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Hydration of Sulphobetaine (SB) and Tetra(ethylene glycol) (EG4)-Terminated Self-Assembled Monolayers Studied by Sum Frequency Generation (SFG) Vibrational Spectroscopy

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Abstract

Sum frequency generation (SFG) vibrational spectroscopy is used to study the surface and the underlying substrate of both homogeneous and mixed self-assembled monolayers (SAMs) of 11-mercaptoundecyl-1-sulphobetainethiol ($\text{HS}(\text{CH}_2)_{11}\text{N}^+(\text{CH}_3)_2(\text{CH}_2)_3\text{SO}_3^-$, SB) and 1-mercapto-11-undecyl tetra(ethylene glycol) ($\text{HS}(\text{CH}_2)_{11}\text{O}(\text{CH}_2\text{CH}_2\text{O})_4\text{OH}$, EG4) with an 11-mercapto-1-undecanol ($\text{HS}(\text{CH}_2)_{11}\text{OH}$, MCU) diluent. SFG results on the C–H region of the dry and hydrated SAMs gave an in situ look into the molecular orientation and suggested an approach to maximize signal-to-noise ratio on these difficult to analyze hydrophilic SAMs. Vibrational fingerprint studies in the 3000–3600 cm^{-1} spectral range for the SAMs exposed serially to air, water, and deuterated water revealed that a layer of tightly-bound structured water was associated with the surface of a non-fouling monolayer but was not present on a hydrophobic N-undecylmercaptan ($\text{HS}(\text{CH}_2)_{10}\text{CH}_3$, UnD) control. The percentage of water retained upon submersion in D_2O correlated well with the relative amount of protein that was previously shown to absorb onto the monolayers. These results provide evidence supporting the current theory regarding the role of a tightly-bound vicinal water layer in the protein resistance of a non-fouling group.

Keywords

water structure; non-fouling; interfacial water; biomaterial surface; protein resistance

Introduction

The role of water structure and the interfacial hydration layer in the interactions of biomaterial surfaces with biomolecules and cells has long been debated. In some instances, water is relegated to being an inert player, but on closer inspection one might discover that its overtly ‘simple’ and unique construction of 2 H-bond donor sites and 2 H-bond acceptor sites makes it an exceptionally active contributor and catalyst.^{1–5} The structure of water is such that it allows for a large degree of mobility and versatility.^{2–4} The surprisingly complex, dynamic structure also makes it difficult to study.^{1,6} The consequence of this is that while the gross molecular structure of water is reasonably understood,^{2–4} water dynamics and organization is less clear — particularly in its associations with surfaces and macromolecules. Because water structure presents us with ambiguities, there is a tendency to relegate it into the category of an

inert, surrounding matrix, which we know it is not.^{7–9} Thus, we minimize its contribution to the functions and properties of many materials, particularly biologics, where this water solvent strongly contributes to the reactivity of key compounds, such as proteins.^{10,11}

Previous studies in our laboratory found that the protein adsorption trends on a zwitterionic sulphobetaine (SB) SAM did not follow the same rules as an tetra(ethylene glycol) (EG4) SAM in terms of the surface characteristics that lent themselves to protein resistance.¹² The commonalities between the two surfaces were their hydrophilicity and apparent dependence on interchain water. The experimental work presented herein utilizes sum-frequency generation (SFG) spectroscopy to characterize and compare both the strength and nature of the interfacial water network associated with these two non-fouling moieties.

There are many probes that are capable of studying liquid interfaces at the microscopic level, including Fourier transform infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR),^{1,2,5,13,14} X-ray diffraction¹⁵, X-ray reflection,^{15,16} atomic force microscopy,¹⁷ ultrafast electron crystallography,¹⁸ and others.^{19,20} However, SFG may be the most successful and versatile of these techniques as it can be utilized *in situ* while maintaining a strict surface specificity due to the invisibility of bulk centrosymmetric materials, *i.e.* the bulk phases are not measured. Some initial work has been done studying the interactions of hydrophobic and a few hydrophilic monolayers on silica substrates in contact with water.^{20–23} However, little work has been performed on the SAM/water interface of SAMs on gold due to the metallic substrate's high non-resonant background and the delicate balance between the signal-to-noise ratio vs. gold ablation.^{22,24} Of those studies, the majority have used compounds that contain methyl groups. Only one study is known to have been performed on a non-fouling surface which does not contain sum-frequency-active methyl groups,^{22,24} and none have been performed through the backside of the gold substrate. There are many advantages to a backside study in SFG spectroscopy. The primary advantage for this study was the ability to separate the SAM/water interface from the water/air interface *in situ* with a high degree of confidence.

This work furthers the study of the interfacial water region utilizing SFG spectroscopy to qualitatively compare the level of more tetrahedrally coordinated hydrogen-bound water ("tightly-bound" water) to less-organized water ("loosely-bound" water) in the water layer immediately adjacent to the surface. Towards these ends, we have developed novel protocols including a substrate preparation protocol which has afforded us a reasonable signal-to-noise ratio allowing us to study the SAM interface on gold through the backside of the gold layer (without visible signs of gold or SAM damage). These substrates were then employed in the study of the interfacial water zone using an approach based on H/D isotopic substitution.^{1,25,26} The results of these studies show that there is a layer of the tightly-bound water that is associated with the surface of a non-fouling monolayer but is not present on a hydrophobic control. These observations are consistent with an overall hypothesis on the role of a tightly-bound interfacial water region in non-fouling materials.

Experimental Methods

Substrate Preparation

IR-grade fused silica windows (Esco products, Oakridge, NJ) were cleaned by sonication in a 4% Isopanol solution for 10 minutes and then rinsed with copious amounts of ultrapure water (Millipore, Milli-Q water with at least 18 MΩ·cm resistivity). The samples were then sonicated for an additional 10 minutes each in fresh ultra pure water, acetone, and then methanol. Samples were then dried under a stream of argon (Airgas) and stored in Parafilm sealed petri dishes. Thin films of polycrystalline gold were prepared by thermal evaporation of 20 nm of gold of 99.999% purity onto one side of the silica windows. Evaporation was performed at a base pressure of better than 3×10^{-7} Torr and a deposition rate of 2 nm/s. Substrates not immediately

used were stored under argon. Gold uniformity was verified using atomic force microscopy (AFM) prior to use. The substrates exhibited uniform surface coverage with root means square roughnesses of the gold films below 1 nm.

Chemicals

The 11-mercaptoundecyl-1-sulpho-betainethiol (SB, $\text{HS}(\text{CH}_2)_{11}\text{N}^+(\text{CH}_3)_2(\text{CH}_2)_3\text{SO}_3^-$) was synthesized in house through procedures adapted from Holmlin *et al.*²⁷ and Ostuni *et al.*²⁸ A perdeuterated dodecanthiol (dDDT, $\text{HS}(\text{CD}_2)_{11}\text{CD}_3$) for use as an SFG control was also prepared in house. (N-undecylmercaptan (UnD, $\text{HS}(\text{CH}_2)_{10}\text{CH}_3$), and 11-mercapto-1-undecanol (MCU, $\text{HS}(\text{CH}_2)_{11}\text{OH}$) were purchased from Aldrich Chemical Co. (Milwaukee, WI). The (1-mercapto-11-undecyl) tetra(ethylene glycol) (EG4, $\text{HS}(\text{CH}_2)_{11}\text{O}(\text{CH}_2\text{CH}_2\text{O})_4\text{H}$) was acquired from Asemblon Corp. (Redmond, WA). Absolute ethanol was purchased from Aaper (Shelbyville, KY). All purchased chemicals were used as received.

SAM Preparation

SAM surfaces were assembled from 0.5 mM solutions in absolute ethanol for either 1 hour (EG4 samples) or 24 hours (all other thiols) at room temperature. After the specified assembly time, the samples were removed from the solution and rinsed thoroughly with copious ethanol. Samples were blown dry with argon, placed in clean petri-dishes, and stored under argon prior to analysis. Samples were always used within 24 hours.

SFG Setup

The SFG spectra were obtained by overlapping visible and tunable IR laser pulses (25 ps) in time and space at incidence angles of 60° and 54°, respectively. The visible beam with a wavelength of 532 nm was delivered by an EKSPLA Nd:YAG laser operating at 50 Hz, which was also used to pump an EKSPLA optical parametric generation/amplification and difference frequency unit based on barium borate and AgGaS_2 crystals to generate tunable IR laser radiation from 1000–4000 cm^{-1} . The bandwidth was 1 cm^{-1} for the visible pump pulses and 1–6 cm^{-1} for the IR laser radiation (1 cm^{-1} for 2750–3000 cm^{-1} and 6 cm^{-1} for higher wave numbers). Both beams were unfocussed and had a diameter of approximately 2 mm at the sample. The energy for both beams was 130 μJ per pulse. The SFG signal generated at the sample was then analyzed using filters and a monochromator, detected with a gated photomultiplier tube and stored in a computer. The spectra were collected with 600 shots per data point in 4 cm^{-1} increments. The Fresnel coefficients of a probed metal surface cause the x- and y-component contributions of the IR electric field at the surface to be smaller than the z-component.²⁹ Thus, the SF emission only occurs when using a polarization combination with a perpendicular component (namely *ssp*, or *ppp*). Since the *ppp* polarization combination probes a greater combination of tensor elements that can either constructively or destructively interfere, it often generates a larger signal value on metallic surfaces than the *ssp* polarization combination.^{29,30,31} All spectra were recorded in *ppp* (sum, visible, and infrared) polarization combination.

Data Collection

A total of four spectra were obtained from two replicates per sample group. The spectra were all acquired with the input beams traveling through the silica/gold substrate to the SAM film (see Figure 1). Three spectra were acquired from one sample in each group. The first spectrum was acquired at the SAM/air interface. Then, a second spectrum was acquired at the SAM/water interface after the SAM was placed in contact with ultrapure water. Finally, the same sample was briefly dried with a stream of nitrogen and placed into contact with fresh deuterated water and a third spectrum was acquired. The fourth spectrum was acquired separately from the second replicate after it was placed directly from an inert argon atmosphere into deuterated

water. For analysis of the C–H and water regions of the EG4, SB/MCU and SB SAMs, the acquired spectra were normalized to the signal from the dDDT/D₂O interface to minimize signal effects from the nonresonant background.

Data Fitting

The fitting routine for the SFG data is described in literature^{32,33} and uses the following expression for the SFG intensity I_{SFG} :

$$I_{SFG} \propto |\chi^{(2)}|^2 = \left| \chi_{NR}^{(2)} e^{i\varphi} + \sum_v \int_{-\infty}^{\infty} \frac{A_v e^{i\varphi_v} e^{-[\omega_L - \omega_v/\Gamma_v]^2}}{\omega_{IR} - \omega_L + i\Gamma_L} d\omega_L \right|^2. \quad (1)$$

Here, $\chi_{NR}^{(2)}$ is the second order nonlinear susceptibility of the nonresonant background, is the A_v strength of the v^{th} vibrational mode, φ denotes the phase of the respective mode and ω_{IR} refers to the frequency of the incident IR field. The integral is over Lorentzian lines, centered at ω_L with a width of Γ_v , having a Gaussian distribution. In the experiments, the Lorentzian line widths were set to 2 cm⁻¹ and Γ_v was allowed to vary since the two contributions to the total line width could not be separated within the accuracy of the measurements. Phase values are reported as relative values to the nonresonant gold background and identical phases were assumed for stretches related to identical molecular groups. The starting frequencies of the C–H region spectra were initially estimated from control spectra and were allowed to shift during peak fitting. The spectra were fit with the SFG equation using identical positions and peak-widths for the tightly-bound (“ice-like”) and the loosely-bound water in all water spectra. The position and width were 3200 cm⁻¹ and 77 cm⁻¹ for tightly-bound (TB) water, and 3400 cm⁻¹ and 191 cm⁻¹ for loosely-bound (LB) water.

Results

Demonstrating that SFG spectra originate from the SAM/Air interface

As this work represents (to the best of our knowledge) the first attempt to acquire a signal through a gold underlayer, we needed to verify that the spectra obtained came from the SAM/Air interface with no significant contribution from the gold/silica or silica/air interfaces. This was achieved by comparing a spectrum of an undecanethiol (UnD) SAM taken in the backside geometry with a spectrum acquired from the frontside. These spectra along with equation 1 line-fitting are displayed in Figure 2. Both spectra are similar with the only difference being a somewhat lower signal to noise ratio in the spectrum acquired through the backside of the gold film.

The spectra exhibited three resonances related to the terminal methyl unit. These were the symmetric stretching mode (r^+) at 2860 cm⁻¹, the methyl Fermi resonance (r^+_{FR}) at 2940 cm⁻¹, and the asymmetric stretching mode (r^-) at 2968 cm⁻¹. For the peak fitting we kept the phase ϕ_{NR} of the nonresonant background constant at a value of $-\pi/2$ to decrease the number of independent parameters.^{34,35} The presence and spectral positions of these peaks are similar to the results found on the more commonly studied octadecanethiol (ODT) SAM and are consistent with a well-formed SAM with a uniform all-trans chain.^{20,21,36} The amplitudes of these peaks in both the front-side and back-side geometries were found to conform to the previously reported tendency for the r^+ resonances to take precedence over the intensity of the r^- mode in odd-n alkanethiolates.³⁷ This tendency is further quantified in a calculation of the average orientation of the terminal methyl group from the ratio of intensities of the r^- and r^+ modes (A_{r^-}/A_{r^+})³⁷:

$$\frac{A_{r-}}{A_{r+}} = D \left(\frac{\langle \cos\theta - \cos 3\theta \rangle}{\cos\theta} \right), \quad (2)$$

where D is the proportionality constant which includes the hyperpolarizabilities of a methyl group and the Fresnel coefficients and θ refers to the angle between the Au/surface normal and the central methyl axis. We assumed $D=3.5$ in accord with the published procedure.³⁷ The A_{r-}/A_{r+} value was found to be ca. 0.7 corresponding to an effective tilt angle of $\theta=25^\circ$ for the UnD SAM with front-side geometry. For the UnD SAM signal obtained with the backside geometry this value was calculated to be ca. 0.8 corresponding to a θ value of 27° . Both of these values are sufficiently close to a reported literature value of 27° .³⁷

SFG C–H region from the three low-fouling SAMs

Before examining the solvent effect on the water region, the effect of the media type was monitored in the C–H region. Vibrational spectra in the 2800–3000 cm^{-1} C–H region of a homogeneous EG4 SAM, a 50:50 SB:MCU mixed SAM and a homogeneous SB SAM exposed to air and deuterated water are shown in Figure 3(a, b, and c), respectively. The spectra shown are normalized to the C–H region of a dDDT SAM and have been fit to the SFG equation (Equation 1). The relative amplitudes of the C–H peaks obtained are low when compared to the previously obtained values for the UnD SAM. This was expected, however, because it has been shown that methylene chains tend to make weaker contributions to the SFG signal due to their predominately centrosymmetric nature in well-ordered monolayers.³⁶ Nonetheless, several interesting contributions of the species to their respective C–H region have been observed as noted below.

There are three main spectral features found in the C–H region of the EG4 SAM (Figure 3(a)) in air. Two dominant peaks are located at 2891 and 2950 cm^{-1} which represent the symmetric (o^+) and asymmetric (o^-) blue-shifted frequencies of a CH_2 group bound to an oxygen atom.³⁸ A symmetric CH_2 peak (d^+) at around 2846 cm^{-1} is also noted in the air spectrum. The $A_{\text{o}^-}/A_{\text{o}^+}$ ratio obtained from the curve-fit air spectrum is approximately 0.68 resulting in a θ value, and subsequent φ value (estimated from Equation 2) to be around 25° . These ratios appear to be similar to the results obtained by Zolk et al.³⁸ on a methoxy tri(ethylene glycol) sample with a perdeuterated methyl group. The S/N from the sample in air is lower than it would normally be due to the employment of the backside geometry. However, the signal-to-noise ratio and sample peak clarification was improved on our sample with the employment of the dDDT normalization. Upon exposure to the deuterated water, the $A_{\text{o}^-}/A_{\text{o}^+}$ ratio increases to a value of 0.96 and a $\theta \approx \varphi$ value of 32° . This is slightly higher than the typical tilt for a well-ordered monolayer. The absence of the methylene stretch vibrations of the alkane chains (d^+) in the EG4 SAM in D_2O can indicate either complete disorder or order in the monolayer. Given previous literature results, it most likely indicates that the hydrated EG4 SAM is in a state of complete disorder.^{39,40}

Figure 3(b) shows the spectra obtained on the 50:50 SB:MCU (V/V) mixed SAMs. Two peaks located at 2847 and 2909 cm^{-1} dominate the C–H region for this SAM in air. We assign these peaks to the symmetric and asymmetric stretching modes (d^+ and d^-) of the terminal CH_2 in the SB end-group. The methylene peaks of a well-ordered SAM are typically not visible in SFG spectroscopy (as seen in Figure 2).^{36–38} Thus, we theorize that the methylene signal most likely comes from the SB end-group, which was shown in previous experiments to have a media-dependant orientation.¹² If there were an even n of CH_2 groups present in SB, we might expect that there would be no signal as the CH_2 groups would cancel each other out. However, the odd n SB moiety used in our experiments allows for the determination of the overall chain conformation. The relative $A_{\text{asym}}/A_{\text{sym}}$ ratio of these CH_2 peaks for the mixed SB SAM is

relatively constant upon exposure to all media with a slight preference for d^- upon exposure to the deuterated water. From experimental calculations, we know that a value of A_{asym}/A_{sym} of 1 indicates a θ value of $\sim 32^\circ$ for the element in question. Since the methylene segment of SB is approximately perpendicular to the CH_2 rotational axis, this gives us an orientation of $\phi \approx 58^\circ$ indicating that the SB headgroups are tilted. This is most likely the result of an overlapping anti-parallel conformation found in our earlier experiments.¹² However, it is also noted that the S/N ratio of this sample is rather low overall. So, it may be possible that only a minority of these groups are tilted or otherwise disordered.

In contrast to the mixed SAM, the homogeneous SB spectra show marked differences in the CH_2 symmetric and asymmetric modes and a much higher signal-to-noise ratio (see Figure 3 (c)). In air, the d^- mode dominates with an amplitude 3.1 times as high as the d^+ mode. This is the equivalent of a θ value of around 62° and ϕ of 28° indicating an upright orientation that is close to being in line with the alkane chain and serves to explain its low signal. This changes to a ratio of about 0.44 in deuterated water. This is estimated from Equation 2 to be the equivalent of a θ orientation of about 20° from the normal and a ϕ of about 70° . Thus, when hydrated the SB terminal group appears to have its dipole vector oriented at about 70° to the surface normal forming the conformation that allows for greater charge interactions with its neighboring SB end-groups. In addition to the CH_2 modes present, there is a third peak at 2872 cm^{-1} which is assigned to the CH_3 sym peak (r^+). The absence of a corresponding asymmetric peak suggests that these methyl groups are in line with the normal. It is hypothesized that the symmetry axis of the methyl peaks is oriented away from the gold underlayer due to the relative peak phase. It is notable that some slight frequency shifts were observed in the peak locations upon exposure to water. These blue-shifts are attributed largely to the changing polarity of the C–H environment.^{23,41}

SFG H/D exchange test

Since the majority of our non-fouling surfaces contain hydroxyl or amine groups that appear in the $3100\text{--}3800\text{ cm}^{-1}$ water region, a method for assessing the quantity of irremovable water was needed. This was achieved through the use of a two sample system. The primary sample was exposed to humid air (RH $\sim 60\%$), followed by H_2O and then D_2O . In previous FTIR-ATR experiments, it was observed that a layer of H_2O was present on our surfaces even after D_2O submersion.¹² Since D_2O is not SF active in this region, we would expect zero signal if the H_2O molecules were removable. The second sample consisted of a substrate from the same stock as the first but whose surface had been maintained in an inert argon atmosphere prior to the acquisition of a spectrum under D_2O . This spectrum should have all of the signature peaks of a hydrated sample without the water contribution. Thus, the D_2O spectrum was used to normalize each acquired spectra in the water region.

Much work has been published on the use of SFG for studying water on bare quartz and quartz coated with hydrophobic monolayers.^{19,20,42–44} The OH stretching region extends from $3000\text{--}3800\text{ cm}^{-1}$. Three peaks have been previously identified as being SF active in this region. A narrow peak, typically located at 3700 cm^{-1} , has been identified as belonging to dangling free OH bonds – or — OH bonds that are not hydrogen bonded. It is usually associated with hydrophobic surfaces. Then there are the more common broad peaks located at ~ 3200 and $\sim 3400\text{ cm}^{-1}$. The peak at 3200 cm^{-1} can be attributed to the coupled symmetric OH stretch mode of the tetrahedrally coordinated, hydrogen-bonded water molecule. Since this is the dominant peak in an SFG spectrum of ice, it is commonly referred to as the more ordered “ice-like” peak or as strongly-bound water. The 3400 cm^{-1} peak can be attributed to the OH stretch mode of asymmetrically bonded water molecules. These water molecules typically have one strong hydrogen bond and one weak hydrogen bond with their neighbors. Since this is the dominant peak in bulk water, it is typically referred to as less than tetrahedrally coordinated

hydrogen bonded water or the loosely-bound water peak. All three types of water are normally found on hydrophobic surfaces.^{20,45} However, we are measuring the amount and nature of an irremovable vicinal water layer. Thus, we would expect that for hydrophobic surfaces there should be little, if any, ordered or disordered water present on the surface.

To verify that our H/D exchange protocol was measuring only very tightly retained water, we performed a test on a hydrophobic UnD SAM control. The resulting spectra are shown in Figure 4. Prior to using it for normalization, the spectrum from sample 2 was smoothed with a 5-pt average so that the resulting “noise” (shown in the upper spectrum of Figure 2) would not impact the normalization. Overall, few changes are observed in the water region. The sample only exhibited a water peak when in contact with water. No water peak is apparent when the sample has been transferred into D₂O. Thus, all H₂O molecules have been effectively replaced and no irremovable water exists on the hydrophobic sample.

The UnD/H₂O SAM spectrum obtained was fitted with the SFG equation. The resulting area of the tightly-bound water peak obtained was observed to be higher than any other sample group (see Table 1). However, as both water peaks disappear when transferred to the D₂O, this ordered water appears to be only weakly held to the surface. Previous literature has theorized that hydrophobic surfaces tend to drive a water structure that is more ordered but weaker than bulk water as the water molecules orient themselves to form hydrogen-bonds with themselves rather than the material surface.⁴⁶ These weaker bonds explain the disappearance of the high loosely-bound water peak in D₂O. The results from the UnD sample correspond with the conclusion that the water associated with a hydrophobic surface, even if it is ordered, is not strongly hydrogen bonded to the surface. Having obtained these results as our null background, we are now prepared to discuss the nature of the irremovable water obtained on our non-fouling SAMs.

SFG H/D exchange on low-fouling SAMs

H/D exchange was performed on each of the low-fouling SAM moieties. The spectra were then normalized to smoothed spectra from a second sample placed directly in contact with D₂O from an inert atmosphere, similar to the UnD control. The resulting spectra for the EG4, 50:50 SB:MCU SAM, and 100:0 SB:MCU SAM are shown in Figure 5(a), (b), and (c), respectively. The spectra were fit with the SFG equation using identical positions and widths for the tightly-bound (“ice-like”) and the loosely-bound water in all spectra. The position and width were 3200 cm⁻¹ and 77 cm⁻¹ for tightly-bound (TB) water, and 3400 cm⁻¹ and 191 cm⁻¹ for loosely-bound (LB) water. The intensity summations ($A_{TB} + A_{LB}$) are then used as an indication of overall retained or irremovable water on the D₂O samples; while, the area ratios (A_{TB}/A_{LB}) are used as an indication of hydrogen bond strength and order. In order to avoid normalization issues, we focus primarily on the use of the peak intensity ratios.

In comparing the EG4 SAM spectra (Figure 5(a)) with the UnD SAM control, it is immediately apparent that not only is there a high degree of non-displaceable vicinal water present in the H/D exchange spectra (labeled H₂O → D₂O), but the majority of the water that is retained is tightly-bound (see Figure 6 for a more detailed explanation). An examination of the EG4 SAM peak fits (Table 1) confirms this. The sample begins with a high A_{TB}/A_{LB} ratio of 2.4 in humid air. When it is exposed to water, the ratio decreases to 1.6. This is expected since it has been placed into contact with a large body of loosely-bound water. Upon soaking in D₂O, the loosely-bound water’s intensity drops and the A_{TB}/A_{LB} ratio increases to 3.2. Approximately 91% of the tightly-bound water and 45% of the loosely-bound water present on the EG4 SAM in contact with water was retained when the sample was exposed to D₂O.

The overall trends in SB-containing SAMs (Figure 5(b) and (c)) were similar to the EG4 SAM. The 50:50 SB: MCU mixed SAM in air had a high tightly-bound to loosely-bound water ratio

(3.9). Upon contact with water, the intensity of the 3400 cm^{-1} loosely-bound water peak increased, causing the $A_{\text{TB}}/A_{\text{LB}}$ ratio to drop to a low 1.4. In comparing the values obtained, it was determined that approximately 88% of the water signal obtained from the sample in water was retained upon soaking in D_2O and, based on the $A_{\text{TB}}/A_{\text{LB}}$ ratio of the SAM/ D_2O spectrum, the water that is retained is primarily tightly-bound water. For the homogenous SB SAM, the $A_{\text{TB}}/A_{\text{LB}}$ ratio decreased from 7.7 to 1.1 in water and increased back to 4.8 in D_2O . The relative amount of interfacial water retained was approximately 40%. Thus, although it was still found to consist of tightly-bound water, of all the low-fouling SAMs it retained the lowest amount of water.

In our earlier published protein adsorption studies, it was determined that the 50:50 mixed SB:MCU SAM adsorbed less than 20 ng/cm^2 of fibrinogen, albumin and lysozyme from 1% plasma concentration and single-protein solutions.¹² These overall adsorption values were comparable to the EG4 SAMs.¹² In contrast, the homogeneous SB SAM was less non-fouling as it consistently adsorbed around 50 ng/cm^2 of protein. For comparison, the UnD SAM adsorbed 180 ng/cm^2 of protein. In comparing the amounts of irremovable water obtained on the surface with these protein adsorption values, one can see several trends in the H/D exchange results that are comparable to the protein adsorption results. Namely, the two ultra non-fouling SAMs (EG4 and 50:50 SB:MCU) retained the highest amount of water overall with the low-fouling homogeneous SB SAM retaining less water and the fouling UnD control retaining no water. According to the data fits, the type of water retained overall tended to be the more structured and tightly-bound water. These findings are consistent with a conclusion that a tightly-bound, irremovable water layer is essential for protein resistance.

Conclusions

A novel method for the examination of thiols on gold utilizing a backside sample geometry was tested and its stability and accuracy was demonstrated with an UnD SAM. The response of several non-fouling SAMs to exposure to humid air, water and deuterated water was studied in both the carbon and hydroxyl bonding regions. Our SFG C–H region studies gave us valuable orientational information that corroborated earlier FTIR-ATR and XPS experiments.¹² A new method for the measurement and detection of an irremovable interfacial water layer with SFG using D/H isotopic substitution was explored. By this method, the percentage of water retained (91%, 88%, and 40%) on the EG4 SAM, 50:50 mixed SB: MCU SAM, and homogeneous SB SAM, respectively, is roughly inversely proportional to the amount of protein adsorbed. The predominant type of water retained upon exposure to D_2O was found to be tightly-bound water. Overall, these results were found to support a conclusion that a strongly-bound irremovable water layer is present at the interface of non-fouling SAMs and –to a lesser degree– the low-fouling homogeneous SB SAM, but not on a hydrophobic UnD control. The results on the UnD control were found to be in agreement with the structure of water in contact with air obtained by Shen and coworkers.⁴⁷ Ongoing work is focused on the characterization of the effects of the interfacial water order to the ordering of the water sublayers.

This study represents the first efforts to perform a D/H substitution study with SFG in a manner that allows the quantification and characterization of a tightly-bound water layer. SFG has been proven to be a powerful new technique for elucidating the structure of the interfacial water layer on a molecular scale. A hypothesis for the mechanism by which non-fouling surfaces function to resist protein pickup starts with the understanding that all surfaces structure water to varying degrees. The driving force for proteins to adsorb to a surface will be the melting or displacement of surface structured water. If the water is so tightly-bound and structured that the protein cannot displace it, then there is no driving force for the protein adsorption. The results obtained in this study are supportive of this hypothesis.

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Figure 1.
Schematic plot of the SFG setup

Figure 2.
Comparison of SFG spectra of the UnD SAM/air interface taken at the frontside (top) and through the backside (bottom) of the gold film. The solid line represents a fit to Equation 1.

Figure 3.

SFG C–H region spectra of EG4 SAMs (a); 50:50 SB:MCU SAMs; (b) and SB SAMs (c) on gold are shown in contact with air (bottom) and deuterated water (top). Prominent absorption peaks are indicated. The dotted lines are provided as a guide for the eyes. The bolded solid line represents a fit to Equation 1.

Figure 4.

The effect of $\text{D}_2\text{O}/\text{H}_2\text{O}$ adsorption onto an UnD SAM control is shown. The spectra have been normalized by a 5 pt averaged spectrum from a second sample exposed to D_2O . The overall system 'noise' removed is shown in the upper spectrum. The vertical lines mark the tetrahedrally coordinated hydrogen-bound water ("ice-like", 3200 cm^{-1}) and the loosely-bound water ("liquid-like", 3400 cm^{-1}). The solid line represents a fit to Equation 1. Dashed lines represent data which could not be fit due to lack of peaks.

Figure 5.

The resulting spectra from the SFG H/D exchange test on the low-fouling (a) EG4, (b) 50:50 SB:MCU, and (c) 100:0 SB:MCU SAMs are shown. The solid lines represent a fit of the data by Equation 1. The spectra are labeled by contact media and have been displaced for clarity

Figure 6.

A schematic of the D/H substitution process. The solid triangles represent H_2O molecules which are visible in the $2800\text{--}3600\text{ cm}^{-1}$ SFG region. The open triangles represent D_2O molecules that are not detected by SFG. Upon introduction of the hydrophilic samples into deuterated water, the easily replaced water molecules are substituted by D_2O molecules and become ‘invisible.’ This leaves only those molecules tightly bound to the surface detectable by SFG.

Table 1
SFG Water Peak Fits

The amplitude (A_q) values obtained from a fitting of the SFG equation to the water region of the spectra shown in Figure 5 is given.

	Tightly-Bound Water	Loosely-Bound Water	$A_{TB} + A_{LB}$	A_{TB}/A_{LB}
	A_{TB} (a.u.)	A_{LB} (a.u.)		
UnD				
Air	N/A	N/A	N/A	N/A
H ₂ O	4.6	6.7	11.3	0.7
D ₂ O	N/A	N/A	N/A	N/A
EG4				
Air	2.9	1.2	4.1	2.4
H ₂ O	3.2	2	5.2	1.6
D ₂ O	2.9	0.9	3.8	3.2
50:50 SB: MCU				
Air	3.1	0.8	3.9	3.9
H ₂ O	2	1.4	3.4	1.4
D ₂ O	2.5	0.5	3.0	5.0
100:0 SB: MCU				
Air	5.4	0.7	6.1	7.7
H ₂ O	3	2.7	5.7	1.1
D ₂ O	1.9	0.4	2.3	4.8