Solid-Supported Amphiphilic Triblock Copolymer Membranes Grafted from Gold Surface

EKATERINA RAKHMATULLINA,1 ALEXANDRE MANTION,1 THOMAS BÜRGI,2 VIOLETA MALINOVA,1 WOLFGANG MEIER1

1 Department of Chemistry, University of Basel, Klingelbergstrasse 80, CH 4056, Basel, Switzerland
2 Institute of Microtechnology, University of Neuchatel, Av. de Bellevaux 51, CH 2009, Neuchatel, Switzerland

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ABSTRACT: Gold-supported amphiphilic triblock copolymer brushes composed of two hydrophilic poly(2-hydroxyethyl methacrylate) (PHEMA) blocks and a hydrophobic poly(n-butyl methacrylate) (PBMA) middle part were synthesized using a surface-initiated ATRP. Attenuated total reflectance Fourier transform infrared spectroscopy, polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS), ellipsometry, contact angle measurements, and atomic force microscopy were used for the characterization of PHEMA-co-PBMA-co-PHEMA brushes. The PM-IRRAS analysis revealed an increase of the chain tilt toward the gold surface during growth of the individual blocks. We suggest that the orientation of the amphiphilic polymer brushes is influenced by both the chain length and the interchain interactions. Additionally, a detachment of the polymer membranes from the solid support and subsequent gel permeation chromatography analyses allowed us to establish their compositions. We applied block-selective solvents (water and hexane) as well as a good solvent for the whole polymer chain (ethanol) to study the morphology and solvent responsive behavior of the amphiphilic brushes. The presented results could serve as a good starting point for the fabrication of functional solid-supported membranes for biosensing applications. © 2008 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 47: 1–13, 2009

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INTRODUCTION

Within the recent years, the design of model membrane systems mimicking the properties of natural biomembranes has received a lot of scientific attention. This is due to the fact that such systems may allow for a better understanding of membrane-related processes as well as for preparation of biosensors.1 To date, the most commonly used model system is based on the so-called tethered bilayer lipid membrane (tBLM),2 which consists of a lipid bilayer that is tethered via a hydrophilic polymer,3–5 lipopolymer,6,7 or peptide8–10 to a solid support [Scheme 1(b)]. Often gold is chosen as a support material because it is suitable for surface analytical techniques and can serve as electrode to study the dielectric properties of the system. To provide the system with
robustness and stability, the tethered spacers (polymers, peptides) have to be covalently linked to the substrate. Such membranes are commonly prepared from self-assembled monolayers,5 by Langmuir-Blodgett (LB) transfer11,12 or by fusion of lipid vesicles onto “activated” surfaces.13 Besides the delicate and complex route of preparation and aspects such as the polymer-lipid bilayer interactions that have to be carefully considered, the final system is often deficient of stability.

On the other hand, amphiphilic block copolymer membranes have proven to be a very useful model of the cellular membrane permitting also an incorporation of functional proteins and enzymes.14 Block copolymers are known to form chemically and mechanically more stable membranes than conventional lipids.15,16 They allow tailoring of their properties for a specific purpose by introducing desired functional groups, which is not possible with lipids to such extent. Moreover, the membrane thickness can be easily controlled through controlling the polymer molecular weight.17 These hallmarks make amphiphilic block copolymer membranes highly attractive also as model systems for biosensing. Typically, for such applications solid-supported membrane structures are required. Recently, we demonstrated that similar to conventional phospholipid vesicles18,19 also block copolymer vesicles with charged hydrophilic blocks can fuse on mica and glass surfaces to solid-supported, planar block copolymer membranes.20 Previous experiments showed also that amphiphilic diblock copolymer brushes could successfully be grown from silicon and gold surfaces using a surface initiated ATRP.21 However, a closer mimicking of the characteristic morphology of a biological membrane requires a sequence of hydrophilic, hydrophobic, and again hydrophilic polymer blocks [Scheme 1(a)], that is, an amphiphilic ABA’ or ABC triblock copolymer.22–24 Here, we applied a “grafting from” approach25 to synthesize amphiphilic ABA’ triblock copolymer membranes by surface-initiated ATRP. This technique provides a good control over the brush thickness through a control of the polymer molecular weights26 and allows preparation of block copolymers by simply activating the functional chain end in the presence of different monomers.27,28 As a model, we synthesized poly(2-hydroxyethyl methacrylate)-co-poly(n-butyl methacrylate)-co-poly(2-hydroxyethyl methacrylate) (PHEMA-co-PBMA-co-PHEMA) block copolymers with the first PHEMA block being anchored to the gold surface while the other PHEMA block is exposed to the outer interface [Scheme 1(a)].

The hydrophilic PHEMA blocks of the biomimetic membranes are not fully water-soluble, but retain (hold) water and considerably swell in aqueous media. It should be noted that the pendant hydroxy groups of this polymer can conveniently be functionalized,29 which allows further tuning of their properties. Here, we prepared block copolymer brushes with different block lengths and characterized them both on the gold surfaces and—after detaching them from the solid support—in solution. Notably, we also monitored the orientation of the amphiphilic copolymer brushes during their growth since this might be an important parameter regarding the insertion of different biological objects into the membrane.

**EXPERIMENTAL**

**Materials**

n-Butyl methacrylate (n-BMA) (Fluka; 99%) and 2-hydroxyethyl methacrylate (HEMA) (Aldrich; 98%) were purified by passing through a column of activated basic alumina before use for removal of the inhibitor. Copper (I) bromide (Aldrich; 98%) was purified according to the method of Keller and Wycoff.30 The ligand N,N,N’,N’’,N’’’-pentamethyldiethylenetriamine (≥98%) (PMDETA) (Fluka) was used without further purification. The disulfide initiator (BrC(CH3)2 COO(CH2)11 S)2 was synthesized by a coupling reaction between PMDETA and a disulfide compound (BrC(CH3)2 COO(CH2)11 S)2.
synthesized according to a literature procedure. Filters Durapore-PVDF were acquired from Millipore Corp. All other chemicals were obtained from Fluka and used without any further purification.

**Preparation of the Gold Substrates**

**Caution!** Piranha solution reacts violently with most organic materials and thus, must be handled with extreme care.

Silicon slides were cleaned in piranha solution ($\text{H}_2\text{SO}_4$:$\text{H}_2\text{O}_2$, 3:1, vol %) using sonication. Wafers were rinsed thoroughly with bidistilled water, then sonicated in bidistilled water, washed once more with water, ethanol, and dried under a stream of nitrogen. Clean silicon slides were directly used for the sputtering of 15 nm chromium adhesion layer followed by 50 nm of gold (Baltec SCD 050 for Cr 120 mA, 0.05 mbar; Baltec MED 020 for Au 50 mA, 0.02 mbar, all in argon atmosphere). We did not perform a thermal treatment of the gold surfaces since the roughness of the synthesized polymer brushes was expected to be higher than that of the bare surface.

**Functionalization of Gold Surfaces by ATRP Initiator Monolayer**

THF solution of ATRP initiator (BrC(CH$_3$)$_2$COO(CH$_2$)$_{11}$S)$_2$ (2 wt %) was passed through a Durapore-PVDF filter. To form self-assembled monolayers (SAM) of the initiator molecules on the gold surface, gold substrates were immersed into the prepared initiator solution overnight at room temperature. After that the samples were washed with THF, ethanol, and dried in a nitrogen stream.

**Growth of Polymer Brushes from Immobilized Initiator SAM**

**Synthesis of the First PHEMA Block**

To a degassed HEMA solution (2 M) in methanol:water (1:1, vol %), CuBr (144 mg, 1 mmol), CuBr$_2$ (22.4 mg, 0.1 mmol), Cu (6.3 mg, 0.1 mmol), and PMDETA (300 mg, 1 mmol) were added under a flow of nitrogen. The mixture was degassed using three freeze-pump-thaw cycles and stirred until a homogeneous blue solution was formed.

The initiator modified substrates were placed into another flask equipped with a rubber septum. The flask was degassed, filled with the above described polymerization solution, and the reaction was carried out at 35 °C for 35 min (experiment 1) and 50 min (experiment 2). After this time, the reaction was quenched by injecting an ethanol/water solution of CuBr$_2$ and PMDETA (1:1 molar ratio, 0.02 M CuBr$_2$) to preserve the end-functionality of the PHEMA block. The substrates were consecutively cleaned with ethanol, water, ethanol, and dried under a nitrogen stream.

**Synthesis of the Second PBMA Block**

The same procedure as the one described for HEMA was applied for BMA polymerization, but dimethylformamide (DMF) was used as a polymerization solvent. The reaction was carried out for 50 min (experiment 1) and 70 min (experiment 2) and quenched by addition of a CuBr$_2$/PMDETA solution.

The substrates were cleaned with DMF and several portions of ethanol, and then dried under a nitrogen stream.

**Synthesis of the Third PHEMA Block**

A similar procedure as described for the synthesis of the second PBMA block was applied for HEMA copolymerization. Reactions were carried out for 1 h (experiment 1) and 1.5 h (experiment 2) and stopped without quenching.

The substrates were cleaned with DMF and several portions of ethanol, and then dried under a nitrogen stream.

**Solvent Treatment for Atomic Force Microscopy Study**

The polymer functionalized gold substrates were immersed into ethanol overnight. The samples were dried at room temperature prior to atomic force microscopy (AFM) investigation. To investigate the influence of selective solvents, the same samples were reimmersed into hexane overnight, dried at room temperature, and further analyzed by AFM. For the AFM analysis of amphiphilic copolymer brushes in water, the sample was immersed into ethanol for 2 h, ethanol/water (1:1, vol %) mixture for 2 h, and left in aqueous medium overnight.

**Detachment of the Copolymer Brushes from the Gold Substrate**

Gold substrates with polymer brushes were immersed for 15 h in flasks containing iodine...
solution (5%) in THF. The flasks were wrapped into aluminum foil. Then the slides were taken out and the residual THF solution was passed through a column with neutral alumina.

Measurement Methods

$^1$H NMR

Spectra were recorded in THF-d8 on a 400.1300 MHz Varian Unity 400 NMR spectrometer with sweep width of 8278.146 Hz and a 22° pulse width of 2.96 μs.

Gel Permeation Chromatography

Agilent Technologies Gel Permeation Chromatography (GPC) instrument with a ODS Hypersil column (5 μm) and polystyrene standards were used. The data obtained were corrected for poly-methacrylate standards as described by Mori. A refractive index detector was applied for sample detection. Tetrahydrofuran was used as eluent.

Contact Angle Determination

All measurements were performed using the plate method with a Tensiometer K100MK2, Krüss Gmbh. Bidistilled water was applied for the analysis. The presented results were taken as average values from five measurements.

Ellipsometer

Film thicknesses were determined using a spectroscopic multi angle ellipsometer (SENTECH SE 850-STE, Sentech Instruments Gmbh) measuring at three angles of 45°, 55°, and 65°. Measurements were carried out with dried samples.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) measurements were performed using a FTIR-8400S spectrophotometer applying Golden Gate ATR setup, Shimadzu. Spectra were recorded with 128 scans repetition for the blank gold surface and the sample, with 2 cm$^{-1}$ resolution.

Atomic Force Microscopy

Contact mode AFM was performed using PycoLE System, Molecular Imaging, and gold coated silicon nitride cantilevers ($k = 0.12$ N/m). Images were recorded at a scan speed of 0.8 line/s, force set point 0.147 V, in topography and friction modes with a pixel number of 512 × 512. All measurements were done at room temperature.

Polarization Modulation Infrared Reflection Absorption Spectroscopy

The sample was mounted in the complementary setup for polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) measurements within the compartment of a Bruker PM 50, connected to an external beam port of a Bruker Tensor 27 Fourier Transform Infrared spectrometer. The detector was a photovoltaic MCT element cooled with liquid nitrogen. Polarization was modulated with a photoelastic modulator (Hinds, PEM 90) at a frequency of 50 kHz. Demodulation was performed with a lock-in amplifier (Stanford Research, SR830 DSP). All spectra were recorded with a resolution of 1 cm$^{-1}$. Bare plasma cleaned gold surface served as a reference for the PM-IRRAS spectra. Data analysis was performed using Fytik. Peaks were modeled using Gaussian curves. Reference spectra for PBMA and PHEMA were recorded using commercial non crystalline polymers.

The angle $\theta$ shows the orientation of the bisector of the two transition dipoles of the methacrylate ester group to the normal of the surface and was determined using the Debe method. The azimuthal factor was taken into the relative concentration factor and was further ignored. We selected vibrations from the ester functionality ν(C=O) at 1732 cm$^{-1}$ and ν(C–C–O) at 1080 cm$^{-1}$ for the analysis.

RESULTS AND DISCUSSION

The synthesis of the triblock PHEMA-co-PBMA-co-PHEMA copolymer brushes included an immobilization of initiator molecules on gold substrates followed by ATRP of HEMA and BMA monomers. Subsequent analyses of the copolymer brushes on surface and a characterization of the detached polymers are described below.

Initiator SAM

As we showed earlier, anchoring of the initiator molecules (BrC(CH$_3$)$_2$ COO(CH$_2$)$_{11}$ S)$_2$ resulted in a densely packed homogeneous self-assembled
monolayer (SAM) on the gold surface. Therefore, we used the same procedure to functionalize gold with initiator molecules. Ellipsometric measurements showed that the obtained initiator layer had a thickness of 1.6 ± 0.2 nm (Table 1).

The functionalization of gold changes the surface polarity which is directly reflected in the wetting properties. Hence, we performed contact angle measurements on gold slides before and after initiator immobilization. Basically, the more hydrophilic the surface, the larger is the contact area of a water droplet on it and, therefore, the smaller is the value of the contact angle. Thus, we detected a change in the contact angle from 75° (gold) to 70° (gold with initiator layer, Table 2). Freshly prepared and analyzed initiator-modified gold substrates were directly used for the following polymerization step.

**Synthesis of the Amphiphilic Triblock PHEMA-co-PBMA-co-PHEMA Brushes**

Scheme 2 represents the surface-initiated ATRP of HEMA and BMA applied for the preparation of the triblock copolymer brushes on gold. The mechanism and kinetics of ATRP are well established. A methanol/water solvent mixture was chosen for the polymerization of the first PHEMA block because it allowed a fast growth of the PHEMA brushes and provided a good solubility of the copper salts. However, we used DMF as a solvent for copolymerization of both the PBMA and the third PHEMA-blocks. It was shown that DMF is a good and nonselective solvent for the triblock copolymers, thus providing accessible polymer chain ends for the subsequent growth reactions.

To preserve the chain end functionality, each polymerization step was quenched by addition of 

The presented values are an average from five measurements of each sample obtained in the experiment 1 and 2. Bidistilled water was used for the analysis.

### Table 1. Thickness, Number Average Molecular Weights ($M_n$) and Polydispersity Indexes (PDI) of the Polymer Chains on the Gold Surface

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Reaction Time (min)</th>
<th>Sample</th>
<th>Thickness (nm)</th>
<th>$M_n$ (g/mol)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>Initiator SAM</td>
<td>1.6 ± 0.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHEMA</td>
<td>3.9 ± 0.5</td>
<td>2,800</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>PHEMA-PBMA</td>
<td>6.0 ± 0.4</td>
<td>7,700</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>PHEMA-PBMA-PHEMA</td>
<td>8.1 ± 0.5</td>
<td>11,800</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>PHEMA</td>
<td>7.2 ± 0.6</td>
<td>6,000</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>PHEMA-PBMA</td>
<td>12.2 ± 0.8</td>
<td>14,300</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>PHEMA-PBMA-PHEMA</td>
<td>15.1 ± 0.8</td>
<td>17,800</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Polydispersity index as a ratio of the mass average molecular weight to the number average molecular weight.

### Table 2. Contact Angle Measurements of the Functionalized Gold Surfaces

<table>
<thead>
<tr>
<th>Sample</th>
<th>Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold</td>
<td>75 ± 1</td>
</tr>
<tr>
<td>Initiator SAM</td>
<td>70 ± 1</td>
</tr>
<tr>
<td>PHEMA brushes</td>
<td>61 ± 1</td>
</tr>
<tr>
<td>PHEMA-co-PBMA</td>
<td>64 ± 1</td>
</tr>
<tr>
<td>PHEMA-co-PBMA-co-PHEMA</td>
<td>63 ± 1</td>
</tr>
</tbody>
</table>

*The presented values are an average from five measurements of each sample obtained in the experiment 1 and 2. Bidistilled water was used for the analysis.*

**Scheme 2.** Synthesis of PHEMA-co-PBMA-co-PHEMA triblock copolymer brushes via surface initiated ATRP.
a CuBr₂/PMDETA solution after the corresponding reaction time. The CuBr₂/PMDETA solution was prepared in the same solvent as the one applied for the ATRP. Additionally, we added a small amount of metallic copper to reduce Cu (II) to Cu (I) and thus to minimize the deactivation process. The corresponding polymer brushes (PHEMA and PHEMA-co-PBMA) containing active bromine atoms at the chain ends served successfully as macrorinitiators for the next synthetic step.

Characterization of the Copolymer Brushes

After each modification step, a subsequent change in the surface wetting behavior was detected. We performed two experiments, that is, experiments 1 and 2, which differed in the applied polymerization time (see Experimental part). Because the ATRP provides a linear growth of the polymer chain with time, the resulting polymer brushes from experiments 1 and 2 differed only in the molar mass of the respective polymer blocks while the chemical composition remained the same. Hence, the contact angle values for the polymer brushes from the two experiments were identical within experimental error. Averaged values of the angles are presented in Table 2. The contact angle decreased from 70° (initiator SAM) to 61° (Table 2) after the first PHEMA block was grown on the surface. Thus, in spite of the identical bromine end groups, the PHEMA layer exhibited a slightly more hydrophilic surface compared with the initiator SAM. It is known that the contact angles are very sensitive to the topmost surface composition changes, with a sensing depth of ~0.5–1.0 nm. Therefore, the ester- and hydroxyl side groups of the PHEMA block can potentially contribute to the overall hydrophilicity of the film.

The contact angles for the subsequent diblock brushes (64°) did not differ significantly from those measured for PHEMA layers (61°) (Table 2). However, we noted that the diblock brushes in all samples were consistently slightly more hydrophobic than the PHEMA layer. This could be due to the hydrophobic n-butyl side groups of the PBMA blocks, which can be partially exposed outside of the layer. Note that all samples were rinsed with ethanol and dried prior to contact angle analysis. Ethanol is a good solvent for both PHEMA and PBMA blocks thus providing solvation and a subsequent stretching. The contact angle value of the triblock copolymer layer was found to be 63°, and also it did not change drastically from the contact angle of the diblock PHEMA-co-PBMA brushes. Thus, we assume that the difference in the side groups of the polymer chains does not provide a considerable change in the contact angle values since order and packing of the macromolecules also play an important role for the wetting behavior of the complete polymer layer. Additionally, both PHEMA and PBMA blocks have identical back-bones which can also contribute to the rather similar contact angles.

The experiments with different reaction times should yield block copolymers with various chain lengths and hence, a variation of the overall layer thicknesses. Indeed ellipsometric measurements showed a significant increase of the film thickness after growth of each polymer block (Table 1). Polymerization of the first PHEMA block resulted in the increase of the layer thickness from 1.6 nm to 7.2 nm (experiment 1) and 12.2 nm (experiment 2), respectively. This corresponds to 2.3 and 5.6 nm thick PHEMA layers. A subsequent growth of the polymer brushes upon BMA polymerization caused a further increase of the polymer layer thickness to overall 6.0 nm (experiment 1) and 12.2 nm (experiment 2). After addition of the third block-PHEMA, the polymer layer thickness raised up to 8.2 nm (experiment 1) and 15.1 nm (experiment 2). Hence, the last copolymerization step provided ~2.2 nm (experiment 1) and 2.9 nm (experiment 2) nm thick PHEMA layers. These results confirm the successful growth of the individual blocks of the polymer brushes.

We further investigated the copolymer brushes using ATR-FTIR spectroscopy to prove the growth of the individual blocks. For the samples from both experiments, an increase of the peak intensity of the main characteristic functional groups was detected. Figure 1 shows a representative example of the ATR-FTIR spectra of the polymer brushes obtained in experiment 2.

The PHEMA spectrum [Fig. 1(a)] demonstrated characteristic absorption bands for ν(CH₃) and ν(CH₂) at around 2880–2990 cm⁻¹ and δ(CH₃) and δ(CH₂) at around 1360 cm⁻¹ and 1470 cm⁻¹, respectively. The ester groups of the methacrylate copolymer chains were confirmed by the strong signal ν(C=O) at 1740 cm⁻¹. After copolymerization of the PBMA block, the ATR-FTIR spectrum [Fig. 1(b)] showed an increase of the intensity of all characteristic peaks. The most significant change of the peak intensity corresponded to the carbonyl signal at 1740 cm⁻¹. An additional
intensity increase of CH, CH₂, CH₃, and C=O absorption bands [Fig. 1(c)] was detected also after growth of the third PHEMA block. The broad peak at about 3500 cm⁻¹ belongs to the OH groups of the PHEMA where the two bands at 3414 and 3515 cm⁻¹ can be clearly attributed to hydrogen bonds between hydroxyl groups of the PHEMA blocks.

Moreover, we applied PM-IRRAS to detect the change in the orientation of the methacrylate ester group toward the surface during chain growth. The ester group is directly connected to the main polymer chain (Fig. 2), thus the results obtained in the PM-IRRAS experiment give a qualitative indication of the chain tilt toward the gold surface. A signal in PM-IRRAS is observed when the transitory dipole moment of a molecule is perpendicular to the surface; otherwise, the signal remains invisible. PM-IRRAS was proved to be an effective method to determine the molecular orientation at surfaces, not only for small molecules,⁴³,⁴⁴ but also for polymers.⁴⁵,⁴⁶

The polymer samples synthesized in experiment 2 were used for the analysis. The angle conventions are given in Figure 2. Angle θ shows the tilt of the bisector of the two transition dipoles of the methacrylate ester group with respect to the normal of the surface, while τ represents the orientation of the main molecular chain toward the gold surface. We took into consideration the dipolar moment associated with the ester functionality, because the signal attribution was already performed in similar systems.⁴⁷,⁴⁸ Furthermore, the vibrations from the methacrylate ester groups ν(C=O) at 1732 cm⁻¹ and ν(C—C—O) at 1080 cm⁻¹ (Fig. 2) have orthogonal, unambiguous character and do not overlap with other groups. This makes their interpretation easier compared with the signals of methyl and ethyl groups.⁴⁵ Taking into account the coordinate analysis of the dipolar moment changes, and deconvolution of the PM-IRRAS spectra, the angles θ were determined for polymer brushes grown in the experiment 2. The results are summarized in Table 3. Obviously, the angle θ increased with increasing number of polymer blocks in the row PHEMA → PHEMA-co-PBMA → PHEMA-co-PBMA-co-PHEMA. This means that the tilt of the methacrylate ester groups toward the gold surface increased with polymerization of each new block. It seems that the increase of the angle value depended also on the chemical nature of the corresponding polymer blocks. Thus, a 5 nm increase in thickness after addition of the second PBMA block induced a 8° change of the ester group orientation toward the gold surface, whereas further growth of the

![Figure 1. ATR-FTIR spectra of the first PHEMA block (a), PHEMA-co-PBMA diblock (b), and PHEMA-co-PBMA-co-PHEMA triblock (c) brushes (experiment 2) on the gold support.](image1)

![Figure 2. Set of axes refers to the surface and not to the main molecular axis of the polymer chain. The molecule is oriented with respect to the surface as indicated in the scheme: the chain is tilted by the angle τ (dotted line), while angle θ shows the orientation of the bisector of the two transition dipoles of the methacrylate ester group to the normal to the surface. The transient dipolar moments of the ester group are presented in vectors. Note the orthogonal character of the two chosen vibrations.](image2)
third PHEMA block (only 2.9 nm) provided an additional 10° tilting. Most probably, interchain interactions such as hydrogen bonding between the hydroxyl groups of PHEMA play also an important role for the final orientation of the ester groups. It is also possible that a premature termination caused a local decrease of the grafting density and induced a subsequent change of the tilt angle between neighboring brushes that was also directly reflected in the orientation of the ester side groups of the chains. Note that the angles given by this method are averages that are also influenced by the polydispersity of the brushes.

Because the methacrylate ester group is connected to the main polymer chain, its increasing tilt to the surface upon addition of polymer blocks might be also related to the change of polymer chain orientation. This could explain the differences in the PM-IRRAS (Fig. 3) and the ATR-FTIR (Fig. 1) signals. Thus, upon the brush growth we observed an increase of the peak intensities in the ATR-FTIR spectra, whereas the characteristic signals had similar intensities in PM-IRRAS spectra. This discrepancy can be attributed to the change in the chain orientation, that is, the angle $\tau$. As the polymer chains grow, the number of functional groups increases and thus, their total absorption increases in ATR-FTIR (Fig. 1). However, the tilt of the polymer chains toward the surface also increases. Therefore, the intensity of the measured signal of the growing chain remains almost constant in PM-IRRAS spectra. Another important feature of the PM-IRRAS spectra is the absence of a signal for the H-bonded hydroxyl groups of the first PHEMA block, despite its clear presence in ATR-FTIR. This is presumably a result of hydrogen bonds oriented in a plane parallel (or only slightly tilted) to the gold surface. Most likely, these interactions are intermolecular (because they are coplanar and not collinear) and provide a tight binding mode and a densely coated polymer surface, respectively. Addition of the second PBMA block led to the appearance of two very small peaks at positions identical to those measured in ATR-FTIR and identified as hydrogen bonds. The detection of these two small bands indicated a change of hydrogen bond orientation toward the gold surface upon addition of the second PBMA block. We suppose that the shift of the hydrogen bond orientation was rather minor since the adsorption peaks were very small. However, a significant increase of the bands at 3414 and 3515 cm$^{-1}$ was detected after the last polymerization step. Thus, further growth of the third PHEMA resulted in the intensity increase of two hydrogen bond peaks indicating perhaps a formation of different hydrogen bond network compared with the first PHEMA brushes. We assume that in the case of triblock copolymer brushes steric effects of the second PBMA block prevent the formation of a dense network of hydrogen bonds in a plane parallel to the surface and shifts the interactions in a direction significantly tilted from the surface.

The surface analysis of the polymer films, however, did not provide information about the molecular weight and the polydispersity of the polymer brushes. To determine these parameters after each polymerization step, we detached the polymer chains from the solid substrate and performed GPC and $^1$H NMR studies. Oxidation of the thiol groups by iodine resulted in a cleavage of the Au-S bond and a release of the polymer brushes. This procedure was already described.

![Figure 3](image)

**Table 3. Values of the Angle $\theta$ for the Copolymer Brushes (Experiment 2)**

<table>
<thead>
<tr>
<th>Block Structure</th>
<th>$\theta$ (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHEMA</td>
<td>30</td>
</tr>
<tr>
<td>PHEMA-co-PBMA</td>
<td>38</td>
</tr>
<tr>
<td>PHEMA-co-PBMA-co-PHEMA</td>
<td>48</td>
</tr>
</tbody>
</table>

Angle $\theta$ indicates orientation of the bisector of the two transition dipoles of the methacrylate ester group to the normal to the surface (see Fig. 2).
A dimerization via disulfide-bridge formation was not observed.\textsuperscript{50} The chemical compositions of the detached PHEMA, PHEMA-co-PBMA, and PHEMA-co-PBMA-co-PHEMA triblock polymer chains were verified by $^1$H NMR (Fig. 4). The main characteristic protons of PHEMA (a–4.03 and b–3.74 ppm) and PBMA (c–3.95 and d–0.88 ppm) were clearly seen in the spectra of the diblock copolymers [Fig. 4(1)]. The copolymerization of the third PHEMA block was reflected in a decrease of the ratio between the corresponding PHEMA [Fig. 4(2,a and b)] and PBMA (Fig. 4(2,c)] peaks.

The number average molecular weights ($M_n$) and the polydispersity indexes (PDI) were measured by GPC and are shown in Table 1 (the GPC curves are presented in the supporting information). The first PHEMA as well as the PHEMA-co-PBMA diblock copolymer brushes from both experiments had relatively narrow polydispersities, that is, 1.1 and 1.2, respectively. However, the triblock copolymer chains revealed broader PDIs, especially for experiment 2 (PDI = 1.6) where longer polymer chains were synthesized. This result can be explained by the fact that the probability of termination processes increases with increasing chain length during conventional surface-initiated ATRP,\textsuperscript{38,51,52} thus leading to broader polydispersities of polymers with higher molecular weights.

Having information about the molecular weights, we established the copolymer compositions to be PHEMA$_{21}$-co-PBMA$_{34}$-co-PHEMA$_{31}$ (experiment 1) and PHEMA$_{46}$-co-PBMA$_{58}$-co-PHEMA$_{27}$ (experiment 2), respectively, (Table 1). Thus, the increase of the polymer chain lengths corroborates the increase of the layer thicknesses measured by ellipsometry.

**AFM Investigations**

The grafted triblock copolymer brushes have an amphiphilic structure where hydrophilic (PHEMA) and hydrophobic (PBMA) blocks of the polymer chain might have different response toward solvent treatment. To test their solvent-responsive behavior, we used block-selective solvents as well as a good common solvent for both block types (experiment 2). Ethanol was chosen as a good solvent for the triblock copolymer chains, whereas hexane and water selectively swell the PBMA and PHEMA blocks, respectively.

Figure 5(A) shows the topography (contact mode) of the polymer sample treated in ethanol. Globular domains with an average size of around 70 nm were seen on the images. The analysis of different areas of the sample showed similar topography indicating homogeneity of the polymer film. An analogous surface morphology was reported for polystyrene-co-poly(methyl methacrylate...
The authors demonstrated that the average size of the domains and roughness of the polymer surface depended on the interchain interactions as well as on the polymer block lengths that were assumed to be proportional to ellipsometric thicknesses. Although we describe an amphiphilic polymer system with different brush thicknesses than the one discussed in literature, similar effects could explain the appearance of the observed nanomorphologies. Briefly, as ethanol is a good solvent for the triblock brushes, we assume that the polymer chains are stretched away from the gold support forming a brush-like structure. Removing ethanol from the polymer layer leads to a collapse of the polymer chains and creation of nanodomains. Additionally, we also suggest that the removal of ethanol supports hydrogen bond formation between neighboring PHEMA chains that contribute the nanodomain morphology of the surface.

Overnight incubation of the amphiphilic copolymer membrane in hexane resulted in a completely different topography of the surface. Figure 5(B) shows a ripple surface of the polymer film. The cross-section analysis [Fig. 5(B,2)] revealed a different surface profile in contrast to the results obtained after sample treatment with ethanol [Fig. 5(A,1)]. The surface histogram demonstrated an increase of the height deviation [Fig. 5(B,b)] compared with the histogram of the polymer film treated with ethanol [Fig. 5(A,a)]. Thus, change in the solvent polarity caused a reorganization of the polymer brushes. Supported by similar previous observations, we propose that triblock copolymers rearranged so that the more hydrophobic PBMA part was exposed toward hexane thus shielding the PHEMA from an unfavorable

**Figure 5.** AFM analysis of the amphiphilic triblock copolymer brushes treated with ethanol (A) and hexane (B). The cross-section profiles of the polymer brushes stored in ethanol (1) and hexane (2) show different surface interface. The histograms of the polymer brushes treated with hexane (b) reveals an increase of the surface roughness compared to the surface roughness of the polymer brushes treated with ethanol (a).
contact to the poor solvent. Drying of the sample caused a collapse of these shielding PBMA loops and a featureless surface was formed correspondingly.\textsuperscript{55}

Finally, the amphiphilic copolymer brushes were subsequently immersed in ethanol, ethanol/water (1:1, vol %), and water and left overnight in water prior to the AFM measurements. Commonly, the PHEMA blocks considerably swell in water, whereas the hydrophobic PBMA block tends to avoid contact to the aqueous surrounding. Figure 6(a) shows the 3D topography image of the wet copolymer chains on the gold surface. The brush-like structure of the macromolecules is in agreement with a stretching of the PHEMA chains upon swelling with water. After drying the sample acquired again a nanodomain topography of the surface [Fig. 6(b)], which was similar to the surface of the polymer brushes treated in ethanol [Fig. 5(a)]. Most probably, also here drying caused a collapse of the polymer brushes and thus, formation of the nanodomains. This observation corroborates well with the data on polymer brushes reported before.\textsuperscript{56}

Interestingly, all these phase segregation processes were reversible because a reimmersion of the sample into one of three tested solvents resulted in reproducible morphologies as described earlier. This proves not only the covalent attachment of the block copolymer layer but could potentially also be used to create responsive surfaces.

To detect the change of uppermost surface composition after membrane immersion into block-selective solvents, we performed contact angle measurements. However, no significant difference of contact angle values was found for the samples treated in ethanol, water, and hexane. These results can indicate that either the amphiphilic polymer chains adjust their conformation immediately after exposure to bidistilled water applied for the contact angle measurements or the different side groups of methacrylate polymer backbones do not cause considerable changes of surface wetting properties. Hence, the contact angle measurements did not provide us any information about reorganization of brushes. Other techniques such as X-ray surface diffraction analysis and X-ray reflectivity will be applied to study the solvent-responsive behavior of amphiphilic polymer membrane in more details. The results will be published elsewhere.

**CONCLUSIONS**

ATRP was successfully applied for grafting of amphiphilic ABA'-triblock copolymer membranes from gold supports. Our experiments indicated that the length of the individual blocks could be controlled by varying the polymerization time. In addition, we confirmed the formation of PHEMA-co-PBMA-co-PHEMA brushes by a variety of different analytical techniques that did not only give information about layer thickness and surface topography but allowed also a preliminary estimation of the orientation of methacrylate ester groups directly connected to the polymer backbone. The amphiphilic character of the triblock copolymer brushes provided a responsive surface that showed a solvent dependent arrangement of the block copolymer chains, which was also reflected in the morphologies of the dried films.

Most interestingly the polymer brushes with a hydrophilic–hydrophobic–hydrophilic sequence can be regarded as a first example of a solid supported, biomimetic block copolymer membrane that has been prepared by a "grafting from" approach.
Upon insertion of membrane proteins these systems could allow for the preparation of mechanically and chemically robust and, potentially, even air-stable biosensor devices. It should be noted that the insertion of membrane proteins and preservation of their functionality requires also a certain mobility of the polymer molecules within the membranes. Because, here, all the individual chains are covalently attached to the solid support they cannot undergo lateral diffusion. However, block copolymer membranes are known to be highly flexible and compressible, which, in our system, can additionally be influenced by the grafting density of the polymer chains. Therefore, we expect that a solubilization of functional building blocks might still be possible. Further experiments to investigate protein insertion and lateral diffusion of “guest-molecules” within the membranes are in progress.

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REFERENCES AND NOTES
