

Effect of Surface Composition on the Adsorption of Photosystem I onto Alkanethiolate Self-Assembled Monolayers on Gold

B. Samuel Ko,[†] Brian Babcock,[†] G. Kane Jennings,^{*,†} S. Grey Tilden,[‡]
Rachel R. Peterson,[‡] and David Cliffler^{*,‡}

Department of Chemical Engineering and Department of Chemistry, Vanderbilt University,
Nashville, Tennessee 37235

Elias Greenbaum

Chemical Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008,
Oak Ridge, Tennessee 37831-6194

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We have used self-assembled monolayers (SAMs) prepared from ω -terminated alkanethiols on gold to generate model surfaces and examine the effect of surface composition on the adsorption of Photosystem I (PSI), stabilized in aqueous solution by Triton X-100. Triton-stabilized PSI adsorbs to high-energy surfaces prepared from HO- and HO₂C-terminated alkanethiols but does not adsorb to low-energy surfaces. The inhibition of PSI adsorption at low-energy surfaces is consistent with the presence of a layer of Triton X-100 that adsorbs atop the hydrophobic SAM and presents a protein-resistant poly(ethylene glycol) (PEG) surface. While the presence of the PEG surface prevents the adsorption of PSI, the displacement of the inhibiting layer of Triton X-100 by dodecanol, a more active surfactant, greatly enhances the adsorption of PSI. This inhibiting effect by Triton X-100 can be extended to other protein systems such as bovine serum albumin.

Introduction

Photosystem I (PSI) is a supramolecular protein complex (MW~300 kDa) that drives photosynthesis.¹ PSI contains a core antenna used to funnel light to a reaction center that triggers a series of electronic excitations and ultimately creates a reducing potential across the photosynthetic membrane.² In this manner, PSI functions as a nanoscale photovoltaic structure that enables unidirectional electron flow.³ PSI exhibits a quantum yield of almost unity and can generate photoinduced charge separation within 10–30 ps across its 6 nm length.⁴ The promising electronic properties of PSI have led to increasing interest in extracting it from the thylakoid membrane of plants and utilizing its properties as a photovoltaic structure and molecular diode in order to produce novel electronic devices.^{3,5} Clearly, to integrate PSI with these materials, we must first develop a fundamental understanding of how PSI interacts with the surfaces of various materials.

Greenbaum and co-workers^{3,5,6} have successfully isolated PSI from spinach leaves to study its properties ex

vivo and have begun to develop methods for integrating it with other materials. They have shown that PSI adsorbs without denaturation to HO₂C- and HO-terminated surfaces, and the orientation of its electron-transfer vector depends on the underlying surface composition.³ PSI adsorbs onto mercaptoethanol monolayers with ~70% of the electron-transfer vectors oriented normal to the surface (upward) while on mercaptoacetic acid monolayers, ~80% of the electron-transfer vectors are oriented parallel to the surface.³ Importantly, PSI retains its activity upon adsorption at these surfaces. On the basis of the characterization of individual PSI complexes by Kelvin force microscopy (KFM), the potential atop the oriented reaction centers is 1.2 V more negative upon excitation by 670-nm light than when in dark.⁵ These results demonstrate the ability for extracting PSI from the plant, interfacing it with both organic and inorganic materials, and measuring its photoinduced, vectorial electron-transfer properties.

Methods to direct the adsorption of PSI to specific regions of a surface may be important for the future development of biomolecular electronic devices. Since PSI adsorbs to unfunctionalized gold substrates as well as various hydroxyl- and acid-terminated SAMs, surfaces must be identified that inhibit or prevent PSI adsorption and thereby guide its directed assembly. Previous studies on protein adsorption at model surfaces have identified two methods for preventing the adsorption of proteins. In the most commonly employed method, a surface is modified with adsorbates containing terminal groups such as oligo(ethylene glycol) (OEG) that are highly resistant to protein attachment.^{7,8} Another method to inhibit protein adsorp-

* To whom correspondence should be addressed: e-mail kane.g.jennings@vanderbilt.edu, fax 615-343-7951; e-mail d.cliffler@vanderbilt.edu, fax 615-322-4936.

[†] Department of Chemical Engineering.

[‡] Department of Chemistry.

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tion is to add molecules or polymers to the protein solution that preadsorb onto low-energy surfaces and provide a protein-resistant layer. For example, poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) copolymers are reported to interact with low-energy surfaces such that the PPO adsorbs to the surface while the PEO chains extend into solution forming a brush configuration that limits protein adsorption.^{9–11} This latter method offers the advantage of convenience since OEG-terminated adsorbates do not have to be synthesized. The inhibiting agent is merely added to the contacting aqueous protein solution.

This paper combines reflectance–absorption infrared spectroscopy (RAIRS), quartz crystal microbalance (QCM), wetting measurements, and cyclic voltammetry to investigate the adsorption of PSI at several model surfaces derived from the adsorption of ω -functionalized and *n*-alkanethiols on gold. Through this work, we are able to identify surfaces that inhibit and/or prevent the adsorption of PSI and explore the fundamental reasons behind the inhibition. The inhibiting effect investigated here is also extended to other protein systems to develop a general method for generating protein-resistant surfaces.

Experimental Section

Materials. Gold shot (99.99%) and silicon (100) wafers were obtained from J&J Materials and Montco Silicon, respectively. Chromium-coated tungsten rods were obtained from R. D. Mathis. Gold electrodes used for electrochemical analysis were purchased from CH Instruments. Deionized water (16.7 M Ω) was purified with a Modu-Pure system. 1-Hexanethiol, 1-heptanethiol, 1-octanethiol, and 1-dodecanethiol were obtained from Aldrich Chemical and were used as received. 11-Hydroxyundecanethiol (Aldrich) and 11-mercaptoundecanoic acid (Aldrich) were each recrystallized in hexane prior to use. Triton X-100 was obtained from Fisher Scientific and was used as received. Bovine serum albumin was obtained from Sigma and was used as received. Phosphate-buffered saline powder (Sigma) was mixed with deionized water to produce a 10 mM solution. Ethyl alcohol (100%, Aaper) was used as received. Sulfuric acid and 30% hydrogen peroxide were purchased from Fisher Scientific. CF₃CF₂–CH₂O(CH₂)₁₁SH was available from previous studies.¹² Polished gold-coated QCM crystals were purchased from Maxtek, Inc. Compressed N₂ gas was purchased from J&M Cylinder Gases, Inc.

Preparation and Activity of Protein Solutions. The PSI solution (approximately 20 μ g/mL) used in all studies except electrochemical characterizations was a gift from James Lee and prepared according to a literature procedure.^{3,13} Briefly, PSI reaction centers were extracted from spinach leaves by Triton X-100 (0.8 mM) solubilization in 0.2 M phosphate (pH 7.0) followed by hydroxylapatite column purification. The PSI solution used in electrochemical studies (approximately 60 μ g/mL) was prepared by a related published procedure as a gift from Jennifer Millsaps.¹⁴ A solution of bovine serum albumin (BSA; 100 μ g/mL) was prepared in 10 mM phosphate-buffered saline (PBS) (pH = 7.4) with and without Triton-X 100 (1 mM).

Preparation of SAMs on Gold. Gold-coated silicon (100) wafers were prepared by first evaporating a 100 Å layer of chromium followed by a 1250 Å layer of gold at a base pressure of 3×10^{-6} Torr using a CVC-PSM66 metal atom evaporator.

The gold-coated wafers were then cut into 1.5 cm \times 1 cm strips, rinsed thoroughly with ethanol, dried in a stream of nitrogen, and immersed in a 1 mM solution of the alkanethiol in ethanol for 24 h at room temperature (22°C). Immediately after removal from the solution, the sample was rinsed with ethanol and dried in a stream of nitrogen.

PSI Adsorption. PSI solutions were prepared by diluting the original PSI solution (20 μ g/mL) 2-fold with deionized water. SAM-coated gold substrates were placed into the diluted PSI solution at room temperature for 8 h. After the adsorption, the samples were thoroughly rinsed with deionized water, dried in a stream of nitrogen, and then characterized.

Quartz Crystal Microbalance (QCM). The kinetics of protein adsorption on SAMs was measured by the change in resonant frequency using a QCM system purchased from Maxtek, Inc. The system consists of a 5 MHz piezoelectric quartz crystal (diameter of 1 in.), a plating probe (KPS 550) containing an internal oscillator circuit mounted close to the crystal, a flow cell (\approx 0.1 mL flow chamber), and a plating monitor (PM 710) that indicates frequency change and plating rate. The system was interfaced to a personal computer.

A polished gold-coated piezoelectric quartz crystal was first exposed to piranha solution (4:1 (v:v) H₂SO₄/30% H₂O₂(aq)) for 2 min before immersion in an ethanolic solution containing 1 mM alkanethiol for 24 h and was subsequently washed thoroughly with ethanol and deionized water and dried in a stream of nitrogen. The quartz crystal was placed on the plating probe, and the flow cell was sealed and tightened on top of the quartz crystal. An aqueous solution containing 0.1 M phosphate (pH 7.0) and 0.4 mM Triton X-100 was fed into the flow cell containing the quartz crystal by a microflow peristaltic pump (Control Co., model 3383). Once a stable baseline was achieved, the pump was reversed and a solution of PSI (10 μ g/mL), 0.1 M phosphate (pH 7.0), and 0.4 mM Triton X-100 was fed into the flow cell. As the protein was adsorbed onto the surface, the computer connected to the plating monitor recorded the frequency change as a function of time.

RAIRS. IR spectra were obtained in a single reflection mode with a Bio-Rad Excalibur infrared spectrometer equipped with a universal reflectance attachment. The p-polarized light was incident at 80° from the surface normal. The reflected light was detected with a narrow-band MCT detector cooled with liquid nitrogen. Spectral resolution was 2 cm⁻¹ after triangular apodization. Spectra were referenced to those of SAMs prepared on gold from octadecanethiol-*d*₃₇, and 1000 scans of both sample and reference were collected. The presence of water was minimized by flowing ultrahigh purity nitrogen gas through the sample compartment at a flow rate of 9 L/min for at least 20 min. A water spectrum was created by rescanning the perdeuterated background sample in the presence of water vapor introduced from the ambient air by lifting the FTIR cover for 3–4 s after the initial background spectrum was taken. The water spectrum was subtracted from the spectrum of each sample to better resolve the peaks of interest in the 1400–1900 cm⁻¹ region.

Contact Angle Measurements. Advancing contact angles were measured on a static drop of deionized water on various surfaces with a Rame-Hart manual goniometer. The contacting water drop was advanced (1 μ L/s) slowly across the surface prior to measurement via an attached syringe supplied by Rame-Hart. Two measurements of the contact angles were performed on opposite edges of a \sim 5 μ L drop with the pipet tip immersed in the drop. The angles measured for drops on samples from at least three independent preparations were then averaged for the reported contact angles.

Cyclic Voltammetry Studies. Using PSI solutions prepared by a method previously described,¹⁴ 1.0 mL of the concentrated PSI solution was emptied into a 5 mL volumetric flask and diluted to the mark with a 100 mM phosphate buffer titrated with NaOH to pH 7.2. A measurement versus background of the diluted sample was taken using a UV–vis spectrometer to determine the approximate chlorophyll concentration.

Cyclic voltammogram measurements were made in fresh 100 mM phosphate buffer adjusted to pH 7.2. Cyclic voltammograms were obtained using a CH Instruments 660A potentiostat with a platinum wire counter electrode, a 2 mm diameter gold disk working electrode, and a Ag/AgCl/sat'd KCl(aq) reference elec-

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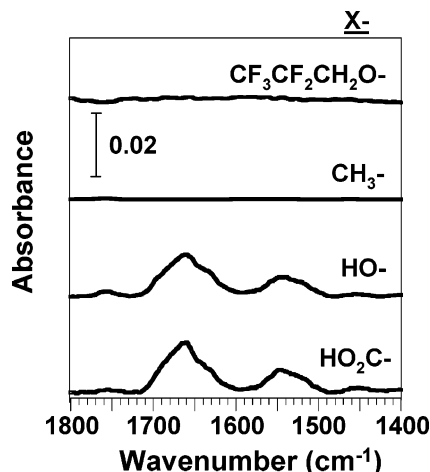


Figure 1. Reflectance infrared spectra of SAMs prepared from alkanethiols with different terminal groups (X) after 8 h of exposure to an aqueous solution containing PSI (10 $\mu\text{g/mL}$).

trode, all from CH Instruments. Oxygen was removed from the sample cell during the cyclic voltammogram measurements by bubbling nitrogen gas through the chamber. The gold electrode was polished with alumina, sonicated for a few seconds in ultrapure water, and then electrochemically polished in 0.5 M H_2SO_4 until the typical cyclic voltammogram for clean gold was obtained. A SAM-coated gold working electrode was prepared by leaving this gold electrode overnight in a 1.0 mM solution of appropriate thiol in ethanol. After a background voltammogram, the working electrode was immersed in the PSI solution for 90 min in a 4 $^\circ\text{C}$ refrigerator, removed, and rinsed with ultrapure water and new cyclic voltammograms were recorded in fresh pH 7.2 phosphate buffer.

Results and Discussion

Effect of Surface Composition on the Adsorption of PSI. To investigate the effect of surface composition on the adsorption of PSI, we obtained IR spectra after exposing SAMs with different terminal groups on gold to aqueous PSI solutions for 8 h at room temperature. These solutions consisted of 0.1 M phosphate-buffered saline, PSI (10 $\mu\text{g/L}$) containing 40 chlorophylls per active P700 reaction center, and Triton X-100 (0.4 mM) as a stabilizing agent used in the extraction of PSI since it is insoluble in water. The SAMs were prepared by adsorbing $\text{HS}(\text{CH}_2)_{11}\text{OH}$ or $\text{HS}(\text{CH}_2)_{10}\text{CO}_2\text{H}$ onto gold to form high-energy surfaces that exhibit low advancing contact angles with water ($\theta_a \sim 20^\circ$) or $\text{HS}(\text{CH}_2)_{11}\text{CH}_3$ or $\text{HS}(\text{CH}_2)_{11}\text{OCH}_2\text{CF}_2\text{CF}_3$ to form low-energy surfaces that exhibit high contact angles with water ($\theta_a \sim 115^\circ$). As a test of whether PSI adsorbed onto the different surfaces, we examined the IR spectra of the exposed samples for the appearance of Amide I and Amide II bands. The Amide I band is primarily due to the carbonyl stretching mode of the amide functional group within proteins whereas the Amide II band arises from N-H bending and C-N stretching vibrations.¹⁵ Figure 1 shows that SAMs with high-energy surfaces (HO- and HO_2C -) exhibit Amide I and Amide II bands at ~ 1660 and ~ 1545 cm^{-1} , respectively, in the IR spectrum after exposure to PSI, consistent with its presence on the surface, while those with low-energy surfaces (F_3C - and H_3C -) do not exhibit these peaks and thereby seem to prevent or inhibit PSI adsorption. These results were surprising since previous research^{7,8} indicates that several model proteins adsorb onto low-energy

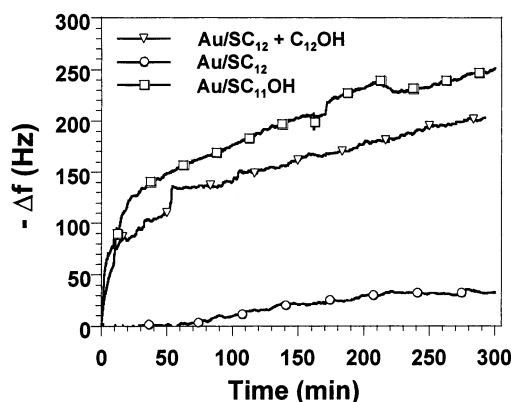


Figure 2. Time-dependent resonant frequency change upon exposure of $\text{Au}/\text{S}(\text{CH}_2)_{11}\text{OH}$ or $\text{Au}/\text{S}(\text{CH}_2)_{11}\text{CH}_3$ (with and without C_{12}OH) to an aqueous solution containing PSI (10 $\mu\text{g/mL}$).

surfaces to a much greater extent than onto high-energy surfaces. Therefore, the ability of low-energy surfaces to prevent or inhibit PSI adsorption, as shown in Figure 1, is highly unusual in comparison with the behavior of other protein-surface combinations and becomes the focus of the remainder of this paper.

We also exposed PSI to methyl-terminated SAMs prepared from shorter-chained alkanethiols ($n = 6-8, 10$) and observed no adsorption on the basis of Amide I and Amide II band intensities (not shown). These shorter-chained adsorbates produce less crystalline SAMs due to weaker van der Waals interactions between the hydrocarbon chains.^{16,17} The nonadsorption of PSI onto these surfaces suggests that a high degree of structural order is not required to prevent PSI from adsorbing at low-energy surfaces.

A possible explanation for the lack of amide signals for PSI on low-energy surfaces using ex situ IR spectra is that the PSI actually does adsorb but is rinsed away during or after removal from solution. To confirm or eliminate this explanation, we examined the adsorption of PSI onto HO- and H_3C -terminated SAMs with a quartz crystal microbalance (QCM), an in situ characterization method that provides real-time information on the adsorption process (Figure 2). The negative change in frequency ($-\Delta f$) during the QCM experiment is consistent with the adsorption of PSI onto the HO-terminated, SAM-modified crystal. The rate of adsorption is rapid during the initial minutes but decreases as the adsorption time progresses, similar to other QCM studies of protein adsorption.¹⁸ In contrast to that for the hydroxyl surface, the frequency change is much smaller upon exposure of a H_3C -terminated crystal to PSI, indicating a true inhibition of PSI adsorption at low-energy surfaces.

Effect of Surface Energy on PSI Adsorption. To investigate the effect of a broader range of surface energy on PSI adsorption, we exposed PSI to mixed SAMs formed onto gold from a binary solution of HSC_{12} and HSC_{11}OH in ethanol. By varying the mole fraction of HSC_{11}OH in solution, we created surfaces that span the range of wettability, exhibiting advancing water contact angles from $\sim 15^\circ$ on a pure HO-terminated SAM to 113° on a pure H_3C -terminated SAM. We estimated the surface mole fraction ($\chi_{\text{surf}}^{\text{OH}}$) of the hydroxyl component within the

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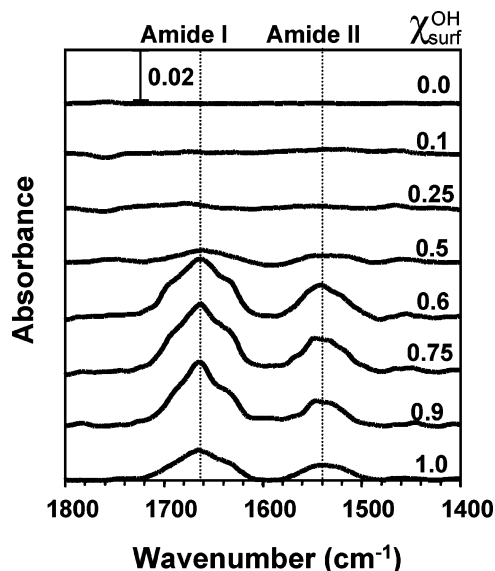


Figure 3. Reflectance infrared spectra showing the effect of hydroxyl surface coverage on the adsorption of PSI. Mixed SAMs containing HSC₁₁OH and HSC₁₂ were exposed to an aqueous solution containing 10 $\mu\text{g/mL}$ of PSI for 8 h.

mixed monolayer by Cassie's equation

$$\chi_{\text{surf}}^{\text{OH}} = \frac{\cos \theta_{\text{meas}} - \cos \theta_{\text{CH}_3}}{\cos \theta_{\text{OH}} - \cos \theta_{\text{CH}_3}} \quad (1)$$

where θ_{meas} , θ_{CH_3} , and θ_{OH} are the advancing contact angles on a mixed monolayer, a pure H₃C-terminated SAM (from C₁₂SH), and a pure HO-terminated SAM (from HOC₁₁-SH), respectively. Equation 1 has been shown to accurately predict the binary composition of HSC₁₁OH/HSC₁₂ mixed monolayers.¹⁹ As shown in Figure 3, barely any PSI adsorbed onto surfaces with $\chi_{\text{surf}}^{\text{OH}} \leq 0.5$ ($\theta_{\text{a}}(\text{H}_2\text{O}) > 73^\circ$) whereas significant adsorption was found for $\chi_{\text{surf}}^{\text{OH}} \geq 0.6$ ($\theta_{\text{a}}(\text{H}_2\text{O}) < 65^\circ$). The Amide I and II bands actually exhibit a maximum absorbance at $\chi_{\text{surf}}^{\text{OH}} = 0.75$, suggesting that the adsorption of PSI does not continually increase with surface energy and that other factors may play a role.

Proposed Explanation for the Nonadsorption of PSI at Low-Energy Surfaces. The unique nonadsorption of PSI at low-energy surfaces is most likely related to interactions between the surface and either PSI or solution additives. The PSI solution as prepared contains Triton X-100 (0.4 mM) to stabilize the nearly insoluble PSI in water. Except at the donor and acceptor ends, which are charged, PSI exhibits a predominantly hydrophobic surface²⁰ that is likely encapsulated by Triton X-100 in water and thereby rendered hydrophilic. This surface modification by Triton X-100 greatly enhances the solubility of PSI in water. This presence of Triton X-100 in solution may then inhibit the adsorption of PSI onto low-energy surfaces.

A plausible reason for the nonadsorption of PSI is that Triton X-100, present in the PSI solution, adsorbs to the low-energy surface to produce a film that inhibits adsorption. The hydrophilic group of Triton X-100 is a poly(ethylene glycol) (PEG) group $(-\text{OCH}_2\text{CH}_2)_n\text{OH}$ where $n \sim 10$, which is similar to those used to prepare surfaces that are highly resistant to protein adsorption.^{7,8} Upon

exposure to a low-energy surface in water, Triton X-100 is known to adsorb,²¹ most likely through hydrophobic interactions to produce an oriented monolayer film with the protein-resistant PEG group at the SAM/water interface. Ward et al.²² have demonstrated the assembly of similar PEG-terminated surfactants into monolayer films atop low-energy methyl surfaces in water. The presence of this PEG surface likely inhibits the adsorption of PSI.

Since Triton X-100 cannot be removed from solution due to its role as a stabilizer of PSI, we have added a very dilute concentration (0.1 mM) of 1-dodecanol to the PSI solution to replace Triton X-100 at the low-energy surface. Due to its poor solubility in water, 1-dodecanol is a much more active surfactant than Triton X-100 and is known to readily displace various surfactants from low-energy surfaces, as reported by Ward et al. using sum frequency generation.²³ Addition of 1-dodecanol to the PSI solution in contact with a H₃C-terminated SAM should therefore effect the displacement of the PEG-terminated Triton X-100 monolayer from the low-energy methyl surface in favor of the more surface-active 1-dodecanol. Upon addition of 1-dodecanol to the PSI solution, a significant amount of PSI becomes adsorbed at the surface, as indicated by the rapid frequency change in QCM (Figure 2). Displacement of the inhibiting monolayer of Triton X-100 by the more active alcohol greatly enhances the adsorption of PSI. In fact, the kinetics of the adsorption process is similar to that on the HO-terminated SAM, suggesting that the addition of 1-dodecanol indeed produces a HO-terminated layer atop the H₃C-terminated SAM and that this outer molecular group governs the adsorptive behavior of the surface. This experiment provides supporting evidence for the role of Triton X-100 in the inhibition of PSI adsorption at low-energy surfaces.

Triton X-100 also likely adsorbs onto high-energy surfaces, similar to the behavior of other nonionic surfactants. Grant et al.²⁴ used atomic force microscopy to show that octaethylene glycol monododecyl ether (C₁₂E₈), a nonionic surfactant similar to Triton X-100, adsorbs onto surfaces of low, intermediate, and high energies prepared by mixed CH₃/HO-terminated SAMs on gold. The surfactants adopt a well-ordered monolayer atop the lower energy, predominately CH₃ surfaces, but form less-ordered surface micelles atop higher energy surfaces with 50% or greater OH content. These previous results for C₁₂E₈, in combination with those shown here for mixed surfaces in Figure 3, suggest that a densely packed monolayer of nonionic surfactant resists protein adsorption whereas less densely packed surface micelles do not, perhaps due to insufficient coverage or more isotropic orientation of the oxyethylene groups at the surface. Therefore, in Figure 3, the large increase in PSI adsorption from $\chi_{\text{surf}}^{\text{OH}} = 0.5$ – 0.6 is likely due to a changing conformation of the Triton X-100 layer to contain less ordered micellar aggregates. The decrease in PSI adsorption upon increasing $\chi_{\text{surf}}^{\text{OH}}$ from 0.75 to 1.0 is consistent with the adsorptive behavior of other proteins⁸ (i.e., less adsorption on higher energy surfaces) as the inhibiting effect of Triton X-100 is greatly reduced. Since ex situ IR spectra (see Supplemental Figure 1) show no evidence for Triton X-100 on the surfaces of SAMs containing PSI, as

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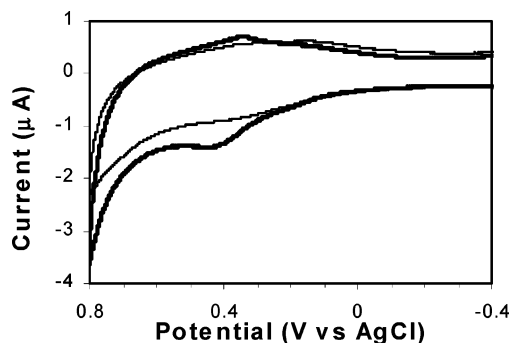


Figure 4. Cyclic voltammogram of a $\text{HO}(\text{CH}_2)_{11}\text{SH}$ -coated gold electrode at 0.1 V/s in phosphate buffer before (bold) and after (thin) adsorption of PSI.

indicated by the absence of an ether stretching band at $\sim 1130\text{ cm}^{-1}$, we assume that PSI displaces surface micelles of Triton X-100 to maximize interactions with the surface of the SAM. Any residual Triton X-100 remaining on the surface after PSI adsorption may then be rinsed away upon removal from solution.

Voltammetric Changes of PSI Adsorption onto Electrodes. Cyclic voltammetry is a diagnostic tool for studying surface changes on an electrode.²⁵ Direct electrochemistry of PSI thylakoid membranes has been demonstrated,^{26,27} but no surface electrochemistry of Triton-stabilized PSI adsorption has been performed. Changes in electrode capacitance resulting from adsorption are reflected in the nonfaradaic current in a cyclic voltammogram, while adsorption onto the surface also interferes with the formation and stripping of metal oxide surface layers. We studied the adsorption of PSI onto four electrode surfaces: hexanethiol (low-energy surface), dodecanethiol (low-energy surface), and 11-hydroxyundecanethiol (high-energy surface) coated gold electrodes and bare gold (high-energy surface). Cyclic voltammograms for bare gold in phosphate buffer electrolyte showed the expected gold oxide formation and stripping and exhibited a nonfaradaic background current of $0.4\text{ }\mu\text{A}$ indicating a large double layer capacitance as expected for a bare electrode (Supplementary Figure 2). Upon dipping into PSI solution, the adsorption of the protein complex onto the high-energy surface results in the disappearance of the gold oxide surface peaks and a reduction in the nonfaradaic capacitive charging current, consistent with electrochemical measurements of thylakoid membranes by Carpentier et al.^{26,27} For both of the low-energy SAM-coated gold electrodes, as modified by hexanethiol and dodecanethiol, the cyclic voltammograms were completely unchanged after dipping in PSI indicating no change in the electrode capacitance that should result if adsorption occurred (Supplementary Figures 3 and 4). Neither of the *n*-alkanethiol coated electrodes showed any gold oxide response indicating that the ordered SAM blocks surface gold electrochemistry. The 11-hydroxyundecanethiol coated electrode did show small gold oxide formation and stripping peaks before PSI exposure indicating a less organized monolayer as expected for SAMs with polar terminal groups (Figure 4). After immersion in the PSI solution, the gold oxide peaks disappeared indicating additional adsorption blocking the electrode, but little change in the electrode capacitance. The electrode ca-

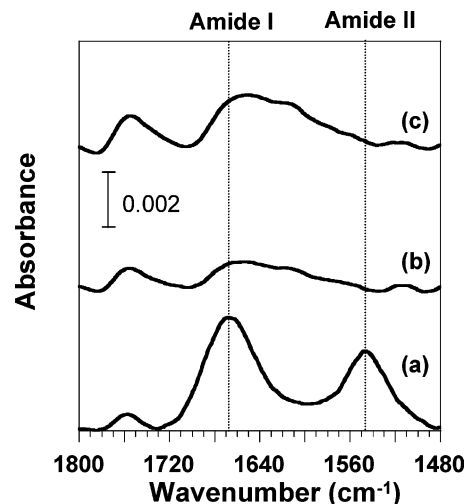


Figure 5. Reflectance infrared spectra of SAMs on gold prepared from $\text{CH}_3(\text{CH}_2)_{11}\text{SH}$ (a and b) or $\text{HO}(\text{CH}_2)_{11}\text{SH}$ (c) after exposure to an aqueous solution of BSA ($100\text{ }\mu\text{g/mL}$) with (b) and without (a and c) 1 mM Triton X-100 (c) for 8 h.

pacitance was already greatly reduced by the long alkyl chain in 11-hydroxyundecanethiol so the lack of change in capacitance is reasonable. Nevertheless, a clear change in the electrode surface accessibility is indicated, and this is attributed to PSI adsorption. We conclude that the adsorption of PSI and Triton X-100 onto this surface results in the complete suppression of the metal oxide electrochemistry. This change in the electrochemistry of the hydroxyl-terminated working electrode was permanent insofar as sonication of the electrode in phosphate buffer did not show any changes in the voltammograms (Supplementary Figure 5). These results are consistent with the presented model that PSI strongly adsorbs onto bare gold and hydroxyl-terminated SAMs but has no affinity to adsorb onto methyl-terminated SAM electrodes.

Extension of Results to Other Protein Systems. The ability to render low-energy surfaces resistant to the adsorption of proteins by merely adding small quantities of Triton X-100 to the contacting solution could lead to more efficient and straightforward methods for preparing biocompatible surfaces and could impact applications such as blood filtration and assays where protein solutions may come in contact with various materials.²⁸ To assess whether the addition of Triton X-100 can affect the nonadsorption of another protein at a low-energy surface, we exposed a methyl-terminated surface prepared from C_{12}SH on gold to a solution of bovine serum albumin (BSA; $100\text{ }\mu\text{g/mL}$) with and without Triton X-100 for 8 h (Figure 5). We have chosen BSA as a model protein due to its tendency to adsorb to many surfaces and the abundance of studies focusing on this protein.⁸ The IR spectra in Figure 5 show that the presence of Triton X-100 at 1 mM greatly reduces the adsorption of BSA on the methyl-terminated surface, as indicated by the lower intensities of the Amide I and Amide II peaks for the SAM exposed to the Triton X-100-spiked BSA solution. The intensities of the IR bands for the methyl surface in the presence of Triton X-100 are even less than those for BSA adsorption onto a HO-terminated SAM (Figure 5 spectrum c), which is known to inhibit the adsorption of BSA and other model proteins in comparison with lower energy surfaces.⁸ This result for BSA, combined with those for PSI, suggests that, in general, the addition of Triton X-100 to an aqueous

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protein solution can greatly reduce the adsorption of that protein upon prolonged exposure to hydrophobic surfaces.

Conclusions. Through a series of in situ and ex situ experiments, this report demonstrates that the non-adsorption of PSI on low-energy surfaces is caused by an adsorbed layer of Triton X-100, which is used as an additive to stabilize PSI in the aqueous solution. The adsorbed layer of Triton X-100 presents a surface composed of PEG groups, which are known to greatly resist protein adsorption. Displacement of these Triton X-100 molecules at the surface by a more surface active molecule greatly enhances the adsorption of PSI. Our work shows that, in general, addition of Triton X-100 to an aqueous protein solution enables the modification of typically adsorptive, low-energy surfaces to protein-resistant, PEG-terminated surfaces. These results demonstrate a new and general method for creating protein-resistant surfaces from highly adsorptive low-energy surfaces.

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Supporting Information Available: RAIRS of a HO-(CH₂)₁₁S-modified gold surface before and after exposure to PSI, cyclic voltammograms of a bare Au electrode, dodecanethiol-coated Au electrode, and hexanethiol-coated Au electrode before and after exposure to PSI, and cyclic voltammograms of PSI-coated HOC₁₁S/Au electrode before and after sonication. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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