

# Reversible Activation of Diblock Copolymer Monolayers at the Interface by pH Modulation, 1: Lateral Chain Density and Conformation

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This study focuses on the design of chemically regulated surfaces that allow for reversible control of the interactions between biological matter (cells and proteins) and planar substrates. As a tunable interlayer, we use a monolayer of a near-monodisperse poly[2-(dimethylamino)ethyl methacrylate-*block*-methyl methacrylate] (PDMAEMA-PMMA) diblock copolymer. Owing to the relatively large fraction (50%) of the hydrophobic PMMA block, this copolymer forms a stable Langmuir monolayer at the air/water interface. Both *in situ* and *ex situ* film balance experiments suggest that the hydrophilic PDMAEMA block adsorbs to the air/water interface in its uncharged state (pH 8.5), but stretches into the subphase in its charged state (pH 5.5). Optimization of the preparation protocols enables us to fabricate stable, homogeneous diblock copolymer films on hydrophobized substrates via Langmuir–Schaefer transfer at well-defined lateral chain densities. Ellipsometry and X-ray reflectivity studies of the transferred films confirm that the film thickness can be systematically regulated by the lateral chain densities. The transferred copolymer films remain stable in water for about a week, suggesting that they are promising materials for the creation of pH-controlled solid substrates for the support of biological matter such as proteins and cells.

## Introduction

The design of compatible interlayers between hard solid matter and soft biological matter is a cross-disciplinary challenge.<sup>1–3</sup> Practical applications where such interlayers are required include the fabrication of novel biofunctional hybrid materials by means of stress-free immobilization of proteins and proliferation of cells onto solid-based devices. In these applications, and also for the study of transmembrane protein functions on planar solid substrates (glass, semiconductor, and metal),<sup>4–7</sup> biocompatible interlayers are required to prevent the direct protein–substrate contact, which often results in denaturation of proteins.<sup>8</sup> In fact, interactions between cells and tissues in nature are mediated by the hydrated layers of biopolymers such as the extracellular matrix (ECM)<sup>9</sup> and the cell–surface glycocalix.<sup>10</sup>

To date, several different strategies have been proposed for the design of interfacial layers that mimic generic roles of ECM and glycocalix. A straightforward strategy is the direct use of

natural ECM components such as laminin, fibronectin, and hyaluronan.<sup>5,9</sup> Although many studies demonstrate that they can bridge cells and solid substrates without denaturing proteins or causing cell death (apoptosis), the physical properties of these natural macromolecules are not well defined. Another promising approach is to coat the surfaces with hygroscopic polymer films or hydrogels such as dextran<sup>11</sup> and poly(ethylene glycol) (PEG) brushes.<sup>12</sup> Because of steric (entropic) forces, these films can resist nonspecific adsorption of proteins.<sup>13–16</sup> On the other hand, such layers are known to destabilize the membrane–surface contact due to large induced fluctuations, so that cells and lipid membranes are repelled from the surface.<sup>17</sup>

Recently, many studies have been conducted to utilize multilayers of strong polyelectrolyte films as polymer interlayers. Although this type of films can easily be grown by alternative deposition of cationic and anionic polyelectrolytes,<sup>18,19</sup> this approach has fundamental drawbacks. Most of these polyelectrolyte complexes are based on strong polyelectrolytes, which carry extremely high charge densities over wide pH ranges. Such highly charged surfaces often attract cells and proteins too strongly when they are oppositely charged. In fact, we have previously found that adult mammal cell membranes (derived from human erythrocytes) were strongly pinned on the surface of a commonly used polycation (poly-L-lysine), which can be explained as the dissipation of the anionic cell envelope (glycocalix with sialic acid residues) to a surface bearing a high density of positive charges.<sup>20</sup>

To alter the balance of forces across the polymer layers by external stimuli, weak polyelectrolytes seem more advantageous against strong polyelectrolytes, since the degree of ionization

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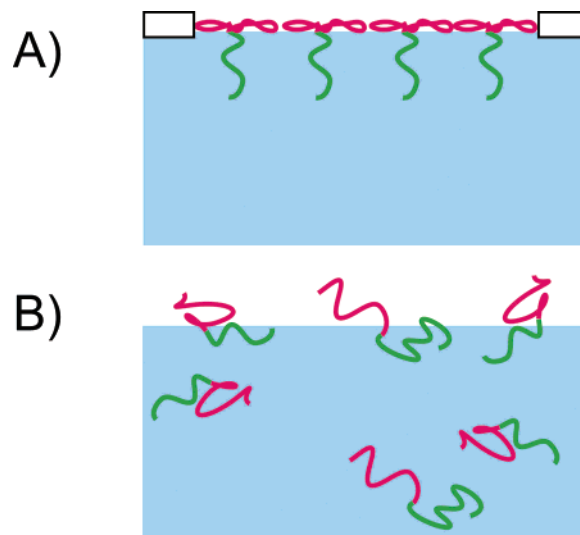
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(d.i.) can be flexibly adjusted by pH titration.<sup>21–24</sup> As chemically switchable polymer material, we chose a diblock copolymer monolayer based on poly[2-(dimethylamino)ethyl methacrylate-*block*-methyl methacrylate], (PDMAEMA-PMMA), which comprises an equal number of monomer repeat units ( $n = 36$ ) for the hydrophobic PMMA and hydrophilic PDMAEMA blocks (designated sample code: DB 50). The hydrophilic PDMAEMA block is a weak polyelectrolyte brush, whose degree of ionization (d.i.) can be switched at around physiological conditions. These seem to be more promising materials than conventional strong polyelectrolytes because: (i) the degree of ionization (i.e., the charge density) is lower than that of fully charged polyelectrolytes, and (ii) the switching between the “charged” and “uncharged” states can occur around neutral pH. Here, the charging/decharging of the PDMAEMA block is expected to reversibly alter, not only the degree of ionization (electrostatic forces), but also the polymer conformation (entropic forces), volume fraction of water (hydration forces), and surface free energy of the film surface (dispersive forces).

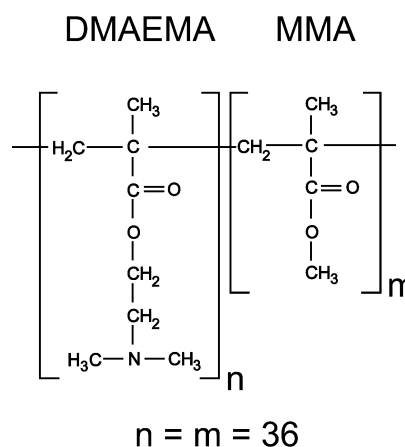
Previously, An et al. fabricated similar diblock copolymer monolayers containing either 70 or 80 mol % of PDMAEMA block (designated DB 70 and DB 80, respectively), which were adsorbed from the subphase to the air/water interface.<sup>25,26</sup> Neutron reflectivity experiments conducted at the air/water interface revealed that the thickness of the Gibbs monolayer, i.e., the amount of polymer absorbed to the interface, strongly depended on the solubility of copolymers (and thus on the solution pH). An et al. also measured the thickness of diblock copolymer films physisorbed on hydrophobic substrates as a function of pH by using neutron reflectivity at the solid/liquid interface.<sup>27</sup> Although varying the solution pH systematically affected the resulting film thickness, the grafting density created by adsorption was relatively low (1300 Å<sup>2</sup> per polymer chain).<sup>27</sup> This can be attributed to the limited compressibility of self-assembled glassy PMMA backbones, whose statistical segment length persists over 5–6 monomers.<sup>28,29</sup>

In the present study, we employed a similar diblock copolymer containing equal number of monomers ( $n = 36$ ) from each block (DB 50). In contrast to DB 70 and DB 80, which form soluble Gibbs monolayers at the air/water interface, the larger fraction of hydrophobic PMMA block in DB 50 was designed to form insoluble Langmuir monolayers at the air/water interface, where the accessible area per molecule and lateral density can be precisely controlled, as depicted in Figure 1. Instead of physisorption from solution, one can transfer the Langmuir monolayer from the air/water interface onto a solid surface simply by horizontal dipping of the hydrophobized substrate (Langmuir–Schaefer transfer).<sup>30</sup> By using Langmuir isotherms (surface pressure vs area at a constant temperature), we can transfer the films at well-defined lateral chain densities and reach higher polymer chain densities by compressing the monolayer to higher surface pressures.

In the first part of this paper, the Langmuir isotherms of DB 50 monolayers at the air/water interface are examined at different pH conditions. Deviations in the global shape of the isotherms can be interpreted in terms of differences in the lateral compressibility, especially at low surface pressures. When the PDMAEMA block is charged by pH titration at a constant surface area, we observe a decrease in the surface pressure. This decrease in the surface pressure clearly indicates desorption of charged polymer chains from the interface. In the second part, the DB 50 monolayers are transferred onto hydrophobized substrates at several lateral pressures. The dry DB 50 films deposited on silicon wafers are characterized by ellipsometry



**Figure 1.** Schematic illustrations of (A) an insoluble Langmuir type monolayer with a well-defined area per molecule, and (B) a Gibbs monolayer where molecules are in solution as well as adsorbed at the interface.



**Figure 2.** Chemical structure of the PDMAEMA-PMMA diblock copolymer (DB 50,  $M_w = 9.3$  kDa;  $M_w/M_n < 1.10$ ). The hydrophobic PMMA block provides the stability of monolayers at the interface, while the degree of ionization of the PDMAEMA block ( $pK_a = 7.3$ ) can be modulated by varying the solution pH.

and specular X-ray reflectometry, indicating a homogeneous transfer of DB 50 monolayers at each surface pressure. In fact, the film thickness remained constant even after intensive rinsing of the films with water, confirming their mechanical and thermodynamical stability.

## Experimental Section

**Materials.** The synthesis of the PDMAEMA-PMMA diblock copolymer (DB 50,  $M_w = 9.3$  kDa;  $M_w/M_n < 1.10$ ) followed the protocol described elsewhere.<sup>31</sup> This copolymer comprised 50 mol % DMAEMA and 50 mol % MMA; each block has a mean degree of polymerization of 36. The chemical structure is shown in Figure 2. The water used throughout this study was purified by using a Milli-Q device (Millipore, Molsheim, France).

To adjust the subphase conditions, a phosphate buffered saline (PBS) with 10 mM NaH<sub>2</sub>PO<sub>4</sub> and 100 mM NaCl was used. Fully deuterated poly(methyl methacrylate) (d8-PMMA) with a molar mass of 35 kDa and a polydispersity index of 1.10 (PSS, Mainz, Germany) and octadecyltrimethoxysilane (ODTMS) (ABCR, Karlsruhe, Germany) were used for hydrophobization of the

TABLE 1: Material Constants Used in This Study

|               | $n$<br>( $\lambda = 633$ nm)   | $V$<br>[ $\text{\AA}^3$ ] <sup>26</sup> | $\rho$<br>[ $10^{-5} \text{\AA}^{-2}$ ] | $\text{Im}\{\rho$ [10 <sup>-7</sup> $\text{\AA}^{-2}$ ] |
|---------------|--------------------------------|-----------------------------------------|-----------------------------------------|---------------------------------------------------------|
| silicon       | 3.868 - $i0.024$ <sup>33</sup> |                                         |                                         |                                                         |
| silicon oxide | 1.462 <sup>a</sup>             |                                         | 1.98                                    | 1.18                                                    |
| d-PMMA        | 1.49 <sup>34</sup>             | 140                                     | 1.36                                    | 1.41                                                    |
| DB 50         | 1.5                            | 365                                     |                                         |                                                         |

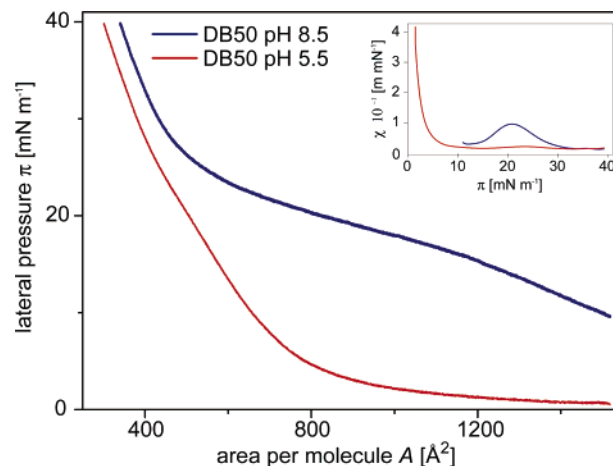
<sup>a</sup> The refractive index of silicon oxide was fitted together with the thickness of the approximately 150 nm oxide layer with the homemade "Fitpaket" program.

silicon wafer surfaces. The silicon (100) substrates with 150 nm thermal oxide were a gift from Wacker Siltronic (Burg-hausen, Germany). All other chemicals were purchased from Sigma-Aldrich (Munich, Germany) and used without further purification.

**Langmuir Isotherms.** Langmuir monolayers of DB 50 were prepared on two types of film balances: (a) a homemade Teflon trough with a total surface area of 1008 cm<sup>2</sup>, and (b) a  $\mu$ -trough with an area of 120 cm<sup>2</sup> (Kibron, Helsinki, Finland). Prior to each experiment, the troughs were cleaned with a 2% Hellmanex solution (Hellmanex, Germany) at 40 °C for 1 h, and rinsed intensively with water. Prior to compression, a 1.2 mg mL<sup>-1</sup> solution of DB 50 in CHCl<sub>3</sub> was spread onto the water subphase. After 15 min, the film was compressed with a single moving barrier at a velocity of  $v = 100 \mu\text{m s}^{-1}$ . The titration experiment was performed after compression of the film to a surface pressure of 20 mN m<sup>-1</sup>. The pH of the subphase was titrated by injecting HCl into the subphase. Changes in the surface pressure were monitored as a function of time while keeping the film area constant.

**Film Transfer onto Solid Supports.** The solid substrates were cleaned by using an abbreviated RCA method and were hydrophobized with two types of organic films: (i) self-assembled monolayers of octadecyltrimethoxysilane (ODTMS) and (ii) spin-coated films of d8-PMMA. The ODTMS monolayer was chemically grafted according to a protocol reported elsewhere.<sup>32</sup> For spin-coating, a 10 mg mL<sup>-1</sup> solution of d8-PMMA in toluene was spread onto the silicon substrate until it fully covered the surface, then the wafer was spun for 60 s at 3500 rpm. After that, the samples were annealed for 1 h at 120 °C. For the Langmuir-Schaefer (LS) transfer, the silicon wafer was placed onto the subphase, while the film balance kept the pressure constant at 20 °C. The sample orientation was adjusted precisely horizontal before the film transfer, and the sample was approaching the surface at a velocity of  $v = 100 \mu\text{m s}^{-1}$ . After contact to the subphase, we let the sample rest for 60 s before lifting it up. Subsequently, it was dried at 70 °C for 1 h, then rinsed with Millipore water and dried again with a jet of N<sub>2</sub>.

**Characterization of the Transferred Films.** The contact angles of sessile droplets of PBS at pH 5.5 and pH 8.5 on the deposited DB 50 films were measured with a FTA 200 contact angle meter (First Ten Ångströms, Portsmouth, VA). The droplet shapes were analyzed with an ellipsoidal fit. Thickness of the deposited polymer layers as well as of the silicon oxide was measured by using a conventional PCSA (polarizer, compensator, sample, and analyzer) ellipsometer (Plasmos GmbH, Germany) at an angle of incidence of 70° and wavelength of 632.8 nm (He-Ne laser). The refractive indices used for the analysis are given in Table 1. Specular X-ray reflectivity measurements were performed with a homemade triple-axis diffractometer at HMI, Berlin. The primary "white" beam from a sealed X-ray tube (Cu anode) was defined by a line focus of 0.04 mm  $\times$  8 mm width. After hitting the sample, the beam passed through an aperture of 2 mm and was monochromatized



**Figure 3.** Langmuir isotherms of DB 50 monolayers measured at 20 °C for pH 8.5 (upper curve) and pH 5.5 (lower curve). Clear changes in the area per molecule can be attributed to protonation/deprotonation of the PDMAEMA block. The inset represents the lateral compressibility of the diblock copolymer monolayer  $\chi$ , as given by eq 2.

at a pyrolytic graphite crystal, which reflected it into the detector. The crystal was set to reflect the Cu K $\alpha$  doublet ( $\lambda = 1.541 \text{\AA}$ ). For the first scan region, including the total reflection ( $\theta < 0.3^\circ$ ), a Ni absorber was inserted into the reflected beam to protect the detector. For the analysis of the reflectivity data, we normalized the measured intensity with the incident intensity  $I_0$  and applied a footprint correction to account for the sample illumination at different angles of incidence. The experimental resolution of the setup was  $\Delta q = 3 \times 10^{-3} \text{\AA}^{-1}$ . To determine the thickness and roughness of the polymer layers, a least-squares fit was applied with the software Parratt32 (available from the Hahn-Meitner Institute Berlin via [http://www.hmi.de/bensc/instrumentation/instrumente/v6/refl/parratt\\_en.htm](http://www.hmi.de/bensc/instrumentation/instrumente/v6/refl/parratt_en.htm)) by assuming a four-box model (air, DB 50, PMMA, silicon oxide). The real and imaginary parts of the electron densities used for this fitting are shown in Table 1. Since the silicon oxide layer was 150 nm thick, the underlying silicon was not taken into account.

The transferred momentum  $q$  at an angle  $\theta$  of specular reflection can be expressed as:

$$q = \frac{4\pi}{\lambda} \sin \theta \quad (1)$$

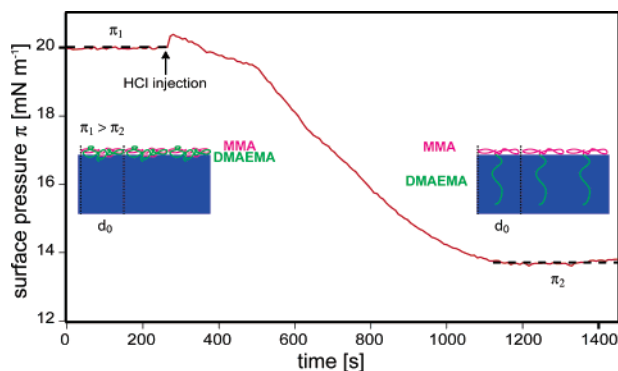
The total film thickness,  $d$ , was extracted from a linear fit to the positions of the maxima ( $\theta_n$ ) of the Kiessig fringes,  $n$ , according to eq 2:

$$n = \frac{2d}{\lambda} \sin \theta_n \quad (2)$$

## Results and Discussion

**Effects of Subphase pH on Langmuir Isotherms, In Situ pH Titration.** Figure 3 shows Langmuir isotherms recorded at different pH subphase conditions at 20 °C. As shown in the figure, the overall shapes of the two curves at pH 8.5 (blue line) and pH 5.5 (red line) are clearly different. The DB 50 monolayer always occupies a bigger area per molecule at pH 8.5 than at pH 5.5 at all the measured lateral pressures. The deviation between these two pH conditions is particularly significant in the lower pressure region, where  $\pi < 25 \text{ mN m}^{-1}$ . At a lateral pressure of  $\pi = 20 \text{ mN m}^{-1}$ , the area per molecule is  $825 \pm 10 \text{\AA}^2$  at pH 8.5 and  $505 \pm 10 \text{\AA}^2$  at pH 5.5. This difference decreases with further compression of the monolayer





**Figure 4.** In situ surface pressure measurements on PDMAEMA-PMMA diblock copolymer monolayers during pH titration. The film was first compressed to  $\pi = 20 \text{ mN m}^{-1}$  at pH  $\sim 8$ , and HCl was injected to adjust the pH to around 6. A clear drop in the surface pressure coincides with desorption of the charged PDMAEMA block from the interface. The schematic views of polymer chain conformations are presented as insets.

at higher lateral pressures, resulting in a consistent shape of the isotherm above  $\pi > 25 \text{ mN m}^{-1}$ . This can be attributed to the difference in the isothermal lateral compressibility of the molecule  $\chi$ :

$$\chi = -\frac{1}{A} \left( \frac{\partial A}{\partial \pi} \right)_T \quad (3)$$

As presented in the inset of Figure 3, the lateral compressibility of a DB 50 monolayer at low surface pressures strongly depends on the adsorption of the PDMAEMA block to the air/water interface. For example, the compressibility for the charged (d.i. = 85% at pH 5.5) polymer at  $20 \text{ mN m}^{-1}$  is  $\chi = 2.9 \times 10^{-1} \text{ m mN}^{-1}$ , while  $\chi = 1.0 \times 10^{-1} \text{ m mN}^{-1}$  for the neutral (d.i. = 12% at pH 8.5) monolayer. On the other hand, the compressibility is largely dominated by the glassy PMMA block at high surface pressures ( $\pi > 30 \text{ mN m}^{-1}$ ), where the  $\chi$  values for uncharged and charged monolayers are identical:  $\chi = 2.4 \times 10^{-2} \text{ m mN}^{-1}$ .

To further investigate the adsorption/desorption of PDMAEMA chains to/from the air/water interface, a pH titration experiment was carried out at a constant film area (Figure 4). Throughout this study, the pH condition of the subphase was adjusted with the aid of a conventional pH meter (Schott GmbH, Hofheim, Germany) within an accuracy of  $\pm 0.1$ . As explained in the previous section, the uncharged DB 50 film was first compressed to  $\pi = 20 \text{ mN m}^{-1}$ . During the titration of the subphase pH with HCl, the surface pressure decreases, reaching a new equilibrium pressure of  $\pi = 13 \text{ mN m}^{-1}$ . The subphase pH was also checked after the entire experiment, confirming that there is no pH drift after reaching the new equilibrium. We also verified that the surface pressure change due to the slight increase in the subphase volume was negligible. Since the hydrophobic PMMA block is insensitive to the subphase pH conditions, the observed change in the surface pressure does reflect the conformational change of the PDMAEMA block upon the pH titration.

The conformational change of the PDMAEMA block is dominated by the charging (protonation) and decharging (deprotonation) of its tertiary amine side groups. This block has a bulk  $pK_a$  of 7.3 and a surface  $pK_a$  of 6.7, where the  $pK_a$  is defined as the pH at which 50% of the tertiary amine groups are charged. The expected degrees of ionization (d.i.) for PDMAEMA at the surface for several pH conditions are presented in Table 2 and described in refs 36–38. Thus one can calculate that 85%

**TABLE 2: Degree of Ionization (d.i.) of the PDMAEMA Block at the Surface at Different pH Conditions<sup>35–37</sup>**

| d.i. | pH  |
|------|-----|
| 0.12 | 8.5 |
| 0.23 | 7.5 |
| 0.61 | 6.5 |
| 0.85 | 5.5 |

of the tertiary amine groups are charged at pH 5.5, while only 12% of these amine groups are charged at pH 8.5. This significant difference has a dramatic effect on the polarity of the PDMAEMA block. When the d.i. is 12% (at pH 8.5), the PDMAEMA block is only weakly hydrophilic. This leads to poor phase separation of the PDMAEMA block and the PMMA block at the air/water interface. As schematically depicted in the inset of Figure 4, the uncharged PDMAEMA block adsorbs to the air/water interface and becomes intermixed with the PMMA block. On the other hand, the two blocks become segregated when the d.i. of the PDMAEMA reaches 85% at pH 5.5. Here, the charged PDMAEMA block tends to desorb from the air/water interface, resulting in the observed reduction in surface pressure (Figure 4).

Previously, Ahrens et al.<sup>38</sup> reported the condensation of counterions in diblock copolymer monolayers, where the hydrophilic block is a strong polyelectrolyte [poly(sodium 4-styrenesulfonate)]. By using specular X-ray reflectometry at the air/water interface, they postulated the condensation of counterions in charged polymer brushes.<sup>39</sup> In fact, the isotherms of the charged PDMAEMA-PMMA monolayer (pH 5.5, stabilized with 10 mM of phosphate buffer) showed a slight difference in the presence or absence of 100 mM NaCl. On the other hand, at lateral pressures  $\pi > 10 \text{ mN m}^{-1}$ , no significant deviations could be observed (see Supporting Information). This result suggests that, at pressures  $\pi > 10 \text{ mN m}^{-1}$  (corresponding to the average intermolecular distance of less than 25 Å), the electrostatic interaction between the charged neighboring PDMAEMA chains are screened, probably due to the condensation of counterions.

**Characterization of the Dry PDMAEMA-PMMA Diblock Copolymer Films on Solid Substrates.** To optimize the film transfer onto solid supports, we need to screen two preparation conditions: (i) the precursor films for surface hydrophobization, and (ii) the subphase pH conditions during film transfer.

We studied two precursor films that render the surface hydrophobic: (a) self-assembled monolayers of alkylsilane (ODTMS), and (b) spin-coated films of deuterated (d8-PMMA). The first system (thickness of  $\sim 2.5 \text{ nm}$ ) resulted in a water contact angle of  $\theta > 95^\circ$ , suggesting that the monolayer consists of crystalline-like alkyl chains. On the other hand, the spin-coated PMMA films exhibit a slightly smaller contact angle ( $\theta \sim 70^\circ$ ), which coincides with the amorphous PMMA chains on the solid surface. Interestingly, despite the excellent hydrophobicity, we often observe partial loss of the transferred diblock copolymer films from the ODTMS surface when the films are intensively rinsed with pure water. In contrast, the copolymer films deposited onto PMMA precursors seem to be stable against intensive rinsing or long-term ( $\sim 1$  week) storage under water. This can be explained in terms of the interdigitation or entanglements between the PMMA block and the PMMA precursor, which stabilizes the diblock copolymer films better than the more-ordered ODTMS interlayer. Thus, we focus on d8-PMMA precursors in the following experiments.

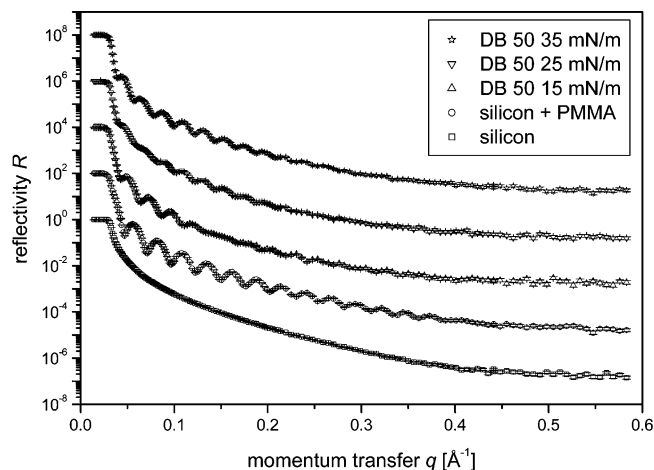
The second parameter, the subphase pH condition at the film transfer, is important for highlighting the effect of preorganization (phase separation) of the hydrophobic and hydro-

**TABLE 3: Contact Angle  $\theta$  for Sessile Droplets of PBS at Different pH**

| pH of LS transfer | droplet pH 5.5 | droplet pH 8.5 |
|-------------------|----------------|----------------|
| 5.5               | 52°            | 55°            |
| 8.5               | 88°            | 78°            |

philic blocks at the air/water interface on the quality of the transferred films. Such segregation is desired for the effective switching of PDMAEMA chains by pH adjustment. Here, the films were transferred at pH 5.5 and pH 8.5, where PDMAEMA chains are charged (d.i. = 85%) and uncharged (12%), respectively. Water contact angles were measured by deposition of sessile droplets of phosphate buffered saline (10 mM PB and 100 mM NaCl) adjusted at pH 8.5 and pH 5.5. To maximize the segregation between the two blocks, the copolymer monolayers were transferred at 35 mN m<sup>-1</sup>. As presented in Table 3, the films transferred at pH 8.5 showed a large contact angle of  $\theta = 88^\circ$  in contact with a droplet at pH 8.5. Such a high contact angle is actually comparable to that of a hydrophobic PMMA precursor. When the same film is in contact with a droplet at pH 5.5, the contact angle decreased to  $\theta = 78^\circ$ ; this can be explained by a slight increase in the polarity of the PDMAEMA block due to protonation of its tertiary amine groups. However, the high contact angles observed here imply that the intermixing of the PMMA and PDMAEMA blocks at the air/water interface (as demonstrated in Figures 3 and 4) was preserved even after the film transfer. In fact, the contact angle measurements showed hysteresis in receding contact angles and pinning centers deforming the contact line, suggesting that there may be chemical heterogeneity on the surface due to intermixing of PMMA and PDMAEMA blocks. On the other hand, the films transferred at pH 5.5 (d.i. = 85%) had a significantly smaller contact angle of  $\theta = 55^\circ$  when in contact with a droplet at pH 8.5. The contact angle decreased further to  $\theta = 52^\circ$ , when the surface is in contact with a droplet at pH 5.5. The lower contact angles observed for the films transferred at pH 5.5 indicate that separation of the two blocks before transfer is the key to maximizing the presence of the hydrophilic blocks at the surface. In fact, the difference between static and receding contact angles on the copolymer films prepared at pH 5.5 was smaller than on those prepared at pH 8.5. Therefore, we prepared all films at a subphase of pH 5.5 and transferred them onto silicon wafer surfaces coated with d8-PMMA.

The thicknesses of dry diblock copolymer films transferred at 15, 25, and 35 mN m<sup>-1</sup> were first measured by ellipsometry. The three transfer pressures correspond to areas per molecule,  $A$ , of  $576 \pm 10 \text{ \AA}^2$  (15 mN m<sup>-1</sup>),  $435 \pm 7 \text{ \AA}^2$  (25 mN m<sup>-1</sup>), and  $338 \pm 5 \text{ \AA}^2$  (35 mN m<sup>-1</sup>), respectively. In comparison to the lateral densities of physisorbed DB 70 and DB 80 diblock copolymers (1300  $\text{\AA}^2$  per molecule), we can achieve densities that are 2–3 times higher by transfer of compressed Langmuir monolayers from the air/water interface. The thicknesses of silicon dioxide, d8-PMMA, and the DB 50 copolymer were measured after the deposition of each layer by using the refractive indices given in Table 1. The mean values of the diblock copolymer layer thicknesses from more than five independent measurement points are summarized in Table 4. The film thicknesses obtained from macroscopically large surfaces (laser beam diameter of 1 mm, the separation between individual measurement points is 2 mm) are very uniform, with a thickness variation of only  $\pm 5 \text{ \AA}$  throughout the experiments. These errors are comparable to those of the underlying silicon dioxide and d8-PMMA, suggesting that the homogeneity of DB 50 copolymer films is independent of the transfer pressures. In fact, the rms roughness values of the DB 50 copolymer films

**Figure 5.** The reflectivity curves ( $I/I_0$  vs  $q$ ) of a bare silicon wafer (squares), a silicon wafer coated with d8-PMMA (circles), silicon wafers with d8-PMMA and copolymer DB 50 transferred at 15 mN m<sup>-1</sup> (upright triangles), 25 mN m<sup>-1</sup> (upside down triangles), and at 35 mN m<sup>-1</sup> (stars).**TABLE 4: Area Per Molecule  $A_{\text{Mol}}$  at the Air/Water Interface and Thickness  $h$  of DB 50 at the Air/Solid Interface at Different Pressures  $\pi$ , Respectively**

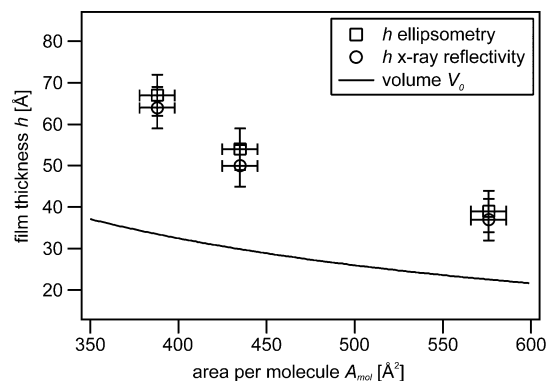
| $\pi$ [m N m <sup>-1</sup> ] | $A$ [ $\text{\AA}^2$ ] | $h$ [ $\text{\AA}$ ] ellipsometry | $h$ [ $\text{\AA}$ ] X-ray |
|------------------------------|------------------------|-----------------------------------|----------------------------|
| 15                           | $576 \pm 10$           | $39 \pm 5$                        | $37 \pm 5$                 |
| 25                           | $435 \pm 7$            | $54 \pm 5$                        | $50 \pm 5$                 |
| 35                           | $338 \pm 5$            | $67 \pm 5$                        | $64 \pm 5$                 |

obtained by atomic force microscopy (tapping mode AFM for  $1 \times 1 \mu\text{m}^2$  and  $5 \times 5 \mu\text{m}^2$ ) are between 5 and 10  $\text{\AA}$  (data not shown).

The transferred DB 50 copolymer films were also characterized by specular X-ray reflectivity experiments. In comparison to ellipsometry, which can determine only the layer thickness by assuming a refractive index, X-ray reflectivity reports the layer thickness and the roughness of the interface independently from the electron densities of individual layers. Figure 5 represents the reflectivity curves ( $I/I_0$  vs  $q$ ) of a bare silicon wafer (squares), a silicon wafer coated with d8-PMMA (circles), silicon wafers with d8-PMMA, and DB 50 copolymer transferred at 15 mN m<sup>-1</sup> (triangles), 25 mN m<sup>-1</sup> (inverse triangles), and at 35 mN m<sup>-1</sup> (stars). According to the four-box model (air, DB 50, d8-PMMA, and silicon dioxide) and the real and imaginary parts of the electron densities (Table 1), the thickness of DB 50 copolymer films can be calculated to be  $h = 37 \text{ \AA}$  (15 mN m<sup>-1</sup>),  $50 \text{ \AA}$  (25 mN m<sup>-1</sup>), and  $64 \text{ \AA}$  (35 mN m<sup>-1</sup>), respectively. These calculated values are in excellent agreement with those obtained by ellipsometry (Table 4), indicating an increase in film thickness according to the film compression. If one assumes a constant volume  $V_0$  for the copolymer, a systematic dependence of the dry DB 50 layer thickness  $h$  on the area per molecule  $A$  (Figure 6) can be simply written as:

$$h = \frac{V_0}{A} \quad (4)$$

By taking the molecular volumes of 225  $\text{\AA}^3$  for DMAEMA monomer and 140  $\text{\AA}^3$  for MMA monomer,<sup>26</sup> the total volume,  $V_0$ , of the DB 50 copolymer can be roughly estimated to be  $1.3 \times 10^4 \text{ \AA}^3$ .<sup>25</sup> If one plots the  $h$  vs  $A$  relationship based on this value for  $V_0$  (a line given in Figure 6), an obvious deviation is observed between the predicted thickness and the experimental results. This discrepancy from an ideal (simple) system is



**Figure 6.** Dry film thickness measured by ellipsometry (squares) and X-ray reflectivity (circles) plotted vs area per molecule  $A_{mol}$ , indicating a systematic dependence of film thickness on the lateral chain density. A clear deviation from the simple constant volume model can be explained by the finite compressibility of glassy polymer chains, which differ from either simple solids or liquids.

explained by the finite compressibility of glassy polymer chains, which are different from either simple solids or liquids.

## Conclusions

We demonstrate that we can create stable Langmuir monolayers comprising an amphiphilic PDMAEMA-PMMA diblock copolymer at the air/water interface under various subphase pH conditions. Clear changes in the global shapes of the Langmuir isotherms suggest the adsorption/desorption of the hydrophilic PDMAEMA block to/from the air/water interface. Desorption of charged PDMAEMA chains from the interface induced by the decreasing pH can be monitored as a decrease in the lateral pressure while keeping the film area constant. Optimization of the key preparative parameters enables us to fabricate stable and homogeneous diblock copolymer films with well-defined lateral chain densities. Ellipsometry and X-ray reflectivity studies confirm that the resulting film thicknesses depend on the lateral chain densities, i.e., the surface pressures during the transfer. Since the transferred films showed sufficient stability against intensive rinsing with water or long-term ( $\sim 1$  week) storage under aqueous solution, the system described here is a promising candidate for designing a “command interface” that enables interactions between solid substrates and biological matter (proteins, cells) to be modulated.

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**Supporting Information Available:** Langmuir isotherms for a PDMAEMA-PMMA diblock copolymer monolayer. This

material is available free of charge via the Internet at <http://pubs.acs.org>.

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