LANGMUIR

Article

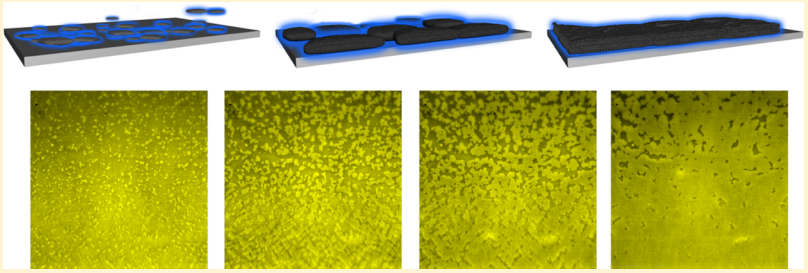
pubs.acs.org/Langmuir

Optimizing the Formation of Supported Lipid Bilayers from Bicellar Mixtures

Kavoos Kolahdouzan,[†,∥] Joshua A. Jackman,[‡,∥] Bo Kyeong Yoon,[‡] Min Chul Kim,[‡] Malkiat S. Johal,[†] and Nam-Joon Cho*[,‡,§]

[†]Department of Chemistry, Pomona College, 645 North College Avenue, Claremont, California 91711, United States
[‡]School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore
[§]School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, 637459, Singapore

Supporting Information



ABSTRACT: Supported lipid bilayers (SLBs) are widely studied model membrane platforms that are compatible with various surface-sensitive measurement techniques. SLBs are typically formed on silica-based materials, and there are numerous possible fabrication routes involving either bottom-up molecular self-assembly or vesicle adsorption and rupture. In between these two classes of fabrication strategies lies an emerging approach based on depositing quasi-two-dimensional lamellar, bicellar disks composed of a mixture of long-chain and short-chain phospholipids to promote the formation of SLBs. This approach takes advantage of the thermodynamic preference of long-chain phospholipids to form planar SLBs, whereas short-chain phospholipids have brief residence times. Although a few studies have shown that SLBs can be formed on silica-based materials from bicellar mixtures, outstanding questions remain about the self-assembly mechanism as well as the influence of the total phospholipid concentration, ratio of the two phospholipids (termed the "*q*-ratio"), and process of sample preparation. Herein, we address these questions through comprehensive quartz crystal microbalance-dissipation, fluorescence microscopy, and fluorescence recovery after photobleaching experiments. Our findings identify that optimal SLB formation occurs at lower total concentrations of phospholipids than previously used as short-chain phospholipids behave like membrane-destabilizing detergents at higher concentrations. Using lower phospholipid concentrations, we also discovered that the formation of SLBs proceeds through a two-step mechanism involving a critical coverage of bicellar disks akin to vesicle fusion. In addition, the results indicate that at least one cycle of freeze−thaw−vortexing is useful during the sample preparation process to produce SLBs. Taken together, the findings in this work identify optimal routes for fabricating SLBs from bicellar mixtures and reveal mechanistic details about the bicelle-mediated SLB formation process, which will aid further exploration of bicellar mixtures as tools for model membrane fabrication.

INTRODUCTION

Planar-supported lipid bilayers (SLBs) are widely used experimental platforms that mimic the architecture of biological membranes and are compatible with various surface-sensitive measurement techniques.[1,2] SLBs are primarily used to study membrane-associated interaction processes involving biomolecules (e.g., lipids, proteins, sugars, and nucleic acids).[3] Although there are numerous fabrication techniques used to prepare micron-scale lipid bilayers on solid supports, such as air-bubble collapse,[4] spin coating,[5] and dip-pen nanolithography,[6] the formation of homogenous, planar SLBs across longer length scales is more challenging and limited to fewer techniques. The most commonly used method for fabricating planar SLBs is the adsorption and spontaneous rupture of phospholipid vesicles as well as Langmuir-type deposition processes, the latter of which requires specialized instrument setups.[7,8] Vesicle adsorption and rupture has increasingly become the most commonly used method; however, the technique requires well-prepared vesicle suspensions with tuned properties and typically works on only a subset of hydrophilic surfaces, including silicon dioxide, borosilicate glass, and mica.[9−13] Furthermore, successful bilayer formation depends on many parameters such as ionic strength,[14] addition of divalent cations,[15] and solution pH,[10] which must be carefully considered for the particular system

Received: January 20, 2017
Revised: April 28, 2017
Published: April 29, 2017

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

under investigation. In addition, spontaneous rupture of adsorbed vesicles works only for vesicles with membrane compositions that are amenable to the fusion−rupture mechanism—cholesterol at relatively high concentrations (25 mol % or higher) is a common component that impedes the formation of SLBs via this mechanism.[16] As a result, such limitations have motivated the further exploration of additional user-friendly methods to fabricate SLBs that require less preparation and use other means of phospholipid self-assembly.

Toward this goal, the solvent-assisted lipid bilayer (SALB) formation method has emerged as a robust, bottom-up approach to form SLBs without requiring lipid vesicles.[17] Early work by Hohner et al. discovered that lipids in isopropanol can be deposited on a silica substrate and that gradual exchange with water−isopropanol mixtures containing increasing water fractions induces a series of phase transitions, which ultimately yields an SLB.[18] Toward a more practical implementation of this concept, Tabaei et al. developed the SALB method in which lipids in a water-miscible organic solvent (i.e., isopropanol, ethanol, or methanol) are deposited on a solid support and then subjected to a rapid solvent exchange with aqueous buffer solution to form an SLB.[17] Importantly, the SALB approach has proven successful in forming SLBs on a wide range of substrates, including silicon oxide, gold, aluminum oxide, and titanium oxide—the latter four of which are intractable to conventional vesicle adsorption and spontaneous rupture.[19,20] It has also enabled the formation of SLBs containing high fractions of cholesterol (up to 60 mol %).[21] At the same time, the SALB approach is sensitive to the solvent-exchange flow conditions and bulk lipid concentration, requiring a minimum lipid concentration for successful the formation of SLBs (0.1 mg/mL or higher) and lipid deposition in organic solvents.[22,23]

Alternative SLB fabrication strategies that also bypass conventional vesicle preparation needs while further enabling operation under fully aqueous conditions would be advantageous. To this end, one promising approach involves the formation of SLBs from bicellar mixtures composed of long-chain and short-chain phospholipids.[24] It is widely understood that bicellar mixtures form disklike aggregates, with edges stabilized by short-chain phospholipids, and these so-called "bicelles" have proven useful in structural biology studies.[25] Bicellar mixtures can form a variety of structures depending on factors such as the total lipid concentration, temperature of the bicellar mixture, and the molar ratio of the two phospholipids (commonly referred to as the q-ratio and defined as [long-chain phospholipid]/[short-chain phospholipid]).[26−29] Given their two-dimensional, disklike membrane properties, bicellar mixtures have also proven to be an interesting tool to fabricate SLBs. Zeineldin et al. first reported the bilayer formation on silicon chips from a bicellar mixture of zwitterionic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine ($DHPC_7$) lipids with a q-ratio of 2.8.[30] Although the formation of SLBs was achieved, the lipid concentrations used in the study were very high (equivalent to 28 mM DPPC and 10 mM $DHPC_7$), and the fabricated SLBs were heterogenous consisting of gel-phase regions rich in DPPC and fluid-phase regions rich in $DHPC_7$. Following this work, Tabaei et al. recorded quartz crystal microbalance-dissipation (QCM-D) measurements to characterize the mass and viscoelastic properties of adsorbed bicellar mixtures of 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC) and 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine ($DHPC_6$, hereafter referred to as DHPC) at a q-ratio of 2.8 and discovered that the structures adsorb onto the silica surface and aggregate because of the lack of electrostatic repulsion.[31,32] To form a single SLB, it was necessary to add a small fraction of a water-soluble cationic surfactant (CTAB) to the bicellar mixtures, effectively preventing bicelle aggregation and allowing the formation of SLBs based on the fusion of adsorbed, planar bicelle fragments. More recently, Morigaki et al. have reported that zwitterionic mixtures of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and DHPC across a wide range of q-ratios (0.05−10) can form SLBs on silica and discussed how the inclusion of short-chain phospholipids not only accelerates the formation of SLBs but can also perturb SLBs because of surfactant-like membrane disruption.[33] Together, the aforementioned studies demonstrate the potential of using bicellar mixtures for the formation of SLBs. At the same time, there is an excellent opportunity to continue studying the formation of SLBs from bicellar mixtures for many fundamental and practical reasons. The existing studies used relatively high lipid concentrations compared with other fabrication methods, and identifying the lower bound of acceptable lipid concentrations would be advantageous, especially with respect to how these values were compared with the typical lipid concentrations used in other fabrication methods. Furthermore, using lower lipid concentrations and hence slowing the observed SLB formation kinetics, it might be possible to temporally distinguish the steps involved in adsorption, fusion, and rupture of bicellar structures on solid supports, and characterization efforts in this direction would contribute mechanistic insight into the SLB formation process as different possible scenarios are discussed in the literature.

Within this scope, inspiration can be gained from previous studies involving the formation of SLBs from phospholipid−detergent mixtures. Tiberg et al. first demonstrated the potential of using detergents such as *n*-dodecyl-*β*-D-maltopyranoside (DDM) to facilitate the deposition of phospholipids onto a silica surface.[34] In this process, mixed micelles of phospholipid and detergent are formed, and the soluble detergent facilitates phospholipid adsorption at the solid−liquid interface. This process takes advantage of the large difference in solubility properties of the phospholipid and detergent by lowering the total concentration, considerably below the critical micelle concentration (CMC) of the detergent. The result is that, as mixed micelles adsorb onto the surface and form bilayer fragments, the more soluble detergent has a short residence time in the bilayer and returns to the bulk solution while the phospholipids remain in the bilayer patches, which gradually fuse together and form an SLB. Vacklin et al. further demonstrated that this concept can be applied to prepare 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) SLBs through a seven-stage adsorption process with a 1:6 mixture of DOPC and DDM, followed by progressively more dilute lipid−detergent mixtures and a subsequent rinsing step.[35] Although this method proves to be effective, it needs to be performed at extremely dilute concentrations to keep DDM below its CMC (0.2 mM), in turn adversely affecting the rate of SLB formation. Nevertheless, studies in the phospholipid−detergent field provide inspiration as they were completed at much lower phospholipid concentrations than the aforementioned bicellar studies and point to the possibility of forming SLBs from bicellar mixtures at much lower concentrations than previously realized—a feature that would prove attractive for

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

SLB fabrication protocols alongside the ease with which bicellar mixtures can be prepared.

Herein, we identify optimal conditions for bicelle-mediated SLB formation by investigating the dependence on total lipid concentration at various q-ratios (DOPC/DHPC, where DHPC is 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine) as well as different processing methods and to further understand the mechanistic underpinnings of SLB formation via this pathway. To achieve these objectives, QCM-D, fluorescence microscopy, and fluorescence recovery after photobleaching (FRAP) measurements were recorded to track the kinetics of the SLB formation and characterize the resultant mass, viscoelastic, and morphological properties of the fabricated SLBs. In addition to studying the formation of SLBs, the effect of short-chain phospholipids as destabilizing agents against the preformed SLBs was also investigated as a function of the short-chain phospholipid concentration. Taken together, our findings contribute optimal strategies to use bicellar mixtures for SLB preparation and provide detailed insights into how various experimental parameters influence the SLB formation process. We also discuss competitive advantages of forming SLBs from bicellar mixtures, including simple sample preparation and the flexibility to use lower phospholipid concentrations than that required for competing fabrication approaches.

MATERIALS AND METHODS

Preparation of DOPC/DHPC Bicellar Mixtures. DOPC and DHPC were purchased from Avanti Polar Lipids (Alabaster, AL). DOPC dissolved in chloroform was dried with a gentle stream of nitrogen and evaporated in a vacuum desiccator overnight. The DOPC film was hydrated with a buffer solution (10 mM Tris, 150 mM NaCl, pH 7.5) that contained the appropriate DHPC concentration to make 1 mL of 1 mM DOPC stock solutions for different q-ratios (as defined by [DOPC]/[DHPC]). Subsequently, the sample was plunged into liquid nitrogen for 1 min, followed by a 5 min incubation period in a 60 °C water bath and vortexing for 30 s. This freeze−thaw−vortex cycle was repeated five times unless otherwise noted. The final bicellar mixture was optically clear at room temperature. Immediately before the experiment, the stock solution was diluted in buffer (10 mM Tris, 150 mM NaCl, pH 7.5) to the desired final lipid concentration. As a control, we also tested the effect of dilution several hours before the experiment and observed identical measurement responses, indicating that the bicellar solutions are at equilibrium.

QCM-D Experiments. QCM-D experiments were conducted using a Q-Sense E4 instrument (Biolin Scientific AB, Stockholm, Sweden) to monitor the SLB formation process. The QCM-D is a label-free measurement technique that is sensitive to the mass and viscoelastic properties of an adsorbate on the surface by measuring the changes in the resonance frequency and energy dissipation of an oscillating, piezoelectric quartz crystal sensor chip.[36] The sensor chip had a fundamental frequency of 5 MHz and a sputter-coated 50 nm thick layer of silicon dioxide (model no. QSX 303, Biolin Scientific AB). Before the experiment, the sensor chips were thoroughly rinsed with ethanol, dried with nitrogen gas, and treated with oxygen plasma for 1 min. In the experiment, a baseline signal with aqueous buffer solution (10 mM Tris, 150 mM NaCl, pH 7.5) was established shortly before injection of the bicellar mixture under continuous-flow conditions. Following the SLB formation, 50 μM bovine serum albumin (BSA) was added under continuous-flow conditions for 10 min to quantify the degree of bilayer completeness. Throughout the experiment, liquid samples were injected at 50 μL/min using a peristaltic pump (Reglo Digital, Ismatec, Glattbrugg, Switzerland). The temperature was maintained at 25.0 ± 0.5 °C. The experiments were carried out at odd-numbered overtones ranging from the 3rd to 11th odd overtone by using the Q-Soft software package (Biolin Scientific AB), with the presented data recorded at the 5th odd overtone. Data processing and analysis were completed using the QTools (Biolin Scientific AB) and OriginPro 8.5 (OriginLab, Northampton, MA) software packages.

Fluorescence Microscopy Experiments. Epifluorescence microscopy was performed to directly observe the SLB formation process.[37] For these experiments, the long-chain phospholipid composition included a mixture of 99.5 mol % DOPC and fluorescently labeled 0.5 mol % 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) lipid. An Eclipse TI-U inverted optical microscope (Nikon, Japan) with a 60× magnification (NA = 1.49) oil-immersion objective lens (Nikon) was used, and images were recorded using an iXon 512 pixel × 512 pixel EMCCD camera (Andor Technology, Northern Ireland). The pixel size was 0.267 × 0.267 μm^2. A fiber-coupled mercury lamp (Intensilight C-HGFIE, Nikon) was used to illuminate the fluorophores with a TRITC filter. SLBs were formed inside of a microfluidic flow-through chamber (sticky slide VI 0.4, Ibidi, Germany) through the injection of a bicellar mixture under continuous-flow conditions at 40 μL/min. After the formation, the lipid bilayer was rinsed with buffer solution (10 mM Tris, 150 mM NaCl, pH 7.5). The process of SLB formation, followed by buffer rinsing, was captured through time-lapsed image recording every 5 s. The initial time, t = 0 s, was defined by when the bicellar mixture reached the channel inlets. For each measurement recorded, the fluorescence intensity of each image was normalized using a custom-written script for the Python(x,y) 2.7.5 software program.

Fluorescence Recovery after Photobleaching. FRAP measurements were recorded by a 5 s photobleaching of a circular spot of 20 μm diameter within the fabricated SLBs using a 532 nm, 100 mW laser (Klastech Laser Technologies, Dortmund). Subsequently, fluorescence micrographs were imaged for 120 s at 1 s intervals to calculate lateral diffusion coefficients using the Hankel transform method.[38]

RESULTS AND DISCUSSION

Design Rationale. In general, bicellar mixtures are composed of a mixture of vesicles containing both long-chain and short-chain phospholipids, which are in dynamic equilibrium with micelles composed of the short-chain phospholipid.[24] Past studies about using bicellar mixtures to form SLBs have used either DMPC or DPPC as the long-chain phospholipid in the bicellar composition.[30,31] Herein, we chose to use the DOPC lipid instead, and it is also capable of forming bicelles, as previously reported.[39,40] Whereas DMPC and DPPC have gel-to-fluid phase-transition temperatures (T_m) of 23 and 41 °C, respectively, DOPC has a lower T_m of −20 °C, which ensures fluid-phase membrane states at room temperature. This stable existence in the fluid phase is important because fluid-phase membranes permit dynamic structural rearrangements, and the formation of SLBs can become inhibited at or near the T_m of a phospholipid.[41] Furthermore, when bicellar mixtures are considerably above their transition temperature, they form fluid-phase membranes in which some DHPC molecules are in fast exchange with long-chain phospholipids,[42] and this exchange causes membrane softening, which decreases the bending moduli of membranes.[43,44]

Another important consideration in our experimental design involves selecting the range of total lipid concentration. As described in the Introduction, previous studies on phospholipid−detergent mixtures have shown that, in a binary system of a low-solubility phospholipid (low CMC) and high-solubility surfactant (high CMC), adsorption of the surfactant decreases as the total lipid−detergent concentration is lowered.[45] Mimicking this approach with bicellar mixtures, we explored whether successful SLB formation is possible at lower phospholipid concentrations than that reported in previous bicellar studies. In particular, by lowering the total phospholipid concentration, phospholipid aggregates in bulk solution and

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

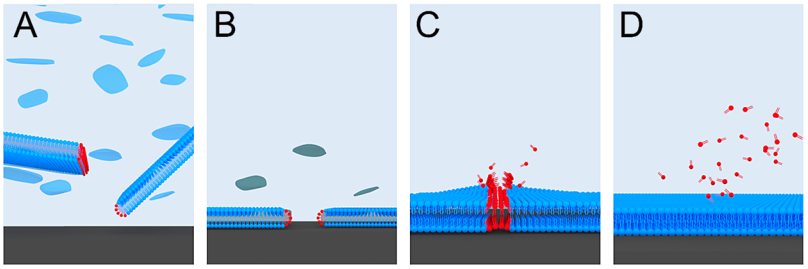


Figure 1. Strategy to form SLBs from bicellar mixtures. (A) Bicelles composed of long-chain (blue) and short-chain (red) phospholipids in bulk solution are presented. (B) Bicelles adsorb onto the silica surface. (C) Bicelles fuse and join together. (D) Upon fusion, the short-chain phospholipids return to the monomeric state in bulk solution because their concentration is below the corresponding CMC value.

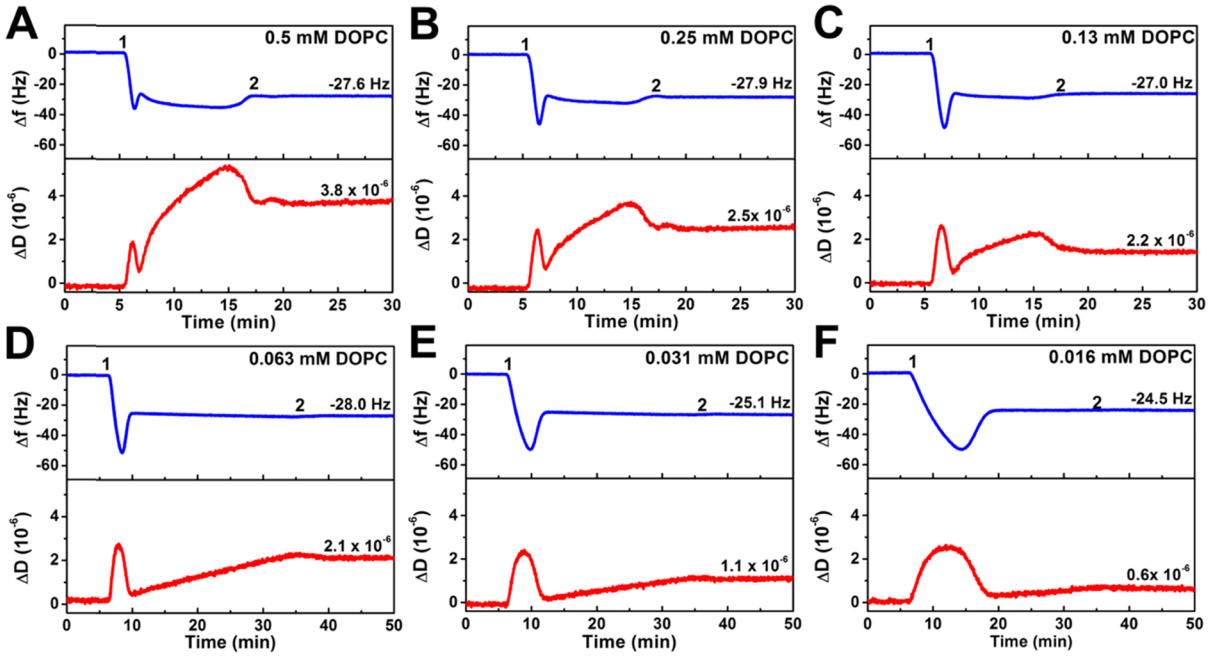


Figure 2. QCM-D measurement of the SLB formation from bicellar mixtures prepared at a q-ratio of 0.05. Δf (blue line) and ΔD (red line) shifts as a function of time are presented for (A) 0.5 mM DOPC, (B) 0.25 mM DOPC, (C) 0.13 mM DOPC, (D) 0.063 mM DOPC, (E) 0.031 mM DOPC, and (F) 0.016 mM DOPC. Bicellar mixtures were added at t = 5 min (step 1), and a washing step was then performed (step 2).

those adsorbed onto the surface would be mainly composed of DOPC phospholipids because DHPC phospholipids would be preferentially in the monomeric state. The conceptual basis for our experimental strategy is presented in Figure 1.

Hence, it is necessary to find the optimal range whereby the bicellar mixture facilitates efficient SLB formation (i.e., DHPC concentration is sufficiently high to promote SLB formation) while minimizing the deleterious effects of short-chain phospholipid detergents (i.e., keeping the DHPC concentration below a critical concentration that causes membrane destabilization).[46] Of note, because DOPC and DHPC are in a mixed micelle system, the CMC of DHPC is affected by the presence of DOPC and hence CMC values of DHPC alone are insufficient to describe the system under investigation, especially with varying q-ratios,[47] in the context of preventing surfactant-induced perturbations in the SLB structure. Indeed, Morigaki et al. previously showed that using short-chain phospholipids at concentrations below their independent CMC values is insufficient by itself to yield defect-free SLBs.[33] Adding to the complexity of the system is the fact that the long-chain and short-chain phospholipids in bicelles exhibit nonideal mixing and the corresponding minimum total lipid concentrations at which bicelles form at different q-ratios cannot be readily predicted from theory alone.[47] To approach this problem, we therefore take an empirical route, whereby we systematically investigate how the SLB formation process depends on total lipid concentration at various q-ratio values.

QCM-D Monitoring of Bicelle Adsorption onto Silicon Dioxide. To investigate the formation of SLBs through the adsorption of bicellar mixtures composed of DOPC and DHPC phospholipids, the QCM-D technique was used to monitor the bilayer formation process on silicon dioxide. The QCM-D technique is a label-free acoustic sensor that measures the mass and viscoelastic properties of a thin film adsorbed based on recording the changes in resonance frequency and energy dissipation.[36] It is commonly used for lipid adsorption studies, especially involving vesicle adsorption and ruptures leading to the formation of SLBs, and can distinguish between different morphological states of adsorbed phospholipid molecules.[48] The effects of total DOPC/DHPC lipid concentration on bicelle adsorption and SLB formation were tested at three different q-ratios: 0.05, 0.25, and 2.5, as described below. Before the QCM-D experiments, dynamic light scattering (DLS) experiments were conducted on the stock preparations, and it

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

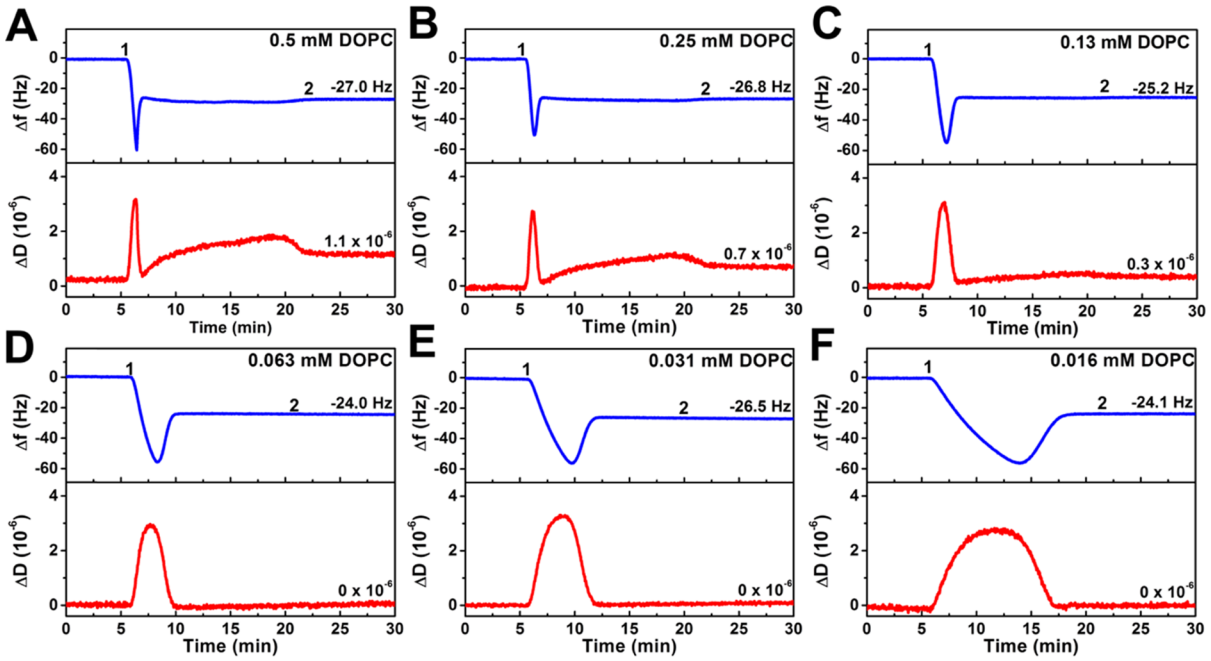


Figure 3. QCM-D measurement of the SLB formation from bicellar mixtures prepared at a *q*-ratio of 0.25. Δ*f* (blue line) and Δ*D* (red line) shifts as a function of time are presented for (A) 0.5 mM DOPC, (B) 0.25 mM DOPC, (C) 0.13 mM DOPC, (D) 0.063 mM DOPC, (E) 0.031 mM DOPC, and (F) 0.016 mM DOPC. Bicellar mixtures were added at t = 5 min (step 1), and a washing step was then performed (step 2).

was observed that the bicelles had larger sizes with an increasing *q*-ratio. The average diameters were 423, 472, and 575 nm for bicellar mixtures at *q*-ratios of 0.05, 0.25, and 2.5, respectively, and this range of values agrees with past literature reports.[31] As mentioned above, it should be noted that the larger bicelles likely coexist with DHPC monomers or micelles depending on the dilution.[47]

q-Ratio of 0.05. Figure 2 shows the Δ*f* and Δ*D* shifts for different total lipid concentrations at a fixed *q*-ratio of 0.05 (20-fold greater amount of DHPC than DOPC). The concentration is reported as the DOPC concentration while the effective *q*-ratio is fixed (proportional change in DHPC concentration as well). The arrows labeled 1 and 2 correspond to the bicelle injection and buffer-washing steps, respectively, and both steps were performed under continuous-flow conditions. At lipid concentrations of 0.13 mM DOPC and above, there was a rapid, initial increase in Δ*f* to an inflection point approximately −38 to −47 Hz, which was mirrored by an increase in Δ*D* up to 2.6 × 10^{-6}, indicating lipid adsorption on the surface (Figure 2A−C). The large increases in Δ*f* and Δ*D* were quickly reversed, leading to values of ∼−25 Hz and ∼0.6 × 10^{-6}, respectively, within a few minutes, which suggests that the inflection point corresponds to a critical coverage of adsorbed bicelles. These measurement values are in good agreement with the expected values for a DOPC SLB, and the two-step adsorption kinetics resembled the SLB formation via vesicle adsorption and rupture.[36,49,50] However, the SLB formation in this high phospholipid concentration range was hindered by a subsequent increase in Δ*f* and Δ*D* values, with particularly large Δ*D* shifts up to approximately 5.5 × 10^{-6}, which increased according to the total lipid concentration and is suggestive of surfactant-induced morphological perturbations.[46] Following a buffer-washing step, the Δ*f* values decreased to final values of ∼−27.5 Hz while Δ*D* values declined to the range of 2 to 4 × 10^{-6}, which is significantly higher than the expected dissipation values for good-quality SLBs (∼0.5 × 10^{-6} or lower).

By contrast, at lower lipid concentrations (0.063 mM DOPC and below), a similar adsorption behavior was observed and there were a larger Δ*f* shift at the critical coverage (inflection point) (approximately −50 Hz) and Δ*D* shifts approximately 2.5 × 10^{-6} (Figure 2D−F). After reaching the critical coverage, the Δ*f* and Δ*D* signals again began to return to typical SLB values of Δ*f* and Δ*D* shifts approximately −25 Hz and 0.3 × 10^{-6}, respectively. In this lower concentration regime, variations in the measurement time until reaching the critical coverage were more apparent and it was approximately 3 min postaddition at 0.063 mM DOPC, 5 min at 0.031 mM DOPC, and 10 min at 0.016 mM DOPC, suggesting that bicelle adsorption is diffusion-limited, as is the case for phospholipid vesicles and large proteins.[51] In accordance with the total lipid concentration and the corresponding DHPC concentration, there were also additional measurement responses attributed to detergent-induced membrane destabilization, leading to final Δ*f* values of approximately −24 to −28 Hz and Δ*D* values up to 2.1 × 10^{-6}. Unlike at higher total lipid concentrations, a subsequent buffer-washing step did not change the measurement responses in this lower concentration regime. At 0.016 mM DOPC, the final Δ*f* and Δ*D* values were −24.5 Hz and 0.6 × 10^{-6}, respectively, which are within the expected range of SLBs. Nearly identical adsorption behavior with slower kinetics was also observed at 0.008 mM DOPC, and the residual DHPC detergent did not appear to influence the SLB formation in this case (Figure S1). The final Δ*f* and Δ*D* values were −26.0 Hz and 0.4 × 10^{-6}, respectively. Hence, at a *q*-ratio of 0.05, we observed that using lower total lipid concentrations improves the SLB quality, with optimal results obtained at approximately 0.016 mM DOPC and below.

For SLBs prepared under all tested conditions, 50 μM BSA was added after the buffer-rinsing step to estimate the degree of SLB completeness across the sensor surface. Although BSA adsorbs prodigiously onto silicon dioxide, there is scant adsorption onto zwitterionic SLBs,[52] and the difference in Δ*f*

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

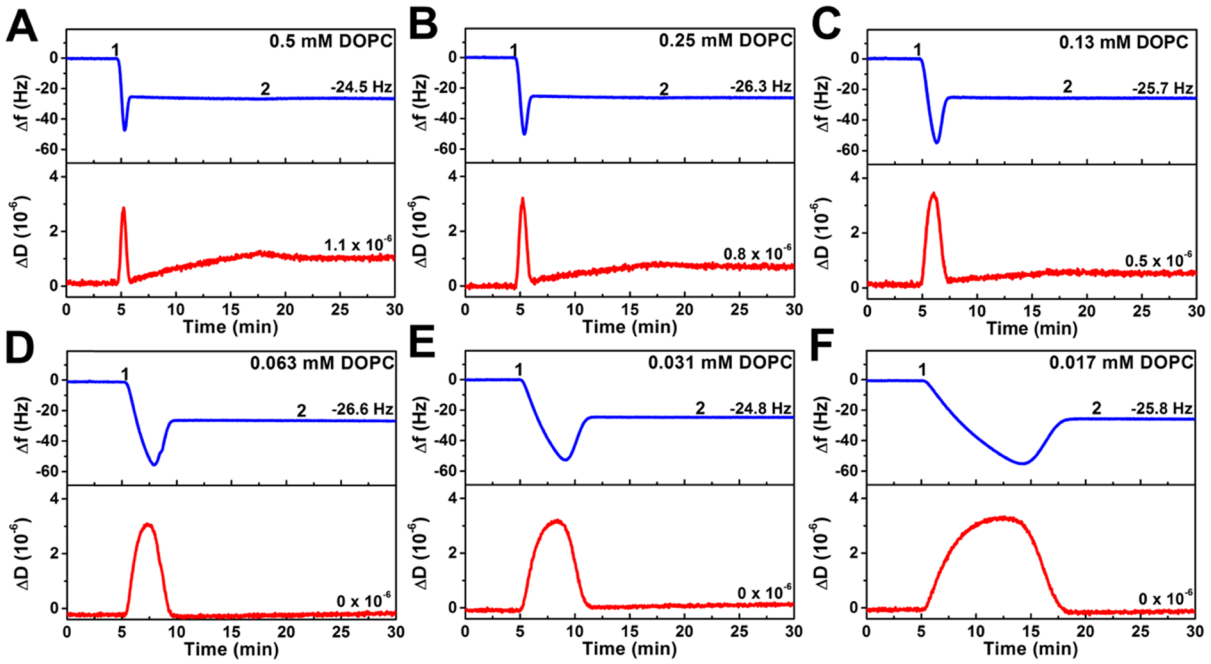


Figure 4. QCM-D measurement of the SLB formation from bicellar mixtures prepared at a *q*-ratio of 2.5. Δ*f* (blue line) and Δ*D* (red line) shifts as a function of time are presented for (A) 0.5 mM DOPC, (B) 0.25 mM DOPC, (C) 0.13 mM DOPC, (D) 0.063 mM DOPC, (E) 0.031 mM DOPC, and (F) 0.017 mM DOPC. Bicellar mixtures were added at t = 5 min (step 1), and a washing step was then performed (step 2).

shifts can be used to estimate the percentage of completeness. Whereas BSA adsorption onto bare silicon dioxide substrate yielded Δ*f* shifts approximately −25 Hz, BSA adsorption onto SLB-coated silicon dioxide caused much smaller Δ*f* shifts of −1.6 ± 0.2 Hz, indicating high surface coverage (~94%) of the SLBs on silicon dioxide.

q-Ratio of 0.25. Figure 3 shows the Δ*f* and Δ*D* shifts for different total lipid concentrations at a fixed *q*-ratio of 0.25 (4-fold greater amount of DHPC over DOPC). At all tested lipid concentrations, two-step adsorption kinetics indicative of a critical coverage leading to the formation of SLBs were observed. Bicelle adsorption at the critical coverages corresponded to Δ*f* and Δ*D* shifts approximately −50 to 55 Hz and 3 × 10^{-6}, respectively, and the time scale again depended on the bulk lipid concentration. At 0.13 mM DOPC and above, similar adsorption kinetics were observed, as described above, with the formation of SLBs followed by moderate DHPC-induced membrane destabilization (Figure 3A−C). At 0.5 mM DOPC, the final Δ*f* and Δ*D* values were −27.0 Hz and 1.1 × 10^{-6}, respectively, whereas the final Δ*f* and Δ*D* values were −25.2 Hz and 0.3 × 10^{-6}, respectively, when SLBs were prepared using 0.13 mM DOPC. The latter values are in excellent agreement with the anticipated values for an SLB and indicate an upper bound concentration value for preparing SLBs with DOPC/DHPC bicelles at a *q*-ratio of 0.25.

By contrast, at lower DOPC concentrations of 0.063 mM and lower, the effect of residual DHPC on SLB quality was negligible, and the Δ*f* and Δ*D* shifts were −24.8 ± 0.7 Hz and 0 × 10^{-6}, respectively (Figure 3D−F). These values are indicative of high-quality SLBs, and further BSA adsorption studies indicate that the fabricated SLBs have greater than 94% completeness. As there was no energy dissipation shift in these cases, it is possible to use the Sauerbrey model[53] to estimate the acoustic thickness of the DOPC SLBs. On the basis of the measured frequency shifts approximately −25 ± 1 Hz, the Sauerbrey model calculations indicate that the bound mass is 443 ± 18 ng/cm^2 and, taking into account the known DOPC density of 781.9 cm^3/mol at 25 °C,[54] the DOPC SLB thickness is in the range of 4.4 ± 0.2 nm, which agrees with the literature values[50,55] for a single DOPC bilayer (~4.5 nm in ref 55).

Of note, the Δ*f* shifts at the critical coverage for phospholipid adsorption at a *q*-ratio of 0.25 were larger than the corresponding Δ*f* shifts for bicelles at a *q*-ratio of 0.05 because the total lipid concentration at a given DOPC concentration is lower in the former case (e.g., for 1 mM DOPC, there is 20 and 4 mM DHPC for *q*-ratios of 0.05 and 0.25, respectively). This agrees with the expected trend of greater adsorption at lower total lipid concentrations even when the same concentration of long-chain phospholipid is present in the system, as previously reported for phospholipid−detergent mixtures.[34]

q-Ratio of 2.5. Figure 4 shows the Δ*f* and Δ*D* shifts for different total lipid concentrations at a fixed *q*-ratio of 2.5 (2.5-fold greater amount of DOPC over DHPC). As described above for bicelles at other *q*-ratios, two-stage adsorption kinetics were observed in all cases. At DOPC lipid concentrations of 0.13 mM and above, the critical coverage corresponded to Δ*f* shifts ranging from −45 at 0.5 mM DOPC to −58 Hz at 0.13 mM DOPC (Figure 4A−C). Moderate DHPC-induced membrane destabilization was observed, and the final Δ*f* and Δ*D* values ranged from −24.5 Hz and 1.1 × 10^{-6}, respectively, at 0.5 mM DOPC to −26.7 Hz and 0.5 × 10^{-6}, respectively, at 0.13 mM DOPC.

On the other hand, at lower DOPC concentrations of 0.063 mM and below, the final Δ*f* and Δ*D* values were −25.7 ± 0.9 Hz and 0.0 × 10^{-6}, respectively (Figure 4D−F). Hence, similar to the bicellar mixtures at a *q*-ratio of 0.25, the optimal concentration range for the SLB formation for the bicellar mixtures at a *q*-ratio of 2.5 appeared to be 0.063 mM DOPC and lower. BSA adsorption experiments confirmed that the SLBs are at least 94% complete in this case as well.

To better understand the effect of DHPC on destabilizing the fabricated SLBs, control experiments were conducted,

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

investigating the effect of adding DHPC molecules at various concentrations to the preformed DOPCs prepared using the SALB method (Figure S2). At 5 mM DHPC and higher, significant membrane destabilization was observed, whereas appreciably smaller measurement responses were observed upon addition of 2.5 mM DHPC or lower. This suggests that the CMC value of DHPC alone in the buffer solution is approximately 2−5 mM, which agrees well with the previous measurements that estimate the CMC value of DHPC to be approximately 20 mM in water[56] and is supported by fluorescence spectroscopy experiments (Figure S3). The lower estimated CMC observed in our case arises from salts in the buffer solution, which reduce electrostatic repulsion among the short-chained, zwitterionic phospholipids and, in turn, permit micellar aggregation at lower concentrations.[57] Importantly, no measurement shifts in response to DHPC addition to SLBs are observed at 0.31 mM DHPC and lower. On the basis of the recommended DOPC concentration ranges (0.063 mM DOPC) to prepare SLBs from bicelles at q-ratios of 0.25 and 2.5, this finding is in excellent agreement with the fact that the corresponding concentrations of DHPC in the bulk solution would be no greater than ∼0.25 mM DHPC in such cases and further supported by recent findings of Saleem et al. that showed that resultant SLBs have negligible DHPC components.[58] Furthermore, DHPC adsorption onto bare silicon dioxide substrates is nearly negligible in this concentration range (Figure S4). Hence, DOPC SLBs formed at lower total lipid concentrations from bicelles at q-ratios of 0.25 and 2.5 are most likely free of DHPC and have high structural integrity because the DHPC concentrations in the bulk solution are sufficiently low to not cause membrane destabilization.

Evidence of a Critical Bicelle Concentration. The aforementioned QCM-D experiments support that a critical coverage of adsorbed bicelles is necessary to induce the SLB formation and that the process likely occurs via a membrane rupture mechanism that bears similarities to the conventional vesicle adsorption and spontaneous rupture. To scrutinize this feature, we performed a stop-flow experiment, in which bicelles were injected until reaching a surface coverage below the critical coverage, and then, the bicelle solution was exchanged with buffer solution (without bicelles). For this experiment, the bicelles had a q-ratio of 2.5 and a DOPC concentration of 0.031 mM. As presented in Figure 5, the bicelles were added at approximately t = 5 min and the Δf shift decreased to approximately −30 Hz with a corresponding ΔD shift of 4 × 10^{-6} before the addition of bicelles was stopped. At this point, the Δf and ΔD values immediately changed with no subsequent measurement shifts until bicelles were again added at approximately t = 15 min. Upon the addition of bicelles , the Δf shift further decreased to a critical coverage approximately −65 Hz with a corresponding ΔD shift of 6.6 × 10^{-6}. Afterward, bicelle rupture occurred, leading to the formation of SLBs based on the final Δf and ΔD values of −26.0 Hz and 0 × 10^{-6}, respectively. This experimental finding reinforces that a critical coverage of adsorbed bicelles is necessary for the SLB formation.

Next, we constructed time-independent plots of energy dissipation shifts as a function of frequency shifts based on the QCM-D measurements tracking the SLB formation process from the adsorbed bicelles at the various tested q-ratios (0.05, 0.25, and 2.5: 0.063 mM DOPC lipid concentration) as well as 0.063 mM DOPC lipid vesicles that were similarly processed

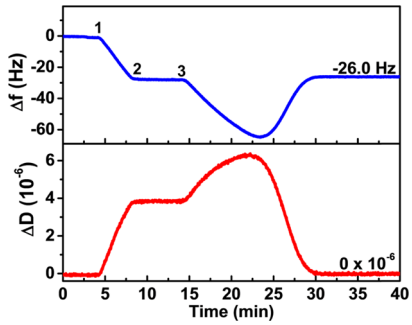


Figure 5. QCM-D measurement of the SLB formation under stop-flow conditions. Δf (blue line) and ΔD (red line) shifts as a function of time are presented for a bicellar mixture prepared at a q-ratio of 2.5, and the experiment was conducted at a 0.031 mM DOPC lipid concentration. The bicellar mixture was added at approximately t = 5 min (step 1), and the flowing solution was then exchanged with buffer solution (without bicelles) at t = 10 min (step 2). The bicellar mixture was then reintroduced at t = 15 min (step 3).

with freeze−thaw treatment (Figure 6). The Δf−ΔD plots are useful for analyzing structural transformations in the adsorbate

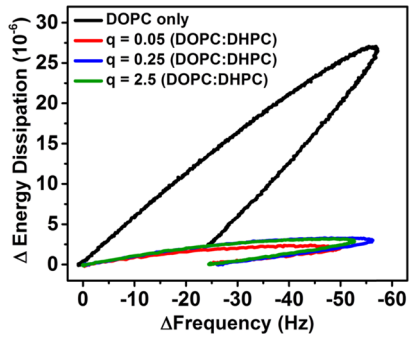


Figure 6. Δf−ΔD analysis of the SLB formation on silicon dioxide from DOPC/DHPC bicellar mixtures. The bicelle adsorption process on silicon dioxide is presented as a function of the energy dissipation shift versus the frequency shift for bicellar mixtures prepared at different q-ratios. The control experiment contained the same preparation process for DOPC phospholipid vesicles only. The DOPC concentration was fixed at 0.063 mM.

properties independent of time.[13] Following this approach, striking differences in the structural transformations of DOPC lipid vesicles (average diameter: 325 nm) versus DOPC/DHPC bicelles were observed. The adsorption profile for DOPC lipid vesicles had a much greater slope, indicating a larger viscoelastic contribution of the adsorbed species relative to the adsorbate mass. For lipid vesicles, this large viscoelastic contribution is attributed to the high amount of coupled solvent inside of the vesicles as well as the hydrodynamically coupled solvent between the adsorbed vesicles. By contrast, the adsorption profile of the DOPC/DHPC bicelles had an appreciably smaller slope, which supports that each adsorbed species has a smaller viscoelastic contribution, likely because of a smaller amount of coupled solvent. This finding is consistent with the previous findings that detergent-like molecules such as DHPC can cause membrane softening on account of reduced membrane stiffness.[43]

As a result, the incorporation of short-chain phospholipids with a positive bending modulus into DOPC lipid membranes leads to an intermediate structure between spherical vesicles

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

and mixed micelles, highlighting the various changes in membrane organization that lipids with different curvatures can undergo.[59] This behavior is consistent with the experimental findings and supports that DOPC/DHPC bicelles exhibit an adsorption profile similar to that of DOPC vesicles, although the geometrical configuration of the adsorbed phospholipid molecules is different and the corresponding viscoelastic contribution of the adsorbed bicelles versus vesicles varies. Furthermore, the membrane softening would increase the deformation of the adsorbed bicelles on the silicon dioxide surface and in turn decrease the corresponding viscoelastic contribution of the adsorbed species. Hence, the inclusion of DHPC molecules facilitates the SLB formation by inducing membrane softening, which favors membrane rupture in the presence of attractive bicelle−substrate interactions.

Direct Observation of the SLB Formation from Bicellar Mixtures. To directly observe bicelle fusion process leading to the formation of SLBs, time-lapsed fluorescence microscopy experiments were performed under continuous-flow conditions on glass substrates using bicelles prepared at the different q-ratios. Specifically, the objective was to capture the bicelle rupture process when the critical bicelle concentration is reached (after several minutes of bicelle adsorption), and the first micrograph in each series presented below corresponds to the time when fusion/rupture processes and the ensuing SLB formation begin. On the basis of the previous QCM-D measurements, a specific DOPC concentration of 0.031 mM was chosen for further investigation based on the optimal conditions, which yielded high-quality SLBs. The experimental results obtained for each q-ratio are described below, and t = 0 min is defined as the time point when bicelles first reached the measurement chamber.

q-Ratio of 0.05. As shown in Figure 7 and Video S1, the adsorption of fluorescently labeled bicelles onto the glass

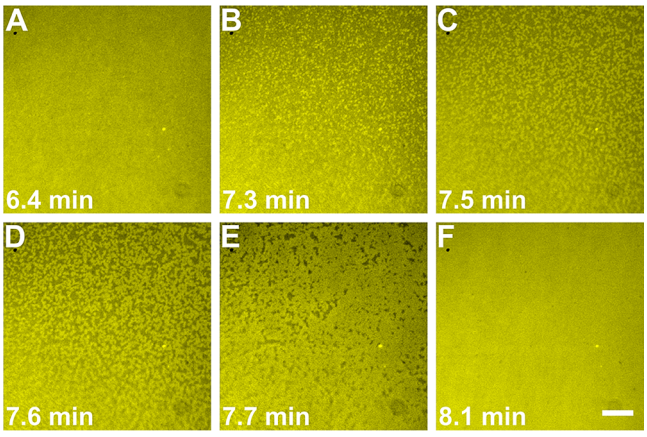


Figure 7. Microscopic observation of SLB formation on a glass surface from a bicellar mixture prepared at a q-ratio of 0.05. (A−F) Image snapshots at various time points depict the rupture of absorbed bicelles as part of the SLB formation process. t = 0 min corresponds to when bicelle addition was begun, and t = 6.4 min approximates when the CBC value was reached. The scale bar is 20 μm.

substrate is indicated by an increase in the fluorescence intensity within the probing volume near the substrate and when adsorption reaches a critical point after approximately 6.4 min (Figure 7A). This time point corresponds to the critical bicelle concentration as the formation of small, bright spots soon begins, indicating bicelle rupture and the formation of planar bilayer fragments (Figure 7B,C). Although it took over 6 min for the surface coverage of adsorbed bicelles to reach the critical value, the resultant SLB formation process was rapid and took less than 2 min. The process continued as the SLB formation propagated across the entire substrate, resulting in an SLB with uniform fluorescence intensity (Figure 7D−F). These observations support the interpretation of the QCM-D measurement results, and the kinetic stages bear similarities to the process of SLB formation via vesicle adsorption and rupture, in which case the hydrophobic edges of bilayer fragments catalyze rupture processes to rapidly complete the SLB formation process.[7]

q-Ratio of 0.25. A similar process of bicelle rupture and SLB propagation was observed using bicelles at a q-ratio of 0.25 as well (Figure 8 and Video S2). In this case, the critical bicelle

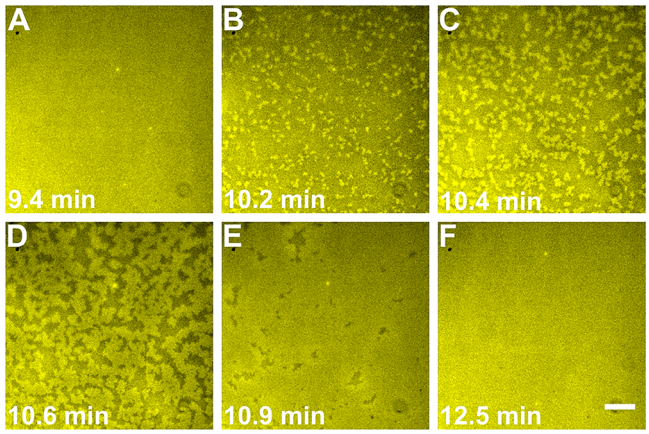


Figure 8. Microscopic observation of the SLB formation on a glass surface for a bicellar mixture prepared at a q-ratio of 0.25. (A−F) Image snapshots at various time points depict the rupture of the absorbed bicelles as part of the SLB formation process. t = 0 min corresponds to the time when bicelle addition was begun, and t = 9.4 min approximates to the time when the CBC value was reached. The scale bar is 20 μm.

concentration was reached at approximately t = 9.4 min, and this longer time scale compared with bicelles at a q-ratio of 0.05 is attributed to the fact that the average bicelle size increases with a greater q-ratio,[33] as verified by the DLS measurements, and hence diffusion-limited adsorption onto the substrate would take longer (Figure 8A). Upon bicelle rupture, the formation of larger SLB fragments was observed than in the case of bicelles with a q-ratio of 0.05 (Figure 8B,C). SLB propagation was again fast and occurred over a few minutes, yielding a complete SLB with uniform fluorescence intensity (Figure 8D−F). The observed patterns in SLB propagation appear to indicate two-dimensional Ostwald ripening and are reminiscent of the decomposition of the adsorbed lipid vesicles leading to SLB island growth.[60,61]

q-Ratio of 2.5. SLB formation using bicelles at a q-ratio of 2.5 followed a trend similar to that of the cases described above (Figure 9 and Video S3). The critical coverage of the adsorbed bicelles was reached at approximately t = 12.7 min, again consistent with the larger size of bicelles in this case as compared with those at lower q-ratios (Figure 9A). The subsequent rupture process yielded larger bilayer fragments than in the aforementioned cases, which is reasonable considering that the bicelle size increases at larger q-ratios (Figure 9B,C). Once again, completion of the SLB process

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

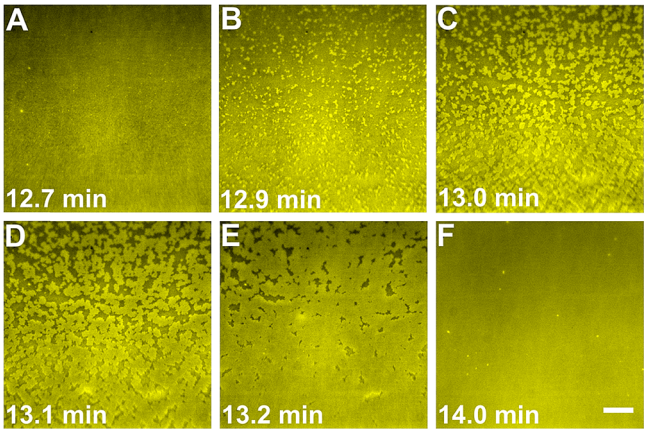


Figure 9. Microscopic observation of the SLB formation on a glass surface for a bicellar mixture prepared at a q-ratio of 2.5. (A–F) Image snapshots at various time points depict the rupture of the absorbed bicelles as part of the SLB formation process. t = 0 min corresponds to the time when bicelle addition was begun, and t = 12.1 min approximates to the time when the CBC value was reached. The scale bar is 20 μm.

occurred quickly within a few minutes, yielding a complete SLB (Figure 9D–F). Hence, independent of q-ratio within the tested range, SLB occurred following a similar process and the key parameters that varied, the time scale until reaching the critical coverage and the surface area of bilayer fragments, obeyed the expected dependence on bicelle size in the bulk solution, as verified by the DLS measurements.

In summary, the fluorescence microscopy experiments support the QCM-D experiments by identifying that SLB formation occurs upon reaching a critical number of adsorbed bicelles, which induces bicelle rupture. Although the kinetic stages of the process are similar to those of the vesicle adsorption and spontaneous rupture after reaching a critical coverage, it is important to recall that DHPC causes membrane softening and also likely further aids the formation of SLBs by activating the edges of the bilayer patches upon contact with adsorbing bicelles. In this case, DHPC would function as edge activators, as seen in so-called "elastic" vesicles,[62,63] and aid bilayer propagation until the SLB formation process is complete.

FRAP Characterization. The lateral lipid diffusion of the fabricated SLBs was also characterized by FRAP measurements for the bicelles prepared at the various q-ratios (with fixed DOPC concentration at 0.031 mM). Time-lapsed FRAP micrographs indicated that nearly complete fluorescence recovery is observed within 2 min (Figure 10). On the basis of the recovery profiles, the Hankel transform method was applied to extract the diffusion coefficient of the lateral lipid mobility within the SLBs.[38] The diffusion coefficients were nearly identical for SLBs at the different q-ratios: 2.22 ± 0.03 $\mu m^2/s$ at q = 0.05, 2.27 ± 0.05 $\mu m^2/s$ at q = 0.25, and 2.24 ± 0.04 $\mu m^2/s$ at q = 2.5. These values agree well with the expected values for a fluidic DOPC SLB on glass.[17] As surfactant-like molecules can influence lateral lipid diffusion in SLBs, it should be noted that the lack of dependence of the measured diffusion coefficient on the q-ratio further supports that DHPC does not affect the DOPC SLB properties, as discussed above. Taken together, the combination of QCM-D, fluorescence microscopy, and FRAP measurement results supports that high-quality DOPC SLBs can be formed at low total phospholipid concentrations, and it is particularly recommended to use bicelles with q-ratio values of 0.25 or 2.5.

Influence of Bicelle Preparation Conditions. Last, we investigated how the preparation of DOPC/DHPC bicellar mixtures (q-ratio of 0.25; 0.063 mM DOPC) influences the formation of SLBs on silicon dioxide, as determined by QCM-D measurements (Figure 11). Although the conventional method to prepare bicelles is relatively simple and involves freeze–thaw–vortex cycles, we wanted to determine whether other treatment conditions (all of which yielded optically clear solutions[64]) could be used instead. When DOPC/DHPC phospholipids were mixed in aqueous buffer to the desired q-ratio without additional treatment, the aggregates had an average diameter and polydispersity of 931 nm and 0.273, respectively, and adsorption led to the final Δf and ΔD values

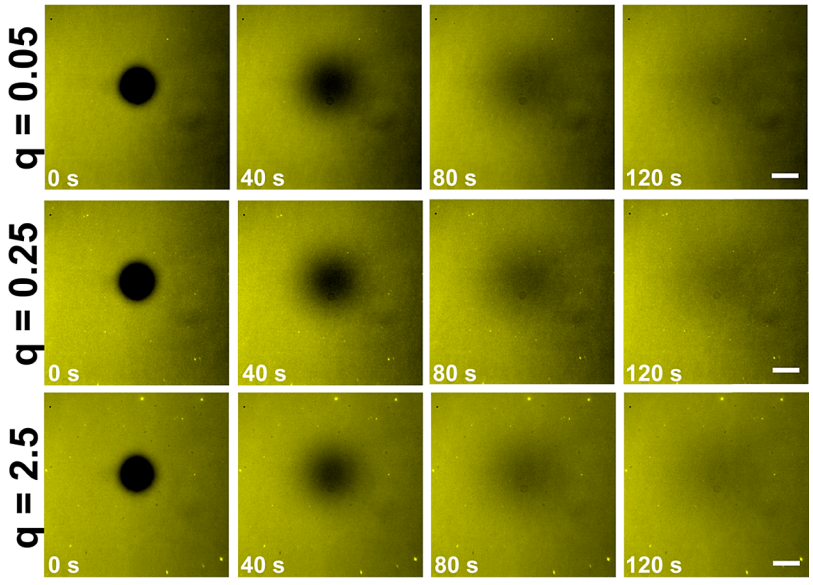


Figure 10. FRAP snapshots of SLBs prepared from bicellar mixtures at different q-ratios. At t = 0 min, an image was recorded immediately after photobleaching, followed by time-lapsed recording of the fluorescence recovery process.

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052–5064

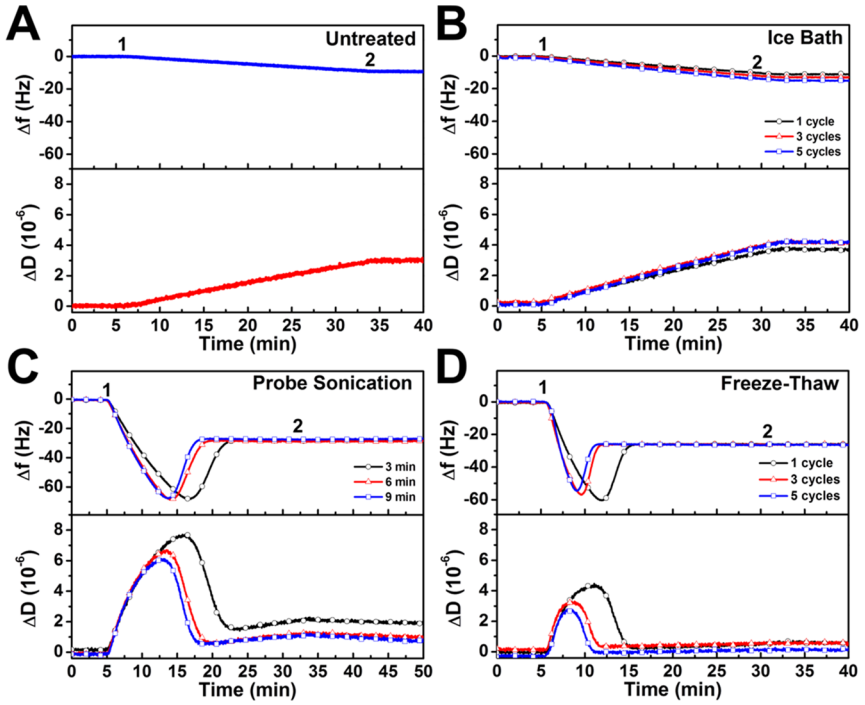


Figure 11. QCM-D evaluation of different preparation methods to prepare SLBs from bicellar mixtures. (A) Untreated sample. (B) Freeze–thaw–vortex cycling with ice bath incubation. (C) Treatment with probe sonication for varying durations. (D) Freeze–thaw–vortex cycling with liquid nitrogen incubation. In all cases, the bicellar mixtures were prepared at a *q*-ratio of 0.25, and the DOPC concentration was fixed at 0.063 mM.

of −8.0 Hz and 3×10^{-6}, respectively, which indicated that the SLB formation was unsuccessful (Figure 11A). Incubation of the DOPC/DHPC bicellar mixtures in an ice bath as part of ice–thaw–vortex cycling was similar, even though this procedure has been reported to form bicelles in solution (Figure 11B).[30,64,65] It is likely that the solution temperature in the ice bath never reached below the eutectic temperature of the bicellar mixture that is necessary to cause fragmentation.[66] Indeed, the average diameters of the ice-bath-treated samples were approximately 900 nm. On the other hand, probe sonication of bicellar mixtures appeared to be more promising and yielded SLBs with final Δf values of approximately −25 Hz, although the ΔD shifts were greater than 1×10^{-6} (Figure 11C). In this case, the probe-sonicated samples had average diameters approximately 300–400 nm, which are within a size range similar to that of the freeze–thawed samples. Altogether, these experiments identified that freeze–thaw–vortex cycling is particularly useful, likely because it causes larger, multilamellar aggregates to fragment into smaller, unilamellar aggregates, as has also been seen to improve the SLB formation when using zwitterionic lipid vesicles.[66,67] Indeed, treatment with one cycle of freeze–thaw–vortexing yielded SLBs with final Δf and ΔD values of approximately −25 Hz and 0.8×10^{-6}, respectively (Figure 11D). An increasing number of freeze–thaw–vortexing cycles did not have much effect on the aggregate size but did further improve the SLB quality based on lower energy dissipation values, presumably by reducing the number of unruptured adsorbed bicelles on the substrates. Collectively, the findings support that freeze–thaw–vortex cycling is a useful step in bicelle preparation to prepare high-quality SLBs, and tip sonication might be further considered as it is possible to prepare bicellar aggregates with similar size distributions in the bulk solution and support the bicelle fusion/rupture process.

Looking forward, using bicellar mixtures to prepare SLBs is an intriguing approach that deserves further exploration as an alternative fabrication strategy. On the basis of the results obtained in this study, we have newly identified that bicelles adsorb onto silica surfaces until reaching a critical coverage and then fuse/rupture to form bilayer islands, which propagate until forming a complete SLB. Briefly, bicelle adsorption initially causes a decrease in the frequency shift and increase in the dissipation shift until reaching critical surface coverages, at which point the bicelles fuse and rupture to yield an SLB while DHPC monomers return to the bulk solution because of their short residence times in bilayer configurations.[67] The bicelle-to-bilayer structural transformation is denoted by inflection points in the QCM-D measurement responses, at which stage bicelle fusion and SLB assembly become the predominant processes that are taking place across the sensor surface. Compared with the past works in the field, our findings reveal for the first time the detailed steps involved in the bicelle-mediated SLB formation process—including the requirement of a critical bicelle concentration for bicelle fusion and SLB propagation to occur—as well as demonstrate how treating bicelles as a mixed micelle system can be used to optimize the SLB formation process. Importantly, these findings led us to identify that appreciably lower phospholipid concentrations than that previously explored are in fact superior for SLB fabrication. Although other methods such as vesicle fusion and SALB are able to prepare SLBs on silica surfaces, one of the most striking features of the bicelle approach is that, when it is used with low lipid concentrations in the appropriate range of *q*-ratio values, the formation of SLBs is extremely of high-quality as evidenced by the characteristic zero energy dissipation shifts, as recorded in the QCM-D measurements and further substantiated using the fluorescence microscopy and FRAP measurements. By contrast, and inevitably, a small fraction of adsorbed vesicles

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052–5064

will nearly always remain intact on a silica surface and not rupture even for relatively small vesicles (<100 nm diameter), resulting in non-zero energy dissipation shifts because of the viscoelastic contribution of the adsorbed vesicles.[37] Similar challenges arise with the SALB process, wherein the SLB formation process involves a complex nucleation process, which is highly dependent on bulk lipid concentration.[19] The utility of the bicellar approach is particularly noteworthy when considering that the bicelles are approximately 400 nm diameter; large vesicles in a similar size range are typically quite poor at forming SLBs, and a large fraction of adsorbed, intact vesicles remain.[68] Another apparent benefit is that bicelles are relatively easier to prepare than vesicles and do not require extrusion as the bicelle size does not appear to be a critical parameter, whereas highly controlled size distributions are an important requirement for vesicle fusion. Although freezing with liquid nitrogen appears to be useful for preparing bicellar mixtures that form SLBs in our experiments, the experimental evidence suggests that sonication methods might also be useful to prepare bicelles for this application, and further attention is warranted at identifying the simplest methods to prepare bicellar mixtures that are suitable for the formation of SLBs.

CONCLUSIONS

Herein, we have identified optimal conditions for preparing SLBs on silicon dioxide using bicellar mixtures. Although previous bicelle-related studies have used high phospholipid concentrations (between 0.25 and 0.8 mg/mL long-chain phospholipid) to prepare SLBs, our findings reveal that SLB quality is optimal when using bicellar mixtures at appreciably lower concentrations of 0.024 mg/mL long-chain phospholipid or lower. In this concentration range, the free-DHPC concentration is sufficiently low to prevent membrane-induced destabilization, resulting in high-quality SLBs via bicelle adsorption and spontaneous rupture after reaching a critical surface coverage. At the same time, the presence of DHPC in the bicellar mixture is important for membrane softening and the disklike bicellar aggregates exhibit greater deformation in the adsorbed state than conventional vesicles. Furthermore, compared to vesicle preparation methods, bicelle preparation is simple and requires only freeze−thaw−vortex cycling, and the concentration range used to form high-quality SLBs is approximately 5−10 times lower than the minimum lipid concentration required for the SALB method (~0.1 mg/mL). In summary, our findings have identified that bicellar mixtures are excellent model membrane tools for SLB fabrication and deserve further exploration as competitive technologies alongside other fabrication techniques such as vesicle adsorption and rupture as well as the SALB method.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b00210.

Descriptions of additional SLB formation experiments, concentration-dependent interaction of DHPC with SLBs, CMC measurements on DHPC, and DHPC adsorption onto silicon dioxide surfaces along with Videos S1−S3, showing the time-lapsed recording of SLB formation using bicellar mixtures at different q-ratios (PDF)

Time-lapsed recording of the SLB formation from bicellar mixtures prepared at a q-ratio of 0.05 (AVI)

Time-lapsed recording of the SLB formation from bicellar mixtures prepared at a q-ratio of 0.25 (AVI)

Time-lapsed recording of the SLB formation from bicellar mixtures prepared at a q-ratio of 2.5 (AVI)

AUTHOR INFORMATION

Corresponding Author

*E-mail: njcho@ntu.edu.sg.

ORCID

Nam-Joon Cho: 0000-0002-8692-8955

Author Contributions

‖K.K. and J.A.J. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by a National Research Foundation Proof-of-Concept Grant (NRF2015NRF-POC001-019) and an A*STAR-NTU-NHG Skin Research Grant (SRG/14028). K.K. was funded by the Pomona College Summer Undergraduate Research Program (SURP).

REFERENCES

(1) Chan, Y.-H. M.; Boxer, S. G. Model membrane systems and their applications. *Curr. Opin. Chem. Biol.* **2007**, *11*, 581−587.

(2) Jackman, J. A.; Knoll, W.; Cho, N.-J. Biotechnology applications of tethered lipid bilayer membranes. *Materials* **2012**, *5*, 2637−2657.

(3) Sackmann, E. Supported membranes: scientific and practical applications. *Science* **1996**, *271*, 43.

(4) Mager, M. D.; Melosh, N. A. Lipid bilayer deposition and patterning via air bubble collapse. *Langmuir* **2007**, *23*, 9369−9377.

(5) Mennicke, U.; Salditt, T. Preparation of solid-supported lipid bilayers by spin-coating. *Langmuir* **2002**, *18*, 8172−8177.

(6) Lenhert, S.; Sun, P.; Wang, Y.; Fuchs, H.; Mirkin, C. A. Massively parallel dip-pen nanolithography of heterogeneous supported phospholipid multilayer patterns. *Small* **2007**, *3*, 71−75.

(7) Richter, R. P.; Bérat, R.; Brisson, A. R. Formation of solid-supported lipid bilayers: an integrated view. *Langmuir* **2006**, *22*, 3497−3505.

(8) Tamm, L. K.; McConnell, H. M. Supported phospholipid bilayers. *Biophys. J.* **1985**, *47*, 105.

(9) Weirich, K. L.; Israelachvili, J. N.; Fygenson, D. K. Bilayer edges catalyze supported lipid bilayer formation. *Biophys. J.* **2010**, *98*, 85−92.

(10) Cremer, P. S.; Boxer, S. G. Formation and spreading of lipid bilayers on planar glass supports. *J. Phys. Chem. B* **1999**, *103*, 2554−2559.

(11) Zasadzinski, J. A.; Helm, C. A.; Longo, M. L.; Weisenhorn, A. L.; Gould, S. A.; Hansma, P. K. Atomic force microscopy of hydrated phosphatidylethanolamine bilayers. *Biophys. J.* **1991**, *59*, 755.

(12) Egawa, H.; Furusawa, K. Liposome adhesion on mica surface studied by atomic force microscopy. *Langmuir* **1999**, *15*, 1660−1666.

(13) Keller, C. A.; Glasmästar, K.; Zhdanov, V. P.; Kasemo, B. Formation of supported membranes from vesicles. *Phys. Rev. Lett.* **2000**, *84*, 5443.

(14) Boudard, S.; Seantier, B.; Breffa, C.; Decher, G.; Félix, O. Controlling the pathway of formation of supported lipid bilayers of DMPC by varying the sodium chloride concentration. *Thin Solid Films* **2006**, *495*, 246−251.

(15) Dacic, M.; Jackman, J. A.; Yorulmaz, S.; Zhdanov, V. P.; Kasemo, B.; Cho, N.-J. Influence of Divalent Cations on Deformation

and Rupture of Adsorbed Lipid Vesicles. *Langmuir* **2016**, *32*, 6486−6495.
(16) Sundh, M.; Svedhem, S.; Sutherland, D. S. Influence of phase separating lipids on supported lipid bilayer formation at SiO_2 surfaces. *Phys. Chem. Chem. Phys.* **2010**, *12*, 453−460.
(17) Tabaei, S. R.; Choi, J.-H.; Zan, G. H.; Zhdanov, V. P.; Cho, N.-J. Solvent-assisted lipid bilayer formation on silicon dioxide and gold. *Langmuir* **2014**, *30*, 10363−10373.
(18) Hohner, A. O.; David, M. P. C.; Rädlera, J. O. Controlled solvent-exchange deposition of phospholipid membranes onto solid surfaces. *Biointerphases* **2010**, *5*, 1−8.
(19) Jackman, J. A.; Saravanan, R.; Zhang, Y.; Tabaei, S. R.; Cho, N.-J. Correlation between Membrane Partitioning and Functional Activity in a Single Lipid Vesicle Assay Establishes Design Guidelines for Antiviral Peptides. *Small* **2015**, *11*, 2372−2379.
(20) Jackman, J. A.; Tabaei, S. R.; Zhao, Z.; Yorulmaz, S.; Cho, N.-J. Self-assembly formation of lipid bilayer coatings on bare aluminum oxide: overcoming the force of interfacial water. *ACS Appl. Mater. Interfaces* **2014**, *7*, 959−968.
(21) Tabaei, S. R.; Jackman, J. A.; Kim, S.-O.; Liedberg, B.; Knoll, W.; Parikh, A. N.; Cho, N.-J. Formation of cholesterol-rich supported membranes using solvent-assisted lipid self-assembly. *Langmuir* **2014**, *30*, 13345−13352.
(22) Yorulmaz, S.; Tabaei, S. R.; Kim, M.; Seo, J.; Hunziker, W.; Szebeni, J.; Cho, N.-J. Membrane attack complex formation on a supported lipid bilayer: initial steps towards a CARPA predictor nanodevice. *Eur. J. Nanomed.* **2015**, *7*, 245−255.
(23) Yorulmaz, S.; Jackman, J. A.; Hunziker, W.; Cho, N.-J. Supported Lipid Bilayer Platform To Test Inhibitors of the Membrane Attack Complex: Insights into Biomacromolecular Assembly and Regulation. *Biomacromolecules* **2015**, *16*, 3594−3602.
(24) Sanders, C. R.; Oxenoid, K. Customizing model membranes and samples for NMR spectroscopic studies of complex membrane proteins. *Biochim. Biophys. Acta, Biomembr.* **2000**, *1508*, 129−145.
(25) Ram, P.; Prestegard, J. H. Magnetic field induced ordering of bile salt/phospholipid micelles: new media for NMR structural investigations. *Biochim. Biophys. Acta, Biomembr.* **1988**, *940*, 289−294.
(26) van Dam, L.; Karlsson, G.; Edwards, K. Direct observation and characterization of DMPC/DHPC aggregates under conditions relevant for biological solution NMR. *Biochim. Biophys. Acta, Biomembr.* **2004**, *1664*, 241−256.
(27) Glover, K. J.; Whiles, J. A.; Wu, G.; Yu, N.; Deems, R.; Struppe, J. O.; Stark, R. E.; Komives, E. A.; Vold, R. R. Structural evaluation of phospholipid bicelles for solution-state studies of membrane-associated biomolecules. *Biophys. J.* **2001**, *81*, 2163−2171.
(28) Nieh, M.-P.; Raghunathan, V. A.; Glinka, C. J.; Harroun, T. A.; Pabst, G.; Katsaras, J. Magnetically alignable phase of phospholipid "bicelle" mixtures is a chiral nematic made up of wormlike micelles. *Langmuir* **2004**, *20*, 7893−7897.
(29) Triba, M. N.; Devaux, P. F.; Warschawski, D. E. Effects of lipid chain length and unsaturation on bicelles stability. A phosphorus NMR study. *Biophys. J.* **2006**, *91*, 1357−1367.
(30) Zeineldin, R.; Last, J. A.; Slade, A. L.; Ista, L. K.; Bisong, P.; O'Brien, M. J.; Brueck, S. R.; Sasaki, D. Y.; Lopez, G. P. Using bicellar mixtures to form supported and suspended lipid bilayers on silicon chips. *Langmuir* **2006**, *22*, 8163−8168.
(31) Tabaei, S. R.; Jönsson, P.; Brändén, M.; Höök, F. Self-assembly formation of multiple DNA-tethered lipid bilayers. *J. Struct. Biol.* **2009**, *168*, 200−206.
(32) Losonczi, J. A.; Prestegard, J. H. Improved dilute bicelle solutions for high-resolution NMR of biological macromolecules. *J. Biomol. NMR* **1998**, *12*, 447−451.
(33) Morigaki, K.; Kimura, S.; Okada, K.; Kawasaki, T.; Kawasaki, K. Formation of substrate-supported membranes from mixtures of long- and short-chain phospholipids. *Langmuir* **2012**, *28*, 9649−9655.
(34) Tiberg, F.; Harwigsson, I.; Malmsten, M. Formation of model lipid bilayers at the silica−water interface by co-adsorption with non-ionic dodecyl maltoside surfactant. *Eur. Biophys. J.* **2000**, *29*, 196−203.
(35) Vacklin, H. P.; Tiberg, F.; Thomas, R. K. Formation of supported phospholipid bilayers via co-adsorption with beta-D-dodecyl maltoside. *Biochim. Biophys. Acta, Biomembr.* **2005**, *1668*, 17−24.
(36) Cho, N.-J.; Frank, C. W.; Kasemo, B.; Höök, F. Quartz crystal microbalance with dissipation monitoring of supported lipid bilayers on various substrates. *Nat. Protoc.* **2010**, *5*, 1096−1106.
(37) Kim, M. C.; Gunnarsson, A.; Tabaei, S. R.; Höök, F.; Cho, N.-J. Supported lipid bilayer repair mediated by AH peptide. *Phys. Chem. Chem. Phys.* **2016**, *18*, 3040−3047.
(38) Jönsson, P.; Jonsson, M. P.; Tegenfeldt, J. O.; Höök, F. A method improving the accuracy of fluorescence recovery after photobleaching analysis. *Biophys. J.* **2008**, *95*, 5334−5348.
(39) Morrison, E. A.; Henzler-Wildman, K. A. Reconstitution of integral membrane proteins into isotropic bicelles with improved sample stability and expanded lipid composition profile. *Biochim. Biophys. Acta, Biomembr.* **2012**, *1818*, 814−820.
(40) Rodríguez, G.; Rubio, L.; Barba, C.; López-Iglesias, C.; de la Maza, A.; López, O.; Cócera, M. Characterization of new DOPC/DHPC platform for dermal applications. *Eur. Biophys. J.* **2013**, *42*, 333−345.
(41) Seantier, B.; Breffa, C.; Félix, O.; Decher, G. In situ investigations of the formation of mixed supported lipid bilayers close to the phase transition temperature. *Nano Lett.* **2004**, *4*, 5−10.
(42) Sternin, E.; Nizza, D.; Gawrisch, K. Temperature dependence of DMPC/DHPC mixing in a bicellar solution and its structural implications. *Langmuir* **2001**, *17*, 2610−2616.
(43) Otten, D.; Brown, M. F.; Beyer, K. Softening of membrane bilayers by detergents elucidated by deuterium NMR spectroscopy. *J. Phys. Chem. B* **2000**, *104*, 12119−12129.
(44) Triba, M. N.; Warschawski, D. E.; Devaux, P. F. Reinvestigation by phosphorus NMR of lipid distribution in bicelles. *Biophys. J.* **2005**, *88*, 1887−1901.
(45) Brinck, J.; Tiberg, F. Adsorption behavior of two binary nonionic surfactant systems at the silica−water interface. *Langmuir* **1996**, *12*, 5042−5047.
(46) Yoon, B. K.; Jackman, J. A.; Kim, M. C.; Cho, N.-J. Spectrum of membrane morphological responses to antibacterial fatty acids and related surfactants. *Langmuir* **2015**, *31*, 10223−10232.
(47) Beaugrand, M.; Arnold, A. A.; Hénin, J.; Warschawski, D. E.; Williamson, P. T. F.; Marcotte, I. Lipid concentration and molar ratio boundaries for the use of isotropic bicelles. *Langmuir* **2014**, *30*, 6162−6170.
(48) Keller, C. A.; Kasemo, B. Surface specific kinetics of lipid vesicle adsorption measured with a quartz crystal microbalance. *Biophys. J.* **1998**, *75*, 1397−1402.
(49) Reimhult, E.; Höök, F.; Kasemo, B. Vesicle adsorption on SiO_2 and TiO_2: Dependence on vesicle size. *J. Chem. Phys.* **2002**, *117*, 7401−7404.
(50) Zwang, T. J.; Fletcher, W. R.; Lane, T. J.; Johal, M. S. Quantification of the layer of hydration of a supported lipid bilayer. *Langmuir* **2010**, *26*, 4598−4601.
(51) Zhdanov, V. P.; Keller, C. A.; Glasmästar, K.; Kasemo, B. Simulation of adsorption kinetics of lipid vesicles. *J. Chem. Phys.* **2000**, *112*, 900−909.
(52) Glasmästar, K.; Larsson, C.; Höök, F.; Kasemo, B. Protein adsorption on supported phospholipid bilayers. *J. Colloid Interface Sci.* **2002**, *246*, 40−47.
(53) Sauerbrey, G. Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung. *Z. Phys.* **1959**, *155*, 206−222.
(54) Murugova, T. N.; Balgavý, P. Molecular volumes of DOPC and DOPS in mixed bilayers of multilamellar vesicles. *Phys. Chem. Chem. Phys.* **2014**, *16*, 18211−18216.
(55) Tristram-Nagle, S.; Petrache, H. I.; Nagle, J. F. Structure and interactions of fully hydrated dioleoylphosphatidylcholine bilayers. *Biophys. J.* **1998**, *75*, 917−925.
(56) Tausk, R. J.; Karmiggelt, J.; Oudshoorn, C.; Overbeek, J. T. Physical chemical studies of short-chain lecithin homologues. I. Influence of the chain length of the fatty acid ester and of electrolytes on the critical micelle concentration. *Biophys. Chem.* **1974**, *1*, 175−183.

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064

(57) Palladino, P.; Ragone, R. Ionic strength effects on the critical micellar concentration of ionic and nonionic surfactants: The binding model. *Langmuir* **2011**, *27*, 14065−14070.
(58) Saleem, Q.; Zhang, Z.; Petretic, A.; Gradinaru, C. C.; Macdonald, P. M. Single Lipid Bilayer Deposition on Polymer Surfaces Using Bicelles. *Biomacromolecules* **2015**, *16*, 1032−1039.
(59) Nawa, E.; Yamamoto, D.; Shioi, A. Chemotactic Amoeboid-Like Shape Change of a Vesicle under a pH Gradient. *Bull. Chem. Soc. Jpn.* **2015**, *88*, 1536−1544.
(60) Evert, L. L.; Leckband, D.; Israelachvili, J. N. Structure and dynamics of ion-induced domains in free and supported monolayers and bilayers. *Langmuir* **1994**, *10*, 303−315.
(61) Zhdanov, V. P.; Kasemo, B. Nontraditional models of Ostwald ripening on solid surfaces: From physics to biology. *Surf. Sci.* **1999**, *437*, 307−316.
(62) Rodríguez, G.; Cócera, M.; Rubio, L.; López-Iglesias, C.; Pons, R.; de la Maza, A.; López, O. A unique bicellar nanosystem combining two effects on stratum corneum lipids. *Mol. Pharm.* **2012**, *9*, 482−491.
(63) Choi, M. J.; Maibach, H. I. Elastic vesicles as topical/transdermal drug delivery systems. *Int. J. Cosmet. Sci.* **2005**, *27*, 211−221.
(64) Lu, Z.; Van Horn, W. D.; Chen, J.; Mathew, S.; Zent, R.; Sanders, C. R. Bicelles at low concentrations. *Mol. Pharm.* **2012**, *9*, 752−761.
(65) De Angelis, A. A.; Opella, S. J. Bicelle samples for solid-state NMR of membrane proteins. *Nat. Protoc.* **2007**, *2*, 2332−2338.
(66) MacDonald, R. C.; Jones, F. D.; Qui, R. Fragmentation into small vesicles of dioleoylphosphatidylcholine bilayers during freezing and thawing. *Biochim. Biophys. Acta, Biomembr.* **1994**, *1191*, 362−370.
(67) Yoon, B. K.; Jackman, J. A.; Kim, M. C.; Sut, T. N.; Cho, N.-J. Correlating Membrane Morphological Responses with Micellar Aggregation Behavior of Capric Acid and Monocaprin. *Langmuir* **2017**, *33*, 2750−2759.
(68) Jackman, J. A.; Kim, M. C.; Zhdanov, V. P.; Cho, N.-J. Relationship between vesicle size and steric hindrance influences vesicle rupture on solid supports. *Phys. Chem. Chem. Phys.* **2016**, *18*, 3065−3072.

DOI: 10.1021/acs.langmuir.7b00210
Langmuir 2017, 33, 5052−5064