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Temperature-activated PEG surface segregation controls the protein repellency of polymers

N. Sanjeeva Murthy[†], Wenjie Wang[§], Sven D. Sommerfeld[†], David Vaknin[§], Joachim Kohn[†]

[†]New Jersey Center for Biomaterials, Rutgers - The State University of New Jersey, Piscataway, NJ 08854, U.S.A.

[§]Ames Laboratory, Iowa State University, Ames, IA 50011

Abstract

Poly(ethylene glycol) (PEG) is widely used to modulate the hydration states of biomaterials and is often applied to produce non-fouling surfaces. Here, we present X-ray scattering data which show that it is the surface segregation of PEG, not just its presence in the bulk, that makes this happen by influencing the hydrophilicity of PEG-containing substrates. We demonstrate a temperature-dependent trigger that transforms a PEG-containing substrate from a protein-adsorbing to a protein-repelling state. On films of poly(desaminotyrosyl-tyrosine-co-PEG carbonate) with high (20 wt %) PEG content, in which very little protein adsorption would have been expected, quartz crystal microbalance (QCM) data showed significant adsorption of fibrinogen (Fg) and bovine serum albumin (BSA) at 8 °C. The surface became protein-repellent at 37.5 °C. When the same polymer was iodinated, the polymer was protein adsorbent, even when 37 wt % PEG was incorporated into the polymer backbone. This demonstrates that high PEG content by itself is not sufficient to repel proteins. By inhibiting phase separation either with iodine or by lowering the temperature, we show that PEG must phase separate and bloom to the surface in order to create an antifouling surface. These results suggest an opportunity to design materials with high-PEG content that can be switched from a protein-attractant to a protein-repellent state by inducing phase separation through a brief exposure to temperatures above their glass transition temperature.

Keywords

Protein adsorption; PEG; Temperature activated adsorption; Antifouling; Quartz crystal microbalance; X-ray scattering; Langmuir isotherms

Corresponding Authors: kohn@dls.rutgers.edu.

Current address for SDS: Translational Tissue Engineering Center, Wilmer Eye Institute, Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

Author Contributions

N.S.M carried out XRD and QCMD measurement and wrote the paper. W.W and D.V carried out LB and XRD measurements, and reviewed the paper. S.D.S did some of the QCMD measurements, and reviewed the paper. J.K reviewed all the data and contributed to the writing of the paper.

Supporting Information

PDF files of an additional figure and data analyses details.

Notes

The authors declare no competing financial interests.

INTRODUCTION

Tissue response to implanted biomedical devices is determined by the physical and chemical properties of the device-tissue interface.^{1,2} Specifically, the surfaces of blood-contacting devices such as catheters, pacemakers, and stents are designed to resist thrombogenesis and biofilm formation.^{3, 4} These properties are realized by using materials that resist protein adsorption at the interface³. A widely used strategy for inhibiting protein adsorption,⁵ and thereby reducing bacterial and cell adhesion,^{6–8} is to utilize a hydrophilic polymer such as poly(ethylene glycol) (PEG) as coatings,^{8, 9} self-assembled brushes,^{10,11} co-blocks,^{3,12,13} covalent attachments,¹⁴ and surface grafts.¹⁵ PEG-rich surfaces are used for the prevention of biofilm formation on biomedical devices,⁸ to increase the circulation times of drug molecules,¹⁶ and to provide inert surfaces for subsequent activation with appropriate molecular cues.¹⁷

At the molecular level, PEG's protein-repellency arises from the water molecules that are bound to two neighboring ether oxygen atoms on the polymer chain (7_2 helix)^{18, 19} that cannot be readily displaced by a protein.²⁰ However, the macroscopic mechanism by which PEG repels proteins when present as a copolymer in a polymer matrix is not well understood.^{21, 22} PEG's effectiveness in inhibiting protein adsorption depends on its molecular weight, concentration, and the grafting density.¹⁵ The concentration of PEG required for inhibiting protein adsorption decreases with increasing molecular weight of the grafted PEG.¹⁵ Inhibition also depends on graft density, being stronger at low concentrations and weaker at higher concentrations.¹⁵ While some reports claim higher molecular weight PEG leads to greater protein resistance,^{8, 11} surfaces containing as few as two monomer units have been found to be effective.⁹ Short PEG chains were also shown to be able of provide full resistance to bioadhesion.⁷ For a given molecular weight, higher concentrations of PEG have been reported to increase resistance to bioadhesion.¹² The manner in which PEG is bonded to a surface and the distribution of PEG at surfaces have been shown to affect cell adhesion. For instance, covalent binding was more effective in reducing bacterial adhesion,¹⁴ while microstructuring was found to dramatically improve fibroblast adhesion²³.

In order to understand the effects of PEG surface concentration and its surface distribution on protein adsorption, we investigated adsorption of the blood clotting protein fibrinogen (Fg) onto several PEG-containing polymers using quartz crystal microbalance with dissipation monitoring (QCM-D). To confirm the hypothesis that the changes in protein adsorption are due to differences in the distribution of PEG at the polymer surface, we examined the surface structure using X-ray reflectivity (XRR) and grazing incidence wide-angle scattering (GIWAXS) of Langmuir films on aqueous surfaces.²⁴ A tyrosine-derived polycarbonate synthesized from desaminotyrosyl-tyrosine ethyl ester (DTE), (abbreviated as PC) was used as the hydrophobic polymer (Figure 1). A PEGylated version of this polymer, poly(DTE-co-8%PEG carbonate) (PC8%PEG), was used as the hydrophilic polymer.

EXPERIMENTAL

Materials:

The polymers used in this study, derived from poly(DTE-*co*-PEG carbonate) (Figure 1), were synthesized and characterized as described previously.¹³ PEG content in the polymers was confirmed with ^1H NMR. The polymers used in these experiments are listed in Table 1. The non-iodinated homopolymer is identified as PC, and the iodinated version, poly(I₂DTE carbonate), as I₂PC. The respective PEGylated versions are poly(DTE-*co*-PEG carbonate) and poly(I₂DTE-*co*-PEG carbonate), abbreviated as PC-PEG and I₂PC-PEG. Except when noted, the molecular weight of PEG is 1 kDa.

QCMD:

Protein adsorption was measured using the QCM-D apparatus (Q-Sense E4) from Biolin Scientific, Sweden. The polymers were spin-coated at 3000 rpm from a 1.5 to 4 % (w/v) tetrahydrofuran (THF) solution. The concentration of the polymer makes little difference in the protein adsorption results. A fibrinogen (Fg) solution (0.1 mg/ml) in phosphate buffered saline (PBS) was used at a flow rate of 25 $\mu\text{l}/\text{min}$. Changes in frequency (Δf) and dissipation (ΔD) were recorded during the QCM-D measurements. The increase in dissipation was less than 1×10^{-6} per 20 Hz drop in frequency ($\sim 5\%$). Therefore dissipation could be neglected, the adsorbed proteins regarded as rigid and laterally homogeneous, and the Sauerbrey relation (Eqn. 1) can be used for estimating the adsorbed mass (Δm).²⁵

$$\Delta m = -nC/\Delta f; C(\text{mass sensitivity of the crystal}) = 17.7 \text{ ng/cm}^2 \cdot \text{s}^{-1}$$

Small-angle x-ray scattering (SAXS):

SAXS measurements were carried out as previously described.²⁶ Briefly, the data were collected on the 5ID-D beam line of the DND-CAT at the Advanced Photon source, Argonne National Laboratory in transmission mode using X-rays of wave length 1.0332 Å, and a 100 $\times$ 100 mm Roper camera with a sample-to-detector distance of 2897 mm and exposure times of 2–20 s.

Pressure-area isotherms.

The surface pressure (II) - area (A) isotherms were obtained by using a standalone Langmuir trough (NIMA technology Ltd, Coventry, England). Polymer solutions prepared in 3:1 chloroform/methanol mixture were spread on an aqueous medium (PBS) in a Langmuir trough. The π -A isotherms were obtained at a compression rate 20 cm^2 per minute. The isotherms are reported as π - A_{mass} , where A_{mass} (m^2/g) is the interfacial-area occupied by a gram of material at a given surface pressure. This allowed multiple isotherms (produced with distinct spreading concentrations) to be combined and displayed as a single continuous function. Five isotherms for PC8%PEG and six isotherms for I₂PC8%PEG with different amounts of deposited materials were used to construct complete isotherms to overcome the limited compression ratio of the trough. The binning and averaging of overlapping data from

distinct isotherms reduced the measurement error. The averaged data set was splined to produce a smooth Π - A_{mass} isotherm.

XRR and GIWAXS.

X-ray measurements were carried out using synchrotron radiation (X-ray energy $E=13.474$ keV, wavelength $\lambda=0.92$ Å) at beamline 9ID-C, Advanced Photon Source (APS), Argonne National Laboratory. A liquid surface spectrometer (LSS) equipped with a smaller trough than that used for Π - A measurements was used. After spreading the polymer on the Langmuir trough, the trough was sealed and purged with water-saturated helium gas to reduce the scattering from the air and minimize radiation damage.²⁴

The details of the XRR and GIWAXS measurements and analysis of data were described in a previous publication.²⁴ Briefly, scattered intensities were recorded at an exit angle α_f of the X-rays incident on the liquid surface at a grazing incident angle α_i with respect to the surface. For specular XRR, $\alpha_f = \alpha_i$, and α_i is changed systematically from below the critical angle for total reflection, α_c , to above α_c . Reflectivity R at each α_i is recorded as a function of the z -component of the scattering vector, Q_z , where $Q_z = (4\pi/\lambda) \sin \alpha_i$. The reflectivity $R(Q_z)$ is further normalized by the calculated Fresnel reflectivity, R_F , of an ideally sharp and smooth air-water interface. The R/R_F data is used to extract the electron density profile, $\rho(z)$, along the surface normal (z -axis). In this study, an effective-density model²⁷ is used to construct $\rho(z)$ and calculate the reflectivity by a recursive dynamic matrix method.²⁸ For GIWAXS, α_i is fixed just below $0.9\alpha_c$, and the scattered intensity $I(Q)$ is recorded as function of $Q = (4\pi/\lambda) \sin \alpha_f$.

RESULTS AND DISCUSSION

Effect of temperature on protein adsorption:

The changes in the frequency response in QCM-D measurements as the polymer are exposed to Fg are shown in Figure 2. A decrease in the frequency (Δf) in these figures corresponds to an increase in mass (Δm) resulting from protein adsorption ($\Delta m = -nC/\Delta f$; n is the harmonic used in the calculation, and C is the mass sensitivity of the crystal, a constant equal to $17.7 \text{ ng/cm}^2 \cdot \text{s}$).²⁹ Figure 2a shows that both PC homopolymer and PC8% PEG copolymer adsorb Fg when cooled to $< 15^\circ \text{C}$ and then hydrated. When hydrated at 37.5°C (physiological temperature), the homopolymer adsorbs Fg whereas the PEGylated version does not (Figure 2b). Similar results, adsorption at 8°C and repellency at 37.5°C , were also obtained with bovine serum albumin (BSA) (results not shown). Interestingly, there was no protein adsorption when PC8% PEG was cooled to 15°C after being hydrated at 37.5°C (Figure 2c). We also found that protein that was adsorbed at 15°C was not desorbed when the temperature was raised to 37.5°C (Figure 2d) showing the protein adsorption was also not reversible. The sequence of hydration and temperature change did not affect protein adsorption for the other polymers shown in the figure.

Phase behavior in the bulk using SAXS:

Among the factors that contribute to the behavior at the interface, distribution of PEG segments in the polymer is the most likely to affect protein adsorption at the polymer-

aqueous interface.²⁶ Small-angle x-ray scattering (SAXS) was used to understand the influence of hydration on the distribution of PEG segments (Figure 3). PEG is molecularly dispersed in the hydrophobic matrix when dry. Therefore, in the absence of water, the polymers do not show any SAXS interference peak indicating homogeneous distribution of PEG in the polymer (Figure 3a).^{5,26} Upon hydration, at temperatures above the glass transition temperature (T_g , 26 °C; Table 1), an intense interference peak appears at $q = 0.05 \text{ \AA}^{-1}$ (Figure 3b). This peak is attributed to the phase separation of PEG segments.²⁶ In the presence of water, PEG-rich segments become more mobile, phase separate, and entrap water giving rise to hydrated regions separated by $\sim 100 \text{ \AA}$ (Figure 3c). However, the PEG blocks immobilized by the hydrophobic blocks that are anchored to the surface would be expected to limit the phase segregation in the deposited film deposited on QCM-D substrates. But, the spin-coated substrate is 150 nm thick (as measured by the frequency shift in QCM-D before and after polymer deposition), and therefore there can be at least ten layers of PEG-rich domains across the thickness of the spin-coated sample. Investigations of the depth dependency of T_g have shown that the polymer chains near the substrate are constrained, but approach the behavior in the bulk in about 2 nm.^{30, 31} Therefore, we would expect only the first layer of PEG-rich domains, $\sim 10 \text{ nm}$ layer of the film close to the substrate to be immobilized. This effect would dissipate over the $\sim 100 \text{ nm}$ distance between the gold and the aqueous surfaces. Furthermore, 3D network of chains in the bulk anchor the PEG domains in the same way as immobilized hydrophobic chains on the substrate; otherwise there will be macroscopic phase separation, which is not observed. Thus, the phase behavior in the spin-coated film at the polymer-water interface, $\sim 150 \text{ nm}$ from the interface, is similar to that in the bulk and would be expected to bring about protein repulsion.

The QCM-D result that protein adsorption in PC8%PEG observed at 8 °C does not occur if the film is cooled from 37.5 °C can be attributed to irreversibility of the phase separation that occurs at 37.5 °C. Thus, it appears protein adsorption at 8 °C is due to the sample being trapped in a non-equilibrium local minimum. Once the PC8%PEG film is brought out of the local minima to a thermodynamically stable, phase-separated state by hydration and heating from 8 to 37.5 °C, it cannot be taken out of this equilibrium, and surface remains protein repellant. We have tested different thermal/kinetic states of the polymer. For example, we have carried out SAXS experiments on “solvent annealed” samples after equilibration for 48 h.³² We have also done kinetics of phase separation during hydration starting with dry films that show phase separation occurs within a matter of minutes and is complete in 2 h.³³ These results show that spin-coated film behaves like bulk polymer, but not like an annealed polymer.

Experiments with iodinated polymer:

To verify the hypothesis that phase separation causes protein repulsion, an iodinated version of the PEGylated polymer, I₂PC8% PEG, was investigated. Iodine has been known to negate the inhibitory effect of PEG.^{12, 34, 35} Figure 3 shows that the SAXS interference peak is absent in the iodinated polymer, even at 60 °C (Figure S1), indicating that the PEG segments do not phase separate in I₂PC8% PEG as they do in PC8% PEG. QCMD measurements show that in contrast to the non-iodinated polymer, Fg is adsorbed onto the surface of

I₂PC8% PEG at both 8 and 37.5 °C reflecting the absence of phase separation upon hydration (Figure 2). Note that this polymer has the same amount of PEG (8 mol%; Table 1) as the non-iodinated counterpart that did not adsorb protein at 37.5 °C.

The amount of PEG that counterbalances the effect of iodine was estimated by testing a I₂PC polymer with 15% PEG_{2k} (37 wt% or 50 vol%). This polymer, which undergoes phase separation,²⁶ did not show protein adsorption (Figure 4). Since I₂PC-24% PEG_{1k} (34 wt% or 47 vol% PEG) also shows phase separation in SAXS at room temperature,²⁶ it appears that iodine can compensate for the tendency of PEG to phase-separate only at PEG less than about 35 wt% (48 vol%). This effect most likely arises from the increased rigidity of the polymer due to the presence of iodine in the polymer chain in the same way as achieved by lowering the temperature.

Macroscopic Surface Effects of PEGylation in Pressure-area (II-A) Isotherms.

The influence of the PEG on the polymer in the bulk discussed above is expected to have an effect on the surface structure that directly affects protein adsorption. These surface structural features were monitored using Langmuir films on aqueous surfaces. II-A isotherms were obtained to identify the gaseous-like, liquid-expanded and condensed phases of the polymer in the films. Macroscopic effects of PEG in the non-iodinated and iodinated polymers can be deduced from their II-A isotherms shown in Figure 5. The results upon recompression were similar to those during the first compression in both the PEG-containing polymers as in other flexible polymers such as PDLLA.⁴ This was not so in the absence of PEG.⁴ This is most likely because copolymerizing with PEG makes the polymers more flexible.

In PC 8% PEG, there appears a section in the II-A isotherm with an intermediate slope when the pressure is increased (Figure 5). This can be attributed to an intermediate phase in which the PEG segments appear to have reorganized into domains in the aqueous subphase. After what appears to be squeezing out all the PEG segments into the aqueous subphase, a condensed phase appears at the air-water interface, further compression results in a sharp increase in surface pressure.

Iodination alters the mobility of the polymer chains as indicated by the large differences in the II-A isotherms for the non-iodinated and iodinated polymers⁴. This could be because the PEG-water interactions alter the surface free energy.⁹ The polymers, especially I₂PC, reaches condensed phase at much lower pressures with PEG (see GIWXS data, Figure 7).

As the surface pressure is increased, the molecular area decreases by ~42% in poly(PC8% PEG and by ~13% in I₂PC8%PEG. This suggests that PEG changes the spreading behavior by altering the polymer-polymer, polymer-water interactions. These differences can be attributed to the higher stiffness of the iodinated films even in the presence of PEG as also seen by the higher T_g of polymer (Table 1). The DTE segments, which are more flexible, are more tightly packed than the I₂DTE segments, and are also more affected by PEG.

Phase behavior at the interface by XRR:

SAXS data show the phase separation of PEG in the bulk, and says little about the composition at the substrate surface where cell-material interactions take place. XRR measurements were carried out on Langmuir films to understand the influence of phase separation on the distribution of PEG at the surface and on subsequent protein adsorption (Figure 5). Reflectivity (R) normalized to Fresnel reflectivity (R_F), R/R_F is shown for polymers with and without PEG at two surface compression ratios (Figs. 6a and 6d). Electron density profiles were derived from these curves using previously described methods.²⁴ (Tables S1 and S2).

The electron density profiles in Figure 6 show that the data from the self-assembled film in Langmuir trough refers to the structure within the ~ 5 nm surface layer at the polymer-water interface, far less than the ~ 150 nm, thickness of the film used in the QCMD experiments. As discussed earlier (Section “Phase behavior in the bulk using SAXS”), any anchoring effect of the polymer at the substrate-polymer interface would have dissipated within < 20 nm from the substrate. Additionally, data in figures 6b and 6e show that the thickness of the PC film is ~ 20 Å in both the iodinated and the non-iodinated versions. This corresponds to ~ 5 layers of polymer chains. Therefore, the inferences that can be made here about the 5 nm -thick layer at the polymer-aqueous interface, such as the redistribution of the PEG segments, would be applicable to similar interfaces in the film deposited on the substrate in QCM measurements.

The electron density profiles change in the presence of PEG and upon hydration due to the differences in the electron densities of PC, PEG and water (0.416 , 0.369 , and 0.334 e/Å³, respectively). Additionally, as PEG becomes hydrated and swells the polymer, the profiles invariably broaden (Figs. 6b and 6e). There are differences in how the broadening occurs in the non-iodinated and the iodinated polymers. The electron density profiles become more asymmetric upon compression. The air-polymer interface remains relatively sharp and changes little upon compression, while the swelling of the polymer segments into the aqueous subphase extends the polymer-water interfaces 20 to 50 Å into the aqueous subphase in both iodinated and the non-iodinated polymers (Figure 6). Pressure-area isotherms (Figure 5) suggest that the non-iodinated polymers are soft and fluid-like even without PEG.²⁴ In the presence of PEG, an intermediate phase is formed in which the PEG segments are likely reorganized into domains in the aqueous subphase. In contrast, the iodinated films are in condensed phase without PEG that become soft or fluid-like only in the presence of PEG.

In the non-iodinated polymers, the electron density at the air-polymer interface remains unchanged upon PEGylation, and increases at lower pressures (Figure 6b). We attribute this to the migration of PEG segments from the air-polymer interface towards the polymer-water interface. At low pressures, as the air-polymer interface gets depleted of the lower electron density PEG, the electron density due to the higher electron density PC at the interface increases. At higher pressures, pressure-area isotherms show that a condensed phase is formed (unpublished), and XRR shows the maximum electron density for the layer in the direct vicinity of the air-monolayer interface approaches that of PC. The electron density at the air-polymer interphase does not change as PEG migrates towards the aqueous subphase.

Since PEGylated polymers can be considered as polymeric amphiphiles, migration from air-polymer to polymer-water interface is expected.⁵ The formation of this PEG-rich layer at the polymer-water interface contributes to the observed protein-repency. Decrease in protein adsorption to the point of complete protein repency has recently been reported in PEG grafted onto model phospholipid monolayers.³⁶

In the iodinated non-PEG polymer films, the peak electron density remains unchanged as the film is compressed as if the polymer had reached the maximum density, but decreases in the PEGylated version of the polymer (Figure 6e). We attribute this to the intermixing of the lower electron density PEG and water with the higher density I₂DTE segments throughout the thickness of the film. This is true even at lower pressures. This observation is consistent with the isotherm data that shows the iodinated polymer without PEG is in the condensed phase at these pressures, and is in the soft or fluid-like in the presence of PEG. This is also reflected in the increase in the effective thickness of the PEGylated film, which is inversely proportional to the reduction of area upon compression (Table S2). Thus, in the iodinated version, the phase separation similar to that in the non-iodinated polymer either does not occur, or is incomplete, and the exposed I₂DTE segments at the polymer-water interface facilitate the adsorption of the protein onto this polymer.

Structure at the air-polymer interface by GIWAXS:

The above conclusions based on the electron density profiles derived by XRR were confirmed using GIWAXS obtained at both low and high pressures (Figure 7). GIWAXS was used to identify the different components at the surface using their XRD signature (molecular structure).

At low pressures (Figures 7a and 7c), the polymer peak is weak or absent in the non-PEGylated films, suggesting that the air-polymer interface is mostly water. In the non-iodinated PEGylated samples, the polymer peak is intense and the water peak is weaker, indicating that PEG drives the non-hydrated segments to the air-polymer interface. In contrast, in the iodinated PEGylated samples both the polymer and the water peak are more intense. Polymers, especially the iodinated version, reached condensed phase at much lower pressures with PEG than without as seen by the presence of a clear $1.5 \text{ \AA}^{-1}$ diffraction peak. Once the condensed phase is formed, further compression would increase the film thickness, but not increase the peak intensity.

At high pressures (Figures 7b and 7d), the polymer peak is intense in all, PEGylated and non-PEGylated, and the iodinated and non-iodinated, polymers. However, this is the only peak that is clearly visible in non-iodinated polymer, suggesting that the air-polymer interface film is primarily made of the DTE segments. In contrast, there is also the $2 \text{ \AA}^{-1}$ peak from water in the iodinated film.

The peak at $2 \text{ \AA}^{-1}$, which sometimes appears as a shoulder, is from O-O correlations in water clusters,³⁷ corresponding to the average distance between water molecules.³⁸ The peaks at $1.35 \text{ \AA}^{-1}$ in the non-iodinated film and at $1.45 \text{ \AA}^{-1}$ in the iodinated film arise from the inter-chain distance of 4.7 and 4.3 Å, respectively, in the two polymers.^{24, 26} The

interchain separation is smaller in the iodinated polymer due to the higher packing density of chains in I₂PC.^{24, 26}

The only peak that is clearly visible in non-iodinated film is that due to the polymer, suggesting that the air-polymer interface with this polymer is primarily made of the DTE segments. This observation, consistent with XRR data, shows that PEG segments migrate towards the polymer-water interface in the non-iodinated polymer leaving the air-polymer interface rich with hydrophobic DTE, and thus with little water. Therefore, there is no water scattering from the air-polymer interface. Since PEG by itself does not significantly alter the packing of the polymer chains²⁶, loss of PEG from the polymer at the air-polymer interface does not change the polymer interchain distance (i.e., the position of the GIWAXS peak). Migration of PEG segments to the polymer-water interface keeps this interface hydrated, making the non-iodinated polymer protein-repellant. In contrast, there is also the 2 Å⁻¹ peak from water in the iodinated polymer. This observation, consistent with the XRR results, shows that the phase separation is absent or incomplete, and both PEG and DTE segments are present throughout the thickness of the film. As a result, the DTE segments at the polymer-water interface in the iodinated polymer make it protein-absorptive.

Conclusions.

It is possible to design PEG-rich polymers that have a protein repellency switch. These polymers can adsorb protein at low temperatures. A short exposure to higher temperatures (above the glass transition temperature, T_g) causes a phase change that makes the polymer surface protein-repellant. This is a one-way switch since lowering the temperatures below the T_g does not reinstate protein adsorption. X-ray scattering and Langmuir isotherm data show that the presence of PEG is not sufficient for protein repellence; PEG repels protein only when it blooms to the surface and forms a hydrophilic layer. In hydrophilic-hydrophobic copolymers, this surface segregation follows the phase separation of PEG-rich chains. Strong interactions between PEG with the hydrophobic polymer matrix, for instance by iodination of the polymer chain, can preclude this phase separation, and raising the temperature or weakening the PEG-guest polymer interactions promotes this phase separation. Temperature- and composition-dependent processes of the type discussed here provide a means to transform a polymer from protein-adsorptive to a protein-repellant state, thereby imparting antifouling characteristics to polymer surfaces on demand.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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X=H, Poly(DTE -co-PEG carbonate); X=I, Poly(I₂DTE-co-PEG carbonate)

Figure 1.
Chemical structure of the PEG-containing tyrosine polycarbonates.

Figure 2.

Assessing Fg adsorption onto various substrates using QCM-D. (a) 15 °C and (b) 37.5 °C. (c) Substrates hydrated at 37.5 °C before Fg adsorption at 15 °C. The initial decrease in the frequency is due to the shift in the oscillation frequency of the crystal as it was being cooled. For (a-c), PC, which adsorbs protein at both temperatures, was used as a control. The time of Fg injection is indicated by an arrow, (d) Fg was allowed to adsorb onto PC8% PEG and I₂PC8% PEG, and then the temperature was raised to 37.5 °C. The increase in the frequency seen in both instances is due to the thermal dependency of oscillation frequency, and not to any change in the mass. The retention of protein, as deduced from the control sample, I₂PC8% PEG that adsorbs proteins at both temperatures, suggests that the adsorption is irreversible.

Figure 3.

PEG phase separates upon hydration, (a) Schematic of the distribution of PEG segments in the dry polymer, (b) SAXS data from hydrated PC8% PEG and I₂PC8% PEG. (c) Formation of PEG-rich hydrated domains in upon hydration in PC8%PEG at ambient temperatures.

Figure 4.

The protein repellency is not observed at 15% PEG even at 15 °C. Comparison of the QCM data at 15 °C from I₂PC15% PEG_{2k} (blue squares circles) and PC8% PEG_{1k} (red triangles). PC8%PEG_{1k} was used as a positive control. The gradual decrease in frequency in I₂PC15% PEG_{2k} starting from time zero is due to the continuous swelling that is often observed

Figure 5.

Iodinated tyrosine polycarbonate is more rigid than its non-iodinated counterpart. Schematic of the X-ray experimental setup with the Langmuir trough (left). Five isotherms for PC8% PEG and six isotherms for I₂PC8%PEG with different amounts of deposited material were used to construct complete isotherms to overcome the limited compression ratio of the trough. Pressure-area isotherms that show difference in the spreading behavior of non-iodinated and iodinated PCs (right). G- Gaseous, L_E – Liquid expanded, C- Condensed

Figure 6.

PEG blooms to the surface of the polymer upon hydration. XRR data and the derived differences in the phase separation behavior of PEG in PC8% PEG (top panel) and I₂PC8% PEG (bottom panel). Reflectivity curves (a and b) and electron density profiles (c and d) are shown, (e and f) Illustration of the distribution of the polymer chains (red) and PEG (blue) for a phase-separated (e) and non-phase-separated (f) substrates. All experiments were carried out at 20 °C.

Figure 7.

Phase separation changes the surface structure and is influenced by the surface pressure.

Grazing-incidence wide-angle x-ray scattering data for the non-iodinated polymers (a and b) and for the iodinated polymers, (a) and (c) are at low pressures, and (b) and (d) are at higher pressures. The scattering curves from the dry polymer (dashed red line) and water (green) are overlaid on the data for comparison.

Table 1.

Compositions and the (T_g s) of tyrosine-derived polycarbonates used in this study.

Polymer	Abbreviation	PEG content			Wet T_g (°C)
		Mol %	Wt %	Vol %	
poly(DTE carbonate)	PC	0	0	0	64.0
poly(DTE- <i>co</i> -8%PEG carbonate)	PC8% PEG	8.0	19.6	22.1	25.7
Poly(I ₂ DTE carbonate)	I ₂ PC	0	0	0	113.2
poly(I ₂ DTE- <i>co</i> -8%PEG carbonate)	I ₂ PC8% PEG	8.0	12.5	19.9	44.7