

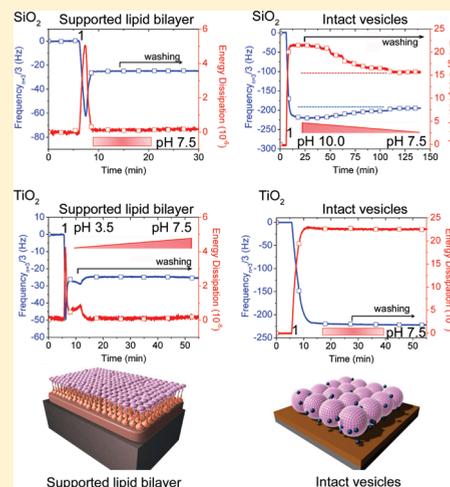
pH-Driven Assembly of Various Supported Lipid Platforms: A Comparative Study on Silicon Oxide and Titanium Oxide

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Supporting Information

ABSTRACT: Supported lipid platforms are versatile cell membrane mimics whose structural properties can be tailored to suit the application of interest. By identifying parameters that control the self-assembly of these platforms, there is potential to develop advanced biomimetic systems that overcome the surface specificity of lipid vesicle interactions under physiological conditions. In this work, we investigated the adsorption kinetics of vesicles onto silicon and titanium oxides as a function of pH. On each substrate, a planar bilayer and a layer of intact vesicles could be self-assembled in a pH-dependent manner, demonstrating the role of surface charge density in the self-assembly process. Under acidic pH conditions where both zwitterionic lipid vesicles and the oxide films possess near-neutral electric surface charges, vesicle rupture could occur, demonstrating that the process is driven by nonelectrostatic interactions. However, we observed that the initial rupturing process is insufficient for propagating bilayer formation. The role of electrostatic interactions for propagating bilayer formation differs for the two substrates; electrostatic attraction between vesicles and the substrate is necessary for complete bilayer formation on titanium oxide but is not necessary on silicon oxide. Conversely, in the high pH regime, repulsive electrostatic interactions can result in the irreversible adsorption of intact vesicles on silicon oxide and even a reversibly adsorbed vesicle layer on titanium oxide. Together, the results show that pH is an effective tool to modulate vesicle–substrate interactions in order to create various self-assembled lipid platforms on hydrophilic substrates.



INTRODUCTION

The design of new combinations of model membrane platforms and substrates holds promise for a wide range of biotechnological and medical applications including (1) biochips for medical diagnostics and drug screening, (2) high electrical resistance films for biosensor design, (3) biomimetic photosynthesis platforms, and (4) biocompatible, nonfouling surfaces.^{1–3} In order to realize such applications, which may depend critically on the model membrane's architecture, an improved understanding of the forces that guide the self-assembly of these platforms is needed. Although biological membranes exhibit a complex architecture of lipids, proteins, and sterols, among other components, solid-supported model membranes derived solely from phospholipids are an excellent mimic with comparable physiochemical properties, including lateral phospholipid diffusion and ion permeability.^{1,3} One particular advantage of model membrane platforms is their self-assembling nature.⁴ The adsorption of lipid vesicles onto a solid substrate can result in a wide range of platforms depending on controllable parameters that include lipid composition,^{5–7} vesicle concentration,⁸ temperature,⁹ osmotic pressure,¹⁰ vesicle size,¹¹ and surface chemistry.¹²

In this paper, we focus on understanding how the interactions of lipid vesicles with two popular and industrially attractive hydrophilic substrates, silicon and titanium oxides,^{13,14} can be modulated in order to control the self-assembly of targeted model membrane platforms. The electrical properties of silicon oxide have led to its importance in electronic device manufacturing, while titanium oxide's biocompatibility makes it a popular choice for medical implants.¹³ Furthermore, the high refractive index of titanium oxide is ideal for optics-based sensing applications.¹³ For hydrophilic substrates in general, there are two primary types of solid-supported lipid platforms that can self-assemble depending on surface properties: a planar lipid bilayer^{4,8,10,12,14–18} (e.g., glass, mica, and silicon oxide) or a layer of adsorbed intact vesicles (e.g., titanium and aluminum oxides).^{9,11} Depending on the intended application, each platform and substrate has its respective advantages. However, there has been only limited success at developing methods to control the self-assembly of different lipid platforms because of the difficulty in overcoming surface-specific interactions.

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Past studies have shown that vesicles composed of phosphatidylcholine lipids, the most abundant component of the extracellular leaflet of mammalian cell membranes and, thus, of significant interest for model systems, adsorb and rupture to form a bilayer on silicon oxide, whereas they adsorb and remain intact on titanium oxide.^{11,12} Recent work has developed strategies to form planar lipid bilayers on titanium oxide by using positively or negatively charged vesicles, suggesting the importance of electrostatic interactions.^{5,7} On the basis of these findings, we developed a new strategy to form a planar lipid bilayer consisting of zwitterionic phosphatidylcholine lipids on titanium oxide by using pH to modulate the electrostatic interaction between vesicles and the substrate, thereby promoting vesicle rupture and spreading.¹⁹ Here, we extend beyond this one fabrication example to provide a more comprehensive and fundamental understanding of how solution pH can be used to tune electrostatic interactions between oxide surfaces and lipid vesicles and, as a result, promote the self-assembly of different types of model membrane platforms.

Since Keller and Kasemo's seminal work that identified the surface-specific adsorption kinetics of lipid vesicles onto solid supports,¹² the quartz crystal microbalance dissipation (QCM-D) has been a popular tool to probe the mass and viscoelastic properties of supported lipid platforms. We used the QCM-D technique to investigate how pH affects the interaction between vesicles and silicon and titanium oxide substrates. In general, acidic pHs promote formation of a planar lipid bilayer, whereas basic pHs lead to the adsorption of unruptured vesicles.¹⁰ In the case of planar lipid bilayer formation, the vesicle rupturing kinetics varied as a function of pH, supporting previous findings that surface charge density is important for controlling the adsorption and fusion of zwitterionic lipid vesicles.^{10,15} Furthermore, the stability of intact vesicle layers varied as a function of pH. These observations suggest that the self-assembly of lipid platforms encompasses a spectrum of adsorption kinetics and pathways rather than just the discrete possibilities of the formation of a planar bilayer or intact vesicles having uniform physical properties.

We employed this spectrum of possible interactions between vesicles and the solid support in order to form both a planar lipid bilayer and a layer of intact vesicles on silicon and titanium oxides, all at the near-physiological pH of 7.5. After first allowing specific platforms to self-assemble in favorable pH ranges at either low or high pH, gradual pH adjustments were made to raise either the alkalinity or acidity of the solution until pH 7.5 was reached. From this work, we propose that pH can adjust the interaction between vesicles and oxide films not only to promote vesicle rupture and spreading but also to hinder such a self-assembly process with adsorbed vesicles remaining unruptured. By readjusting the solution pH after self-assembly, we demonstrate that a wider range of model membrane platforms and substrates can be matched to design the optimal combination at the appropriate pH as well as the ability to use simple chemistry to control the design of nanoscale macromolecular assemblies on solid supports.

MATERIALS AND METHODS

Lipid Vesicle Preparation. Extruded unilamellar lipid vesicles (referred to as vesicles) composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Avanti Polar Lipids, Alabaster, AL) were prepared by the extrusion method.²⁰ Vesicle adsorption kinetics is highly

dependent on the experimental conditions. Therefore, in order to focus solely on the effects of pH, we fixed the other key parameters, including vesicle size, ionic strength, temperature, lipid phase, and lipid composition. POPC was selected because phosphocholine has a gel–fluid phase transition temperature (~ -10 °C) well below the experimentally convenient temperature of 24 °C, thereby permitting us to ignore lipid phase transition effects.²¹ Further, throughout the study, we only varied the pH (10 mM Tris, 200 mM NaCl). First, lipid films were prepared by drying the as-supplied lipids in chloroform under a gentle stream of nitrogen gas. The dried lipid films were then stored in a vacuum desiccator for at least 24 h to remove any residual chloroform. Afterward, the lipid films were initially hydrated in a stock Tris buffer (10 mM Tris, 150 mM NaCl, pH 7.5) at a nominal lipid concentration of 5 mg mL⁻¹ before periodic vortexing for 3 min. The multilamellar vesicles formed were subsequently passed through a 100 nm polycarbonate track-etched membrane 27 times with a minitruder (Avanti Polar Lipids), resulting in a monodisperse vesicle population as confirmed by dynamic light scattering measurements. The size distribution and polydispersity of the vesicles were 110 ± 3.4 nm and 0.34 ± 0.12 , respectively (data not shown). Before experiment, the vesicles were diluted in the desired titrated stock buffer to a fixed concentration of 0.125 mg mL⁻¹ and used within 1 day following extrusion. For preparation of the pH buffers used in this study, the original stock buffer was titrated with either sodium chloride or hydrochloric acid, and the solution pHs were measured before and after experiment to verify their stability. All solutions were prepared with 18.2 M Ω ·cm Milli-Q water (Millipore, Billerica, MA).

Quartz Crystal Microbalance Dissipation. Adsorption kinetics and the properties of the adsorbed layer were monitored in situ using a Q-Sense E4 (Q-Sense AB, Gothenburg, Sweden). AT-cut crystals (Q-Sense AB) with 14 mm diameter and 50 nm thermally evaporated silicon oxide or titanium oxide coats were used for all experiments. Each crystal was treated with oxygen plasma using a Plasma Prep 5 plasma cleaner (GaLa Instrumente GmbH, Bad Schwalbach, Germany) at ~ 70 W for 2 min immediately before the experiment. The changes in resonance frequency, Δf_n , and energy dissipation, ΔD_n , were simultaneously recorded as a function of time at three different overtones ($n = 3, 5,$ and 7). Data presented throughout the main text were obtained at the third overtone. A peristaltic pump was used to flow sample into the measurement chamber, and the temperature of each flow cell was set at 25.0 °C and controlled by a Peltier element within a 0.02 °C range.

Viscoelastic Modeling. One of the advantages of the QCM-D technique for probing thin films is its capability to detect an adsorbed mass, Δm , in the nanogram range, using a linear relationship between the added mass and the change in resonance frequency of the quartz crystal, as described by Sauerbrey.²² With the assumption of a homogeneous adlayer having a spatially uniform density, the Sauerbrey equation, written to include harmonic resonances, is

$$\Delta m = -C_{\text{QCM}} \frac{\Delta f_n}{n} \quad (1)$$

where Δm is the adsorbed mass on the surface, C_{QCM} is the mass sensitivity constant ($17.7 \text{ ng cm}^{-2} \text{ Hz}^{-1}$ at $f = 5 \text{ MHz}$), and Δf_n is the change in the resonance frequency at the n th harmonic. In this study, the interpretation of physical properties of the adsorbed film is based on the model presented by Voinova et al.²³ in which the adsorbed film is represented by a single Voigt element. Further, it is assumed in the Voigt Voinova model that the thickness, t_f , and the density, ρ_f , of the film are uniform, that the viscoelastic properties of shear modulus and viscosity are frequency-independent, and that there is no slip between the adsorbed layer and the crystal during shearing (see ref 3 and references therein). The adsorbed layer is described as a homogeneous film on the sensor surface using four unknown parameters ($\rho_f, t_f, \mu_f, \eta_f$). Above the adsorbed film is a semi-infinite bulk liquid (ρ_l, η_l). We employed the

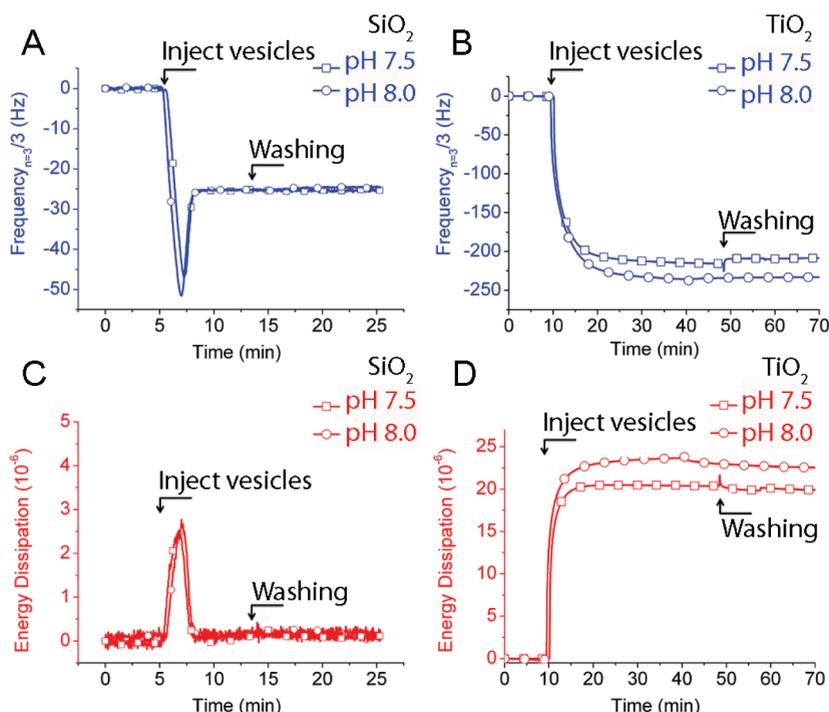


Figure 1. Vesicle adsorption kinetics at near-physiological pH. QCM-D was used to monitor vesicle adsorption kinetics as a function of time at pHs 7.5 and 8.0. After an initial stabilization period, vesicles were injected and the frequency response was recorded on (A) silicon oxide and (B) titanium oxide. Corresponding energy dissipation responses are presented for (C) silicon oxide and (D) titanium oxide. After the frequency and dissipation responses reached stable values, a buffer wash was performed, resulting in no significant changes in the platform's mass or viscoelastic properties.

Voigt–Voinova model to evaluate the experimentally measured Δf_n and ΔD_n for the bound mass in order to gain insight into the structural changes of the adsorbed film on the solid support. The adsorbed layer is represented by a frequency-dependent complex shear modulus, which is defined by

$$G = G' + iG'' = \mu_f + 2\pi if\eta_f = \mu_f(1 + 2\pi if\tau_f) \quad (2)$$

where G' describes energy storage, G'' describes energy dissipation, f is the oscillation frequency, μ_f is the elastic shear (storage) modulus, η_f is the shear viscosity (loss modulus), and $\tau_f = \eta_f/\mu_f$ is the characteristic relaxation time of the film.

RESULTS

Intact Vesicles or Planar Bilayer on Hydrophilic Solid Supports at Intermediate pH. To investigate how pH affects the interactions between lipid vesicles and silicon and titanium oxide substrates, we first measured the adsorption kinetics for these interactions by QCM-D monitoring at the near-physiological pH conditions of 7.5 and 8.0. As we expected, characteristic two-step kinetics of vesicle adsorption onto silicon oxide were observed with final frequency and dissipation shifts of -25.5 Hz and 0.2×10^{-6} , respectively, which correspond to the formation of a planar lipid bilayer,¹² as shown in Figure 1A,C. The small pH difference had no significant effect on the adsorption kinetics or the resulting bilayer's mass and viscoelastic properties. Likewise, Figure 1B,D demonstrates typical exponential vesicle adsorption kinetics on titanium oxide at both pHs.¹¹ However, in this case, there is a deviation between the final frequency and dissipation shifts at pH 7.5 ($\Delta f_n = -215$ Hz and $\Delta D_n = 20 \times 10^{-6}$) and pH 8.0 ($\Delta f_n = -230$ Hz and $\Delta D_n = 23 \times 10^{-6}$) for the layer of adsorbed, intact vesicles, which had an average diameter of

110 ± 3.4 nm based on dynamic light scattering measurements. Further, a more detailed investigation of vesicle diameter as a function of pH across the range of 2–10 demonstrated that vesicle diameter is largely independent of this parameter, with a less than 3% size change across this range.

As a tool to probe this model membrane system, the QCM-D acoustic wave sensor uniquely detects a resonance frequency response related to the change in adsorbed mass of both lipids and hydrodynamically coupled solvent, while its dissipation response measures the change in the adlayer's viscoelastic properties.²⁴ At pH 8.0 versus 7.5 on titanium oxide, there is a 6.8% decrease in the final frequency shift and a corresponding 13.0% decrease in the dissipation, which together demonstrate that vesicle adsorption at the lower pH results in an adlayer with less bound mass and a corresponding decrease in energy dissipation (Figure S1). These physical property differences, in particular, the decrease in film viscoelasticity, are likely due to less coupled solvent. Moreover, given the nonlinear relationship between mass and frequency response for adsorbates with high energy dissipation,²³ the mass difference between pHs 8.0 and 7.5 suggests increased vesicle flattening, likely a result of more attractive vesicle–substrate interactions at lower pH. On the basis of this trend of increased vesicle flattening (i.e., nonspherical shape due to interactions with the substrate^{18,25,26}) at lower pH, we sought to further evaluate how pH can be used to control the physical properties of lipid assemblies on solid supports.

Evidence of More Attractive Vesicle–Substrate Interactions at Lower pH. Our attention then shifted to lower pH regimes to examine how the adsorption kinetics is affected. Although the types of interactions varied between silicon and titanium oxides, there was a consistent trend toward more attractive interactions at lower pH. On silicon oxide, the

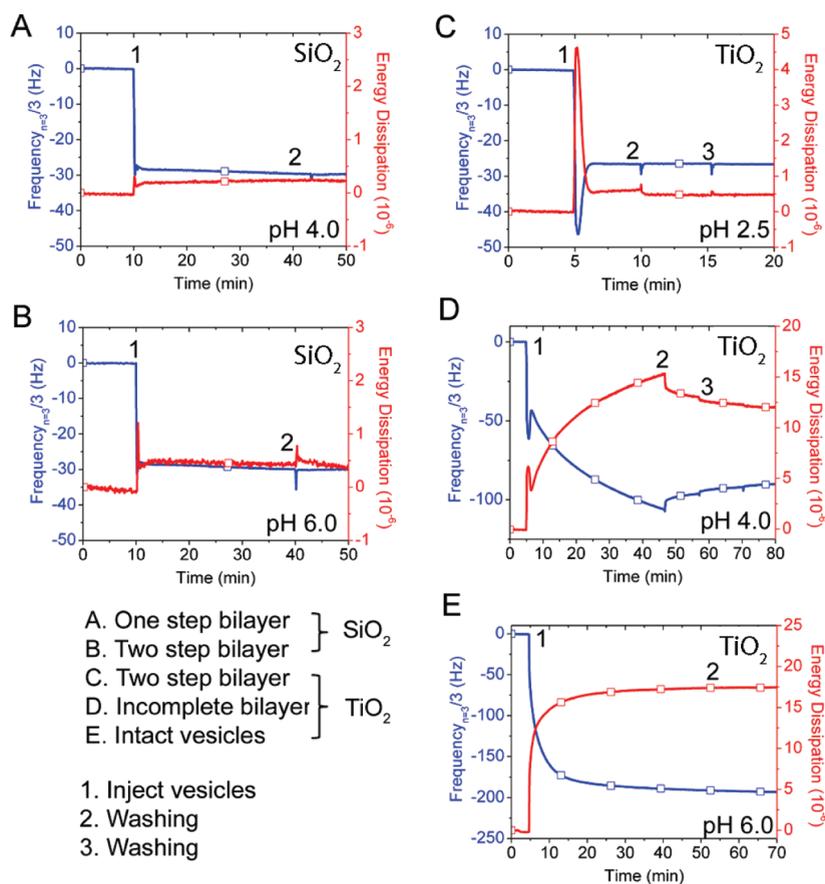


Figure 2. pH effects alter vesicle rupturing kinetics. QCM-D frequency and dissipation response kinetics vary depending on the pH. On silicon oxide, vesicles rupture at (A) pH 4.0 and (B) pH 6.0 to form a planar lipid bilayer in one step, as opposed to the two-step kinetics seen at higher pHs. On titanium oxide, vesicles rupture to form a bilayer in two steps at (C) pH 2.5. Vesicles also appear to rupture at (D) pH 4.0, but the process does not result in a complete bilayer. Instead, vesicles continue to adsorb, as indicated by the subsequent frequency decrease and dissipation increase. Buffer washes further affected the QCM-D responses, resulting in a frequency increase and dissipation decrease that suggest the adlayer is unstable and likely to be a combination of intact vesicles and bilayer patches. At (E) pH 6.0, a stable layer of intact vesicles adsorbs.

adsorption of vesicles at pH 4.0 (Figure 2A) and pH 6.0 (Figure 2B) resulted in a planar lipid bilayer, as expected. However, the adsorption kinetics approached one-step behavior. Compared to the two-step kinetics observed at higher pH, the observation of one-step kinetics suggests that there are more attractive interactions at lower pH.²⁷

Likewise, increasingly acidic pHs altered the adsorption kinetics on titanium oxide. At pH 6.0, there was irreversible adsorption of an intact vesicle layer (Figure 2C) with final frequency and dissipation shifts of -180 Hz and 18.0×10^{-6} , respectively. Compared with similar measurements made at pH 7.5 and pH 8.0 (Figure 1B,D), the results suggest increased vesicle flattening as a result of more attractive vesicle–substrate interactions (Figure S1). As discussed below, the adsorption kinetics at even lower pHs indicate that vesicle adsorption on titanium oxide can result in the self-assembly of a planar lipid bilayer.

At pH 4.0, an incomplete planar bilayer was formed (Figure 2D). The adsorption kinetics at first appeared to demonstrate typical two-step kinetics (Figure 2D), leading to bilayer formation with a maximum frequency shift of -60 Hz corresponding to the critical vesicle coverage (see Figure S2 for magnified view). After reaching the point of critical vesicle coverage, there was a 29.0% mass decrease based on the

QCM-D frequency response that corresponded to vesicle rupturing. However, there was then a continuous frequency decrease corresponding to intact vesicle adsorption upon the presumed bilayer patches. The monotonic frequency and dissipation kinetics of this latter adsorption behavior resembled the exponential kinetics of intact vesicle adsorption despite the presence of bilayer patches.¹⁷ A buffer wash at 46 min affected the QCM-D responses, demonstrating that the coexistence of bilayer patches and adsorbed, intact vesicles on titanium oxide is not stable, as evidenced by the removal of mass with buffer washing. This mixture of kinetic behavior at pH 4.0 suggests that the vesicle–substrate interactions are attractive enough to cause vesicle rupture in isolated events but not to propagate this action and form a complete planar bilayer. Interestingly, we have observed similar complicated adsorption kinetics on silicon oxide at pH 9.0 (Figure S3), suggesting that while the two substrates have different surface chemistries at any one pH value, their trends across the pH scale are similar, likely due to their different surface pK_a s.

On the basis of these results, we tested whether the strong attractive forces generated by acidic pHs could promote vesicle rupture and bilayer propagation in order to form a complete, planar bilayer based on the QCM-D resonance frequency and energy dissipation responses ($\Delta f = -24.5 \pm 1.5$ Hz and

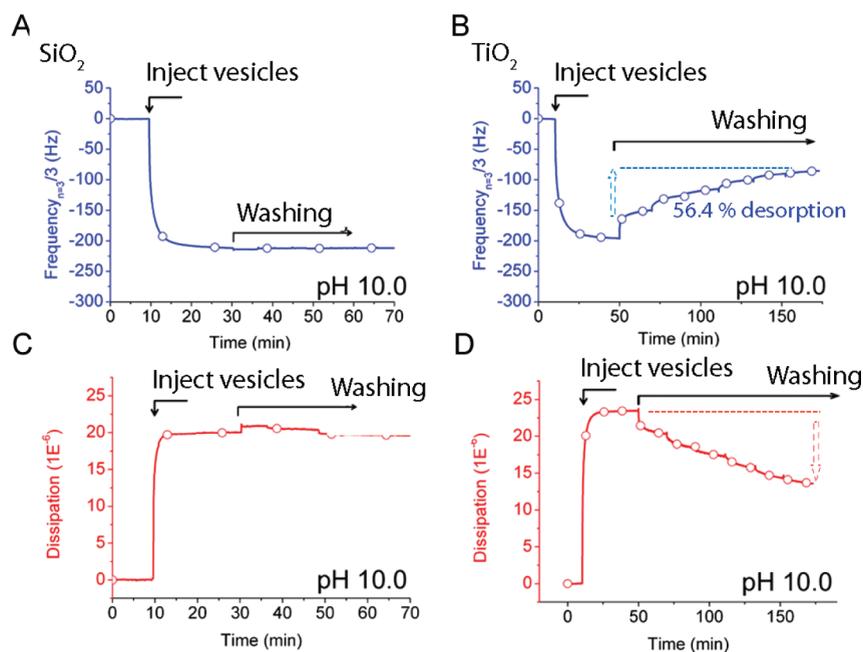


Figure 3. Intact vesicle platforms on silicon and titanium oxides at pH 10. After the QCM-D responses stabilized in buffer at pH 10, vesicles were injected. On silicon oxide, the (A) frequency and (B) energy dissipation responses indicate a stable adlayer that was unaffected by a series of washing steps. By contrast, the (C) frequency and (D) energy dissipation responses on titanium oxide show that the adlayer is more weakly bound. Each subsequent washing step resulted in a frequency increase and an energy dissipation decrease.

$\Delta D < 2.0 \times 10^{-6}$).^{14,28} Formation of a complete, planar bilayer has previously been demonstrated from POPC vesicles with 45 nm diameter.¹⁹ Here, we investigated whether attractive vesicle–substrate interactions could also promote complete bilayer formation from the much larger 110 nm vesicles used in this study. Whereas the smaller vesicles fused to form a complete planar bilayer at pH 3.5, complete bilayer formation from the larger vesicles required lower pH conditions. At pH 2.5 (Figure 2E), there was two-step vesicle adsorption kinetics leading to bilayer formation, which resembled the typical two-step bilayer formation signature on silicon oxide (Figure 1A,C), as indicated by final frequency and dissipation shifts of -26 Hz and 0.5×10^{-6} , respectively. This interplay of vesicle size and pH conditions indicates that many factors are involved in the self-assembly of lipid platforms on solid supports. Nonetheless, we show that pH is an effective modulator of these self-assembly processes.

Evidence of Increasing Electrostatic Repulsion at Higher pH. On the basis of the observed effects of acidic pH, we next investigated the effects of alkaline conditions on the vesicle adsorption kinetics in order to understand how less attractive vesicle–substrate interactions might hinder bilayer formation. Surprisingly, at pH 10.0, a layer of intact vesicles adsorbed onto silicon oxide (Figure 3A) with final frequency and dissipation shifts of -207 Hz and 19×10^{-6} , respectively. Subsequent buffer washes did not affect the layer, demonstrating its irreversible adsorption. Contrarily, reversible adsorption of an intact vesicle layer was observed on titanium oxide (Figure 3B), albeit with similar final frequency and dissipation shifts of -205 Hz and 23×10^{-6} , respectively. A series of buffer washes gradually removed the adlayer with subsequent mass desorption after each washing step (Figure S4). After a total of six washing steps, the final frequency shift increased to -80 Hz and the final dissipation shift decreased to 13×10^{-6} , corresponding to a 56.4% mass

loss. This result suggests that at pH 10.0 the titanium oxide substrate has significantly weaker attractive forces than silicon oxide. Further, the vesicle adlayer had a higher dissipation factor (15.3% deviation) on titanium oxide versus silicon oxide despite less mass, supporting the view that there is less electrostatic attraction between vesicles and titanium oxide at pH 10.0, as compared to the silicon oxide case.

pH as a Simple Modulator To Control the Self-Assembly of Lipid Platforms. From experiments across the pH range of 2.5–10.0, we characterized the types of interactions that can occur on each substrate. On silicon oxide, there are three possible types of results: (1) bilayer formation in one step (pH < 6.0); (2) bilayer formation in two steps (pH ~ 7.5); and (3) irreversible adsorption of an intact vesicle layer (pH ≥ 10.0). On titanium oxide, there are also three types of possible results: (1) bilayer formation in two steps (pH ≤ 2.5); (2) irreversible adsorption of an intact vesicle layer (pH ~ 7.5); and (3) reversible adsorption of an intact vesicle layer (pH ≥ 9.0). Taken together, four different lipid platforms can be created by using pH to modulate the self-assembly process. On the basis of these possibilities, we employed these different self-assembly pathways to form different model membrane platforms (a planar lipid bilayer or a layer of intact vesicles) on the same substrate (silicon or titanium oxide) at the same pH. Since there is growing interest in the application of these platforms for investigating biomacromolecular interactions, we chose pH 7.5 to create the four different possible platforms.

First, we show that a planar bilayer can be formed at pH 7.5 on silicon oxide, as expected (Figure 4A). However, in order to form an intact vesicle layer on silicon oxide, it was necessary to develop a new strategy based on the initial adsorption of vesicles onto the substrate at a higher pH. At pH 10.0, we again created an intact vesicle layer (Figure 4B). Instead of washing the bilayer with buffer at pH 10.0, however, we introduced a series of increasingly

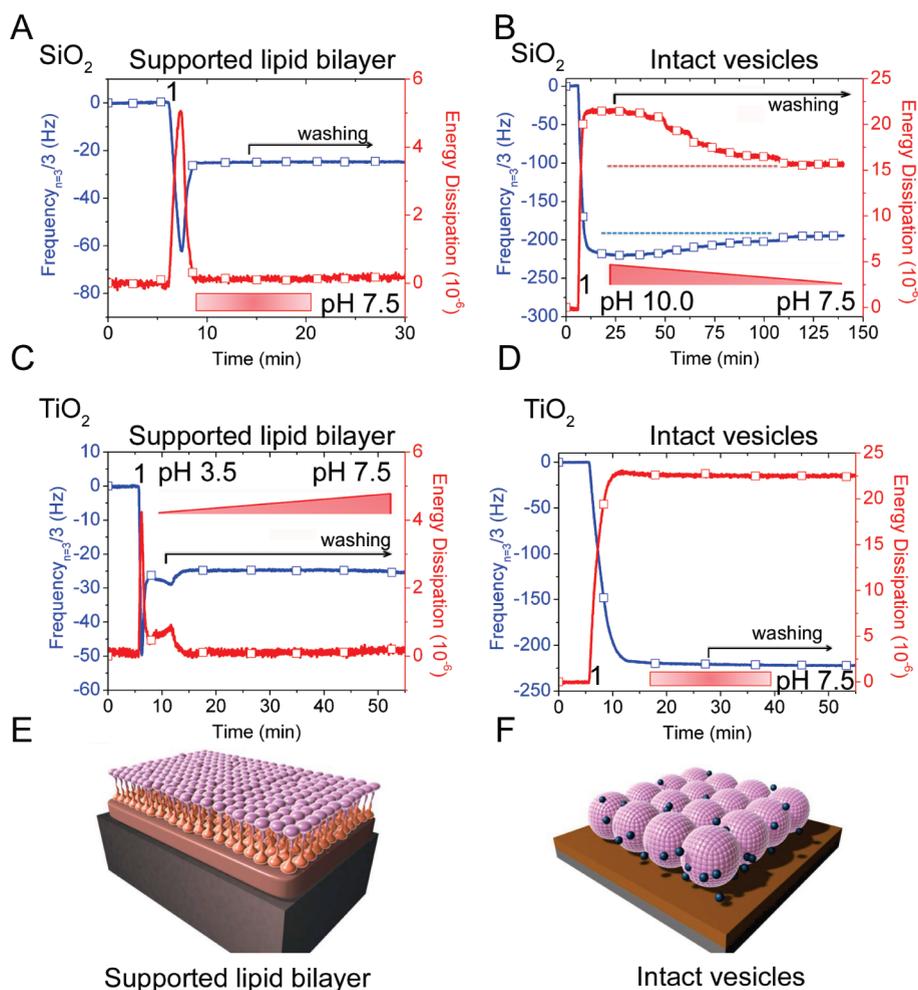


Figure 4. Alternate pathways lead to two different model membrane platforms on both silicon and titanium oxides at pH 7.5. QCM-D frequency and dissipation response kinetics for the self-assembly of four different platforms. After initial stabilization, vesicles were injected at 5 min. (A) On silicon oxide, a planar lipid bilayer was formed in two steps at pH 7.5. (B) A layer of intact vesicles first adsorbed at pH 9.5 on silicon oxide. A series of increasingly acidic buffer washes gradually lowered the pH to 7.5 (Figure S1). Note the structural rearrangement of the adsorbed vesicle layer is due to pH adjustment, as indicated by the frequency and dissipation changes. (C) A planar lipid bilayer was formed on titanium oxide at pH 2.5 before raising the pH to 7.5 as a result of a series of increasingly alkaline buffer washes. After the initial buffer wash, which removed weakly adsorbed species, the subsequent wash steps had no effect on the bilayer's mass or viscoelastic properties. (D) At pH 7.5, a layer of intact vesicles adsorbed on titanium oxide and the subsequent buffer wash had no effect on the platform's stability. Schematics of (E) a supported lipid bilayer and (F) a layer of adsorbed, intact vesicles. Note that the red bars at the bottom of panels A–D correspond to pH adjustments (in 0.5 pH unit increments) made over the measurement period. No change in the bar thickness indicates no pH adjustment. An increase in the bar thickness over time corresponds to a pH increase to the specified value. Likewise, a decrease in the bar thickness over time corresponds to a pH decrease to the specified value.

acidic buffers (in 0.5 pH unit increments) over a period of 100 min. Strikingly, the adsorbed vesicles did not rupture as the vesicle–substrate interactions became increasingly attractive when the solution pH within the measurement chamber was reduced to 7.5. However, the series of buffer washes did affect the adlayer properties; the frequency shift increased from -215 to -180 Hz, and the dissipation shift decreased from 22×10^{-6} to 16×10^{-6} . This 16.2% mass decrease and corresponding decrease in film viscoelasticity are attributed to a structural rearrangement of adsorbed vesicles. Repeated washing at one pH value did not affect the adlayer's properties, whereas the introduction of solutions with increasingly lower pH values caused the aforementioned property changes. Furthermore, the magnitudes of the property changes were significantly smaller than those previously observed for vesicle rupture within an adlayer of intact vesicles.²⁹ These observations suggest that the structural

rearrangement of the vesicle adlayer is due to morphological changes as a result of increasingly attractive vesicle–substrate interactions at lower pH.

On titanium oxide, a similar strategy based on pH adjustment after platform self-assembly was employed to form a planar bilayer at pH 7.5. At pH 2.5, the vesicle adsorption behavior displayed two-step kinetics with final frequency and dissipation shifts of -26 Hz and 0.2×10^{-6} , respectively, indicating formation of a complete, planar bilayer (Figure 4C). In order to adjust the pH of the measurement chamber to 7.5, a series of increasingly alkaline buffer solutions were introduced in 0.5 pH unit increments over a period of 100 min. These buffer exchanges had no effect on the QCM-D responses, indicating that the platform remained a complete, planar bilayer. Compared to planar bilayer formation on titanium oxide at pH 7.5, a simpler approach was taken to form a layer of intact vesicles. As seen in

Figure 4D, vesicle adsorption kinetics at pH 7.5 displayed monotonic behavior that resulted in the self-assembly of an irreversibly adsorbed layer of intact vesicles with final frequency and dissipation shifts of -230 Hz and 23×10^{-6} , respectively. Together, the results demonstrate that, while vesicle adsorption kinetics are surface-specific, pH is an appropriate tool to modulate vesicle–substrate interactions in order to control the self-assembly pathway.

DISCUSSION

Macromolecular interactions with model membranes depend critically on the architecture of the platform. Certain proteins and peptides exhibit high binding affinity to a particular model membrane platform, thereby making its use either requisite or highly advantageous. Because of the previously identified surface specificity of the lipid vesicle self-assembly pathway, only a limited number of substrates can serve as the solid support for a particular platform. For sensor applications, this substrate limitation can also restrict the type of measurement techniques that can be employed and consequently the physical properties of the model membrane that can be probed. Therefore, a better understanding of the parameters that guide the self-assembly pathway of model membrane platforms on solid supports is needed in order to control the fabrication of these structures. We have demonstrated that solution pH is an effective tool to guide the self-assembly of either a planar lipid bilayer or an intact vesicle adlayer on silicon and titanium oxides. While pH is indeed a modulator of oxide surface charge density, its role in controlling these self-assembly pathways is complex, as we discuss below. Nonetheless, the sum of these interactions can be harnessed to yield an exciting array of new platforms, which overcome intrinsic material properties and surface-specific kinetics at physiological pH. As such, the approach pursued in these studies is simple enough to become widely used for applied studies and yet is also important from a fundamental standpoint since it provides much-needed insight into the spectrum of lipid structures that can result from vesicle–substrate interactions.

Low pH Maximizes Adhesion Energy. As the surface charge density decreased with lower pH, the bilayer formation kinetics on silicon oxide approached nearly a single step starting at pH 6.0 (Figure 2A,B). Although observation of one-step kinetics does not necessarily indicate individual vesicle rupture upon adsorption, it does demonstrate that a critical degree of vesicle–vesicle interactions is not the limiting step for vesicle rupturing. It is important to note that a wide range of experimental parameters can also influence the adsorption kinetics. However, as previously discussed, all other parameters were fixed, demonstrating that, under the given experimental conditions, the shift from two-step to one-step kinetics is caused by pH effects. Vesicle–substrate interactions and the corresponding edge effect of bilayer patches are sufficient to promote vesicle rupturing, which leads to bilayer formation. The change in adsorption kinetics from two-step to one-step behavior is interesting because the zeta potential of POPC lipid vesicles asymptotes near -2 mV (effectively charge neutral) below pH 6.0,¹⁹ suggesting that charge–dipole interactions make vesicle–substrate interactions more attractive.¹⁵ Another factor that influences vesicle–substrate interactions is the degree of ionization on the silicon oxide substrate, which is reduced at lower pH.³⁰ On the basis of the combination of effectively charge-neutral vesicles and the

decreased surface charge density on the substrate, which has an isoelectric point of ~ 2 ,²⁹ repulsive electrostatic interactions are minimized with the possibility of attractive charge–dipole interactions. Therefore, van der Waals attractive interactions as well as hydration and steric forces predominate, strengthening the adhesion energy.^{8,26}

Strikingly, in contrast to the kinetics effects observed on silicon oxide, the vesicle adsorption pathway on titanium oxide was affected by lower pH. Below pH 3.0, there was a structural transformation from a layer of intact vesicles to a planar bilayer (Figure 2C). Interestingly, this occurred below the substrate's isoelectric point of $\sim 4-5$,²⁹ possibly a result of an increase in crystallinity of the titanium oxide layer at low pH.³¹ Compared to planar bilayer formation on silicon oxide where the combination of van der Waals, steric, and hydration forces is sufficient to promote bilayer formation, our data support that electrostatic attraction is also required to induce vesicle rupture on titanium oxide. Near the isoelectric point at pH 4, there was incomplete bilayer formation, as indicated by QCM-D kinetics suggestive of two-step vesicle rupturing kinetics, but with final mass and dissipation values that did not correspond to a complete, planar bilayer (Figure 2D). Thus, bilayer propagation must be influenced by electrostatic interactions in addition to the hydrophobic edge effect,⁸ at least in the case of titanium oxide. At pH 2.5, complete, planar bilayer formation was observed (Figure 2C). The substrate's positive surface charge likely played a role in promoting bilayer formation via attractive interactions between positively charged oxide groups and the slightly negatively charged vesicles. Together, the results suggest that minimizing repulsive electrostatic interactions is insufficient for propagating bilayer formation on titanium oxide. Electrostatic attraction is also necessary to promote complete bilayer formation by increasing the adhesion energy.²⁶

We identified that electrostatic interactions, which are modulated by changes in the solution pH, play a key role in controlling the self-assembly process through two parameters: kinetics and the overall pathway. On silicon oxide, minimizing repulsive electrostatic interactions can shift the bilayer formation kinetics from two-step to one-step. By contrast, pH adjustments can make electrostatic interactions between vesicles and titanium oxide shift from repulsive at higher pH to attractive at lower pH, thereby promoting the formation of a planar bilayer in the lower pH regime.³² Moreover, on silicon oxide, more alkaline environments can hinder the bilayer formation process, resulting in an irreversibly adsorbed layer of intact vesicles.

Two Types of Vesicle Adsorption at High pH. At pH 10.0, we observed two types of intact vesicle adlayers. On silicon oxide, the layer of intact vesicles was irreversibly adsorbed, as demonstrated by QCM-D frequency and dissipation values that were unaffected by a series of buffer washes (Figure 3A,C). This observation is in contrast to previous findings that zwitterionic vesicle interactions with silicon oxide under ambient temperature conditions result in either planar bilayer formation or no lipid adsorption.^{10,12,27} While these interactions generally result in planar bilayer formation under near-physiological conditions (pH ~ 7.5 , $I \sim 150$ mM), certain experimental conditions (pH ~ 12 , $I \sim 50$ mM) have been shown to result in no lipid vesicle adsorption.¹⁰ At low temperatures, Reimhult et al. showed that adsorbed vesicles can remain intact on the substrate, demonstrating that the vesicle rupture process is thermally activated.²⁹ Here, we observed another activation barrier that is related to the degree of electrostatic repulsion between vesicles

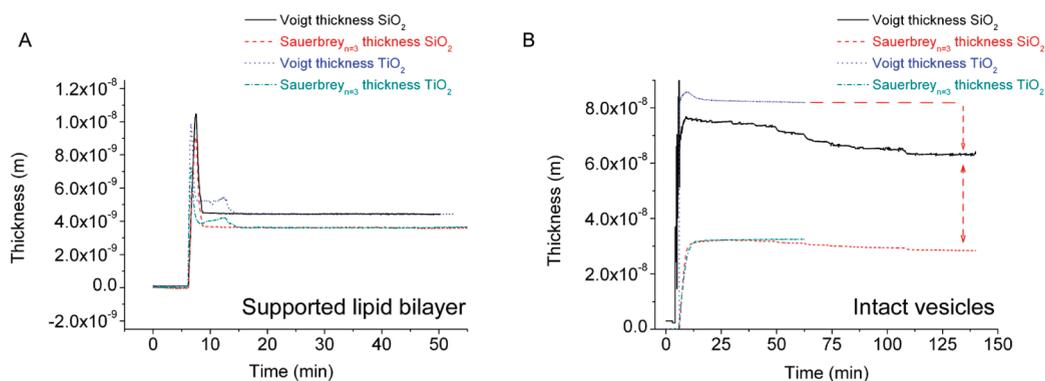


Figure 5. Viscoelastic modeling of model membrane platforms. A Voigt element-based viscoelastic model was fit to the QCM-D data in order to determine the thickness of the planar lipid bilayers and layers of intact vesicles at pH 7.5. (A) For bilayers on silicon oxide and titanium oxide, the Voigt–Voinova viscoelastic model fit resulted in identical bilayer thicknesses on both substrates. Note that the Sauerbrey relationship underestimates the bilayer thickness by 1 nm. (B) For layers of intact vesicles, the Sauerbrey relationship fails to properly capture the viscoelastic adlayer’s properties. The viscoelastic model fit indicates that the layers have similar Voigt thicknesses after their initial self-assembly. While the layer on titanium oxide was formed at pH 7.5, the layer on silicon oxide was formed at pH 10.0 (Figure 4B). Note that a series of buffer washes gradually lowered the pH of the vesicle adlayer on silicon oxide, resulting in the adlayer’s structural rearrangement, as indicated by a 13 nm decrease in the calculated Voigt thickness.

and the substrate. At higher pH, both the vesicle zeta potential (-17 mV at pH 10.0 vs -6 mV at pH 8.0) and the substrate’s negative surface charge density are increased,²¹ leading to more repulsive electrostatic interactions. Silicon oxide has a second pK_a value around pH 9;¹⁰ in this pH range, we have observed that a planar bilayer, an intact vesicle layer, or a combination of the two can form on the substrate (Figure S3). Cremer et al. also identified phase instability in this pH range at intermediate ionic strengths.¹⁰ Consequently, at pH values above this second pK_a value, where significant ionization of the substrate occurs, the adhesion energy is too low to promote vesicle rupture and spreading.³⁰

Another consideration is hydration of the substrate. The silicon oxide–buffer interface becomes more ordered with increasing pH, which creates a poor lubricant layer that does not facilitate bilayer formation.¹⁰ Nonetheless, the adhesion energy is sufficient enough so that the layer of vesicles is irreversibly adsorbed on silicon oxide at pH 10.0. By contrast, a layer of intact vesicles on titanium oxide reversibly adsorbs at pH 10.0 (Figure 3B,D). Subsequent washing affected the adlayer’s properties, as observed by mass removal as well as a decrease in energy dissipation. As is the case on silicon oxide, the pH increase results in more electrostatic repulsion that lowers the adhesion energy. Although the physical properties of the adlayers differed, the intact vesicle platform on each substrate displayed similar adsorption kinetics as well as initial mass and viscoelastic properties upon self-assembly. However, the two platforms showed different physical stabilities when washed with buffer solution. Thus, while lipid vesicles followed the same self-assembly process on both substrates at pH 10.0, the intact vesicle layers’ properties were surface-specific. We sought to further understand how lipid platform properties varied on the two substrates by employing a viscoelastic model to better capture the film properties.

Flattening of Adsorbed, Intact Vesicles on Silicon Oxide.

In Figure 5, the QCM-D frequency and dissipation responses from Figure 4 were fit to the Sauerbrey and Voigt–Voinova models, the latter of which captures the interaction of soft, intact vesicles with the substrate, in order to better understand the adlayers’ properties. The calculated thicknesses are in good agreement for planar lipid bilayer formation on both substrates

(Figure 5A). While the two models provide comparable results for the two bilayers, confirming that the planar bilayers formed on both substrates have similar mass, thickness, and viscoelastic properties in agreement with past results, there is a significant deviation between the models for the more viscoelastic adlayer of intact vesicles since only the Voigt model accounts for the viscoelastic effects on the QCM-D responses. The calculated thickness of the intact vesicle platform on titanium oxide at pH 7.5 varied between the two models (Figure 5B). The Sauerbrey thickness was 33 nm, whereas the Voigt thickness was 84 nm for the vesicle adlayer on titanium oxide. The initial thicknesses of the intact vesicle platform on silicon oxide at pH 10.0 (prior to pH adjustment) were in good agreement with these values; the Sauerbrey thickness was 33 nm, whereas the Voigt thickness was 75 nm. The difference in Voigt thicknesses is likely due to increased vesicle flattening (i.e., less coupled solvent per area) on silicon oxide.

Strikingly, as the pH of the intact vesicle platform on silicon oxide was adjusted from 10.0 to 7.5, the Voigt thickness decreased by 12 nm, while the Sauerbrey thickness only decreased by 3 nm. While there was a 10.5% decrease in frequency, there was only a 26.2% decrease in energy dissipation. However, the shear modulus of the film only decreased from 9.1 to 8.9 kPa, demonstrating that the adlayer retains its viscoelastic properties, which change only slightly as a result of the structural rearrangement process induced by pH changes. As the pH of the silicon oxide substrate was lowered to 7.5, the surface charge density decreased, resulting in more attractive electrostatic interactions between vesicles and the substrate. This increased the adhesion energy as well as the vesicle membrane tension.²⁶ However, the QCM-D kinetics demonstrates that the adhesion energy was not strong enough to promote vesicle rupture. Rather, minor changes in the QCM-D responses were observed during each pH adjustment as the adlayer thickness decreased due to increased vesicle flattening brought upon by more attractive vesicle–substrate interactions.²⁵ Steric forces may also play a role in this structural rearrangement process.⁸ As vesicles become more flattened, it is possible that some vesicles desorb from the substrate due to packing constraints. Nonetheless, the vesicle adlayer maintains its physical properties, and its stability permits its use as a sensing platform.

CONCLUSION

The self-assembly of lipid platforms on solid supports is governed by a wide range of parameters. Here, we focused on the effects of pH on vesicle adsorption kinetics and the self-assembly pathway. By modulating pH, the silicon and titanium oxide substrates had varying surface charge densities, which enabled the self-assembly of one of two platforms, a layer of intact vesicles or a planar lipid bilayer, in a controlled fashion. Depending on the substrate, we determined that the role of electrostatic interactions varies for bilayer formation. Furthermore, repulsive electrostatic interactions led to the self-assembly of intact vesicle adlayers on both substrates at alkaline pHs. After self-assembly of the desired lipid platform at the appropriate pH, we demonstrated that the pH could be adjusted to physiological levels in order to use the platform for biological studies, while maintaining the platform's structural integrity. This study is the first demonstration of the self-assembly of two lipid platforms with significantly different viscoelastic properties on silicon and titanium oxides under identical experimental conditions. We foresee that the ability to tune surface-specific properties in order to promote self-assembly of a desired lipid platform will lead to the design of improved platforms for a wide range of biosensing and biomaterial applications.

ASSOCIATED CONTENT

S **Supporting Information.** Figures S1–S4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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REFERENCES

- (1) Hook, F.; Kasemo, B.; Grunze, M.; Zauscher, S. Quantitative Biological Surface Science: Challenges and Recent Advances. *ACS Nano* **2008**, *2* (12), 2428–2436.
- (2) Kanazawa, K. K.; Cho, N.-J. Quartz Crystal Microbalance as a Sensor to Characterize Macromolecular Assembly Dynamics. *J. Sens.* **2009**.
- (3) Sackmann, E. Supported membranes: Scientific and practical applications. *Science* **1996**, *271* (5245), 43–48.
- (4) Tamm, L. K.; McConnell, H. M. Supported phospholipid bilayers. *Biophys. J.* **1985**, *47* (1), 105–13.
- (5) Kunze, A.; Sjoval, P.; Kasemo, B.; Svedhem, S. In situ preparation and modification of supported lipid layers by lipid transfer from vesicles studied by QCM-D and TOF-SIMS. *J. Am. Chem. Soc.* **2009**, *131* (7), 2450–1.
- (6) Kunze, A.; Svedhem, S.; Kasemo, B. Lipid transfer between charged supported lipid bilayers and oppositely charged vesicles. *Langmuir* **2009**, *25* (9), 5146–58.
- (7) Rossetti, F. F.; Textor, M.; Reviakine, I. Asymmetric distribution of phosphatidyl serine in supported phospholipid bilayers on titanium dioxide. *Langmuir* **2006**, *22* (8), 3467–73.
- (8) Anderson, T. H.; Min, Y.; Weirich, K. L.; Zeng, H.; Fygenon, D.; Israelachvili, J. N. Formation of supported bilayers on silica substrates. *Langmuir* **2009**, *25* (12), 6997–7005.
- (9) Reimhult, E.; Hook, F.; Kasemo, B. Temperature dependence of formation of a supported phospholipid bilayer from vesicles on SiO₂. *Phys. Rev. E* **2002**.
- (10) Cremer, P. S.; Boxer, S. G. Formation and spreading of lipid bilayers on planar glass supports. *J. Phys. Chem. B* **1999**, *103* (13), 2554–2559.
- (11) Reimhult, E.; Hook, F.; Kasemo, B. Vesicle adsorption on SiO₂ and TiO₂: Dependence on vesicle size. *J. Chem. Phys.* **2002**, *117* (16), 7401–7404.
- (12) Keller, C. A.; Kasemo, B. Surface specific kinetics of lipid vesicle adsorption measured with a quartz crystal microbalance. *Biophys. J.* **1998**, *75* (3), 1397–1402.
- (13) Kasemo, B.; Lausmaa, J. Aspects of surface physics on titanium implants. *Swed. Dent. J. Suppl.* **1985**, *28*, 19–36.
- (14) Keller, C. A.; Glasmaster, K.; Zhdanov, V. P.; Kasemo, B. Formation of supported membranes from vesicles. *Phys. Rev. Lett.* **2000**, *84* (23), 5443–6.
- (15) 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* (4), 1270–4.
- (16) Johnson, J. M.; Ha, T.; Chu, S.; Boxer, S. G. Early steps of supported bilayer formation probed by single vesicle fluorescence assays. *Biophys. J.* **2002**, *83* (6), 3371–9.
- (17) Richter, R. P.; Brisson, A. R. Following the formation of supported lipid bilayers on mica: a study combining AFM, QCM-D, and ellipsometry. *Biophys. J.* **2005**, *88* (5), 3422–33.
- (18) Schonherr, H.; Johnson, J. M.; Lenz, P.; Frank, C. W.; Boxer, S. G. Vesicle adsorption and lipid bilayer formation on glass studied by atomic force microscopy. *Langmuir* **2004**, *20* (26), 11600–6.
- (19) Cho, N. J.; Frank, C. W. Fabrication of a planar zwitterionic lipid bilayer on titanium oxide. *Langmuir* **2010**, *26* (20), 15706–15710.
- (20) Mayer, L. D.; Hope, M. J.; Cullis, P. R. Vesicles of variable sizes produced by a rapid extrusion procedure. *Biochim. Biophys. Acta, Biomembr.* **1986**, *858* (1), 161–168.
- (21) Ichimori, H.; Hata, T.; Matsuki, H.; Kaneshina, S. Effect of unsaturated acyl chains on the thermotropic and barotropic phase transitions of phospholipid bilayer membranes. *Chem. Phys. Lipids* **1999**, *100* (1–2), 151–164.
- (22) Sauerbrey, G. Verwendung Von Schwingquarzen Zur Wagung Dunner Schichten Und Zur Mikrowagung. *Z. Phys.* **1959**, *155* (2), 206–222.
- (23) Voinova, M. V.; Jonson, M.; Kasemo, B. Missing mass effect in biosensor's QCM applications. *Biosens. Bioelectron.* **2002**, *17* (10), 835–41.
- (24) Rodahl, M.; Hook, F.; Fredriksson, C.; Keller, C. A.; Krozer, A.; Brzezinski, P.; Voinova, M.; Kasemo, B. Simultaneous frequency and dissipation factor QCM measurements of biomolecular adsorption and cell adhesion. *Faraday Discuss.* **1997**, No. 107, 229–246.
- (25) Dimitrievski, K.; Kasemo, B. Influence of Lipid Vesicle Composition and Surface Charge Density on Vesicle Adsorption Events: A Kinetic Phase Diagram. *Langmuir* **2009**, *25* (16), 8865–8869.
- (26) Seifert, U.; Lipowsky, R. Adhesion of vesicles. *Phys. Rev. A* **1990**, *42* (8), 4768.
- (27) Seantier, B.; Breffa, C.; Felix, O.; Decher, G. Dissipation-Enhanced Quartz Crystal Microbalance Studies on the Experimental Parameters Controlling the Formation of Supported Lipid Bilayers. *J. Phys. Chem. B* **2005**, *109* (46), 21755–21765.
- (28) Cho, N.-J.; Frank, C. W.; Kasemo, B.; Hook, F. Quartz crystal microbalance with dissipation monitoring of supported lipid bilayers on various substrates. *Nature Protocols* **2010**, *5* (6), 1096–1106.
- (29) 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* **2002**, *19* (5), 1681–1691.

(30) Ong, S. W.; Zhao, X. L.; Eiseenthal, K. B. Polarization of water-molecules at a charged interface - 2nd harmonic studies of the silica water interface. *Chem. Phys. Lett.* **1992**, *191* (3–4), 327–335.

(31) Penn, R. L.; Banfield, J. F. Morphology development and crystal growth in nanocrystalline aggregates under hydrothermal conditions: insights from titania. *Geochim. Cosmochim. Acta* **1999**, *63* (10), 1549–1557.

(32) Fortunelli, A.; Monti, S. Simulations of Lipid Adsorption on TiO₂ Surfaces in Solution. *Langmuir* **2008**, *24* (18), 10145–10154.