



Preparation and characterization of asymmetric planar supported bilayers composed of poly(bis-sorbylphosphatidylcholine) on *n*-octadecyltrichlorosilane SAMs

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ABSTRACT

Planar supported lipid bilayers (PSLBs) have been widely studied as biomembrane models and biosensor scaffolds. For technological applications, a major limitation of PSLBs composed of fluid lipids is that the bilayer structure is readily disrupted when exposed to chemical, mechanical, and thermal stresses. A number of asymmetric supported bilayer structures, such as the hybrid bilayer membrane (HBM) and the tethered bilayer lipid membrane (tBLM), have been created as an alternative to symmetric PSLBs. In both HBMs and tBLMs, the inner monolayer is covalently attached to the substrate while the outer monolayer is typically composed of a fluid lipid. Here we address if cross-linking polymerization of the lipids in the outer monolayer of an asymmetric supported bilayer can achieve the high degree of stability observed previously for symmetric PSLBs in which both monolayers are cross-linked [E.E. Ross, L.J. Rozanski, T. Spratt, S.C. Liu, D.F. O'Brien, S.S. Saavedra, *Langmuir* 19 (2003) 1752]. To explore this issue, HBMs composed of an outer monolayer of a cross-linkable lipid, bis-sorbylphosphatidylcholine (bis-SorbPC), and an inner SAM were prepared and characterized. Several experimental conditions were varied: vesicle fusion time, polymerization method, and polymerization time and temperature. Under most conditions, bis-SorbPC cross-linking stabilized the HBM such that its bilayer structure was largely preserved after drying; however these films invariably contained sub-micron scale defects that exposed the hydrophobic core of the HBM. The defects appear to be caused by desorption of low molecular weight oligomers when the film is removed from water, rinsed, and dried. In contrast, poly(bis-SorbPC) PSLBs prepared under similar conditions by Ross et al. were nearly defect free. This comparison shows that formation of a cross-linked network in the outer leaflet of an asymmetric supported bilayer is insufficient to prevent lipid desorption; inter-leaflet covalent linking appears to be necessary to create supported poly(lipid) assemblies that are impervious to repeated drying and rehydration. The difference in stability is attributed to inter-leaflet cross-linking between monolayers which can form in symmetric bis-SorbPC PSLBs.

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1. Introduction

Planar supported lipid bilayers (PSLBs) are useful as biomembrane models as well as a biomimetic matrix for design of biosensors based on ligand binding to incorporated receptors [1]. In addition to providing a biocompatible environment for receptors, a PSLB composed of phosphorylcholine (PC) lipids is highly resistant to surface “fouling” due to nonspecific protein adsorption [2–4].

A major limitation of fluid PSLBs formed from naturally occurring lipids is that the lipid molecules are self associated by

relatively weak intermolecular interactions [5]. These forces are insufficient to maintain the bilayer structure under chemical, mechanical, or thermal stress. Consequently, partial or complete loss of the bilayer structure occurs upon exposure to surfactants or organic solvents, as well as upon removal from water [5–7]. Therefore, interest in developing stabilized lipid films while maintaining their inherent biocompatibility has been a focus of research efforts since the early 1980s [3,8–11].

Covalent polymerization of lipid monomers containing reactive moieties has been widely used to create stable lipid bilayers [3, 9,10,12,13]. Although a diverse array of polymerizable lipids have been synthesized and characterized during the past three decades (see [13–15] for reviews), relatively few have been used to make lipid films on solid supports. Most of the early work in this field

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utilized diacetylene lipids [3,13,16,17]. More recently, Ross et al. prepared PSLBs composed of dienoyl-functionalized lipids on silica and glass that were cross-linked using either UV photopolymerization or redox-initiated radical polymerization. In some cases, highly stable PSLBs were produced that were unaffected by repeated air drying or exposure to organic solvents [6,7,18].

In recent years, several types of asymmetric supported bilayer structures, in which the composition of the two monolayers is different, have been created as alternatives to symmetric PSLBs. One example is the hybrid bilayer membrane (HBM) [19–23]. The inner monolayer in a HBM is an alkyl self-assembled monolayer (SAM) upon which an outer lipid monolayer is deposited by either Langmuir–Schaefer or vesicle fusion methods. A more sophisticated type of asymmetric supported bilayer is the tethered bilayer lipid membrane (tBLM) in which the SAM is replaced with an inner phospholipid monolayer that is tethered to the underlying support, usually through a hydrophilic spacer group [24–28]. Tethering the inner monolayer in an asymmetric supported bilayer enhances its structural stability. However when the outer monolayer is composed of fluid lipids that are associated solely via noncovalent interactions, it is relatively unstable, similar to a fluid-phase PSLB [7,29].

Several studies have described preparation of asymmetric bilayers using polymerizable lipids in an attempt to achieve stabilization through monolayer polymerization [29–38]. For example, Chaikof and coworkers formed HBMs on several modified surfaces, including alkyl-terminated SAMs, using monoacrylate-functionalized lipids [29,35–38]. These HBMs were subjected to thermally- or photo-initiated radical polymerization to yield linear polymers. Relative to an unpolymersized HBM, polymerization enhanced the stability of the membrane; however the water contact angle gradually increased during incubation in surfactants, indicative of lipid desorption [38]. These results are consistent with studies of vesicles showing that linear lipo-polymers are generally less stable to dissolution in surfactants than cross-linked lipo-polymers [8,39–41]. In addition, significant protein adsorption and platelet adhesion occurred on these HBMs [29,36], presumably due to defects in lipid film.

In this work, we address if cross-linking polymerization in the outer lipid monolayer of an asymmetric supported bilayer can achieve the high degree of stability observed previously [7] for symmetric PSLBs in which both monolayers are cross-linked. To explore this issue, HBMs composed of an outer monolayer of a cross-linkable lipid, bis-sorbylphosphatidylcholine (bis-SorbPC) [42, 43], and an inner SAM were prepared and characterized. The influence of a number of different preparation conditions, such as polymerization method, on the resulting HBM structure was examined. In most cases, cross-linking the lipid layer stabilized the HBM such that its bilayer structure was largely preserved after drying. However, these films invariably contained a higher density of sub-micron scale defects relative to poly(bis-SorbPC) PSLBs prepared under similar conditions [7,44]. This difference is attributed to inter-leaflet cross-linking, which can take place in a symmetric PSLB because both leaflets are composed of bis-SorbPC, whereas such cross-linking is restricted to the upper leaflet in a HBM.

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids and used without further purification. The polymerizable lipid, bis-SorbPC, was prepared in the Chemical Synthesis Facility at the University of Arizona by a modification of the procedure reported by Lamparski et al. [45]. The

Table 1

Ellipsometric thickness, static water contact angle, and AFM rms roughness measurements on dried Si wafer substrates, OTS SAMs, and unpolymersized HBMs

	Ellipsometric thickness (Å) ^a	Static water contact angle (degrees)	rms roughness (nm)
Native SiO ₂ layer on Si wafer ^b	19.3 ± 0.5	<10	0.10 ± 0.02
OTS SAM layer on SiO ₂	26 ± 1	109 ± 2	0.13 ± 0.04
DOPC monolayer on OTS/SiO ₂ ^c	3 ± 3	97 ± 8	0.5 ± 0.3
Bis-SorbPC monolayer on OTS/SiO ₂ ^c	23 ± 6	98 ± 2	1.7 ± 0.4

^a Thickness values refer to individual layers, not the multilayer structure.

^b Optical parameters of $n = 3.874$ and $k = 0.016$ were used for silicon [69] and $n = 1.46$ and $k = 0$ for SiO₂ [70].

^c Lipid monolayers were deposited by vesicle fusion for 8 h.

lipid structure and purity were established by ¹H NMR, high-resolution mass spectrometry, and thin layer chromatography. 95% *n*-octadecyltrichlorosilane (OTS) (Gelest, Inc., PA) and 99% dicyclohexyl (Aldrich, WI) were used as received. The water used in rinsing and the polymerization experiments, hereafter referred to as deionized (DI) water, was obtained from a Barnstead Nanopure system with a measured resistivity of 18.3 MΩ cm and total organic content specified as less than 10 ppb. Silicon wafer substrates (1,1,1) were purchased from Wacker. All other reagents and solvents were purchased from standard commercial sources and used as received.

2.2. Surface preparation

Silicon wafers were cleaned by soaking in 3:7 (v/v) H₂O₂:H₂SO₄ (Piranha) solution for ca. 30 min, followed by rinsing several times with DI water. The cleaned substrates were then sonicated in DI water for 10 min, rinsed again in DI water, and dried under flowing N₂. This procedure produced a 19 Å thick, hydrophilic SiO₂ layer with a static water contact angle of less than 10° (Table 1). The substrates were used immediately after cleaning.

2.3. OTS SAM and lipid monolayer formation

OTS SAMs were deposited on cleaned Si substrates at 22–23 °C (ca. 72 F) and at 20–30% humidity in a controlled atmosphere box [46]. Silane solution was prepared by dissolving OTS in dicyclohexyl at a concentration of 1 μL/mL. The substrates were then dipped into the silane solution for 6 h. The solution was not stirred and fresh solutions were prepared for each experiment. Upon removal, silane-coated substrates were sonicated sequentially in chloroform, ethanol, and in DI water for 5, 7 and 10 min, respectively. The samples were blown dry with a nitrogen stream immediately prior to lipid deposition.

Supported bis-SorbPC monolayers were prepared by vesicle fusion. Bis-SorbPC was handled under yellow light to avoid photo-initiated polymerization. Lipids from stock benzene solution were dried under a stream of argon followed by drying for ca. 4 h under vacuum. The lipids were hydrated in DI water to a final concentration of 0.5–1 mg/mL and then vortexed several times at 35 °C. Small unilamellar lipid vesicles (SUVs) were prepared by sonicating the solution to clarity at 35 °C for ca. 30 min, then used immediately for vesicle fusion [6,7,21,47,48]. Drops of SUV solution were deposited on the dried OTS-coated substrate until the surface was fully covered with the solution (approximately 100 μL for 1 cm² surface). Substrates were left in a 97% relative humidity environment prepared from a saturated K₂SO₄ solution enclosed in a desiccator [49]. The fusion time was varied as described later. The lipid-coated substrate was then transferred to a jar filled with freshly prepared redox mixture for redox polymerization or to a shallow crystallization dish for UV polymerization. Caution was

taken to prevent excessive mechanical shock and exposing unpolymerized HBMs to air prior to initiating polymerization.

DOPC HBMs were prepared in a similar manner except that during vesicle fusion, the OTS-coated substrate was dipped into a well in a plate filled with DOPC vesicles [50]. After vesicle fusion, they were removed from the fusion well and rinsed several times with DI water, then dried with a gentle flow of Ar.

2.4. Polymerization

UV polymerization was performed by exposure to a low-pressure mercury pen lamp (Fisher Scientific) with a rated intensity of $4500 \mu\text{W}/\text{cm}^2$ at 254 nm for ca. 30 min. A 260–400 nm FWHM bandpass filter (U-330, Edmund Optics) was used to remove the shorter wavelength UV that was found to cause degradation of the lipid film [51]. Redox-initiated polymerization was performed with degassed and then Ar purged solutions of 20 mM potassium persulfate and 10 mM sodium hydrogen sulfite. The two solutions were mixed together immediately before HBMs were immersed. The polymerization reaction was allowed to proceed under positive Ar pressure for at least 2 h, except where noted. After polymerization, HBMs were removed from solution, rinsed several times with DI water, and dried under flowing Ar.

2.5. Surface characterization

Thickness measurements on HBMs were performed by ellipsometry as described previously [7], assuming a uniform refractive index profile of 1.46 for OTS and lipid films. Measurements were made at several locations on each sample and on a minimum of three independently prepared samples for each type of film. Static contact angles of DI water on substrates were measured as described previously [7], using a volume of $1 \mu\text{L}$ at multiple sites on each sample, and on at least three independently prepared samples for each film.

The surface morphology of films was examined using atomic force microscopy (AFM) in tapping mode on a Nanoscope III (Digital Instruments, Santa Barbara, CA). Tapping mode etched silicon probes (TESP from Digital) were tuned to between 200 and 400 kHz for measurements performed in air. Measurements on samples immersed in DI water were performed in a Digital solution cell using contact mode probes (NP-20) tuned to approximately 33 kHz in tapping mode configuration. A minimum of three different samples for each film type were prepared and imaged. For each sample, images were acquired at several locations (at least three). The images presented below are representative of multiple measurements on the respective samples. Roughness analyses were also performed on some samples, using DI software. The root-mean-square (rms) surface roughness was calculated as the standard deviation of all the height values within an image area of $1 \mu\text{m}^2$ unless stated otherwise. The surface roughness data listed in the tables in this article were calculated from measurements made on a minimum of three samples (i.e., a minimum of nine locations that were $1 \mu\text{m}^2$ in size).

3. Results and discussion

HBMs were prepared using bis-SorbPC, a polymerizable lipid used in several previous studies from this group to form poly(PSLBs) on glass and silica [6,7,18,44,52,53]. The polymerization parameters and film characterization methods that were used in the previous studies provided a starting point for conducting comparative studies on bis-SorbPC HBMs. Lipid deposition and polymerization methods and parameters were varied and the resultant effects on film structure were examined.

3.1. OTS SAMs on SiO_2

OTS SAMs were prepared on oxidized Si wafers and evaluated by measuring water contact angle, film thickness, and film morphology. Summary data are presented in Table 1. The water contact angle of 109 ± 2 degrees confirmed generation of a hydrophobic surface compared to the initial hydrophilic SiO_2 surface (contact angle <10 degrees). AFM images of SAMs were featureless and nearly indistinguishable from that of clean SiO_2/Si wafers (Fig. 1). However, the rms roughness of the SAM was slightly greater than that of SiO_2/Si (Table 1). These data and the ellipsometric thickness of $26 \pm 1 \text{ \AA}$ are consistent with measurements of OTS SAMs on SiO_2 reported previously [46,54,55].

3.2. Redox polymerization of bis-SorbPC HBMs as a function of vesicle fusion time

Vesicle fusion to form a lipid film is slower on hydrophobic surfaces than on hydrophilic surfaces [56–60]. Furthermore, Orban et al. reported that acrylate-PC lipid vesicles take 8 h to fuse on OTS-coated substrates, as indicated by a decrease in water contact angle over the initial 8 h of fusion time, after which no significant changes were observed [38]. Therefore, in an effort to determine the appropriate deposition conditions to create poly(bis-SorbPC) HBMs, the fusion time was varied while keeping other film preparation conditions constant. In these experiments: (i) both vesicle fusion and redox polymerization were performed at room temperature ($23\text{--}25^\circ\text{C}$); and (ii) the polymerization time was 4 h in 20 mM potassium persulfate and 10 mM sodium hydrogen sulfite, which previous studies have shown is sufficient for $>90\%$ of monomers to react [7].

Dried HBMs were evaluated by measuring water contact angle, film thickness, and film morphology. Summary data are presented in Table 2. The water contact angle remained relatively constant, in the range of $69^\circ\text{--}75^\circ$, over the range of 2–26 h. These values are much greater than the 32° measured for a highly uniform poly(bis-SorbPC) bilayer [7] but considerably less than that of an OTS film (109°). The intermediate value of ca. 70° suggests that these poly(HBMs) contain defects that expose the interior of the film to water droplets. As shown in Table 2, a gradual increase in ellipsometric thickness was observed with fusion time. The mean thickness values at 8 and 11 h were 30 and $26 \text{ \AA}$, respectively, close to the value of ca. $27 \text{ \AA}$ expected for a bis-SorbPC monolayer [6,44]. At longer times the thickness increased further to $36 \text{ \AA}$.

AFM imaging of these HBMs was generally found to be more difficult than the redox-polymerized poly(PSLBs) described in our previous papers [6,7,18,44], due to adhesion of material from the film on the AFM tip. Representative AFM images and line scans measured as a function of fusion time are shown in Fig. 2. In all cases, the outer surface of HBMs contained holes with an apparent depth of $15\text{--}20 \text{ \AA}$, although the number per unit area declined as the fusion time increased. These defects likely exposed the interior of the lipid monolayer, and possibly the underlying OTS layer, which is consistent with the water contact angle data discussed above. Beginning at 11 h, small spherical shapes adsorbed to the HBM, most likely unfused vesicles, were observed with increasing frequency (Figs. 2d–2f). Extensive rinsing did not remove these structures. Their higher surface coverage in the 15 and 26 h films, relative to the 11 h films, is consistent with the increased thickness measured for these films. The tendency of phospholipids to form adsorbed multilayers of intact vesicles at relatively long fusion times has been reported by others [29,61,62].

Ross et al. [7] prepared poly(bis-SorbPC) PSLBs using a variety of conditions, some of which produced films that contained defects similar to those shown in Fig. 2. In that work, it was shown

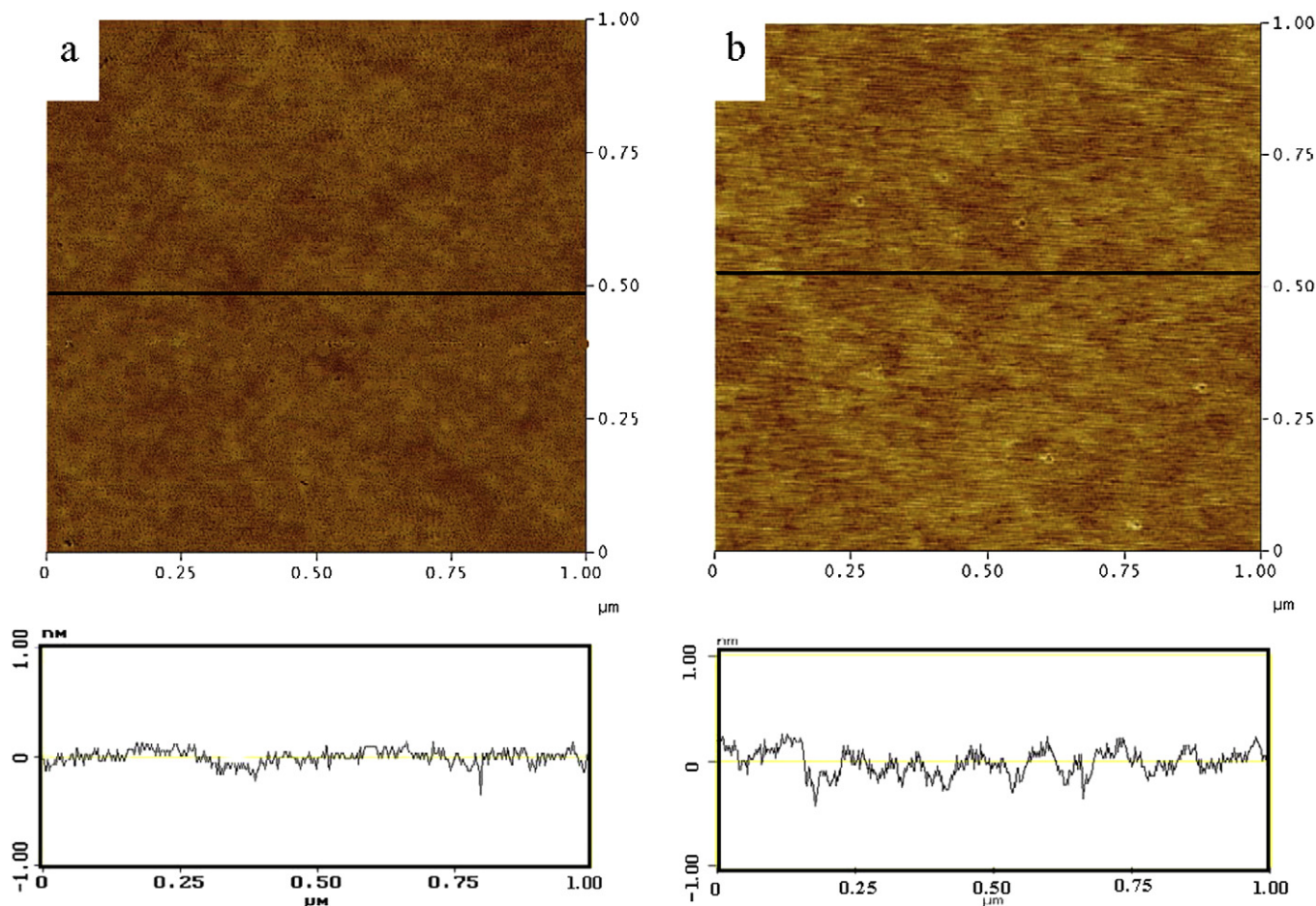


Fig. 1. Representative AFM images and line scans of: (a) a clean SiO₂/Si wafer and (b) an OTS SAM on a SiO₂/Si wafer. Both images were acquired in air and have a height scale of 2 nm. The dark line across each AFM image shows the position from which the line scan was taken.

Table 2

Ellipsometric thickness, static water contact angle, and AFM rms roughness of dried, redox polymerized HBMs measured as a function of vesicle fusion time

Fusion time (h)	Ellipsometric thickness (Å)	Static water contact angle (degrees)	rms roughness (nm)
2	17 ± 2	72 ± 1	0.73 ± 0.06
5	22 ± 3	72 ± 3	0.7 ± 0.1
8	30 ± 2	69 ± 2	0.47 ± 0.03
11	26 ± 5	76 ± 1	0.5 ± 0.1
15	36 ± 10	73 ± 2	0.37 ± 0.03
26	36 ± 10	75 ± 1	0.4 ± 0.1

that the defects appeared upon transfer of a poly(bis-SorbPC) film across the air–water interface, presumably due to desorption of bis-SorbPC oligomers of low molecular weight and possibly unreacted monomers. To determine if this process also produced defects in bis-SorbPC HBMs, AFM images were acquired on a redox-polymerized HBM before and after it was removed from water. The underwater image (Fig. 3a) shows that the poly(bis-SorbPC) surface was continuous and smooth (rms roughness of ca. 0.1 nm), similar to results reported by Winger and Chaikof for PC lipid-based HBMs prepared on OTS SAMs [63]. Fig. 3b is an AFM image of the same poly(bis-SorbPC) HBM shown in Fig. 3a after it was removed from water, subjected to several drying and rehydration cycles, and dried. The film morphology is similar to that shown in Figs. 2b–2d; it contained numerous holes and was much rougher (rms roughness of ca. 0.4 nm) than before rinsing and drying. These data

show that a continuous bis-SorbPC monolayer is deposited on an OTS SAM by vesicle fusion for 8 h, and that the defects in the film form upon drying, likely due to desorption of oligomers and monomers.

Prior experiments done on poly(bis-SorbPC) PSLBs indicate that formation of these defects is irreversible. When poly(PSLBs) composed of 98.5% bis-SorbPC and 1.5% of a nonpolymerizable fluorescent lipid (rhodamine-DOPE) were subjected to repeated drying and rehydration, the measured fluorescence intensity decreased with each cycle [7]. Thus desorbed lipids do not reinsert into the PSLB upon rehydration. In another set of experiments, fusion of fluid DOPC SUVs containing rhodamine-DOPE was performed in an attempt to fill defects in poly(bis-SorbPC) PSLBs that had been dried and rehydrated. Incorporation of lipids into the PSLB was not detected (data not shown), suggesting that the defects are too small to accommodate vesicle adsorption and fusion.

3.3. Unpolymerized HBMs

For comparison to polymerized HBMs, unpolymerized HBMs were formed by vesicle fusion of bis-SorbPC and DOPC for 8 h, then removed from water and characterized. Table 1 lists the ellipsometric thickness, rms roughness from AFM imaging, and water contact angle data. For DOPC HBMs, the measured thickness of the lipid layer, 3 ± 3 Å, was minimal, consistent with reports that near-quantitative lipid desorption occurs when a fluid lipid film is transferred across the air/water interface [5,7]. AFM imaging

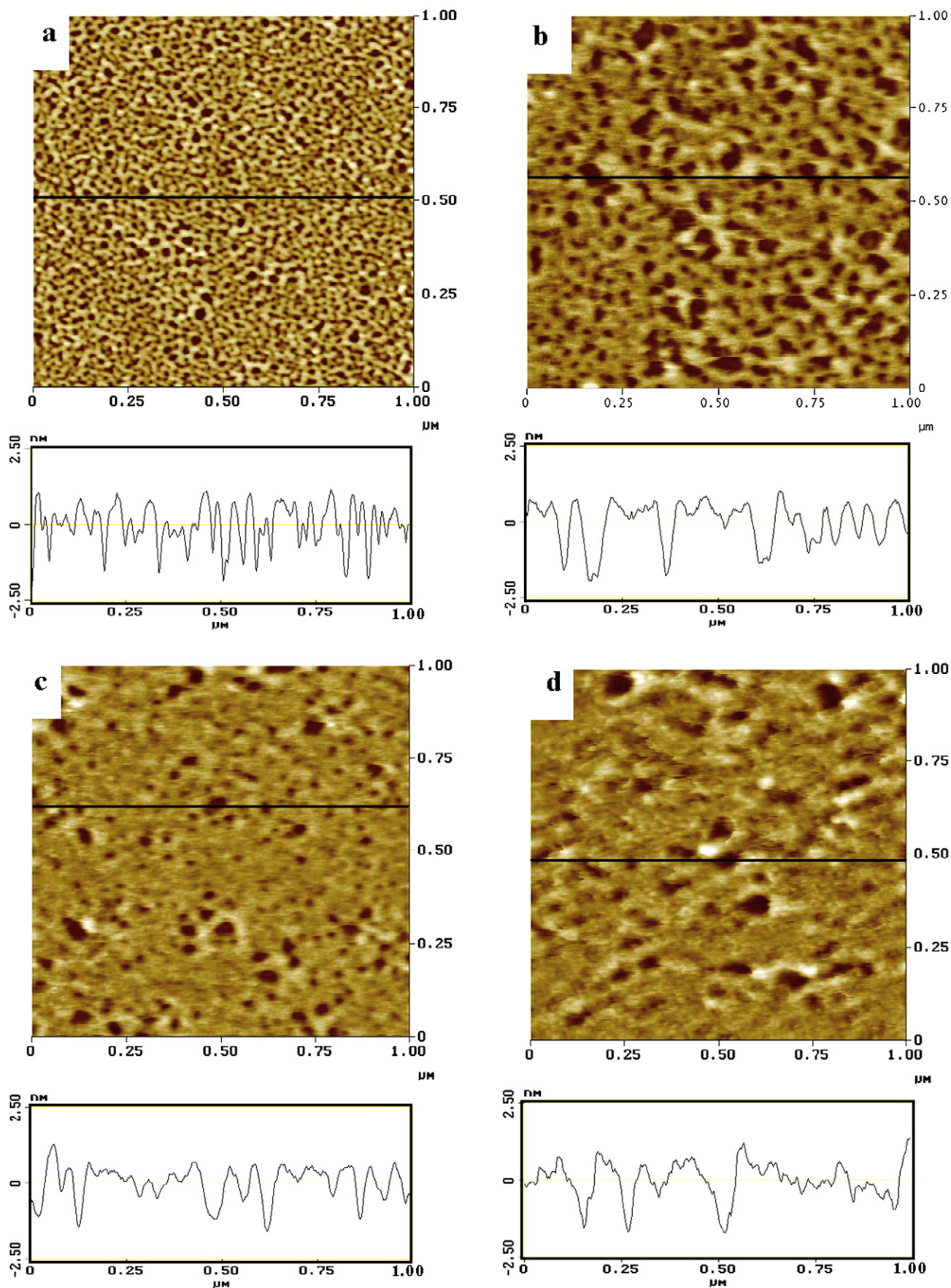


Fig. 2. Representative AFM images and line scans of dried, redox polymerized HBMs that were formed with different vesicle fusion times: (a) 2 h, (b) 5 h, (c) 8 h, (d) 11 h, (e) 15 h, and (f) 26 h. All images and line scans have a height scale of 5 nm. The dark line across each AFM image shows the position from which the line scan was taken.

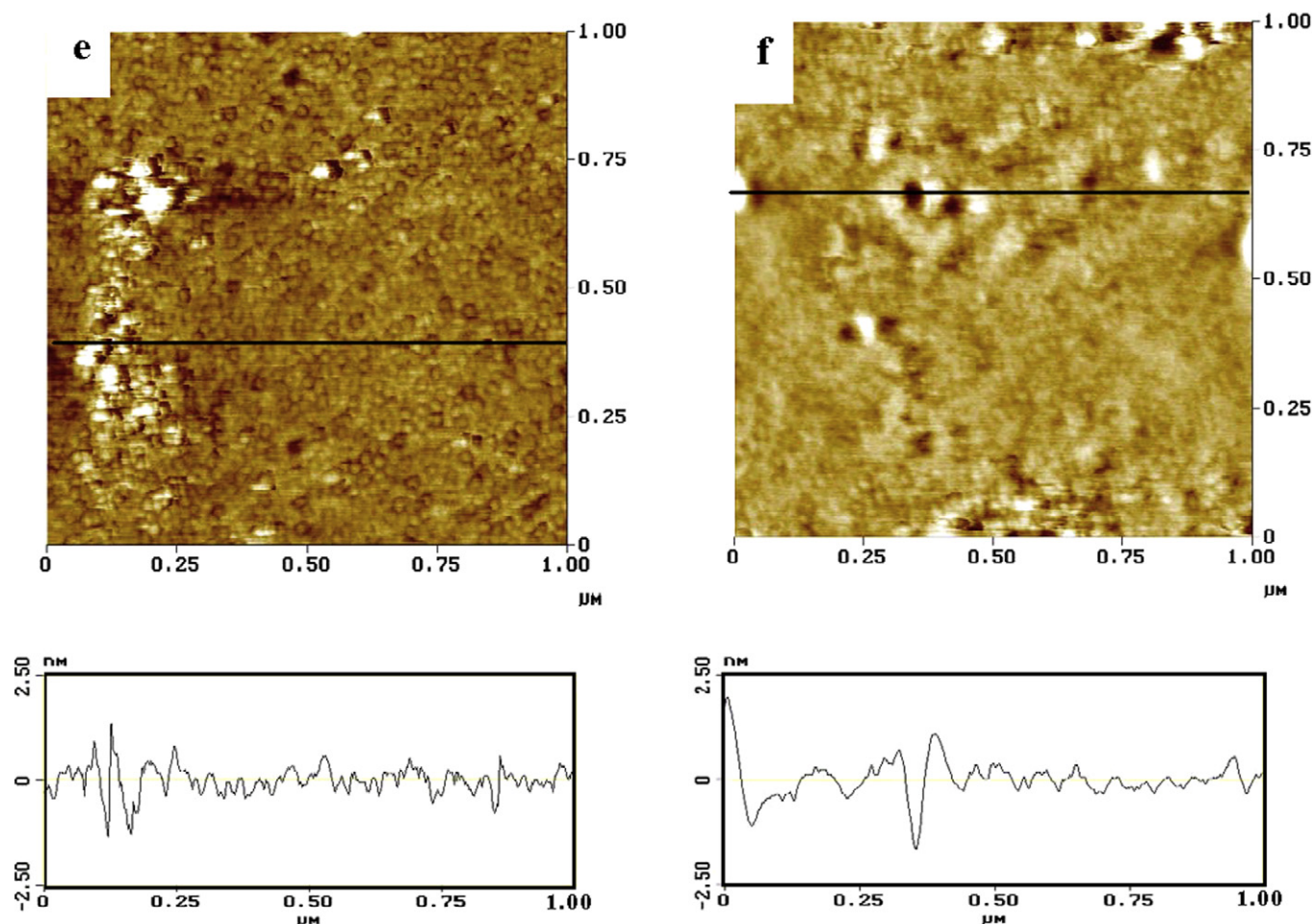


Fig. 2. (continued)

showed some patches of adsorbed material (apparent heights for imaged domains range from ca. 30–70 Å), presumably DOPC, scattered over the underlying SAM surface (Fig. 4a). Since the length of a DOPC molecule is ca. 28 Å [64], the measured thickness of 3 Å is interpreted as a submonolayer composed of domains of lipid that are much thicker than 3 Å separated by large areas of the SAM that are nearly devoid of lipid. The water contact angle (97°) is consistent with the ellipsometry and AFM data.

For bis-SorbPC HBMs, the measured thickness of 23 ± 6 Å is surprisingly high given that the film was not polymerized (the length of a bis-SorbPC molecule is about 27 Å). Clearly the retention of bis-SorbPC on OTS is substantial; in contrast, when a bis-SorbPC PSLB on glass is removed from water, near quantitative desorption occurs [7]. However the AFM image and line scan (Fig. 4d) show that the structure of the bis-SorbPC monolayer is not preserved when the unpolymerized HBM is removed from water. The surface was very rough (rms = 1.7 nm) and composed of irregularly shaped domains with an apparent height ranging up to ca. 40 Å. Upon drying, it appears that the lipids form aggregates that in some cases are thicker than one lipid monolayer. The reason that unpolymerized bis-SorbPC is largely retained on OTS upon drying, rather than the desorption that occurs in the case of DOPC, is unknown.

3.4. Effects of other variables on redox-initiated polymerization

In an effort to create bis-SorbPC HBMs with fewer defects than those shown in Fig. 2, two other variables that affect lipid polymer-

ization, temperature and polymerization time, were investigated. For these studies, the fusion time was held constant at 8 h because the results described above showed that this time period is sufficient to form a uniform lipid monolayer. In addition, the initiator concentrations were not varied from those described above. This choice was based on previous work showing that highly uniform bis-SorbPC PSLBs were produced by redox polymerization at initiator concentrations ≥ 0.01 M, regardless of the oxidant/reductant ratio [7].

Orban and coworkers found that longer photopolymerization periods were accompanied by lower water contact angles for HBMs formed using monoacrylate-lipids [38]. Based in part on this report, the effect of polymerization time on the properties of bis-SorbPC HBMs was investigated. The results are presented in Table 3. These studies were performed at room temperature (RT). Little or no change in ellipsometric thickness, water contact angle, and rms surface roughness from AFM analysis was observed over 2–15 h. A typical AFM image and line scan of a HBM polymerized for 4 h is shown in Fig. 2c; the 2 and 15 h images were similar (data not shown). Although these data demonstrated that no more than 2 h are required for redox-initiated polymerization of HBMs, for consistency a polymerization time of 4 h was used in all further studies.

Other research groups have reported that for radical polymerization of lipids using $K_2S_2O_8/NaHSO_3$ initiation, increasing the temperature above the main phase transition temperature (T_m) of the lipid increases the degree of polymerization (X_n) [65,66]. The increase in X_n was attributed to enhanced lateral diffusion of

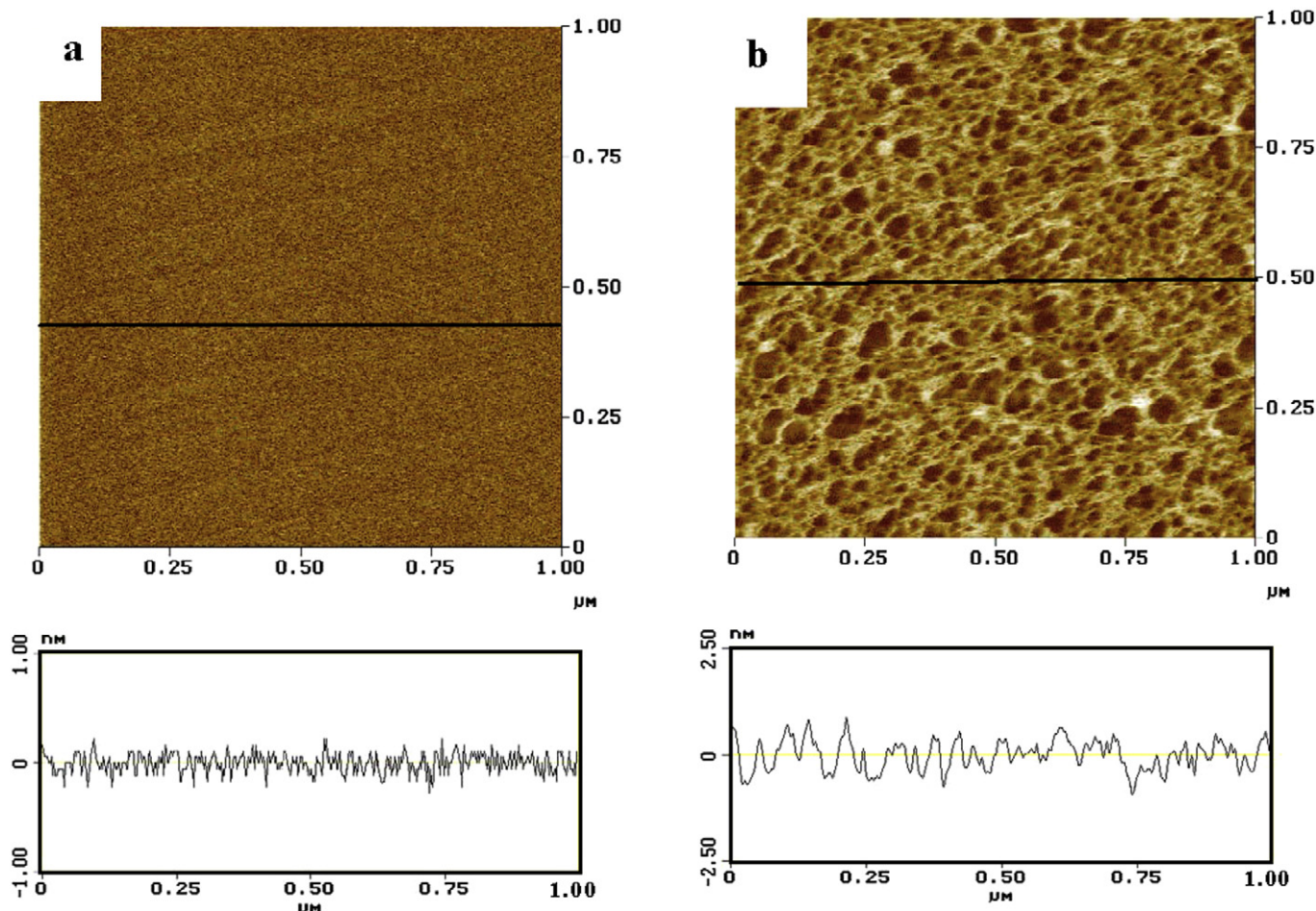


Fig. 3. Representative AFM images and line scans of: (a) a HBM after redox polymerization but before removal from water, and (b) the same HBM after several drying and rehydration cycles, and imaged in air. Vesicle fusion time was 8 h. The height scale is 2 nm in (a) and 5 nm in (b). The dark line across each AFM image shows the position from which the line scan was taken.

lipid molecules and improved permeability of free radicals into the bilayer. Based on these studies, the effect of temperature on the redox polymerization of bis-SorbPC HBMs was examined. The T_m of bis-SorbPC measured in vesicles is 28.8 °C [43] (although for a substrate-supported film, the T_m may be shifted) [67]. A temperature range of 10°–45° was therefore chosen to ensure that data were obtained at temperatures both above and below the T_m . The characterization data are summarized in Table 4 and the corresponding AFM images and line scans are shown in Fig. 5. There was no difference in ellipsometric thickness, water contact angle, and surface topography of HBMs polymerized at RT and 30 °C. For films polymerized at 10 °C, the thickness and wettability were similar to the RT and 30 °C films; however the surface topography was much different. The image in Fig. 5a shows a surface with a higher density of defects of smaller apparent size, suggesting that polymerization occurs on smaller length scales. There are two likely causes: At lower temperatures, lateral diffusion of lipid monomers is slower. In addition, the radicals formed at RT or higher are thought to be primarily 'OH, whereas at low temperatures, they are thought to be primarily ionic species, which are expected to have relatively lower permeability into the hydrophobic lipid membrane [66].

When the polymerization temperature was raised to 40 °C, the average thickness was unaffected but the water contact angle and the rms surface roughness increased to 88° and ca. 1.6 nm, respectively. The AFM image (Fig. 5c) shows that the films contained comparatively large defects, consistent with the higher contact an-

gle, and indicate that significant lipid desorption occurred. Winger et al. [29] also reported significant loss of lipid from HBMs when they were heated to 14 °C above the T_m of the lipid, consistent with our observations, and suggested that input of thermal energy causes vesicle formation and detachment. Additional support for this hypothesis is provided by the results observed when the reaction temperature was further increased to 45 °C. A negligible film thickness of 1 ± 2 Å was obtained, and AFM imaging (Fig. 5d) showed no evidence for retention of a lipid film on OTS.

3.5. UV polymerization

Bis-SorbPC HBMs formed by vesicle fusion for 8 h were also polymerized by direct UV irradiation. The lipid film thickness measured by ellipsometry was 20 ± 3 Å and the water contact angle was $93^\circ \pm 4^\circ$. These data indicate incomplete lipid coverage of the underlying OTS, and the AFM data support this interpretation. A representative image and line scan are shown in Fig. 6. The surface is composed of globular domains separated by gaps with an apparent width of 10–80 nm. The majority of domains have an apparent diameter of 50–80 nm with an apparent height ranging up to ca. 9 nm, which are reflected in a relatively high rms surface roughness of 2.7 ± 0.2 nm. In comparison to redox polymerized HBMs (e.g., Fig. 2c and corresponding data in Table 2), the UV polymerized films were less uniform with a higher density of defects. Similar trends have been observed in a comparison of bis-SorbPC

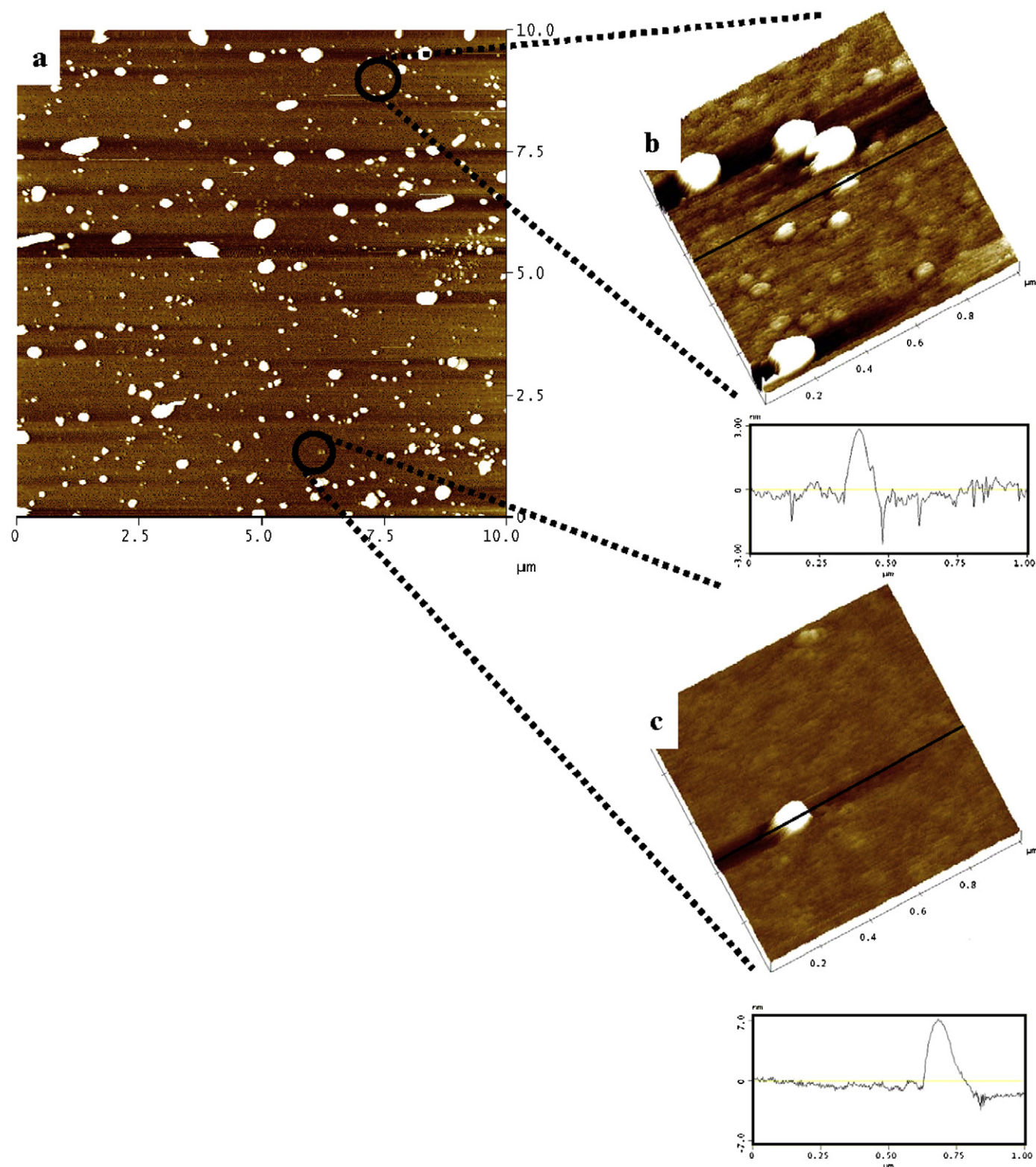


Fig. 4. Representative AFM images and line scans of unpolymerized, dried HBMs: (a) composed of DOPC, (b) and (c) expanded areas of (a) to show structural details, and (d) composed of bis-SorbPC. In both cases, the lipid film was deposited by vesicle fusion for 8 h before removal from water. The height scale is 10 nm in (a) and 5 nm in the other images. The dark line across the images in (b)–(d) shows the position from which the line scan was taken. The height scale on the line scans is 6 nm in (b), 14 nm in (c), and 5 nm in (d).

PSLBs polymerized by UV and redox methods [7]. The most probable cause is a difference in X_n , which is reported to be 3–10 for UV polymerization and 50–500 for radical polymerization of lipid vesicles [42]. It is reasonable to assume that the trends in X_n ob-

served in lipid vesicles also extend to planar supported lipid films [7]. The lower molecular weight polymers should be more easily desorbed upon withdrawal of the lipid film from water, creating a higher density of defects [7].

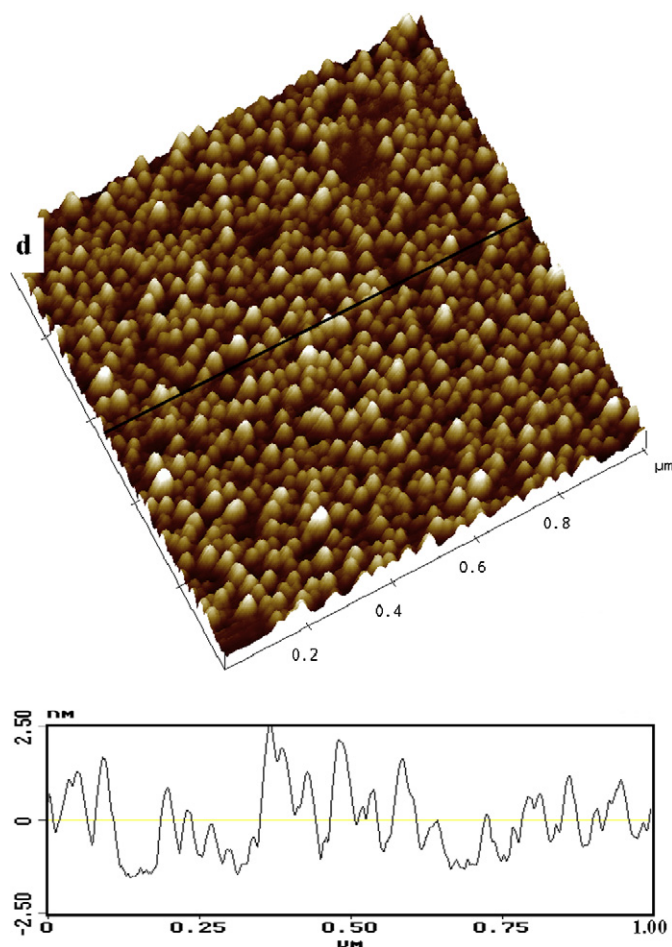


Fig. 4. (continued)

Table 3

Ellipsometric thickness, static water contact angle, and AFM rms surface roughness of dried, redox polymerized HBMs measured as a function of polymerization time^{a,b}

Polymerization time (h) ^b	Ellipsometric thickness (Å)	Static water contact angle (degrees)	rms roughness (nm)
2	26 ± 2	74 ± 3	0.47 ± 0.07
15	30 ± 1	74 ± 2	0.47 ± 0.03

^a Lipid films were deposited by vesicle fusion for 8 h and polymerized at room temperature (23–25 °C).

^b Data for HBMs polymerized for 4 h are listed in Table 2.

3.6. Comparison of HBMs to PSLBs

In an earlier paper, Ross et al. [7] examined experimental conditions for preparation of polymerized bis-SorbPC PSLBs on silica and glass substrates. A combination of vesicle fusion and redox polymerization conditions were identified that produced air stable, supported bilayers that were nearly defect free, whereas several other sets of experimental conditions generated bilayers that contained numerous defects.

In the present work, a number of different experimental conditions to prepare poly(bis-SorbPC) HBMs were assessed, using the work of Ross et al. [7] as a starting point. Although most conditions did form an air stable poly(lipid) film on OTS, none were identified that produced films comparable in quality to the nearly defect free PSLBs created by Ross et al. [7]. The major difference between bis-SorbPC PSLBs and HBMs is that in the latter, cross-linking polymerization can occur only in the upper leaflet of the

Table 4

Ellipsometric thickness, static water contact angle, and AFM rms roughness of dried, redox polymerized HBMs measured as a function of the temperature at which the polymerization was performed^{a,b}

Polymerization temperature (°C) ^b	Ellipsometric thickness (Å)	Static water contact angle (degrees)	rms roughness (nm)
10	32 ± 4	75 ± 3	0.6 ± 0.2
30	29 ± 2	74 ± 2	0.4 ± 0.1
40	29 ± 4	88 ± 5	1.6 ± 0.4
45	1 ± 2	88 ± 4	0.4 ± 0.1

^a Lipid films were deposited by vesicle fusion for 8 h and polymerized for 4 h.

^b Data for HBMs polymerized at room temperature (23–25 °C) are listed in Table 2.

bilayer. In a PSLB, cross-linking occurs in both leaflets; more importantly interdigitation of lipid chains should allow cross-linking *between* the leaflets. Formation of inter-leaflet covalent bonds between oligomers in the upper and lower leaflets would make it more difficult to desorb these structures when the bilayer is exposed to destabilizing conditions [7,68]. Conversely, the absence of inter-leaflet cross-linking should create a less stable lipid film. This is the most probable cause for our inability to create nearly defect free HBMs comparable to the PSLBs reported by Ross et al. [7]. However this is a hypothesis because we cannot directly detect inter-leaflet cross-links.

4. Summary

In this study, experimental conditions to form HBMs composed of a monolayer of a cross-linkable lipid, bis-SorbPC, deposited on an OTS SAM were investigated. Several parameters were varied: vesicle fusion time, polymerization method, and (for the redox-initiated method) polymerization time and temperature. Most conditions produced an air stable poly(lipid) film on OTS. The optimum conditions were fusion for 8 h followed by redox-initiated radical polymerization for 4 h at a temperature of 20–30 °C; these largely preserved the overall HBM structure (lipid film on SAM) after removal from water, rinsing, and drying. However no conditions were identified to generate films that did not contain a significant number of defects, i.e., gaps in the lipid film that exposed the hydrophobic core of the HBM. The gaps are thought to be caused by desorption of low molecular weight polymers upon drying. Desorption from HBMs is more likely to occur than from PSLBs prepared under similar experimental conditions because inter-leaflet cross-links can form in PSLBs but not in HBMs.

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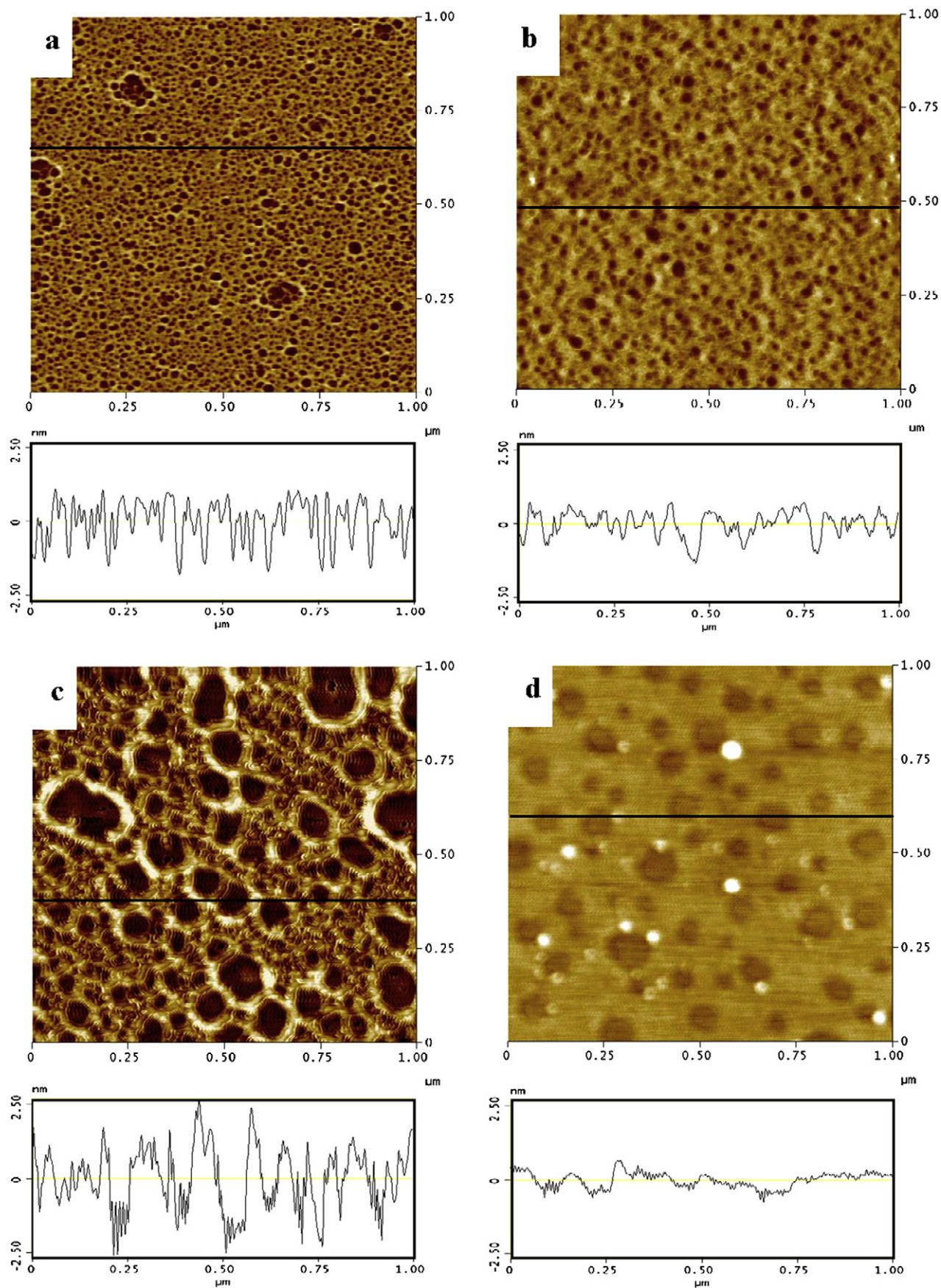


Fig. 5. Representative AFM images and line scans of dried, redox polymerized HBMs that were prepared at different temperatures: (a) 10 °C, (b) 30 °C, (c) 40 °C, and (d) 45 °C. Data for a HBM polymerized at room temperature are shown in Fig. 2c. In all cases, lipid films were formed by fusion for 8 h at room temperature. All the images and line scans have a height scale of 5 nm. The dark line across each AFM image shows the position from which the line scan was taken.

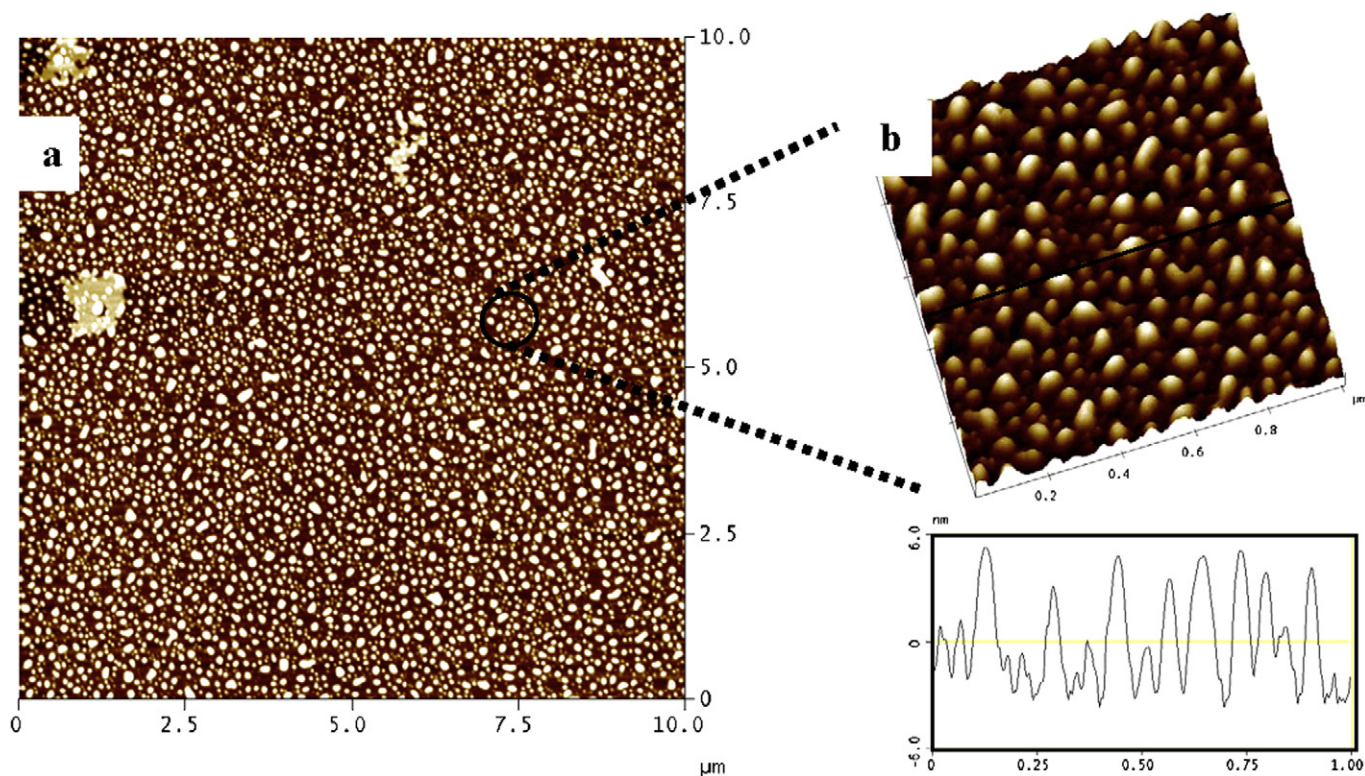


Fig. 6. Representative AFM data obtained on a dried bis-SorbPC HBM that was deposited by vesicle fusion for 8 h and polymerized for 30 min by UV illumination: (a) image of 10 $\mu\text{m} \times 10 \mu\text{m}$ scan area, (b) angled view and line scan of a 1 $\mu\text{m} \times 1 \mu\text{m}$ area of (a). Both images have a height scale of 10 nm while the line scan has a height scale of 12 nm. The dark line across the AFM image in (b) shows the position from which the line scan was taken.

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