Surveying for Surfaces that Resist the Adsorption of Proteins

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This contribution describes an experimentally straightforward procedure for preparing and screening surfaces for their ability to resist the adsorption of proteins from solution. For brevity, we call surfaces "protein resistant" when they are resistant to the adsorption of proteins from solution. We have used this procedure to identify several functional groups that had not previously been recognized as protein resistant. This work both identifies a number of functional groups that will be useful in designing resistance to the adsorption of biomolecules into devices used in sensing and in cell biology,[1−3] and contributes to an understanding of the mechanism of action of protein resistant surfaces by correlating this property with molecular-scale structure.[4−6]

We combined self-assembled monolayers (SAMs)[7,8] and surface plasmon resonance (SPR) spectroscopy[9] into a system that enabled us to screen a number of functional groups rapidly for their ability to resist the adsorption of proteins. The surfaces were prepared by the "anhydride method" (Figure 1).[10,11] This reaction generates a "mixed" SAM that comprises an ∼1:1 mixture of −CONRR′ and CO_2H/CO_2^- groups.[10,12] (We have not defined the state of the ionization of the CO_2H groups in these SAMs.) The ease with which this class of mixed SAMs can be prepared by the anhydride method (relative to the synthesis of the functionalized alkanethiols $HS(CH_2)_nR'$ normally used for the preparation of single-component SAMs) makes this route efficient for exploratory and screening work.[11,13]

We have examined the adsorption of two proteins to these surfaces: fibrinogen, a large (340 kD) blood plasma protein that adsorbs strongly to hydrophobic surfaces, and lysozyme, a small protein (14 kD, pI = 12) that is positively charged under the conditions of our experiment (phosphate buffered saline, PBS, pH 7.4). Fibrinogen is used as a model for "sticky" serum proteins;[11,14,15] lysozyme is often used in model studies of electrostatic adsorption of proteins to surfaces.[16,17] Since lysozyme has a substantial net positive charge ($Z_p = +7.5$ at pH 7.4, 100 mM KCl),[18] it allowed us to examine attractive electrostatic interactions with CO_2^- groups on the surface.

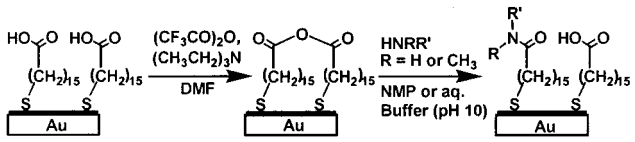

Figure 1. Schematic illustration of the synthesis of mixed SAMs that present a 1:1 mixture of −CONRR′ and CO_2H/CO_2^- groups using the anhydride method.[13] This scheme is idealized and not drawn to scale.

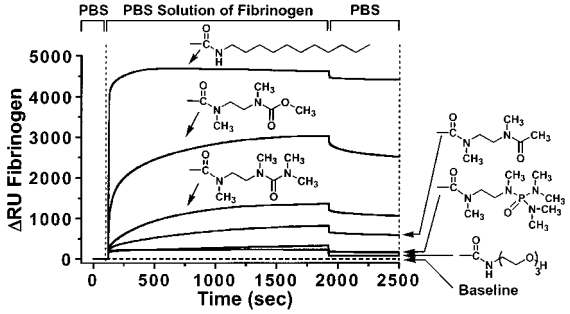

Figure 2. Representative SPR data for the adsorption of fibrinogen to mixed SAMs that were prepared by the anhydride method (Figure 1). ΔRU was determined by subtracting the value of RU measured at the vertical dashed line prior to the injection of protein from the value of RU measured 10 min after the completion of the protein injection.

We have prepared more than 50 surfaces, each presenting a different functional group, using the anhydride procedure, and surveyed them for protein resistance.[19] Table 1 summarizes selected results from this survey. The amount of protein adsorbed (ΔRU = change in response units) as measured by SPR was determined by subtracting the value of RU prior to the injection of protein from the value of RU measured 10 min after the completion of the protein injection; for clarity, these points are each labeled with a vertical dashed line in Figure 2. The value of ΔRU was used to calculate the percentage of a monolayer (%Monolayer) of that protein using eq 1.[11,20] We define "%ML" according to eq 1 strictly to simplify the comparison between surfaces.

$$\%\ \mathrm{ML} = \frac{\Delta \mathrm{RU}_{\text{mixed SAM (–CONRR')}}}{\Delta \mathrm{RU}_{\text{mixed SAM (–CON(CH}_2)_{10}\mathrm{CH}_3)}} \times 100 \quad (1)$$

The functional groups used in this study resulted in mixed SAMs that ranged substantially in their tendency to adsorb protein (Figure 2). SAMs that present oligo(ethylene glycol)$_n$ ($n = 3-6$) groups are currently the most protein-resistant surfaces available.[6,14,21] They are the standard against which we have judged new protein-resistant surfaces. We compare the adsorption of proteins to our mixed SAMs to that of a mixed SAM that presents a 1:1 mixture of tri(ethylene glycol) groups (−COHN(CH_2-

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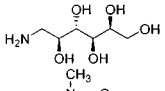
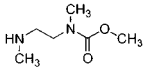
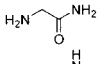
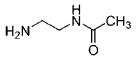
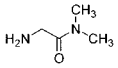
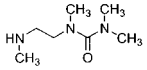
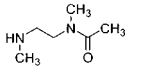
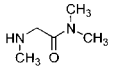
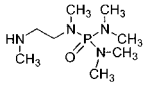
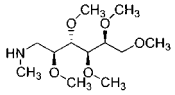
Table 1. Amount of Fibrinogen (Fib) and Lysozyme (Lys) Adsorbed to Mixed SAMS

Entry No.	HNRR′	% ML Fib[a, b, c]	% ML Lys[a, b, c]	θ_a[d]
1	$H_2N(CH_2)_{11}CH_3$	100	100	163[e]
2		68	20	37
3		58	43	75
4		58	30	39
5		40	5	49
6		33	15	61
7		25	11	62
8		12	4	53
9		9	2	65
10		4	< 1	66
11		3	6	81
12	$H_2N(CH_2CH_2O)_3H$	2	1	54

[a] %ML = "%Monolayer" is defined by eq 1. The experiments involved flowing a 1 mg/mL solution of protein over the surface for 30 min at 10 μL/s. [b] The uncertainty in %ML for both fibrinogen and lysozyme is $\leq \pm 4\%$ absolute value; for values of %ML less than 12%, the uncertainty is $\leq \pm 1\%$ absolute value. [c] Arrows have been added to identify comparable functional groups. [d] The advancing contact angle, θ_a, was measured using droplets of water, with the surface immersed in cyclooctane. [e] This value refers to the sessile contact angle.

$CH_2O)_3H$) and $-CO_2H/CO_2^-$ groups (entry 12, Table 1). On the basis of this comparison, we have identified four functional groups that show useful resistance to the adsorption of proteins when presented on SAMs mixed ~1:1 with $-CO_2H/CO_2^-$ groups: entries 8, 9, 10, and 11 (Table 1). We also confirmed that *homogeneous SAMs* made using alkanethiols terminated with each of these four groups resisted the adsorption of proteins more than mixed SAMs made by reaction of the amine derivatives of the four functional groups with anhydride SAMs.[19] These functional groups share four molecular characteristics: (i) they contain polar functional groups, (ii) they incorporate hydrogen bond accepting groups, (iii) they do *not* contain hydrogen bond donating groups, and (iv) they have no net charge. The most protein-resistant surfaces were hydrophilic; there is, however, no clear correlation between %ML and the advancing contact angle of water (under cyclooctane)[22] on the SAMs (Table 1).

Elimination of hydrogen bond donor groups appears to be a key structural element in protein-resistant surfaces. Smaller amounts of proteins adsorbed to surfaces that presented compounds with NCH_3 and OCH_3 groups than to surfaces that presented their more polar analogues with NH and OH groups (Table 2). SAMs that presented OCH_3- and OH-terminated oligo(ethylene glycol) groups adsorbed indistinguishable quantities of the two test proteins, but the quantities were too small to compare.[11]

Table 2. Comparison of Protein Adsorption to Mixed SAMs that Present Unmethylated and Methylated Functional Groups, CONRR′

	% ML (R = H)[a]/% ML (R = CH_3)	
−CONRR′	fibrinogen	lysozyme
$-CONRCH_2(CH(OR))_4CH_2OR$	23	3
$-CONRCH_2CON(R)_2$	6	15
$-CONRCH_2CON(CH_3)_2$	4	6
$-CONRCH_2CH_2NRCOCH_3$	3	1.5
$-CONH(CH_2CH_2O)_3R$[b]	1	1

[a] These terms are defined by eq 1. [b] Reference 11.

The presence of the CO_2H/CO_2^- groups in these mixed SAMs complicates the analysis in two ways: first, the carboxyl group (especially as carboxylate anion) is, in principle, able to interact with proteins; second, this group is smaller than most of the groups ($-COONR\mathbf{R}'$) we examined, and its presence as a component of the monolayer influences the packing of the amide groups. For one functional group, $-CONH(CH_2CH_2O)_nR$ ($n = 3$ or 6; R = H or CH_3), we have compared protein resistance of single-component SAMs to that of mixed SAMs containing CO_2H/CO_2^- groups[11] or CH_3 groups,[14] and concluded that levels of protein resistance in all three cases were comparable. We do not know if this conclusion applies to the other groups that we examined.

The most important conclusion from the present work is that a substantial number of different types of organic functional groups can form the basis for SAMs that prevent the adsorption of proteins. This conclusion is important for two reasons. (i) It rests on the demonstration of alternatives to derivatives of oligo(ethylene glycol) as the basis for protein resistant surfaces. Oligo(ethylene glycol) derivatives are effective—in fact, still the most effective of the group examined—but they tend to autoxidize.[21,23,24] Table 1 identifies several useful alternatives to oligo(ethylene glycol): one, $HN(CH_3)CH_2CON(CH_3)_2$, is available commercially, and others, $HN(CH_3)CH_2(CH(OCH_3))_4CH_2OCH_3$, $HN(CH_3)CH_2CH_2N(CH_3)PO(N(CH_3)_2)_2$, and $HN(CH_3)CH_2CH_2N(CH_3)COCH_3$, are straightforward to synthesize. (ii) This conclusion also clarifies the mechanism(s) that underlie protein resistance. It is clear that the theory of DeGennes/Andrade[4,5] (developed to describe the behavior of poly(ethylene glycol) at surfaces) does not describe protein adsorption on the SAMs listed in Table 1. Simple physical parameters—polarity, wettability, conformational mobility—also do not correlate with the ability of surfaces to resist the adsorption of protein. Grunze, we, and Laibinis have suggested that the formation of structured or tightly bound water at the interface may be important.[6] The data in Table 1 are compatible with this hypothesis, but do not demand it.

The observation (Table 2) that the elimination of hydrogen bond donor moieties in the functional groups reduces (by factors of 3 to 24) the adsorption of fibrinogen to these SAMs was the most surprising result from this work.

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Supporting Information Available: SPR protocol, synthetic procedures for some of the compounds listed in Table 1, and reaction conditions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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