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RESEARCH ARTICLE

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Resistance of Galactoside-Terminated Alkanethiol Self-Assembled Monolayers to Marine Fouling Organisms

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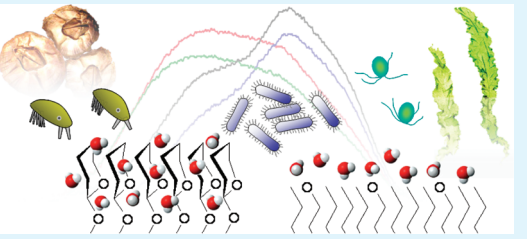
Supporting Information

ABSTRACT: Self-assembled monolayers (SAMs) of galactoside-terminated alkanethiols have protein-resistance properties which can be tuned via the degree of methylation [*Langmuir* **2005**, *21*, 2971−2980]. Specifically, a partially methylated compound was more resistant to nonspecific protein adsorption than the hydroxylated or fully methylated counterparts. We investigate whether this also holds true for resistance to the attachment and adhesion of a range of marine species, in order to clarify to what extent resistance to protein adsorption correlates with the more complex adhesion of fouling organisms. The partially methylated galactoside-terminated SAM was further compared to a mixed monolayer of ω-substituted methyl- and hydroxyl-terminated alkanethiols with wetting properties and surface ratio of hydroxyl to methyl groups matching that of the galactoside. The settlement (initial attachment) and adhesion strength of four model marine fouling organisms were investigated, representing both micro- and macrofoulers; two bacteria (*Cobetia marina* and *Marinobacter hydrocarbonoclasticus*), barnacle cypris larvae (*Balanus amphitrite*), and algal zoospores (*Ulva linza*). The minimum in protein adsorption onto the partially methylated galactoside surface was partly reproduced in the marine fouling assays, providing some support for a relationship between protein resistance and adhesion of marine fouling organisms. The mixed alkanethiol SAM, which was matched in wettability to the partially methylated galactoside SAM, consistently showed higher settlement (initial attachment) of test organisms than the galactoside, implying that both wettability and surface chemistry are insufficient to explain differences in fouling resistance. We suggest that differences in the structure of interfacial water may explain the variation in adhesion to these SAMs.



KEYWORDS: self-assembled monolayer, marine biofouling, *Cobetia marina*, *Marinobacter hydrocarbonoclasticus*, *Balanus amphitrite*, *Ulva linza*

INTRODUCTION

Biofouling, or the unwanted accumulation and growth of biomass on surfaces, compromises the function and reliability of equipment and processes in a number of fields. In natural waters, this pertains to water desalination and purification plants, ship hulls, aquaculture, or the operation of instruments deployed in natural waters, to mention but a few. The problems associated with biofouling in industrial environments such as pharmaceutical bioreactors or in food processing plants are very different in kind but perhaps not in severity. Diagnostic devices, medical implants, or life-supporting equipment in contact with physiological environments are subject to yet other kinds of biofouling, with potential impact on the health or even survival of patients. This diversity means that research on biofouling involves several disciplines, and the problems related to the prevention and removal of biofouling are usually addressed individually, on a case-by-case basis. Despite this, many of the mechanisms and processes involved in biofouling are similar, and there is presumably much to gain from interaction between the disciplines. In this vein, we are looking into what we can learn in marine biofouling from knowledge obtained in biomedically oriented fouling research, and more specifically, to what extent protein-resistant surfaces are also efficient in reducing marine biofouling; issues which are usually treated in isolation.[1] The rationale of this approach is the occurrence and roles of proteinaceous materials in marine biofouling; many marine foulers use proteins for

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attachment to surfaces. For example, proteins are a major component of the extracellular polymeric substances (EPS) secreted by microorganisms, by which they adhere to surfaces, and which may also influence subsequent attachment by other species. Previous efforts to relate low nonspecific adsorption of model proteins to resistance to adsorption, settlement (attachment), or adhesion strength of marine fouling organisms have yielded mixed results, and in the cases where a good correlation has been demonstrated (eg Schilp et al.[2]), the coatings have usually exhibited poly(ethylene glycol) (PEG) chains in some form. We have demonstrated that thin photografted PEG-based hydrogel coatings display excellent protein-rejecting properties both in model protein assays and in complex biofluids,[3] and also inhibit the adhesion of a range of marine species in laboratory assays.[4] Ostuni et al. tested six different protein-resistant SAMs for their ability to resist adsorption of bacteria and mammalian cells, but found that the resistance of surfaces to protein adsorption did not correlate with their resistance to bacterial or mammalian cell adhesion (and neither did the resistance to the adhesion of bacterial cells correlate with the resistance to the adhesion of mammalian cells).[5] Side-group modified polystyrene-based surface-active block copolymers modified with either PEG or semifluorinated segments were both protein resistant and reduced the attachment of algal zoospores, as compared with glass controls,[6] and certain PEG-modified fluoropolymers resistant to proteins and other biomacromolecules also reduce algal zoospore attachment and facilitate zoospore release upon exposure to shear forces.[7] Statz et al. have demonstrated that a set of peptidomimetic polymers provide effective short-term resistance to proteins as well as to cell and bacterial fouling, and for peptoids with PEG side-chains also long-term resistance to cell attachment.[8] In a study of the fouling resistance of surface-anchored anionic oligosaccharides, Cao et al. found that although the coatings were resistant to proteins and inhibit cell and tissue adhesion, algal spores and barnacle cyprids adhered to the polysaccharides.[9]

For many years, the use of biocides has been a common and effective means to prevent marine biofouling, notably on ship hulls. However, as legal and environmental restrictions continuously limits the use of biocides, there is a pressure to develop more environmentally benign alternatives for fouling prevention. This pressure has stimulated research in, for example, the use of secondary metabolites and other presumably nontoxic antifouling chemicals from natural sources,[10] various biomimetic or bioinspired — and frequently antiepibiotic — approaches,[11] or the utility of physical and chemical methods in the design of antifouling and nonadhesive surfaces.[12] Thus, a number of physicochemical surface properties and their importance for the adsorption, adhesion, attachment or colonization of selected foulers have been studied, including properties such as topography and morphology, surface energy or wettability, surface charge, color, and many more parameters (see Prendergast[13] for an extensive summary). In cases where, for example, surface chemistry, wettability, molecular conformation, or functional substituends are in focus, the advantages of SAMs as model surfaces are well-established. SAMs permit close control of surface properties and chemical composition on the same type of supporting substrate, while also enabling monitoring of the monolayer structure, which has been exploited in a number of marine biofouling studies.[14−19]

In biomedical applications, adsorbed proteins may assist in the attachment of cells or bacteria and subsequent biofilm growth onto implants and catheters, induce inflammatory response, or interfere with the operation of a sensor. This implies a need for protein-resistant surfaces, and great efforts have been devoted to understanding how proteins adsorb to surfaces, how surfaces denature proteins, and how adsorption may be prevented. For biomaterials and biosensing, the use of high-molecular-weight poly(ethylene glycol) (PEG),[20] polysaccharides,[21] and in particular dextrans[22,23] as fouling-prevention coatings is widespread, although each of these are also associated with particular problems in real applications. However, high-molecular-weight polymers with large conformational freedom are not necessary to make a surface protein-resistant; this can also be obtained with, for example, oligo(ethylene glycol)-terminated alkylthiol SAMs.[24−26] It was found that helical EG chains with gauche defects adsorb water strongly, acting as templates for water nucleation, whereas all-trans chains interact only weakly with water molecules. This difference is essential because the latter are less effective in preventing protein adsorption.[26,27] A great number of differently terminated alkylthiol SAMs have been investigated and found to be protein- or cell-resistant.[28−33] Some of the general observations that have been inferred from these are, for example, that protein adsorption increases with decreasing wettability of the SAMs,[34] the hypothesis that a successful protein-resistant surface should be (i) charge neutral, (ii) hydrophilic (polar), and (iii) should have hydrogen-bond acceptors (but not hydrogen-bond donors),[29,31] or that surfaces exposing kosmotropes are effective in this respect.[32]

Of particular interest to us is that among the surfaces and surface chemistries that have been investigated for protein resistance, we also find saccharide SAMs terminated by maltose[28] and mannitol[35] groups, as well as physisorbed[23] or covalently attached[9] polysaccharides. In a previous publication,[36] it was demonstrated that mixed SAMs of methylated and nonmethylated galactoside-terminated alkylthiols prepared on gold substrates resulted in very low levels of fibrinogen and lysozyme adsorption on the mixed monolayers where the advancing water contact angles were between 24° and 45°, and below 45°, respectively. Further, a monomethylated galactoside-terminated compound with wettability corresponding to the low protein adsorption regime of the mixed SAMs also showed very low levels of protein adsorption, demonstrating that the mixed monolayer could effectively be replaced by a single-component SAM of galactoside-terminated thiols with the appropriate ratio of methyl and hydroxyl groups.[36] These results support the hypothesis that hydrophilicity and surface neutrality are important for successful design of protein-rejecting surfaces, though they are not in agreement with the notion that the presence of hydrogen-bond acceptors, but not hydrogen-bond donors, is important for protein resistance.[29,31]

The purpose of the current study was to investigate whether these protein-resistant galactoside-terminated SAMs also are effective in inhibiting the attachment (settlement) of marine fouling organisms, using four different models in laboratory assays, representing the settling (attaching) stages of common micro- and macrofoulers. For the former, we selected two bacteria that are frequently found in marine bacterial biofilms: *Cobetia marina*, which is Gram-negative and rodlike, and *Marinobacter hydrocarbonoclasticus*, which has a single flagellum and may grow either in planktonic form or in biofilms. The models representing macrofoulers are the zoospores of the most important fouling macroalga, *Ulva linza*, and cypris larvae of the barnacle *Balanus amphitrite*. All the test organisms represent the stages of the life cycle that are responsible for initial colonization

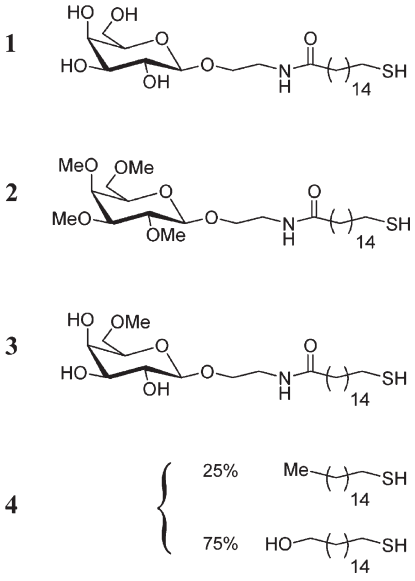

Figure 1. Used SAMs were single-component monolayers formed from one of the three galactoside-terminated thiols (1−3), and a mixed monolayer prepared from a solution of 25% methylated and 75% hydroxylated alkylthiols (4).

of surfaces; thus preventing the attachment of cells, spores, and larvae is the key to preventing biofouling. In contrast to the protein adsorption study,[36] which used a range of mixed galactoside SAMs, we compare the attachment and adhesion strength of organisms onto single-component monolayers formed from the nonmethylated (**1**), fully methylated (**2**), and the monomethylated (**3**) galactoside-terminated alkylthiols (Figure 1). The monomethylated galactoside **3** performed better than **1** and **2** in the protein resistance assays, and whether this is also true for the marine foulers is of considerable interest to us. Many marine and other biological adhesives are proteins, or proteinaceous, and the hypothesis on which this study relies is that these will not adhere strongly to a protein-rejecting surface, and it is thus an attempt to generalize properties inferred from the protein adsorption assays to a wider range of fouling species. The SAMs formed from the monomethylated galactoside (**3**) are also compared to SAMs formed from a mixture of methyl- and hydroxyl-terminated alkylthiols (**4**) with wetting characteristics (referring to both advancing and receding contact angles) and surface methyl to hydroxyl ratio closely resembling that of the monomethylated galactoside SAM (**3**). These two latter SAMs could thus be expected to have very similar surface properties, and we also ask whether the differences are still enough to cause differences in the attachment or adhesion of marine biofouling species.

MATERIALS AND METHODS

The synthesis and characterization of the three galactoside compounds, *N*-(16-mercapto-hexadecanoyl)-2-aminoethyl-β-D-galactopyranoside (**1**), *N*-(16-mercapto-hexadecanoyl)-2-aminoethyl-2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside (**2**), *N*-(16-mercapto-palmitoyl)-2-aminoethyl-6-*O*-methyl-β-D-galactopyranoside (**3**) (Figure 1), as well as details about the characterization of the SAMs formed from these three compounds, were thoroughly described by Hederos et al.[36] Properties of mixed methyl- and hydroxyl-terminated alkylthiol SAMs (**4**, Figure 1) have also been described earlier[37,38] and in the following we provide only the most relevant characteristics of these SAMs.

SAM Preparation. Two types of borosilicate glass slides, 76 × 25 mm^2, thickness 1 mm, were used as SAM supports. Standard slides for microscopy (VWR) were TL1-cleaned (5/7 H_2O, 1/7 30% H_2O_2, and 1/7 25% NH_3 at 80 °C for 10 min) before metal deposition, whereas cleanroom-cleaned Nexterion Glass B slides (Schott) were used as received (no differences were observed as a result of switching between these two types). A resistively heated vacuum evaporation system with a base pressure of less than 3×10^{-6} Torr was used to coat the slides with a 25 Å chromium adhesion layer at a rate of 0.3−0.5 Å/s and an additional 2000 Å gold layer (at 5−10 Å/s). The gold-coated slides were TL1-cleaned before 24 h incubation in thiol solutions. Saccharide-terminated thiols were adsorbed from 50 μM solutions of the thiols in ethanol, whereas the mixed monolayers **4** were adsorbed from a solution containing 25 mol % 16-thiohexadecane (Aldrich 99%) and 75 mol % 16-thiohexadecanol (gift from Pharmacia Biosensor, >99.5%), with a total thiol concentration of 1 mM. After incubation, the SAMs were sonicated for 2 min in ethanol, rinsed with ethanol, dried with dry nitrogen gas, and either subject to physicochemical characterization or packed under nitrogen for overnight shipment to the biological test laboratories. SAMs for characterization by IRAS and some of the ellipsometric measurements were prepared on 20 × 40 mm^2 pieces cut from a silicon wafer with native oxide, TL1-cleaned, and coated in a electron-beam UHV system (base pressure <5×10^{-9} Torr) with a titanium adhesion layer preceding the gold layer, but with layer thicknesses as described above. All water used during SAM preparation was 18.2 MΩ cm Milli-Q water (Millipore).

Samples for barnacle assays were prepared in 24-well polystyrene cell culture plates, coated by a 300 Å thick gold layer to keep them semitransparent. The wells were gold-coated on the inside by mounting the plates at a variable angle from the horizontal on a rotating sample holder, and then evaporated under the conditions described above, whereas gradually varying the tilt angle of the sample holder to ensure that both the bottom and the sides of the wells were evenly coated. The plates were sterile when mounted, and to avoid the TL1 cleaning steps, the wells were filled with the thiol solutions immediately after removal from the evaporator. For contact angle measurements, flat portions were cut from a well plate, coated as described above, and incubated with thiol solution in the slightly larger well of an uncoated 6-well plate.

SAMs intended for biological assays were packed under inert gas (N_2) immediately after preparation, and shipped overnight for assay. All biological assays were performed at least twice on samples prepared in different batches, and with different batches of organisms.

Contact Angle Measurements. A semiautomatic optical contact angle meter (KSV CAM 200) was used to determine advancing and receding contact angles of water on the SAMs. A manual dispenser was used to expand or retract a droplet, while the sessile drop profile was video-recorded. Drop shape analysis of the video images using the software supplied with the instrument provided the contact angles. For each batch of samples, data were averaged from three samples, two measurements were made on each sample, and each measurement consisted of at least ten images for the advancing and receding angles, respectively.

Ellipsometry. An ellipsometer set at 70° angle of incidence at a fixed wavelength of 632.8 nm (Rudolph Research AutoEL) was used to determine SAM thicknesses. The refractive index of the clean gold substrate was obtained immediately after the TL1-cleaning step, prior to incubation.A three layer optical model (ambient/organic film/gold) was used to determine the SAM thickness, assuming an isotropic, transparent organic layer with the refractive index of $n = 1.50$. Both the properties of the substrate and the film thicknesses were calculated by averaging values from five different spots on each sample, and at least three samples from each batch were compared to ensure consistency.

Infrared Reflection−Absorption Spectroscopy (IRAS). IRAS spectra were recorded in a grazing angle (85°) reflection setup in a Bruker IFS66 system using a N_2-cooled MCT detector. The system

Table 1. Advancing and Receding Contact Angles, and Ellipsometric Thicknesses[a]

	description	advancing angle (deg)	receding angle (deg)	thickness (Å)
1	hydroxylated galactoside	<10	<10	27.8 ± 0.7
2	methylated galactoside	76 ± 3	60 ± 2	29.5 ± 0.3
3	monomethylated galactoside	50 ± 3	24 ± 4	28.7 ± 0.7
4	CH_3-/OH-terminated alkylthiols	52 ± 2	22 ± 3	22.3 ± 0.7

[a] Errors for contact angle data are maximum deviations for all measured samples; the thickness errors are standard deviations.

was continuously purged with nitrogen before and during the measurement. All spectra were acquired at 2 cm^{-1} resolution between 4000 and 700 cm^{-1}, as a summation of 3000 scans. A three-term Blackmann-Harris apodization was applied to the interferograms before Fourier transformation. Background spectra were acquired using a deuterated hexadecanethiol SAM.

***Ulva* Zoospore Attachment and Adhesion Strength Assay.** Reproductive thalli of the green macroalga *Ulva linza* (formerly *Enteromorpha linza*) were collected from Llantwit Major beach, Glamorgan, Wales (52° 23′ N; 3° 30′ W). Zoospores were released into artificial seawater (ASW) at pH 8.0 and 32‰ ('Tropic Marin', Aquarientechnik GmbH), and prepared for assay as described in Callow et al. (1997). Samples were assayed within 24 h of receipt, and packaging was opened immediately prior to assay; contact time with air was <5 min. Attachment assays followed the principles outlined in Callow et al.[39] and Finlay et al.[40] In brief, each SAM surface (6 replicates of each chemistry) was placed in a separate compartment of a Quadriperm plate (Greiner Bio-One Ltd.) to which ten milliliters of a suspension containing 1.0 × 10^6 mL^{-1} zoospores were added. Zoospores were allowed to settle (attach) onto the surfaces for 60 min, in the dark, before the residual suspension was aspirated and the slides gently washed. Three replicate slides were fixed in 2.5% (v/v) glutaraldehyde in seawater, washed in deionized water, and air-dried as described in Callow et al.[39] The other 3 replicates were exposed to a wall shear stress of 52 Pa in a water channel,[41] and then fixed as described above. The density of adhered spores was determined using a ×10 objective with a Zeiss Kontron 3000 image analysis system attached to a Zeiss epifluorescence microscope and video camera as described in Callow et al.[42] Thirty fields of view were counted at 1 mm intervals along the length of each of 3 replicate slides before and after exposure in a flow channel. Zoospore removal data are expressed as a percentage of the initial density (i.e., before exposure to flow) of attached spores. Percentage data were arcsine transformed prior to statistical analysis. A nested ANOVA with pairwise Tukey comparison was performed in Minitab 14. $N = 90$ throughout.

Barnacle Settlement Assay. Cypris larvae were obtained from adult *Balanus amphitrite* and maintained in the laboratory as described elsewhere.[43] The 24-well plates were rinsed gently with artificial seawater (Tropic Marine, Germany) to remove any remaining solvent and were then immersed in a tank of artificial seawater (ASW) for one hour before the assay was started. Ten three-day-old cyprids were introduced to each well contained within 2 mL of ASW (6 replicates for assay 1 and 12 for assay 2). The plates were incubated in darkness at 28 °C for 48 h in total, and settlement was determined after 24 and 48 h of incubation. A blank polystyrene 24-well plate was included in the assay as an internal laboratory standard to gauge settlement behavior of the cyprids. The results are expressed as the percentage of the settlement, with 95% confidence intervals. The data were analyzed statistically using the Kruskal–Wallis test with post hoc comparisons of treatment means made with the Dunn's multiple comparison test.

Bacterial Attachment and Adhesion Assays. The methods are explained in detail in D'Souza et al.[44] In brief, cultures of *Marinobacter hydrocarbonoclasticus* and *Cobetia marina* (formerly *Halomonas marina*) were subcultured and grown for 18–22 h. Cells were collected by centrifugation at 8000 rpm for 8 min, washed and resuspended in sterile ASW to give a turbidity of 0.2 OD. Six replicate slides of each sample were used for each assay; two were treated as blanks and four were inoculated with bacteria (two to quantify attachment and two to quantify adhesion strength).

The test slides were placed in Quadriperm dishes with 8 mL of culture and incubated at 30 °C for 1 hour for initial attachment of cells. Slides were washed by dipping twice in two beakers of ASW and then placed in new Quadriperm dishes containing 8.8 mL of enriched seawater (SW) for 4 h at 30 °C with gentle shaking. Slides were rinsed by dipping in two consecutive beakers of ASW to remove loosely attached cells. The slides that were to be used for release measurements were mounted on the drum of a rotor apparatus, which was immersed in SW and rotated at 12 knots for 10 min. To assess the density of bacteria on the test surfaces, the slides were partially air-dried at 30 °C, and then four drops of the fluorescent nucleic acid label SYTO-13 (Invitrogen) (1.5 μM) added and a thin coverslip glass was placed on top. The slides were kept in darkness for 10 min after which fluorescence was measured in a plate reader. The SAMs of each chemistry were exposed without bacteria under otherwise the same conditions as the other samples, serving as coating blanks. The fluorescence from these blanks was subtracted from the results obtained in the bacterial assays. Significance was tested using nested ANOVA with pairwise Tukey comparison, $N = 45$.

RESULTS

SAM Characterization. Samples were routinely characterized with ellipsometry and contact angle goniometry before being shipped for biological assays. IRAS was used to verify the structure of the monolayers, and also to ensure the integrity of the SAMs after some of the experimental procedures. The results summarized in Table 1 are averages for 11 samples from different batches and substrates. The relatively large errors for the contact angle measurements reflect the fact that contact angles for the SAMs tend to be slightly different depending onto which substrate the gold film is deposited (glass, silicon or polystyrene), but the deviations within each batch of samples were smaller. The results in Table 1 and the IRAS spectra (see the Supporting Information, Figures S1 and S2) are in good agreement with the results obtained previously on these SAMs.[36] To verify the stability of the SAMs in ASW, IRAS, ellipsometry and contact angle measurements were performed on samples after 60 h immersion in ASW. Results before and after immersion were similar, with deviations within experimental error (see the Supporting Information, Figure S3).

***Cobetia marina* Attachment.** Results for the formation of bacterial biofilms of *Cobetia* are shown in Figure 2. The amount of biofilm differs significantly between the samples ($p = 0.001$, nested ANOVA analysis). Further, each surface is significantly different from every other ($p = 0.01$), with the exception of **2** and **4** (Tukey's pairwise comparison). The biofilm formation on each surface type is plotted against advancing contact angle in Figure 3 (circles), and there is no significant relationship with contact angle (whether **4** is included or not). No parametric testing of

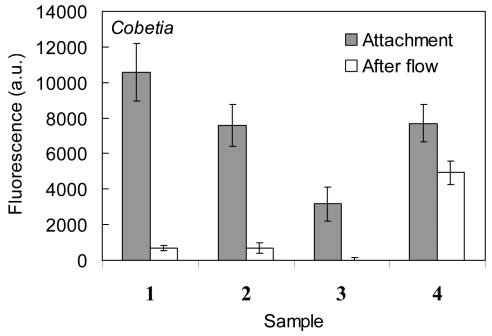

Figure 2. *Cobetia* initial bacterial attachment and after exposure to flow. The error bars represent 2 × standard error.

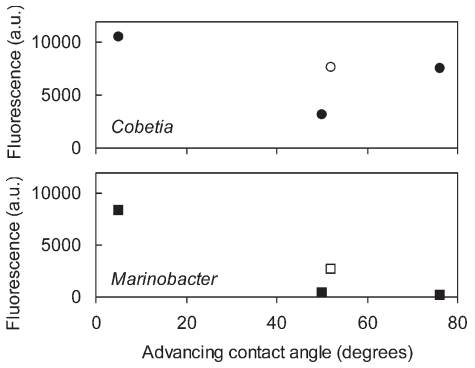

Figure 3. Bacterial attachment versus advancing contact angles for *Cobetia* (top panel, circles) and *Marinobacter* (bottom panel, squares). In both panels, samples 1−3 are represented by filled symbols and the mixed methyl-/hydroxyl-terminated SAM 4 with open symbols.

differences in biofilm release were performed since the release was almost complete for all surfaces but **4**. Epifluorescence images of representative areas of the slides before and after exposure to flow are included in the Supporting Information.

***Marinobacter hydrocarbonoclasticus* Attachment.** *Marinobacter* is a Gram-negative, motile, rod-shaped, hydrocarbon-degrading bacteria. Data for biofilm formation onto the four tested chemistries are provided in Figure 4. Biofilm development was relatively poor on the partly and fully methylated samples. Nested ANOVA analysis shows significant differences in attachment (p = 0.001). Each surface is significantly different from every other (p = 0.01) with the exception of **2** and **3** (NS) according to a Tukey's pairwise comparison test. For *Marinobacter* biofilm formation, there is a significant relationship with the contact angle of the surfaces (p = 0.001 with **4**, and p = 0.05 without **4**), see Figure 3. Again, because removal of the biofilm was complete from all surfaces, no parametric tests of differences in biofilm release were made. Fluorescence images of the slides before and after exposure to flow are included in the Supporting Information (Figures S4 and S5).

***Ulva* Assays.** The results of the *Ulva* attachment and removal assays in Figure 5 show that there are marked differences in the attachment density of *Ulva* spores on the different SAMs. One way nested ANOVA analysis shows that the attachment density differs significantly with surface type (p = 0.001), and the posthoc Tukey's test shows that each surface type differs significantly from all the others (p = 0.01). Attachment is greatest on the methylated

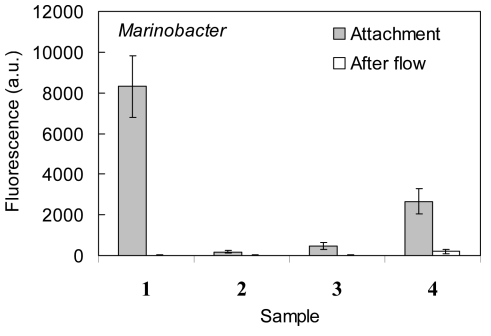

Figure 4. *Marinobacter* initial attachment and after exposure to flow. The error bars represent 2 × standard error.

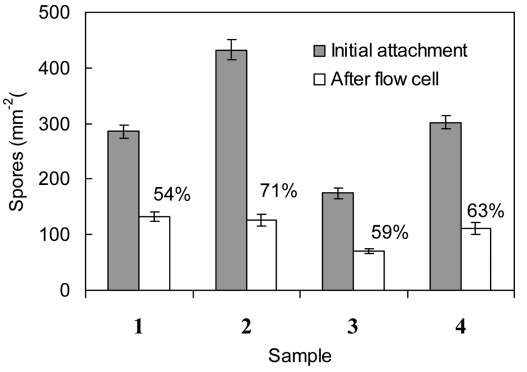

Figure 5. Density of *Ulva* spores after 45 min attachment and after 45 min attachment and exposure to 52 Pa wall shear stress in a flow cell. The numbers indicate the % removal of the spores from each sample. N = 90, error bars = ±2 × standard error.

galactoside SAM (**2**) and least on the partially methylated galactoside SAM (**3**).

Attachment and removal are expressed as a function of advancing contact angle in Figure 6. The galactoside SAM series is shown with filled markers and the mixed methyl-/hydroxyl-terminated SAM with open markers. The extent of spore removal differs significantly with surface type (p = 0.01). Tukeys test shows that **1** is significantly different to **3** (p = 0.05), as well as to **2** and **4** (p = 0.01 for the last two). Removal from **3** is not significantly different to **4**, but is significantly different to **2** (p = 0.01). Also **4** and **2** are significantly different (p = 0.01) in this respect. According to a model 1 regression, there is no significant relationship between advancing contact angle and spore attachment density (Figure 6, top panel), whereas the extent of spore removal varies significantly with contact angle (Figure 6, bottom panel); if the regression is performed on all samples p = 0.001, whereas if **4** is excluded and the regression is performed only on the galactosides, then the significance of the relationship is slightly less certain (p = 0.01). Clearly, *Ulva* attachment does not vary systematically with the wettability of the surfaces, whereas the removal follows a clear trend of increasing removal with decreasing wettability.

Barnacle Cyprid Assays. The result of the barnacle cyprid settlement assay is that either there is no, or very little settlement on any of the tested SAMs at both time intervals. Figure 7 shows data from two independent assays performed on different occasions, and also the attachment onto a polystyrene (PS) sample, used as an internal laboratory standard to evaluate

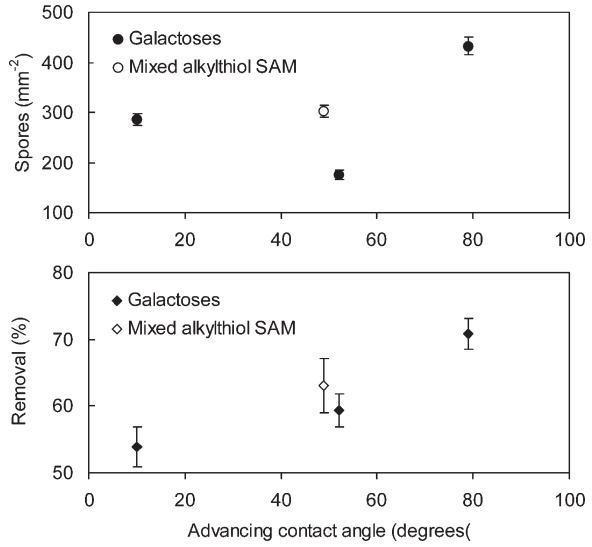

Figure 6. Top: Density of *Ulva* spores as a function of the advancing contact angle of the SAMs. Bottom: Removal of *Ulva* spores by 52 Pa wall shear stress, as a percentage of the initial attachment density and shown as a function of the advancing contact angle of the SAMs. For both data sets, filled symbols represent galactoside SAMs, while the open symbols represent the mixed CH_3-/OH-terminated alkylthiol SAM. $N = 90$, error bars = $\pm 2 \times$ standard error.

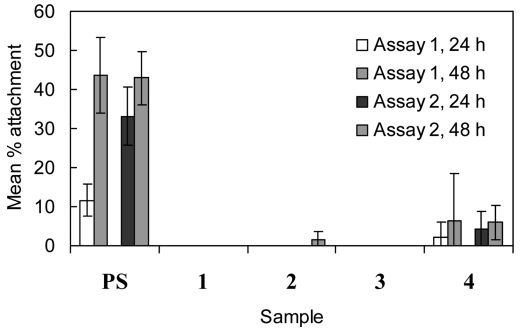

Figure 7. Mean percentage attachment of barnacle cyprid larvae after 24 and 48 h. Error bars show 95% confidence intervals, $N = 6$ for assay 1, and 12 for assay 2. The attachment onto a polystyrene (PS) internal laboratory standard is included to demonstrate the viability of cyprids in each assay.

consistency between different assays. Statistical evaluation of the results shows that attachment on the four tested SAMs cannot be distinguished from each other ($p < 0.05$) even though settlement is higher on **4** compared to the galactoside-terminated SAMs **1**−**3**. The significantly higher settlement on the PS standard demonstrates that the low settlement on the SAMs cannot be attributed to an inability of the cyprids to settle and that they were healthy. As well as settlement, the percentage mortality of the cyprids used in the assay was calculated; no, or very low, mortality was detected on the test surfaces, and no differences were observed between the test surfaces and the polystyrene standard in this regard.

DISCUSSION

This section is organized as follows: first, the SAM structure and each of the biological assays will be discussed, thereafter two particular issues will be highlighted, that is (i) whether the monomethylated saccharide **3**, which is more effective than **1** and **2** in preventing protein adsorption, also is more effective in preventing or reducing the attachment of marine fouling organisms, and (ii) to what extent the wettability-matched surfaces **3** and **4** yield different results in the fouling assays, and the possible causes of any differences.

SAM Structure. From IRAS data, the degree of order and the orientation of the molecules in the SAMs can be determined. The peak positions of the CH_2 stretching modes are indicative of the molecular organization and packing of the alkyl chains, and the relative contributions from the amide I (whose origin is predominantly C═O stretching) and amide II bands (C−N stretching combined with C−N−H in-plane bending) are indicative of the alkyl chain orientation relative to the surface normal, as well as the degree of order in the alkyl chain layer. The IRAS data is in excellent agreement with previously published results (see the Supporting Information for IRAS spectra). In the following, we summarize the results, and refer to previous publications for peak assignments and further details of their analysis.[36,37] Peak frequencies for the symmetric (ν_s) CH_2-stretching mode range from 2918 cm^{-1} for a perfectly ordered all-trans alkyl chain, to 2928 cm^{-1} for a liquidlike gauche-rich chain; similarly, the asymmetric (ν_{as}) mode shifts from 2850 to 2856 cm^{-1} with increasing disorder. The asymmetric mode appears at 2850 cm^{-1} for all four SAMs, while the symmetric mode shifts from 2918 cm^{-1} in the mixed alkylthiol SAM **4** to 2919 cm^{-1} in **1** and **3**, and 2920 cm^{-1} in **2**. This may be interpreted as a slightly less ordered alkyl chain layer in **2**, but the suppression of the amide I band intensity relative to that of amide II (due to orientation of the C═O stretching mode dipole moment parallel to the surface) is more pronounced for **2** than in either **1** or **3** (which are very similar in this respect). This indicates that the orientation of the alkyl chains is, on average, somewhat more upright in **2** than in **1** and **3**, and any slight disorder in the alkyl chain layer in SAMs of **2** is thus not propagated to the saccharide headgroups. The CH_3/OH ratio in **4** was chosen to match the contact angles of **3**, but since it is not certain that the composition in a mixed monolayer may be matched to both the advancing and receding angles simultaneously for any given surface, it is of interest that this is indeed a ratio that gives a good match. Since a large hysteresis range usually is indicative of surface disorder or heterogeneity, the fact that the hysteresis of **3** is similar to that of the crystalline SAM **4**, also supports the interpretation that the saccharide-terminated SAMs form well-ordered monolayers with little room for reorganization of terminating saccharide moieties. The slight variations in contact angles between SAMs on different substrates are most likely caused by differences in substrate surface roughness.

Taken together, the IRAS, ellipsometry and wetting data show that all four SAMs are well-ordered, and have their alkyl chains arranged in a crystalline layer dominated by all-trans configurations.

Bacterial Attachment. In situations where specific cell-surface interactions are missing, the attachment of bacteria to surfaces is initially governed by the physicochemical properties of both the bacteria and the surface (as well as of the intervening medium), where interfacial energy (or wettability) and surface charge are the dominant properties, with van der Waals interactions, polymer bridging and hydrophobic interactions giving relatively weaker contributions. Soon after biofilm formation commences, EPS produced by the bacteria will effectively reduce

the influence of initial surface properties, and the properties of the EPS will instead dictate the suitability for further attachment of the cells. In the evaluation and comparison of bacterial biofilm formation on different substrata, this process is further complicated by variability in the composition of EPS produced by the same type of bacteria, if it is attached to different substrates.[45] The relevance of thermodynamic predictions in explaining bacterial attachment and adhesion is well documented,[46−48] indicating that hydrophobic bacteria should adsorb to a greater extent onto hydrophobic materials, and hydrophilic bacteria should prefer hydrophilic materials, and also that surface hydrophobicity (of either the cell or the substratum) correlates experimentally with adhesion. The application of these predictions is complicated by specific effects of surface chemistry, variability, and changes in bacterial physiology, and in the determination of surface properties of adhering cells, which makes it difficult to systematically investigate deviations from predicted behavior.

Cobetia marina. Although bacterial attachment is greatest on the most hydrophilic sample (**1**), there is no significant overall relationship with contact angle (or the cosine of the contact angle, not shown), emphasizing the role of surface chemistry in favor of wettability in this case. This contrasts with results from Ista et al., who found preferential attachment of *Cobetia* onto hydrophobic surfaces, with similar results for mixed SAMs formed from either OH/CH_3- and COOH/CH_3-terminated alkylthiols, and which demonstrated a negative linear relationship between *Cobetia* attachment and the cosine of the advancing contact angle, thus suggesting that cell attachment is in (at least qualitative) agreement with thermodynamic models.[49] We note that the assays in Ista et al. were performed in a flow system attached to a chemostat, so that the samples were also conditioned with culture medium ingredients, while in our assays bacteria were resuspended in ASW before assay, which may contribute to the observed differences. The bacterium used by Ista et al. were also selected for their hydrophobicity before use, and this may be a very important point, since published data for the wettability of *Cobetia marina* vary considerably, with water contact angles ranging from 15[50] to 75°.[51] To some extent, differences in reported bacterial properties may be explained by the difficulties involved in the determination of bacterial surface properties,[48] but bacterial physiology may vary with time and growth or culture conditions, and thus reflect natural or selectively cultured variations in surface properties. Akesso et al. found that *Cobetia* attachment increased with increasing surface energy,[50] and because their bacterial assays were performed in the same laboratory as in our study, it is pleasing to note that the results are not inconsistent with ours, and we thus suggest that deviations between our study and previously published results can be attributed to differences in surface properties of the used bacteria, or possibly to differences in assay conditions.

Marinobacter hydrocarbonoclasticus. The data presented in Figures 3 and 4 show a significant increase in bacterial attachment for hydrophilic surfaces, equivalent to increasing biofilm formation upon increasing surface energy. Literature data indicate that *Marinobacter* are relatively hydrophobic bacteria; reported advancing contact angles are 85[52] and 81.5° (surface energy 25.4 mJ/m^2).[50] In a study by Bakker et al., it was concluded that the influence of substrate surface energy on the deposition of bacteria onto solids was "largely in line with surface thermodynamics", with low surface energy bacteria preferentially adhering to low-energy surfaces. In particular, *Marinobacter* showed a greater preference for hydrophobic coatings.[52] A similar observation was made by Akesso et al., in that *Marinobacter* biofilm formation decreased as the surface energy increased.[50] Both these studies predict trends that are opposite to our data, for which there is a significant negative correlation between bacterial attachment and the water contact angle (see Figures 3 and 4). Again, it is difficult to pinpoint the reason for this deviation, but because it is obvious for both the bacterial assays that attachment onto **3** and **4** are different, wettability is not a suitable parameter for predicting adsorption to these surfaces; this is discussed in detail later.

***Ulva* Zoospore Attachment.** Mature plants of *Ulva* produce motile zoospores (7−8 μm in length), which lack a cell wall, and move with the aid of flagella. The random swimming of the zoospores through the water column turns to surface exploration behavior as zoospores approach a surface. During this stage, a spore makes brief and repeated contact with surfaces, and apparently responds to a variety of cues in a selective manner. Eventually, and as some appropriate cue is sensed, irreversible morphological changes associated with permanent attachment of the spore commence.[39,53] The response of *Ulva* zoospores to a range of cues has been investigated, of particular interest to this discussion is a series of studies using mixed SAMs of methyl- and hydroxyl-terminated[14,49,54] and methyl- and carboxylic acid-terminated alkylthiols.[49]

In the studies using mixed OH-/CH_3-terminated alkylthiol SAMs, spores clearly and consistently preferred to settle onto the hydrophobic surfaces dominated by methyl groups, with an approximately 10-fold increase of the number of settled spores on the fully methylated surfaces ($\theta_a \approx 100°$) relative to the hydroxylated surfaces ($\theta_a \approx 20°$).[14,49,54] Adhesion was weaker on the hydrophobic than on the hydrophilic surfaces, decreasing monotonically with increasing contact angle, and overall, the results were consistent with thermodynamic models based on estimated interfacial free energies in the system.[54] Our data presented above agree qualitatively with these studies to some extent, in that the fully methylated SAM **2** was the sample with highest attachment, but attachment corresponds to only an approximately ×1.5 increase over that on the fully hydroxylated surface, and while the attachment increases monotonically with the surface CH_3−group content (and contact angle) in the studies using OH-/CH_3-terminated SAMs,[14,49,54] we note that attachment onto the monomethylated galactoside **3** is the smallest in our study. It is also clear from Figure 6 that attachment onto our samples does not vary monotonically with either surface composition or advancing contact angle, and that attachment thus cannot be explained simply in terms of the wettability of the surfaces, but must be determined by other factors. In our data there is a small but significant trend of decreasing adhesion with increasing contact angle, with spore removal being lower for hydrophilic surfaces, again in qualitative, but not quantitative agreement with the results from the OH/CH_3 SAMs in Finlay et al.[54] In the latter, removal ranges from approximately 25% for the fully hydroxylated surface, to 90% for the methylated. The corresponding range in our data is 54−71%. It is of interest that removal from our mixed OH/CH_3 sample **4** (63% removal) is in reasonable agreement with the interpolated result for the SAM in Finlay et al.[54] with corresponding contact angle (approximately 50% removal; the difference is within the range of normal variations between assays), indicating that the observed differences between the saccharide SAMs in this study and the previous results obtained using OH-/CH_3-terminated SAMs cannot be

attributed merely to differences in the spore quality, but rather must reflect a difference in response to the different surfaces.

There was a clear difference between the trends observed on OH/CH_3 and $COOH/CH_3$ mixed SAMs;[49] whereas the variation in attachment with the cosine of the advancing contact angle (which is proportional to the surface energy) is nearly linear in the former case, it clearly is not in the latter, although the variation is still monotonic in both cases. Also, for the mixed-composition SAMs zoospore attachment is lower on $COOH/CH_3$ mixtures than on the OH/CH_3 SAMs.[49] The introduction of surface charges and the ensuing sensitivity of both the surface and the spores to variations in the pH complicates the interpretation for mixed $COOH/CH_3$ SAMs, and simple thermodynamic models fail to (quantitatively) account for the obtained results. The lesser attachment to $COOH/CH_3$ mixtures was attributed to the interfacial properties of the adhesive-SAM interface, but whether the differences in attachment are related to phenomena of importance during the exploratory "pre-attachment" phase or by factors affecting cell-to-cell signaling or other indirect effects were not clear. Our results similarly indicate a nontrivial relationship between surface composition and both the quantity and the strength of the attachment. Of the three galactoside SAMs, the sample with intermediate advancing contact angle (**3**) has the lowest attachment, while the attachment is significantly greater onto the mixed OH/CH_3 SAM **4** with similar contact angles and approximately the same ratio of OH and CH_3 groups. However, the linear relation between the cosine of the contact angle and spore removal (not shown), and the similarity between the removal data of the two contact-angle matched SAMs **3** and **4**, suggests that at least the removal of the spores could be dictated by thermodynamic properties, and that the observed data are consistent with adhesive failure at the interface, whose physicochemical properties would determine the result of applied shear stress.

Barnacle Cyprid Attachment. The low, and indistinguishable, attachment of barnacle cyprid larvae onto the SAMs is encouraging only in the sense that they have excellent antifouling properties to cyprids (this result precluded adhesion measurements), and as such the results are in good agreement with a similar study of other mono- and oligo-saccharide-terminated SAMs.[55] However, from a physicochemical point of view it is disturbing that surfaces with contact angles ranging from <10 (**1**) up to 76° (**2**), and consisting of hydroxyl- and methyl-terminated glucosylic (**3**) and alkylthiol (**4**) SAMs alike, have equally little appeal to a cyprid. This is in contrast to previous studies where *Balanus amphitrite* cyprids displayed a preference for hydrophilic surfaces.[56,57] Our data clearly do not follow this pattern; not only is the attachment equally low on all SAMs but attachment onto the SAMs is also much lower than on the hydrophobic PS surface (uncoated wells in the PS well plate, advancing contact angle approximately 75−80°). It could be hypothesized that the different optical properties play a role; all SAMs appear visually as shiny gold surfaces with high reflectance, but because attachment assays are performed in the dark, this cannot explain the deviation neither within the presented results nor from published data.

Sugars are known to affect barnacle larval attachment, and for example, D-mannose, D-glucose, and D-galactoside dissolved in solution were all demonstrated to have an inhibitory effect on the attachment of cyprids of five different barnacle species.[58] Later, it was demonstrated that other sugars may aid in the attachment,[59,60] and D-mannose has also been shown to result in higher metamorphosis of settling cyprids.[61] These observations are clearly of interest to us, but of little relevance to the study at hand. The steric availability of the sugar moieties in the close-packed SAMs is very limited, and it is difficult to conceive how any specific interactions could take place with the surface-bound sugars. The surface characterization shows beyond doubt that the SAMs are well-ordered with molecules arranged in an upright conformation. This does not exclude the presence of pinholes, line defects at ledges and dislocations, and other defects in the monolayers, but these will not considerably improve the steric availability of the saccharide groups, but only marginally increase their exposure. Taking into account that sugars interact weakly with their receptors, typically requiring the whole sugar moiety to be available for binding,[62] it does not seem likely that specific interactions occur with sugars in these SAMs. This, however, may be possible via surface dilution of the sugars in the SAM,[63] which is also the topic of a forthcoming paper investigating antifouling properties of other mono- and oligosaccharides.[55]

In a recent study of covalently attached polysaccharide coatings prepared from hyaluronic acid, alginic acid, and pectic acid, Cao et al. found that only hyaluronic was efficient in reducing the attachment of barnacle cyprids, compared to a glass reference surface, but this effect was smaller than the differences in resistance of the surfaces against proteins.[9] However, direct comparison of these results with our data is difficult since the three polysaccharides were all charged, and considerable impact of bivalent ions on the fouling resistance were reported due to structural changes in the adsorbed polysaccharide films. Our samples were not explicitly tested for sensitivity to bivalent ions, but these are certainly present in the artificial seawater salt mixtures used for the assays (and for the SAM stability tests), although the lack of ionizable groups and the much more rigid structure of our coatings also reduce any structural effects of ions on the surface layers, and we expect divalent ions to play a lesser role in the attachment to the SAMs.

Does the monomethylated SAM 3 prevent attachment of marine organisms better than 1 and 2? In the protein adsorption study by Hederos et al.,[36] it was found that fibrinogen adsorption was near zero onto **3**, but much greater onto **1** and **2**, and that lysozyme adsorption was prevented by **3** and **1** but not by SAMs of **2** (this is by no means a coincidence; **3** was designed to reproduce the protein-resistant properties of a 75/25 mixture of **1** and **2**). This pattern of **3** being more fouling-resistant is weakly reflected in this study; clearly in the *Ulva* zoospore and *Cobetia* biofilm formation assays, whereas the results of the *Marinobacter* and *Balanus* assays are ambiguous — at least they do not contradict this conclusion, in that the results for **2** and **3** are similar in the two latter cases. These observations support the hypothesis that high protein resistance also results in low attachment of marine organisms for these surfaces, but given the limited range of test organisms and the somewhat ambiguous result in two of the assays, this statement is rather weak.

Differences between the Monomethylated SAM (3) and the Mixed Alkylthiol SAM (4). It is evident from the results that the two wettability-matched surfaces **3** and **4** perform distinctly differently across all the assays, and the obvious question to ask in light of this is: What is it really that is different between the surfaces **3** and **4**?

The results for *Ulva* attachment, and the biofilm formation for both of the tested bacteria, *Marinobacter* and *Cobetia*, were significantly different between the SAMs **3** and **4**. In the barnacle assay, there is nominally some attachment onto **4** but

Table 2. Contact Angle Data for the Four SAMs Using Water (W), Diiodomethane (DI), and Ethylene Glycol (EG)

	advancing contact angles (deg)			surface energy components (mJ/m^2) [a]				
	θ^{W}	θ^{EG}	θ^{DI}	γ^{LW}	γ^{AB}	γ^{+}	γ^{-}	total γ_{sv}
1	5.8 ± 0.6	13.3 ± 0.8	26.5 ± 0.6	45.6	0.85	0.00	67.0	46.4
2	73.6 ± 0.9	68.4 ± 0.3	63.1 ± 0.2	26.8	2.08	0.05	21.9	28.9
3	48.7 ± 0.7	41.7 ± 0.1	46.0 ± 1.5	36.5	1.27	0.01	39.4	37.7
4	52.0 ± 0.4	32.3 ± 0.5	45.1 ± 1.2	37.0	6.36	0.34	29.6	43.3

[a] The surface energy components to the surface free energy are: γ^{LW} = Lifshitz−van der Waals component, γ^{AB} = acid−base component, γ^{+} = Lewis acid, γ^{-} = Lewis base.

not on **3**, while this difference is not significant at the 95% level. Superficially, **3** and **4** have different chemistries, but advancing and receding contact angles are very similar for the two, and both SAMs expose similar functional groups, and at the same ratio (with the reservation that the surface concentration of thiols in mixed SAMs does not exactly reflect the solution composition, though in this case, for a 75/25 ratio of OH/CH_3, the difference is rather small[37]). In addition, the roughness of these samples is dominated by the graininess of the gold coating (with grain sizes typically about 30−50 nm), but since substrates for different samples in each of the biological assays were always coated in parallel, this is not a likely cause of differences either. Of course, the differences in molecular structure of the SAMs **3** and **4** result in structural differences on the subnanometer length scale (which would in principle be accessible by scanning probe microscopic methods), but if this is a cause of the attachment differences, it raises serious and difficult questions about the mechanisms for distinguishing between these surface chemistries or surface structures in bacteria and *Ulva* zoospores. Specific interactions with the galactoside moieties is a possibility in principle, but this is an unlikely cause of the difference since both bacterial biofilm formation and *Ulva* attachment is lower on the saccharide SAM, and further, access to the sugar moieties in the close-packed SAM is very limited because of steric hindrance, as was discussed above. The differences in molecular structure between these two samples are obviously of critical importance, either directly or indirectly, though it is not obvious what the relevant physical differences between these samples are.

In a study, where CH_3-terminated alkylthiols were mixed with either OH- or COOH-terminated molecules of similar length, it was found that for similar wettabilities, the attachment of *Cobetia marina* cells was indifferent to the chemistry of the hydrophilic component of the SAM, but determined solely by the wettability.[49] The structural differences between CH_3/OH-SAMs and CH_3/COOH-SAMs are minor, and as our data demonstrate significant differences in bacterial attachment (for both bacteria) between samples **3** and **4**, with similar wettabilities, this again emphasizes the importance of other parameters than wettability.

Of some interest in this respect is the study of Bowen et al., comparing the adhesion of the diatom *Navicula perminuta* and spores of *Ulva linza* onto SAMs formed from alkylthiols with hydrocarbon chains varying from C_8 to C_{18} in length.[16] The differences in wettability between the samples (and presumably also surface energy, though this is not explicitly proved) are minute, only two degrees. However, the SAM structure changes from amorphous to crystalline at chain lengths around C_{12}−C_{14}, and this is accompanied by a distinct change in adhesion for both organisms. Adhesion correlates well with friction coefficients for the surfaces, and it was thus suggested that lubricity of the surfaces was important for the adhesion. This is less likely to be the cause of the observations for the saccharide SAM surfaces discussed here; the surface characterization unambiguously demonstrate that all four SAMs form close-packed and well-ordered monomolecular films, where there is no room for significant within the layer for any of the samples.

Surface Energy. The water contact angles are very similar for the surfaces **3** and **4**, but this does not mean that their interfacial free energies are similar. Young's equation relates the contact angle θ to the interfacial free energies γ_{sv}, γ_{sl}, and γ_{lv}, where l, s, and v represent the liquid, solid, and vapor phases, respectively

$$\cos\theta = \frac{\gamma_{sv} - \gamma_{sl}}{\gamma_{lv}}$$

From this relation, it is clear that the contact angles, probed on two surfaces with the same liquid, could be similar even if the surface free energies are different, as long as the difference $\gamma_{sv} - \gamma_{sl}$ remains constant. Thus, we cannot infer from the contact angles of one liquid whether the surfaces have similar free energies. Several semiempirical relations for estimating surface energies from contact angle data have been suggested, and we have chosen the Good-van Oss-Chaudhury (GvOC) approach[64] on the assumption that since it uses data from three different test liquids, it is more robust than models requiring only one or two liquids. It should also be mentioned, that any attempt to determine solid surface free energies from wetting data is dependent on the choice of liquids.[65] The contact angles for three different liquids − water (W), ethylene glycol (EG) and diiodomethane (DI) − are presented in Table 2. Using these data and the properties of these liquids as tabulated in Good,[64] we may proceed to calculate the surface free energy of each SAM (see the Supporting Information for details).

The calculation of surface energy components and the total surface energy shows that there are indeed differences between the surfaces, although moderate, and particularly that the SAMs formed from **3** and **4** do not have the same surface free energies. The dispersive component γ^{LW} is very similar for the two surfaces, and the difference is mainly in the polar, or acid−base, component γ^{AB}. In relative terms, the difference between the two surfaces' Lewis acid terms (γ^{+}) is greatest, but in absolute terms the difference is greater for the Lewis base (γ^{-}) component. However, since in the GvOC model $\gamma^{AB} \propto (\gamma^{-}\gamma^{+})^{1/2}$ (see the Supporting Information), γ^{AB} is more sensitive to the relative difference in γ^{+} than the absolute difference in γ^{-}. The Lewis base (γ^{-}, electron donor) component of a hydrogen-bonding pair is the hydrogen-bond acceptor, and in view of this, it may seem puzzling that OH-terminated surfaces have much more pronounced electron-donor (hydrogen-acceptor) than electron-acceptor (hydrogen-donor) character, which is true for all of the

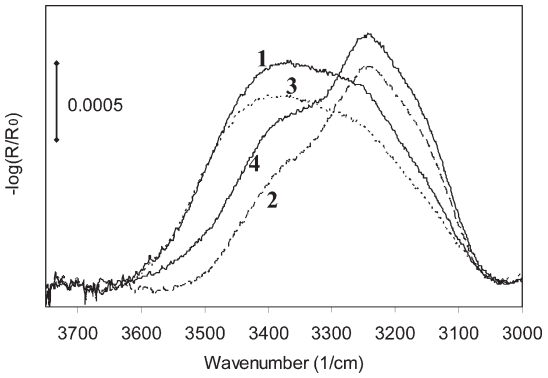

Figure 8. Hydrogen-bonding region of the IR spectra for the four SAMs.

SAMs **1**−**4**. Indeed, it has been noted that solid surfaces systematically have greater basic than acidic components, and if this is true it makes it difficult to assess, for example, the claim that protein-resistant surfaces should have hydrogen-bond acceptors but not hydrogen-bond donors,[29,31] but the cause of this imbalance is debated.[66] Irrespective of this, we note that the total surface energies of **1**−**3** are rather similar to their Lifshitz−van der Waals components, while this is not the case for **4**, because it has a sizable acid−base component due to the relatively larger Lewis acid term; the difference in absolute terms is small, but the relative difference to the Lewis acid components of the other SAMs is considerable. Whether this difference is of relevance to the observed variations in antifouling performance is not clear to us, but it would imply that **4** is a surface more prone to act as hydrogen-bond donor than the other SAMs. It may also be of relevance that **4** is different from **1**−**3** in the barnacle assay, but we cannot propose a mechanistic basis for this difference. Before leaving the topic of surface energies, we also note that in the *Marinobacter* assay (and only in this assay), the attachment is smallest on the surface with surface free energy nearest the minimum in the Baier curve.

Interfacial Water Structure. A recurring theme in the analysis of protein resistance of SAMs, and in particular the performance of oligoethylene-terminated SAM, is the role of water structure at the surface, and this is also the basis of the "chemical view" of protein resistance. The hypothesis being that surfaces which strongly bind and/or coordinate water, have a higher resistance against protein adsorption due to the unfavorable energetics involved in displacing the water from the surface. This was recently advanced also to explain the resistance of zwitterionic polymer coatings to barnacle cyprid settlement.[67] Proper determination of hydrogen-bond structure at the interface could be performed with, for example, sum-frequency generation spectroscopy, and although this would be of considerable interest, we leave this for future work, and consider instead some indirect information available from the acquired IRAS data. The IRAS results show that the alkyl chains in the saccharide SAMs are close-packed, with only marginal disorder as compared to those in the mixed SAM **4**. This implies that even if the average area occupied by each alkyl chain in **3** and **4** is only approximately equal, the surface density of hydroxyl groups is still very different. Where the mixed SAM **4** has three OH groups per four alkyl chains, the saccharide **3** has three OH-groups per alkyl chain, which results in a difference in hydroxyl surface density by a factor of 4. If this difference is propagated into an equal difference in hydrogen bonding to surrounding water molecules, it is clear that the enthalpic cost of replacing the bound water by other molecules should be much greater on **3** than on **4**, and that this could be an explanation of the observed differences in fouling properties.

The symmetric and antisymmetric water OH-stretching modes are well-defined in gas phase (ν_{ss} = 3657 cm^{-1} and ν_{as} = 3756 cm^{-1}), but broaden into a band stretching over 3000−3600 cm^{-1} in condensed phases, as a result of variability in the hydrogen-bonding environment. The IRAS data shows strong absorption in this region, indicating the presence of adsorbed water, see Figure 8 (the IRAS spectra are acquired on "dry" surfaces under N_2 purging). The spectra are dominated by broad absorptions centered at 3230 and 3400 cm^{-1}. The former has its origin in strongly coordinated water (sometimes referred to as "icelike" or tetrahedral), and the higher energy band at 3400 cm^{-1} represents water with lower coordination (or "liquidlike").[68,69] All four spectra have contributions from both bands, but there is a qualitative difference in that SAMs **1** and **3**, i.e., the fully and partly methylated sugars, are dominated by the "liquidlike" contribution, while the hydroxylated sugar **2** and the mixed SAM **4** are dominated by the "icelike" band. Thus, the structure of hydrogen-bonded water is different on samples **3** and **4**. However, water adsorbed onto **4** is more strongly coordinated ("icelike") than the lower-coordinated water in the partly hydroxylated saccharide layer. If the energy of displacing bound water is related to resistance to fouling, it is not obvious why the weakly coordinated water molecules on **3** are more difficult to remove from the surface than the strongly coordinated water on **4**. A possible explanation could be that the water adsorbed onto **3** resides inside the layer of saccharide headgroups. The nonpolar close-packed alkyl chain region of the SAMs is a very unfavorable environment for water molecules, and water adsorbed to the mixed alkylthiol SAM **4** must reside on top of the monolayer, where coordination to other water molecules would be relatively easy, whereas water may well penetrate into the polar saccharide headgroup region of the monomethylated galactoside, from where it would be much more difficult to remove, and where coordination to other water molecules may be sterically hindered. This explanation is consistent with the conclusions by Herrwerth et al. regarding the protein resistance of oligoether-terminated thioalkyl monolayers, that both internal and terminal hydrophilicity of the terminating chains are important, and that penetration of water to the interior of the SAM is a necessary prerequisite for protein resistance.[70] This pertains also to recent work on the antifouling properties of chiral polyol monolayers, where it was found that protein adsorption onto either of two enantiomers was higher than adsorption to the racemic mixture.[19] This difference was speculated to be caused by variations in water solvation at the interfaces. Clearly, the arrangement of water molecules adsorbed to, and within, the layer of polyols is expected to be different for either of the enantiomers, as compared to a mixture.

SUMMARY AND CONCLUSIONS

The opening question of this study was to what extent protein-resistant surfaces are also efficient in reducing the attachment of marine fouling organisms, and specifically, whether the higher resistance to nonspecific protein adsorption by SAMs exposing a partially methylated galactoside headgroup, as compared to that of the fully hydroxylated or fully methylated counterparts,[36] can be generalized to resistance to attachment and adhesion of

marine fouling organisms. Such a generalization is weakly supported by the obtained results, but the small number of test organisms and the ambiguous data in some of the assays limits the general validity of such a claim.

Our results corroborate the view that wetting behavior is a poor determinant for attachment of marine organisms, and since surfaces with similar functional groups (and in similar ratios) perform differently in the marine organism assays, it is clear that the exposed functional groups on the surface only indirectly affect marine biofouling. It appears unlikely that differences in fouling between **3** and **4** are related to an ability of organisms to discriminate between, and respond to, differences in the molecular structure of these two SAMs. We exclude the possibility that differences in fouling can be explained by specific interactions with the sugar moieties on our surfaces; the monolayers are well-ordered and close-packed, and access to the sugar rings is sterically hindered. Other structural properties such as water adsorption or uptake in the layer emerge as much more likely candidates for determining the antifouling properties of these molecular films. In this respect, there are similarities between our results and conclusions drawn from the rather extensive work performed on OEG SAMs, and their antifouling properties.[71]

The intention of this study was to investigate general (nonspecific) surface properties of galactoside-terminated SAMs for antifouling purposes, but the fact that the SAMs consist of sugars is in itself of some interest. Many sugars are substrates for specific recognition by receptors, but considering that specific binding of carbohydrates to lectins or other receptors usually is weak, and that carbohydrate substrates need to fit very well into their receptors,[62] surfaces exposing sugar moieties need to be carefully designed and optimized for these purposes. We foresee that saccharide-terminated SAMs could be used for studies of specific interactions in the future, provided that steric availability of the sugar moieties is improved. Work to this end, using ethoxylated alkylthiols as filler molecules to control the surface density of sugar groups has been carried out, and will be published elsewhere.[55]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. IRAS data for the four used SAMs (**1**−**4**), covering the amide and C−H stretching regions. Fluorescence images from the bacterial assays. Details about the calculation of surface free energies using the Good−van Oss−Chaudhury model. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Banerjee, I.; Pangule, R. C.; Kane, R. S. *Adv. Mater.* **2011**, *23*, 690–718.
(2) Schilp, S.; Rosenhahn, A.; Pettitt, M. E.; Bowen, J.; Callow, M. E.; Callow, J. A.; Grunze, M. *Langmuir* **2009**, *25*, 10077–10082.
(3) Larsson, A.; Ekblad, T.; Andersson, O.; Liedberg, B. *Biomacromolecules* **2007**, *8*, 287–295.
(4) Ekblad, T.; Bergström, G.; Ederth, T.; Conlan, S. L.; Mutton, R.; Clare, A. S.; Wang, S.; Liu, Y.; Zhao, Q.; D'Souza, F.; Donnelly, G. T.; Willemsen, P. R.; Pettitt, M. E.; Callow, M. E.; Callow, J. A.; Liedberg, B. *Biomacromolecules* **2008**, *9*, 2775–2783.
(5) Ostuni, E.; Chapman, R. G.; Liang, M. N.; Meluleni, G.; Pier, G.; Ingber, D. E.; Whitesides, G. M. *Langmuir* **2001**, *17*, 6336–6343.
(6) Youngblood, J. P.; Andruzzi, L.; Ober, C. K.; Hexemer, A.; Kramer, E. J.; Callow, J. A.; Finlay, J. A.; Callow, M. E. *Biofouling* **2003**, *19*, 91–98.
(7) Gudipati, C. S.; Finlay, J. A.; Callow, J. A.; Callow, M. E.; Wooley, K. L. *Langmuir* **2005**, *21*, 3044–3053.
(8) Statz, A. R.; Barron, A. E.; Messersmith, P. B. *Soft Matter* **2008**, *4*, 131–139.
(9) Cao, X.; Pettit, M. E.; Conlan, S. L.; Wagner, W.; Ho, A. D.; Clare, A. S.; Callow, J. A.; Callow, M. E.; Grunze, M.; Rosenhahn, A. *Biomacromolecules* **2009**, *10*, 907–915.
(10) Qian, P.-Y.; Xu, Y.; Fusetani, N. *Biofouling* **2010**, *26*, 223–234.
(11) Salta, M.; Wharton, J. A.; Stoodley, P.; Dennington, S. P.; Goodes, L. R.; Werwinski, S.; Mart, U.; Wood, R. J. K.; Stokes, K. R. *Philos. Trans. R. Soc., A* **2010**, *368*, 4729–4754.
(12) Vladkova, T. In *Marine and Industrial Biofouling*; Flemming, H.-C., Murthy, P. S., Venkatesan, R., Cooksey, K. E., Eds.; Springer: Heidelberg, Germany, 2009; pp 135−163.
(13) Prendergast, G. S. In *Biofouling*; Dürr, S., Thomason, J. C., Eds.; Wiley-Blackwell: Chichester, U.K., 2010; pp 30−59.
(14) Callow, M. E.; Callow, J. A.; Ista, L. K.; Coleman, S. E.; Nolasco, A. C.; Lopez, G. P. *Appl. Environ. Microbiol.* **2000**, *66*, 3249–3254.
(15) Callow, J. A.; Callow, M. E.; Ista, L. K.; Lopez, G. P.; Chaudhury, M. K. *J. R. Soc., Interface* **2005**, *2*, 319–325.
(16) Bowen, J.; Pettitt, M. E.; Kendall, K.; Leggett, G. J.; Preece, J. A.; Callow, M. E.; Callow, J. A. *J. R. Soc. Interface* **2007**, *4*, 473–477.
(17) Schilp, S.; Kueller, A.; Rosenhahn, A.; Grunze, M.; Pettitt, M. E.; Callow, M. E.; Callow, J. A. *Biointerphases* **2007**, *2*, 143–150.
(18) Ederth, T.; Nygren, P.; Pettitt, M. E.; Östblom, M.; Du, C.-X.; Broo, K.; Callow, M. E.; Callow, J.; Liedberg, B. *Biofouling* **2008**, *24*, 303–312.
(19) Bandyopadhyay, D.; Prashar, D.; Luk, Y.-Y. *Langmuir* **2011**, *27*, 6124–6131.
(20) *Poly(ethylene glycol) Chemistry—Biotechnical and Biomedical Applications*; Harris, J. M., Ed.; Plenum Press: New York, 1992.
(21) Morra, M.; Cassellini, C. In *Water in Biomaterials Surface Science*; Morra, M., Ed.; John Wiley & Sons: New York, 2001; pp 353−387.
(22) Löfås, S.; Johnsson, B. *J. Chem. Soc., Chem. Commun.* **1990**, 1526–1528.
(23) Holland, N. B.; Qiu, Y.; Ruegsegger, M.; Marchant, R. E. *Nature* **1998**, *392*, 799–801.
(24) Pale-Grosdemange, C.; Simon, E. S.; Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 12–20.
(25) Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 10714–10721.
(26) Harder, P.; Grunze, M.; Dahint, R.; Whitesides, G. M.; Laibinis, P. E. *J. Phys. Chem. B* **1998**, *102*, 426–436.
(27) Wang, R. L. C.; Kreuzer, H. J.; Grunze, M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 3613–3622.
(28) Prime, K. L.; Whitesides, G. M. *Science* **1991**, *252*, 1164–1167.

(29) Chapman, R. G.; Ostuni, E.; Takayama, S.; Holmlin, R. E.; Yan, L.; Whitesides, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 8303–8304.
(30) Holmlin, R. E.; Chen, X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 2841–2850.
(31) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605–5620.
(32) Kane, R. S.; Deschatelets, P.; Whitesides, G. M. *Langmuir* **2003**, *19*, 2388–2391.
(33) Chen, S.; Liu, L.; Jiang, S. *Langmuir* **2006**, *22*, 2418–2421.
(34) Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, *120*, 3464–3473.
(35) Luk, Y.-Y.; Kato, M.; Mrksich, M. *Langmuir* **2000**, *16*, 9604–9608.
(36) Hederos, M.; Konradsson, P.; Liedberg, B. *Langmuir* **2005**, *21*, 2971–2980.
(37) Bertilsson, L.; Liedberg, B. *Langmuir* **1993**, *9*, 141–149.
(38) Ederth, T.; Liedberg, B. *Langmuir* **2000**, *16*, 2177–2184.
(39) Callow, M. E.; Callow, J. A.; Pickett-Heaps, J. D.; Wetherbee, R. *J. Phycol.* **1997**, *33*, 938–947.
(40) Finlay, J. A.; Callow, M. E.; Schultz, M. P.; Swain, G. W.; Callow, J. A. *Biofouling* **2002**, *18*, 251–256.
(41) Schultz, M. P.; Finlay, J. A.; Callow, M. E.; Callow, J. A. *Biofouling* **2000**, *15*, 243–251.
(42) Callow, M. E.; Jennings, A. R.; Brennan, A. B.; Seegert, C. E.; Gibson, A.; Wilson, L.; Feinberg, A.; Baney, R.; Callow, J. A. *Biofouling* **2002**, *18*, 237–245.
(43) Hellio, C.; Marechal, J.-P.; Véron, B.; Bremer, G.; Clare, A. S.; Le Gal, Y. *Mar. Biotechnol.* **2004**, *6*, 67–82.
(44) D'Souza, F.; Bruin, A.; Biersteker, R.; Donnelly, G.; Klijnstra, J.; Rentrop, C.; Willemsen, P. *J. Ind. Microbiol. Biotechnol.* **2010**, *37*, 363–370.
(45) Maki, J. S.; Ding, L.; Stokes, J.; Kavouras, J. H.; Rittschof, D. *Biofouling* **2000**, *16*, 159–170.
(46) Absolom, D. R.; Lamberti, F. V.; Policova, Z.; Zingg, W.; van Oss, C. J.; Neumann, A. W. *Appl. Environ. Microbiol.* **1983**, *46*, 90–97.
(47) Fletcher, M.; Pringle, J. H. *J. Colloid Interface Sci.* **1985**, *104*, 5–14.
(48) van Loosdrecht, M. C. M.; Norde, W.; Zehnder, A. J. B. *J. Biomater. Appl.* **1990**, *5*, 91.
(49) Ista, L. K.; Callow, M. E.; Finlay, J. A.; Coleman, S. E.; Nolasco, A. C.; Simons, R. H.; Callow, J. A.; Lopez, G. P. *Appl. Environ. Microbiol.* **2004**, *70*, 4151–4157.
(50) Akesso, L.; Pettitt, M. E.; Callow, J. A.; Callow, M. E.; Stallard, J.; Teer, D.; Liu, C.; Wang, S.; Zhao, Q.; D'Souza, F.; Willemsen, P. R.; Donnelly, G. T.; Donik, C.; Kocijan, A.; Jenko, M.; Jones, L. A.; Guinaldo, P. C. *Biofouling* **2009**, *25*, 55–67.
(51) Mitik-Dineva, N.; Wang, J.; Truong, V. K.; Stoddart, P. R.; Malherbe, F.; Crawford, R. J.; Ivanova, E. P. *Biofouling* **2009**, *25*, 621–631.
(52) Bakker, D. P.; Huijs, F. M.; de Vries, J.; Klijnstra, J. W.; Busscher, H. J.; van der Mei, H. C. *Colloids Surf., B* **2003**, *32*, 179–190.
(53) Callow, J. A.; Callow, M. E. In *Biological adhesives*; Smith, A. M., Callow, J. A., Eds.; Springer: Berlin, 2006; pp 63−78.
(54) Finlay, J. A.; Callow, M. E.; Ista, L. K.; Lopez, G. P.; Callow, J. A. *Integr. Comp. Biol.* **2002**, *42*, 1116–1122.
(55) Fyrner, T.; Lee, H.-H.; Mangone, A.; Ekblad, T.; Pettitt, M. E.; Callow, M. E.; Callow, J. A.; Conlan, S. L.; Mutton, R.; Clare, A. S.; Konradsson, P.; Liedberg, B.; Ederth, T. Submitted.
(56) Rittschof, D.; Costlow, J. D. *Sci. Mar.* **1989**, *53*, 411.
(57) Finlay, J. A.; Bennett, S. M.; Brewer, L. H.; Sokolova, A.; Clay, G.; Gunari, N.; Meyer, A. E.; Walker, G. C.; Wendt, D. E.; Callow, M. E.; Callow, J. A.; Detty, M. R. *Biofouling* **2010**, *26*, 657–666.
(58) Neal, A. L.; Yule, A. B. *J. Mar. Biol. Assoc. U. K.* **1996**, *76*, 649–655.
(59) Matsumura, K.; Mori, S.; Nagano, M.; Fusetani, N. *J. Exp. Zool.* **1998**, *280*, 213–219.
(60) Matsumura, K.; Nagano, M.; Fusetani, N. *J. Exp. Zool.* **1998**, *281*, 12–20.
(61) Khandeparker, L.; Anil, A. C.; Raghukumar, S. *J. Exp. Mar. Biol. Ecol.* **2002**, *281*, 77–88.
(62) Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719–728.
(63) Svedhem, S.; Öhberg, L.; Borrelli, S.; Valiokas, R.; Andersson, M.; Oscarson, S.; Svensson, S. C. T.; Liedberg, B.; Konradsson, P. *Langmuir* **2002**, *18*, 2848–2858.
(64) Good, R. J. In *Contact Angle, Wettability and Adhesion*; Mittal, K. L., Ed.; VSP: Utrecht, The Netherlands, 1993; pp 3−36.
(65) Radelczuk, H.; Hołysz, L.; Chibowski, E. *J. Adhes. Sci. Technol.* **2002**, *16*, 1547–1568.
(66) Della Volpe, C.; Siboni, S. In *Water in Biomaterials Surface Science*; Morra, M., Ed.; John Wiley & Sons: Chichester, U.K., 2001; pp 183−214.
(67) Aldred, N.; Li, G.; Gao, Y.; Clare, A. S.; Jiang, S. *Biofouling* **2010**, *26*, 673–683.
(68) Du, Q.; Freysz, E.; Shen, Y. R. *Phys. Rev. Lett.* **1994**, *72*, 238.
(69) Kim, J.; Cremer, P. S. *J. Am. Chem. Soc.* **2000**, *122*, 12371–12372.
(70) Herrwerth, S.; Eck, W.; Reinhardt, S.; Grunze, M. *J. Am. Chem. Soc.* **2003**, *125*, 9359–9366.
(71) Rosenhahn, A.; Schilp, S.; Kreuzer, H. J.; Grunze, M. *Phys. Chem. Chem. Phys.* **2010**, *12*, 4275–4286.

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