Imparting Nonfouling Properties to Chemically Distinct Surfaces with a Single Adsorbing Polymer: A Multimodal Binding Approach

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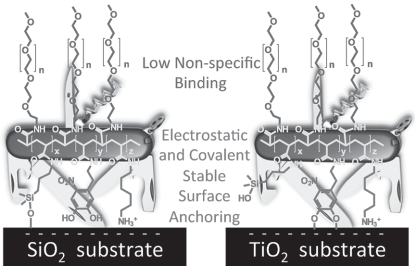
Surface-active polymers that display nonfouling properties and carry binding groups that can adsorb onto different substrates are highly desirable. We present a postmodification protocol of an active-ester-containing polymer that allows the creation of such a versatile platform. Poly(pentafluorophenyl acrylate) has been postmodified with a fixed grafting ratio of a nonfouling function (mPEG) and various combinations of functional groups, such as amine, silane and catechol, which can provide strong affinity to two model substrates: SiO_2 and TiO_2. Adsorption, stability and resistance to nonspecific protein adsorption of the polymer films were studied. A polymer was obtained that maintained its surface functionality under a variety of harsh conditions. EG surface-density calculations show that this strategy generates a denser packing when both negatively and positively charged groups are present within the backbone, and readily allows the fabrication of a broad combinatorial matrix.



Biomaterials that can resist the nonspecific binding of biological entities such as proteins, bacteria, fungi, or cells are unquestionably of both interest and importance. Their broad spectrum of applications can range from biomedical devices and implants, biosensors or drug-delivery approaches[1–3] to water-purification systems,[4] marine installations (ship hulls, fishing nets, or any other underwater structure)[5,6] to packaging and equipment in the food industry.[7,8] The most promising nonfouling behavior in coatings has been achieved through the use of certain uncharged hydrophilic or zwitterionic polymers,[9–11] that are able to resist the first step in biofouling, which is the nonspecific adsorption of proteins and other macromolecules. The criteria for polymers being nonfouling include the existence of hydrogen-bond acceptor and polar functional groups, and the absence of net charge or hydrogen-bond donor groups.[12,13] Another common feature of this type of coating is the strongly bound hydration layer that is generated at the surface. This occurs due to hydrogen bonding with water molecules for hydrophilic materials or electrostatic interactions for zwitterionic ones,[14] creating both a physical and an energetic barrier to protein adsorption. Since a high degree of hydration implies an increased total binding energy for the water molecules, this will also increase the enthalpic penalty necessary to remove them and allow proteins to remove the water layers and adhere.[15]

One highly successful example of such biomaterial coatings is poly(l-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG)—a hydrophilic graft polymer that spontaneously adopts a brush-like conformation upon surface adsorption onto negatively charged surfaces in an aqueous environment.[16] Its highly protein-resistant

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properties have been widely reported and are easily tunable via the chain length of both backbone and nonfouling entity or its grafting density.[16–20] However, an important limitation of this system is its surface-binding mechanism. The purely electrostatic interactions (Coulombic forces), which are essential for the PLL backbone to bind to the surface and allow the PEG chains to adopt a brush-like conformation, are sensitive to variations in both pH and ionic strength. This compromises the stability of the coating under certain conditions, and limits its use in various applications. A broad range of chemical functionalities can provide specific adhesion to (oxide) surfaces, as has recently been reviewed,[21] but most self-assembly systems carry just one of these functionalities—mainly due to synthetic or conformational limitations. The ability to achieve stabler, more durable brush-like or hydrogel-like layers, by means of polymers that can bind to a wide variety of different substrates, under a broad range of conditions, is a continuing and worthwhile challenge in biomaterials.

With the goal of meeting this challenge, we have developed a flexible platform for readily synthesizing brush-forming polymers with improved stability, thanks to the presence of different surface binding groups (electrostatic and covalent) that are targeted at different substrates. In the first example, we have targeted SiO_2 and TiO_2 and to this end postmodified a reactive backbone (poly(pentafluorophenyl acrylate)—pPFPAc), with the following chemical functionalities: (i) amine-functionalized PEG, to impart water solubility and protein resistance, (ii) N-boc-hexanediamine as a long-range electrostatic component, suggested to be crucial for polymer adsorption in the right conformation,[22] (iii) nitro-catechol groups, to form covalent/coordinative bonds to titanium oxide,[23] and (iv) silane-based groups, to form covalent bonds to silicon oxide (see Scheme 1 for the reaction conditions).

The desired final polymer should have the following properties: its surface adsorption should occur through self-assembly from a dilute aqueous solution, should bind multimodally on two model substrates, SiO_2 and TiO_2, and should provide protein resistance to the substrates after being challenged by different harsh chemical treatments (salt, pH, detergents).

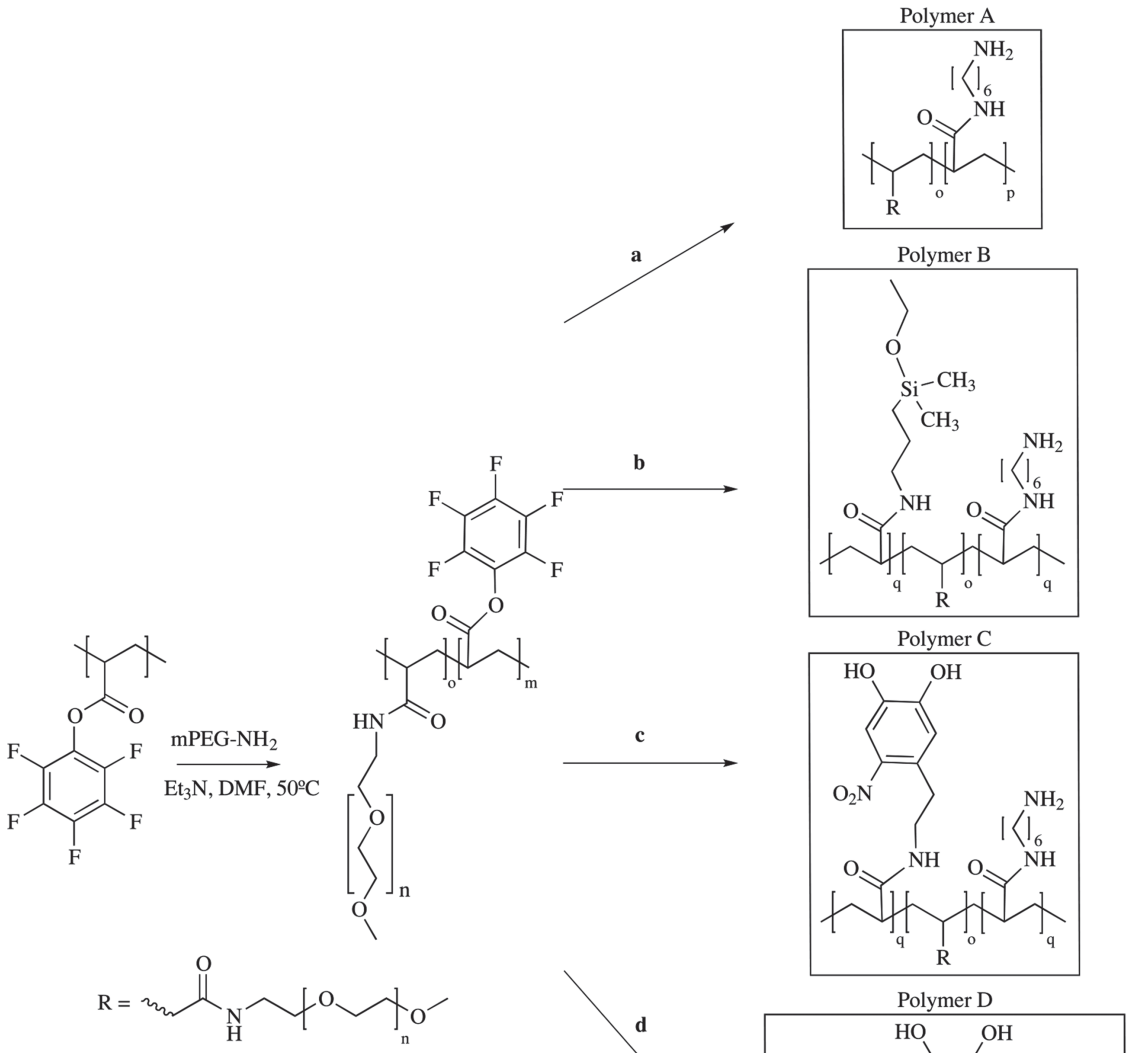
As for comparison with the PLL-*g*-PEG system, it was necessary to first determine the optimal grafting density of PEG at which this polymer would assume a brush-like conformation upon adsorption and thus achieve nonfouling behavior. For that purpose an analog of a molecule, known to be effective at inhibiting fouling: PLL(20)-*g*[3.5]-PEG(2), was synthesised, where the numbers refer to PLL MW (in kDa), grafting density (PEG chains per lysine), and PEG MW (in kDa), respectively (see Figure 1).

It has been shown that, for PLL-*g*-PEG, the grafting density, *d*PEG, that provides the lowest protein uptake from human blood serum and least cell attachment lies between 0.25 and 0.33. If the grafting density lies outside this interval, the antifouling performance is compromised as a result of lower surface PEG density.[24] After considering the different lengths of both backbone repeat units (acrylate vs lysine), an equivalent grafting density of 0.20 for the PAA-*g*-PEG architecture was estimated. A series of poly(acrylamide)-*g*-(PEG, 1,6-hexanediamine) polymers with different grafting densities around the estimated value ($0 < d\text{PEG} < 0.23$) was then synthesized in order to verify the influence of surface charge and PEG density on polymer adsorption, and the resulting reduction of nonspecific protein adsorption. Silicon oxide and titanium oxide surfaces were coated with these polymers by immersion in low-concentration aqueous solutions at pH 7.4. The coated samples were then immersed in HEPES II buffer (10×10^{-3} M 4-(2-hydroxyethyl)piperazine-1-(2-ethanesulfonic acid) (HEPES) and 150×10^{-3}M NaCl, pH = 7.4) for ≥ 16 h to test for stability and finally exposed to human serum for 30 min to test for protein resistance. Between each step, ellipsometry was used to assess thickness variations, and the results are shown in Figure 2.

As can be observed for both substrates, in the absence of PEG ($d = 0$) there is a minimum in adlayer thickness and maximum in protein uptake, as expected. As soon as some PEG is grafted on the polymer backbone, the adlayer thickness increases, reaching a maximum when $d = 0.20$ on SiO_2 and $d = 0.15$ on TiO_2. After exposure to the HEPES II buffer for 16 h, the thicknesses decrease (except for $d = 0$) on both substrates. The smallest loss and the thickest remaining layers are obtained with a PEG grafting density of 0.15. This is a consequence of the optimized ratio between binding positive charges and brush-forming PEG chains, leading to the most stable surface interaction. The average protein uptake values are lowest between $d = 0.10$ and $d = 0.20$ for both substrates. Based on these findings, four polymers with a PEG grafting density of $d = 0.15$ and the following combinations of amine, catechol, and silane binding groups were synthesized and tested on both silicon oxide (SiO_2) and titanium oxide (TiO_2) surfaces:

- Polymer A: poly(acrylamide)-*g*-(PEG, 1,6-hexanediamine) (2000:116.2 M_r; 0.15:0.85 *d*);
- Polymer B: poly(acrylamide)-*g*-(PEG, 1,6-hexanediamine, 3-aminopropyldimethylethoxysilane) (2000:116.2:161.3 M_r; 0.15:0.425:0.425 *d*);
- Polymer C: poly(acrylamide)-*g*-(PEG, 1,6-hexanediamine, nitrodopamine) (2000: 116.2:198.2 M_r; 0.15:0.425:0.425 *d*);
- Polymer D: poly(acrylamide)-*g*-(PEG, 1,6-hexanediamine, 3-aminopropyl-dimethylethoxysilane, nitrodopamine) (2000:116.2:161.3:198.2 M_r; 0.15:0.425:0.2125: 0.2125 *d*).

The quoted grafting densities of the four polymers are based on the stoichiometric amounts of reagents used for the synthesis. To be able to assess the resilience of the



a. 1) N-Boc-1,6-hexanediamine hydrochloride
Et_3N, DMF, 50ºC
2) DCM/TFA 4:1

b. 1) N-Boc-1,6-hexanediamine hydrochloride
Et_3N, DMF, 50ºC
2) 3-aminopropyldimethylethoxysilane
Et_3N, DMF, 50ºC
3) DCM/TFA 4:1

c. 1) N-Boc-1,6-hexanediamine hydrochloride
Et_3N, DMF, 50ºC
2) nitrodopamine
Et_3N, DMF, 50ºC
3) DCM/TFA 4:1

d. 1) N-Boc-1,6-hexanediamine hydrochloride
Et_3N, DMF, 50ºC
2) 3-aminopropyldimethylethoxysilane
Et_3N, DMF, 50ºC
3) nitrodopamine
Et_3N, DMF, 50ºC
4) DCM/TFA 4:1

Scheme 1. Synthesis scheme for preparation of the proposed polymer formulations. Et_3N = triethylamine, DMF = dimethylformamide, DCM = dichloromethane, and TFA = trifluoroacetic acid.

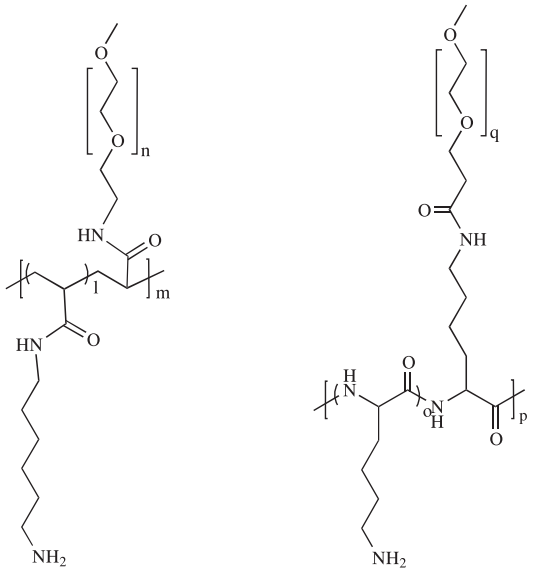


Figure 1. Left: structure of the PAA-*g*-(PEG; hexanediamine) used for the grafting density studies; right: structure of the analogous PLL-*g*-PEG model system.

coatings, film thickness and protein uptake were measured via ex situ ellipsometry following sample exposure to both high- and low-ionic-strength media (NaCl 2 M and 0.16 M) (functionalization and stability test protocols are described in the Supporting Information). Results (see Figure 3) show formation of a polymeric film, whose thickness depends on the type of chemistry used for binding. When in the presence of electrostatic binding groups only (Polymer A—solely positively charged ammonium groups), an initial adlayer is formed on both substrates, as they are both negatively charged at the pH = 7.4 of incubation. Although some thickness loss is measured after 16 h exposure to both low- and high-ionic-strength media, it is noticeable that at low ionic strength the remaining film thickness is ≥1 nm and the protein resistance is maintained. In 2 M NaCl, the electrostatic interaction between the film and the substrate is screened. Upon losing the electrostatic attraction to the surface, the polymers begin to coil and eventually desorb from the surface, leading to a loss of bound polymer and as a consequence also the protein resistance.[25] Concerning the polymers comprising both the electrostatic component and a selective covalent binding group to the surface (Polymer B for SiO_2 and Polymer C for TiO_2), the two behave remarkably well at low ionic strength on both substrates, but at 2 M NaCl the covalent binding group is the determining factor, as it becomes the only linker responsible for retaining the polymeric coating on the surface. Supporting this finding are the protein-uptake results: full resistance of Polymer B is observed on SiO_2 but not TiO_2

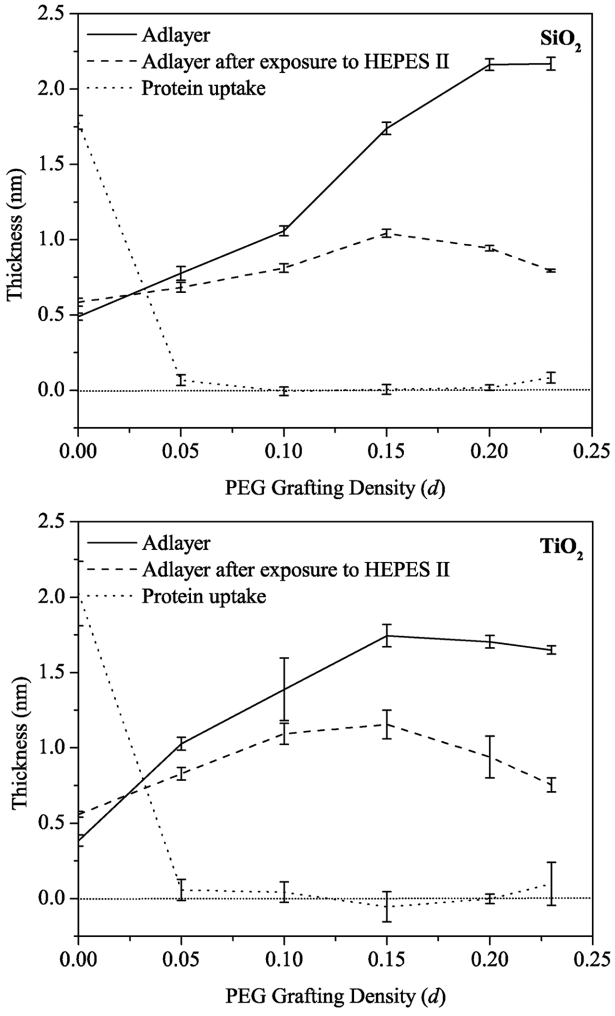


Figure 2. Adsorption, stability (exposure to HEPES II solution for 16 h) and protein-resistance ellipsometry results on silicon oxide and titanium oxide surfaces for six postmodified polymers: poly(acrylic acid)-*g*-(PEG, 1,6-hexanediamine) was synthesized with different grafting densities (0 < dPEG < 0.23). Grafting densities are calculated assuming 100% reaction yield.

while Polymer C demonstrates an analogous behavior by maintaining its resistance on TiO_2 but not SiO_2. Polymer D, having all three adhesive groups grafted to the backbone, resists protein adsorption on both substrates, even after an exposure to the high-ionic-strength medium.

Based on these results, a series of studies was performed in order to assess the stability and protein resistance of Polymer D under different conditions: (i) a cationic surfactant (CTAB), (ii) an anionic surfactant (SDS), and (iii) an acid solution (pH = 2.4) (see the Supporting Information). Polymer A was used as a control.

As can be observed in graphs a and b of Figure 4, Polymer A's exposure to a cationic surfactant (CTAB) has a larger effect on the adlayer on SiO_2 than on TiO_2. In the first case the polymer thickness obtained after the

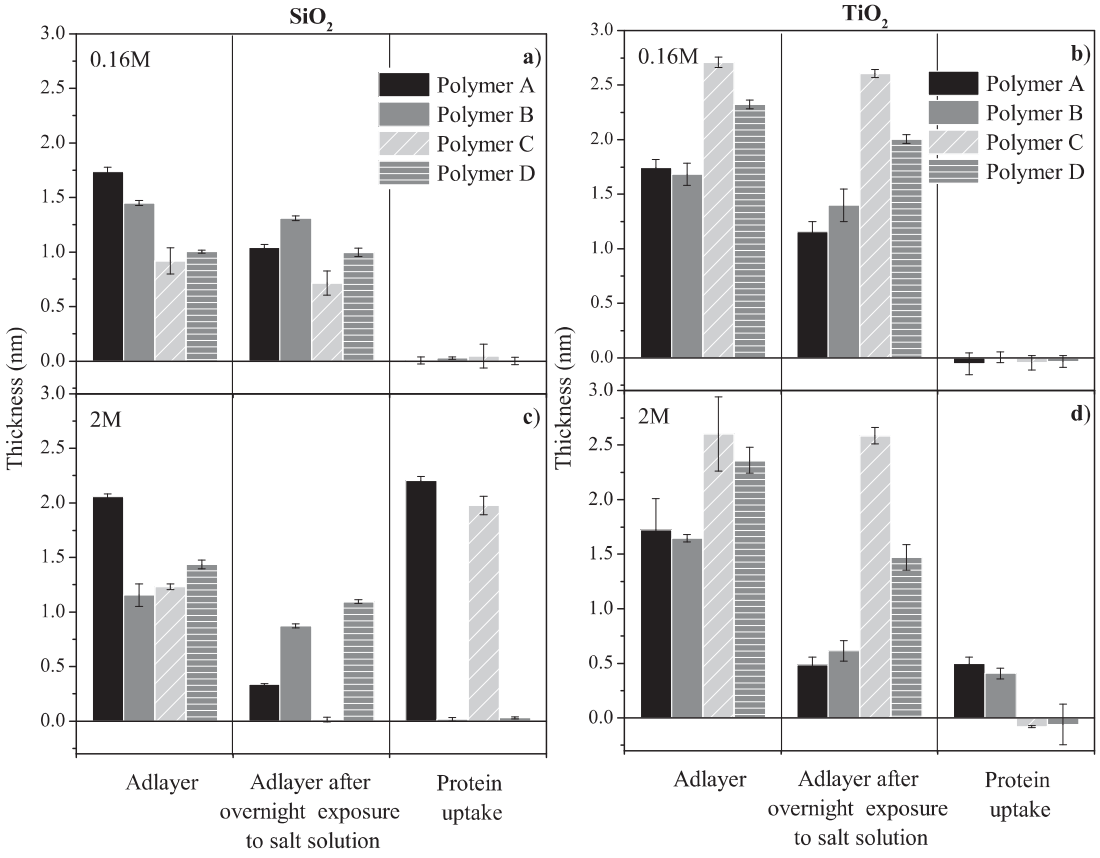


Figure 3. Adsorption, stability (exposure to salt solution overnight), and protein-resistance results on silicon oxide and titanium oxide surfaces for four postmodified polymers: polymer A, polymer B, polymer C, and polymer D. The graphs a,b) show the results for the polymeric coatings when exposed to a low-ionic-strength medium (HEPES II 0.16 M) during the stability-test step for SiO_2 and TiO_2 substrates, respectively, while in graphs c,d) the coatings were exposed to a high-ionic-strength medium (2 M NaCl).

test was below 1 nm, which translated into subsequent protein uptake, while in the case of titanium oxide the thickness before and after CTAB exposure did not differ significantly, allowing the coating to maintain its protein resistance. The loss of polymer may be due to the higher mobility and the positive charge of the surfactant molecules, which may easily displace the amine groups in the polymer backbone from the negatively charged SiO_2. This is less marked in the TiO_2 case because of the low net charge (it is near to its isoelectric point) when exposed to the CTAB solution. The negative protein uptake peaks shown in Figure 4 graphs b and f further indicate that under those specific experimental conditions, thickness loss of the polymeric films is still being observed, although not to an extent that compromises their ability to resist protein uptake.

In the case of SDS (see Figure 4 graph c and d), the Polymer A results show a considerable decrease in thickness on both substrates (again more pronounced in the SiO_2 case), which explains the protein uptake. In this case, the cationic polymer adlayer is now removed from the substrate by the anionic surfactant. Although the polymer layer is just bound electrostatically to both metal surfaces, it is clear that in the two cases the layer structure is more stable on TiO_2 than on SiO_2.

However, when Polymer D on SiO_2 or TiO_2 is exposed to the two surfactants, the graphs a–d in Figure 4 clearly show that the stability of the polymeric coating is not compromised and it maintains its protein resistance. This is due to the covalent bonds formed (silane on SiO_2, nitrodopamine on TiO_2), which prevent significant polymer desorption from the surface during surfactant exposure.

The results of the influence of pH on stability/desorption of these polyelectrolytes are displayed in Figure 4 graphs e and f. The data presented reveal that both polymer combinations on both substrates suffer a reduction in their absolute thickness after exposure to the acid solution. Nevertheless, if an adlayer of ≥ 1 nm remains after the stability test, as is the case for Polymer D on both substrates and Polymer A on TiO_2, protein resistance is maintained. This confirms the importance of having a combination of electrostatic and covalent binding to both stabilize and maintain the nonfouling ability of the coating.

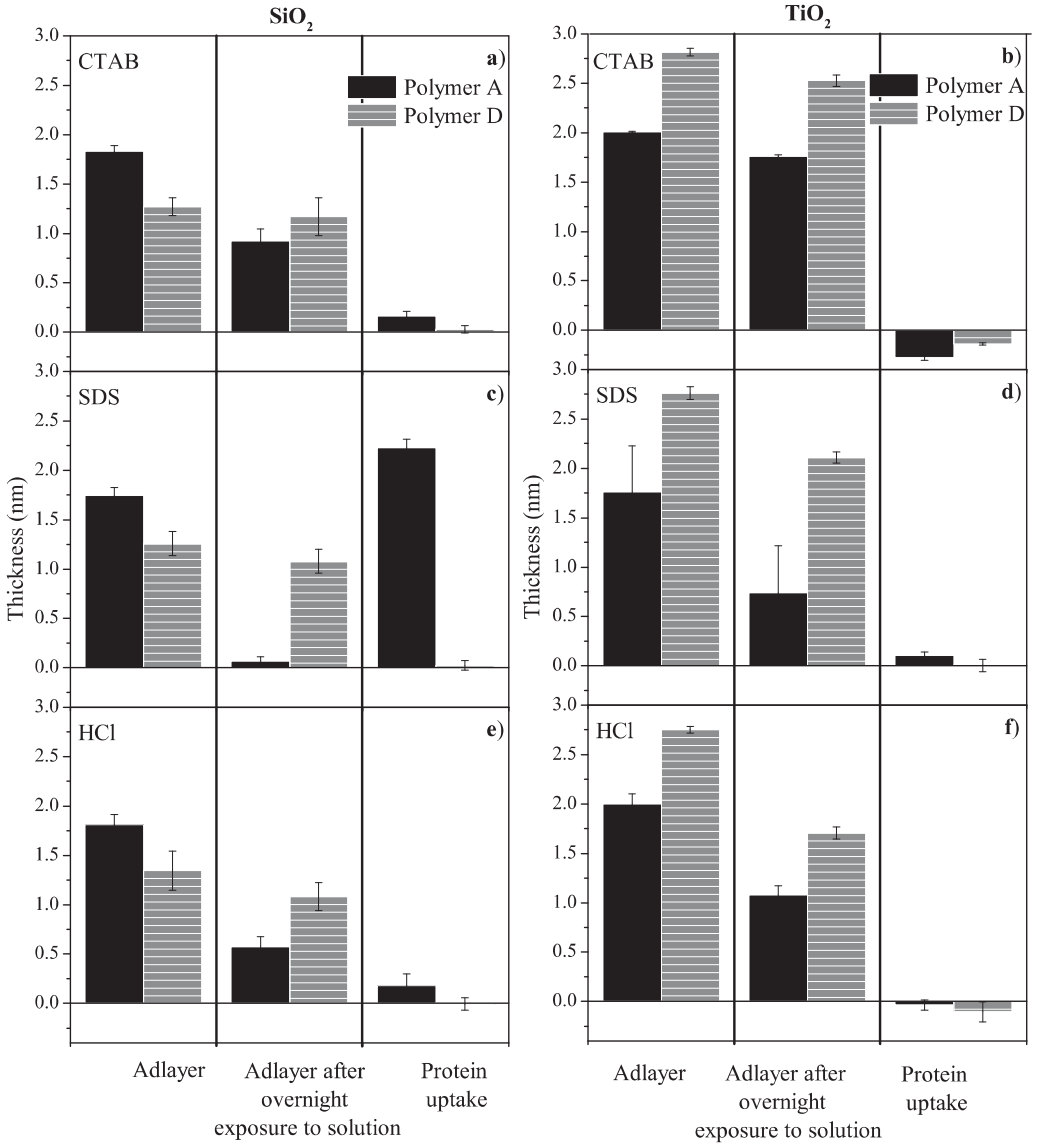


Figure 4. Adsorption, stability (exposure overnight to test solution), and protein-resistance results for polymers A and D.

Further analysis of the data also suggests some interesting observations with respect to the initial thicknesses obtained. The differences in these values, which correlate either with different polymers themselves and/or between substrates, are a strong indication that the polymer conformations are far from being similar. In fact, denser packing leads to further stretching of the PEG chains, resulting in overall higher thicknesses, and, vice versa—a slightly more coiled conformation caused by a lower polymer surface density generates lower thicknesses. This is an indication that a different type of surface packing is involved in these two extreme cases, as indicated by the calculated values of surface density of PEG shown in Table 1.

In the SiO_2 example, a higher surface-grafting-density regime is found when more positively charged amines are present in the backbone (Polymer A vs Polymers B, C, and D). This behavior can be due to charge repulsion between the negative charges present on the covalent surface binding groups and the substrate, which compromises the amount of polymer adsorbed. In the case of nitrodopamine, at the incubation pH each of these entities possess one negatively charged oxygen (as their

Table 1. Initial PEG-surface-density values.

	PEG surface density σ (chains nm^{-2})	
	SiO_2	TiO_2
Polymer A	0.55	0.55
Polymer B	0.44	0.51
Polymer C	0.25	0.73
Polymer D	0.35	0.67

pKas are below 7.4) and in the case of the silanol groups, the same effect would be observed. However, this trend is not observed in the TiO_2 case, as the nitrodopamine will bind covalently to the surface as soon as it is driven toward it and a higher PEG surface density results than in all other studied cases (Polymer C and D vs Polymer A and B). In this scenario, we hypothesize that the nitrodopamine's negative charge is randomly surrounded by the positive amines, already creating a more compact polymer structure in solution than the linear stretched conformation, due to charge attraction. This packed conformation then allows for a larger amount of adsorbed polymer, leading to a consequently greater stretching of the PEG,and thus a thickness increase. As for why this adsorption is still possible despite the theoretical zero net charge of both these polymeric designs, there are two possible explanations. One relates to the probability of the polymers rearranging themselves in a way that the charges would be cancelled out and no longer be available to drive the polymer to the surface. This is very unlikely due to the stiffness of the alkyl chain present in the backbone and/or steric hindrance. The fact that it is a random copolymer also contributes to our lack of knowledge as to how these charges are distributed along the backbone, which leads us to the other potential explanation: the pKa values. The value obtained for the single nitrodopamine molecule not grafted to a polymer is pKa1 = 6.3.[23] Once this group is added to the backbone it may or may not be surrounded by the positive charges of the amines, which affects differently the acidity of the hydroxyl protons. This is one of the reasons why there is not a sharp transition in acid/base titration curves of polyelectrolytes.[26]

In summary, with the results presented in this work we have demonstrated that the adsorption, stability, and nonspecific protein-resistance behaviors of the synthesized polymers were well predicted by the rationales used for their chemical design. The combinations that contained both positively charged entities and groups that bind covalently to the model surfaces used, prevent the irreversible desorption of the electrostatically bound groups, while still retaining their nonfouling abilities under various conditions. This additional feature also allowed for a denser packing to be formed on the surfaces under mild conditions (room temperature, physiological pH and low ionic strength), providing these groups contained partially negatively charged monomers. By adding groups that targeted different chemistries, a multipurpose binding polymer was obtained (Polymer D) that still maintained its surface functionality on both silica and titania under various harsh conditions. Moreover, the postmodification possibilities of the PFPAc backbone according to our protocol are virtually limitless and only require the existence of a charged group. We suggest that besides the nonfouling function of PEG, one could graft other entities (e.g., polymers, fluorescence markers, single-stranded DNA fragments, or antibodies), and as far as anchors are concerned, an analogous approach could be employed simply by adding anchors with chemistries specific to different types of substrates.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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