Zwitterionic SAMs that Resist Nonspecific Adsorption of Protein from Aqueous Buffer

R. Erik Holmlin, Xiaoxi Chen, Robert G. Chapman, Shuichi Takayama, and George M. Whitesides*

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

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This paper describes the use of surface plasmon resonance spectroscopy and self-assembled monolayers (SAMs) of alkanethiols on gold to evaluate the ability of surfaces terminating in different combinations of charged groups to resist the nonspecific adsorption of proteins from aqueous buffer. Mixed SAMs formed from a 1:1 combination of a thiol terminated in a trimethylammonium group and a thiol terminated in a sulfonate group adsorbed less than 1% of a monolayer of two proteins with different characteristics: fibrinogen and lysozyme. Single-component SAMs formed from thiols terminating in groups combining a positively charged moiety and a negatively charged moiety were also capable of resisting the adsorption of proteins. Single-component SAMs presenting single charges adsorbed nearly a full monolayer of protein. The amount of protein that adsorbed to mixed zwitterionic SAMs did not depend on the ionic strength or the pH of the buffer in which the protein was dissolved. The amount of protein that adsorbed to single-component zwitterionic SAMs increased as the ionic strength of the buffer decreased; it also decreased as the pH of the buffer increased (at constant ionic strength). Single-component zwitterionic SAMs composed of thiols terminating in *N*,*N*-dimethyl-amino-propane-1-sulfonic acid ($-N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$) groups were substantially more effective at resisting adsorption of fibrinogen and lysozyme from buffer at physiological ionic strength and pH than single-component zwitterionic SAMs composed of thiols terminating in phosphoric acid 2-trimethylamino-ethyl ester ($-OP(O)_2^-OCH_2CH_2N^+(CH_3)_3$). Several of these zwitterionic SAMs were comparable to the best known systems for resisting nonspecific adsorption of protein.

Introduction

This paper characterizes the ability of self-assembled monolayers (SAMs) on gold that present positively and negatively charged groups to resist the nonspecific adsorption of protein from aqueous buffer.[1] Throughout this paper, we use two condensed phrases for brevity: (i) the phrase "zwitterionic SAMs" refers both to SAMs formed from a 1:1 mixture of positively and negatively charged thiols and to SAMs formed from thiols that combine a positively charged moiety and a negatively charged moiety in the same molecule and (ii) the phrase "inert surface" refers to any surface that resists nonspecific adsorption of protein from aqueous buffer.[1]

Nonspecific adsorption of proteins to synthetic surfaces is an important consideration in a range of areas; examples include substrates for cell culture,[2,3] materials for protein purification,[4] prostheses,[5] contact lenses,[6,7] in-dwelling sensors,[8] and catheters.[8] Here, we have combined surface plasmon resonance (SPR) spectroscopy and SAMs to study the adsorption of proteins to zwitterionic SAMs. The objective of the work was to guide the design of new materials that resist the adsorption of protein.

Surfaces That Resist Protein Adsorption. *PEG.* Poly(ethylene glycol) (PEG) is a water soluble, electrically neutral polyether that has been widely used as a coating for biomedical devices.[9] Incorporating PEG as a copolymer in polymeric materials or grafting it onto the surface of biomaterials increases the biocompatibility of these surfaces.[9,10] Surfaces covered with a low density of PEG high molecular weight ($(EG)_n$, $n \sim 30$) have the characteristic that they resist nonspecific adsorption of protein;[10] this low protein adsorption[11,12] is one key aspect of the biocompatibility that PEG affords. Although PEG has been a useful component of biomaterials, it is susceptible to autoxidation in the presence of dioxygen and transition metal ions.[13−15] In addition, the terminal hydroxyl group of PEG can be oxidized by alcohol dehydrogenase to an aldehyde; there is concern that this aldehyde may react with proteins in vivo and with other molecules having amine groups. The aldehyde can be oxidized further by aldehyde dehydrogenase.[16,17] There is great interest in identifying alternatives to PEG.[18]

* To whom correspondence should be addressed. E-mail: gwhitesides@gmwgroup.harvard.edu.

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Other Surface-Grafted Polymers That Resist the Adsorption of Proteins. Polymers other than PEG have been explored for biomedical applications. Rabinow et al.[19] compared the protein resistance of PEG, poly(vinyl alcohol) (PVA), polyethyloxazoline (PEOX), and poly(vinylpyrrolidone) (PVP) when the polymers were incorporated into polymer films. PVP, PEOX, and PVA all reduced the amount of protein adsorption relative to the amount that adsorbed on unmodified polymer films. In these comparisons, PEG performed the best. Several groups have examined surfaces derivatized with carbohydrates. Österberg et al.[20] found that derivatives of cellulose grafted to polystyrene were nearly as effective as PEG at preventing protein adsorption. Polysaccharides crosslinked to poly(ethylenimine) (PEI) bound noncovalently to polystyrene also reduced the amount of protein adsorption to the polymer surface.[21] Preadsorption of methyl cellulose or poly(vinyl methyl ether) to hydrophobic membranes used for ultrafiltration reduced the amount of protein that adsorbs to the pore walls of the membrane.[22] Chapman et al. grafted PEI to SAMs and acylated the free amine groups to give polymer films containing high concentrations of various *N*-acyl groups.[23] The functionalized PEI films formed inert surfaces either when the *N*-acyl derivative contained an oligomer of EG or when it contained an acetyl group.

SAMs That Resist the Adsorption of Proteins. SAMs of alkanethiols terminated in different functional groups have been used to study the physical-organic chemistry of the adsorption of proteins to synthetic surfaces. SAMs that present a high density of short oligomers of EG ($(EG)_n$, $n = 3-6$) resist the adsorption of proteins to the same extent as surfaces coated with a relatively low density of high molecular weight PEG.[24,25] SAMs on gold terminated in oligomers of tripropylene sulfoxide are comparable to SAMs presenting $(EG)_3$ in their ability to block nonspecific protein adsorption[26] found. Using SPR spectroscopy in combination with a procedure for rapidly incorporating terminal functional groups on the surface of SAMs on gold, we have screened a variety of groups for their ability to render surfaces inert to the adsorption of proteins.[27,28] This screening method identified several new groups that were previously unrecognized for their ability to resist protein adsorption.

Considerations for Design of Organic Surfaces that Resist Protein Adsorption. $(EG)_n$ is not the only organic group that can be used to generate inert surfaces. A variety of functional groups, when presented as terminal groups on the surface of SAMs on gold, are comparable to oligomers of EG in resisting protein adsorption.[27] In general, these groups share four features: they are (i) hydrophilic, (ii) electrically neutral, (iii) hydrogen bond acceptors, and (iv) not hydrogen bond donors. This set of properties seems to describe many but not all inert surfaces. In a notable and unexpected exception, Mrksich has reported that SAMs terminating in mannose groups (a hydrogen bond donor) are inert.[29] Conformational flexibility is also a characteristic of many of these groups, but it does not seem to be essential.

The origin of resistance to protein adsorption is not yet clear. When a protein approaches an interface, the energetics of interaction between them contains contributions from van der Waals, electrostatic, and hydrogen-bonding terms.[30] Electrical neutrality may be important in minimizing the electrostatic interactions, and the absence of hydrogen bond donors may be important in minimizing the hydrogen bonding interactions, but it has been difficult to disentangle the individual contributions to account for the general properties of inert surfaces.

This paper explores protein adsorption to SAMs composed of alkanethiols functionalized with terminal charged groups. Charged groups such as quaternary ammonium groups and alkyl sulfonates have been used extensively in materials for ion-exchange chromatagraphy. In these applications, charged analytes (proteins, nucleic acids, and small molecules) adsorb electrostatically to resins and are eluted by buffers with high ionic strength. Quaternary ammonium groups and alkyl sulfonates share three of the four common characterstics of groups that resist protein adsorption; the exception is that they are not electrically neutral. We hypothesized that combining positively charged and negatively charged groups in a 1:1 ratio at the SAM−buffer interface, either by coadsorbing two different thiols or by synthesizing a thiol containing both moieties in the headgroup, might render the surfaces electrically neutral and might give a new class of inert surfaces. The idea that zwitterionic surfaces might be biocompatible (and perhaps resistant to protein adsorption) has a precedent in biology: most cell-surface lipids are zwitterionic, and a variety of studies have shown that grafting different phosphorylcholine derivatives to surfaces reduces protein adsorption.[31,32] We wished to examine zwitterionic surfaces in detail using SAMs as model surfaces.

Experimental Design. *SAMs.* Chart 1 shows the structures of the thiols we have used. Some of the different SAMs that can be formed from them are pictured schematically in Figure 1. Adsorption of protein to neutral SAMs formed from thiols **1**−**3** has been studied extensively,[1,25] and we use these SAMs as a basis for comparing adsorption to new surfaces. Using thiols **4**−**7**, we have prepared SAMs that can be classified into one of three categories: (i) single-component SAMs with single charges (SAMs of **4** or **5**), (ii) zwitterionic mixed SAMs (SAM of **4**

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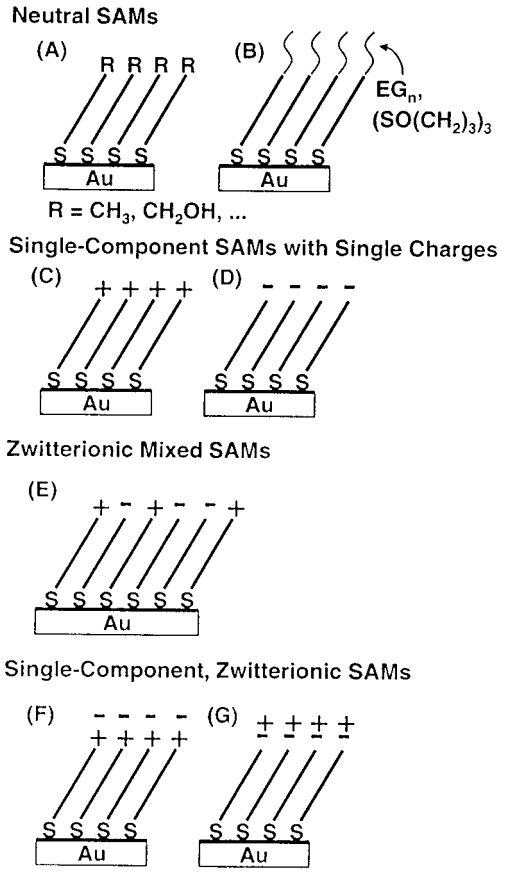
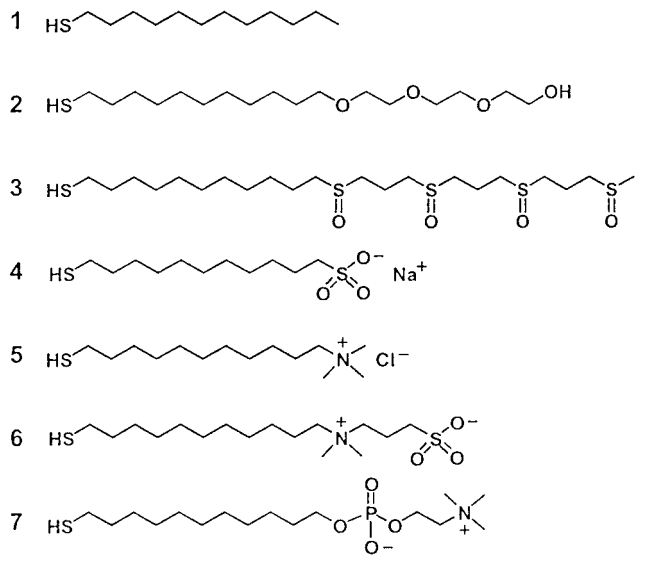


Figure 1. Schematic illustrations of SAMs formed from thiols shown in Chart 1.

Chart 1



and **5** in a 1:1 ratio), and (iii) single-component zwitterionic SAMs (SAMs of **6** or **7**).

Proteins. We surveyed a panel of proteins that vary in molecular weight and pI to establish the scope of the ability of zwitterionic SAMs to resist adsorption of protein (Table 1). We have examined two of these proteins, fibrinogen and lysozyme, in detail. Fibrinogen is a large (340 kD, pI = 6.0) protein present in blood plasma that adsorbs strongly to hydrophobic surfaces.[25,28,33] Lysozyme [E.C. 3.2.1.17] is a small protein (14 kD, pI = 12) that is positively charged under physiological pH (7.0−7.4) where we have carried out most of our studies.[34,35] Lysozyme has been used widely in model studies of electrostatic adsorption of proteins to surfaces.[36]

Table 1. Panel of Proteins Used for Studies of Adsorption

protein	E.C. no.	subunits[a]	MW (kD)[a]	pI[a]
albumin, bovine serum[b]		1	69	4.8
β-galactosidase[b]	3.2.1.23	4	468	5.0
carbonic anhydrase II (bovine)[b]	4.2.1.1	1	29.3	5.9
fibrinogen		1	340	6.0
myoglobin		1	17.6	6.8
ribonuclease A[b]	3.1.27.5	1	13.7	9.8
cytochrome *c*		1	12.4	10.0
lysozyme	3.2.1.17	1	14.3	10.9

[a] See SWISS-PROT database: http://expasy.hcuge.ch/sprot/sprot-top.html; see also: Colton, I. J.; Anderson, J. R.; Gao, J.; Chapman, R. G.; Isaacs, L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1997**, *119*, 12701−12709. [b] We use the following abbreviations in the text and figures to refer to these proteins: albumin, bovine serum (BSA); β-galactosidase (β-Gal); carbonic anhydrase II (bovine) (CA); ribonuclease A (RNAse A).

SPR Spectroscopy. SPR spectroscopy provided a convenient technique with which to monitor the interaction of protein with the synthetic surfaces and to measure the amount of protein that adsorbs to these surfaces.[33] SPR is an optical technique that detects changes in the refractive index above a thin film of gold.[37] In our experiments, we allowed a solution of protein in buffer to flow over the SAM on gold and monitored its adsorption as a function of time; we then replaced the solution of protein with buffer to wash away free and weakly bound protein to reveal the amount of protein irreversibly adsorbed on the SAM. The amount of protein adsorbed was measured in response units (RU, 10 000 RU corresponds to a shift of 1° in the angle of incidence of light that excites surface plasmons in the gold). We report values of ΔRU, which is the change in RU relative to the response for the clean surface exposed to buffer without protein ($\Delta RU = RU^{buffer+protein} - RU^{buffer}$). Equation 1 relates the percentage of a full monolayer of protein (% Monolayer, % ML) adsorbed to a surface to values of ΔRU measured by SPR.[27]

$$\%\ ML = \%\ Monolayer = \frac{\Delta RU_{SAM}}{\Delta RU_{DT}} \times 100 \qquad (1)$$

This equation is an empirical relation that uses the estimation by Mrksich et al.[33] that the value of ΔRU for protein exposed to a methyl-terminated surface reflects the response for adsorption of a full monolayer of protein (here, we use dodecanethiol (DT)). Using it simplifies comparisons of different proteins adsorbing to the same surface.

Results and Discussion

Synthesis of Thiols. Alkanethiol **5** was prepared using procedures described previously.[38] In the preparation of **6** (Scheme 1), dimethylamine reacted with 11-bromo-

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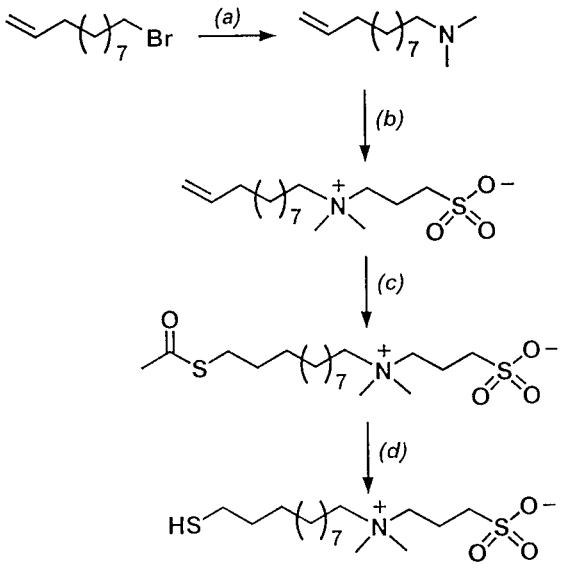
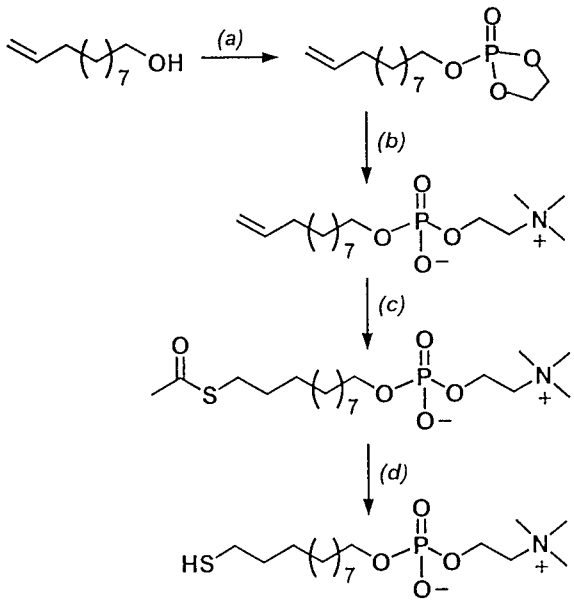
Scheme 1. Reagents and Conditions[a]



[a] (a) Trimethylamine, THF, RT, 8 h. (b) 1,3-Propanesulfone, acetone, RT, 24 h. (c) $CH_3C(O)SH$, ABCN, CH_3OH, hν, RT, 16 h. (d) NaOH, H_2O, RT, 2 h.

Scheme 2. Reagents and Conditions[a]



[a] (a) 2-Chloro-[1,3,2]dioxaphospholane 2-oxide, triethylamine, THF, −15 °C, 2 h. (b) Trimethylamine, CH_2Cl_2, 55 °C (sealed bomb), 2 days. (c) $CH_3C(O)SH$, AIBN, CH_3OH/H_2O (9:1), hν, RT, 16 h. (d) NaOH, H_2O, RT, 2 h.

undec-1-ene to afford *N*,*N*-dimethyl-undec-10-enyl-amine in 99% yield. The resulting tertiary amine reacted with 1,3-propanesultone to give 3-(*N*,*N*-dimethyl-undec-10-enyl-amino)-propane-1-sulfonic acid in 79% yield. We introduced the thiol as the thioacetate by irradiating the alkene in the presence of thioacetic acid;[39] the free thiol was obtained by hydrolysis of the thioacetate with NaOH in a rigorously degassed aqueous solution. To synthesize **7** (Scheme 2), 2-undec-10-enyloxy-[1,3,2]dioxaphospholane 2-oxide formed (72%) on reaction of 2-chloro-[1,3,2]-dioxaphospholane-2-oxide with undec-10-en-1-ol in the presence of triethylamine. Reaction of 2-undec-10-enyloxy-[1,3,2]dioxaphospholane-2-oxide with trimethylamine opened the phospholane to afford phosphoric acid 2-trimethylamino-ethyl ester undec-10-enyl ester.[40] The thiol functionality was introduced using the procedure described for **6**.

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Table 2. Ellipsometric Thicknesses and Advancing Contact Angles of Water and Buffer at pH 10 for SAMs Formed from Thiols Terminated in Charged Groups

	ellipsometric thickness		contact angles	
thiol used to form SAM[a]	estimated (Å)[b]	measured (Å)[c]	θ^{CO} (H_2O)[c,d]	θ^{CO} (pH 10)[c,e]
4	16	15 ± 2	48 ± 5	35 ± 4
5	16	13 ± 1	52 ± 8	67 ± 4
4 + **5** (1:1)	16	11 ± 1	<15	<15
6	20	9 ± 1	<15	<15
7	19	19 ± 1	49 ± 11	<15

[a] Schematic illustrations of thiols **4**, **5**, **6**, and **7** are shown in Chart 1. [b] The thicknesses of the SAMs were estimated as the sum of the Au−sulfur bond, the length of the thiol in extended trans conformation. The lengths of the thiolates were calculated with Chem 3D using MM2; these lengths were multiplied by cos 32° = 0.84 to account for the tilt angle of the thiol on gold. We did not include any contribution by counterions to the estimated thicknesses of SAMs formed from **4** or **5**. [c] The uncertainty is the largest difference between the average of three independent measurements and the value of each measurement. [d] θ^{CO} (H_2O) is the advancing contact angle of deionized water under cyclooctane. [e] θ^{CO} (pH 10) is the advancing contact angle of 25 mM sodium carbonate buffer at pH 10 under cyclooctane. SAMs were soaked in the buffer at pH 10 for 10 min before measuring the contact angle.

Fabrication and Characterization of SAMs. Evaporating a thin layer of gold (40 nm) onto glass microscope slides that had been primed with an adhesion layer of titanium (1 nm) provided chips for SPR spectroscopy.[33] SAMs formed on the gold films upon soaking them in solutions of the appropriate thiol (or mixture of thiols) for ∼24 h. To prepare single-component SAMs with single charges and mixed zwitterionic SAMs, distilled deionized water was the solvent that dissolved the thiols;[38] ethanol dissolved zwitterionic thiols for preparing single-component zwitterionic SAMs.

The thicknesses of SAMs of **4**, **5**, and **7**, measured by ellipsometry (Table 2), were reasonably close to the values expected for films in which the thiols adopted configurations typical of these monolayers.[41] SAMs formed from a 1:1 mixture of **4** and **5** and SAMs formed from **6** were not as thick as expected. For SAMs of **6**, thinner films may reflect disorder in the zwitterionic groups arising from intermolecular interactions between oppositely charged moieties or the difference in size between the terminal groups and the polyethylene chains. Another explanation for the observation of thin films could be that the optical properties of the films are different from the standard optical properties assumed when calculating the thickness from ellipsometry; we assumed a refractive index of 1.45 for the monolayer. This refractive index was chosen on the basis of the refractive indexes of hydrocarbons (hexadecane (liquid) = 1.435, octacosane (solid) = 1.452) and mercaptans (decanethiol (liquid) = 1.457, octadecanethiol (solid) = 1.464).[42] These results establish that we have not formed multiple layers of thiols on the gold films. They also suggest that these SAMs may be somewhat disordered; this suggestion is not surprising

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given the size of the headgroups that terminate the alkanethiols.

To establish the wettability of the SAMs, we measured the advancing contact angle of water under cyclooctane (θ^{CO/H_2O}) and that of buffer (pH 10) under cyclooctane ($\theta^{CO/pH\ 10}$) (Table 2). Sigal et al.[43] have suggested that values of θ^{CO/H_2O} provide a more sensible wettability scale for studies of protein adsorption than do contact angles of water under air. We have adopted their procedure for this work. Single-component SAMs with single charges (SAMs of **4** or **5**) were moderately hydrophilic. Mixed zwitterionic SAMs formed from a 1:1 mixture of **4** and **5** and single-component SAMs formed from **6** were wet significantly better than the single-component SAMs with single charges. The value of θ^{CO/H_2O} for SAMs of **7** was similar to that of the single-component charged SAMs; the value of $\theta^{CO/pH\ 10}$ resembled that of the zwitterionic SAMs. We presume that the effect of pH on the wettability of SAMs of **7** reflects deprotonation of some fraction of phosphodiester to generate zwitterionic groups. Although the pK_a values of these phosphodiester groups are low enough that one would expect the SAMs to be fully deprotonated at pH 7.4,[44] the pK_a values of acidic groups on surfaces are known to be higher than their pK_a values in solution.[45] For SAMs of **7**, the phosphodiester is buried in the low dielectric medium of the alkyl monolayer, so the pK_a would be expected to have increased not only as the result of being packed in the monolayer but also as a result of poor solvation of the anion by water. The pK_a of methane sulfonic acid, for example, increases by 3.2 units in going from water (−2.0) to DMSO (1.2).

Adsorption of Fibrinogen and Lysozyme to SAMs. *Single-Component Charged SAMs and Mixed Zwitterionic SAMs.* Figure 2 compares the adsorption of fibrinogen and lysozyme to SAMs of **4**, SAMs of **5**, and mixed SAMs formed from a 1:1 mixture of **4** and **5**. The proteins were dissolved in phosphate buffer (1 mg/mL) at pH 7.4 with an ionic strength of 170 mM. SAMs of **4** and SAMs of **5** each adsorbed nearly full monolayers of fibrinogen. By contrast, only ~1% of a monolayer of fibrinogen adsorbed on a SAM formed from a 1:1 mixture of **4** and **5**. This low level of adsorption shows that zwitterionic SAMs can be resistant to protein adsorption.

Lysozyme also adsorbed to the single-component SAMs with single charges. Even though it has a substantial net positive charge under the conditions of this experiment ($Z_p = +7.5$ at pH 7.4, 100 mM KCl),[36] lysozyme adsorbed to the SAM of **5**, which presents a positively charged terminal group. The mixed zwitterionic SAM was essentially completely resistant to adsorption of lysozyme.

Single-Component Zwitterionic SAMs. Figure 2 also shows sensorgrams for the adsorption of fibrinogen and lysozyme to SAMs of **6** and **7**. SAMs of **6** adsorbed more protein than did the mixed zwitterionic SAM; this amount of adsorption was, nevertheless, substantially less than that of single-component SAMs of **4** or **5**. SAMs of **7** adsorbed more protein than did SAMs of **6**. One explanation for the increased adsorption to SAMs of **7** is that some of the phosphodiester is protonated under the conditions of this experiment. Because SAMs of **7** adsorbed substantially less protein than did SAMs of **5** (**5** and **7** have the same terminal group), we conclude that most of the phosphodiester groups of **7** are deprotonated at pH 7.4.

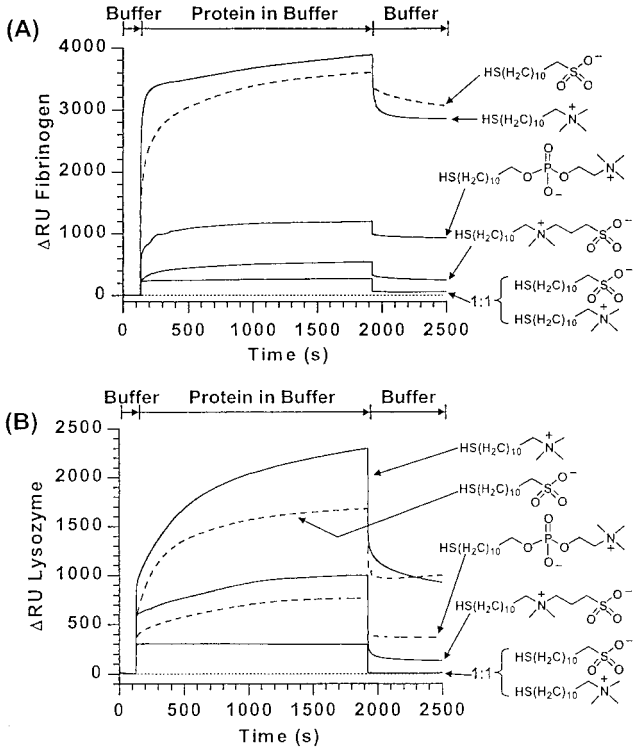

Figure 2. Plots of ΔRU as a function of time for the adsorption of fibrinogen (top) and lysozyme (bottom) to different SAMs. The SAM that corresponds to each curve is defined on the plots. The curves for each protein flowing over the different SAMs were adjusted vertically to have the same value of ΔRU at the time when the protein began to flow over the SAM. The region of time during which protein was present in the buffer is indicated above the plot. The buffer conditions for these experiments were as follows: 10 mM sodium phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.4. All proteins were dissolved in buffer at 1 mg/mL.

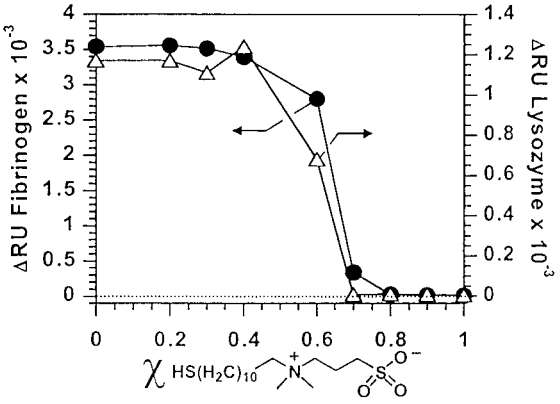

Figure 3. Plots of ΔRU as a function of the mole fraction of **6** in solution with $HS(CH_2)_{11}CH_3$ for adsorption of fibrinogen (●) and lysozyme (△) to mixed SAMs presenting methyl groups and zwitterionic groups. The values of ΔRU in the plot are those taken after a solution of protein flowed over the SAM for 3 min followed by PBS buffer for 5 min.

SAMs Formed from Mixtures of $HS(CH_2)_{11}CH_3$ and ***6****.* Figure 3 shows isotherms for fibrinogen and lysozyme adsorbing to mixed SAMs made up of methyl-terminated thiolates and thiolates that terminate in a zwitterion (**6**). The values of ΔRU in the plot are those taken after a solution of protein flowed over the SAM for 3 min followed by PBS buffer for 5 min; this procedure is different from

(43) Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, *120*, 3464−3473.

(44) The values of pK_a of *n*-alkyl phosphodiesters range from 1.3 to 1.7 for the homologous series of methyl (1.3), ethyl (1.4), propyl (1.6), and butyl (1.7) (see: Kumler, W. D.; Eiler, J. J. *J. Am. Chem. Soc.* **1943**, *65*, 2355−2361.).

(45) Yan, L.; Marzolin, C.; Terfort, A.; Whitesides, G. M. *Langmuir* **1997**, *13*, 6704−6712.

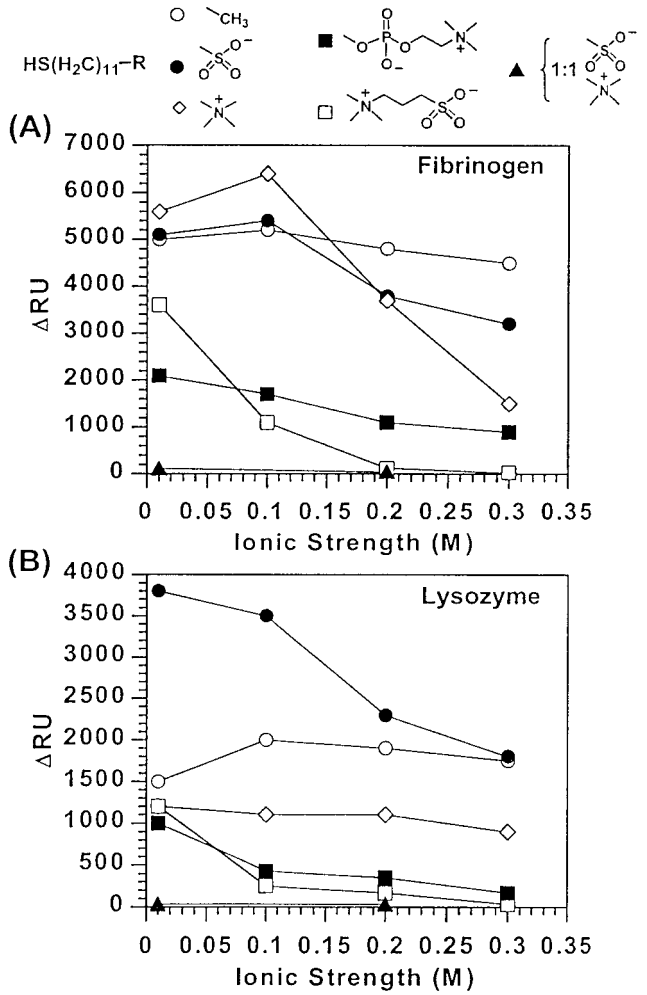

Figure 4. Plots of ΔRU for irreversible adsorption of fibrinogen (A) and lysozyme (B) to different SAMs as a function of the ionic strength of the buffer dissolving the protein. The buffer was 4.4 mM phosphate (pH = 7.4, ionic strength = 10 mM), and the ionic strength was adjusted by dissolving NaCl in the appropriate concentrations. The symbols corresponding to the different functional groups presented at the SAM−buffer interface are defined above the plots.

the one that we used for all other experiments in this paper (a solution of protein flowed over the SAM for 30 min followed by buffer for 10 min) and is used to shorten the time required for the entire experiment. The plots are of ΔRU against the mole fraction of **6** *in the solution used to form the SAM*; that is, we assume that the mole fraction of **6** in the monolayer is that in solution and have not measured it directly. This assumption is undoubtedly incorrect in detail, but the *trends* we observed will still be reliable. They have the same shape for fibrinogen and lysozyme; the amount of protein that adsorbs to a mixed SAM of methyl-terminated thiol and **6** does not decrease until the fraction of **6** is in the range of 0.5−0.6. At mole fractions of **6** > ∼0.7, very little protein adsorbed to the surfaces. Dodecanethiol SAMs diluted with **2** or **3** resisted the adsorption of fibrinogen when the mole fraction of **2** or **3** was ∼0.4−0.5.[24,25]

Parametric Sensitivities of the Adsorption of Fibrinogen and Lysozyme to Zwitterionic SAMs. *Ionic Strength.* We hypothesized that ionic strength would affect the amount of protein that adsorbed to SAMs comprising charged groups. Figure 4 compares the amounts of fibrinogen and lysozyme that adsorbed irreversibly to single-component SAMs of **1**, **4**, **5**, **6**, and **7** and to mixed SAMs formed from a 1:1 mixture of **4** and **5** as a function of the ionic strength of the buffer that dissolved the protein. These plots show that the amount of protein that adsorbs to neutral SAMs does not depend on the ionic strength of the buffer; SAMs presenting charged groups, however, adsorbed substantially more protein at lower than at higher ionic strength. The mixed zwitterionic SAMs were inert to protein adsorption at all ionic strengths that we tested; single-component zwitterionic SAMs adsorbed protein at lower ionic strengths. The dipole moment of a single headgroup of **6** and **7** results from the unit charges at the N and S (or P and N) atoms being separated by a distance of approximately 4 Å. In the cases where these dipoles are oriented perpendicular to the surface or where the dipole vectors have a perpendicular component to the surface, there will be a net electric field associated with these dipoles at the surface. Such electric fields have been studied for several zwitterionic systems; an example is the zwitterionic micelles of 3-(*N*-hexadecyl-*N*,*N*-dimethylamino)-propanesulfonate (positive inner surface, negative outer).[46] These electric fields may be at least partially screened by the counterions at high ionic strength; at low ionic strength, they may promote adsorption of protein through electrostatic interactions. The observation that mixed zwitterionic SAMs are inert even at low ionic strength suggests that the charged groups are fairly evenly distributed throughout the surface and that they generate an interface that is overall electrically neutral.

pH and Ionic Strength. Figure 5 compares protein adsorption to SAMs from buffers with values of pH = 7.4 and 10.4 and values of ionic strength of 10 mM and 170 mM. At pH = 10.4, SAMs of **6** and SAMs of **7** adsorbed ≤6% of a monolayer of fibrinogen and <1% of a monolayer of lysozyme; the amount of protein adsorbed did not depend on ionic strength when the pH of the buffer was 10.4. At pH = 7.4, SAMs of **6** adsorbed protein only at low ionic strength (10 mM); SAMs of **7** adsorbed significant amounts of protein even at high ionic strength (170 mM). Regardless of pH or ionic strength, mixed zwitterionic SAMs formed from **4** and **5** were inert (data not shown in Figure 5).

These results do not point out a simple trend for protein adsorption to single-component zwitterionic SAMs as a function of pH. They are consistent with the suggestion that SAMs of **7** adsorb protein at pH 7.4 because these SAMs are not fully zwitterionic; at pH 10.4, where all of the phosphodiesters should be deprotonated (as suggested by contact angle), they are inert.

The puzzling behavior is that of SAMs of **6** as a function of pH with buffers of low ionic strength (10 mM) (Figure 5). We regard the increase in the amount of protein that adsorbed to SAMs of **6** at low ionic strength (pH = 7.4) to reflect the electrostatic dipoles at the buffer−SAM interface (negative pole at the interface); these dipoles could plausibly promote protein adsorption by electrostatic interaction. We did not expect this adsorption to disappear when the pH of the buffer with ionic strength of 10 mM was raised to 10.4. In this analysis, however, it is important to note that the charge of the protein becomes substantially less positive at higher pH, so it will tend to be less attracted to the surface electrostatically as the pH increases.

Survey of the Adsorption of Different Proteins to Zwitterionic SAMs. We measured the adsorption of eight different proteins to two zwitterionic SAMs to examine

(46) (a) Baptista, M. D.; Cuccovia, I.; Chaimovich, H.; Politi, M. J.; Reed, W. F. *J. Phys. Chem.* **1992**, *96*, 6442−6449. (b) Beschiaschvili, G.; Seelig, J. *Biochim. Biophys. Acta* **1991**, *1061*, 78−84. (c) Seelig, J.; Gally, H.-U.; Wohlgemuth, R. *Biochim. Biophys. Acta* **1977**, *467*, 109.

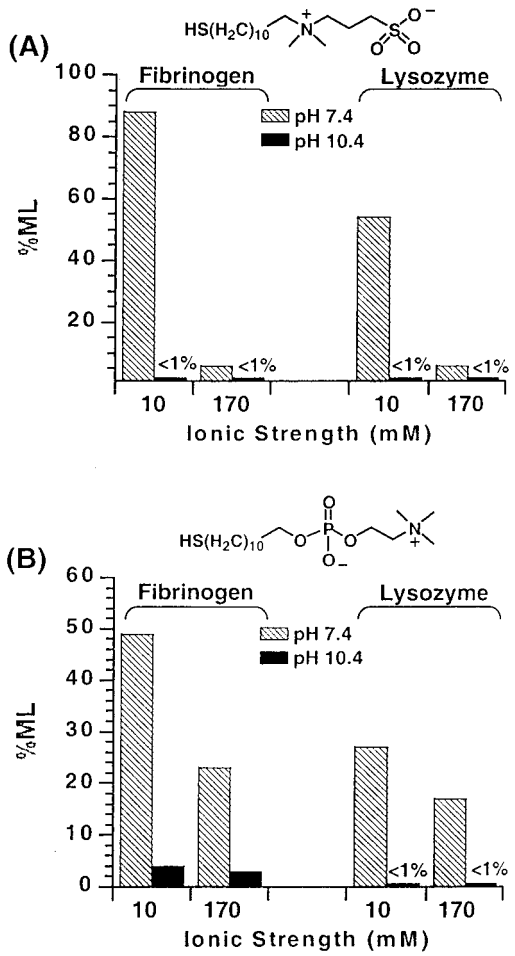

Figure 5. Plots of % Monolayer for the irreversible adsorption of fibrinogen and lysozyme to SAMs of **6** (A) and to SAMs of **7** (B). The plots compare the amount of protein adsorbed at two different pH values and at two different ionic strengths.

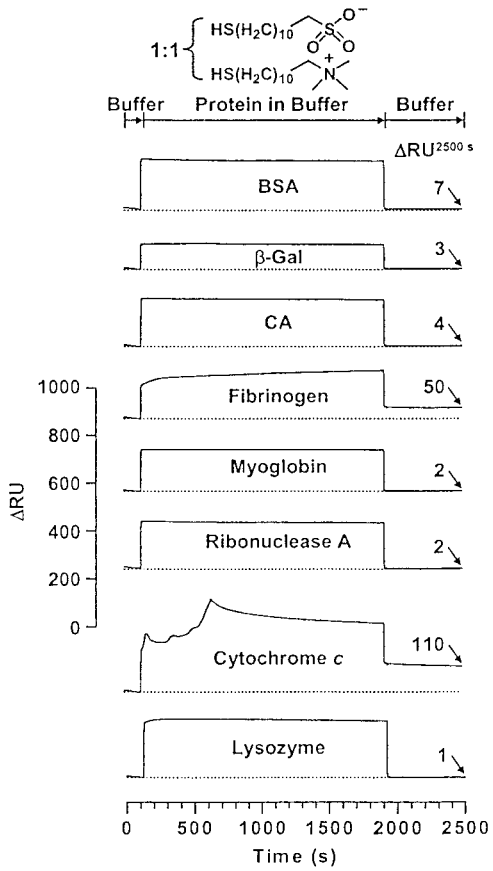

Figure 6. Plots of ΔRU as a function of time for the adsorption of a panel of eight proteins to SAMs formed from a 1:1 mixture of **4** and **5**. The proteins that were present in each experiment are indicated on the plot. The region of time during which protein was present in the buffer is indicated above the plot. The experimental conditions were the same as those described in the Figure 2 caption.

their versatility as inert surfaces (Figures 6 and 7 and Table 3). We selected the mixed zwitterionic SAMs and the single-component zwitterionic SAMs composed of **6** for further testing because they adsorbed the least amounts of protein under conditions that are relevant to biochemistry (pH 7.4, $\mu = 170$ mM). The physical properties of the proteins in this panel span a wide range: the values of their pI range from 4.8 to 10.9 and their molecular weights range from 12.4 to 468 kD. All of the proteins are globular with a single subunit, except for β-galactosidase, which has four subunits. Despite this range of characteristics, only cytochrome *c* adsorbed more than 5% of a monolayer to either zwitterionic SAM. The sensorgram of cytochrome *c* showed an irregular response during the first 10 min that the solution of protein flowed over the surface of the mixed zwitterionic SAM. We do not understand the origin of these changes and have not studied them in enough detail to speculate. In general, these results indicate that the ability of zwitterionic SAMs to form inert surfaces does not depend strongly on the physical characteristics of the proteins in solution above the surface.

Conclusions

This paper shows that inert surfaces, in the form of SAMs on gold, can be fabricated from thiols that terminate in charged groups. Single-component SAMs with single charges (all positive or all negative) adsorb nearly full monolayers of fibrinogen and lysozyme. SAMs formed from a 1:1 mixture of thiols terminated in a negatively charged group and in a positively charged group adsorb <1% of a monolayer of protein. Single-component SAMs formed from thiols terminating in groups having both a positively charged moiety and a negatively charged moiety were also capable of resisting the adsorption of proteins. These SAMs are comparable to the best known systems for resisting the nonspecific adsorption of protein from aqueous buffer.[1] The ability of zwitterionic surfaces to resist protein adsorption supports the notion that one of the essential characteristics in the design of an inert surface is that it be electrically neutral. The surfaces that we have studied also demonstrate that conformational flexibility (which is present in the $(EG)_n$ SAMs but is probably less in the zwitterionic SAMs) is not a requirement of functional groups that resist protein adsorption. One of the advantages of inert surfaces based on charged groups over those based on EG is that they are probably more stable to oxidation. Another advantage is their sensitivity to pH and ionic strength. These properties may provide a strategy to use experimental conditions to control protein adsorption: under certain conditions, proteins adhere to the surface, and under others they do not.

Experimental Section

Materials. All chemicals used were reagent grade unless stated otherwise. Fibrinogen (from bovine plasma, Sigma no. F8630), lysozyme (egg white, E.C. 3.2.1.17, Sigma no. L6876), cytochrome *c* (horse heart, Sigma no. C7752), β-galactosidase

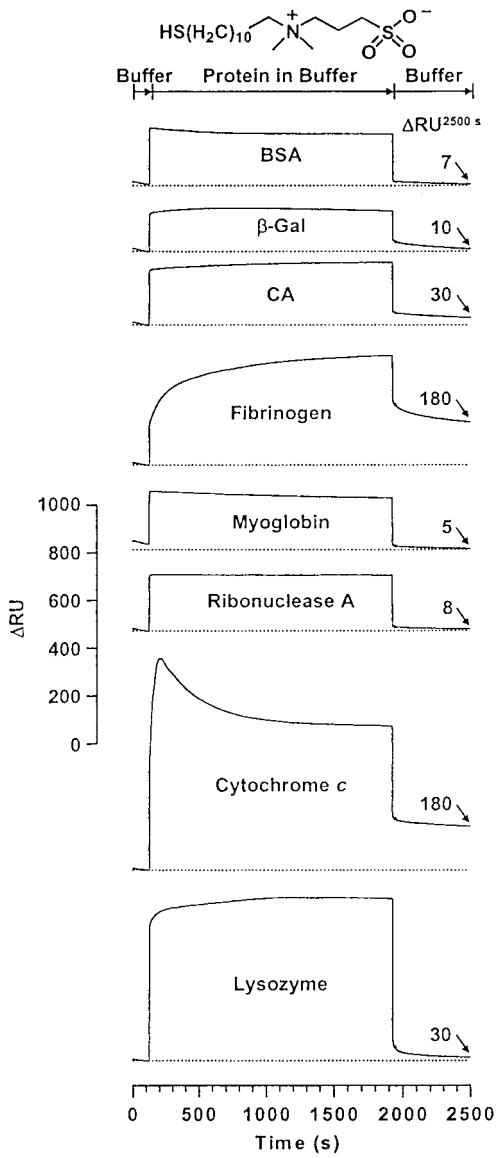

Figure 7. Plots of ΔRU as a function of time for the adsorption of a panel of eight proteins to SAMs formed from **6**. The proteins that were present in each experiment are indicated on the plot. The interval of time during which protein was present in the buffer is indicated above the plot. To allow comparison, the *y*-scale is the same as that in Figure 6. The experimental conditions were the same as those described in the caption of Figure 2.

(β-Gal) (grade VI, *Escherichia coli*, E.C. 3.2.1.23, Sigma no. G6008), carbonic anhydrase II (CA) (bovine erythrocytes, E.C. 4.2.1.1, Sigma no. C3934), myoglobin (horse heart, Sigma no. M1882), ribonuclease A (RNAse A) (bovine pancreas, type III-A, Sigma no. R5125), and sodium dodecyl sulfate were purchased from Sigma (St. Louis, MO). Trimethylamine, 1,3-propanesultone, dimethylamine (1 M in THF), thioacetic acid, 2-chloro-[1,3,2]-dioxaphospholane 2-oxide, AIBN, 4,4′-azobis(4-cyanopentanoic acid) (ABCN) were purchased from Aldrich (Milwaukee, WI) and used as received. 1-Undec-10-en-1-ol and 11-bromo-undec-1-ene were purchased from Pfatz & Bauer (Watersburg, CT). Anhydrous THF, anhydrous *N*,*N*-dimethyl formamide (DMF), and triethylamine were purchased from EM Science (Gibbstown, NJ). Absolute ethanol was purchased from Pharmco Products (Brookfield, CT). Alkanethiol [$HS(CH_2)_{11}N(CH_3)_3$]Cl was synthesized as previously described.[38] The ^{1}H NMR spectra were recorded at 400 MHz on a Bruker spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent ($CHCl_3$ = 7.26 ppm).

Table 3. Survey of the Adsorption of Different Proteins to Zwitterionic SAMs

		ΔRU$^{irreversible\ b}$ for SAM formed from		% ML^d for SAM formed from	
entry	protein[a]	**4** + **5** (1:1)[c]	**6**[c]	**4** + **5** (1:1)[c]	**6**[c]
1	BSA	7	7	<1	<1
2	β-Gal	3	10	<1	<1
3	CA	4	30	<1	2
4	fibrinogen	50	180	1	4
5	myoglobin	2	5	<1	<1
6	RNAse A	2	8	<1	<1
7	cytochrome *c*	110	180	10	16
8	lysozyme	1	30	<1	1

[a] The molecular weight, pI, and number of subunits for each protein are listed in Table 1. [b] The values of ΔRU for irreversible adsorption are taken from sensorgrams after the solution of protein (1 mg/mL) was allowed to flow over the SAM for 30 min and buffer was allowed to flow over the SAM and adsorbed protein for 10 min. [c] The structures of these thiols are shown in Chart 1. [d] % ML, the percent of a full monolayer of protein adsorbed to the surface, is given by eq 1.

Buffers and Solutions of Proteins. We used buffers with the following compositions: (i) 10 mM sodium phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.4, ionic strength (μ) = 170 mM; (ii) 4.4 mM potassium phosphate, pH 7.4, μ = 10 mM; (iii) 4.4 mM potassium phosphate, 90 mM NaCl, pH 7.4, μ = 100 mM; (iv) 4.4 mM potassium phosphate, 190 mM NaCl, pH 7.4, μ = 200 mM; 4.4 mM potassium phosphate, 290 mM NaCl, pH 7.4, μ = 300 mM.[47] Buffers were prepared in distilled, deionized water and filtered through 0.22 μM filters prior to use. Solutions of protein were prepared by dissolving solid protein (10 mg) in the appropriate buffer (10 mL) at room temperature. After the protein dissolved, the solutions were filtered through 0.22 μM filters; the first 2 mL of the filtrate was discarded and the remaining 8 mL of filtrate was used for experiments.

Surface Plasmon Resonance Spectroscopy. SPR was performed on a Biacore 1000 instrument (Biacore). The substrate containing the SAM to be analyzed was mounted in a SPR cartridge as previously described.[33] Our SPR protocol for measuring the adsorption of protein to SAMs consisted of (i) flowing a solution of sodium dodecyl sulfate (40 mM in PBS) over the SAM surface for 30 min followed by rinsing the surface with a solution of PBS buffer for 10 min and (ii) flowing PBS buffer for 2 min, then substituting the flow with a solution of protein (1 mg/mL in PBS) for 30 min, and finally injecting PBS buffer for an additional 10 min. The flow rate used for all experiments was 10 μL/min. Table 4 summarizes the % ML of fibrinogen and lysozyme that adsorbed to SAMs examined in this study under all experimental conditions.

Ellipsometry. Ellipsometry was done on a Rudolf Research Type 43603-200E ellipsometer using a wavelength of 6328 Å (He−Ne laser) and an incident angle of 70°. Samples were washed with ethanol and blown dry with nitrogen before the measurements were taken. Three separate points were measured on each sample, and the readings were then averaged. Readings were taken on the clean gold, to establish the optical constant for the bare substrate, and after monolayer formation. SAMs comprising **4** and **5** were prepared from solutions of the appropriate thiol or mixture of thiol (2 mM SH) in deionized water; they were washed with nonionic surfactant (Triton X), deionized water, and ethanol and then blown dry with a stream of nitrogen prior to measuring their thickness. SAMs of **6** and **7** were prepared from solutions of thiol (2 mM) in ethanol; they were rinsed with ethanol and dried with nitrogen prior to measuring their thickness. The thicknesses were calculated by using a parallel, homogeneous three-layer model with an assumed refractive index of 1.45 for the monolayer.

Contact Angles. Contact angles were measured under water-saturated cyclooctane[43] using a Ramé-Hart model 100 contact angle goniometer. We measured advancing contact angles of a drop of water delivered to the surface using a Matrix Technologies Microelectropipette. The values reported are the average of three

(47) Collinsen, M.; Bowden, E. F. *Langmuir* **1992**, *8*, 1247−1250.

Table 4. Summary of the Adsorption of Fibrinogen and Lysozyme to SAMs

entry	thiol(s) used to form SAM[a]	buffer[b] pH	buffer[b] ionic strength (mM)	% Monolayer of protein adsorbed[c] fibrinogen	% Monolayer of protein adsorbed[c] lysozyme	% of adsorbed protein removed by SDS[d] fibrinogen	% of adsorbed protein removed by SDS[d] lysozyme
1	**2**	7.4	170	<1	<1	nm[e]	nm[e]
2	**3**	7.4	170	<1	<1	nm[e]	nm[e]
3	**4**	7.4	170	80	40	90	97
4	**4**	7.0	10	120	98	nm[e]	nm[e]
5	**4**	10.4	10	8	25	nm[e]	87
6	**5**	7.4	170	70	44	69	67
7	**5**	7.0	10	290	nm[e]	nm[e]	nm[e]
8	**5**	10.4	10	170	78	79	57
9	**4** + **5** (1:1)	7.4	170	1	<1	100[f]	100[f]
10	**4** + **5** (1:1)	7.0	10	3	<1	100	100[f]
11	**4** + **5** (1:1)	10.4	10	<1	<1	100[f]	100[f]
12	6	7.4	170	6	6	88	78
13	**6**	10.4	170	<1	<1	100[f]	100[f]
14	**6**	7.0	10	88	54	nm[e]	nm[e]
15	**6**	7.0	100	27	11	nm[e]	nm[e]
16	**6**	7.0	200	3	7	nm[e]	nm[e]
17	**6**	7.0	300	<1	1	nm[e]	nm[e]
18	**6**	10.4	10	<1	<1	100[f]	100[f]
19	**7**	7.4	170	23	17	74	76
20	**7**	10.4	170	3	<1	100	100[f]
21	**7**	7.0	10	49	27	nm[e]	nm[e]
22	**7**	7.0	100	42	19	nm[e]	nm[e]
23	**7**	7.0	200	27	16	nm[e]	nm[e]
24	**7**	7.0	300	22	8	nm[e]	nm[e]
25	**7**	10.4	10	4	<1	100	100[f]

[a] Structures of thiols are shown schematically in Chart 1. [b] See Experimental Section for composition of buffers. [c] Percentage of a full monolayer of protein that adsorbed irreversibly to the SAM after flowing a solution of protein (1 mg/mL, 10 μL/min) over the surface for 30 min followed by flowing buffer over the surface for 12 min. The values were calculated with eq 1. The uncertainty in % Monolayer adsorbed for both fibrinogen and lysozyme is $\leq \pm 4\%$. [d] The % adsorbed protein removed by SDS was calculated by comparing the amount of protein that remained on a SAM after flowing SDS in buffer over the surface for 10 min and flowing buffer over the surface for 2 min to the amount of protein that had adsorbed irreversibly to the SAM; a value of 100% in this column means that all of the adsorbed protein was removed from the SAM by treatment with SDS in buffer. [e] This value was not measured (nm). [f] The uncertainty in % Monolayer adsorbed is a result of the inhomogeneity of the surface rather than the SPR response. In the case when less than 1% ML adsorbed was measured, the uncertainty is actually much less than $\pm 4\%$. Because we observed reproducible ΔRUs and flat baselines in the SPR sensorgrams, we conclude that the % of adsorbed proteins removed by SDS is close to 100%.

measurements taken on each of three different drops in contact with three different locations of the SAM surface.

Synthesis. *N,N-Dimethyl-undec-10-enyl-amine.* 11-Bromo-undec-1-ene (10 mL, 43 mmol) was added to a solution of dimethylamine in THF (100 mL, 1 M) and the reaction mixture was stirred for 8 h at ambient temperature. The reaction mixture was concentrated in vacuo, NaOH (200 mL, 1 M) was added, and the slurry was extracted with methylene chloride (2 × 200 mL). The combined organics were dried with anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford *N,N*-dimethyl-undec-10-enyl-amine as a colorless oil (8.8 g, 99% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.25−1.43 (m, 14H), 1.99 (m, 2H), 2.17 (s, 6H), 2.20 (m, 2H), 4.90 (m, 2H), 5.77 (m, 1H). HRMS−FAB: *m/z* 198.2213 ([M + H]$^+$; calcd for $C_{13}H_{28}N$, 198.2222).

3-(N,N-Dimethyl-undec-10-enyl-amino)-propane-1-sulfonic Acid. *N,N*-Dimethyl(11-mercaptoundecyl)amine (3.0 g, 15 mmol) was added to a solution of 1,3-propanesultone (1.6 mL, 18 mmol) in anhydrous acetone (100 mL), and the reaction mixture was stirred for 24 h at ambient temperature. The reaction mixture was filtered, and the resulting solid was washed with acetone (2 × 10 mL) and dried in vacuo to afford 3-(*N,N*-dimethyl-undec-10-enyl-amino)-propane-1-sulfonic acid as a white solid (4.1 g, 79% yield). ^{1}H NMR ($CDCl_3$/MeOD 75:25, 400 MHz): δ 1.20−1.48 (m, 14H), 2.01 (m, 2H), 2.11 (m, 2H), 2.77 (t, J = 7.2 Hz, 2H), 2.92 (s, 6H), 3.12 (m, 2H), 3.35 (m, 2H), 4.95 (m, 2H), 5.87 (m, 1H). HRMS−FAB: *m/z* 342.2087 ([M + Na]$^+$; calcd for $C_{16}H_{33}NO_3SNa$, 342.2079).

$CH_3C(O)S(CH_2)_{11}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$. Nitrogen gas was bubbled through a solution of $CH_2CH(CH_2)_9N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$ (2.7 g, 7.9 mmol) and $CH_3C(O)SH$ (5.0 mL) in methanol (10 mL), ABCN (50 mg, 0.2 mmol) was added, and the solution was irradiated in a photoreactor for 16 h at ambient temperature. Anhydrous acetone (200 mL) was added, and the resulting precipitate was filtered and dried in vacuo. The product was recrystallized from acetone/methanol to afford $CH_3C(O)S(CH_2)_{11}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$ as a white solid (2.1 g, 67%). ^{1}H NMR (D_2O, 400 MHz): δ 1.16−1.23 (s, 14H), 1.42 (m, 2H), 1.63 (m, 2H), 2.08 (m, 2H), 2.22 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 2.83 (t, J = 7.2 Hz, 2H), 2.97 (s, 6H), 3.19 (m, 2H), 3.33 (m, 2H). HRMS−FAB: *m/z* 418.2044 ([M + Na]$^+$; calcd for $C_{18}H_{37}NO_4SNa$, 418.2062).

3-[(11-Mercapto-undecyl)-N,N-dimethyl-amino]-propane-1-sulfonic Acid, $HS(CH_2)_{11}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$. Nitrogen gas was bubbled through a solution of $CH_3C(O)S(CH_2)_{11}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$ (1.0 g, 2.5 mmol) in H_2O (10 mL) for 10 min, NaOH (5 mL, 1.0 M) was added, and the solution was stirred for 2 h. The solution was acidified with HCl (6 mL, 1 M), filtered into a scintillation vial, frozen, and lyophilized to afford $HS(CH_2)_{11}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$ as a white solid (2.5 g, 99%). ^{1}H NMR (D_2O, 400 MHz): δ 1.15−1.22 (s, 14H), 1.43 (m, 2H), 1.61 (m, 2H), 2.07 (m, 2H), 2.40 (t, J = 7.2 Hz, 2H), 2.83 (t, J = 7.2 Hz, 2H), 2.94 (s, 6H), 3.17 (m, 2H), 3.32 (m, 2H). HRMS−FAB: *m/z* 376.1952 ([M + Na]$^+$; calcd for $C_{16}H_{35}NO_3SNa$, 376.1956).

Phosphoric Acid 2-Trimethylamino-ethyl Ester Undec-10-enyl Ester. 2-Chloro-[1,3,2]dioxaphospholane 2-oxide (2.66 mL, 29 mmol) was added dropwise over 10 min to a solution of triethylamine (4.1 mL, 29 mmol) and undec-10-en-1-ol (5.0 g, 29 mmol) in anhydrous THF (100 mL) at −15 °C. The reaction mixture was allowed to warm to ambient temperature over 2 h. The reaction mixture was cooled to −15 °C and filtered. The filtrate was concentrated in vacuo, cold anhydrous diethyl ether (100 mL) was added, and the mixture was again filtered to remove the remaining triethylammonium chloride salts. The filtrate was concentrated in vacuo to afford 2-undec-10-enyloxy-[1,3,2]dioxaphospholane 2-oxide as an oil (5.8 g, 72% yield). 2-Undec-10-enyloxy-[1,3,2]dioxaphospholane 2-oxide was used without further purification. A solution of 2-undec-10-enyloxy-[1,3,2]dioxaphospholane 2-oxide (2.8 g, 10.8 mmol) and trimethylamine (1 mL, 11 mmol) in anhydrous methylene chloride at −15 °C was sealed in a bomb, and the reaction was heated to 55 °C and stirred for 2 days.[40] The reaction mixture was cooled and filtered

to give 0.5 g of a white solid. The product was loaded onto a silica gel gravity column (20 g) and eluted with CH_2Cl_2/MeOH/NH_4OH 80:20:1 to 65:30:5 to afford phosphoric acid 2-trimethylaminoethyl ester undec-10-enyl ester as a white solid (0.35 g, 10%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.25−1.35 (m, 12H), 1.55 (m, 2H), 2.03 (m, 2H), 3.39 (s, 9H), 3.77 (m, 4H), 4.26 (m, 2H), 4.95 (m, 2H), 5.79 (m, 1H). HRMS−FAB: *m/z* 336.2323 ($[M + H]^+$; calcd for $C_{16}H_{35}NO_4P$, 336.2304).

$CH_3C(O)S(CH_2)_{11}OP(O^-)(O)OCH_2CH_2N^+(CH_3)_3$. Nitrogen gas was bubbled through a solution of $CH_2CH(CH_2)_9OP(O)(O^-)OCH_2CH_2N^+(CH_3)_3$ (0.35 g, 1.0 mmol) and $CH_3C(O)SH$ (1.0 mL) in methanol/H_2O (9:1, 10 mL), a catalytic amount of AIBN was added, and the solution was irradiated in a photoreactor for 16 h at ambient temperature. The solution was concentrated in vacuo, and the residual oil was loaded onto a reverse phase silica gel gravity column (10 g) and eluted with MeOH/H_2O 80:20 (v/v) to afford $CH_2CH(CH_2)_9OP(O^-)(O)OCH_2CH_2N^+(CH_3)_3$ as a colorless glassy solid (0.32 g, 80%). ^{1}H NMR ($CDCl_3$/MeOD 95:5, 400 MHz): δ 1.12−1.18 (m, 14H), 1.44 (m, 4H), 2.19 (s, 3H), 2.72 (t, J = 7.3 Hz, 2H), 3.15 (s, 9H), 3.56 (m, 2H), 3.68 (q, J = 6.6 Hz, 2H), 4.11 (m, 2H). HRMS−FAB: *m/z* 434.2116 ($[M + Na]^+$; calcd for $C_{18}H_{38}NO_5PSNa$, 434.2106).

$HS(CH_2)_{11}OP(O^-)(O)OCH_2CH_2N^+(CH_3)_3$. Nitrogen gas was bubbled through a solution of $CH_3C(O)S(CH_2)_{11}OP(O)(O^-)OCH_2CH_2N^+(CH_3)_3$ (0.23 g, 0.56 mmol) in H_2O (3 mL) for 10 min, NaOH (1 mL, 1.0 M) was added, and the solution was stirred for 4 h. The solution was acidified with HCl (1.2 mL, 1 M) and then frozen and lyophilized to afford $HS(CH_2)_{11}OP(O^-)(O)OCH_2CH_2N^+(CH_3)_3$ as a white solid (0.20 g, 96%). ^{1}H NMR (MeOD, 400 MHz): δ 1.31−1.39 (m, 14H), 1.56 (m, 2H), 1.69 (m, 2H), 2.48 (t, J = 7.2 Hz, 1H), 3.24 (s, 9H), 3.73 (m, 4H), 4.04 (q, J = 6.6 Hz, 2H), 4.45 (m, 2H). HRMS−FAB: *m/z* 370.2173 ($[M + H]^+$; calcd for $C_{16}H_{37}NO_4PS$, 370.2181).

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Supporting Information Available: Three figures showing SPR sensorgrams for fibrinogen and lysozyme adsorbing to different SAMs under different buffer conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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