. Murray PA, Prakobphol A, Lee T, Hooper CI, Fisher SJ (1992) Adherence of oral Streptococci to salivary glycoproteins. Infect Immuno 60:31–38
11. Scott JR, Zahnner D (2006) Pili with strong attachments: Gram-positive bacteria do it differently. Molec Microbio 62:320–330
12. Sharma PK, Rao KH (2002) Analysis of different approaches for evaluation of surface energy of microbial cells by contact angle goniometry. Adv Colloid Interfac 98:341–463
13. van Oss CJ (2006) Interfacial Forces in Aqueous Media. Taylor and Francis, Boca Raton
14. Absolom DR, Lamberti FV, Policova Z, Zingg W, van Oss CJ, Neumann AW (1985) Surface thermodynamics of bacterial adhesion. Appl Environ Microbio 46:90–97
15. Baez RE, Meyer AE, Nastiella JR, Nastiella RR, Carter JM (1984) Surface properties determine bioadhesive outcomes - methods and results. J Biomed Mater Res 18:337–355
16. Berg JM, Eriksson LGE, Claesson PM, Borve KG (1994) 3-component Langmuir-Bodgett-films with a controllable degree of polarity. Langmuir 10:1225–1234
17. Buzan J, Buzan J (1988) Additive and nonadditive surface-tension components and the interpretation of contact angles. Langmuir 10:2177–2184
18. van Oss CJ, Good RJ, Chaudhury MK (1988) Additive and nonadditive surface-tension components and the interpretation of contact angles. Langmuir 10:2177–2184
19. Bellon-Fontaine M-N, Rault J, van Oss CJ (1996) Microbial adhesion to solvents a novel method to determine the electron-donor/electron-
acceptor or Lewis acid–base properties of microbial cells. Colloid Surface B 7:47–53
20. van Oss CJ (2002) Use of the combined Lifshitz-van der Waals and Lewis acid–base approaches in determining apolar and polar contributions to surface and interfacial tensions. J Adhes Sci Technol 16:669–677
21. Mao YJ, Subramaniam PK, Taefiq K, Chen G (2011) microbial biofouling: a mechanistic investigation. J Adhes Sci Technol 25:2155–2168
22. Strevett KA, Chen G (2003) Microbial surface thermodynamics and applications. Res Microbiol 154:329–335
23. Dorobantu LS, Bhattacharjee S, Fohgt JM, Gray MR (2009) Analysis of Force Interactions between AFM Tips and Hydrophobic Bacteria Using DLVO Theory. Langmuir 25:6968–6976
24. Liu C, Zhao Q (2011) The CQ ratio of surface energy components influences adhesion and removal of fouling bacteria. Biofouling 27:275–285
25. Ista LK, Lopez GP (2012) Interfacial tension analysis of oligo(ethylene glycol) terminated self-assembled monolayers and their resistance to bacterial attachment. Langmuir 28:12844–12850
26. Pale-Grosdemange C, Simon ES, Prime KL, Whitesides GM (1991) Formation of self-assembled monolayers by chemisorption of derivatives of oligo (ethylene glycol) of structure HS(CH2)nO(CH2CH2)meta-OH on gold. J Am Chem Soc 113:12–20
27. Prime KL, Whitesides GM (1993) Adsorption of proteins onto surfaces containing end-attached oligo(ethylene oxide): a model system using self-assembled monolayers. J Am Chem Soc 115:10714–10721
28. Ostuni E, Chapman RG, Holmlin RE, Takayama S, Whitesides GM (2001) A survey of structure–property relationships of surfaces that resist the adsorption of protein. Langmuir 17:5605–5620
29. Schlip S, Kuehler A, Rosenhahn A, Grunze M, Pettitt ME, Callow ME, Callow JA (2007) Settlement and adhesion of algal cells to hexa (ethylene glycol)-containing self-assembled monolayers with systematically changed wetting properties. Biointerphases 2:43–150
30. Balamurugan S, Ista LK, Yan J, Lopez GP, Himmelhaus M, Grunze M (2002) Reversible protein adsorption and biofoulsion on monolayers terminated with mixtures of oligo(ethylene glycol) and methyl groups. J Am Chem Soc 127:14548–14549
31. Ista LK, Fan H, Baca O, Lopez GP (1996) Attachment of bacteria to model solid surfaces: oligo(ethylene glycol) surfaces inhibit bacterial attachment. FEMS Microb Lett 142:59–63
32. Schlip S, Rosenhahn A, Grunze M, Pettit ME, Allow ME, Callow JA (2007) Settlement and adhesion of algal cells to hexa (ethylene glycol)-containing self-assembled monolayers with systematically changed wetting properties. Biointerphases 2:43–150
33. Liu YT, Strauss J, Camesano TA (2007) Thermodynamic investigation of Staphylococcus epidermidis interactions with protein-coated substrata. Langmuir 23:7134–7142
34. Konsters K (1992) The genus Delays. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The Prokaryotes. Springer-Verlag, New York
35. Ista LK, Perez-Luna VH, Lopez GP (1999) Surface-grafted, environmentally sensitive polymers for biofilm release. Appl Environ Microbiol 65:1603–1609
36. Ubbink, J, Schard-Zammaretti P (2007) Colloidal properties and specific interactions of bacterial surfaces. Curr Opin Colloid Interface Sci 12:263–270
37. Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image Processing with Image J. Biophotonics Int 11:36
38. Adamson AW (1990) Physical Chemistry of Surfaces. John Wiley and Sons, New York
39. Ista LK, Callow ME, Finlay JA, Coleman SE, Nolasco AC, Callow JA, Lopez GP (2004) Effect of substratum surface chemistry and surface energy on attachment of marine bacteria and algal spores. Appl Environ Microbiol 70:4151–4158
40. Liu Y, Gallardo-Moreno AM, Pinzon-Arango PA, Reynolds Y, Rodriguez G, Camesano TA (2008) Cranberry changes the physicochemical surface properties of E. coli and adhesion with uroepithelial cells. Colloid Surface B 65:35–42
41. Krüss Corporation G (2004) Surface tensions of solids and liquids
42. Lee LH (1996) Correlation between Lewis acid-base surface interaction components and linear solvation energy relationship solvatochromic alpha and beta parameters. Langmuir 12:1681–1687
43. Khan MM, Ista LK, Lopez GP, Schuler AJ (2011) Experimental and theoretical examination of surface energy and adhesion of nitrifying and heterotrophic bacteria determined using self-assembled monolayers. Environ Sci Technol 45:1055–1060
44. Gonzalez-Martín ML, Janczuk B, Labajos-Broncano L, Brueque JM (1997) Determination of the carbon black surface free energy components from the heat of immersion measurements. Langmuir 13:5991–5994
45. Klausen M, Aaes-Jorgensen A, Molin S, Tolker-Nielsen T (2003) Involvement of bacterial migration in the development of complex multicellular structures in Pseudomonas aeruginosa biofilms. Mol Ecobiol 50:61–68
46. Klausen M, Heydorn A, Raga P, Lambertsen L, Aaes-Jorgensen A, Molin S, Tolker-Nielsen T (2003) Biofilm formation by Pseudomonas aeruginosa wild type, flagella and type IV pili mutants. Mol Microbiol 48:1511–1524
47. Shea C, Lovelace LJ, Smith-Thornberry HE (1995) Delays marina as a model organism for studies of bacterial colonization and biofilm formation. J Ind Microbiol 15:290–296
48. Paul JH, Jeffrey WH (1985) Evidence for separate adhesion mechanisms for hydrophilic and hydrophobic surfaces in Vibrio proteolytica. Appl Environ Microbiol 50:431–437
49. Bowman GR, Lykoytovata AI, Shapiro L (2011) Bacterial polarity. Curr Opin Cell Biol 23:71–77
50. Breech B, Gubner R, Zinkevich V, Hanjangsit L, Avci R (2000) Characterisation of conditioning layers formed by exoplymeric substances of Pseudomonas NCIMB 2101 on surfaces of AISI 316 stainless steel. Biofouling 16:93–104
51. Gubner R, Breech IB (2000) Characterisation of conditioning layers formed by exoplymeric substances of Pseudomonas NCIMB 2101 on surfaces of AISI 316 stainless steel. Biofouling 16:93–104
52. Tolker Nielsen T, Brinch UC, Raga PC, Anderson JB, Jacobson CS, Molin S (2000) Development and dynamics of Pseudomonas sp. biofilms. J Bacteriol 182:6482–6489
53. Jain A, Bhose N (2009) Biochemical composition of the marine biofilm conditioning cell: implications for bacterial adhesion. Biofouling 25:13–19
54. Dalton HM, March PE (1998) Molecular genetics of bacterial attachment and biofouling. Curr Opin Biotechnol 9:252–255

doi:10.1186/1559-4106-8-24

Cite this article as: Ista and López: Thermodynamic analysis of marine bacterial attachment to oligo(ethylene glycol)-terminated self-assembled monolayers. Biointerphases 2013, 8:24.

Submit your manuscript to a SpringerOpen journal and benefit from:

▸ Convenient online submission
▸ Rigorous peer review
▸ Immediate publication on acceptance
▸ Open access: articles freely available online
▸ High visibility within the field
▸ Retaining the copyright to your article

Submit your next manuscript at ▶ springeropen.com