Langmuir
Letter
pubs.acs.org/Langmuir

Fabrication of a Planar Zwitterionic Lipid Bilayer on Titanium Oxide

Nam-Joon Cho[†,‡] and Curtis W. Frank*[,†]

[†]*Department of Chemical Engineering and* [‡]*Department of Medicine, Division of Gastroenterology and Hepatology, Stanford University, Stanford, California 94305*

Received April 16, 2010. Revised Manuscript Received September 6, 2010

There is great demand to fabricate planar phosphlipid bilayers on biocompatible materials. The preferred method of forming bilayers on these substrates is the spontaneous adsorption and rupture of phospholipid vesicles. However, in the case of titanium oxide, model vesicles composed solely of zwitterionic phospholipids do not follow this self-assembly pathway under physiological conditions, prompting the use of complex bilayer materials and less-facile methods. Herein, we report a novel pH-based strategy for fabricating zwitterionic bilayers on titanium oxide in a simple and robust manner. Depending on the pH conditions under which lipid vesicles adsorb onto titanium oxide, quartz crystal microbalance-dissipation (QCM-D) monitoring demonstrated that the self-assembly pathway can in fact result in planar bilayer formation. The pH of the solution could then be adjusted to physiological levels with no effect on the mass and viscoelastic properties of the bilayer. Moreover, fluorescence recovery after photobleaching (FRAP) measurements indicated a high degree of lateral lipid diffusivity within the bilayer at physiological pH, commensurate with its role as a cell membrane mimic. Compared to existing protocols, this strategy permits the fabrication of a more diverse array of planar bilayers on titanium oxide by tuning the self-assembly pathway of lipid vesicle adsorption onto solid substrates.

Introduction

The cell membrane has a complex architecture composed of phospholipids, sterols, sphingolipids, proteins, and other biomaterials.[1,2] Key structural features of the membrane architecture help regulate many cellular processes, including the transport of inorganic and organic species into and out of the cell as well as signal transduction pathways.[1,2] The membrane's important role in such biological processes has sparked interest into better understanding how its building blocks function. By employing a bottom-up approach focused on the design and investigation of simplified lipid-based model membranes, a high level of insight has been gained about how the lipid composition and experimental conditions affect the membrane's physical properties such as the phase and electrical surface charge, establishing parameters that are useful for the design of biosensors.[3,4] In particular, solid-supported planar lipid bilayers (SLB) offer a well-defined planar geometry for characterizing the architectural features of the membrane.[5] Furthermore, the SLB platform can be employed to monitor biological interactions such as antibody binding[6] and enzymatic reactions.[7,8]

Spontaneous vesicle adsorption and rupture is a simple and robust method of fabricating SLBs by taking advantage of the lipid reassembly process that occurs as a result of lipid vesicle adsorption onto certain hydrophilic substrates.[9] Although spontaneous vesicle adsorption can occur on a relatively wide range of substrates, only a subset has the appropriate surface properties (e.g., polarizability and hydrophilicity) to promote vesicle rupture and subsequent SLB formation.[9−12] SLBs consisting of single-component zwitterionic phospholipids can self-assemble from a dispersion of lipid vesicles under physiological conditions (e.g., ionic strength 140 mM, pH 7.4) on substrates including glass, silicon oxide, and mica.[9,10] In contrast, zwitterionic vesicles adsorbed on titanium oxide remain intact and do not rupture.[11]

Although an SLB on titanium oxide represents an attractive platform for a number of applications including medical implants[13] and biosensors,[4] the fundametal characterization of the substrate's interaction with lipid vesicles has only recently received attention.[4,14] In this letter, we present a new strategy based on controlling the electrostatic interaction between vesicles and the substrate in order to form a single-component zwitterionic bilayer that is both fluid and planar on a titanium oxide substrate. Using quartz crystal microbalance dissipation (QCM-D) and fluorescence recovery after photobleaching (FRAP), we characterized the properties of SLB platforms that resulted from the interaction of vesicles with a titanium oxide substrate across a range of pH values.

*Author to whom correspondence should be addressed. E-mail: curt.frank@stanford.edu

(1) Sackmann, E.; Tanaka, M. Supported membranes on soft polymer cushions: fabrication, characterization and applications. *Trends Biotechnol*. **2000**, *18*, 58–64.
(2) Sackmann, E. Supported membranes: scientific and practical applications. *Science* **1996**, *271*, 43–48.
(3) Tu, R. S.; Tirrell, M. Bottom-up design of biomimetic assemblies. *Advanced Drug Delivery Rev*. **2004**, *56*, 1537–1563.
(4) Hook, F.; Kasemo, B.; Grunze, M.; Zauscher, S. Quantitative Biological surface science: challenges and recent advances. *ACS Nano* **2008**, *2*, 2428–2436.
(5) Tanaka, M.; Sackmann, E. Supported membranes as biofunctional interfaces and smart biosensor platforms. *Physica Status Solidi A* **2006**, *203*, 3452–3462.
(6) Patel, A. R.; Kanazawa, K. K.; Frank, C. W. Antibody binding to a tethered vesicle assembly using QCM-D. *Anal. Chem*. **2009**, *81*, 6021–6029.
(7) Cho, N. J.; Cheong, K. H.; Lee, C.; Frank, C. W.; Glenn, J. S. Binding dynamics of hepatitis C virus' NS5A amphipathic peptide to cell and model membranes. *J. Virol*. **2007**, *81*, 6682–6689.
(8) Jackman, J. A.; Cho, N. J.; Duran, R. S.; Frank, C. W. Interfacial binding dynamics of bee venom phospholipase A2 investigated by quartz crystal microbalance and dynamic light scattering. *Langmuir* **2010**, *26*, 4103–4112.
(9) Keller, C. A.; Kasemo, B. Surface specific kinetics of lipid vesicle adsorption measured with a quartz crystal microbalance. *Biophys. J*. **1998**, *75*, 1397–1402.
(10) Keller, C. A.; Glasmastar, K.; Zhdanov, V. P.; Kasemo, B. Formation of supported membranes from vesicles. *Phys. Rev. Lett*. **2000**, *84*, 5443–5446.
(11) Reimhult, E.; Hook, F.; Kasemo, B. Vesicle adsorption on SiO_2 and TiO_2: dependence on vesicle size. *J. Chem. Phys*. **2002**, *117*, 7401–7404.
(12) Reimhult, E.; Hook, F.; Kasemo, B. Intact vesicle adsorption and supported biomembrane formation from vesicles in solution: influence of surface chemistry, vesicle size, temperature, and osmotic pressure. *Langmuir* **2003**, *19*, 1681–1691.
(13) Kasemo, B.; Lausmaa, J. Biomaterial and implant surfaces: on the role of cleanliness, contamination, and preparation procedures. *J. Biomed. Mater. Res., Part A* **1988**, *22*, 145–158.
(14) Kanazawa, K. K.; Cho, N.-J. Quartz crystal microbalance as a sensor to characterize macromolecular assembly dynamics. *J. Sens*. **2009**.

On the basis of our findings, we identified an effective pH range for SLB formation. After bilayer formation, we demonstrate that the pH can be readjusted back to physiological levels, making our strategy a simple and effective way to create SLBs on titanium oxide for biological applications.

Experimental Section

Substrate Preparation. AT-cut crystals (Q-Sense) of 14-mm-diameter silicon oxide with 50 nm thermally evaporated titanium oxide coats were used for all studies. Prior to experiment, each sensor crystal was treated with oxygen plasma at ~80 W for ~5 min (March Instruments, California). The reported isoelectric points for silicon oxide and titanium oxide are ~2 and ~4, respectively.[11,15]

Vesicle Preparation. Small unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with or without 1,2-di-(9*Z*-octadecenoyl)-*sn*-glycero-pyrophosphate (DGPP) (Avanti Polar Lipids, Alabaster, AL) were prepared by the extrusion method. A fluorescently labeled lipid, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt (Texas red-DHPE) (Molecular Probes, Eugene, OR), was added for FRAP measurements such that a molar ratio of 99.5% POPC or DGPP/POPC to 0.5% Texas red-DHPE was prepared. After the desired molar ratio was mixed in chloroform, the solvent was evaporated under a gentle stream of nitrogen gas in order to form a dried lipid film at a nominal lipid concentration of 5 mg·mL^{-1}. After a minimum of 5 h in a vacuum desiccator to remove any residual chloroform, the lipid film was hydrated in 18.2 MΩ·cm Milli-Q water (MilliPore, Billerica, MA) and then vortex mixed for 5 min to form large multilamellar vesicles. Afterwards, the vesicles were subsequently sized by a miniextruder (Avanti Polar Lipids) through a series of track-etched polycarbonate membranes with decreasing 100, 50, and 30 nm pore sizes. The resulting small unilamellar vesicles were diluted to 0.125 mg·mL^{-1} in the appropriate buffer before both QCM-D and FRAP experiments and were generally used within 24 h of preparation. A Tris buffer (10 mM Tris and 150 mM NaCl, pH 7.4) was used to dilute all vesicle solutions. Hydrochloric acid or sodium hydroxide was subsequently added to the vesicle solution to adjust the pH to the desired value for each experiment. All buffer solutions were prepared in 18.2 MΩ·cm Milli-Q water (MilliPore, Billerica, MA).

Within the pH range used in the studies, no significant lipid degradation activity is expected as a result of the solvent conditions (Personal Correpsondence, Avanti Polar Lipids Technical Staff). The QCM-D measurements of SLBs formed at low pH support this finding; no changes in the mass or viscoelasticity of the bilayer film were recorded over the measurement period, indicating that the physical properties of the bilayer and its constitutents phospholipids were unaffected.

Dynamic Light Scattering and Zeta Potential. A 90Plus particle size analyzer (Brookhaven Instruments) with a 658.0 nm monochromatic laser was used to analyze the vesicle size distribution after extrusion. To minimize the reflection effect, the scattering angle was set at 90° and the results were analyzed by digital autocorrelator software. All autocorrelation functions were analyzed by CONTIN and nonnegatively constrained least-squares algorithms to check for multimodal distributions. In addition, a gold electrode set was used to measure the electrophoretic mobility of vesicles in solution as a function of pH in order to calculate the vesicle zeta potential.

Quartz Crystal Microbalance-Dissipation (QCM-D). A Q-Sense D300 (Q-Sense AB, Gothenburg, Sweden) was used to monitor the in situ adsorption of lipid vesicles.[16] The crystal was initially driven at its resonance frequency, and then the drive circuit was short circuited. The exponential decay of the crystal oscillation was recorded and analyzed, yielding frequency and dissipation changes at 5, 15, 25, and 35 MHz. The normalized data presented here was recorded at the third overtone (15 MHz). The temperature of the measurement cell was 25.0 °C with an in-cell Peltier element ensuring thermal fluctuations of no greater than ±0.5 °C.

Fluorescence Photorecovery after Photobleaching (FRAP). SLBs on titanium oxide-coated quartz crystals (Q-Sense) were imaged using a Nikon Eclipse E800 upright microscope with an epifluorescence package. Water-immersion objectives (10× and 40×) were used to acquire images of the SLBs in a Tris buffer solution. Bleaching was performed with a 100 W high-pressure mercury light source using a contracted field diaphragm, a 40× objective lens, and a high-resolution air-cooled CCD camera (Photometrics CoolSNAP, Roper Scientific) to capture subsequent fluorescence images. MetaMorph software (Universal Imaging, CA) was used to collect image stacks in order to analyze the digitized fluorescence counts of the specified regions. Openlab 4.0 (Improvision Inc., England) was used to render 3-D profiling and to provide snapshots of the images. The graphs present the corresponding image traces of normalized fluorescence intensity across the bleaching spots from $t \approx 0$ to 500 s in order to measure the changes in fluorescence recovery.

FRAP analysis was performed to calculate the diffusion coefficients from the temporal fluorescence intensity profiles of selected regions during the measured recovery period. The Axelrod method[17] was employed to obtain the diffusion coefficients from the following equation

$$D = \frac{0.224w^2}{\tau_{1/2}} \quad (1)$$

where D is the fluorophore diffusion constant, w is the radius of the bleached area, and $\tau_{1/2}$ is the half recovery time defined by the recovery fraction $f(t_{1/2}) = [F(t_{1/2}) - F(0)]/[F(t \rightarrow \infty) - F(0)] = 0.5$. $F(t)$ is the fluorescence intensity at time t, which is fitted to a single-exponential function. The immobile fraction in the SLB was determined by $[1 - F(t \rightarrow \infty)] \times 100\%$.

Results and Discussion

Keller and Kasemo[9] first demonstrated that QCM-D is a valuable tool for characterizing interactions between lipid vesicles and a variety of substrates in terms of the mass and viscoelastic properties of the adlayer. Depending on the substrate surface chemistry, they determined that the adsorption of zwitterionic lipid vesicles can result in the formation of (1) an adsorbed layer of unruptured vesicles; (2) a supported lipid monolayer; or (3) an SLB. Recently, Rossetti et al.[18,19] demonstrated that the divalent cation Ca^{2+} can be used to promote the vesicle rupture of phosphatidylserine (PS)-containing vesicles on titanium oxide. They proposed that Ca^{2+} acts as a positively charged bridge to stabilize the interaction between negatively charged PS lipids and the negatively charged substrate. Also, on the basis of electrostatic interactions, Kunze et al.[20] showed that a postively charged SLB

(15) Petronis, S.; Gretzer, C.; Kasemo, B.; Gold, J. Model porous surfaces for systematic studies of material-cell interactions. *J. Biomed. Mater. Res., Part A* **2003**, *66*, 707–721.

(16) Cho, N. J.; Frank, C. W.; Kasemo, B.; Hook, F. Quartz crystal microbalance with dissipation monitoring of supported lipid bilayers on various substrates. *Nat. Protoc.* **2010**, *5*, 1096–1106.

(17) Axelrod, D.; Koppel, D. E.; Schlessinger, J.; Elson, E.; Webb, W. W. Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophys. J.* **1976**, *16*, 1055–1069.

(18) Rossetti, F. F.; Bally, M.; Michel, R.; Textor, M.; Reviakine, I. Interactions between titanium dioxide and phosphatidyl serine-containing liposomes: formation and patterning of supported phospholipid bilayers on the surface of a medically relevant material. *Langmuir* **2005**, *21*, 6443–6450.

(19) Rossetti, F. F.; Textor, M.; Reviakine, I. Asymmetric distribution of phosphatidyl serine in supported phospholipid bilayers on titanium dioxide. *Langmuir* **2006**, *22*, 3467–3473.

(20) Kunze, A.; Svedhem, S.; Kasemo, B. Lipid transfer between charged supported lipid bilayers and oppositely charged vesicles. *Langmuir* **2009**, *25*, 5146–5158.

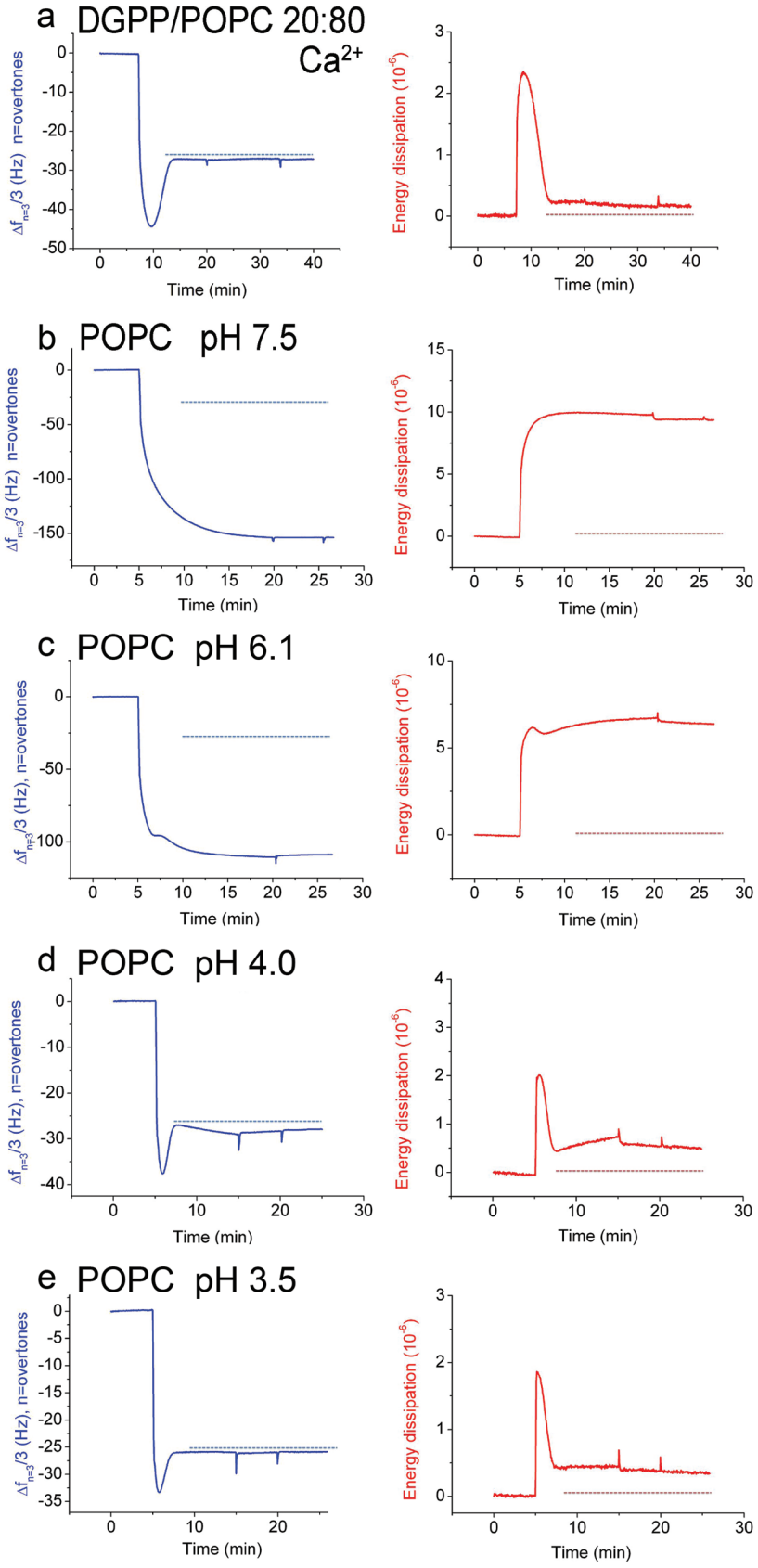

Figure 1. QCM-D responses for two different strategies to form a planar bilayer on titanium oxide. Frequency (blue) and energy dissipation (red) responses are presented as a function of time. Note that the dotted lines in each graph correspond to the ideal SLB frequency and energy dissipation responses of $\Delta f_{n=3}/3 = -25$ Hz and $\Delta D = 0.0 \times 10^{-6}$, respectively. (a) Ca^{2+} promotes SLB formation from negatively charged 20:80 DGPP/POPC vesicles. (b) Unruptured zwitterionic POPC vesicles adsorb at pH 7.0. (c) Unruptured, zwitterionic POPC vesicles still adsorb at pH 6.0, but the mass of the adlayer is lower than at pH 7.0. (d) Incomplete zwitterionic POPC bilayer formation at pH 4.0 (e) Zwitterionic POPC bilayer formation at pH 3.5.

can be formed on a negatively charged titanium oxide substrate. Here, we utilized a doubly negative charged lipid pyrophosphate (DGPP) to similarly promote vesicle rupture via Ca^{2+} bridging, forming a 20:80 DGPP/POPC SLB on titanium oxide at pH 7.5 (Figure 1a).

However, the effects of lipid redistribution during the self-assembly of two-component bilayers are not well understood. Consequently, there has been an effort to form simpler single-component bilayers on titanium oxide that lack the redistribution process, affording more thorough characterization.[18−20] Previous research reported findings of mixed layers of vesicles and bilayers on titanium oxide.[21] Interestingly, recent research[22] suggested the formation of single-component zwitterionic SLBs on titanium oxide at physiological pH, but Boxer[18] has pointed out the possibility of silicon oxide contamination on these rutile substrates. Moreover, the study itself as well as others[21,22] have noted the existence of a large number of defects, presumably intact vesicles, on titanium oxide substrates after adsorption at physiological pH. In comparison to these attempts that failed to fabricate a complete, planar zwitterionic bilayer on titanium oxide, we have previously developed a method to form a complete, single-component zwitterionic SLB on titanium oxide by introducing an amphipathic α-helical (AH) peptide to destabilize a layer of adsorbed vesicles.[23] The AH peptide interaction causes vesicle rupture, and the lipid reassembly processs results in an SLB with final frequency and dissipation values in good agreement with SLBs formed on silicon oxide.[7] However, this method requires the use of a destabilizing agent (AH peptide) to promote the structural transformation of intact vesicles to a planar lipid bilayer. Therefore, we sought to develop a simpler self-assembly strategy to form a zwitterionic SLB on titanium oxide that does not require a foreign agent.

By changing the pH of the bulk solution, we can adjust the surface charge density on both the substrate and vesicles in order to reproducibly form an SLB on titanium oxide. We first used a near-physiological buffer pH (7.0) to demonstrate the typical[9] exponential adsorption kinetic behavior of POPC vesicles on titanium oxide (Figure 1b). The corresponding dissipation response indicates a high degree of viscoelasticity, which is presumably a result of adsorbed, unruptured vesicles. Buffer washing had no effect on the film properties, supporting the conclusion that vesicle adsorption is irreversible. However, the strength of the interaction is still insufficient to promote vesicle rupture at physiological pH.

The most interesting and important behavior was observed when we investigated the kinetics of POPC vesicle adsorption onto titanium oxide under acidic conditions (Figure 1c−e). There are clear differences in the adsorption kinetics between the near-physiological pH and the three acidic pH values such that there are adsorbed, unruptured vesicles at pH 7.0, an intermediate state of adsorbed, unruptured vesicles and bilayer patches at pHs 6.1 and 4.5, and the formation of a planar bilayer at pH 3.5. At pH 6.1, the frequency and dissipation response kinetics indicate that vesicles initially adsorb onto the substrate (Figure 1c). A close inspection of the initial adsorption process suggests that vesicles adsorb until reaching a critical surface coverage and then rupture (kink in Figure 1c). However, the vesicle-rupturing process does not lead to SLB formation, suggesting that there may be insufficiently

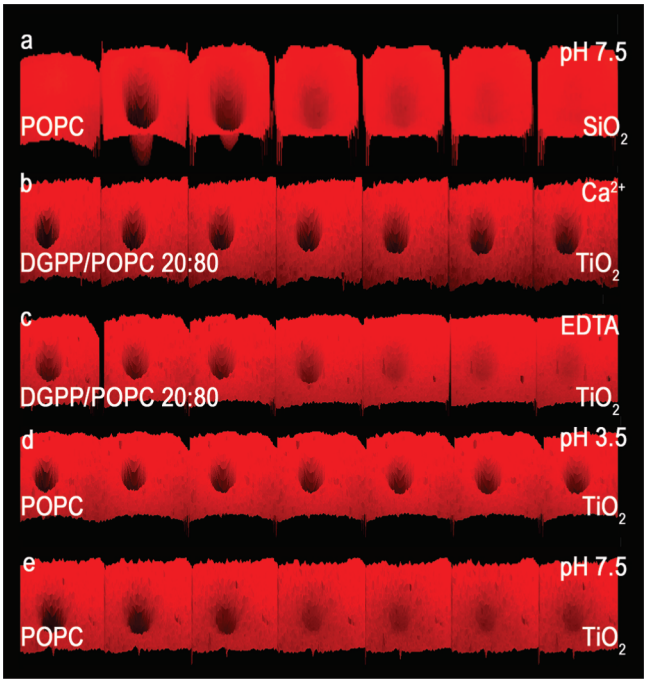

Figure 2. FRAP measurements for SLBs on titanium oxide. (a) POPC SLB on silicon oxide at pH 7.5. (b) 20:80 DGPP/POPC SLB on titanium oxide in the presence of Ca^{2+}. (c) 20:80 DGPP/POPC SLB after washing with the EDTA chelating agent to remove Ca^{2+}. (d) POPC SLB on titanium oxide formed at pH 3.5. (e) POPC SLB on titanium oxide after pH adjustment to 7.5.

strong interactions between vesicles and the substrate. The frequency and dissipation repsonses demonstrate continued viscoelastic mass uptake as a result of further vesicle adsorption. It is likely that the initial rupturing process leads to the formation of bilayer patches but that the energetics of the lipid−substrate interactions are not attractive enough to propagate bilayer formation.[24] Therefore, intact vesicles presumably adsorb onto the substrate and coexist with bilayer patches. Similarly, at pH 4.0, there is two-step vesicle adsorption kinetics that suggests bilayer formation, but the final frequency and dissipation values indicate that the film's mass and viscoelastic properties do not match those of a complete SLB. In marked constrast, there is clear evidence of two-step adsorption kinetics leading to bilayer formation at pH 3.5 (Figure 1e). The final frequency shift of ~25.5 Hz and the low dissipation shift of less than 0.3×10^{-6} indicate the formation of a complete SLB.

However, the mass and viscoelastic properties are only two of the three parameters that we chose to characterize because the SLB's fluidity is also critical to its use as a platform for biological studies.[17] Therefore, corresponding FRAP experiments were performed to verify the SLB fluidity and also to understand better how pH and related factors affect bilayer fluidity. A standard measurement of a POPC SLB on silicon oxide, the model substrate for SLBs,[9] was first recorded at pH 7.5 (Figure 2a) with a mobility value of $1.80 \pm 0.34\ \mu m^2 \cdot s^{-1}$. Measurements before and after the addition of ethylenediaminetetraacetic acid (EDTA), which is a chelating agent of Ca^{2+}, demonstrate that the lateral lipid mobility of the 20:80 DGPP/PC SLB after ($1.64 \pm 0.33\ \mu m^2 \cdot s^{-1}$) but not before EDTA treatment resembles that of the POPC SLB on silicon oxide (Figure 2b,c).[18,19]

(21) Csucs, G.; Ramsden, J. J. Interaction of phospholipid vesicles with smooth metal-oxide surfaces. *Biochim. Biophys. Acta* **1998**, *1369*, 61–70.
(22) Starr, T. E.; Thompson, N. L. Formation and characterization of planar phospholipid bilayers supported on TiO_2 and $SrTiO_3$ single crystals. *Langmuir* **2000**, *16*, 10301–10308.
(23) Cho, N. J.; Cho, S. J.; Cheong, K. H.; Glenn, J. S.; Frank, C. W. Employing an amphipathic viral peptide to create a lipid bilayer on Au and TiO_2. *J. Am. Chem. Soc.* **2007**, *129*, 10050–10051.
(24) Cha, T.; Guo, A.; Zhu, X. Y. Formation of supported phospholipid bilayers on molecular surfaces: role of surface charge density and electrostatic interaction. *Biophys. J.* **2006**, *90*, 1270–1274.

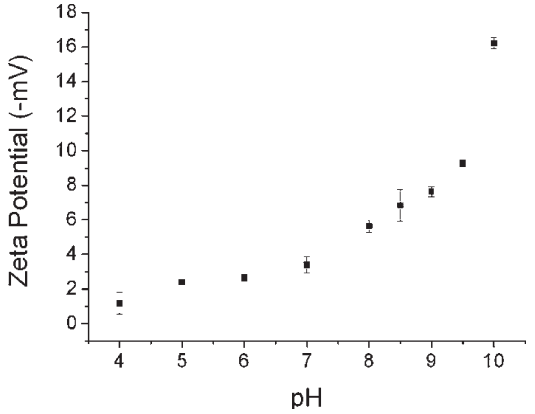

Figure 3. Zeta potential of zwitteroionic POPC lipid vesicles as a function of pH. Each measurement is the average of three independent measurements. Error bars represent the standard deviation. If no error bar is visible, then the standard deviation lies within the height of the data point.

We then measured the lateral lipid diffusivity of the POPC SLB at pH 3.5 (Figure 2d), and it showed a low degree of mobility, possibly as a result of attractive lipid−substrate interactions that are due to low pH. Strikingly, the lateral lipid mobility could be increased by raising the pH of the bulk solution after bilayer formation. Although abrupt changes in the bulk solution pH perturb the bilayer structure (Supporting Information Figure 1), the pH of the bulk solution can be gradually increased by repeated buffer exchanges, each time resulting in a slightly higher pH (~0.5 pH units), without the disruption of the bilayer structure (Figure 2e). At pH 7.5, the SLB had a mobility value of $1.52 \pm 0.29\ \mu m^2 \cdot s^{-1}$, displaying a level of lateral lipid diffusivity comparable to that of an SLB on silicon oxide (Figure 2e). To the best of our knowledge, this is the first time that a single-component zwitterionic bilayer has been self-assembled on titanium oxide with mobility values in good agreement with the silicon oxide model system.

To examine how the pH of the bulk solution affects the interaction between vesicles and the substrate, we next measured the zeta potential of vesicles across a wide pH range (Figure 3). Although the POPC lipid is a zwitterionic amphiphile, POPC lipid vesicles have a negative surface potential because the dipole potential of the zwitterionic headgroup creates a weak external field, resulting in a negative surface potential.[25] Under acidic conditions between pHs 2 and 4, the vesicle zeta potential is roughly constant with a value of −1 mV. Thereafter, the vesicle zeta potential becomes increasingly negative in an exponential fashion with increasing pH. In addition, the pH of the bulk solution affects the substrate's surface chemistry. The isoelectric point of the titanium oxide substrate has previously been reported to be ~4.[11] At pH 4 where both the substrate and vesicles have approximately no charge, there is partial vesicle rupture that is preceded by further vesicle adsorption. This finding suggests that van der Waals interactions play an important role in the vesicle-fusion process because a strong electrostatic interaction is not necessary for vesicle rupture.[26] However, van der Waals interactions are not sufficient for propagating complete SLB formation. At pH values of less than 4 where there is complete bilayer formation, the substrate has a positive surface charge that promotes the fusion of effectively charge-neutral vesicles via charge−dipole interactions.[24] Therefore, the ability to form planar bilayers on titanium oxide at low pH is likely to be a result of both van der Waals and electrostatic interactions, the latter of which can be tuned by adjusting the bulk solution pH in order to promote vesicle fusion, as we have demonstrated.

Conclusions

In this letter, we compare and discuss different strategies for forming planar bilayers on titanium oxide. A new method based on controlling the pH of the bulk solution is introduced, and this is the first time that a fluid zwitterionic bilayer can be formed by self-assembly on titanium oxide under near-physiological conditions. Although titanium oxide at physiological pH favors the adsorption of unruptured vesicles, the substrate's properties can be tuned to promote vesicle fusion, which leads to SLB formation, by controlling the electrostatic interaction between vesicles and the substrate. More importantly, the new strategy gives researchers greater control over the interactions between lipid vesicles and titanium oxide by experimentally demonstrating that solution pH effects can alter the self-assembly pathway.

Acknowledgment. This work was supported by the Center on Polymer Interfaces and Macromolecular Assemblies, an NSF Materials Research Science and Engineering Center. N.-J.C. is the recipient of an American Liver Foundation postdoctoral fellowship, a Global Roche postdoctoral fellowship, and a Stanford Dean's Fellowship.

Supporting Information Available: Materials and epifluorescence microscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

(25) Yang, Y.; Mayer, K. M.; Wickremasinghe, N. S.; Hafner, J. H. Probing the lipid membrane dipole potential by atomic force microscopy. *Biophys. J.* **2008**, *95*, 5193–5199.

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