Abstract: This paper reports a study of the adsorption of four proteins—fibrinogen, lysozyme, pyruvate kinase, and RNase A—to self-assembled monolayers (SAMs) on gold. The SAMs examined were derived from thiols of the structure RSH(CH \textsubscript{2}) \textsubscript{n}O, where R was CH \textsubscript{3}, CH \textsubscript{2}OH, and oligo(ethylene oxide). Monolayers that contained a sufficiently large mole fraction of alkanethiolate groups terminated in oligo(ethylene oxide) chains resisted the kinetically irreversible, nonspecific adsorption of all four proteins. Longer chains of oligo(ethylene oxide) were resistant at lower mole fractions in the monolayer. Resistance to the adsorption of proteins increased with the length of the oligo(ethylene oxide) chain: the smallest mole fraction of chains that prevented adsorption was proportional to \( n^{-0.4} \), where \( n \) represents the number of ethylene oxide units per chain. Termination of the oligo(ethylene oxide) chains with a methoxy group instead of a hydroxyl group had little or no effect on the amount of protein adsorbed. The amount of pyruvate kinase that adsorbed to mixed SAMs containing hexa(ethylene oxide)-terminated chains depended upon the temperature. When the mole fraction of oligo(ethylene oxide) groups in the monolayer was below the level needed to prevent adsorption, more pyruvate kinase adsorbed to the monolayer at 37 °C than at 25 °C. No difference was observed between adsorption at 25 and 4 °C.

Introduction

Materials presenting oligomers or polymers of ethylene oxide \([-\text{CH}_2\text{CH}_2\text{O}]_n\), abbreviated as EO, on their surfaces are promising candidates for use in applications requiring contact with proteins, cells, and other biological systems. \(^5-8\) We have prepared monoo{mercaptoundecyl} ethers of a number of short oligomers of EO and used them to form self-assembled monolayers (SAMs) on gold. These SAMs present EO groups in controlled numbers at the solid–water interface. \(^9\) Here we describe the influence of the length and number of EO chains upon the adsorption of proteins on these SAMs.

When hydrophobic colloids are treated with aqueous solutions containing poly(ethylene oxide) (PEO), the polymer adsorbs spontaneously to the colloid–solution interface. \(^10\) Once the colloid surface has adsorbed significant amounts of PEO, it is stabilized against flocculation. \(^10\)–\(^13\) Similarly, block copolymers formed from PEO and a hydrophobic polymer adsorb to the surfaces of hydrophobic particles and stabilize the dispersion against flocculation. \(^10\) The best stabilization to date— as measured by surface force–balance techniques— has been achieved with chains that are tightly anchored at one end to the hydrophobic surface. \(^13\) The anchoring "head group" must interact more strongly with the surface than does PEO. The needed selectivity has been achieved by physisorption \(^12\)–\(^14\) (as in the case of monoalkylated PEO surfactants, \( \text{CH}_3(\text{CH}_2)_n(\text{OCH}_2\text{CH}_2)_m\text{OH} \)), chemisorption \(^15\)–\(^16\) (e.g., amino-terminated PEO on mica), and covalent grafting. \(^4\)–\(^5\) The repulsive interactions between two surfaces bearing end-attached chains of PEO become significant at larger interparticle separations than those between two surfaces bearing randomly adsorbed PEO. \(^12\)–\(^13\)

Theoretical treatments of the stabilization of colloids by physi- and chemisorbed derivatives of PEO and other solvophilic polymers \(^10\)–\(^11\) are usually based on the concept of "steric repulsion". These theories derive stabilization energies from one or both of two sources. First, as two polymer-coated colloidal particles collide, the polymer layers are compressed. The polymer chains have access to fewer configurations, and the resulting loss in entropy disfavors compression. Second, the collision expels solvent from the polymer surface layer. When the free energy of the solvent is lower in the solvent-swollen polymer layer than in the bulk, the energy required to desolvate the polymer layer disfavors compression of that layer. If these two stabilizing effects are energetic enough, the hydrophobic colloidal particles do not meet and do not flocculate.

The adsorption of proteins to polymer surfaces can be treated as a problem of colloidal stability in which the surfaces of the polymers and the proteins play the role of the hydrophobic colloids. Thus, it comes as no surprise that hydrophobic polymers to which PEO is either adsorbed or chemically grafted are resistant to the adsorption of proteins. \(^17\)–\(^18\)

Recently, Jeon and Andrade proposed a quantitative model for protein adsorption at a hydrophobic polymer surface to which
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PEO chains are terminally attached.\(^{19,20}\) Their model pitted steric repulsion against both van der Waals attraction and the long-range hydrophobic attraction first reported by Pashley and co-workers.\(^{21,22}\) The long-range hydrophobic attraction decays exponentially with a characteristic length of 1.4 nm. They calculated the free energies of adsorption of uniformly hydrophobic proteins—modeled as spheres of different radii—onto a planar hydrophobic surface bearing terminally attached PEO chains 80–120 residues long. The polymer chains were examined in the brush structure, where the average distance \(d\) between the chains was greater than the diameter of the chains in the crystal (0.463 nm) and less than the Flory radius \(R_f\) of the polymer.

Jean and Andrade drew several conclusions: (i) that a weak, long-range hydrophobic attraction between PEO and protein competes with repulsion and determines the resistance of the surface to the adsorption of protein, (ii) that the concentration of PEO chains in the interface required to resist the adsorption of protein decreases as the size of the protein increases, and (iii) that surfaces comprising densely packed, nearly crystalline chains of protein might not resist the adsorption of protein.

Despite the growing body of theoretical and experimental work on these materials, the length and number of EO chains at the solid–solution interface that are required for resistance to protein adsorption have remained poorly defined. We sought a convenient experimental means to test the effects of grafting density and polymer length upon the resistance properties of the surface. Recently, we reported that SAMs containing appropriate concentrations of hexa(ethylene oxide)–terminated chains [S(CH\(_2\)]\(_{11}\)(OCH\(_2\)CH\(_2\))\(_n\)OH, abbreviated as SC\(_11\)E\(_n\)OH, \(1e\)] resist the adsorption of proteins.\(^{23}\) Figure 1 is a schematic illustration of a SAM comprising a mixture of S(CH\(_2\)]\(_{11}\)(OCH\(_2\)CH\(_2\))\(_n\)OH (abbreviated as SC\(_11\)OH) and SC\(_1\)E\(_n\)OH. Water is a good solvent for PEO, and in contact with water, the EO chains in the SAM are self-avoiding and tend to form gauche bonds.\(^{24}\) The monolayer–solution interface of these SAMs corresponds to the interfacial structures of hydrophobic polymers bearing end-grafted PEO chains.

\[
\begin{align*}
\text{HS(CH}_2\text{)}_{11}\text{(OCH}_2\text{CH}_2\text{)}_{n}\text{OR} & \quad \text{HS(CH}_2\text{)}_{10}\text{CH}_3 \\
1a: n = 0, R = H & \quad 2 \\
1b: n = 1, R = H & \\
1c: n = 2, R = H & \\
1d: n = 4, R = H & \\
1e: n = 6, R = H & \\
1f: n = 6, R = \text{CH}_3 & \\
1g: n = 17, R = \text{CH}_3 & \\
\end{align*}
\]

SAMs make particularly good model systems for the study of protein–surface interactions. They are easily prepared.\(^{25}\) Their topology is controlled by the topology of the underlying gold substrate, which is nearly constant from sample to sample. SAMs of \(\omega\)-substituted alkanethiols [S(CH\(_2\)]\(_n\)R, \(n \geq 10, R = \text{a functional group with a cross section no larger than that of a CH}_2 \text{ group}\)] comprise densely packed, pseudocrystalline arrays of predominantly trans-extended chains oriented with their sulfur termini at the gold–SAM interface.\(^{26}\) Thus, the properties of the SAM–solution interface can be controlled by varying \(R\) during synthesis of thiols. Thiols 1a, 1d, 1e, and 1g were available from previous studies.\(^{9}\) Thiols 1b, 1c, 1f, and 1g were prepared according to Scheme I. Details are provided in the supplementary material.

Preparation and Characterization of SAMs. Polycrystalline gold films (200-nm thick) were deposited by evaporation of gold dots.
onto chromium-primed silicon wafers.31 These films were immersed for 12 h to 1 week in ethanol solutions containing mixtures of 1 and 2 in different relative concentrations. The total concentration of thiol in each solution was 0.25 mM.32 Each SAM was characterized by X-ray photoelectron spectroscopy (XPS) and by measurement of its maximum advancing and minimum receding contact angles with water33 (\( \theta_1(\text{H}_2\text{O}) \) and \( \theta_2(\text{H}_2\text{O}) \), respectively). The average thicknesses of mixed SAMs formed from 1g and 2 were measured by ellipsometry.

Since 2 was one of the two components in every mixed SAM described here, we shall use the shorthand phrase "mixed SAM of X" to refer to a SAM prepared from a solution that contained a mixture of X and 2. For the sake of clarity, we shall also use the phrase "pure SAM of X" to refer to a SAM prepared from a solution that contained only X.

The mole fraction of 1 (\( x_1 \)) in each mixed SAM of 1a–f was calculated by comparing the area under the O(1s) photoelectron peak of the mixed SAM (\( I_{\text{O(1s)}} \)) with the area under the O(1s) peak of a pure SAM of 1(\( I_{\text{O(1s)}} \)). Due to attenuation of the photoelectrons by the SAMs, the O(1s) signal from mixed SAMs of 1g was not a linear function of the thickness of the SAM as measured by ellipsometry. Therefore, we determined the values of \( x_1 \) for mixed SAMs of 1g by comparing their ellipsometric thicknesses to those of pure SAMs of 1g and of 2. We estimate that the values of \( x_1 \) derived from XPS and ellipsometry data are accurate to \( \pm 0.05 \).

**Protein Adsorption.** Figure 2 is a schematic representation of the protocol we used for forming the SAMs and measuring the amounts of proteins that adsorbed to them.35 The SAMs were removed from the solutions in which they were prepared, rinsed thoroughly with ethanol, and dried with a stream of nitrogen. They were then immersed for 2 h in solutions containing 1 mg/mL of protein in 10 mM aqueous sodium phosphate buffer at pH 7.5 and 20–25 °C.34 The amount of protein that remained on each SAM after it had been rinsed with water was determined by ellipsometry. Three measurements of \( d_p \) were made at different positions on each SAM; the value reported for each SAM is the average of these three measurements.

The calculation of \( d_p \) from the experimentally obtained ellipsometric constants required that those constants be interpreted in light of some model of the interface. We applied a standard, homogeneous three-layer (substrate–film–ambient) model.36 This model contains three unknowns: the thickness (\( d_p \)), refractive index (\( n_p \)), and extinction coefficient (\( k_p \)) of the adsorbed protein film. (The refractive index and the extinction coefficient are the real and complex parts, respectively, of the complex refractive index \( N_p = n_p + k_p i \).) Either \( n_p \) or \( k_p \), but not both, can be determined analytically from the specific kind of experiment we employed.37 We assumed values of \( n_p = 1.45 \) and \( k_p = 0 \) for all the proteins. This approach offered an uncomplicated, rapid method for determining the amount of proteins adsorbed on the surface without prior knowledge of the optical properties of the film of protein (which are frequently unknown).

Our model assumes that incomplete films of proteins are continuous layers with thicknesses \( d_p = d_{\text{max}} \), where \( \rho \) is the fractional coverage of the surface and \( d_{\text{max}} \) is the thickness observed for pure SAMs of 2. Real protein films are likely to have structures more closely resembling islands with thickness \( d_{\text{max}} \) covering a fraction \( \rho \) of the surface. By assuming a constant thickness for the protein molecule, calculating values of \( n_p \) from the ellipsometric data, and applying the approach of Maxwell Garnett,38 one can calculate the value of \( \rho \) for an island model. With our samples, the difference between the values of \( \rho \) obtained by the continuous and island models was always equal to or smaller than the scatter in our data. Because the two models gave experimentally indistinguishable results, we continued to employ the more convenient continuous model.

The assumption of a common value for \( n_p \) leads to two qualifications to the values of \( d_p \) reported here: First, the values of \( d_p \) for different proteins may not be directly comparable, since the values of \( n_p \) for these proteins may differ slightly. The values of \( n_p \) for proteins adsorbed at interfaces usually fall between 1.35 and 1.55.39 When we calculated values of \( d_p \) using \( n_p = 1.33 \), the values of \( d_p \) were 25% higher than the values of \( d_p \) calculated using \( n_p = 1.45 \). A 25% decrease in the values of \( d_p \) relative to those obtained for \( n_p = 1.45 \) required \( n_p = 1.71 \). We conclude that the true values of \( d_p \) differ from the values we have calculated by no more than \( \pm 25\% \). Second, if \( n_p \) varies with the amount of a given protein that is adsorbed, the shapes of the curves in Figures 3 and 5 will be distorted. Changes in the value of \( n_p \) could occur were the protein to undergo conformational changes upon adsorption, thereby changing the ratio of protein to water in the film. As we noted above, the value of \( d_p \) is relatively insensitive to changes of \( \pm 0.05 \) in \( n_p \); changes in the balance of protein and water in the adsorbed film could change only small changes in the value of \( n_p \). We conclude that it is unlikely that errors arising from variation in \( n_p \) with the amount of adsorbed protein have any significant effect upon the shapes of the curves in Figures 3 and 5.
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and 5. Supporting evidence for these two conclusions is presented in the supplementary material.

The ellipsometric protocol we employed required that the protein films be dried with a stream of nitrogen before being analyzed. This step was required to prevent any physisorbed water from contributing to the value of $d_p$. We do not know the extent to which the drying step perturbed the structures of the proteins. Therefore, the values of $d_p$ are best interpreted as measures of the relative amounts of protein adsorbed to the SAMs, not as measures of the absolute thicknesses of these protein films when in contact with solutions containing proteins.

Stability of the SAMs. We subjected pure SAMs of each of the alkanethiols used in this study to the protein adsorption protocol summarized in Figure 2, omitting the protein from the buffer solution. The ellipsometric thicknesses, XPS spectra, and contact angles of the SAMs were unchanged by this treatment. This result demonstrates that the SAMs remained intact under the conditions of the experiment.

Relationship between Protein Adsorption and Composition of the SAM. Figure 3 shows the nominal thickness of the adsorbed film ($d_p$) as a function of $x_l$. Adsorption of fibrinogen, pyruvate kinase, lysozyme, and ribonuclease A to mixed SAMs of SC$_{11}$E$_n$OR decreases as the number or the length of the SC$_{11}$E$_n$OR chains increases. The nominal thickness $d_p$ of the adsorbed films of the four proteins on mixed SAMs containing SC$_{11}$E$_n$OR is plotted as a function of $x_l$, the mole fraction of SC$_{11}$E$_n$OR chains in the SAM. The values of $d_p$ were determined by ellipsometry and represent the average of three measurements made at different positions on a single sample. The values of $x_l$ were determined prior to the adsorption of protein. For SC$_{11}$E$_n$OR ($n \leq 6$), the values of $x_l$ are the normalized intensities of the O(1s) photoelectron signals from the SAMs; for SC$_{11}$E$_{(17)}$OCH$_3$, they are the normalized thicknesses of the SAMs, as determined by ellipsometry. The diamonds on the horizontal axes denote estimated values of $x_l^\text{est}$. The horizontal and vertical scales are uniform throughout the figure.

Figure 3. Nominal thickness ($d_p$) as a function of $x_l$. Adsorption of fibrinogen, pyruvate kinase, lysozyme, and ribonuclease A to mixed SAMs of SC$_{11}$E$_n$OR decreases as the number or the length of the SC$_{11}$E$_n$OR chains increases. The nominal thickness $d_p$ of the adsorbed films of the four proteins on mixed SAMs containing SC$_{11}$E$_n$OR is plotted as a function of $x_l$, the mole fraction of SC$_{11}$E$_n$OR chains in the SAM. The values of $d_p$ were determined by ellipsometry and represent the average of three measurements made at different positions on a single sample. The values of $x_l$ were determined prior to the adsorption of protein. For SC$_{11}$E$_n$OR ($n \leq 6$), the values of $x_l$ are the normalized intensities of the O(1s) photoelectron signals from the SAMs; for SC$_{11}$E$_{(17)}$OCH$_3$, they are the normalized thicknesses of the SAMs, as determined by ellipsometry. The diamonds on the horizontal axes denote estimated values of $x_l^\text{est}$. The horizontal and vertical scales are uniform throughout the figure.

We highlight several significant observations from these data.

1. SAMs that Contained Appropriately High Concentrations of E$_n$ Chains Resisted the Adsorption of Proteins. This resistance was the most significant feature of the mixed SAMs of 1. We could not detect the presence of protein on these surfaces by ellipsometry, XPS [N(1s)], or contact angle measurement. Some caution in interpreting these results is warranted. We use $d_p = 0$ as the operational definition of resistance to protein adsorption. Whether resistance is observed for any given SAM is therefore dependent upon our protocol for measuring $d_p$. A value of $d_p = 0$ should not be interpreted to mean that no protein adsorbed to the SAM in situ but only that any adsorbed protein was easily removed by gentle rinsing. Indeed, all of the SAMs emerged from the protein solutions completely wet by the solution. SAMs for which $d_p = 0$ regained finite receding contact angles with water—characteristic of SAMs containing high mole fractions of 1—only after the first rinse. We will consider this observation more thoroughly in the Discussion.

2. Longer E$_n$ Chains Resisted Adsorption More Effectively Than Shorter Chains. This trend was manifested in two ways: First, the value of $x_l^\text{est}$ decreased between $n = 1$ and $n = (17)$. Second, the slope of the curve derived from the data at low values of $x_l$ became more negative as $n$ increased.

3. A Terminal Hydroxyl Group Was Not Required To Prevent Adsorption of Proteins. Mixed SAMs of SC$_{11}$E$_n$OCH$_3$ resisted adsorption of proteins almost as well as mixed SAMs of SC$_{11}$E$_n$.
Wettability of mixed SAMs of SC110H and SC11E0H as a function of 1. The wettability of mixed SAMs of SC110H and SC11E0H, the maximum advancing and minimum receding contact angles of water on the SAMs-increased as a function of x1, the mole fraction of SC110H in the SAM. Each symbol in the plot is bounded above by the value of x of SC110H and below by the value of x of SC11E0H. The length of the symbol represents the hysteresis in the contact angle of water, [cos θ of SC110H] - [cos θ of SC11E0H]. The diamonds indicate the values of x of SC110H for each of the four protein studies. The horizontal and vertical scales of the plots are uniform throughout the figure.

protein than mixed SAMs of SC110H (n ≥ 1), although pure SAMs of SC110H were more hydrophilic than pure SAMs of SC11E0H (n ≥ 1). Methoxy-terminated chains resisted adsorption at lower (more hydrophobic) values of cos θ of SC110H than hydroxyl-terminated chains.

Effects of Temperature. The majority of experiments reported here were carried out using solutions at 25 °C. Proteins are often encountered by materials at other temperatures. We examined the dependence of the adsorption of pyruvate kinase onto mixed SAMs of SC110H upon the temperature at which the adsorption

4. Wettability Was Only a General Predictor of Resistance to Protein Adsorption. Mixed SAMs of SC110H adsorbed more

O H; the introduction of a methyl ether had essentially no effect upon the ability of the SAMs to resist the adsorption of proteins.

4. The Results Were Reproducible. The data shown in Figure 3 represent one to three experiments for each curve. Whenever more than one independent experiment was performed, the curves were superimposable within experimental error. The scatter in the ellipsometric thicknesses of adsorbed protein films—as determined from three different locations on the sample—was sometimes two to three times greater than the corresponding scatter in the thicknesses of SAMs of n-alkanethiols, particularly at values of x for which dP was changing most rapidly. Occasionally, this scatter was sufficient to cause the data to fall well away from the curves represented here. We have removed outliers from the figures presented here to improve clarity; in no case do they represent more than 1 in 20 of the measurements made.

Relationship between Wettability and Composition of the SAM. Figure 4 shows the maximum advancing and minimum receding contact angles of water on the SAM as a function of x. Above a certain value of x different for each compound 1a—g—mixed SAMs of I were as wettable as pure SAMs of I. This observation implies that these mixed SAMs of I presented an interface that consisted almost entirely of E groups. Not surprisingly, then, the values of x for each protein occurred near the lowest value of x at which the wettability of the mixed SAM of I was indistinguishable from that of a pure SAM of I.

Others have proposed a number of methods for relating the solid-water interfacial energy of a material to its protein resistance or biocompatibility.41 Real materials sometimes show large differences between θ (H2O) and θ (H2O); it is not clear whether either measurement should provide a predictor of resistance to protein adsorption. Figure 4 shows that mixed SAMs of I on gold that resisted protein adsorption also exhibited low and nearly constant hysteresis. In the following section, we plot dP as a function of cos θ (H2O); examination of the data using cos θ (H2O) yielded no new information.

Relationship of Protein Adsorption to Wettability of the SAMs. Figure 5 shows the thickness, dP, of the adsorbed films of fibrinogen, pyruvate kinase, lysozyme, and ribonuclease A as a function of cos θ (H2O) for the SAMs that we examined. These figures present a number of important observations.

Protein Adsorption Was Frequently More Sensitive Than Wettability to the Presence of the Hydrophobic Component of the Mixed SAMs. This sensitivity was reflected in the upward curvature of many of the plots in Figure 5. For the most hydrophilic surfaces, small changes in the wettability of the surface often produced large changes in the amount of protein adsorbed. In a number of instances, significant increases in protein adsorption occurred before any change in the wettability of the SAMs could be detected.

2. For a Given Hydrophilic Component, Resistance to Protein Adsorption Increased with the Hydrophilicity of the Interface. The amount of adsorbed protein decreased monotonically—although not linearly—in all cases. This observation is consistent with a large body of experimental data42 but does not establish whether the dominant variable is the concentration of the hydrophilic component in the interface or the wettability of the interface.

3. Homologous Hydrophilic Groups Had Similar Properties of Resistance to Protein Adsorption. The curves obtained by plotting dp as a function of cos θ for mixed SAMs of SC11E0H (n = 2, 4, and 6) were almost indistinguishable.

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Figure 5. Nominal thickness (dp) as a function of wettability. Adsorption of proteins to mixed SAMs of SC11E10H is sensitive to the presence of accessible hydrophobic regions. The nominal thickness dp of adsorbed films of fibrinogen, pyruvate kinase, lysozyme, and ribonuclease A on mixed SAMs of SC11E10H is plotted as a function of the wettability—represented by cos β(H2O)—of the SAM. The vertical and horizontal scales are uniform throughout the figure. The solid curves in each column are the same. They are taken from the data for SC11E10H in that column and are reproduced on each graph to aid comparison among them.

Figure 6. Nominal thickness of the film of adsorbed pyruvate kinase, dpK, as a function of the mole fraction of SC11E10H in the SAM. Mixed SAMs of SC11E10H for which x1 > x1^trap adsorbed more pyruvate kinase at 37 °C than at lower temperatures. There was no experimentally significant difference between the adsorption at 4 and 25 °C. The value of x1^trap did not change over the range of temperatures shown.

Discussion

Origin of Resistance of EO SAMs to Adsorption of Proteins.

The most striking observation derived from the data presented in Figure 3 and 5 is the similarity between the shapes of the adsorption curves of the different proteins on each series of mixed SAMs. Lysozyme and ribonuclease are globular proteins with molecular weight approximately 15 000 Da; pyruvate kinase is a noncovalent tetramer of 55 000-Da subunits, and fibrinogen is a structurally complex, flexible molecule of approximately 340 000 Da. The number of EO chains per unit area required to eliminate adsorption was almost the same for each of the four. The similar behavior of different proteins on the same surface suggests that the adsorbance of each mixed SAM is dominated by the interfacial properties of the SAM, not by those of the protein.

We sought to relate a useful parameter of resistance to protein adsorption to some structural property of the SAM. The properties of polymers can frequently be expressed as power laws of the lengths of the polymer chains. We fitted the value of x1^trap to such a power law: x1^trap = k1n^(k2). The values of k1 and k2 were determined by least-squares analysis for each of the four proteins. The average value of k2 was -0.4 ± 0.05; k1 ranged from 0.8 to 1.1 (see Figure 7). In Figure 7, we plotted the value of x1^trap as a function of n for all four proteins combined and

[References]
and that longer chains simply cover the surface more effectively than shorter chains. Recent reports of dramatically reduced protein adsorption to hydrophobic surfaces that areraft-polymerized with tetra(ethylene glycol) derivatives confirm this interpretation.\(^5\)

Then, are our data compatible with the hypothesis that steric repulsion (as applied to the stabilization of colloids by polymers) is important in resisting protein adsorption? Jean and Andrade\(^5\) made three predictions based upon their calculations: (i) the optimal density of PEO chains for resistance to protein adsorption decreases as the size of the protein increases; (ii) that chains of PEO packed near the density of crystalline PEO should be less resistant to protein adsorption than less densely packed chains; and (iii) that at the optimum value of \(\chi_t\), longer chains are more resistant to adsorption. The limited data in this study do not provide a substantial test of these predictions. We observed no optimum value of \(\chi_t\), only a threshold value. The most closely packed chains of EO we observed (\(\chi_t = 1\)) were entirely resistant to adsorption. The packing of these chains is, however, still sufficient to provide substantial volume for water. We did observe that longer chains had lower values of \(\chi_t\) and lower values of \(d_p\), the constant values of \(d_p\).

**Adsorption in Situ.** The technique that we employed to measure \(d_p\) in these studies involved removing the samples from solution and rinsing them. An observation of \(d_p = 0\) means that no protein was able to adsorb strongly enough to resist the shear of rinsing. Is it possible that protein adsorbs weakly to mixed SAMs of EO in situ? Our observations suggest, but do not prove, that such weak adsorption occurs.

When a SAM of 1 that has not been exposed to protein is rinsed with water, the water runs off the sample leaving a dry surface (the value of \(\theta(H_2O)\) is greater than 0\(^\circ\)). This same phenomenon is observed when rinsing protein solutions from mixed SAMs of 1 for which \(d_p = 0\). Water does not run off mixed SAMs of 1 for which \(d_p > 0\). Instead, it forms a thin film \(\theta(H_2O) = 0\). The protein solutions do not run off of any SAM, even those for which \(d_p = 0\). One rationalization of this observation would be the presence of an adsorbed film of protein that persists until the first rinse removes it. Another would be that the dissolved proteins lower \(\gamma_s\) enough to make \(\theta(H_2O) = 0\). We can not presently distinguish between these alternatives.\(^4\)

**Temperature Effects.** There are at least two reasons for thinking that the resistance of mixed SAMs of 1 toward protein adsorption might decrease with increasing temperature. First, the "hydrophobic effect" is expected to increase with increasing temperature.\(^9\) Second, EO chains are known to have lower solubility limits at elevated temperatures,\(^10\) an effect that is usually ascribed to a negative entropy of solvation of EO groups by water. Such an effect would render EO chains at an interface less hydrophilic


**Figure 7.** Values of \(\chi_{\text{resist}}^\text{min}\) plotted as a function of \(n^{0.4}\). The resistance of mixed SAMs of 1 to the adsorption of proteins, as measured by \(\chi_{\text{resist}}^\text{min}\), is proportional to \(n^{0.4}\), where \(n\) is the number of monomer units per chain. The curve fit for the individual proteins were made by least-squares analysis to a line passing through the origin. The curve fit for the combined data was made by least-squares analysis to a line. The slopes \(k_1\) and correlation coefficients \(r\) of the fits are displayed on each figure. The horizontal and vertical axes are uniform in scale and meaning throughout the figure.
protein adsorption on oligo(ethylene oxide) surfaces

we investigated the effect of temperature upon the adsorption of pyruvate kinase to mixed sam's of sc\textsubscript{11}e\textsubscript{2}eo. the results are shown in figure 6. there is no significant difference between the curves for \( T = 4 \) °c and \( T = 25 \) °c. we do not know why are shown in figure 6. there is no significant difference between \( T = 25 \) °c and \( T = 37 \) °c, in which mixed sam's for \( \chi_1 \) \( \chi_2 \) adsorbed more protein at the higher temperature. the value of \( \chi_2 \) remained constant, however.

experimental section

materials. test-grade, 100-mm, singly polished silicon wafers (silicon sense), chromium (99.999\%o, aldrich), and gold (99.999\% as machined pellets, materials research corp.) were used as received.

fibrinogen (fraction i from human plasma, sigma), pyruvate kinase (ec 2.7.1.40, type pk-3 from rabbit muscle, biozyme), lysozyme (ec 3.2.1.17, grade iii from chicken egg white, sigma), and ribonuclease a (ec 3.1.27.5, type iii-a from bovine pancreas) were used as received. buffer solutions were prepared from disodium hydrogen phosphate (0.010 m) and titaed to ph 7.5 with phosphoric acid.

absolute ethanol (us industrial co.) was used as received. deionized water was distilled from glass in a corning ag-lb still.

preparation of gold substrates. gold substrates were prepared by evaporating first cr (10 nm) and then au (200 nm) onto silicon wafers in a two-gun electron-beam evaporator. the evaporations were performed at a pressure of \( 2 \times 10^{-7} \) torr and a rate of 0.5 nm/s for both metals. the cr was evaporated as an adhesion layer between the oxidized si surface and the au. the au substrates were stored in poly(propylene) wafer carriers and immersed in thiol solutions within an hour after they were removed from the vacuum chamber.

solutions of alkane thiols. volumetric flasks were rinsed thoroughly with water, then treated with freshly prepared piranha solution (a 3:7 v/v mixture of concentrated h\textsubscript{2}so\textsubscript{4} and 30\% aqueous h\textsubscript{2}o\textsubscript{2}) for 30 min at 90 °c, rinsed thoroughly with distilled, deionized water, and dried in an oven. disposable glass scintillation vials (20 ml) were used for the adsorption at 4 °c the sam's were first prepared and analyzed by x-ray diffraction as soon as possible.

chcif\textsubscript{2} (chemtronics, ultrajet esl270), removed from the cold room at several molecular weights. this research was supported by the national science foundation (nsf) under the engineering research center initiative and is housed at the harvard university materials research laboratory, an nsf-funded facility. the nmr spectrometer was provided to the chemical laboratories of harvard university through a gift from the nsf (che-8410774). k.l.p. was an nsf predoctoral fellow from 1986 to 1989.

supplementary material available: available: detailed analysis of the methods used for calculating \( d_\phi \) and \( \chi_2 \) and synthesis procedures, spectral data, and analyses of compounds 1b, 1c, 1f, and 1g (19 pages). ordering information is given on any current masthead page.