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Planar Supported Lipid Bilayer Polymers Formed by Vesicle Fusion. 1. Influence of Diene Monomer Structure and Polymerization Method on Film Properties†

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Although fluid lipid films have been used widely in biosensing devices, they lack the high stability desired for technological implementation because the noncovalent forces between the constituent lipids are relatively weak. In this work, polymerized, planar supported lipid bilayers ((poly)PSLBs) composed of diene-functionalized lipids have been prepared and characterized. Several parameters relating (poly)-PSLB structure and stability to observations made in studies of polymerized bilayer vesicles were examined, including a comparison of UV photopolymerization and redox-initiated radical polymerization, the number and location of the polymerizable moieties in the lipid monomer, and a comparison to PSLBs produced with diacetylene lipids. Redox-initiated polymerization of films composed of bis-substituted diene lipids with at least one polymerizable moiety located near the acyl terminus produced dried PSLBs that were highly uniform and stable. All other conditions yielded PSLBs that contained a high density of defects after drying, including those formed from diacetylene lipids. In most cases, defect formation is attributed to desorption of unreacted monomers or low molecular weight polymers when the film was passed through the air/water interface. Studies on highly stable (poly)PSLBs doped with nonpolymerizable lipids showed that 40−80% of the dopants are retained when the film is dried. Thus to ensure quantitative lipid retention upon PSLB removal from water, all of the lipid monomers must be covalently anchored to the polymer network.

Introduction

Supported lipid films are increasingly being implemented as coatings for biosensor transducers.[1−6] They offer the ability to minimize sensor "fouling", that is, the undesirable adsorption of nontarget protein molecules invariably present in biological matrixes, by exploiting the characteristic protein adsorption resistance of the phosphorylcholine (PC) lipid headgroup.[7] Additionally, their well-defined and controllable architecture allows for favorable orientation and minimal denaturation of tethered water-soluble proteins.[6,8,9] Planar supported lipid bilayers (PSLBs) also provide a suitable environment for reconstitution of transmembrane receptors with retained bioactivity.[10] Lipid mono- and bilayers can be self-assembled by vesicle fusion, an important issue for commercial applications, onto a variety of optically and electrically active substrates.[11−13] Furthermore, recent developments in micropatterning techniques illustrate the potential of membrane-based biochips with parallel arrays of sensing elements for high-throughput biological or pharmaceutical screening or sensing.[14,15]

A key problem associated with implementing lipid structures in molecular devices is the inherent lack of stability that arises from the exclusively noncovalent forces that are responsible for the formation of the lipid lamellar phase. As a result, partial or complete loss of the lamellar structure occurs upon exposure to surfactants or organics

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or upon removal from aqueous environments. In addition, planar lipid bilayer instability has been observed upon extended storage in, and exchange of, the contacting buffer, in the presence of soluble proteins, and with variations in buffer pH and temperature.[16,17] These problems compromise the ability to maintain, store, and/or reuse lipid-membrane-based devices and thus pose significant impediments to their reliable implementation.

Interest in developing stabilized lipid films on solid supports has consequently received considerable scientific attention. From a broad perspective, two general approaches have been developed, one that seeks to preserve the ability of the constituent lipids to laterally diffuse and a second that greatly reduces or eliminates lateral diffusion through the introduction of covalent bond networks. The former approach generally involves either the use of "template" molecules covalently attached to a substrate, around which free lipids can organize,[5,18] or the formation of a lipid monolayer on an alkyl-terminated SAM film to create a "hybrid" bilayer.[2,6,11,19] These strategies have been demonstrated to extend the aqueous stability of lipid structures, presumably by increasing the energy barrier for the reformation of more thermodynamically favored spherical bilayers. However, they do not prevent lipid loss upon exposure to harsher conditions such as surfactant solutions or transfer across the air/water interface.

The second approach is inspired by the physical and chemical stability of a cross-linked polymer relative to its constituent monomers. A considerable body of work has shown that lamellar lipid assemblies can be polymerized from lipids containing reactive moieties.[7,20,21] Linear polymerizations of vesicles composed of monosubstituted lipids cause moderate changes in the permeability of the bilayer to water-soluble molecules and resistance to vesicle dissolution in surfactant solutions. Bis-substituted lipids can be cross-linked, resulting in more dramatic changes in bilayer chemical and physical properties.[20a,c] The properties of lipo-polymer vesicles, such as the degree of polymerization, conversion, and cross-linking, are highly dependent upon a number of factors, including the type of polymerizable moiety and the method of polymerization.[20a] All of these factors are likely to also influence the stability/robustness of supported lipo-polymer films.

However, despite the large number of polymerizable lipids that have been synthesized and characterized in liposomes,[20e,22] reported attempts to stabilize phospholipids on solid supports have focused on only a few polymerizable groups. The diacetylene moiety has been frequently used in polymerization of both single- and double-tail amphiphiles. As early as 1980, Ringsdorf and co-workers demonstrated that diacetylene-functionalized lipids, including phospholipids, could be polymerized in liposomes, resulting in enhanced stability.[23] Chapman and co-workers used diacetylene-modified PC lipids to form multilayer films on a variety of medically relevant polymers in work that pioneered the use and study of phosphorylcholine as a "biocompatible" substrate coating.[7a,b] Much of the more recent work with diacetylenic amphiphiles has focused on their use as chemical sensing materials, based on shifts in the absorption spectrum of the conjugated polymer induced by ligand binding to the lipid headgroups.[24]

Aside from Chapman's early work, the use of diacetylenes in supported lipid structures has been limited, although recently Morigaki et al.[25] used regio-selective polymerization to create patterned arrays of diacetylene-based PSLBs. However, diacetylene is not an ideal candidate to stabilize a lipid bilayer because its polymerization is highly sensitive to reactive group packing.[26] The reaction can occur only in the solid analogous phase, which usually produces low degrees of conversion[27] and precludes self-assembly by vesicle fusion (at room temperature).[25]

In contrast, lipids containing alkene-functionalized tails (e.g., acryloyl, methacryloyl, sorbyl, and dienoyl) can be polymerized in either the solid or liquid analogous phases with a high degree of conversion (>90%).[28] Polymerization can be initiated by direct UV irradiation, thermal and redox-generated radicals, or γ irradiation in unilamellar vesicles composed partially or wholly of functionalized lipids.[20a,b,29] O'Brien and co-workers have elucidated polymerization mechanisms for sorbyl- and acryloyl-functionalized lipids and determined reaction conditions that yield high molecular weight linear or cross-linked (poly)vesicles.[30,31] To our knowledge, however, only the methacryloyl and acryloyl functionalities have been used to prepare alkene-lipid-based mono- and bilayer polymer films on planar solid substrates. Regen and co-workers UV-polymerized a monolayer of bis-methacryloyl phosphatidylcholine adsorbed on (poly)ethylene, producing a more hydrophilic surface that was unaffected by exposure to a chloroform/methanol solution.[32] However, their water contact angle data were indicative of a surface more hydrophobic than expected for a uniform array of zwitterionic PC headgroups,[33] suggesting incomplete surface coverage and/or significant film disorder. More recently, Chaikof and co-workers polymerized mono-acryloyl lipid monolayers on several modified surfaces, including alkyl-terminated SAMs, using both thermal and dye-sensitized radical initiation.[34] Polymerization enhanced the stability

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of the lipid monolayer; however, contact angle measurements indicated that lipid loss occurred when films were rinsed with a surfactant (*n*-octyl-*β*-glucopyranoside). This is consistent with the observation by several groups that in vesicles, linear lipo-polymers are generally less stable to dissolution in surfactants and organic solvents than cross-linked polymers.[20b,32,35]

To date, the most commonly used techniques for examining the structure of polymerized supported lipid films have been ellipsometry, contact angle measurements, and IR spectroscopy. These techniques are useful for determining macroscopic film properties but are less well suited to investigating smaller scale structural features, for example, inhomogeneities in the polymer film that could potentially arise from domain formation or incomplete polymerization. A low degree of conversion could lead to desorption of unreacted monomer and low-mass oligomers, exposing the underlying substrate or the hydrophobic interior of the bilayer. Since proteins adsorb readily to most hydrophobic, metal, oxide, and polymeric surfaces,[4,36] an understanding of the nature and origin of defects present in lipo-polymer films is important to develop them for use in applications where the suppression of nonspecific protein adsorption is a significant issue, such as biosensor platforms. One technique with the resolution to observe inhomogeneities in lipid films is atomic force microscopy (AFM), which has been used by several groups to examine defects in fluid supported lipid bilayers immersed in water.[37]

In a recent communication,[38] we described the polymerization of a self-assembled PSLB composed of bis-diene-functionalized PC lipids, producing a highly stabilized film. AFM images revealed a PSLB surface that was devoid of defects on the nanometer scale after exposure to conditions (organic solvents, surfactant solutions) that would destroy a fluid PSLB. Equally important, the resistance to nonspecific adsorption of bovine serum albumin was shown to be equivalent to that of a fluid PC lipid bilayer.

In this work, we expand our initial investigation to several parameters that relate PSLB structure and stability to observations made in studies of polymerized bilayer vesicles. The parameters include a comparison of UV photopolymerization and redox-initiated radical polymerization, the number and location of the polymerizable moiety in the lipid monomer, and a comparison to films produced with diacetylene lipids. We also investigate the stability of nonpolymerizable lipids within the polymer films, which bears on the ease with which polymerized PSLBs can be derivatized with functional (e.g., fluorescent) lipids. In the following paper,[39] we examine the protein adsorption characteristics of the polymer films prepared and characterized in this work.

Experimental Section

Materials. Bis-sorbyl phosphatidylcholine (bis-SorbPC) and mono-sorbyl phosphatidylcholine (mono-SorbPC) were prepared by a modification of the procedure reported by Lamparski et al.[40a] The synthesis of bis-dienoyl phosphatidylcholine (bis-DenPC) was adapted from that reported by Dorn et al.[40b] Dienoyl sorbyl phosphatidylcholine (DenSorbPC) was prepared by the procedure reported by Liu et al.[41] The lipid structures (shown in Figure 1) were established by ^{1}H NMR and high-resolution mass spectrometry (HRMS). In addition, the purity was confirmed by the presence of only one spot on thin-layer chromatography (TLC). Dioleoylphosphatidylcholine (DOPC), 1,2-bis(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphocholine (DAPC), and phosphatidylethanolamine labeled with lissamine rhodamine B (Rh-DOPE) were purchased from Avanti Polar Lipids (see http://www.avantilipids.com for DAPC and Rh-DOPE structures). 2-(4,4-Difluoro-5-octyl-4-bora-3a,4a-diaza-*s*-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (BodipyPC) was purchased from Molecular Probes (see http://www.probes.com for structure). All other reagents and solvents were purchased from standard commercial sources and used as received.

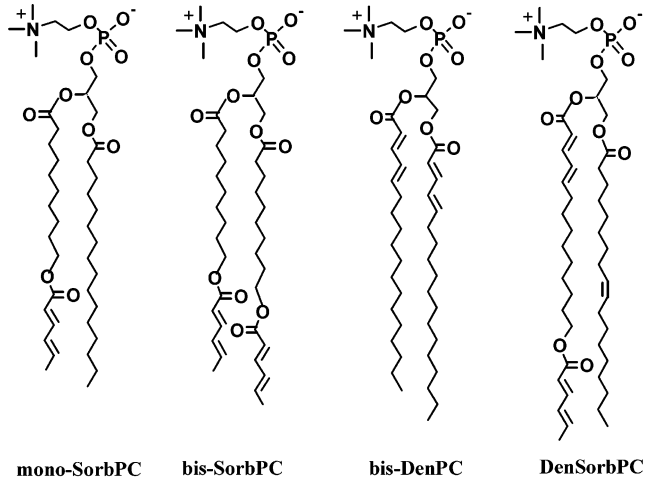

Figure 1. Structures of alkene-functionalized, polymerizable lipids used in this study.

The water used in the polymerization and protein adsorption experiments, hereafter referred to as deionized water, was obtained from a Barnstead Nanopure system with a measured resistivity of 18 MΩ cm and total organic content specified as less than 10 ppb. Silicon wafer substrates (1,1,1) were purchased from Wacker. Fused silica substrates were purchased from Dynasil.

Surface Preparation and Lipid Film Formation. Silicon wafers and fused silica slides were used as substrates. They were cleaned by a 30 min immersion in hot piranha solution, 70% H_2SO_4/30% H_2O_2, followed by extensive rinsing and sonication in deionized water. This procedure produced hydrophilic substrates with a sessile water contact angle of less than 10°. The thickness of the oxide layer on Si wafers, measured by ellipsometry, was 20 ± 2 Å. Substrates were used within 2 h of cleaning and were blown dry with a directed stream of nitrogen immediately prior to lipid deposition.

Supported lipid bilayers were prepared by vesicle fusion or Langmuir−Blodgett−Schaefer (LBS) deposition. Prior to polymerization of reactive lipids, they were handled under yellow light to avoid photoinitiated polymerization. To prepare small unilamellar lipid vesicles (SUVs) for vesicle fusion, lipids from stock chloroform or benzene solutions were dried under flowing Ar to remove the storage solvent, followed by drying overnight under a vacuum. The lipids were then resuspended in deionized water to a final concentration of 0.5 mg/mL and then vortexed and sonicated to clarity in a Branson Sonicator fitted with a cup horn.[42] The temperature was maintained at greater than 10 °C above the lipid main phase transition temperature (T_m). Within

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30 min of preparation, SUVs were used for vesicle fusion. After the substrate was dried under flowing N_2, a few drops (approx 60 μL) of SUV solution were deposited on the substrate. Lipids were fused at temperatures equal to or above their respective T_m for at least 10 min. SUVs readily self-assemble to form a PSLB on hydrophilic glass surfaces.[43a−c] The lipid-coated substrate was then transferred to either a test tube (for redox polymerization) or to a shallow crystallization dish (for UV polymerization). Care was taken to not expose unpolymerized PSLBs to air or to excessive mechanical shocks.

Supported DAPC bilayers were formed using LBS deposition on a Nima model 611D Langmuir trough.[15,44] DAPC (50 μg) was spread from benzene (1 mg/mL) on a deionized water subphase. The solvent was allowed to evaporate for 30 min before the film was compressed. After an additional 5 min equilibrium period, the first monolayer was deposited by a vertical upstroke (10 mm/min) at a surface pressure of 35−40 mN/m, followed by a horizontal Schaefer transfer (500 mm/min) at the same pressure. Transfer ratios for the first monolayer were always near 100%. The bilayer was then transferred under water to a suitable container for polymerization.

Polymerization. Direct UV polymerization was performed by exposing lipid bilayer films to light from a low-pressure mercury pen lamp (Fisher Scientific) with a rated intensity of 4500 μW/cm^2 at 254 nm. The polymerizable lipid monomers used in this work have an absorbance maximum at ∼260 nm.[25,31] A 230 nm cutoff filter (UG5, Schott Glass) was used to block exposure of samples to shorter wavelengths, which can fragment polymer chains into oligomers.[45] In cases where oxygen was to be excluded during polymerization, solutions were purged with flowing Ar for at least 30 min before and during the polymerization procedure. (Note: In the case of DAPC bilayers, polymerization could not be effected with oxygen present in the solution; if the water in contact with the bilayer was not purged with Ar before and during the polymerization procedure, the thickness of the dried lipid film was negligible.) Redox-initiated polymerization was performed with deoxygenated solutions of potassium persulfate and sodium bisulfate; except where noted, the concentration of each component was 0.01 M. The polymerization reaction was allowed to proceed under positive Ar pressure for a minimum of 2 h, except where noted. After UV or redox polymerization, substrate-supported lipid films were removed from solution, rinsed with a stream of deionized water, at least 250 mL, and blown dry with nitrogen before analysis.

Ellipsometry and Contact Angle Measurements. Ellipsometric measurements of lipid film thickness were made with a Rudolph Research model 43603-200E ellipsometer, using the 632.8 nm line of a HeNe laser at a 70° incident angle. The elliptical spot size was approximately 1.5 mm × 3 mm. Initial readings were taken on cleaned, bare Si wafers to determine the substrate optical constants and oxide layer thickness. A uniform refractive index of 1.46 was assumed for all lipid films. The data were used to calculate the film thickness values using DafIBM version 2.0, a computer program supplied by Rudolph Research and implemented on a DOS-based PC system. Measurements were made at several physical locations on each sample and on a minimum of three independently prepared samples for each type of film.[46]

Contact angles of deionized water on bare and lipid-coated substrates were measured using the sessile drop method. Images of multiple 3 μL water droplets on each substrate surface were acquired using a Pulnex TM-7CN video camera and Video Snapshot Snappy. Images were converted into tagged image format using corresponding software, and angles were measured using Image-Pro Plus 1.3 software (Media Cybernetics). Contact angles were also measured with a Krüss model DSA 10 Mk2 drop shape analysis system. Deionized water drops were delivered with a motor-driven syringe. Drop shapes were analyzed with the software Drop Shape Analysis (DSA) for Windows, version 1.70. Static drop analysis was performed with a volume of 1 μL, at multiple sites on each sample. Dynamic advancing and receding contact angles were measured on several still frames from videos of a drop expanding or receding at a rate of 10 μL per minute. Sessile and advancing contact angles were best fit using the Tangent-1 method provided with the DSA software, while receding images were best fit with the Tangent-2 method, per Krüss instructions. Contact angle data are reported herein as the mean of measurements on at least three independently prepared samples for each film type, made at three different physical locations on each sample.

Atomic Force Microscopy. AFM was performed in tapping mode on a Digital Instruments Multimode III instrument. Tapping mode etched silicon probes (TESP) were purchased from Digital Instruments and were tuned to between 300 and 400 kHz for measurements in air. Measurements on samples immersed in deionized water were performed in a fluid cell in tapping mode with contact mode probes (NP-20) tuned to approximately 33 kHz as per supplemental Digital Instruments instructions. A minimum of three separate samples for each film type were prepared and imaged; for each sample, images were acquired at several different locations on each substrate. The images presented below are typical and representative of the measurements on the respective samples.

Absorbance and Fluorescence Spectroscopy. The conversion of bis-SorbPC monomer to polymer by UV- and redox-initiated polymerization was followed by UV−vis spectroscopy. The transmission cell was composed of three fused silica slides that were separated by two U-shaped gaskets and clamped together with clips. This configuration created two cell chambers, with a total of four parallel fused silica surfaces, which allowed light transmission measurements to be performed simultaneously on four supported lipid bilayers. To initiate the experiment, approximately 400 μL of a bis-SorbPC SUV suspension was added to each cell chamber. After vesicle fusion occurred, the chambers were rinsed with water to remove unfused vesicles and polymerization was initiated by the addition of potassium persulfate/sodium bisulfate or by UV irradiation. Absorbance spectra were collected at timed intervals with a Spectral Instruments 440 UV−vis spectrometer. Since the redox initiators were found to absorb light in the UV spectral region, the cell was rinsed with deionized water before each spectrum was acquired and then refilled with initiator to resume polymerization.

Total internal reflectance fluorescence (TIRF) microscopy[44] was used to measure fluorescence emission from Rh-DOPE and BodipyPC incorporated into polymerized bis-SorbPC films. The liquid cell consisted of an O-ring compressed between a hollowed Teflon block and a fused silica microscope slide. Thus polymer films formed on fused silica slides could be dried and rehydrated repeatedly by the sequential addition and removal of water from the cell. Using a fused silica prism and index matching fluid, the 488 or 514 nm line from an argon ion laser was coupled into the slide at an angle that produced total internal reflection. One of the reflections that occurred in the portion of the slide underneath the hollowed Teflon block was used to excite fluorescence from the supported lipid film. Fluorescence emission was collected with a 10× objective, filtered using a 514 long pass filter (BodipyPC) or a 568 ± 20 nm band-pass filter (Rh-DOPE), and measured using a photomultiplier tube as described.[44] Under the conditions in which the measurements were performed, photobleaching was negligible.

(42) (a) Barrow, D. A.; Lentz, B. R. *Biochim. Biophys. Acta* **1980**, *597*, 92−99. (b) The size of the SUVs prepared in this work was not measured. Ultrasonication is reported to produce SUVs with a diameter of about 30 nm (Menger, F. E.; Lee, J. J.; Aikens, P.; Davis, S. *J. Colloid Interface Sci.* **1989**, *129*, 185−191).

(43) (a) Cremer, P. S.; Boxer, S. G. *J. Phys. Chem. B* **1999**, *103*, 2554−2559. (b) Johnson, S. J.; Bayerl, T. M.; McDermott, D. C.; Adam, G. W.; Rennie, A. R.; Thomas, R. K.; Sackmann, E. *Biophys. J.* **1991**, *59*, 289−294. (c) Bayerl, T. M.; Bloom, M. *Biophys. J.* **1990**, *58*, 357−362.

(44) Conboy, J. C.; McReynolds, K. D.; Gervay-Hague, J.; Saavedra, S. S. *J. Am. Chem. Soc.* **2002**, *124*, 968−977.

(45) Meier, H.; Sprenger, I.; Baermann, M.; Sackmann, E. *Macromolecules* **1994**, *27*, 7581−7588.

(46) Although a refractive index of 1.46 was assumed for all films, the actual refractive index of each type of lipid layer is not known; it may vary due to differences in molecular packing and is likely to be anisotropic. The contribution of strongly bound water to the measurement is also unknown. In the case of DAPC, polymerization produces a conjugated material that absorbs in the visible spectrum (ref 57). These factors contribute uncertainty to the accuracy of using ellipsometry to determine the thickness of different types of dehydrated PSLBs. Therefore, when the ellipsometry values listed in Table 1 are compared to each other and to thickness values for hydrated, fluid lipid bilayers measured by other methods, the comparison should be considered semiquantitative.

Table 1. Ellipsometry and Water Contact Angle Measurements on Polymerized, Dried PSLBs

type of PSLB	ellipsometric thickness (Å)		water contact angle (deg)		
	after rinsing and drying	after surfactant[a]	sessile[b]	advancing[b]	receding[b]
unpolymerized bis-SorbPC	5 ± 5 (n = 3)				
redox(poly)bis-SorbPC	46 ± 3 (n = 10)	45 ± 3 (n = 3)	32 ± 3	37 ± 2	8 ± 3
UV(poly)bis-SorbPC	28 ± 5 (n = 5)		59 ± 4	70 ± 5	16 ± 1
redox(poly)mono-SorbPC	33 ± 2 (n = 3)				
redox(poly)mono-SorbPC/bis-SorbPC (7:3 molar ratio)	41.5 ± 3 (n = 3)				
redox(poly)DenSorbPC	45 ± 2 (n = 6)	42 ± 3 (n = 3)	39 ± 3	45 ± 2	15 ± 3
redox(poly)bis-DenPC	52 ± 4 (n = 3)	39 ± 4 (n = 3)	52 ± 4		
UV(poly)DAPC	43 ± 21 (n = 5)		68 ± 10	80 ± 8	27 ± 6

[a] Films were bath sonicated in 2% Triton X-100 for 10 min. [b] For all data points, n = 3.

Results and Discussion

In studies of bilayer vesicles composed of polymerizable lipids, Sisson et al.[20b] determined the gel point by correlating the onset of significantly altered physical properties with the bilayer composition. Bis-substituted lipids were reacted to yield cross-linked vesicles, as characterized by the lateral diffusion of a probe molecule in the bilayer, vesicle stability in the presence of surfactants, and (poly)lipid solubility in organic solvents. Although both direct UV photopolymerization and radical polymerization methods have been used to produce cross-linked (poly)lipid vesicles, a significant difference in the degree of polymerization (X_n) was observed. In studies with lipids functionalized with sorbyl, dienoyl, and acryloyl reactive groups, radical polymerizations were generally found to produce larger polymers (X_n of 40−600) than UV irradiation (X_n of 3−10), which suggests different propagation mechanisms for the two methods.[28,31,61b] These results provided a starting point for initiating comparative studies to examine the effects of several variables, including polymerization method and monomer structure, on the properties of (poly)PSLBs.

Bis-SorbPC Films. PSLBs were formed from bis-SorbPC by vesicle fusion and subjected to redox-initiated radical polymerization before removing the film from water. These films are referred to below as redox(poly)-bis-SorbPC. Table 1 lists the ellipsometric thickness and water contact angle measurements for redox(poly)bis-SorbPC films formed under various conditions, as well as several other types of dried lipid films. Note that if the PSLB was not exposed to conditions that caused polymerization before the Si wafer was removed from water, the measured film thickness was negligible (5 ± 5 Å). AFM images of these surfaces were basically indistinguishable from images of clean Si wafers. This observation is consistent with those of other groups that near-quantitative lipid desorption occurs when a fluid PSLB is transferred across the air/water interface.[43a]

UV−vis spectroscopy was used to monitor the polymerization rate and extent of monomer-to-polymer conversion, since the monomer absorbs at 260 nm whereas (poly)bis-SorbPC is transparent in the UV spectral region.[47] At oxidant and reductant concentrations of 0.01 M, the redox-initiated polymerization reaction appeared complete (>90% conversion) after 30 min (data not shown). A more precise determination of conversion was limited by the insensitivity of making an absorbance measurement on a nearly transparent film. Therefore, to ensure near-quantitative conversion, the reaction was allowed to proceed for 2 h before the PSLB was removed from the test tube in which the polymerization was performed.

The ellipsometric thickness of a dried redox(poly)bis-SorbPC film formed on an Si wafer is 45 Å, which agrees well with the expected thickness for a bilayer composed of fully extended bis-SorbPC molecules.[48] It is also comparable to the thickness determined by a number of analytical methods (e.g., neutron reflectivity and AFM) for fluid bilayers composed of lipids having lengths comparable to that of bis-SorbPC.[37b,43b] The film thickness did not change after bath sonication in a 1% solution of Triton X-100 for 10 min or immersion in common organic solvents (e.g., acetone, chloroform). This stability indicates that the average polymer size in a redox(poly)bis-SorbPC film is sufficiently large to make it insoluble and provides strong evidence that the polymers are cross-linked. In addition, these films were observed to be inert to additional lipid adsorption, meaning that fluid SUVs did not adsorb and spread on a redox(poly)bis-SorbPC surface to form a multilayer.

The sessile water contact angle was 32°, and the dynamic advancing and receding angles were 37° and 8°, respectively. A direct comparison to a reference bilayer (e.g., DOPC) cannot be made since a contact angle cannot be measured on a fluid PSLB that is not air-stable. However, our data are similar to the 28° sessile, 52° advancing, and 8° receding contact angles reported by Cooper et al.[33] for a phosphorylcholine-terminated SAM film on gold. This is strong evidence that the redox(poly)-bis-SorbPC film presents a densely packed array of PC headgroups at the film/air interface.

AFM was used to examine the morphology of dried PSLBs. A typical image and linescan of a redox(poly)-bis-SorbPC film formed on a Si wafer are presented in Figure 2a. As reported in our initial communication, these films are very continuous and smooth (root-mean-square (rms) roughness of ca. 0.15 nm). A linescan across a film deliberately damaged by repeated high-force scanning reveals a thickness of about 44 Å, consistent with the ellipsometry measurement (Figure 2b). No apparent changes in morphology were observed when a previously dried region of a film was imaged after immersion in water. For comparison purposes, an example of an incompletely polymerized film is shown in Figure 2c. A bis-SorbPC PSLB was removed from the polymerization vessel 10 min after initiating the reaction; UV absorbance measurements showed that during this time period, conversion of monomer to polymer was only ca. 80% complete. The AFM image and linescan in Figure 2c reveal a relatively rough surface composed of domains separated by gaps having an apparent depth of 10−25 Å.[49] This morphology was very uniform across the substrate surface; no regions of the substrate were found that were devoid of polymer film. The most likely explanation for the observed topography is that upon transfer of the film across the

(47) Tyminski, P. N.; Ponticello, T. S.; O'Brien, D. F. *J. Am. Chem. Soc.* **1987**, *109*, 6541−6542.

(48) Weiner, M. C.; White, S. H. *Biophys. J.* **1992**, *61*, 434.

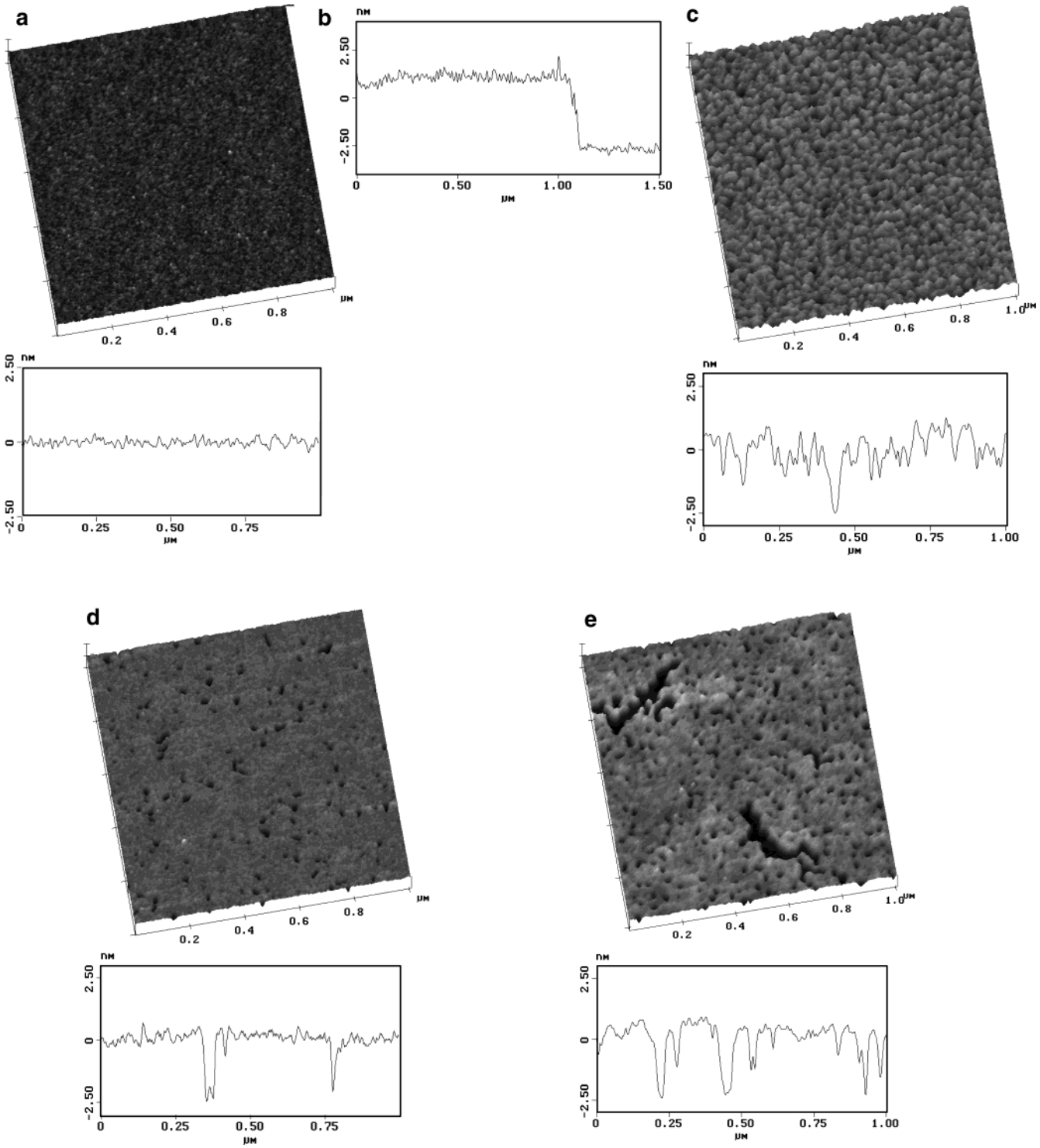

Figure 2. AFM data obtained on dried redox(poly)bis-SorbPC films formed on Si wafers. All the image areas are 1 μm^2 with a height scale of 5 nm. (a) Image and linescan of film polymerized in 10 mM persulfate/bisulfite solution for 2 h. (b) Linescan of an intentionally damaged region of (a) resulting from repeated high-force scanning. (c) Image and linescan of film polymerized in 10 mM persulfate/bisulfite solution for 10 min. (d) Image and linescan of film polymerized in 1 mM persulfate/bisulfite solution for 2 h. No changes were observed in films that were polymerized overnight. (e) Image and linescan of film polymerized in 10 mM persulfate/bisulfite solution for 2 h, with a 1 h delay between bilayer formation and initiation of polymerization.

air/water interface, bis-SorbPC monomers and oligomers were desorbed, creating the observed gaps. Thus it is clear that near-quantitative reaction of the sorbyl groups is required to generate a PSLB that is stabilized to lipid loss upon removal from water.

For diene lipids, the degree of polymerization is known to be influenced by the ratio of monomer to initiator and by the ratio of oxidant to reductant. For example, in lipid vesicles composed of 1-oleoloyl-2-[16-methyl-(*E*,*E*)-2-4-octadecadienoyl]-*sn*-glycero-3-phosphoethanolamine, the degree of polymerization increased from oligomers (X_n = 10 ± 2) at an oxidant/reductant ($S_2O_8^{2-}$/L-cysteine) ratio of 1:1 to greater than 200 when the ratio was increased to 10:1.[50] To assess effects of these variables on PSLBs, polymerizations of bis-SorbPC bilayers were performed at a variety of oxidant/reductant concentrations and molar

ratios, ranging from nearly saturated solutions of persulfate (approximately 1 M) to 1 mM, and with persulfate/bisulfite ratios ranging from 10:1 to 1:1. At all concentrations of ≥0.01 M, regardless of the oxidant/reductant ratio, redox(poly)bis-SorbPC films were indistinguishable as characterized by AFM and ellipsometry. However, when polymerization was initiated with solutions that were 1 mM in both persulfate and bisulfite, significant defects were observed in the film, as illustrated in Figure 2d. This result appears to contradict the hypothesis[20a,30,31] that at lower initiator concentrations, the frequency of chain termination reactions declines, which should increase the degree of polymerization; the higher molecular weight fragments should be less easily desorbed upon drying the film, generating a more stable PSLB. However, this hypothesis assumes that the structure of the unpolymerized lipid bilayer is maintained while reaction proceeds. To address this issue, a bis-SorbPC PSLB was formed by vesicle fusion; 1 h was then allowed to elapse before bilayer polymerization was initiated with 0.01 M persulfate/bisulfite. The AFM image and linescan of the resulting film shown in Figure 2e display defects similar in size to those observed in bis-SorbPC films polymerized with 1 mM initiator (Figure 2d). This result shows that unpolymerized PSLBs of bis-SorbPC are unstable in aqueous solution and must be polymerized immediately after vesicle fusion. We speculate that due to the relatively polar ester groups in the center of the bilayer, the critical micelle concentration of bis-SorbPC is greater than that of a lipid having pure hydrocarbon tailgroups, for example, DOPC.

Although adjusting the ratios and concentrations of the oxidant and reductant changes the solution concentration of initiating radicals, they must diffuse through the bilayer to react with the sorbyl groups. The actual concentration of initiator in the bilayer is therefore unknown. The invariant structure of poly(redox)bis-SorbPC films observed at all concentrations and ratios of oxidant and reductant suggests that radical diffusion limits the initiator concentration in the bilayer such that the polymer molecular weight is only weakly dependent on the aqueous oxidant and reductant concentrations.

Other redox initiators have been shown to effectively polymerize dienoyl groups in vesicles.[29,50] Differences in the oxidizing and reducing strength of the redox couple are known to affect the rates of radical generation and isotropic radical polymerization. To determine if polymerization of bis-SorbPC PSLBs could be effected with other redox couples, experiments were performed using potassium bromate/L-cysteine hydrochloride and $FeCl_2$/*tert*-butyl peroxide as initiators. The bromate/L-cysteine couple yielded PSLBs that were comparable to those generated using persulfate/bisulfite, based on evaluations of film thickness (ellipsometry) and surface topography (AFM). The Fe(II)/*tert*-butyl peroxide couple did not initiate polymerization, as evidenced by the persistence of the bis-SorbPC monomer absorbance band at 260 nm after making several additions of freshly prepared, deoxygenated initiator solution to the transmission cell. In contrast, Tsuchida et al.[29] found that Fe(II)/*tert*-butyl peroxide efficiently polymerized dienoyl lipid vesicles at a temperature of 8 °C. However, at 30 °C, no significant polymerization was observed, which they attributed to the "dead-end polymerization" that occurs when a rapid "explosion" of radical formation occurs, followed by rapid self-annihilation before the radicals can diffuse into the bilayer. This phenomenon is a probable cause for our inability to initiate polymerization of bis-SorbPC PSLBs with Fe(II)/*tert*-butyl peroxide at room temperature. Another possibility may be hindered diffusion of the bulky *tert*-butyl peroxide radical to the center of the bilayer. Consistent with this explanation, at 8 °C Tsuchida et al.[29] observed ca. 50% conversion of dienoyl lipid vesicles, which they interpreted to mean that only the outer leaflet of the bilayer was polymerized.

PSLBs formed by fusion of bis-SorbPC vesicles were also polymerized by direct UV irradiation. These films are referred to below as UV(poly)bis-SorbPC. As for redox(poly)bis-SorbPC films, a variety of characterization techniques was used to examine the properties of UV(poly)bis-SorbPC films. UV−vis spectroscopy was used to monitor the polymerization rate and degree of monomer-to-polymer conversion. Under the illumination conditions described above, the polymerization reaction appeared complete (>90% conversion) after 5 min (data not shown). To ensure near-quantitative conversion, PSLBs were irradiated for 30 min before removal from the crystallization dish in which the polymerization was performed, unless noted otherwise.

Ellipsometry and water contact angle measurements for UV(poly)bis-SorbPC films are listed in Table 1. The film thickness was 28 Å, the sessile contact angle was 59°, and the advancing and receding contact angles were 70° and 16°, respectively. These data show that there are significant differences in the structures of UV(poly)bis-SorbPC and redox(poly)bis-SorbPC films. The film was much thinner than that expected for a bilayer, suggesting that considerable lipid loss occurred upon removal of the PSLB from water, leading to either exposure of the hydrophobic interior of the bilayer or, if the entire bilayer was removed in some areas, exposure of the underlying hydrophilic quartz. Since the contact angles were larger than that measured for redox(poly)bis-SorbPC films, the former interpretation is more plausible.

AFM imaging provided additional support for the former interpretation. A typical image and linescan of UV(poly)-bis-SorbPC formed on a Si wafer are presented in Figure 3. The surface morphology is much rougher (rms roughness of ca. 0.35 nm) than that of redox(poly)bis-SorbPC. The film is composed of domains having an apparent average diameter of 25 nm separated by gaps having an apparent width and depth of 25−50 nm and 10−25 Å, respectively.[49] These features are very uniformly distributed on the substrate surface; no regions were found that were completely devoid of polymer film or where the domains differed appreciably in size.[51a] Given the ellipsometric thickness of 29 Å, the most likely explanation consistent with the observed topography is that upon withdrawal of

(49) The lateral dimensions and depths of domains and defects observed in dried PSLBs are much smaller than the radius of a typical AFM tip. We consequently refer to these sizes as apparent, because their true sizes are unknown, due to well-known tip-broadening effects. See for example: Atamny, F.; Baiker, A. *Surf Sci.* **1995**, *323*, L314−L318. Thus, unless its lateral dimensions are relatively large, a defect that extends through both leaflets of the PSLB to the underlying substrate probably cannot be distinguished from a defect that is confined to only the upper leaflet of the bilayer.

(50) Srisiri, W.; Lee, Y.-S.; Sisson, T.; Bondurant, B.; O'Brien, D. F. *Tetrahedron* **1997**, *53*, 15397−15414.

(51) (a) In contrast to redox(poly)bis-SorbPC films, UV(poly)bis-SorbPC films were difficult to image under water, indicative of interaction between the film and the probe tip. It was found that tips were rapidly contaminated, with material adhering to them shortly after imaging began, producing blurred images. In the best images obtained on hydrated UV(poly)bis-SorbPC films, the gaps seen in dried films were also visible. Inadequate resolution and tip/sample interactions prevented a determination of whether the domains swelled when hydrated. (b) The small amount of material in a lipid bilayer on a ca. 1 cm^2 planar substrate is insufficient to allow the degree of polymerization to be accurately determined.

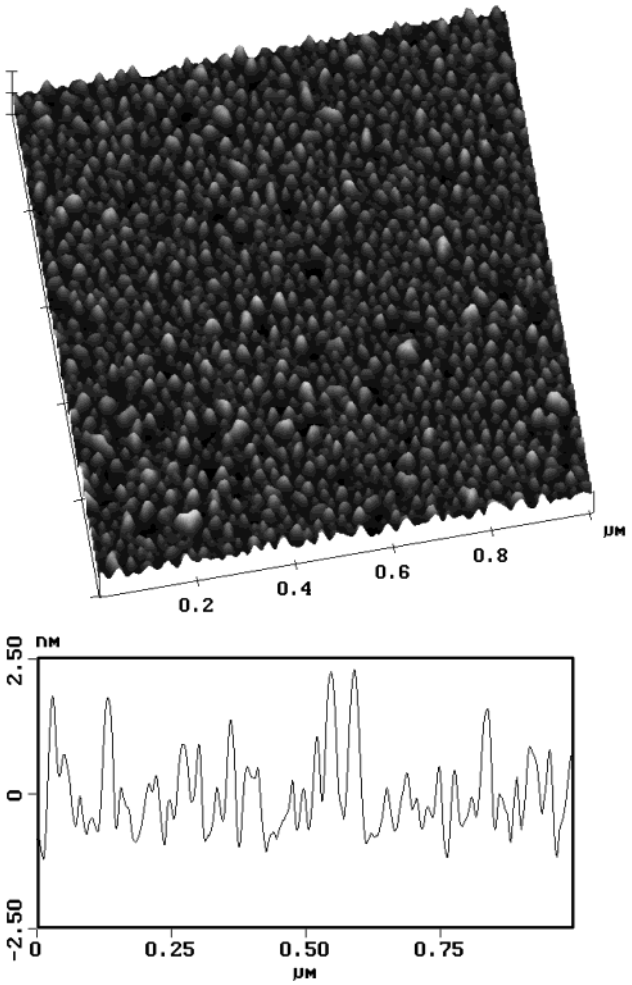

Figure 3. AFM image and linescan of a dried UV(poly)bis-SorbPC film that was photopolymerized for 30 min. The image area is 1 μm^2 with a height scale of 5 nm.

the film from water, lipids or low molecular weight oligomers are desorbed, creating the gaps seen in Figure 3. The apparent depth of the gaps is no greater than the thickness of a lipid monolayer, suggesting that material is desorbed primarily from the upper leaflet of the bilayer.[49]

The possibility was considered that UV photofragmentation during the latter part of the standard 30 min illumination period could generate low molecular weight structures that would be readily desorbed when the film was removed from water (although a 230 nm long pass filter was used specifically to avoid photofragmentation). However, the same morphology was observed for films that were irradiated for only 5 min, eliminating this possibility. Furthermore, UV absorbance measurements showed that both polymerization methods produced an equivalent degree of conversion of monomer to polymer (data not shown). However, as noted above, in (poly)-SorbPC vesicles redox initiation produces a greater degree of polymerization (X_n of 50−500) than UV irradiation (X_n of 5−10). It is reasonable to assume that the trends regarding relative X_n observed in vesicles also extend to PSLBs.[51b] Therefore we attribute the high defect density in UV(poly)bis-SorbPC films, relative to redox(poly)bis-SorbPC films, to the production of *domains* of cross-linked (poly)lipid, rather than a cross-linked (poly)lipid film that is semicontinuous. Neither polymerization method is expected to yield monodisperse polymers; therefore a range of molecular weights should be present in a (poly)bis-SorbPC PSLB. Desorption of the smaller polymers, predominately from the outer leaflet of the bilayer, occurs when a UV(poly)bis-SorbPC film is passed through the air/water interface, producing the defects. In contrast, the polymer molecular weights present in redox(poly)-bis-SorbPC films are apparently large enough to make them highly resistant to desorption, as well as insoluble in surfactants and organic solvents. Evidence from protein adsorption studies[39] provides further support for this interpretation. Drying and rehydrating a UV(poly)bis-SorbPC film caused a significant increase in protein adsorption relative to unpolymerized and UV-polymerized PSLBs that were never removed from water. Thus the film structure is significantly altered by drying rather than polymerization. Lipid desorption is likely driven by the tendency for the polar lipid headgroups to remain strongly hydrated, coupled with the hydrophobic force driving the lipid tails to adsorb at the air/water interface of droplets on the PSLB surface.

The effects of several other variables on UV polymerization of bis-SorbPC were examined. The structure of UV(poly)bis-SorbPC films was found to be insensitive to the presence/absence of oxygen during the polymerization reaction. The rate of polymerization, which was altered by changing the intensity of the UV light used to irradiate the film, also did not have any measurable effect on film properties. The influence of the phase state of the bilayer was also investigated. The main phase transition temperature (T_m) of bis-SorbPC is 28.8 °C.[52] At room temperature, a bis-SorbPC PSLB should therefore consist of coexisting solid- and fluid-phase analogous domains (however, note that the lack of membrane curvature and the existence of intermolecular interactions between the substrate and the bilayer in a PSLB may alter the T_m from that observed in vesicles). To examine if the phase state of the bilayer affected its polymerization, bis-SorbPC PSLBs were polymerized at 4 °C (in the solid-analogous phase) and at 40 °C (in the liquid-analogous phase). No measurable effect on film structure was detected, which eliminates phase segregation as a contributing factor in creation of the film morphology shown in Figure 3.

Incorporation of Nonpolymerizable Lipids into Bis-SorbPC Films. To implement PSLBs in technological applications, for example, as a sensor transducer coating, it will be necessary to develop methods to create PSLBs that are functionalized with dopant molecules that impart some desired chemical properties to the PSLBs. For example, a number of commercially available lipids have modified headgroups that react with amino acid side chains or are recognized as ligands by water-soluble proteins.[53] These lipids can be incorporated into PSLBs, allowing proteins to be covalently[54] or biospecifically[55] tethered to the PSLB surface. The (poly)PSLBs described above lacked such functionality. An obvious strategy to functionalize these films would be synthesis of a lipid with a reactive headgroup and a polymerizable tailgroup. The assumption underlying this strategy is that a minor mole fraction of functional lipids doped into a (poly)PSLB must be covalently attached to the bilayer structure to render the film stable to destabilizing conditions such as drying. The results obtained with UV(poly)PSLBs (Figure 3) and incompletely reacted redox(poly)PSLBs (Figure 2c) provide

(52) Lamparski, H.; Lee, Y.-S.; Sells, T. D.; O'Brien, D. F. *J. Am. Chem. Soc.* **1993**, *115*, 8096−8102.

(53) See for example products available from Avanti Polar Lipids (http://www.avantilipids.com) and Molecular Probes (http://www.probes-.com).

(54) Edmiston, P. L.; Saavedra, S. S. *Biophys. J.* **1998**, *74*, 999−1006.

(55) Edmiston, P. L.; Saavedra, S. S. *J. Am. Chem. Soc.* **1998**, *120*, 1665−1671.

support for this assumption. As a further test, PSLBs were formed from vesicles composed of 7:3 (mol/mol) bis-SorbPC/DOPC and polymerized by redox initiation. A typical AFM image and linescan obtained on one of these films after drying are shown in Figure 4a. The film topography was similar to that observed for UV(poly)-bis-SorbPC films, consisting of interconnected domains of polymer separated by gaps with an apparent depth of approximately one lipid monolayer.[49] These data clearly show that significant desorption of nonpolymerizable lipids occurs when a partially polymerized film is removed from water.

However, these data do not address if a small mole fraction, for example, 1%, of a functionalized, nonpolymerizable lipid can be retained in a PSLB that is composed almost entirely of cross-linked (poly)bis-SorbPC. If true, this would circumvent the apparent need to synthesize functionalized polymerizable lipids, making it relatively easy to create (poly)PSLBs bearing a variety of different lipid headgroups. To address this issue, redox-polymerized PSLBs composed of 98.5 mol % bis-SorbPC and 1.5 mol % of either BodipyPC or Rh-DOPE were prepared on fused silica substrates. TIRF was used to measure the fluorescence intensity of these films as a function of the number of drying and rehydration cycles. The results are plotted in Figure 4b. Before drying, both types of films were observed to be uniformly fluorescent by epifluorescence microscopy at 400× magnification. In the case of Rh-DOPE as the dopant, the measured intensity decreased with each cycle, until stabilizing at about 37% of the initial normalized intensity after 12 cycles. We conjecture that the retained Rh-DOPE is largely confined to the inner leaflet of the PSLB. For BodipyPC-doped films, the loss was less severe and was limited to the first two cycles, after which the retained lipid stabilized at about 83% of the initial amount.

These data show that a significant percentage of each dopant is retained after only one drying. Thus, incorporating a nonpolymerizable lipid into a (poly)PSLB does provide some degree of stability when compared to the nearly quantitative desorption of lipids that occurs when a fluid PSLB is removed from water. However, these results also demonstrate that nonpolymerizable lipids cannot be quantitatively retained when a (poly)PSLB is dried, even when they comprise only a small percentage of the total film. Finally, the extent of dopant loss clearly depends on its structure. The greater loss observed for Rh-DOPE is likely due to the presence of a more surface-active headgroup relative to the phosphorylcholine headgroup of BodipyPC.

Films Containing Mono-SorbPC. The stability of redox(poly)bis-SorbPC films provides strong evidence that these films are composed of cross-linked polymers. A linearly polymerized PSLB should therefore be less stable if trends in (poly)vesicles are followed.[20b] To investigate this issue, PSLBs were formed from mono-SorbPC and redox-polymerized under conditions identical to that used to produce redox(poly)bis-SorbPC films. Dried redox(poly)-mono-SorbPC returned a mean ellipsometric thickness of 31 Å (Table 1), much thinner than that of redox(poly)-bis-SorbPC and comparable to that of UV(poly)bis-SorbPC. A typical AFM image and linescan obtained on one of these films after drying are shown in Figure 5. The film morphology was similar to that observed for UV(poly)-bis-SorbPC films. The domainlike features have an apparent diameter of 25−50 nm and are separated by gaps with an apparent depth of one lipid monolayer (1.5−2 nm).[49] A comparison of Figures 5 and 2a clearly illustrates the effect of the number of reactive groups per lipid on the resulting PSLB structure.

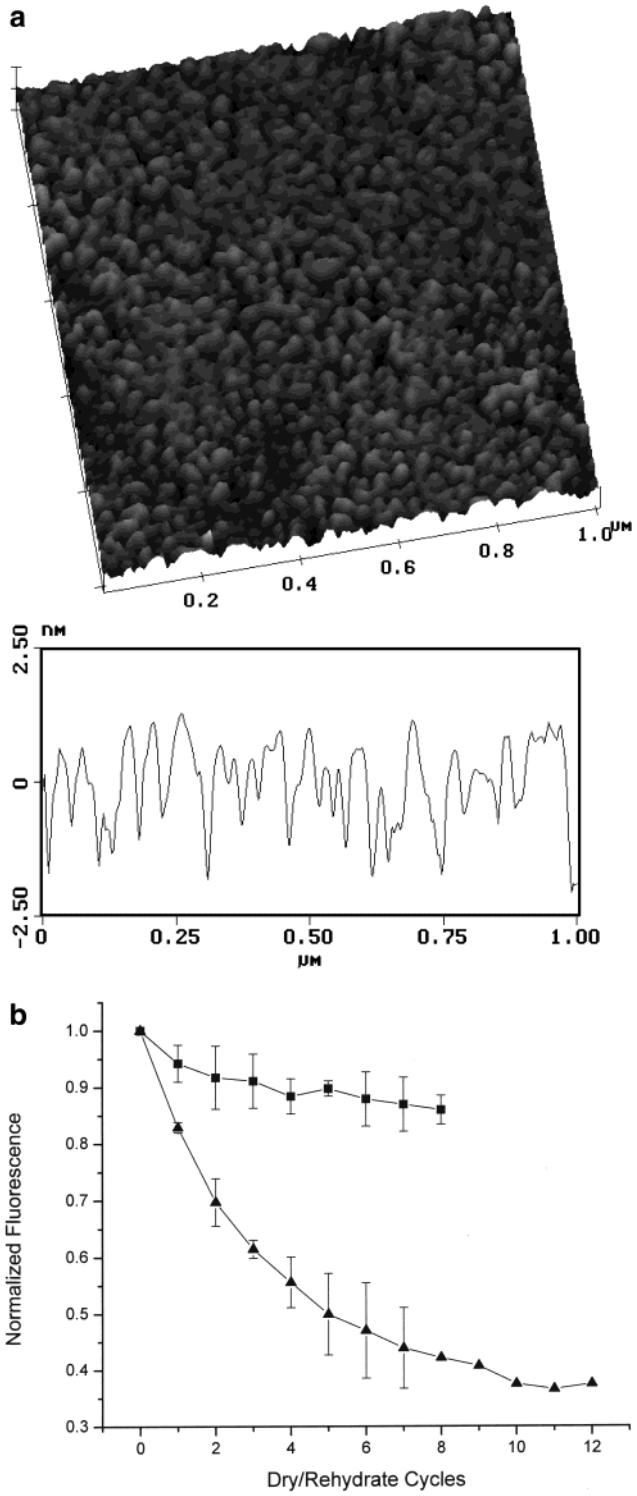

Figure 4. (a) AFM image and linescan of a dried, redox-polymerized PSLB formed from a 7:3 molar ratio of bis-SorbPC/DOPC. The image area is 1 μm^2 with a height scale of 5 nm. (b) Plot of fluorescence observed from dried, redox-polymerized PSLBs composed of 98.5% bis-SorbPC and 1.5% of a fluorescent, nonpolymerizable lipid, either BodipyPC (squares, $n = 3$) or Rh-DOPE (triangles, $n = 2$ where error bars are shown), measured as a function of the number of times the PSLB was dried and rehydrated. The measurements were performed with the films immersed in water. The initial intensity, measured before drying the film once, was set to a normalized value of 1.

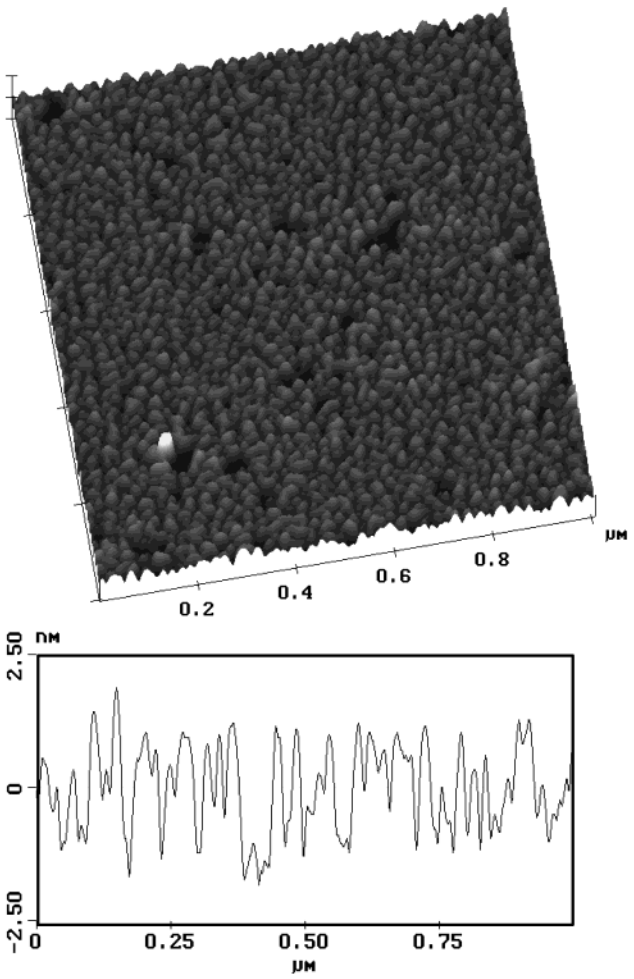

Figure 5. AFM image and linescan of a dried, redox-polymerized mono-SorbPC PSLB. The AFM image area is 1 μm^2 with a height scale of 5 nm.

Regen and co-workers reported that the stability of (poly)vesicles to surfactant lysis increased in the order of unpolymerized monomer < linear polymer < cross-linked polymer.[56] This trend was also observed in sorbyl polymerizations, where cross-linking caused a significant decrease in the solubility of (poly)vesicles in hexafluoroisopropanol.[20b] Consistent with the results of these studies, our results also demonstrate a significant difference in the stability of linear versus cross-linked sorbyl PSLBs. The enhanced stability may be due to a difference in molecular mass between linear and cross-linked fragments or a difference in other physical properties between linear and cross-linked fragments of comparable mass.

Mixtures of mono- and bis-functionalized polymerizable lipids can yield cross-linked, highly stable (poly)lipids. Sisson et al.[20b] found that approximately 30 mol % of bis-SorbPC mixed with mono-SorbPC was sufficient to produce cross-linked bilayer vesicles. To investigate if this observation extends to supported lipid bilayers, PSLBs were formed from 7:3 (mol/mol) mono-SorbPC/bis-SorbPC and redox-polymerized under conditions identical to those used to produce redox(poly)bis-SorbPC films. AFM images of these mixed films (data not shown) were indistinguishable from images of pure redox(poly)bis-SorbPC films. The mean ellipsometric thickness of the mixed film was 42 Å, which also agrees well with the 46 Å measured for pure redox(poly)bis-SorbPC films. Therefore the onset of cross-linking in PSLBs apparently occurs at bis-lipid concentrations equivalent to that which produces cross-linked vesicles, suggesting that a similar polymerization efficiency is achieved in the two types of bilayers. Furthermore, this observation helps to justify our comparisons between the extensively characterized vesicle polymerizations and the relatively less well characterized PSLB polymerizations.

Diacetylene Lipid Films. In their studies of Langmuir−Blodgett (LB) monolayers and multilayers of diacetylenePC lipids, Chapman and co-workers pioneered the use of phosphorylcholine-containing materials as protein resistant coatings.[7a,b] However, supported bilayers of diacetylenePC lipids have not been thoroughly investigated, which may be due to inherent problems in self-assembling and efficiently polymerizing these materials,[26,27] as described above. Specifically, information on the microstructure of diacetylenePC PSLBs, such as film uniformity and defect density, has not been reported in the literature, nor have quantitative data on protein adsorption properties. We therefore prepared polymerized PSLBs using a commercially available diacetylenePC lipid (DAPC) and compared their structural features to those of (poly)PSLBs fabricated from diene lipids.

DAPC PSLBs were deposited using the LBS technique and polymerized by UV irradiation, closely following the procedures described by Morigaki et al.[15,25]

The ellipsometric thickness for films irradiated for 1 h was 43 Å, although significant film thicknesses were observed with irradiation times as short as 15 min. The 43 Å value suggests that a nearly complete bilayer was formed;[46] however, the contact angle and AFM data indicate otherwise. The sessile, advancing, and receding water contact angles were 68°, 80°, and 27°, respectively, which agree well with contact angles of 70−90° reported by Chapman and co-workers for multilayer, headgroup-out (poly)DAPC films.[57] However, all of these values are much greater than expected for a film terminated uniformly with phosphorylcholine groups[33] and are indicative of a disordered and/or incomplete bilayer in which the hydrophobic interior is exposed to the ambient environment.[58]

A typical AFM image and linescan of a DAPC (poly)-PSLB are shown in Figure 6. (Note: To our knowledge, these are the first published AFM data for dried diacetylene-based PSLBs, which is somewhat surprising given the extensive use of DAPC and similar molecules to form polymerized vesicles and supported films.[7b,15,25,27]) The film morphology was rough with numerous defects, similar to that observed for UV(poly)bis-SorbPC films (Figure 3) and redox-polymerized films of 7:3 bis-SorbPC and DOPC (Figure 4a). However, the domainlike features were larger than those observed in diene lipid films. Analysis of linescans showed that the defects could be grouped into two classes, based on apparent depth: 2.5−3 nm and 4.5−6 nm. These may represent, respectively, defects in the outer leaflet of the bilayer and defects that extend through the bilayer to the substrate.[49]

(56) Regen, S. L.; Singh, A.; Oehme, G.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 791−795.

(57) Albrecht, O.; Johnston, D. S.; Villaverde, C.; Chapman, D. *Biochim. Biophys. Acta* **1982**, *687*, 165−169.

(58) The fact that the contact angle on (poly)DAPC films was 20° larger than that measured on UV(poly)bis-SorbPC films (which were thinner) may be due to differences in the structure of the monomer acyl chains. A sessile contact angle of 63° was measured on a bis-Sorb monolayer deposited by the LB transfer. This value is smaller than expected for a lipid monolayer containing hydrocarbon acyl chains and reflects the presence of the ester groups near the acyl chain termini of bis-SorbPC. Thus we expect that defects in a (poly)PSLB that expose the acyl chain termini in the bilayer interior would produce a lower contact angle when the monomer is bis-SorbPC versus DAPC.

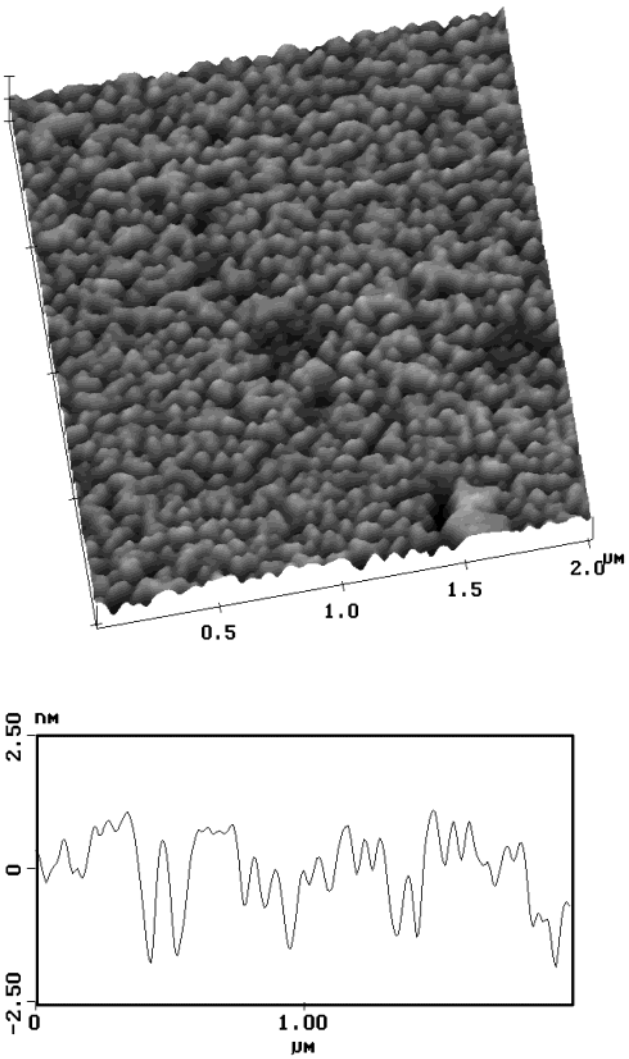

Figure 6. AFM image and linescan of a dried DAPC PSLB deposited by the Langmuir−Blodgett−Schaefer technique and polymerized by direct UV irradiation for 1 h. The AFM image area is 4 μm^2 with a height scale of 5 nm.

Among the multiple samples prepared and analyzed, there was a greater variability in the surface morphology and thickness of (poly)DAPC films as compared to diene lipid films, for example, UV(poly)bis-SorbPC. This may reflect the sensitivity of the polymerization to the packing of the reactive groups in the lipid chains, which could be affected by the transfer process in both the LB and Schaefer deposition steps.

Several experimental conditions were altered in an effort to improve the structural homogeneity of (poly)DAPC films, including (i) varying the intensity of the UV light and the time period that the film was irradiated, (ii) polymerizing using the redox-initiation procedure employed to prepare redox(poly)bis-SorbPC films, (iii) depositing the PSLB by vesicle fusion at ca. 50 °C (at which the bilayer is in the fluid-analogous phase given that the T_m for DAPC is 43.1 °C[59]), and (iv) varying the surface pressure at which the film was deposited, in an attempt to vary the packing of the lipid chains. Several combinations of film deposition and polymerization conditions produced significant coverage (i.e., an ellipsometric film thickness greater than 40 Å). For example, vesicle fusion at 50 °C followed by UV or redox-initiated polymerization at room temperature produced dried PSLBs that were ca. 40 Å thick. However, none of the DAPC films prepared or polymerized under a variety of conditions (more than 10) were devoid of significant defects as revealed by AFM imaging. In summary, LBS deposition followed by UV polymerization produced the "highest quality" films; however, the structural homogeneity of these films was still poor relative to redox(poly)bis-SorbPC films.

Finally, since the DAPC monomer has a relatively low molar absorptivity, the rate and extent of its conversion to polymer could not be measured as was done for bis-SorbPC films. Furthermore, the degree of polymerization is not known for DAPC under the polymerization conditions used here. We therefore cannot provide experimental data to support attributing the defects observed in DAPC films to desorption of either unreacted monomers or low molecular weight oligomers. However, the similarities in morphology between (poly)DAPC films and (e.g.) redox-(poly)bis-SorbPC films containing 30 mol % DOPC (Figure 4a) strongly suggest that the high defect density in (poly)-DAPC films is due to a low conversion efficiency.

Bis-DenPC and DenSorbPC Films. PSLBs were also prepared from bis-DenPC, which contains two reactive dienes located near the glycerol backbone, and DenSorbPC, which contains one sorbyl and one dienoyl moiety. Lipids with polymerizable groups located proximal to the glycerol backbone have been shown to effectively cross-link bilayers, producing vesicles with properties similar to those of (poly)vesicles formed from sorbyl-functionalized lipids.[20a,60] For example, in studies comparing the cross-linking efficiency of diene lipids, only ca. 12 mol % of bis-DenPC monomer was necessary to cross-link vesicles composed of mono-DenPC, compared to the 30% bis-substitution required to cross-link sorbyl-functionalized lipid vesicles.[61] The reactive moieties in heterobifunctional lipids such as DenSorbPC can be polymerized independently of one another by judicious selection of polymerization conditions.[60a] Very stable (poly)DenSorbPC vesicles can be dried and redispersed in water with retention of their spherical structure.[62]

Ellipsometric thickness and sessile water contact angle data for (poly)bis-DenPC and (poly)DenSorbPC PSLBs prepared by vesicle fusion and redox-initiated polymerization are listed in Table 1. Dried redox(poly)bis-DenPC films were 52 Å thick, consistent with the expected thickness of a PSLB. However, after bath sonication in Triton X-100, a significant decrease in film thickness (to 39 Å) was observed. The contact angle was 52°, considerably greater than that on redox(poly)bis-Sorb, indicative of significant structural differences between the two types of bilayers. AFM imaging revealed a film that contained defects located uniformly across its surface (Figure 7a). Analysis of linescans showed that the apparent depth of the defects was less than 3 nm, suggesting that they were formed by loss of material from the outer leaflet of the PSLB.[49]

Polymerization of DenSorbPC produced dried PSLBs that were indistinguishable from redox(poly)bis-SorbPC films by AFM imaging. An example image and linescan are displayed in Figure 7b. The ellipsometric thickness (45 Å) was nearly identical as well, and upon bath sonication in Triton X-100, only a minor decrease was observed. Slightly greater sessile, advancing, and receding contact angles, 39°, 45°, and 15°, respectively, were

(59) Obtained from a LIPIDAT search (http://www.lipidat.chemistry.ohio-state.edu/search.stm).

(60) (a) Sisson, T. M.; Srisiri, W.; O'Brien, D. F. *J. Am. Chem. Soc.* **1998**, *120*, 2322−2329. (b) Liu, S.; Sisson, T. M.; O'Brien, D. F. *Macromolecules* **2001**, *34*, 465−473.

(61) Liu, S.; O'Brien, D. F. *Macromolecules* **1999**, *32*, 5519−5524.

(62) Liu, S.; O'Brien, D. F. *J. Am. Chem. Soc.* **2002**, *114*, 6037−6042.

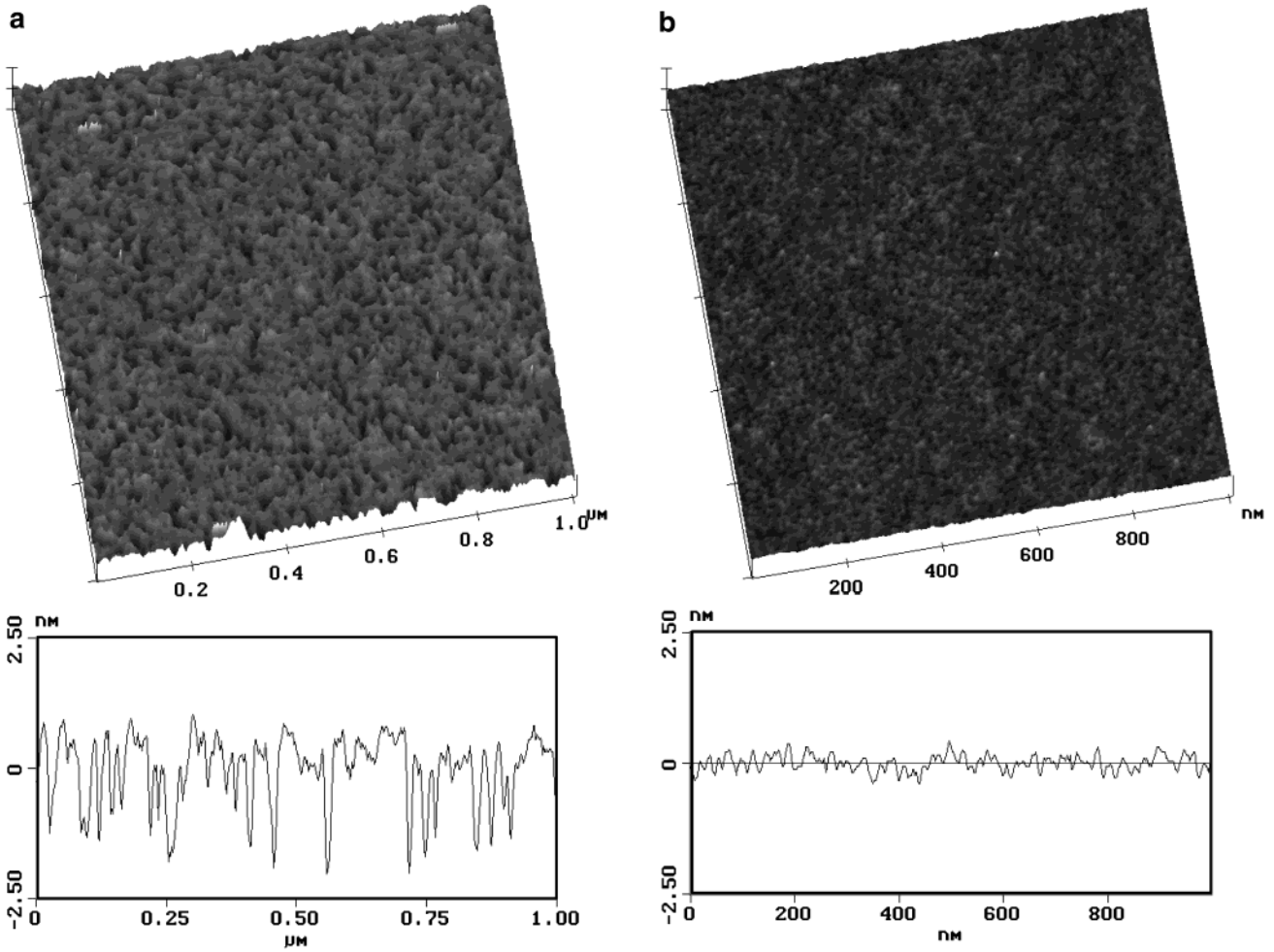

Figure 7. AFM images and linescans of (a) a dried redox(poly)bis-DenPC film and (b) a dried redox(poly)DenSorbPC film. Polymerization was initiated with 10 mM persulfate/bisulfite solution. Both image areas are 1 μm^2 with a height scale of 5 nm.

observed relative to redox(poly)bis-SorbPC films. It unknown whether this difference results from desorption of some material upon drying or if it is a phenomenon associated with the polymerization process. It is possible that formation of a covalent network near the glycerol backbone produces a more rigid polymer network, limiting local headgroup mobility. In other words, the conformational freedom of the PC groups to rearrange during wetting may be restricted, whereas when the covalent network is located in the center of the bilayer (i.e., the case with bis-SorbPC), the headgroups may be less conformationally restricted.

Regarding the differences observed between the three bis-substituted diene lipids examined in this work, we note that redox polymerization of the two sorbyl-functionalized lipids, DenSorbPC and bis-SorbPC, produced highly uniform films with a very low defect density, whereas bis-DenPC did not. On the basis of these observations, a plausible argument can be made that cross-linking across the two leaflets of the bilayer occurs with sorbyl lipids and is important for creating a highly stable PSLB. However, this argument assumes that the degree of polymerization in the PSLB geometry was equivalent for all three lipids. Moreover, the degree of polymerization of (poly)DenPC in vesicles is lower than that of (poly)-SorbPCs for equivalent reaction conditions.[29,31] In that work, it was suggested that cross-linking near the lipid backbone created a greater barrier to monomer diffusion to the growing polymer chain than a linkage near the acyl chain terminus. Therefore, in summary, we cannot solely attribute the defects in redox(poly)bis-DenPC films to either a lower degree of polymerization or to the inherent inability of this monomer to form intermonolayer covalent linkages.

Film Solvation in Hexafluoroisopropanol. One measure used to determine the efficiency of cross-linking in bilayer vesicle polymerizations is the weight percentage of the freeze-dried polymer product that can be solubilized. Hexafluoroisopropanol (HFIP) has been found to be very effective for solvating zwitterionic PC lipid polymers.[20b] Dried (poly)vesicles of bis-SorbPC and DenSorbPC were found to be 30% and 40% soluble in HFIP, respectively, whereas linearly polymerized vesicles were greater than 86% soluble.[20b,60b] Here AFM was used to examine the morphology of redox(poly)bis-SorbPC and redox(poly)-DenSorbPC films after immersion in neat HFIP for various time periods. Striking differences in the behavior of the film types were observed. After immersion in neat HFIP for periods as short as 3 s, the redox(poly)bis-SorbPC film was observed to "wrinkle" (Figure 8a). Immersion for longer periods ($\geq$1 min) appeared to cause areas of the film to partially delaminate from the Si wafer (Figure 8b). However, the film remained contiguous and attached to the substrate at numerous points. The delaminated and wrinkled areas refracted light differently as observed by the video camera used for aligning the probe tip and laser (Figure 8d). An image of a boundary between delaminated and wrinkled regions is shown in Figure 8c. Small "holes" are visible in the delaminated portion of the film, likely representing either solubilized lower molecular weight

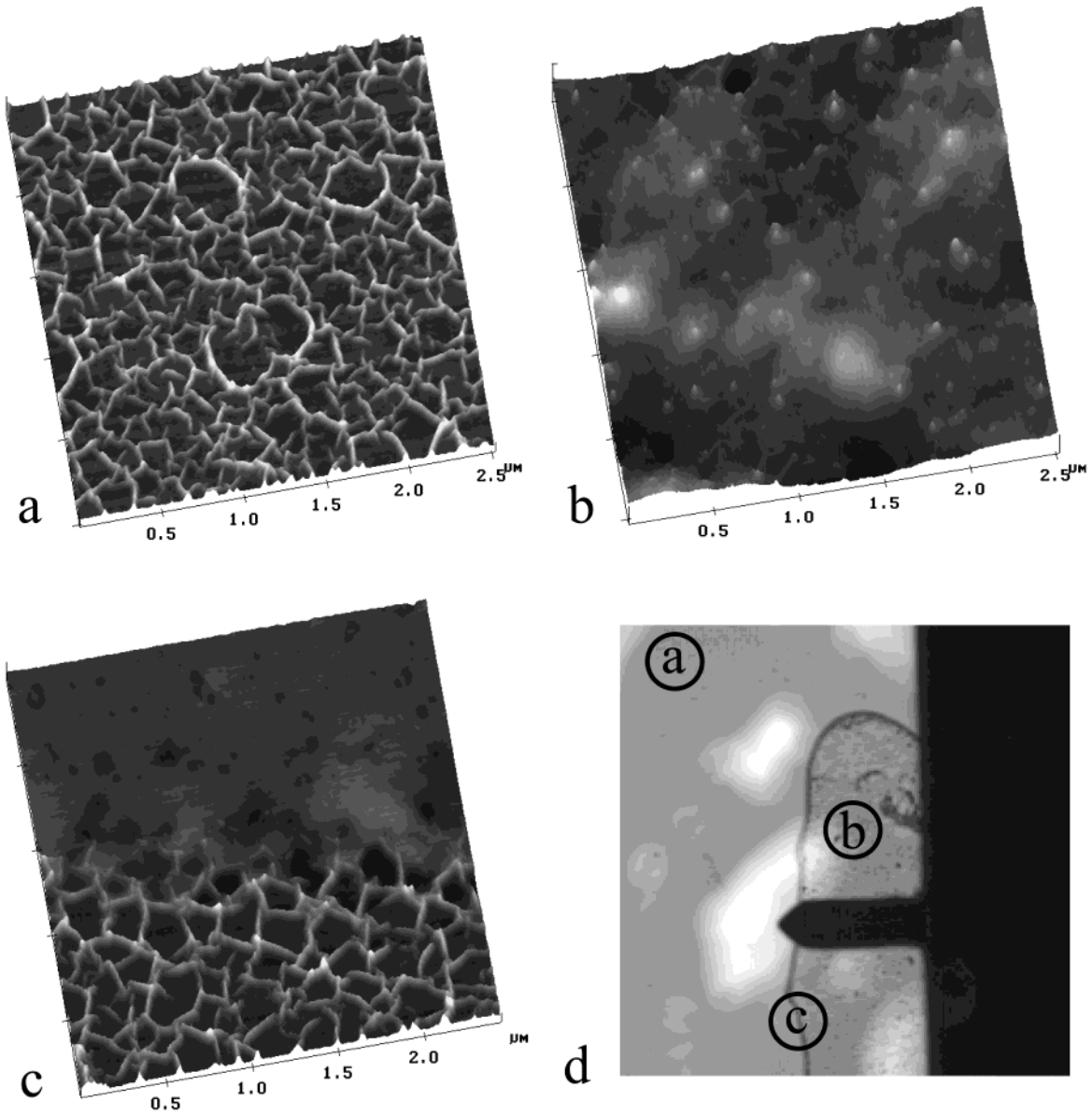

Figure 8. AFM images showing the effect of exposing a redox(poly)bis-SorbPC PSLB to HFIP. After 60 s of immersion in HFIP, the film was withdrawn and imaged in air: (a) an example of a wrinkled region, (b) an example of a delaminated region, and (c) an example of a boundary area between delaminated and wrinkled regions. All the image areas are 2.5 μm × 2.5 μm with a height scale of 10 nm. (d) The delaminated and wrinkled areas refracted light differently as observed by the video camera used for aligning the probe tip and laser. The letters in (d) indicate the regions from which scans a−c were acquired.

fragments or points where the film remained attached to the surface. However, the film appears to be contiguous even after separation from the substrate, which implies a high degree of cross-linking such that the polymer film behaves as a semi-infinite molecular sheet.

In comparison, immersion of a redox(poly)DenSorbPC film into HFIP did not cause wrinkling even after 30 min. The surface roughness increased slightly, but the film morphology remained uniform (data not shown). Note that there are three regions of connectivity in (poly)DenSorbPC: one each near the headgroup plane in each leaflet and one at the center of the bilayer. Creation of a polymer network at three planes may reduce the permeability of solvent molecules into the bilayer and prevent it from being solvated.

Conclusions

The degree of stability required for commercial use of PSLBs will vary with the intended application. Many applications can be envisioned requiring greater stability than afforded by unpolymerized lipid structures, for example, the surface coating of a biosensor intended to have a long shelf life. Other applications will require even greater stability, for example, a reusable, washable surface coating for a sensor that is used in the presence of surfactants and/or organic solvents. Toward this end, developing an understanding of the degree of PSLB alteration or damage upon exposure to these potentially destabilizing conditions is important in the design of robust, lipid-based surface coatings.

We have demonstrated that there are significant differences in the properties and stability of PSLBs formed from polymerizable lipids, depending on the method of initiating polymerization and the number, type, and location of the reactive groups in the lipid monomer. However, determining how these and other parameters affect (poly)PSLB structure and stability is limited by the relatively small amount of polymer product present in a

ca. 50 Å thick coating over a cm^2 area, which precludes determining the degree of polymerization. Consequently it is difficult to determine conclusively if intra- and/or interleaflet cross-linking is required to stabilize a PSLB, or if the instability observed when these linkages are absent (in polymerizations of mono-SorbPC and bis-DenPC, respectively) is due to production of low molecular weight polymer products that readily desorb when the PSLB is dried.

Regardless, we have found that films composed of bis-substituted diene lipids with at least one polymerizable moiety located near the acyl terminus yield highly uniform, robust polymer films. Specifically, redox-initiated polymerization of bis-SorbPC and DenSorbPC produced highly cross-linked films in which the overall bilayer structure and uniformity is preserved upon drying. The results obtained with DAPC demonstrate that the type of polymerizable moiety is also an important parameter. Regardless of the deposition and polymerization method employed, the resulting DAPC PSLB contained a high density of defects. The most likely explanation is that the sensitivity of acetylene polymerization to reactive group packing precluded achieving a high degree of monomer-to-polymer conversion.

Direct photopolymerization of bis-SorbPC produced films having a high defect density, which is attributed to the observation that this polymerization method generates smaller cross-linked domains that are more easily desorbed when the PSLB is dried. However, the fact that UV polymerization yielded any film after drying still represents a significant increase in stability relative to an unpolymerized, fluid PSLB.

Although linear polymerization using pure mono-SorbPC also produced PSLBs having a high density of defects, adding 30 mol % bis-SorbPC generated (poly)-PSLBs that were structurally comparable to those formed from pure bis-SorbPC. This result is significant because it suggests that by using mixtures of mono- and bis-functionalized polymerizable lipids, it may be possible to systematically adjust the elasticity of a (poly)PSLB, while still retaining a cross-link density sufficient to achieve stability. A more conformationally flexible film may place fewer conformational restrictions on incorporated biomolecules, for example, transmembrane receptors, allowing their reconstitution into (poly)PSLBs with higher retention of bioactivity. Incorporation of functional biomolecules, including transmembrane proteins, into polymerized PSLBs is a subject of ongoing research activities in our laboratories.

The results obtained with BodipyPC and Rh-DOPE show that a nonpolymerizable lipid doped into an otherwise highly stable (poly)PSLB is not quantitatively retained when the film is removed from water. These results indicate that when considering strategies to covalently tether a water-soluble protein to the surface of a (poly)PSLB, the lipid that is reactive toward the protein should also contain a polymerizable moiety that allows it to be covalently anchored to the polymer network.

Finally, the inherent resistance to nonspecific protein adsorption is one of the key features of PC-lipid-based films that make them attractive for use in biosensing applications. Thus the protein resistance properties of polymerized versus fluid PSLBs is an important issue. In the following paper,[39] we compare protein adsorption on polymerized and fluid lipid bilayers, showing that cross-linking polymerization does not adversely affect the protein resistance characteristic of a fluid PC lipid bilayer.

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