Synthesis of Free-Standing Quasi-Two-Dimensional Polymers

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Received August 8, 2002. In Final Form: October 1, 2002

This paper describes a synthesis of free-standing, 10−15-nm-thick polymer films of well-defined lateral size and shape. The three key elements of this procedure are (1) formation by microcontact printing (μCP) of a patterned, self-assembled monolayer (SAM) with hydrophobic regions (alkane-terminated) and adsorption-resistant regions (oligo(ethylene oxide)-terminated); (2) initiation of spatially selective growth of films of poly(electrolyte) multilayers by adsorption on the hydrophobic regions of the patterned SAM; and (3) dry transfer of these films to a water-soluble sacrificial backing, from which the films can be released into solution. This technique exploits the hydrophobic effect as an interaction that can be switched off when it is not needed: during the growth of the films in aqueous buffer, the hydrophobic effect anchors the polymers to the surface; once these films have been dried after synthesis, they are bound to the substrate only by van der Waals interactions and can be transferred nondestructively to a sacrificial backing. The growth and final state of the films were characterized using surface plasmon resonance (SPR), polarized infrared external reflectance spectroscopy (PIERS), ellipsometry, fluorescence microscopy, and atomic force microscopy (AFM).

Introduction

This paper describes an efficient method for the synthesis of freely suspended polymer films of controlled composition, thickness (on the scale of tens of nanometers), and lateral dimension and shape (on the scale of microns); we refer to these structures as "quasi-two-dimensional (quasi-2D) polymers". Figure 1 outlines the steps in our approach (Figure 1A): (i) patterning of a surface into adsorbing and nonadsorbing regions using self-assembled monolayers (SAMs) and microcontact printing (μCP);[1] (ii) noncovalent adsorption of an initial, reactive polymer layer from an aqueous solution of polymer onto the adsorbing regions of this patterned surface; (iii) covalent attachment of a second layer of polymer onto this initial, noncovalently adsorbed layer, with consequent formation of a cross-linked, molecularly thin film (step (iii) can be repeated to make thicker, multilayer films); (iv) application of a water-soluble, sacrificial backing; (v) transfer of the cross-linked polymer films to this sacrificial layer; and (vi) release of the films by dissolving the sacrificial polymer support in water. Using this method, we synthesized freely suspended polymer films that have a variety of sizes, shapes, and compositions. The advantages of this synthetic method are (i) it produces a monodisperse sample of quasi-2D polymers with micrometer-scale lateral dimensions and with thickness on the order of ∼10 nm; (ii) it allows for the manipulation and nondestructive release of these polymer films with high yield; and (iii) it allows the composition of the films to be controlled by the stepwise addition of layers.

With two microscopic or macroscopic dimensions and one molecular-scale dimension, these quasi-2D polymers combine the properties of materials, molecules, and colloids: in the lateral dimensions, mechanical moduli can be defined (and potentially measured, although we have not carried out this measurement); in the thin dimension, thermal fluctuations will control conformation on a molecular scale (provided the films are sufficiently flexible); in suspension, a monodisperse sample of films with nanometer-scale thickness and micrometer-scale lateral extent will constitute a new class of complex fluids with the potential to show new types of non-Newtonian behavior.[2] Free-standing, molecularly thin films also have potential to be important in a range of applications: as scaffolds for ligands to promote polyvalent interactions at synthetic and biological surfaces,[3] as scaffolds for the organization of nanoparticles,[4,5] or as anisotropic elements in complex or liquid crystalline fluids.[2] The study and application of quasi-2D polymers will require flexible methods to synthesize them.

Freely suspended thin polymer films have been a theoretical curiosity[6−10] and an experimental challenge[11−15] for several decades. The existing synthetic routes to freely suspended 2D or quasi-2D polymers include polymeri-

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(1) Xia, Y.; Whitesides, G. M. *Annu. Rev. Mater. Sci.* **1998**, *28*, 153−184.
(2) Escalante, J. I.; Hoffmann, H. *Rheol. Acta* **2000**, 39.
(3) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2755−2794.
(4) Schmitt, J.; Decher, G.; Dressick, W. J.; Brandow, S. L.; Geer, R. E.; Shashidhar, R.; Calvert, J. M. *Adv. Mater.* **1997**, 9.
(5) Mamedov, A. A.; Kotov, N. A. *Langmuir* **2000**, *16*, 5530−5533.
(6) de Gennes, G.-P. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.
(7) Kantor, Y.; Kardar, M.; Nelson, D. R. *Phys. Rev. Lett.* **1986**, *57*, 791−794.
(8) Kantor, Y.; Kardar, M.; Nelson, D. R. *Phys. Rev. A* **1987**, *35*, 3056−3071.
(9) Safran, S. A. *Statistical Thermodynamics of Surfaces, Interfaces, and Membranes*; Addison-Wesley Publishing Co.: Reading, MA, 1994; Vol. 90.
(10) Radzihovsky, L.; Toner, J. *Phys. Rev. E* **1998**, *57*, 1832−1863.
(11) Elgsaeter, A.; Stokke, B.; Mikkelsen, A.; Branton, D. *Science* **1986**, *234*, 1217.
(12) Schmidt, C.; et al. *Science* **1993**, *259*, 952.
(13) Hwa, T.; Kokofuta, E.; Tanaka, T. *Phys. Rev. A* **1991**, *44*, R2235.
(14) Spector, M.; Naranjo, E.; Chiruvolu, S.; Zasadzinski, J. *Phys. Rev. Lett.* **1994**, *73*, 2867−2870.
(15) Huck, W. T. S.; Stroock, A.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 1058−1061.

10.1021/la026379f CCC: $25.00
Published on Web 12/07/2002

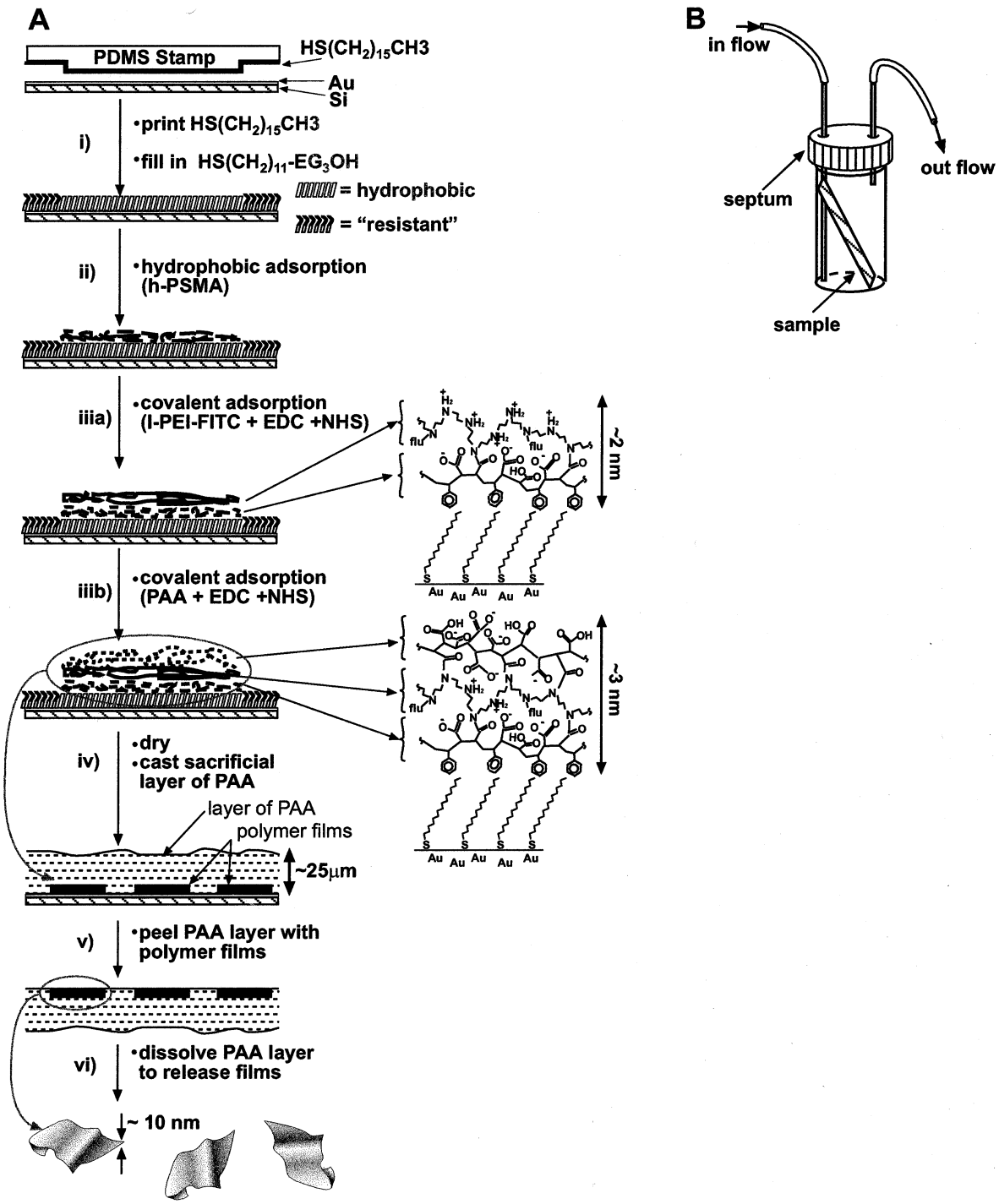

Figure 1. (A) Schematic outline of the preparation of free-standing polymer films of well-defined size and shape. In the drawing of the bilayer in steps iiia and iiib, fluorescein is represented by "flu." (B) Diagram of the flow cell reactor used for the deposition of the polymer films.

zation at fluid−fluid interfaces (flat liquid−air or liquid−liquid interfaces and interfaces of liquid droplets in liquids[16]), polymerization of lipid bilayers[17] or of molecules dissolved in lipid bilayers,[18] growth of multilayers of poly(ions) on colloidal particles that are subsequently dissolved,[19] and deposition of poly(electrolyte) multilayers onto a sacrificial substrate that is subsequently dissolved.[5] None of these methods allow for the synthesis of films with defined shapes, that is, defined, micron-scale lateral dimensions. With the exception of the work of the Möhwald group with multilayer polymer films on colloids,[19−23] these methods offer little or no control of the thickness and molecular structure in the thin dimension.

Our methods are founded on the substantial body of work on solid-supported polymer multilayers by the groups of Decher,[24,25] Rubner,[26,27] and Möhwald[19−23] and, in

(16) Rehage, H.; Veyssie, M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 439−448.
(17) Lee, Y.; Obrien, D. *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 1427−1445.
(18) Hotz, J.; Meier, W. *Langmuir* **1998**, *14*, 1031−1036.
(19) Caruso, F.; Caruso, R. A.; Mohwald, H. *Science* **1998**, *282*, 1111−1114.
(20) Georgieva, R.; Moya, S.; Leporatti, S.; Neu, B.; Baumler, H.; Reichle, C.; Donath, E.; Mohwald, H. *Langmuir* **2000**, *16*, 7075−7081.
(21) Leporatti, S.; Voigt, A.; Mitlohner, R.; Sukhorukov, G.; Donath, E.; Mohwald, H. *Langmuir* **2000**, *16*, 4059−4063.

particular, the work of Hammond on patterned polymer multilayers on patterned SAMs.[28−30] Previous work by our group demonstrated the use of SAMs patterned with μCP for the growth of quasi-2D polymers of well-defined lateral size and shape.[15] In that work, we used ionic interactions to template the adsorption of the initial layer of polymer onto the patterned surface; lifting the films off of the surface required harsh conditions and the destruction of the substrate. The method presented here allows the films to be lifted off in milder conditions and does not destroy the substrate.

Results and Discussion

Preparation of Freely Suspended Polymers Films of Well-Defined Size and Shape. Figure 1a shows the procedure used to synthesize freely suspended polymer films. We present results based on a set of three polymers that form the cross-linked polymer multilayers: hydrolyzed-poly(styrene-*alt*-maleic anhydride) (*h*-PSMA) (M_w 350 000) that acts as the poly(amphiphile) that adsorbs to hydrophobic SAMs by hydrophobic interactions and that forms the initial polymer layer; linear poly(ethylenimine) (*l*-PEI) coupled to fluorescein (*l*-PEI−FITC) (M_w 250 000; ∼0.5% coupling of amines to fluorescein); and poly(acrylic acid) (PAA) (M_w 90 000). The second, third, and subsequent layers are formed, in an alternating fashion, of *l*-PEI−FITC and PAA. The method is not restricted to these polymers.[31] The procedure has six principle steps:

(i) Patterning Substrate with Thiol SAMs. SAMs of hexadecanethiol ($HS(CH_2)_{15}CH_3$) were patterned on gold using μCP to form regions of methyl-terminated, hydrophobic SAMs. SAMs of oligo(ethylene glycol)-terminated thiol ($HS(CH_2)_{11}-(OCH_2CH_2)_3-OH$, EG-thiol) were allowed to form on the remainder of the gold surface. Many polymers[31] having hydrophobic regions or functional groups adsorb onto the methyl-terminated SAMs from aqueous solution via hydrophobic interactions. SAMs of EG-thiols are resistant to the adsorption of many proteins[32] and poly(electrolytes) from aqueous solution.[29,30]

(ii) Patterned, Noncovalent Adsorption of Initial Polymer Layer. The key element in this synthetic approach is the use of hydrophobic interactions at the patterned surface to template the growth of polymer multilayers of well-defined thickness and lateral shape. Hydrophobic interactions have two characteristics that are important for our synthesis: (1) when in water, the hydrophobic interactions between hydrophobic moieties and the methyl-terminated SAMs are sufficiently strong to replace the ionic interactions that are conventionally used to initiate the growth of polymer multilayers on charged surfaces in water;[25] and (2) when removed from water and dried, the hydrophobic interactions between the polymers and the methyl-terminated SAMs no longer exist, and only van der Waals interactions remain (that is, the dry polymer layers can then be removed from the substrate under mild conditions).[33,34] The hydrophobic interaction stabilizes a layer of poly(amphiphiles) so long as the substrate remains submerged in water.[35,36] We used a flow cell reactor (Figure 1b) in order to perform all deposition steps without removing the substrate from water. Another reason to avoid drying the patterned samples between deposition steps is that drying tends to compromise the ability of the SAMs of EG-thiol to resist the adsorption of poly(electrolytes).[29,30]

(iii) Growth of Covalently Cross-Linked Polymer Multilayers on a Noncovalent Initial Layer. The first, noncovalently attached layer bears functional groups (i.e., carboxylic acids in the case of *h*-PSMA) that can be used for the covalent attachment of a second layer. Subsequent layers are formed of alternating layers of poly(amines) (i.e., *l*-PEI−FITC; step iia in Figure 1) and poly(acids) (i.e., PAA; step iiib). For the covalent attachment of the second and subsequent layers, we used carbodiimide coupling to form amides between adjacent layers: coupling reagents were added in excess directly to the solution of the next polymer to be deposited; this solution was allowed to flow through the reactor. In the addition of a poly(amine) to a surface-bound layer of poly(acids) (step iiia), the active esters (intermediates in the carbodiimide coupling) are formed directly on the surface; in the addition of a poly(acid) to a surface-bound layer of poly(amines) (step iiib), the active esters are primarily formed in solution. We performed all steps in the same buffer (200 mM sodium phosphate, pH 8) in order to maintain the same solvent quality throughout the cross-linking steps.

(iv) Drying the Sample and Applying a Sacrificial Layer. Covalently cross-linked bilayers (e.g., *h*-PSMA/*l*-PEI−FITC) or multilayers were stable against desorption when removed from the buffer. Weakly cross-linked films (e.g. noncovalently cross-linked bilayers) would usually crack and recede with the air−water interface during this step. With the films intact, the entire patterned substrate—both the methyl- and the EG-terminated regions—was hydrophilic; some contrast was nonetheless still visible

(22) Mohwald, H. *Colloids Surf., A* **2000**, *171*, 25−31.
(23) Moya, S.; Donath, E.; Sukhorukov, G. B.; Auch, M.; Baumler, H.; Lichtenfeld, H.; Mohwald, H. *Macromolecules* **2000**, *33*, 4538−4544.
(24) Lvov, Y.; Decher, G.; Sukhorukov, G. *Macromolecules* **1993**, *26*, 5396−5399.
(25) Decher, G. *Science* **1997**, *277*, 1232−1237.
(26) Stockton, W.; Rubner, M. *Macromolecules* **1997**, *30*, 2717−2725.
(27) Yoo, D.; Shiratori, S. S.; Rubner, M. F. *Macromolecules* **1998**, *31*, 4309−4318.
(28) Hammond, P. T.; Whitesides, G. M. *Macromolecules* **1995**, *28*, 7569−7571.
(29) Clark, S.; Hammond, P. *Adv. Mater.* **1998**, *10*, 1515−1519.
(30) Clark, S.; Montague, M.; Hammond, P. *Macromolecules* **1997**, *30*, 7237−7244.
(31) We explored several other options for the composition of the polymer films: hydrolyzed poly(octadecene-*alt*-maleic anhydride) (*h*-POMA) (M_w 30 000) (Aldrich), poly(methacrylic acid) (PMA) (M_w 100 000) (Polyscience), poly(acrylic acid) PAA (M_w 90 000) (Polyscience), *l*-PEI−FITC, and the protein alkaline phosphatase (AP) (Sigma) for the noncovalent first layer, and linear PEI (*l*-PEI) (M_w 250 000), branched poly(ethylenimine) (*b*-PEI) (M_w 750 000) (Aldrich), poly(diallyldimethylammonium chloride) (PDDA) (M_w 500 000) (Aldrich), and poly(styrene sulphonate) (PSS) (M_w 1 000 000) (Aldrich) for the second layer. For the first layer, *h*-POMA, PMA, and AP all adsorb to methyl-terminated SAMs to a similar extent as *h*-PSMA, as measured by SPR (Figure 2). All but PMA form stable bilayers when covalently cross-linked with *l*-PEI. PSS does not adsorb to the methyl-terminated SAM. *b*-PEI is not appropriate for cross-linking patterned layers because it adsorbs to the EG-terminated SAMs. PDDA and PSS can be used as noncovalent cross-linking layers.

(32) Mrksich, M., Whitesides, G. M., Eds. *Using Self-Assembled Monolayers That Present Oligo(ethylene glycol) Groups To Control the Interactions of Proteins with Surfaces*, 1st ed.; American Chemical Society: Washington, DC, 1997.
(33) Tanford, C. *Physical Chemistry of Macromolecules*; John Wiley & Sons: New York, 1961.
(34) In water, the free energy of adhesion between two hydrophobic surfaces is $\Delta G_{water} \sim -2\gamma_{oil-water} = -1.0 \times 10^{-5}$ J·cm^{-2}. In air, $\Delta G_{air} \sim -2\gamma_{oil-air} = -4.4 \times 10^{-6}$ J·cm^{-2}, where $\gamma_{oil-water}$ and $\gamma_{oil-air}$ are the interfacial energies of oil (octane) with water and air at room temperature.[33]

(35) Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *Langmuir* **1997**, *13*, 2749−2755.
(36) Unlike the case of adsorption of low molecular weight amphiphiles onto hydrophobic surfaces,[35] high molecular weight polymers with hydrophobic regions or functional groups adsorb irreversibly from water (i.e. the layer does not desorb when the concentration of the polymer in solution is lowered, see Figure 2). Typically, these layers will desorb when the surface is removed from water if the layer has not been further stabilized by cross-linking.

Table 1. Thickness of Dry Films on Methyl-Terminated SAMs as Measured by Ellipsometry[a]

additional layer on gold	total thickness (nm)	change in thickness (nm)
$HS(CH_2)_{16}CH_3$	1.8 ± 0.1	1.8 ± 0.1
+ *h*-PSMA/*l*-PEI−FITC	4.2 ± 0.2	2.4 ± 0.2
+ PAA	4.6 ± 0.3	0.4 ± 0.1
+ *l*-PEI−FITC	5.4 ± 0.4	0.8 ± 0.2
after transfer to sacrificial layer	0.8 ± 0.4	−4.6 ± 0.4

[a] Measurements were performed at three different positions on a single sample with a uniform methyl-terminated SAM. The values reported for the total thickness are the average of the values measured at these positions. The error is the maximum difference from the average (positive or negative) that was measured. The changes in thickness were calculated separately for each position; the average over the three positions is reported. The error is the maximum difference from the average. The optical constants of the gold were measured before the formation of the SAM. The index of refraction was taken to be $n = 1.465$, for all measurements. The layers were deposited by the procedure in Figure 1. After the deposition of the first two layers together and after the depositions of the third and forth layers independently, the sample was dried and the ellipsometry was performed. The dry sample was allowed to rehydrate for 20 min before the deposition of the following layer.

in the condensation pattern[37] between the two regions. Once an intact film was dried, it remained stably attached to the surface in air as well as in solvents.[38] To avoid using harsh chemical (e.g., acidic solutions for etching metals) or mechanical (e.g., sonication) conditions to remove the cross-linked films from the surface, we first transferred them mechanically to a water-soluble solid support of poly(acrylic acid) (PAA; M_w 90 000). To form a layer of PAA on the surface with the intact films, we cast a solution of PAA (10% in a mixture of 90% methanol and 10% water) and allowed it to dry at room temperature for 1 h; the partially dried PAA layer was pliable and had a thickness of ~25 μm.

(v) Transfer of Films to a Sacrificial Layer. The partially dried PAA layer was peeled from the surface manually with tweezers; only the edges of the PAA layer were damaged in this process. The peeled PAA film left no residue on the patterned substrate, and the methyl-terminated regions regained their hydrophobic character once the film was removed. Ellipsometry (Table 1) indicates that the cross-linked films remain with the PAA layer as it is peeled away. Note that the peeling step was possible for both *l*-PEI and PAA-terminated multilayers. We presume that this transfer occurs because the ionic and hydrogen bonding interactions between the PAA and the terminal layer of the cross-linked films are stronger than the van der Waals interaction of the dried films with the methyl-terminated SAMs.

(vi) Release from Sacrificial Layer. The PAA sacrificial layer was dissolved in a basic, aqueous solution (200 mM phosphate buffer at pH 8); dissolution released the cross-linked polymer films.

Characterization. *SPR.* We used surface plasmon resonance (SPR) on *unpatterned* methyl-terminated SAMs and EG_3-terminated SAMs to characterize the adsorption of the first layer of polymer. The use of SPR was necessary because single layers of poly(amphiphiles) on the hydrophobic surface desorb when the surface is removed from water; this desorption makes the use of other techniques (e.g. ellipsometry, AFM) difficult or impossible. For the SPR measurements, the flow cell of the SPR instrument was used as the reaction chamber. Figure 2A shows the sensorgram obtained for *h*-PSMA on a uniform, methyl-terminated SAM. The response indicates that a substantial amount of polymer remained attached to the surface after the polymer solution was flushed from the chamber.[39] The adsorbed film of *h*-PSMA was completely removed by a rinse with SDS (~10 mM at pH 8 in 200 mM phosphate buffer); this behavior is consistent with the hypothesis that the *h*-PSMA layer adsorbs via hydrophobic interactions. Figure 2B and C shows sensorgrams obtained for *h*-PSMA and *l*-PEI−FITC on uniform EG-terminated SAMs. In both cases, there was a small amount of irreversible adsorption of polymer. This adsorption to the EG-terminated surface could eventually lead to the growth of continuous polymer films in these regions, but we have not see any evidence of growth in these regions for films with up to five layers.

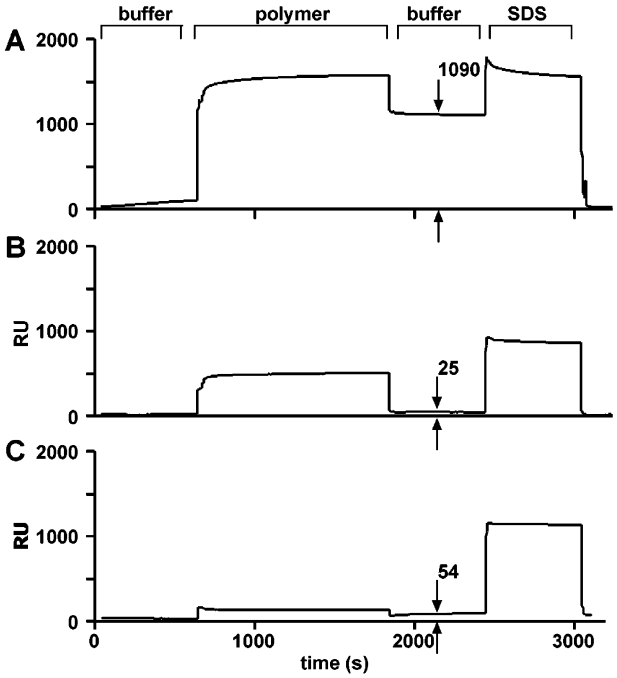

Figure 2. Surface plasmon resonance (SPR) sensorgrams for (A) the adsorption of *h*-PSMA (0.1 wt % in buffer) onto a methyl-terminated SAM, (B) the adsorption of *h*-PSMA (0.1 wt % in buffer) onto a EG-terminated SAM, and (C) the adsorption of *l*-PEI−FITC (0.1 wt % in buffer) onto a EG-terminated SAM. In each experiment, the flow chamber of the SPR machine was flushed for 10 min with clear buffer (0.2 M phosphate, pH 8), for 20 min with the polymer solution, for 10 min with clear buffer, and for 10 min with SDS (10 mM). The adsorption is reported in response units (RU) that are specific to the SPR instrument used. The response for a typical monolayer of protein is 2000 RU.[39] The degree of irreversible adsorption of polymer to the substrate in buffer is indicated by the response that is recorded during the second flushing with buffer. Substantial adsorption (1090 RU) is seen only in part A for *h*-PSMA on methyl-terminated SAM. The observed differences in the response during the flushes of SDS between the three experiments could have several causes: In part A, the SDS associates reversibly with the methyl-terminated surface and leads to a larger response than that for the EG-terminated surfaces in parts B and C.[35] Small differences in the concentration of SDS might also lead to significant changes in the response, as the concentration that was used (10 mM) is near the critical micellar concentration of SDS (~8 mM).

Ellipsometry. Unpatterned cross-linked films were prepared to measure the thickness of the surface-bound

(37) López, G. P.; Biebuyck, H. A.; Frisbie, C. D.; Whitesides, G. M. *Science* **1993**, *260*, 647−649.

(38) The films remained intact after 12 h in ethanol, tetrahydrofuran, and dimethylformamide, and in a 10 mM solution of sodium dodecyl sulphonate (SDS) in water.

(39) Mrksich, M.; Sigal, G. B.; Whitesides, G. M. *Langmuir* **1995**, *11*, 4383−4385.

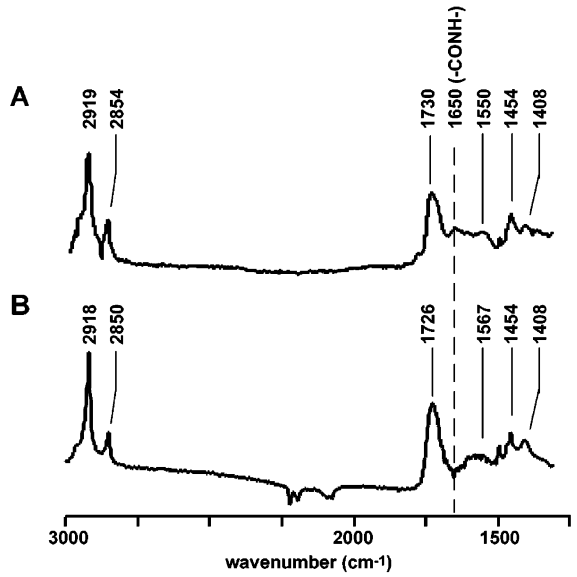

Figure 3. PIERS spectra of (A) covalently cross-linked *h*-PSMA/*l*-PEI−FITC/PAA on a uniform, methyl-terminated SAM and (B) noncovalently cross-linked *l*-PEI−FITC/*h*-PSMA on a uniform, carboxylic acid-terminated SAM.

Table 2. Assignment of Selected Absorption Bands in the Infrared Spectra of Covalently and Noncovalently Cross-linked Layers[a]

wavenumber (cm^{-1})		
SAM-CO_2H + lPEI−FITC/hPSMA	SAM-CH_3 + hPSMA/lPEI−FITC/PAA	assignment
2919	2918	$\nu_{as}(CH_2)$
2854	2850	$\nu_s(CH_2)$
1731	1725	ν(C═O) for acid
1650		amide I or amide stretch
1551	1567	δ(N−H^+) or amide II

[a] Assignments taken from refs 40 and 46.

layers by ellipsometry. For these measurements, the films were not transferred to the PAA layer (i.e. steps 3−6 in Figure 1a were skipped); ellisometry was performed on films bound to the original gold-coated substrate. Table 1 summarizes the results for different numbers of layers. The first bilayer made up of *h*-PSMA and *l*-PEI−FITC was ∼2 nm thick. This thickness of ∼1 nm per layer is comparable to that of covalently and ionically templated layers.[40] After the transfer of the multilayer film from the original substrate to the sacrificial layer, the measured thickness of the remaining material (0.8 nm) is less than that of the original SAM (1.8 nm). This reduced thickness may indicate that some fraction of the methyl-terminated SAM was removed during the transfer or that the optical characteristics of the gold have changed during the proceeding steps.

IR Spectroscopy. Figure 3 shows the IR spectra for covalently cross-linked layers (A) and noncovalently (ionically) cross-linked layers (B). Table 2 lists the key adsorption bands of these samples. The band at 1650 cm^{-1} in part A that we assign to the amide I or the amide stretch confirms the presence of amide cross-links in the sample that was prepared with carbodiimide coupling. We identify the strong peak near 1730 cm^{-1} for both samples as the carbonyl of the acids that are present in large numbers even after cross-linking; only a small fraction of the acids are converted to amides. The stability of the "covalent" layers is likely due to a combination of covalent and ionic interactions between adjacent layers.

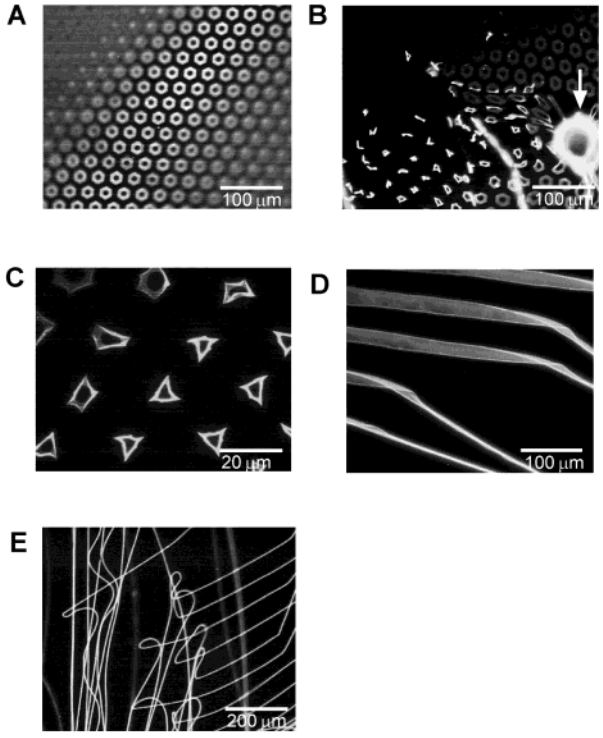

Figure 4. Fluorescence micrographs of trilayer films before, during, and after release from the sacrificial layer. (A) 20 μm hexagons on sacrificial PAA layer. The PAA support layer bends out of the focal plane of the objective on the top left and the bottom right of the image. (B) Release of hexagons (same as in part A) from the PAA layer in basic buffer. On the right of the image, films are still supported by the PAA layer. On the left, films are floating freely in the buffer. Note how the individual films are distorted near the bubble (indicated by a white arrow) on the right side of this image. (C) Higher magnification image of the same release process. (D) Release of the 20 μm line from PAA in buffer. (E) Examples of fully rolled up 20 μm lines.

Fluorescence Microscopy. The fluorescence in the *l*-PEI−FITC layer allowed characterization of the films by fluorescence microscopy (Figure 4). Figure 4 shows fluorescence micrographs of patterned films of *h*-PSMA/*l*-PEI−FITC before, during, and after release from the PAA sacrificial layer. In Figure 4A, films patterned as ∼20 μm hexagonal rings in the center was still bound to a free-standing PAA sacrificial layer (as in Figure 1A, step 4). The PAA layer was bent out of the focal plane of the microscope objective, blurring the image in the top left and bottom right corners. Figure 4B shows the same hexagonal films as they began to release as the PAA layer dissolved in buffer. In the bottom right corner, the films were still bound flat on the PAA layer. The bright circular structure in Figure 4B was due to an air bubble that distorted the PAA film and captured fully released films (blurry, bright region out of plane of focus). On the left side of the image, there are films at various stages of release; the films curled up from the edges as they released and began to float freely in the buffer. Figure 4C shows the hexagonal polymer films as they were releasing from the sacrificial layer. In this and most cases, the films rolled such that the bottom surface of the film (*h*-PSMA) became the inner surface. The hexagonal films reached a stable, triangular conformation like the one seen in Figure 4C. Parts D and E of Figure 4 show two stages of the release of 20 μm wide lines. Upon curling, the lines became thin filaments of micron-scale diameter.

(40) Huck, W. T. S.; Yan, L.; Stroock, A.; Haag, R.; Whitesides, G. M. *Langmuir* **1999**, *15*, 6862−6867.

AFM. Figure 5 shows the characterization of films by atomic force microscopy (AFM) at different stages of synthesis. Figure 5A shows a contact mode topography image of the trilayer films (*h*-PSMA/*l*-PEI−FITC/PAA) that are still attached to the patterned gold surface. We attribute the graininess in this image to grains of gold. The average cross section (averaged between the lines drawn on the image) shows the contrast in the adsorption between the methyl-terminated regions (broad, raised lines) and the EG-terminated regions (narrow lines). The measured thickness of the films (2.6 nm) is comparable to the thickness of a uniform sheet measured by ellipsometry (2.4 nm; Table 1).

Figure 5B shows a tapping mode image of the same type of lines after transfer to the sacrificial layer, release into solution, and deposition onto a polished silicon surface. After deposition, the silicon surface was soaked in buffer (0.2 M phosphate, pH 8) overnight in order to dissolve excess PAA. The surface was then rinsed with deionized water and dried before imaging. We interpret the thick borders on each line as folds; these lines have partially folded onto themselves from the edges. The border regions appear to be two plies thick. The released lines in Figure 5B are $^1/_3$ the width (∼1 μm wide) of the intact lines in Figure 5A (∼3 μm wide) (note the differences in both the in-plane and vertical scales between parts A and B). The narrow folds at the borders cannot explain this change in the width of the lines; the films appear to have shrunken laterally and thickened. The thickness of the films grew by ∼4 times (2.6−12 nm) upon release. The observed thickening and the lateral shrinkage may be due to local creases in the films or to swelling of the films with water once they are released from the substrate. The tapping mode image in Figure 5C shows the more complicated, folded state of the hexagonal shaped films.

Conclusions

Polymer multilayers provide a versatile format around which to organize diverse types of synthetic polymers,[26] biomolecules,[41,42] and inorganic materials.[4] Nonetheless, with a few exceptions,[5,19−23] this work has been limited to the study of uniform multilayer structures that are bound to the solid surface on which they were grown. This article describes a method for (1) controlling the lateral size and shape of polymer multilayers and (2) releasing these structures into solution under mild conditions. Our method can easily be adapted to work with other polymers that form multilayers (e.g., see ref 31). In particular, a multilayer of any pair of water-soluble poly(cations) and poly(anions) can be grown using our method by first adsorbing *h*-PSMA on regions of a methyl-terminated SAM; after the adsorption of *h*-PSMA, the regions that were originally methyl-terminated will present negative charges, and growth of the poly(electrolyte) multilayer can be initiated by adsorbing a layer of the poly(cation) onto these regions. The *h*-PSMA acts as a hydrophobic attachment to the surface; structures that are attached in this way can be transferred to a sacrificial layer in PAA and released into aqueous solution.

Upon release into solution, the polymer structures that are formed with this method represent a new class of materials with one controlled molecular dimension, two controlled microscopic dimensions, and the freedom to move and change conformation. We have observed flat,

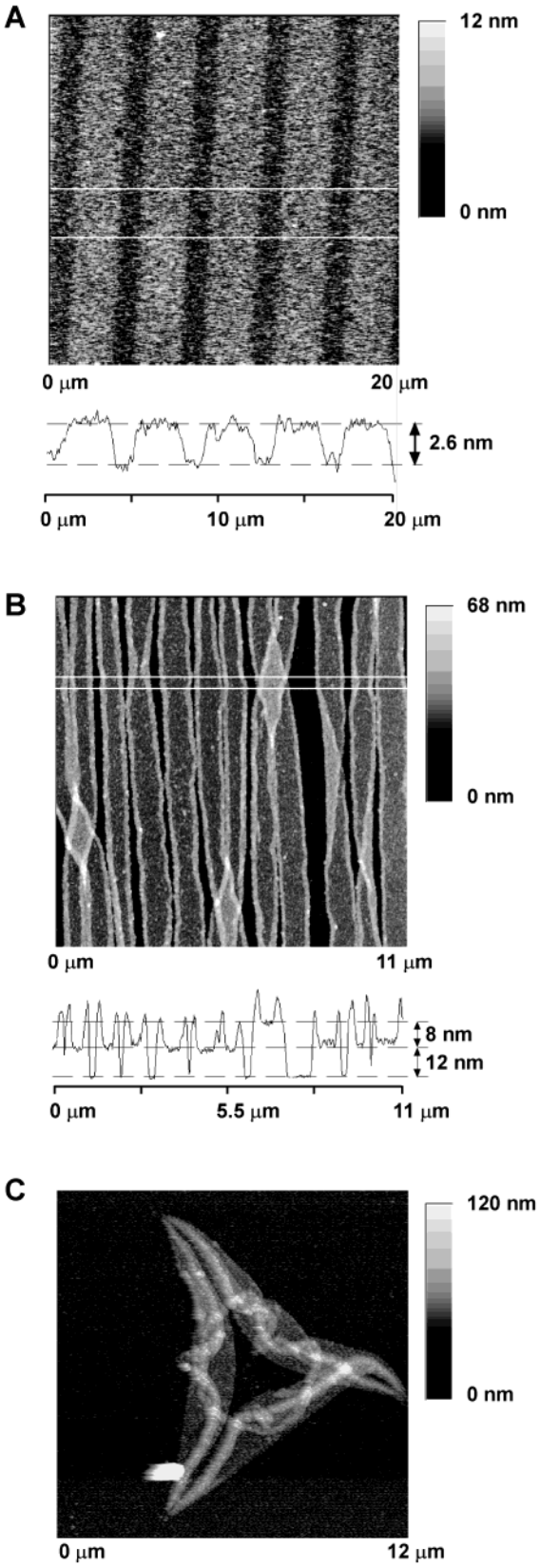

Figure 5. Atomic force micrographs of a trilayer of *h*-PSMA/*l*-PEI−FITC/PAA intact on the original gold surface (A) and deposited after release onto polished silicon (B and C). The films in parts A and B are from the same sample of films that were patterned in the form of ∼3 μm wide lines. The object in part C is a hexagonally shaped film that has folded in on itself into a triangular form like those seen in Figure 3B and C. Part A was acquired in contact mode. Parts B and C were acquired in tapping mode.

(41) Caruso, F.; Mohwald, H. *J. Am. Chem. Soc.* **1999**, *121*, 6039−6046.
(42) Ladam, G.; Schaaf, P.; Cuisinier, F. J. G.; Decher, G.; Voegel, J. C. *Langmuir* **2001**, *17*, 878−882.

folded, and rolled structures. The type of conformational change undergone by a structure appears to depend on the lateral size and shape: lines appeared to roll into tubes (Figure 4E) or remain flat (Figure 5B), while hexagonal rings fold into a stable triangular structure (Figures 4C and 5C). These structures may be related to the tethered membrane models that have recently been used to predict the evolution of flat sheets into tubular forms.[10]

Materials and Methods

Materials. Oligo(ethylene glycol)-terminated alkanethiols ($HS(CH_2)_{11}-(OCH_2CH_2)_3-OH$) were synthesized previously.[43] Linear poly(ethylenimine) (lPEI) (M_w 250 000) was also synthesized previously.[44] Hexadecanethiol was purchased from Fluka (Neu-Ulm, Switzerland). Solvents, poly(styrene-*alt*-maleic anhydride) (PSMA) (M_w 350 000), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), fluorescein isothiocyanate isomer I (FITC), and mono- and dibasic sodium phosphate, were purchased from Aldrich (Milwaukee, WI). Poly(acrylic acid) (PAA) was purchased from Polysciences (Warrington, PA). In building the flow cell reactor, plastic tubing (Tygon R-3603) and stainless steel needles were used for the in-flow and out-flow. All aqueous buffer solutions were prepared in deionized water.

Preparation of Hydrolyzed PSMA. A 0.5 wt % aqueous solution of hydrolyzed PSMA (*h*-PSMA) was prepared as follows: 1.4 g of PSMA was dissolved in 25 mL of acetone with mild heating. This solution was added slowly to 300 mL of 300 mM sodium hydroxide in water with vigorous stirring and allowed to react for 3 h. The pH was then adjusted to 8 with the addition of hydrochloric acid. Finally, the acetone was removed on a rotary evaporator.

Preparation of Fluorescein-Labeled Linear Poly(ethylenimine) (*l*-PEI−FITC). FITC (M_w 389) was allowed to react with ∼200 monomer equivalents of linear PEI (*l*-PEI) (monomer M_w 43) as follows: 200 mg of *l*-PEI was dissolved in 5 mL of 1-methyl-2-pyrrolidinone (NMP) with mild heating. FITC (10 mg) was dissolved in 1 mL of NMP. The solution of FITC was added slowly to the solution of *l*-PEI with vigorous stirring achieved by holding the vial of the solution of *l*-PEI on a vortexer; the FITC and the *l*-PEI were allowed to react for 15 min. NMP and unreacted fluorescein were removed by dialysis.

Preparation of Patterned SAMs. A poly(dimethyl siloxane) (PDMS) stamp with the desired features was prepared according to previously published procedures.[1] The stamp was inked with a 2 mM solution of hexadecanethiol and dried with a stream of nitrogen. The stamp was placed on a freshly evaporated gold substrate—200 nm of gold on a test grade, ⟨100⟩ single-crystal silicon wafer, primed with 5 nm of chromium—for 30 s.[45] The stamp was carefully peeled off the substrate, and the substrate was immersed for 12 h in a 2 mM solution of EG-thiol.

Deposition of Polymer Layers onto Patterned Surface. All solutions were prepared in phosphate buffer (200 mM, pH 8—filtered and degassed). The substrate with patterned SAM was placed in a 5-mL vial (Figure 1B) and submerged in buffer. The top of the vial was then sealed with a rubber septum through which two needles passed. A syringe was used to fill tubing attached to these needles and initiate a flow driven by gravity through the vial. Flows were driven with a 30-cm difference of height between the input and waste containers; this difference in height led to a flow rate of ∼10 mL/min. The incoming solution could be changed by moving the input tubing from one container to another; during this switching step, the input tubing was lowered to the level of the waste container to stop the flow and avoid introducing bubbles into the flow chamber. For deposition, the series of solutions that were flushed through the chamber were (1) 50 mL of SDS (10 mM); (2) 300 mL of buffer; (3) 35 mL of *h*-PSMA (0.1 wt %) followed by a 15 min pause; (4) 300 mL of buffer; (5) 35 mL of *l*-PEI−FITC (0.1 wt %), EDC (30 mg/mL), and NHS (5 mg/mL) followed by a 15 min pause; (6) 300 mL of buffer; (7) 35 mL of PAA (0.1 wt %), EDC (30 mg/mL), and NHS (5 mg/mL) followed by a 15 min pause; (8) 300 mL of buffer. For additional layers, steps 5−8 were repeated. Note that EDC and NHS were added to the solutions of polymer just prior to flushing them through the reaction chamber.

Drying and Release of Polymer Layers with a Sacrificial Layer of PAA. The substrate with the cross-linked, patterned, multilayer films bound was removed from water with tweezers, rinsed briefly with deionized water, and dried in a stream of a dry nitrogen.

Characterization. SPR (BiaCORE 1000), ellipsometry (Rudolph/Auto EL), IR (Nicolet Nexus 870 FT-IR), microscopy (Leica DMRX), AFM (Digital Instruments Nanoscope III).

Acknowledgment. We thank Justin Jiang for the EG-thiol used in this study and Emanuelle Ostuni and Robert Chapman for the *l*-PEI used in this study. This work was supported by DARPA. M.W. was supported by the German Academic Exchange Service (DAAD). S.J.M. was supported by an NIH Postdoctoral Training Grant.

LA026379F

(43) Pale-Grosdemange, C.; Simon, E. S.; Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 12−20.

(44) Chapman, R. G.; Ostuni, E.; Liang, M. N.; Meluleni, G.; Kim, E.; Yan, L.; Pier, G.; Warren, H. S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 1225−1233.

(45) Zhao, X.-M.; Wilbur, J. L.; Whitesides, G. M. *Langmuir* **1996**, *12*, 3257−3264.

(46) Silverstein, R.; Webster, F. *Spectrometric Identification of Organic Compounds*, 6th ed.; Wiley: New York, 1998.