Influence of Poly(propylene sulfide-*block*-ethylene glycol) Di- and Triblock Copolymer Architecture on the Formation of Molecular Adlayers on Gold Surfaces and Their Effect on Protein Resistance: A Candidate for Surface Modification in Biosensor Research

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ABSTRACT: Control of nonspecific interactions between bioanalytical surfaces and proteins in the analyte is critical in the design of biosensor systems. Here we explore poly(propylene sulfide-*block*-ethylene glycol) (PPS−PEG) di- and triblock copolymer adlayers on gold to gain such control. Chemisorption of the PPS block permits a simple dip-and-rinse coating process. We synthesized different architectures of di- and triblock copolymers, varying the molecular weight of PEG between 1.1 and 5 kDa while keeping the PPS block constant at around 4 kDa, thus permitting systematic variations in ethylene glycol surface density in the adlayer. A simple dip-and-rinse process was used to produce PPS−PEG adlayers on gold substrates, which were characterized with surface plasmon resonance (SPR) and further confirmed by ex situ variable angle spectral ellipsometry (VASE), and X-ray photoelectron spectroscopy (XPS). Crowding in the PPS chemisorbed layer seemed to limit the polymer adsorption process. Subsequent exposure of PPS−PEG adlayers to protein adsorption (human serum albumin at 1 mg/mL or full-concentration human serum) was monitored with in situ SPR. Protein adsorption can be reduced up to 97% for human serum albumin and up to 96% for blood serum relative to bare gold substrates. Triblock copolymers were more effective than corresponding diblocks. The possibility to render gold surfaces bioinert is the basis for application in bioanalytical devices.

Introduction

The use of in situ biosensors in pharmaceutical, biochemical, and medical applications has gained importance in the past few years, since it allows monitoring of dynamic interactions between biological species, e.g. antibody−antigen interactions, receptor−ligand interactions, and DNA hybridization. To improve the sensitivity of such biosensors, their surface has to be designed and engineered to minimize nonspecific binding. The present study is aimed to improve the control of nonspecific interactions between the biosensor surface and the analyte and thus to improve the sensitivity while increasing the signal-to-noise ratio for future biochips.

Gold surfaces are widely used for bioanalytic devices, especially those based on surface plasmon resonance (SPR) methods. Its conductivity, resistance to oxidation, and simplicity to produce thin and ultra flat films on inorganic substrates make this material particularly attractive.

As to chemisorption on the gold surface, thiol and thioether containing species are known to chemisorb onto gold, spontaneously forming self-assembled monolayers (SAMs).[1] Regarding the issue of interactiveness of surfaces with biological media, poly(ethylene glycol) (PEG) has been used in numerous biomedically relevant systems to control protein adsorption on surfaces.[2−4] PEG chains in water are highly hydrated and highly flexible, both of which may be important for steric exclusion of potential protein adsorbates. These features, in combination with PEG's nonionic character, are believed to be the bases for the nonfouling properties (protein resistance) of high molecular weight PEG.[5−7] PEG can be attached to surfaces through a variety of different approaches including silanization, plasma cross-linking, physi- and chemisorption, and chemical grafting.

Oligo(ethylene glycol)-functionalized alkanethiols[8−10] and PEG-tethered alkanethiols have been characterized for chemisorption on gold.[11] An important limitation of such systems lies in the relatively poor stability of chemisorbed alkanethiolates. It has been shown that under ambient conditions substantial oxidation and subsequent loss of stability of alkanethiol SAMs takes place within days and the integrity of the adlayer is readily compromised.[12−14] As an alternative, our groups have recently described chemisorption of a poly(propylene sulfide-*block*-ethylene glycol) (PPS−PEG) triblock copolymer.[15] This copolymer demonstrated quite high stability as an assembled monolayer on gold, based on the availability of many chemisorption sites per adsorbate molecule as well as higher stability to oxidation of the sulfides in the chain thioether backbone as compared to thiolates.

The goal of the present study was to perform a structure−function study, to understand the role of architecture (by comparing di- and triblock copolymers, and by varying the MW ratios of the PPS and PEG blocks) as they impact polymer chemisorption and

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Table 1. Architecture and Molecular Weights (MW) of the Polymers Used in This Work[a]

name	architecture	MW(PEG) total (kDa)	MW(PPS) (kDa)	MW(PEG)/ MW(PPS)	MW distribution and polydispersity
5k/4k/5k	triblock	10	4.0	2.48	bimodal
2k/4k/2k	triblock	4	3.9	1.03	1.32
1.1k/4k/1.1k	triblock	2.2	4.2	0.53	1.31
5k/4k	diblock	5	5.2	0.96	1.46
2k/4k	diblock	2	2.2	0.91	bimodal

[a] The molecular weight of poly(ethylene glycol) is given by the supplier. The average molecular weight of poly(propylene sulfide) was calculated from NMR measurements (see text for details). The molecular weight (MW) distribution of the polymer was characterized by gel permeation chromatography; for the polymers with unimodal distribution the calculated polydispersity is listed.

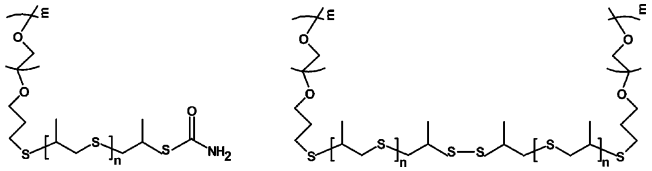

Figure 1. Structure of PPS−PEG diblock (left side) and triblock (right side) copolymers.

subsequent steric stabilization of protein adsorption. While the PPS block was kept more-or-less constant (about MW 4000), the PEG component was varied between 1100 and 5000 Da with the rationale of obtaining different ethylene glycol (EG) surface densities. As shown by Pasche and co-workers, the EG surface density plays an important role in controlling the degree of biointeractiveness of the coated surface.[16] The formation of a chemisorbed adlayer was monitored by surface plasmon resonance (SPR) and further confirmed by ex situ variable-angle spectral ellipsometry (VASE) and X-ray photoelectron spectroscopy (XPS). Finally, the resistance of the surfaces toward protein adsorption was evaluated in situ by SPR.

Materials and Methods

Polymer Synthesis. Copolymer synthesis uses episulfide anionic living polymerization using a PEG-thiolate initiator for generating diblock copolymers (structures shown in Figure 1, architectures and MW combinations formed shown in Table 1). The thiolate at the living end is either blocked, to yield the final diblock copolymer, or allowed to dimerize via disulfide bonding to yield a triblock copolymer. The details of synthesis and purification have been described elsewhere.[17] PEG was purchased from Sigma-Aldrich (Buchs, Switzerland). Further purification of the copolymer was performed by dialysis of copolymer colloidal aggregates in water against water (MWCO 6-8000, Spectrum Laboratories, CA) except for PPS−PEG containing 1100 MW PEG because of the insufficient solubility in water. Water was removed by lyophilization. The final products were characterized with nuclear magnetic resonance spectroscopy (^{1}H NMR) and gel permeation chromatography (GPC). ^{1}H NMR spectra were obtained on a 300 MHz Bruker spectrometer using chloroform-*d* as solvent (stabilized with Ag, 99.8 at. % D, Dr. Glaser AG, Basel, Switzerland). Molecular weights of the PPS blocks were calculated based on NMR, using the manufacturer's specification of PEG MW in the calculation. We calculated the repeating units of PPS by comparing the PEG chain protons at 3.5−3.7 ppm with CH_3 protons in the PPS chain at a chemical shift of 1.35−1.45 ppm. GPC was performed in tetrahydrofuran (THF) on a Polymer Laboratories column with refractive index and UV−vis detectors. M_n and M_w were obtained using a calibration with poly(ethylene glycol) standards (Polymer Laboratories).

Substrates. Here, 50 nm gold layers were deposited on glass substrates (10 × 10 mm, Plano GmbH, Germany) with an intermediate layer of chromium (5 nm) using a Leybold direct current magnetron Z600 sputtering unit (PSI, Villigen, Switzerland). SPR substrates (20 × 20 mm) were obtained from Resonant Probes GmbH, Mainz, Germany, or sputtered at PSI (glass: 20 × 20 mm, Plano GmbH, 1.5 nm Cr and 47 nm Au). Prior to polymer adsorption, the surfaces were cleaned using a UV cleaner (Boekel Ind. Inc., Feasterville, PA) for 30 min.

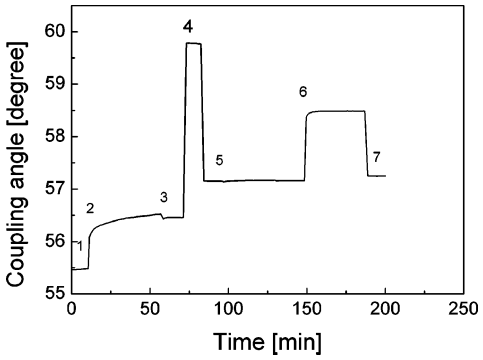

Figure 2. SPR adsorption curve following the coupling angle. After a baseline in methanol was established (1), the PPS−PEG containing solution (1 mg/mL) was injected for 45 min (2) and the adsorbed mass was determined after rinsing in methanol (3). The solution was changed from methanol to HEPES 2 with an intermediate ethanol wash (4) and the system was left to equilibrate (5). Human serum albumin, HSA, (1 mg/mL) in HEPES 2 or full serum was injected for 30 min (6) and rinsed in HEPES 2 in order to determine the amount of adsorbed proteins (7).

Surface Modification. The polymer was assembled onto the surface by dipping the gold substrates in a methanol solution (HPLC purity, ≥99.9% (v/v), Fluka, Buchs, Switzerland) of the copolymer at a concentration of 1 mg/mL for 45 min, followed by methanol rinsing and drying in a stream of filtered nitrogen. In a first study, the kinetics of PPS−PEG adsorption to gold surfaces was investigated. It was found that adsorbed polymer mass leveled off for adsorption times of 15−25 min and that protein resistance did not further improve for times exceeding 45 min. The latter value was therefore chosen for the standard PPS−PEG assembly protocol. The solutions of PPS−PEG in methanol were clear except for the 2k/4k architecture, which was milky, apparently forming a micellar colloidal suspension in methanol rather than a homogeneous solution.

Surface Analysis Methods. Surface Plasmon Resonance (SPR). The SPR system (Resonant Probes GmbH, Goslar, Germany) used a monochromatic laser light source (633 nm) focused onto a gold-coated glass slide (n = 1.5230). Index matching between the 45° prism and the gold coated glass slide was achieved by using index matching oil (n_D^{25} = 1.7000 ± 0.0002, Cargille Labs, Cedar Grove, NJ). The instrumental sensitivity measured for methanol is 0.003° with a baseline shift of 0.001°/min prior to PPS−PEG exposure. The experimental sensitivity is 0.03° if one looks at the methanol baseline rinsed with ethanol followed by a return to methanol. A low instrumental detection limit is particularly important, in that protein adsorption on some of the low-fouling surfaces can be in the range of a few ng/cm^2.[15,18]

An example of a SPR experiment is shown in Figure 2. After a stable baseline was achieved in methanol, the gold substrate was exposed to PPS−PEG in methanol for 45 min and subsequently rinsed with methanol in order to compare the

Table 2. XPS Binding Energy (±0.1 eV) Assignment of PPS−PEG Chemisorbed on Gold Surfaces[a]

polymer type	Au $4f_{7/2}$	O 1s	C 1s	S 2p	I $3d_{3/2\ and\ 5/2}$
diblock	84.0 Au $_{met.}$	532.8 C−**O**	285.5 **C**−S,[b] **C**H_3−C[b] 286.4−286.5 **C**−O[c]	163.5 C−**S**[b]	619 **I**-NH_2
triblock	84.0 Au $_{met.}$	532.8 C−**O**	285.5 **C**−S,[b] **C**H_3−C[b] 286.4−286.5 **C**−O[c]	163.5 C−**S**[b]	

[a] Reference: Au $4f_{7/2}$ gold peak at 84.0 eV ($n = 3$). [b] Functional groups from PPS. [c] Functional groups from PEG.

in-coupling angle before and after PPS−PEG adsorption. After determination of the adsorbed mass of the PPS−PEG adlayer in methanol, an intermediate rinsing step with ethanol, followed by injection of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid-buffered saline solution (HEPES 2, 10 mM with 150 mM NaCl, pH 7.4) was performed. The amount of adsorbed protein was determined from the difference in the baseline in HEPES 2 prior to and after exposure to either human serum albumin (HSA, 1 mg/mL in HEPES 2, Sigma-Aldrich, Buchs, Switzerland) or full-concentration human serum (Roche, Basel, Switzerland), respectively. Changes in mass were recorded after 30 min exposure to the surface, which was sufficient to reach a steady-state adsorbed protein mass. All adsorption steps were performed under stagnant fluid, nonflow conditions. As control surfaces, clean gold surfaces and gold chemisorbed with PPS homopolymer from dichloromethane (1 mg/mL, Fluka, Buchs, Switzerland)) were used.

The amount of adsorbed mass (polymer or protein) was determined by comparison of the coupling angle level in the same solution (methanol or HEPES 2) before and after adsorption or exposure to protein-containing media, respectively. The measured angle shift values were converted in adsorbed mass by use of the following equation.[19,20]

$$m = k\Delta\alpha$$

where

$$m = \text{adsorbed mass of proteins (ng/mm}^2\text{)}$$

$$\Delta\alpha = \text{angle shift (deg)}$$

and

$$k = (10/1.868), \text{ conversion factor}$$

Since the adsorbed mass is proportional to the refractive index increment, we can transform the equation to calculate the adsorbed mass for a polymer if we know the refractive index increment.

$$m_{\text{polymer}} = m_{\text{protein}}\,(\mathrm{d}n/\mathrm{d}n)_{\text{protein}}/(\mathrm{d}n/\mathrm{d}c)_{\text{polymer}}$$

The increment of the refractive index as a function of the polymer concentration ($\mathrm{d}n/\mathrm{d}c$) was measured with a Zeiss refractometer and determined for all PPS−PEG architectures as $(\mathrm{d}n/\mathrm{d}c)_{\text{polymer}} = 0.2685$. The refractive index increment for all proteins was fixed at $(\mathrm{d}n/\mathrm{d}c)_{\text{protein}} = 0.182$.[21]

X-ray Photoelectron Spectroscopy (XPS). XPS analyses were performed at a takeoff angle of 90° using a SAGE 100 instrument (SPECS, Berlin, Germany). Spectra were acquired at a chamber pressure below 1×10^{-7} mbar using an Al Kα source operated at 325 W (13 kV, 25 mA). The electron-energy pass analyzer was set to 50 and 14 eV for low and high-resolution scans. All binding energies are referenced relative to the Au $4f_{7/2}$ peak, set at a binding energy of 84.0 eV (fwhm = 1.16 ± 0.01 eV). For C 1s detail spectra fwhm were assigned at 1.4−1.8. Measured intensities were transferred to normalized intensities by taking into account their respective photoionization cross-section corresponding to Scofield sensitivity factors. Spectra were fitted with the casaXPS software using the sum of 70% Gaussian and 30% Lorentzian function. Table 2 shows the assignments for the high-resolution XPS spectral peaks.

Spectroscopic Ellipsometry (VASE). Ellipsometric data were measured with a variable angle spectrometric ellipsometer (VASE) M-2000F (L.O.T. Oriel GmbH, Darmstadt, Germany). The measurement was conducted in the spectral range of 370−1000 nm at three angles of incidence (65, 70, and 75°) under ambient conditions. VASE measurements were fitted with multilayer models using WVASE32 analysis software. The analysis of optical constants was based on a bulk gold layer, fitted for n and k on glass. After adsorption of the block copolymers, the adlayer thickness was determined using a Cauchy model ($A = 1.45$, $B = 0.01$, $C = 0$).[22]

Results

Polymer Synthesis and Characterization. Poly(propylene sulfide-*block*-ethylene glycol) (PPS−PEG) copolymers (Figure 1) with different architectures were successfully synthesized and characterized by ^{1}H NMR and GPC. Table 1 summarizes the block copolymers that were synthesized and used for this work. In this table, denotation as bimodal indicates some contamination with the diblock and triblock in a predominantly triblock or diblock background, as determined by GPC: the sample 2k/4k only contains minor amounts of the triblock, while the sample 5k/4k/5k is a mixture of diblock and triblock. The polydispersities are in an expected range, except the one polydispersity of 1.46 is perhaps broad taking into account the type of polymerization.[17] Looking at the molecular weight distribution measured by GPC (data not shown), the molecular weight distribution shows the presence of fraction of lower molecular weights. This could potentially influence the adsorption profile of the PPS−PEG adlayer on gold, because smaller molecular weights have a higher diffusion coefficient.

Surface Plasmon Resonance Study for the Adsorption of PPS—PEG on Gold: Kinetics of Adsorption. SPR was used to monitor adsorption in situ from methanol. All the triblocks show a plateau where the PPS−PEG adlayer is saturated and full coverage is reached. The time for saturation is dependent on the molecular weight of the block copolymer, e.g. 1.1k/4k/1.1k reaches the plateau faster than 5k/4k/5k. Considering the form of the adsorption curve from these measurements, we concluded that the whole process exists in a regime of mixed kinetic and diffusion control. For an exclusively diffusion-controlled adsorption, the concentration is too high. A zoomed view of the first 15 min of the polymer absorption is shown in Figure 3.

In the case of the 2k/4k polymer, a completely different behavior is observed and more than a full coverage of a monolayer is reached. This polymer shows the lowest PEG/PPS ratio, and its solubility behavior in methanol differs from that of the others polymers. In fact, at the concentration of 1 mg/mL used in the assembly protocol, the polymer solution was quite turbid. In view of the dual hydrophilic−hydrophobic character of these block copolymers, micelles, vesicles or other aggregates could be formed in methanol. Although we do not have a direct proof, it is likely in this case that such aggregates were adsorbing onto the

Table 3. SPR Angle Shifts (deg) Measured upon Adsorption of PPS−PEG Polymers of Different Architectures, and Subsequent Exposure to Human Serum Albumin (HSA) or Full Human Serum[a]

name	polymer adsorption (deg)	polymer adsorption (ng/cm^2)	HSA adsorption (deg)	HSA adsorption (ng/cm^2)	serum adsorption (deg)	serum adsorption (ng/cm^2)
5k/4k/5k	0.97 ± 0.05	352 ± 17	0.02 ± 0.01	8 ± 7	0.06 ± 0.03	34 ± 16
2k/4k/2k	0.75 ± 0.02	273 ± 8	0.01 ± 0.02	7 ± 12	0.03 ± 0.01	16 ± 5
1.1k/4k/1.1k	0.82 ± 0.09	298 ± 32	0.05 ± 0.02	24 ± 13	0.07 ± 0.03	37 ± 14
5k/4k	0.7 ± 0.20	268 ± 66	0.13 ± 0.03	71 ± 16	0.14 ± 0.06	73 ± 32
2k/4k	1.1 ± 0.20	394 ± 73	0.17 ± 0.05	93 ± 27	0.18 ± 0.06	95 ± 33
PPS	0.91 ± 0.07	331 ± 26	0.28 ± 0.07	150 ± 46	0.4 ± 0.20	198 ± 81
Au bare	0	0	0.40 ± 0.02	212 ± 8	0.71 ± 0.03	380 ± 14

[a] The data represent the average of six measurements for polymer adsorption and three measurements for HSA and serum adsorption. The adsorbed masses were calculated from the measured angle shifts following a procedure described in the text.

Table 4. XPS Normalized Intensities (in at. %) of PPS−PEG Polymers Adsorbed on Cleaned Gold Substrates (n = 3) and Adlayer Thickness (n = 6)[a]

	thickness (VASE) (Å)	Au (4f)	O (1s)	C (1s) C−S	C (1s) C−O	S (2p)	I (3d)
5k/2k/5k	44 ± 2	23 ± 1	11 ± 1	31 ± 1	26 ± 1	9 ± 1	0
2k/4k/2k	34 ± 4	28 ± 1	9 ± 1	28 ± 1	23 ± 1	12 ± 1	0
1.1k/4k/1.1k	40 ± 4	26 ± 2	9 ± 1	32 ± 2	20 ± 1	13 ± 1	0
5k/4k	40 ± 2	24 ± 2	6 ± 1	32 ± 7	21 ± 5	16 ± 1	1 ± 1
2k/4k	50 ± 5	20 ± 1	6 ± 1	43 ± 1	12 ± 3	18 ± 1	1 ± 1
Au (reference)		85 ± 2		15 ± 2 (hydrocarbons)			

[a] The measured peak areas were divided by the corresponding sensitivity factors and normalized to 100% total intensity.

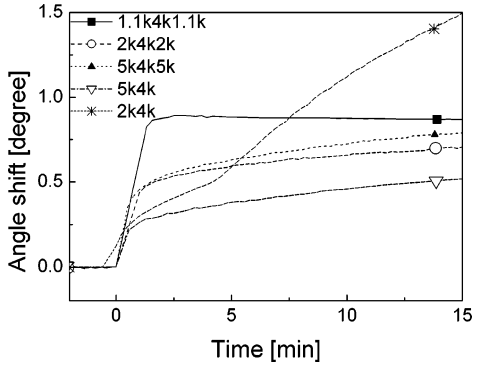

Figure 3. SPR adsorption curves of the adsorption of PPS−PEG with different architectures in methanol during the first 15 min.

surface without (completely) decomposing into a monomolecular assembly film. This would result in a higher average coating thickness compared to the other polymer architectures. After a rinsing step with methanol, some loosely bound PPS−PEG, e.g., in the form of loosely bound micelles, is rinsed away and the angle shift drops down to $\Delta\alpha = 1.1°$ (see Table 3).

From the SPR measurements, it was possible to determine the amount of adsorbed polymer, which varied from a minimum of 268 ng/cm^2 (polymer 5k/4k) to a maximum of 394 ng/cm^2 (polymer 2k/4k) (see Table 3 for details). No simple correlation between copolymer architecture (block number or size) and adlayer surface concentration or thickness was observed. When one compares the triblock copolymers with a 4 kDa PPS central block (5k/4k/5k; 2k/4k/2k; and 1.1k/4k/1.1k), adlayer thickness was higher in the case of the 5k PEG block than with the shorter 2k and 1.1k PEG blocks. Thus, it would seem that for this series, the PPS block volume determines some of the mass adsorption limits, and that for longer PEG blocks, these blocks extend above the PPS block to increase overall thickness, with crowding below determining packing density rather than crowding above. No such correlation was observed for diblock architecture; however the variances of these measurements were much higher.

Adsorbed Amount of PPS—PEG on Gold Surfaces and Comparison to Other Adlayer Characterizations: XPS and VASE. The copolymer adlayers where also characterized by VASE and XPS. For the first, it was observed that the layer thickness varied from 50 ± 5 Å for 2k/4k to 34 ± 4 Å for 2k/4k/2k. The results from XPS indicate that a reverse order between layer thickness and the normalized intensities of gold exists, as would be expected (see Table 4). Furthermore, the presence of iodine as a contaminant is observed in the case of diblocks architectures. This originates from residues of the end-capping agent, i.e., iodoacetamide, which could not be fully removed by dialysis. This contamination could be significant, because iodine is known to chemisorb onto gold substrates.[23] The maximum amount of adsorbed iodine observed was 1 atom-%.

Although the measurements conditions are very different between these methods (ultrahigh vacuum for XPS, air for VASE, methanol for SPR), we observed a good correlation between the different methods for all the different architectures. This correlation was determined by fitting of a linear regression (R^2(XPS vs VASE) = 0.87 and R^2(XPS vs SPR) = 0.84), and it was observed that the increasing intensity ratio of polymer adlayer normalized to gold corresponds to higher amount of PPS−PEG investigated using SPR and to thicker adlayer investigated using VASE (Figure 4).

PPS—PEG Adlayer Exposed to Proteins and Quantitative Analysis of the Adsorption. SPR measurements provide good evidence that the mass of proteins adsorbed from whole serum or HSA solution (at 1 mg/mL) depends on the architecture of the preadsorbed polymer. Therefore, the mass of adsorbed proteins, shown in Table 3, is also expressed in Figure 5 as a function of the density of ethylene glycol monomeric units (EG) at the interface. In general, less adsorption of both HSA and serum is observed in the presence of a PPS−PEG adlayer, and increasing the EG surface

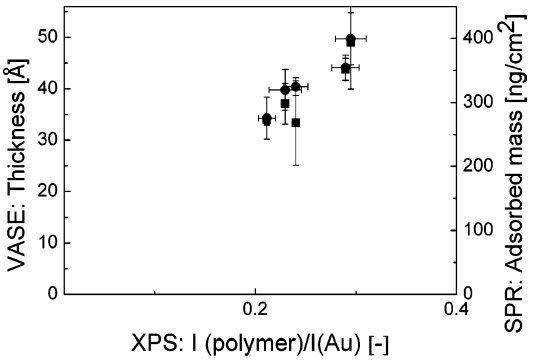

Figure 4. PPS−PEG adlayer thickness on gold, determined by SPR and ellipsometry. Adsorbed mass from SPR measurements (■, right axis) and adlayer thickness determined by VASE (●, left axis) are expressed as a function of the ratio of the sum of carbon, oxygen and sulfur atomic concentrations, I (polymer), to gold atomic concentration, I (Au), determined by XPS.

concentration is beneficial for protein resistance. It is also clearly observed that triblocks are more effective than diblocks in rendering a surface protein resistant, even though the diblock copolymers were effective in providing EG units to the gold surface.

Both for HSA and serum, the lowest protein adsorption levels were observed for 5k/4k/5k and 2k/4k/2k (no significant difference between the two). In comparison to a bare cleaned gold substrate, the HSA adsorption is lessened by 97% and for serum by 96% for 2k/4k/2k.

Discussion

In our approach, a block copolymer containing one (diblock) or two (triblock) PEG chains separated by a poly(propylene sulfide) (PPS) part is used. PPS−PEG in aqueous solution forms vesicles that may be interesting for controlled drug delivery.[24,25] When PPS−PEG is chemisorbed onto gold surfaces, a stable linkage between the sulfur atoms of the PPS thioether and the metal surface is observed. The hydrophilic PEG part forms a dense PEG brush, exposed to the aqueous environment.

Adsorption of PPS—PEG on Gold: Comparison to Alkanethiol Self-Assembled Monolayers (SAM) and Previous Studies of PPS—PEG on Gold. Since the early studies of Nuzzo et al.[1,23,26] about the adsorption of organic disulfides and other organosulfur compounds on gold surfaces, it was demonstrated that the adsorbate−substrate interaction is relatively strong and involves chemisorption via sulfur atoms. Whereas thiols chemisorb via the thiolate to gold,[27−29] the thioether from PPS block possibly binds through one of the electron lone pairs.[15]

The central block of the triblock copolymer used in this study consists of two polysulfide blocks joined in the middle via a disulfide block. Thus, both C−S−C and C−S−S−C exist for chemisorption to the Au surface. We presume that the C−S−C binds without influencing the stability of the C−S bond;[15,30−33] however, whether the central S−S bond is broken is unknown.

When comparing the PPS−PEG technology to other surface systems that are compatible with Au substrates, it should be noted that lower adsorbed protein mass values have been reported in single protein and serum exposure experiments, particularly for oligo(ethylene glycol)-terminated alkanethiol SAMs (with typically 3−6 EG monomer units) on gold.[8−10,34] However, such studies generally refer to short term experiments that do not take into account possible chemical changes of the surface upon storage such as oxidation of alkanethiol SAMs, resulting in a loss of protein resistance with time.[35−39] A specific advantage of the PPS−PEG technology is the excellent shelf life stability of the adlayers, an important aspect in terms of technological applications.

The use of multisite attachment via polysulfides can minimize the loss of functionality due to adlayer degradation, as shown by Bearinger et al.[15] In particular, the use of PPS−PEG triblock copolymers has proven to impart long-term stability against oxidation from air (up to 41 days) and renders surfaces resistant against complex protein solutions, such as full human serum, where a reduction of 95% in comparison to bare gold was reported. In addition, the use of longer PEG chains (EG units: 17 and 9) in comparison to EG functionalized alkanethiols (EG units: 1, 2, 4, 6)[10] can avoid the requirement of an ordered adlayer to obtain EG surface densities and specific EG conformation that allow protein resistance.

Here, we sought to compare symmetric triblock copolymers with diblocks and as well to change the synthesis so as to remove a potentially hydrolytically sensitive ester link that existed between one of the PEG blocks and the central PPS block in the copolymers reported previously by Bearinger et al.[15] and Valentini et al.[40] However, the use of a symmetric triblock with a

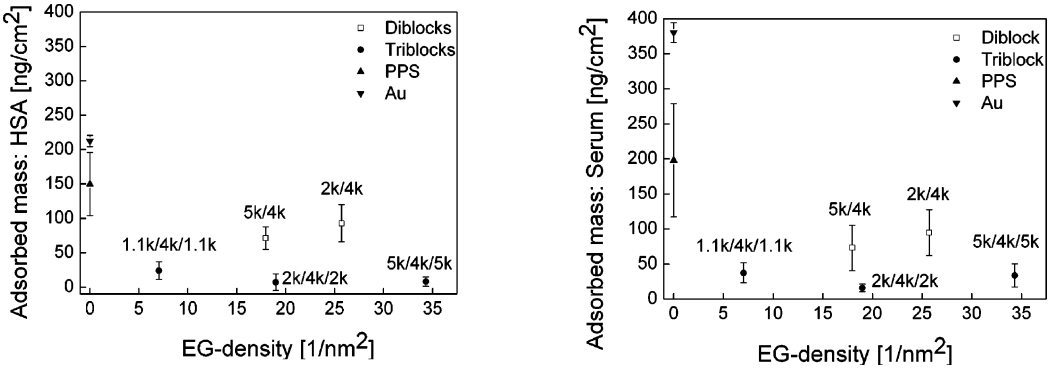

Figure 5. Mass of albumin (left side) and serum (right side) in ng/cm^2 that adsorbs on PPS−PEG-coated gold surfaces upon exposure for 30 min to HSA and human serum, respectively, and subsequent rinsing in 10 mM HEPES buffer (pH 7.4) as a function of the EG monomer surface density, n_{EG}. The corresponding values of m_{Serum} for the uncoated and PPS-coated gold surfaces are also plotted. The adsorbed protein mass was determined by SPR; the EG monomer surface density was determined from the adsorbed polymer mass (SPR) and the polymer architecture (NMR) of the bulk polymer. The data represent the average and standard deviation of 3 independent measurements for the protein adsorption.

disulfide bond in the middle of the PPS part could also present complexities. Indeed, Nuzzo and Allara, and later others, suggest that disulfides chemisorb dissociatively on the gold surface to give a stable surface thiolate.[26] It is thus possible that this disulfide bond is cleaved during chemisorption on the gold surface and diblock molecules are finally present on the complete adlayer. Since there exists competition between the dominant sulfide and the minority disulfide for surface Au, and since we do not know the extent to which all sulfur atoms chemisorb vs exist in loops attached to the surface, we do not know the extent to which this theoretical possibility exists in reality. Moreover, the contrast between results obtained with the pure diblock architectures shows that real differences are indeed observed in respect to solubility and protein resistance.

Summarizing, PPS−PEG block copolymers have these main advantages: no ordering is needed to obtain an optimal EG surface density, in contrast to self-assembled monolayers, and multiadhesion sites are possible in comparison to only one sulfur interacting with the gold surface of the monothiols and thus a greater stability of the adlayer under ambient conditions is achieved. Here we performed a systematic study of different PPS−PEG architectures chemisorbed onto gold; parallel determination of their protein resistance allowed us to determine performance as a function of adsorbed EG density.

To better analyze the structure of PPS−PEG adlayer, we compared the average mean distance between PEG chains and free PEG coils. We assumed that the PPS-part is adsorbed flat onto the surface and that the PEG-blocks form a sphere with the radius of gyration being the radius of the sphere. The radius of gyration is calculated using the following equation [41] based on static light scattering from poly(ethylene oxide) in methanol:

$$R_g = 0.0143 M_w^{0.61\pm0.03}\,\mathrm{nm}$$

where M_w is the molecular weight of the PEG chain. We obtain 1.02, 1.48, and 2.58 nm for 1.1k, 2k, and 5k PEG, respectively.

To calculate the average mean distance L between the PEG chains, we assume that the PEG chains have a spherical shape, are hexagonal closest packed distributed among the PPS−PEG layer, and have a PEG density which correspond to the surface density determined by SPR (n_{PEG}). We can calculate the average mean distance between the PEG chains with[16]

$$L = (2/(n_{PEG}\sqrt{3})^{0.5}$$

Converting our SPR measurements of adsorbed polymer mass by taking into account the corresponding architecture into the spacing L, we end up in a range of 0.18−0.40 nm for the different architectures. A comparison between the L and R_g values shows that L is clearly smaller than $2R_g$. This finding suggests therefore that the adsorbed PPS−PEG adlayer is dense packed and the PEG chains are stretched upward to form a dense polymer brush. This conformation leads to the steric hindrance effect, preventing proteins from adsorbing onto the surface.

Minimizing Protein Resistance. SPR measurements showed a good correlation between the mass of proteins adsorbed from HSA or serum solutions and the type of polymer architecture (di- or triblock) as well as the mass ratio of the different blocks, i.e., MW_{PEG}/MW_{PPS}. The level of protein adsorption reached its lower limit for 5k/4k/5k and 2k/4k/2k polymers, no significant difference being observed between the two architectures. This result is in good agreement with other work that reported that PEG 2000 is the most effective for reducing protein adsorption.[42] In our studies, the triblock architectures performed significantly better than the corresponding diblock copolymers, suggesting different structures of the resulting polymer adlayers.

Although the protein-rejecting properties of PEG-containing coatings are well-known, there has been some discussion about the molecular mechanisms behind this phenomenon. Several theories have been proposed to explain protein resistance, including excluded volume effects, osmotic repulsion, the high mobility of the PEG chain, the high content of water in PEG layers and the low interfacial energy of PEG with water.[6,7,43−45]

EG Surface Density as a General Benchmark for Protein Resistance. Poly(L-lysine)-*grafted*-PEG (PLL-*g*-PEG) represents an attractive system to render surfaces highly resistant to nonspecific protein adsorption, since a dense PEG-brush is formed between the underlying surface and the proteins.[46−48] In particular, PLL-*g*-PEG has been a useful system to study the relationship between PEG surface density and protein resistance in the case of negatively charged substrates such as metal oxides (TiO_2, Nb_2O_5, $Si_{0.4}Ti_{0.6}O_2$, and ITO)[16,49−52] or oxidized polymer surface such as tissue culture polystyrene.[53] In such cases, an optimal use of the electrostatic interaction capabilities of the system leads to ideal polymer surface coverages. Another approach based on immobilizing PEG chains through 2−3 dihydroxyphenylalanine to metal oxide surfaces has been demonstrated to result in exceptionally good stability and high PEG densities.[21] Pluronics, or poloxamers, amphiphilic block copolymers that consist of poly(propylene oxide) flanked by two poly(ethylene oxide) chains, are commonly used to immobilize PEG on hydrophobic surfaces.[54,55] The conformation of the poly(ethylene oxide) molecule as well as the number of ethylene oxide monomers per surface area have been shown to influence protein adsorption.[56−60] The same kind of architecture structure can be achieved with PPS−PEG, where a hydrophilic (PEG) and a hydrophobic (PPS) domain are present. But instead of using hydrophobic−hydrophobic or electrostatic interaction to adsorb the polymer onto the hydrophobic surface, on gold a potential more stable chemisorption of PPS−PEG is taking place.

Malmsten et al. suggested that firmly adsorbed PEG-containing polymers behaved similarly to covalently attached PEG species regarding inhibition of protein adsorption, independently from the nature of the underlying surface.[61] Indeed, when comparing the results obtained from different works, the hypothesis that the platform chosen for surface PEGylation is not a determinant parameter can be further considered, assuming a basic level of stability of the anchoring group when exposed to protein rich environment. As consequence of this, the main concern for obtaining an efficient protein rejection is then only limited by achieving a sufficiently high interfacial chain density, which can be expressed as EG density, i.e., the number of EG units per surface area. To test this hypothesis, Pasche et al.[49] presented a normalization in respect of the EG-density

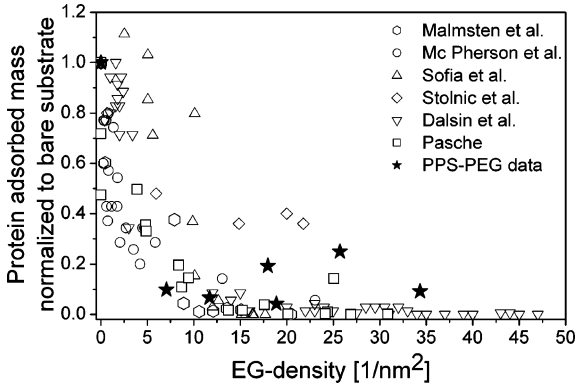

Figure 6. Comparison of experimental protein adsorption data for PEGylated surfaces prepared by using different PEG surface immobilization schemes. Protein adsorbed mass has been normalized to the adsorbed mass on the respective bare substrate and corresponds to the average of the adsorbed masses of all serum proteins presented in the study.[16,21,59,61,63,64]

of results obtained from a number of studies with a number of PEG-grafting or PEG-immobilization methods. Protein adsorption results from this study are expressed as the average of all available protein adsorption data for the different systems, normalized to the adsorption onto the corresponding bare substrate, to compare with our results obtained with PPS−PEG upon exposure to serum. The obtained master curve, along with our data, is presented in Figure 6.

Protein adsorption from serum on PPS−PEG coated surfaces and the various protein adsorption data from literature are in relatively good agreement with the stated hypothesis and are independent from the underlying substrate or anchoring platform as long as the effective PEG layer thickness is high enough to screen electrostatic interactions with proteins due to potentially present interfacial charges.[62] However, the results of our study, which utilized well-characterized and analogous diblock and triblock architectures, show a clear benefit in using the triblock architecture, independent of EG surface density. We hypothesize that this difference relates to the crowding in the PEG overlayer and thus the density and degree of chain extension in the corresponding PEG brush. If the overall amount of copolymer adsorbed is primarily determined by crowding in the PPS-rich domain of the adlayer, and if the PEG-rich domain responds (within the regime tested) by extending more-or-less into the bulk (as suggested by the mass measurements on the 5k/4k/5k, 2k/4k/2k, 1.1k/4k/1.1k series), then the structure of the PEG-rich overlayer does indeed depend on polymer architecture. Given this argument, extension in the diblock overlayer would be much less than in the corresponding triblock overlayer. Measurements of resistance to protein adsorption and divergence of these results as a function of EG surface concentration support that these differences are functionally important.

Conclusions

In this study, we synthesized a number of defined architectures of the block copolymer poly(propylene sulfide-*block*-ethylene glycol), (PPS−PEG), and adsorption on gold surfaces in a controlled manner through a dip-and-rinse process from methanol was studied. A combination of techniques such as surface plasmon resonance (SPR), ex situ variable angle spectrometric ellipsometry and X-ray photoelectron spectroscopy (XPS) permitted quantitative determination of polymer surface coverage, surface density of the PEG chains, and the degree of protein resistance upon contact with human serum albumin (HSA) or full human serum. Results with polymer adsorption suggest that polymer crowding in the PPS-rich region of the adlayer limit the mass of adsorbed copolymer and suggest that the PEG-rich region is more extended in the case of triblock copolymers than diblock copolymers. Results obtained with HSA and serum indicated a close relationship between protein adsorption resistance and polymer architecture: although EG surface density was a key parameter in determining protein resistance, triblock copolymers performed clearly better than diblocks. Among the different possibilities to passivate gold surfaces, PPS−PEG based adlayers have then shown to be a highly interesting technique considering their high stability and their protein resistance. Furthermore, substrates such as gold (and potentially silver and copper) can only be partially modified by PEG-graft polyelectrolytes which require the presence of highly charged substrates, typical for metal oxides and oxidized polymer, to form dense PEG brushes. Therefore, we believe that PPS−PEG is a valuable addition for the surface modification of those metal substrates, widely used in the field of biosensors.

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