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Protein-Induced Changes in Poly(ethylene glycol) Brushes: Molecular Weight and Temperature Dependence

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Terminally grafted chains of poly(ethylene glycol) (PEG) and oligo(ethylene glycol) reduce protein adsorption and cell adhesion on material surfaces. However, previous studies showed that protein−PEG adhesion is induced by the application of pressure. Because polymer behavior can vary with the molecular weight, in this study we directly measured the forces between streptavidin and end-grafted monolayers of PEG of different molecular weights and at grafting densities. The results of these measurements show that grafted PEG chains can exist in two different states: a protein-repulsive state and a protein-attractive state. The attractive state can be induced not only by compression but also by increasing the temperature or by altering the polymer molecular weight. Both the critical applied load to induce the protein-attractive form of PEG and the relaxation time back to the protein-resistant state depend on the molecular weight of the grafted chains. The consequences of the observed behavior for the use of grafted PEG chains as protein antifouling coatings of biomaterials are discussed.

Introduction

Poly(ethylene glycol) (PEG) is used extensively to improve the biocompatibility of foreign materials for both in vivo and ex vivo applications.[1,2] This is primarily because PEG exhibits unusually good protein resistance; it is nonimmunogenic and nonantigenic.[1] Combined with low toxicity and with the presumed biological inertness of the polymer, these properties of PEG provide a solid basis for its use as an inert, relatively nonfouling surface coating in biological environments.[1−7] For example, PEG functionalization of liposomes increases their blood circulation times by nearly an order of magnitude.[7] Similarly, the covalent attachment of PEG to proteins results in a minimal loss of activity, renders the proteins both nonimmunogenic and nonantigenic, and increases their circulation time in the bloodstream.[8,9] Ethylene oxide (EO) surface grafts are used to reduce protein adsorption to the surfaces of biomedical polymers.[1−6,10] This is an important strategy for controlling the biological responses to the latter, because protein adsorption is generally recognized as a first step in the humoral response against foreign materials.[10−12]

The majority of models of grafted poly(ethylene oxide) films attribute its protein resistance to two main ingredients, namely, (i) the steric or osmotic repulsion between proteins and the chains and (ii) the assumption that the EO segments completely repel proteins.[3,13−18] The wide acceptance of this view is based on the limited predictive power of these simple models.[2,3,19−24] If shown to be generally applicable, such theories could be used to establish a priori guidelines for the rational design of protein-repellant biomaterial coatings.

Yet there is now an increasing body of evidence which indicates that PEG does bind proteins. Neutron scattering studies of protein−PEG solutions,[25] the formation of protein−PEG complexes,[26−29] protein adsorption onto grafted chains,[23,24,30,31] and direct surface force measure-

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ments[32] demonstrated protein−PEG attraction, either directly or indirectly. Moreover, poly(ethylene glycol) is soluble in organic solvents,[1] adsorbs at the air−water interface,[33−35] and adsorbs to hydrophobic surfaces.[20,36−39] Aqueous solutions of PEG also exhibit both upper and lower critical solution temperatures.[40] All of these observations are incompatible with the simple polymer theories, which describe polymer chains as comprising structureless, noninteracting segments.[41−43]

To account for the unusual phase behavior of aqueous PEG solutions, alternative polymer models have been developed, which incorporate details of the segment interactions in the polymer description. In contrast to the simple model for PEG, which assumes that the polymer segments exhibit a single monomeric state,[13,15−18,20,44] experimental studies show that ethylene oxide segments can adopt multiple configurations.[45−49] Moreover, the different conformers interact differently with water.[45,46,50] Recent "two-state" polymer models allow the polymer segments to assume two different states within the chain.[51−57] This modification is sufficient to predict the occurrence of both upper and lower critical solution temperatures.[51,57] Additionally, these two-state models can account qualitatively for some of the results from force measurements between protein and grafted poly(ethylene oxide) chains.[58,59]

Thus far, the consequences of these additional segment conformations for biological interactions have been explored only for short chains ($n < 6$).[45] Direct force measurements of PEG interactions with proteins were done with a single molecular weight (MW 2000). Prime and Whitesides[60] suggested that dense oligoethylene oxide (OEG) layers with merely three ethylene oxide segments ($N = 3$) could resist protein adsorption as effectively as long chains. Other experimental data suggest that short oligomers do not resist protein and cell adhesion as effectively as long chains.[61] There is, however, no reason that proteins should interact with both short and long chains by the same mechanism, especially when the phase behavior of PEG/water solutions varies with the molecular weight.[34,40] Nevertheless, there have been few systematic investigations of the molecular weight and grafting-density dependence of the interactions between grafted ethylene glycol layers and proteins. Consequently, the variations in the biological activity of PEG with the polymer molecular weight, the chain density, and temperature remain unexplored. Without addressing the potential impact of these parameters on PEG−protein interactions, the establishment of general design guidelines for protein-resistant, grafted PEG surface coatings will remain elusive.

This paper describes the use of direct force measurements to quantify the dependence of the interactions of grafted ethylene glycol chains with proteins as a function of the molecular weight, grafting density, and temperature. Here, we studied PEG interactions for polymer molecular weights ranging from 120 to 5000. The consequences of these findings for the fabrication of biocompatible PEG coatings are discussed.

Experimental Section

Materials. Conjugates of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) with methoxy-terminated PEG of M_r equal to 120, 750, 2000, and 5000 (DSPE−EO_3, DSPE−EO_{18}, DSPE−EO_{45}, and DSPE−EO_{114}) were purchased in the powder form from Avanti Polar Lipids Inc. High-purity 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), DSPE, 1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine (DTPC), and 1,2-distearoyl-3-trimethylammoniumpropane (DSAP) were also purchased from Avanti. DPPE labeled with fluorescein isothiocyanate (FITC−DPPE) was from Molecular Probes. All salts were high purity (>99.5%) and were purchased from Aldrich. Streptavidin was from Calbiochem. Water was purified with a Milli-Q UV filtration system (Millipore) and exhibited a resistivity of 18 MΩ. HPLC grade methanol and chloroform were from Mallinckrodt.

Preparation of Supported Lipid Bilayers Displaying Grafted PEG Chains. Solutions of PEG−lipid (DSPE−EO_x) and pure DSPE were prepared in 9:1 chloroform/methanol solutions. Mixtures of various molar ratios of DSPE−EO_x with DSPE (from 0.3 to 10 mol % of DSPE−EO_x) were prepared by mixing solutions of pure DSPE and DSPE−EO_x in the appropriate proportions. In cases when the concentration of PEG−lipid in the monolayer was higher than 10 mol %, an equimolar fraction of positively charged lipid, DSAP, was added to a lipid mixture. This was done to avoid high negative surface charges on PEG−lipid membranes that are due to the ionized phosphate groups on the anchoring phospholipid.[32,62] Surface pressure versus area measurements were made with a commercial Langmuir−

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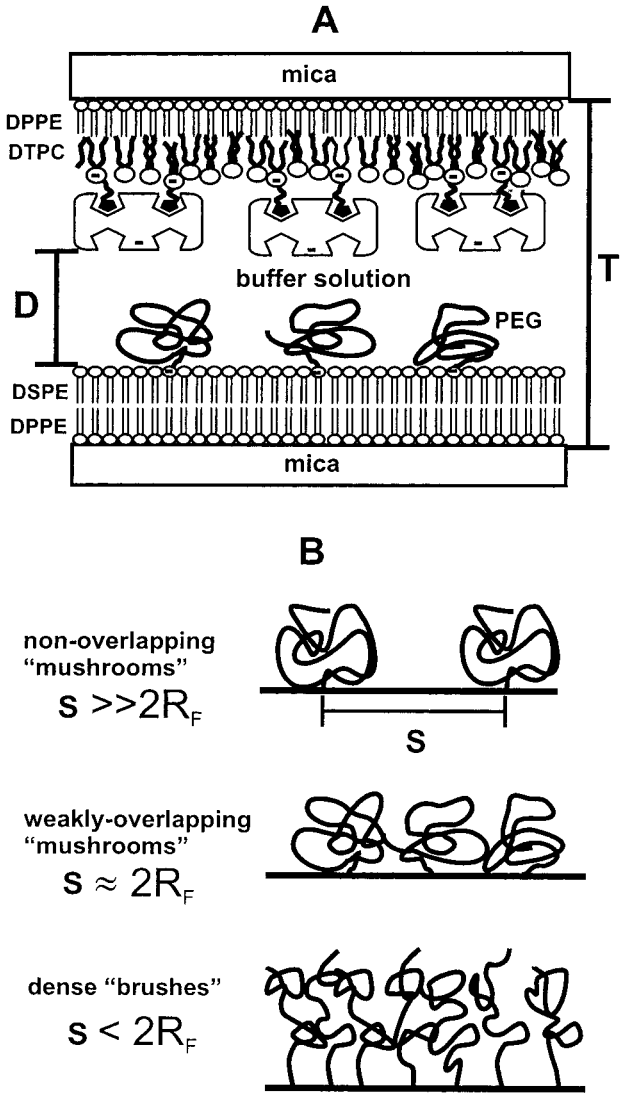

Figure 1. (A) Sample configuration used in the direct force measurements. The distances, *D*, reported in the text refer to the separation between the outer streptavidin (STA) surface and the bare DSPE lipid membrane beneath the grafted PEG layer, as shown here. (B) The three surface concentration regimes of PEG chains: dilute nonoverlapping "mushrooms" (0.3 mol % PEG 5000 and 1.5 mol % PEG 2000); semidilute, weakly overlapping chains (4.5 mol % PEG 2000) and concentrated brushes (10.0 mol % PEG 5000, 9.0 mol % PEG 2000, 30 and 50 mol % PEG 750 and 50% PEG 120).

Blodgett trough (NIMA Technologies, type 611), equipped with a standard Wilhelmy microbalance.

The grafted polymer layers (Figure 1) were prepared by Langmuir−Blodgett deposition of the mixed lipid monolayers onto hydrophobized mica as described elsewhere.[62] After the lipids were spread on the water surface, they were compressed to an average area of 43 Å^2 per lipid. The monolayer was then deposited at constant pressure onto a hydrophobic, crystalline monolayer of DPPE, prepared by its Langmuir−Blodgett deposition from the air−water interface onto freshly cleaved mica. The transfer ratio, that is, the area transferred relative to the area coated by the film, was close to unity in all cases. Adjusting the DSPE−EO_x mole fraction in the monolayer thereby precisely controlled the polymer grafting density. PEG chains in the grafted polymer layers were in dilute, semidilute, or concentrated brush regimes, depending on the ratio of the Flory radius, R_F, to the average distance between polymer chains, *s* (Figure 1B).

The lateral distribution of lipids in the monolayers was tested using epifluorescence microscopy. To visualize the monolayer structure, 0.5 mol % of FITC-labeled DPPE was added to the monolayers. The monolayers were deposited onto freshly cleaved hydrophobized mica sheets and transferred under water into a cell used for fluorescence microscopy. Fluorescence micrographs were acquired with an Olympus BX-60 microscope equipped with an 80× objective (the total magnification of microscope was 1400×). All monolayers used in this study exhibited a homogeneous distribution of fluorescent dye.

Surface plasmon resonance was used to characterize the stability of the Langmuir monolayers of DSPE or DSPE/DSAP, containing various molar fractions of DSPE−EO_x. There was no detectable desorption of lipids from the monolayers, in the absence of exogenous lipid in the bathing medium, for at least 24 h.

Preparation of Oriented Streptavidin Monolayers. Force measurements were conducted with oriented monolayers of the model protein, streptavidin. This protein does not participate in the in vivo recognition of foreign materials, but its surface amino acid composition is similar to that of other soluble proteins found in biological fluids. Homogeneously oriented streptavidin monolayers on a supported lipid bilayer displaying biotin conjugated to 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (Figure 1A) were prepared as described previously.[63,64] Streptavidin bound to these layers self-assembles into two-dimensional crystals.[65] The fractional protein surface coverage of 85 ± 3% was determined both by quantifying the amount of ^{125}I radiolabeled protein bound to the bilayers[66] and by using surface plasmon resonance.[67] Thus, the protein layers are homogeneous and close-packed. In these studies, we are investigating the physical−chemical interactions between proteins and PEG brushes. Although a protein monolayer does differ from the soluble molecules, this construction allows us to directly measure the interactions between PEG and proteins and to identify parameters that alter them.

Force Measurements. Force measurements were conducted with a Mark III surface force apparatus (SFA) (SurForce Co., Santa Barbara, CA).[68] The SFA quantifies the molecular forces between thin films as a function of their separation. The materials are confined to the surfaces of opposed, crossed hemicylindrical silica lenses. A positioning control system varies the spacing between the surfaces, and forces are measured from the deflection of a spring that supports one of the lenses. The force is determined with an accuracy of ±1 nN,[68] and the distance between the bilayers is measured with a resolution of ±2 Å by multiple beam interferometry.[69]

Forces were measured between two bilayer-coated mica sheets (Figure 1). The area probed was ∼100 μm^2, so that the total measured force represents the force integrated over >350 000 protein molecules. The chamber of the instrument housing the samples was filled with a 10 mM sodium phosphate buffer solution (pH 7.0), containing 30 mM KNO_3. Before the chamber was filled, the solution was filtered through a surfactant-free 0.2 μm Durapore membrane (Millipore).

Results

Definition of the Intersurface Distance, *D*. The distances reported refer to the separation between the outer surface of the streptavidin monolayer and the surface of the bilayer membrane supporting the grafted PEG. The difference in the distance of closest intersurface approach determined before and after removal of the organic layers from the mica defines the total thickness, *T*, of both lipid bilayers together with the polymer headgroups and the streptavidin (Figure 1A). This difference is determined after burning away the organic material on the mica by

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Table 1. Streptavidin Interactions with PEG 5000 Monolayers

concn of DSPE−EO_{114} (mol %)	jump-in (Å) from → to	adhesive minimum (Å)	F_{adh}/R (mN/m)	adhesion energy kT/protein
		$T = 25$ °C		
0.3 (dilute)	62 ± 9 → 40 ± 4	45 ± 5	−0.5 to −3.0	−1.5 to −8.6
10 (brush)	173 ± 15 → 125 ± 10	129 ± 10	−0.5 ± 0.2	−1.5
		$T = 37$ °C		
0.3 (dilute)	88 ± 8 → 66 ± 8	68 ± 7	−0.5 to −3.0	−1.0 to −5.8
10 (brush)	189 ± 9 → 137 ± 6	143 ± 10	−0.5 ± 0.2	−1.0

UV irradiation. The compressed polymer layer thickness t_{PEG} was then determined by subtraction of the thicknesses of the two lipid bilayers plus that of the streptavidin (STA) monolayer from the total thickness T (Figure 1). Thus, $t_{PEG} = L = T - (t_{STA} + t_{DSPE} + t_{DTPC} + 2t_{DPPE})$. For these determinations, the thickness of each of the DSPE, DTPC, DPPE, and streptavidin monolayers was 28, 13, 27, and 43 ± 2 Å, respectively.[32,62−64]

Streptavidin Adheres to Grafted PEG 5000 at 25 °C and at 37 °C. Force measurements were conducted between streptavidin and either a brush of PEG 5000 chains (10 mol % DSPE−EO_{114}) or dilute nonoverlapping chains (0.3 mol % DSPE−EO_{114}) at both 25 and 37 °C. The polymer grafting densities were 430 and 14330 Å^2/chain, respectively. At both grafting densities, the PEG chains readily adhered to the streptavidin during the very first contact between unperturbed monolayers (Figure 2A,B; Table 1). Qualitatively the same behavior was observed at both temperatures, with some differences in the range and magnitude of the attraction. Upon approach, the surfaces jumped into adhesive contact at separation distances below 190 and 95 Å for the concentrated and dilute grafted chains, respectively (Figure 2, Table 1). At 25 °C, the surfaces came to rest at 125 ± 10 Å in the case of the concentrated chains and at 45 ± 5 Å at the low grafting density. At 37 °C, the equilibrium separations were slightly farther out at 137 and 68 Å with concentrated and dilute chains, respectively (Table 1).

The theoretical thickness of the concentrated PEG brush is given by $L = nl^{5/3}/s^{2/3}$ where n is the degree of polymerization, l is the monomer length, and s is the distance between grafting sites.[70,71] For these PEG 5000 layers, $L = 110$ Å. For dilute chains, the theoretical polymer layer thickness is the Flory radius $R_F \sim ln^{3/5}$.[70,71] With dilute grafted PEG 5000 chains, $R_F = 60$ Å. Thus, in both cases the surfaces came to rest at the outer edge of the polymer layers, that is, at $D/L = 1.1$ and $D/R_F = 1.1$.

With the dense chains at 25 °C, the surfaces jumped into contact at 125 Å, or $D/L = 1.2$. Upon separation, the surfaces jumped apart from slightly farther out at 143 Å ($D/L = 1.3$). Again, the equilibrium separation and adhesive minima were slightly farther out at 37 °C (Table 1). The adhesive force at the minimum in the curve was −0.5 ± 0.2 mN/m, at both 25 and at 37 °C (Table 1). The normalized force F_{adh}/R corresponds to the adhesion energy per area $E = F/(1.5\pi R) = 1.1 \times 10^{-4}$ J/m^2.[71] If we normalize this by the measured protein density of 5500 Å^2/streptavidin,[72] then the estimated adhesive energy per protein is $-1.4kT$ and $-1.2kT$ at 25 and 37 °C, respectively.

A possible source of the intersurface attraction could be the electrostatic double-layer force, since surfaces with electrostatic potentials of the same sign but different magnitudes can attract.[73] Both the streptavidin and the membrane underlying the PEG monolayer were negatively

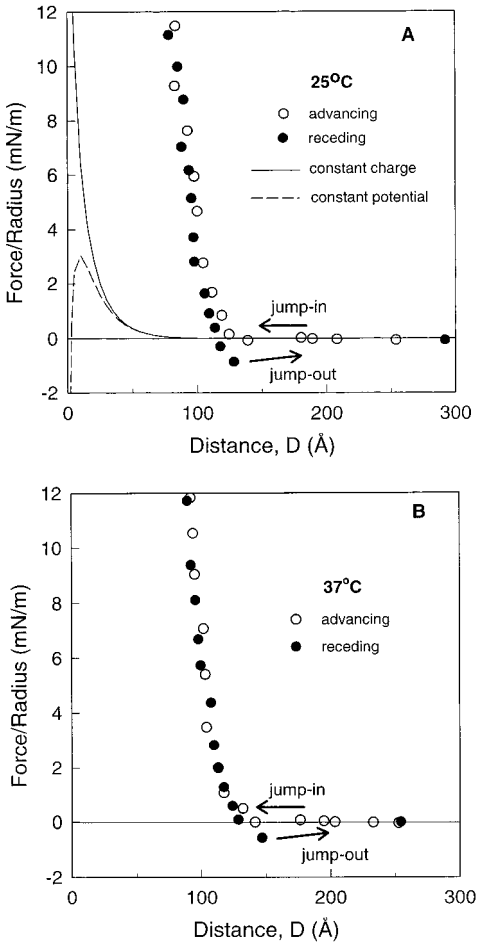

Figure 2. (A) Forces measured during approach (open circles) and separation (filled circles) between an unperturbed, grafted DSPE−EO_{114} brush (10 mol % PEG 5000) and streptavidin monolayer at 25 °C. Electrostatic double-layer forces were calculated using the nonlinear Poisson−Boltzmann equation, for constant charge (solid line) and constant potential (dashed line) boundary conditions (see text for the details). (B) Force−distance profile between a DSPE−EO_{114} brush (10 mol % PEG 5000) and streptavidin monolayer at 37 °C. Both experiments were conducted at pH 7.0 in a buffered solution of 10 mM NaH_2PO_4 and 30 mM KNO_3.

charged.[32,62,63] We therefore calculated the electrostatic double-layer force using the nonlinear Poisson−Boltzmann equation and numerical procedures described elsewhere.[63,72] The outer Helmholtz planes were placed at the outer surface of the streptavidin monolayer and near the negatively charged phosphate of the DSPE. Constant surface charge densities of one negative charge per 430 Å^2 (−37 mC/m^2) or 14330 Å^2 (−1.1 mC/m^2) were used for the boundary conditions for 10 and 1.3 mol % polymer layers, respectively (Figure 2A, solid line).[32] Alternatively, the constant surface potentials used were

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Table 2. Streptavidin Interactions with PEG 2000 Monolayers

surface concn of DSPE−EO_{45} (mol %)	PEG layer thickness[a] (Å)	pull-off distance (Å)	F_{adh}/R (mN/m)	adhesion energy (kT/protein)	compressive energy, E^{comp},[b] (kT/protein)
		$T = 25$ °C[c]			
1.5 (dilute)	35 ± 3	40 ± 10	−0.7 ± 0.3	−2.0	4.4
4.5 (semidilute)	38 ± 3	64 ± 8	−0.8 ± 0.2	−2.2	26
9.0 (brush)	50 ± 5	65 ± 6	−0.6 ± 0.3	−1.7	44
		$T = 37$ °C			
1.5 (dilute)	30 ± 10	51 ± 12	−0.7 ± 0.1	−1.3	0
4.5 (semidilute)	50 ± 10	63 ± 10	−0.7 ± 0.2	−1.3	0
9.0 (brush)	58 ± 6	72 ± 5	−0.8 ± 0.2	−1.5	0

[a] Measured from the range of steric repulsion. [b] Calculated using the Derjaguin approximation. [c] Data from ref 32.

−61 and −2 mV (Figure 2B, dashed line). The charge density and electrostatic potential of the streptavidin monolayer were −16 mC/m^2 and −54 mV, respectively.[72]

Regardless of the boundary conditions, the range of the double-layer force between the brush and streptavidin is <70 Å (D/L < 0.6) (Figure 2A, solid and dotted lines), and the force becomes attractive only at D < 20 Å (D/L < 0.2). Yet the protein and polymer surfaces jumped into contact from distances up to 190 Å, and the adhesive minimum between streptavidin and the PEG brush was at 125 Å at 25 °C (D/L = 1.1). The intersurface attractive force is, therefore, due to protein−PEG adhesion and not to the double-layer force.

The adhesion between streptavidin and concentrated EO_{114}−DSPE chains, measured in successive force runs, was distributed about a mean value, independent of the load or time in contact. On the other hand, the adhesion between dilute EO_{114}−DSPE chains and streptavidin increased during successive force measurements, from −0.5 up to −3 mN/m, where it stabilized (Table 1). The corresponding adhesion energies ranged from −1.4 to −8.5kT/protein. This behavior is similar to streptavidin interactions with dilute EO_{45}−DSPE monolayers.[32] It may be due to the relative ease of protein penetration of the polymer layer and consequently to the formation of a larger number of EO segment/protein contacts in successive measurements. Alternatively, this could be due to possible differences in the segment conformations closer to the membrane surface (see Discussion). The adhesion energy in this case is as high as −8.5kT per protein. This attraction would support stable protein deposition,[13,73] providing the proteins acquired sufficient energy to penetrate the brush.[13,44,73]

Forces between Streptavidin and Grafted PEG 2000 at 25 °C and 37 °C. A previous paper[32] demonstrated that the interactions between streptavidin and grafted EO_{45} at 25 °C depend on the magnitude of the compressive load applied to the surfaces during measurements. At 25 °C, the estimated energies needed to induce protein−PEG 2000 adhesion were ∼4kT, ∼26kT, and ∼44kT per protein at 1.5, 4.5, and 9.0 mol % DSPE−EO_{45}, respectively.[32] The values were determined from the force required to induce adhesion and the Derjaguin approximation.[71] The interaction energy per area $E = F/(2\pi R)$, and the energy per protein is determined by normalizing E by the protein density.

Because the induction of protein−PEG adhesion appears to be an activated process, we remeasured the forces under identical conditions, but at the elevated temperature of 37 °C (Figure 3). In contrast to the interactions at 25 °C, the PEG chains, at all grafting densities, spontaneously adhered to the streptavidin monolayer without the application of any compressive force at 37 °C (Figure 3, Table 2). During approach, the surfaces jumped into adhesive contact. With dilute chains, the jump-in occurred below 76 ± 6 Å (D/R_F < 2.2). The jump-in also occurred

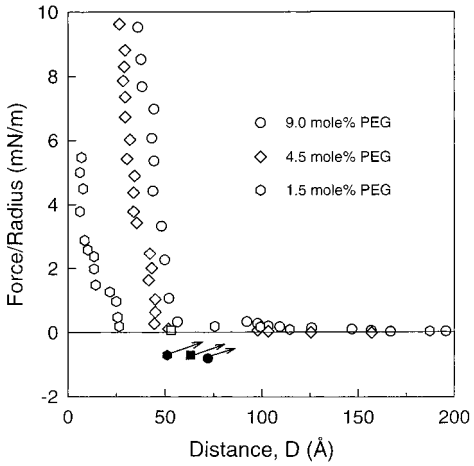

Figure 3. Force−distance profile between streptavidin and PEG 2000 chains at 37 °C. DSPE−EO_{48} was mixed with DSPE at mole fractions of 1.5 mol % (dilute chains, hexagons), 4.5 mol % (semidilute chains, open diamonds), and 9.0 mol % (dense chains, open circles). The measurements were conducted at pH 7.0 in a buffered solution of 10 mM NaH_2PO_4 and 30 mM KNO_3. Despite the presence of the electrostatic repulsion, the surfaces spontaneously jumped into adhesive contact from 75 Å (dilute chains) and 90 ± 10 Å (semidilute and dense chains). The outward directed arrows indicate the positions of the adhesive minima and the point at which the two surfaces jumped out of contact in each case.

below 98 ± 10 Å for both the concentrated chains and the weakly overlapping chains (Figure 3, Table 2). The surfaces came to rest at 30 ± 10 Å at the outer edge of the dilute chains (D/R_F = 0.9). Similarly, the equilibrium separations were at 50 ± 10 and at 58 ± 6 Å in the case of semidilute and concentrated chains, respectively. The range of the repulsive force was only slightly larger at 37 °C relative to that at 25 °C.[32] Upon separation, the surfaces jumped apart at distances slightly farther out from the equilibrium separation; namely, the minima were at 51 ± 12 Å (D/R_F = 1.5), 63 ± 10 Å (D/L = 1.4), and 72 ± 5 Å (D/L = 1.6) in the case of the dilute, semidilute, and concentrated polymer layers, respectively (Table 2, column 3). The average measured adhesive force was −0.7 ± 0.2 mN/m and was roughly the same at all three grafting densities (Table 2).

Forces between Streptavidin and Grafted PEG 750 and PEG 120 at 25 °C and at 37 °C. Due to the interest in the behavior of short ethylene glycol oligomers, we also investigated PEG−lipids with shorter oligo(ethylene glycol) headgroups, namely, DSPE−EO_{18} (PEG 750) and DSPE−EO_3 (PEG 120). The smaller headgroups enabled us to prepare brush layers with much higher grafting densities. To avoid high surface charges on the PEG−lipid membrane, we added positively charged lipid DSAP to the DSPE−EO_n/DSPE (n = 3, 18) mixture. The molar fraction of DSAP was ∼10% lower than that of the

Table 3. Streptavidin Interactions with PEG 750 Monolayers

surface concn of DSPE−EO_{18} (mol %)	PEG layer thickness[a] (Å)	pull-off distance (Å)	F_{adh}/R (mN/m)	adhesion energy (kT/protein)	compressive energy, E^{comp},[b] (kT/protein)
		T = 25 °C			
30 (brush)	25 ± 5	35 ± 5	−0.1 to −0.8	−0.3 to −2.3	23
50 (brush)	25 ± 6	23 ± 5	−0.2 to −0.9	−0.6 to −2.6	2.6
		T = 37 °C			
30 (brush)	25 ± 5	34 ± 5	−0.3 to −1.6	−0.6 to −3.1	5.2
50 (brush)	31 ± 6	28 ± 5	−0.2 to −0.9	−0.4 to −1.8	1.7

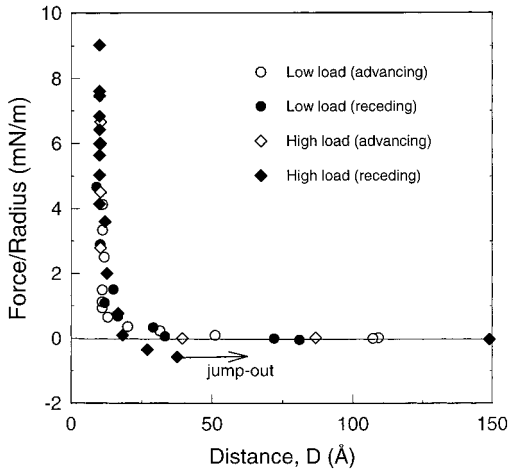

Figure 4. Force profiles between streptavidin and densely grafted PEG 750 (30 mol % DSPE−EO_{45}) at 25 °C. For initial measurements in which the compressive force was kept below 8 mN/m, the force curves were reversible (circles). After applying higher loads (diamonds), the streptavidin and PEG adhered during separation. The outward directed arrow indicates the position of the adhesive minimum and the position at which the surfaces jumped out of contact.

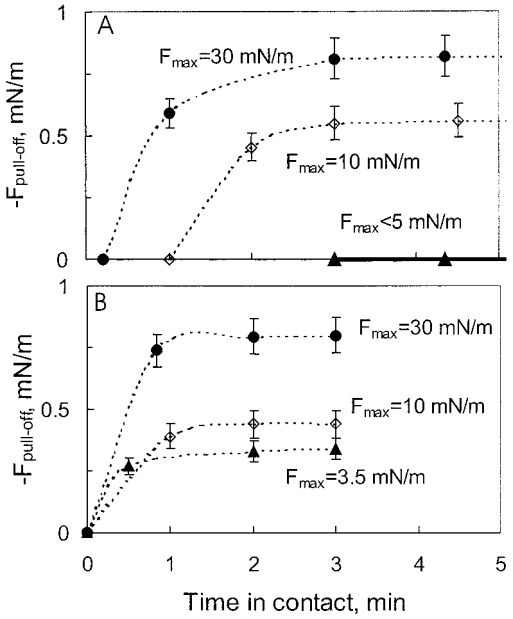

Figure 5. Adhesion between PEG 750 (30% of PEG−lipid) and streptavidin: effect of the applied load and time in contact. Panel A shows data taken at T = 25 °C, and panel B shows data obtained at T = 37 °C.

PEG−lipid, to preserve a low negative charge on the PEG−lipid membrane and to prevent any electrostatic attraction between the PEG-coated surface and the negatively charged streptavidin.

Figure 4 shows the force curve between streptavidin and a monolayer containing 30 mol % of DSPE−EO_{18} at 25 °C. In this system, the volume fraction of EO segments in the brush was 0.17, which is twice as high as in the 9 mol % DSPE−EO_{45} brush. At the ionic strength used, there was no force at D > 40 Å. The onset of the steep steric repulsion at 20 Å agrees with the predicted brush thickness of 25 Å. Similar to the behavior of PEG 2000 (EO_{45}) at 25 °C, the force was repulsive at low compressive loads (<2 mN/m), and the advancing and receding curves were reversible. Nevertheless, the protein adhered to the oligomers with an adhesive force of −0.8 ± 0.1 mN/m (ca. −2kT/protein), at loads greater than 2 mN/m. The surfaces jumped apart from 32 Å (D/L = 1.3). In this case, the critical compressive energy required to induce adhesion, at 23kT/protein, is comparable to that of semidilute DSPE−EO_{45} (Table 2). However, the protein-attractive state of the brush was less stable and relaxed back to the protein-repellant state within 30 min.

The magnitude of the adhesion depends on both the magnitude of the compressive force and the time that surfaces were kept in contact (Figure 5). This behavior was also temperature-dependent. At 37 °C (Figure 5B), the maximum adhesion of −0.8 mN/m was achieved within 1 min at all loads investigated, while it required more than 3 min of incubation under the same load at 25 °C (Figure 5A). At 37 °C, the onset of attraction also occurred at lower loads.

With a 50 mol % PEG 750 lipid monolayer, the interactions between PEG and streptavidin were qualitatively the same. However, at 50 mol % PEG 750 the onset of adhesion occurred at the lower compressive loads of 2.6kT/protein and 1.7kT/protein, at 25 and 37 °C, respectively (Table 3).

The force curves between streptavidin and a monolayer of 50 mol % DSPE−EO_3 (PEG 120 at 86 Å^2/chain) are shown in Figure 6. With these short, densely packed chains, the volume fraction of the EO segments in the brush was 0.29, which is 3 times higher than either 9 mol % PEG 2000 or 10 mol % PEG 5000 brushes. The long-ranged, exponentially decaying repulsive force at D < 50 Å is well described by the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory.[71,73] The best fit of the data to DLVO theory was obtained with constant electrostatic surface potentials of −54 ± 2 mV (−16 mC/m^2) and −32 ± 2 mV (−22 mC/m^2) for the streptavidin and PEG 120 surfaces, respectively. At D < 12 ± 2 Å, the force increased steeply due to the steric repulsion between the protein and ethylene oxide segments. Interestingly, although the interactions were initially repulsive, the streptavidin adhered to the chains when the applied force exceeded 6 mN/m. This does not reflect the load on the polymer directly, since there is substantial electrostatic double-layer repulsion at this distance. It does indicate the facility with which the protein and oligomers bind. The initial adhesion was −0.5 mN/m (−1.4kT/protein), but this increased with the applied load up to the maximum of −1 mN/m (−3kT/protein) at a load of 20 mN/m (Figure 7, Table 3).

With PEG 120 at this density, the magnitude of the adhesion was independent of the time in contact. Additionally, the PEG 120 oligomers converted back to the

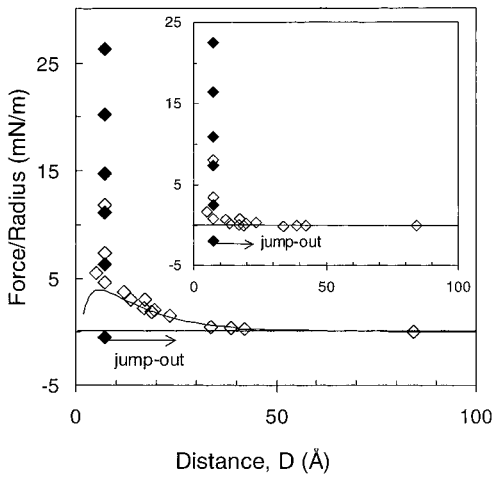

Figure 6. Force profile between streptavidin and PEG 120 (50 mol % DSPE−EO_3) at 25 °C. The experimental conditions are given in the text. The solid line through the data shows the fit of the long-ranged force to DLVO theory, with the best fit parameters of −54 ± 2 and −32 ± 2 mV for the electrostatic potentials of the streptavidin and PEG 120 bilayer, respectively. The outward directed arrow indicates the adhesion between the surfaces. The inset shows the force profile obtained after subtraction of the DLVO force.

protein-repellant state faster than either PEG 750 or PEG 2000. That is, after inducing the adhesion, the layers again repelled when they were brought back into contact within 1 min after separation. At 37 °C, the behavior of the system was qualitatively the same, with the only difference being that the maximum adhesion was achieved at lower loads of ∼10 mN/m (Figure 7, Table 4).

Forces between Lipid Bilayers and Both Streptavidin and PEG Brushes. In control experiments, we measured the forces between (1) streptavidin and bare DSPE monolayers and (2) bare DSPE monolayers and PEG brushes. Between streptavidin and DSPE bilayers, the advancing and receding force curves were identical, and the forces were repulsive. The long-distance repulsive force between the surfaces was well described by DLVO theory[71,73] (data not shown), with the best-fit potentials for both the protein and bilayer surfaces of −54 ± 2 and 0 ± 2 mV. These values agree with previous reports.[63,64] At D < 10 Å, we measured a repulsive force, which is due to the steric hydration layers on both the protein and membrane surfaces. There was no measurable attraction between the streptavidin and bilayer membrane at any separation and no evidence of protein denaturation at compressive pressures up to 50 atm.

To test whether chain compression could induce the behavior observed with streptavidin, forces were measured between bare DSPE monolayers and both 4.5 mol % DSPE−EO_{45} and 10 mol % DSPE−EO_{114}. The measured forces between them were repulsive at all separations (data not shown). Even the application of loads up to 50 mN/m did not alter the force−distance curves.

Forces Between Identical Polymer Layers Are Not Attractive. To determine whether high compressive loads might induce attraction between grafted PEG layers, we conducted force measurements between identical 4.5 or 9 mol % PEG 2000 monolayers and between identical 10 mol % PEG 5000 monolayers. Even when the polymers were held in contact under a load of up to 30 mN/m for 2 h, they did not adhere.

The force curves between identical 50% PEG 120 monolayers are shown in Figure 8. A long-range electrostatic repulsive force dominated the force profile at D < 100 Å. The repulsive force increased steeply at D < 20 Å due to the steric repulsion between the EO_3 headgroups. The best fit of the long-range part of the curve to the DLVO theory was obtained with a constant surface potential of −45 ± 2 mV (−22 mC/m^2). The electrostatic origin of the long-range force was further verified by the substantial increase in the electrostatic surface potential measured between pure PEG 120 layers (no DSAP) and by the ionic strength dependence of the decay length (data not shown). Only fully reversible, repulsive interactions were measured, and there was no adhesion.

Discussion

The measured attraction between streptavidin and grafted PEG layers was clearly due to protein−polymer adhesion in all cases reported in this study. First, all the measurements were conducted at forces much too low to denature streptavidin. Second, neither the polymer compression by a bare bilayer nor the extended incubation with a second polymer layer resulted in similar adhesion or changes in the force curves. Third, although the double-layer force between streptavidin and 1.5 or 4.5 mol % PEG 2000 lipid monolayers is attractive at D < 15 Å, the double-layer force was repulsive at the positions of the adhesive minima.

How do these findings bear on our understanding of PEO interactions with protein solutions? The activation energy for binding and the adhesion energy per protein are two parameters, estimated from these data, which are particularly relevant to protein interactions with PEO. The first will influence the kinetics of the protein adsorption[73] and the second will govern the equilibrium adsorption behavior.[13] Within the context of current views of PEG−protein interactions, the behavior of systems in which the magnitude of the adhesion energy is <1−2kT/protein or which exhibit an activation barrier of >2kT/protein should be well described by the simple polymer models.

These force measurements show that under many conditions the measured interactions agree with the predictions of current theories.[13,17,20,23,32,44,74] Adsorption will occur when the attraction between the protein and surface U_{ads} < −1kT.[13,73] In the majority of cases reported here, the adhesion energy per protein is greater than −2kT. In only a few cases does the magnitude exceed 2kT. Under such conditions, we expect to detect increased protein deposition, if the activation barrier for binding is not too high.

It is important to note that the adhesion energies in Tables 1−3 strictly apply only to streptavidin and that the magnitudes will depend on the protein's identity. Previous studies suggest that more hydrophobic proteins would adhere more strongly than hydrophilic ones.[39] Additionally, adsorption studies show differences in the adsorbed amounts of different proteins on PEG films.[23] Despite such variations, we do expect the qualitative trends in PEG behavior to be similar to those reported here.

Even if the PEG−protein adhesion is substantial, if the activation barrier is high relative to kT, adsorption will be kinetically limited.[13,44,73] For some systems investigated (Tables 1−3), the measured activation energies were significant (>2kT/chain or >10kT/protein). In these cases, the protein adsorption kinetics should be too slow to be of any consequence. However, the magnitude of the activation energy depends on several variables. We have shown that merely raising the temperature or changing

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Table 4. Streptavidin Interactions with PEG 120 Monolayers

surface concn of DSPE−EO_3 (mol %)	PEG layer thickness[a] (Å)	pull-off distance (Å)	F_{adh}/R (mN/m)	adhesion energy (kT/protein)	compressive energy, E^{comp},[b] (kT/protein)
		T = 25 °C			
50 (brush)	15 ± 6	8 ± 5	−0.3 to −1.2	−0.6 to −2.6	2.6
		T = 37 °C			
50 (brush)	13 ± 5	10 ± 5	−0.4 to −1.2	−0.8 to −2.3	1.7

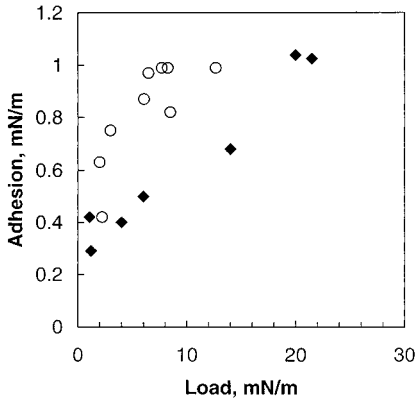

Figure 7. Dependence of the adhesion between PEG 120 (50% DSPE−EO_3) and streptavidin on the applied load, at T = 25 °C (open circles) and at T = 37 °C (filled triangles).

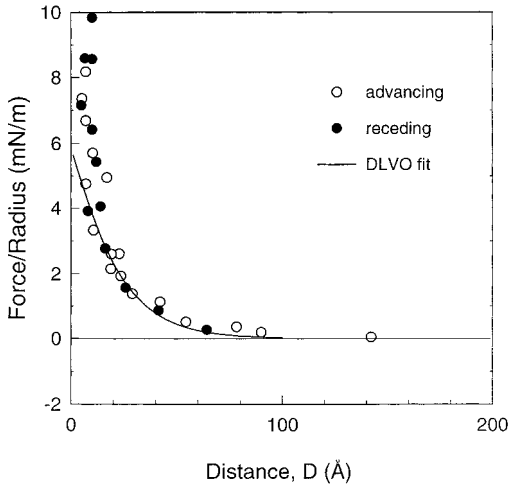

Figure 8. Forces measured during approach (open symbols) and separation (filled symbols) of two identical 50 mol % PEG 120−lipid (DSPE−EO_3) monolayers. The measurement was performed at pH 7.0 and 25 °C in 10 mM NaH_2PO_4 and 30 mM KNO_3. The best fit of the long-ranged, exponentially decaying part of the curve to DLVO theory was obtained with a constant electrostatic surface potential of −45 ± 5 mV for the PEG 120 membranes. The deviation from theory at D < 20 Å is attributed to steric repulsion between the EO_3 headgroups.

the molecular weight or grafting density can substantially reduce or eliminate the energy barrier.

The temperature dependence of the force data is consistent with the temperature dependence of protein adsorption on PEG brushes.[30,31,75] For example, at 25 °C the streptavidin−PEG 2000 (EO_{45}) adhesion energy is ca. −2kT per protein, but the activation barrier to binding is substantial. These same monolayers repel bovine serum albumin (BSA) and bovine pancreatic trypsin inhibitor, and weakly adsorb fibrinogen.[23] At 37 °C, however, the activation barrier is abolished. Correspondingly, fibrinogen adsorption onto grafted PEG 1900 reportedly increases significantly over this temperature range.[31]

The low activation energy for adhesion to the short EO_3 chains was surprising in light of previous reports.[60,76] Despite this, the magnitude of the adhesion energy is low, being on the order of −0.5 to −2kT. These films would therefore not support significant protein adsorption, except perhaps by hydrophobic or denatured proteins.[39] However, long-ranged forces between proteins and the underlying surface could cause protein deposition on surfaces coated with short ethylene oxide chains.[13] Both the measured double-layer force and the calculated van der Waals force extend beyond the 20 Å range of the steric barrier (Figure 8). Proteins could therefore undergo weak secondary adsorption at the outer edge of the brush.[13,59] In agreement with this prediction, a recent study showed that endothelial cells adhere to and form focal contacts on EO_3-terminated alkanethiols on gold.[61] However, this adhesion was abolished when longer chains, that is, thicker films, were used.

An examination of the activation energies in Tables 2 and 3 indicates that at the high grafting densities the barriers also depend on the polymer molecular weight. For short chains, the barrier is low, but it goes through a maximum at MW 2000, and then vanishes at MW 5000. Currie et al.[30] similarly reported that BSA adsorption depends on the chain length. With relatively short chains, protein adsorption decreases monotonically with increasing grafting density, as predicted by simple polymer theories.[20,21,23,30] However, at higher molecular weights BSA adsorbed to the PEG films. This indicates the presence of direct protein−polymer attraction, and the behavior was successfully modeled by including this attractive interaction.[30] It is intriguing that shorter chains behave as noninteracting polymers, but higher molecular weight PEG displays the properties we describe. There is currently no explanation for this. The onset of detectable BSA−PEG attraction occurred with n > 148. In this work, streptavidin readily bound to EO_{114}. The molecular weight at which this transition occurs may, however, depend on the polymer−protein attraction, which will vary with the protein. This still needs to be explored.

The majority of models for protein−PEG brush interactions emphasize the repulsion between the protein and polymer.[13,15,17,74] They therefore do not account for (1) protein−PEG adhesion, (2) the interconversion between protein repulsive and attractive states, (3) the temperature-dependent adhesion, and (4) the dependence of the adhesion on the PEG MW and grafting density.[23,25,26,30−32,39,46,75] Two groups incorporated explicit, attractive protein−polymer interactions.[30,74] This modification can account for some of our observations, as well as those of others, but it does not explain the temperature and MW dependence of the attraction or the apparent interconversion between two states of the polymer brush.

Alternatively, we can rationalize several of our findings qualitatively with two-state models for grafted PEG chains.[51−55,57] Björling and coauthors[52,53] proposed that PEG segments interconvert between polar, trans−gauche−trans (tgt) and nonpolar, gauche configurations, such as

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observed experimentally.[45,50] The different conformers are predicted to segregate within the brush, with nonpolar segments concentrated near the surface and polar segments at the outer edge.[52,53] Halperin,[54] on the basis of n-cluster theory,[55,56] similarly predicted this phase separation within the brush. Both models can qualitatively account for the pressure-induced onset of PEG−protein adhesion, if the protein selectively binds the (nonpolar) inner phase but not the (polar) outer phase. Compression would increase the segment volume fraction in the brush and favor the nonpolar, inner phase.[54] If proteins selectively bind the inner phase, then compression would induce adhesion, once the proteins contact the nonpolar segments.[54,59] This may explain the greater adhesion between streptavidin and more dilute chains (ref 32 and this work), since streptavidin can penetrate the polymer layer and contact the inner segments. The temperature-dependent increase in protein−PEG adhesion is also consistent with this picture, if the protein-attractive phase is favored at high temperature.[77] Spectroscopic studies of EO_3 monolayers indeed showed that the segment equilibrium shifts in favor of the higher energy, protein-attractive all-trans conformer at higher temperatures.[45,50]

The structures and interfacial properties of OEG chains suggest that the interconversion between protein-attractive and protein-resistant PEG is due to segment rearrangements in the polymer chains. The increased protein adhesion and adsorption[31,75] at higher temperatures may be due to a temperature-dependent increase in all-trans conformers in the brush.[45,50] This would reduce the solvent quality at higher temperatures, as observed experimentally, since it increases the population of hydrophobic conformers in the polymer. Such changes would also increase the polymer−protein attraction.[39,52,53] Wang et al.[77,78] calculated the energetics of the different OEG conformational states. Although the measured interconversion rates are somewhat faster than would be predicted on the basis of the calculated energy barriers between the different conformations,[77] helical EO segments convert rapidly to an amorphous state when placed in water.[47] The time scales reported here are consistent with the latter experimental results. The distribution of segment configurations does not, however, appear to be governed by simple Boltzmann statistics since (1) the properties depend on the molecular weight and (2) the chi parameter of PEG depends on the segment density. How these properties influence the distribution of segment conformers within a PEG brush has not been established.

Despite the qualitative agreement between our data and two-state model predictions, the latter do not account for several additional observations. First, they do not predict the maximum in the activation barrier versus molecular weight. Second, at higher temperatures, the outer segments of both 2000 and 5000 MW chains are attractive rather than repulsive. This contrasts with predicted behavior.[13,52,53] Finally, these equilibrium descriptions do not address the dynamics of interconversion. The two-state models embed known characteristics of EO segments and explain some deviations from simple polymer behavior. However, they are clearly not sufficiently developed to account for the complexity of PEG interactions with proteins.

In conclusion, this study explored several parameters that affect the attractive and repulsive interactions between proteins and grafted PEG chains. Despite the apparent contradiction between some of our results and the assumptions of current theories, our results are consistent with other experimental findings. Importantly, we have shown that changes in the molecular weight, temperature, and polymer coverage can alter the interactions in ways that lead to deviations from current predictions of protein−PEG interactions. The latter findings are supported by independent protein adsorption studies. Currently, there is no theoretical model that adequately describes the range of PEG behavior reported by us and by other groups. However, direct force measurements can quantify how various parameters affect the interactions between PEG brushes and proteins and thereby dictate measured adsorption behavior.

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