

# Influence of Osmotic Pressure on Adhesion of Lipid Vesicles to Solid Supports

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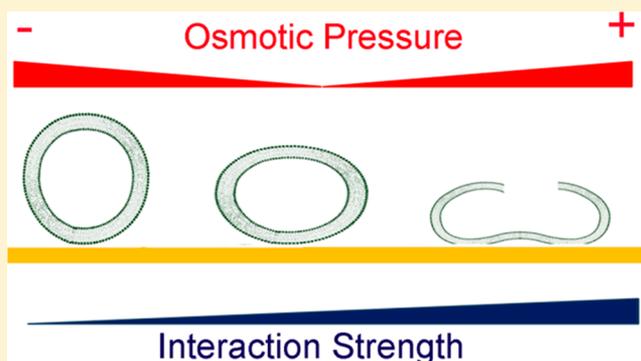
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## S Supporting Information

**ABSTRACT:** The adhesion of lipid vesicles to solid supports represents an important step in the molecular self-assembly of model membrane platforms. A wide range of experimental parameters are involved in controlling this process, including substrate material and topology, lipid composition, vesicle size, solution pH, ionic strength, and osmotic pressure. At present, it is not well understood how the magnitude and direction of the osmotic pressure exerted on a vesicle influence the corresponding adsorption kinetics. In this work, using quartz crystal microbalance with dissipation (QCM-D) monitoring, we have experimentally studied the role of osmotic pressure in the adsorption of zwitterionic vesicles onto silicon oxide. The osmotic pressure was induced by changing the ionic strength of the solvent across an appreciably wider range (from 25 to 1000 mM NaCl outside of the vesicle, and 125 mM NaCl inside of the vesicle, unless otherwise noted) compared to that used in earlier works. Our key finding is demonstration that, by changing osmotic pressure, all three generic types of the kinetics of vesicle adsorption and rupture can be observed in one system, including (i) adsorption of intact vesicles, (ii) adsorption and rupture after reaching a critical vesicle coverage, and (iii) rupture just after adsorption. Furthermore, theoretical analysis of pressure-induced deformation of adsorbed vesicles and a DLVO-type analysis of the vesicle–substrate interaction qualitatively support our observations. Taken together, the findings in this work demonstrate that osmotic pressure can either promote or impede the rupture of adsorbed vesicles on silicon oxide, and offer experimental evidence to support adhesion energy-based models that describe the adsorption and spontaneous rupture of vesicles on solid supports.



## INTRODUCTION

Cellular membranes are one of the most important components of biological systems, and are involved in many different processes and/or functions, including cell protection, cell–cell communication, and signaling.<sup>1–3</sup> In light of the membrane's complexity, there is interest in developing model systems that mimic the fundamental properties of membranes while offering a simplified platform for basic and applied research.<sup>4–6</sup> Among the various model membrane systems, solid-supported lipid assemblies are particularly useful because they are based on a robust sensing platform and are compatible with a wide range of surface-sensitive techniques for analytical characterization.<sup>7</sup> Many different types of solid-supported lipid assemblies exist including a lipid monolayer, planar lipid bilayer, tethered lipid bilayer, intact vesicle layer, and tethered vesicles. Depending on the application, each platform has its own particular advantages, and a common theme in the field is to identify robust methods to produce model membranes.

While early model membrane systems were typically fabricated by the Langmuir–Blodgett transfer process,<sup>8</sup> molecular self-assembly has more recently become the main fabrication strategy.<sup>9</sup> Self-assembly is based on the adsorption of vesicles onto solid supports,<sup>10,11</sup> and understanding of various self-assembly pathways has been aided by real-time monitoring with surface-sensitive tools including quartz crystal microbalance with dissipation monitoring (QCM-D),<sup>12</sup> electrochemical impedance spectroscopy,<sup>13</sup> ellipsometry,<sup>14</sup> and surface plasmon resonance.<sup>15</sup> On a wide range of hydrophilic substrates such as titanium oxide<sup>16</sup> and gold,<sup>12</sup> vesicles adsorb and remain stably intact, resulting in the formation of a layer of surface-bound vesicles. In contrast, on other hydrophilic substrates such as silicon oxide<sup>12</sup> and mica,<sup>17</sup> vesicles typically adsorb until reaching a critical vesicle coverage and then rupture to form a

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planar bilayer. Likewise, on hydrophobic substrates such as self-assembled alkanethiol monolayers on gold, vesicles spontaneously fuse to form a lipid monolayer.<sup>18</sup> While surface-sensitive techniques have been able to identify these different self-assembly pathways, detailed interpretation of the kinetics of vesicle adsorption accompanied by rupture is not straightforward.

In general, the rupturing process is governed by the combination of vesicle–substrate, vesicle–vesicle, and/or vesicle–rupture product interactions taken into consideration along with the membrane tension of the adsorbed vesicles.<sup>19,20</sup> Vesicle rupture may occur via a few channels including the spontaneous one, vesicle fusion followed by rupture, and rupture induced by the boundaries of the lipid bilayer islands formed on a substrate after rupture.<sup>20–25</sup> In cases of strong vesicle–substrate interactions, rupturing may commence upon the adsorption of individual vesicles and in the absence of vesicle–vesicle interactions.<sup>22</sup> For cases with weaker vesicle–substrate interactions, both vesicle–substrate and vesicle–vesicle interactions may be necessary to promote rupturing.<sup>22</sup> The relative role of these channels depends not only on surface-specific properties (including topology<sup>26</sup>) but also on a wide range of experimental parameters related to vesicle preparation and solution conditions. Critical parameters include vesicle size,<sup>27</sup> lipid composition,<sup>28</sup> solution pH,<sup>29</sup> ionic strength,<sup>30</sup> and divalent cations.<sup>31</sup> More recently, the osmotic pressure<sup>27</sup> exerted on a vesicle has been identified as a key parameter that can influence the formation kinetics of a planar bilayer on a solid support.

Osmotic pressure is caused by a difference in the solute concentrations in the two regions separated by a lipid bilayer, as defined by

$$\Delta P = (c_2 - c_1)k_B T \quad (1)$$

If this pressure is induced by a difference in ionic strength (e.g., caused by NaCl concentration), the concentrations  $c_1$  and  $c_2$  should be represented as a sum of the concentrations of both ions. The sign of osmotic pressure can be chosen arbitrarily. In our presentation, we consider that osmotic pressure is either positive or negative if the concentration is higher or lower outside of a vesicle, respectively. Positive osmotic pressure causes vesicle compression whereby the volume of the vesicle decreases in order to equilibrate the solute concentration inside and outside of a vesicle.<sup>32</sup> As a result of vesicle deformation, bilayer bending concomitantly increases and causes membrane destabilization that is expected to decrease the barrier for vesicle rupture.<sup>27</sup> By contrast, negative osmotic pressure promotes expansion of a vesicle, which may counter volume changes associated with deformation.<sup>32</sup> While the balance of adhesion energy between vesicle–substrate interactions and vesicle stretching and bending energies is relatively well characterized, less understood is the degree to which osmotic pressure and related parameters such as ionic strength in the bulk solution can influence the adhesion energy and corresponding adsorption kinetics.

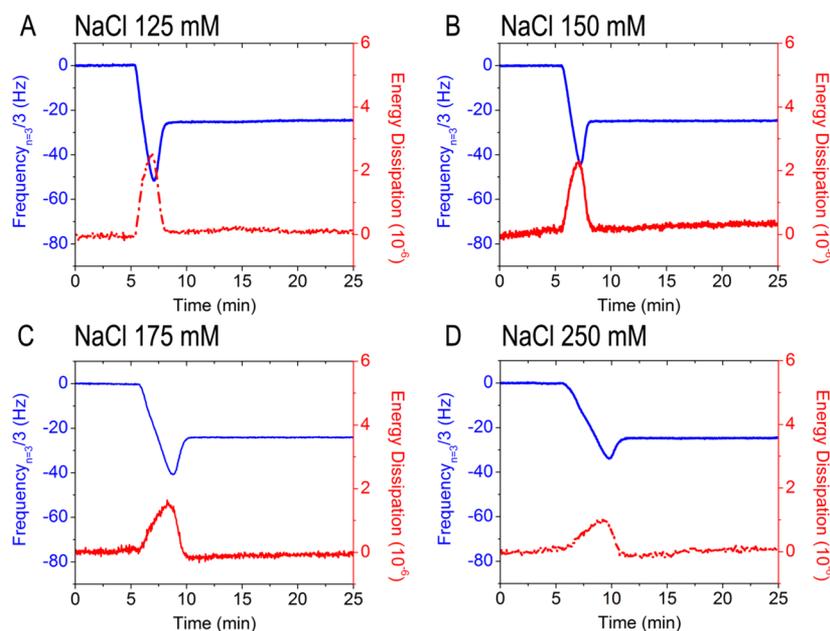
Early work by Cremer et al.<sup>33</sup> investigated the influence of pH and ionic strength on the interaction of zwitterionic vesicles with a glass surface. Ionic strength conditions were varied between 0 and 80 mM NaCl and it was observed by fluorescence recovery after photobleaching (FRAP) that the rupture of vesicles generally occurred under higher ionic strength and lower pH conditions. This work not only demonstrated that ionic strength-related parameters are

important determinants of how vesicles interact with solid supports, but also proposed a mechanistic explanation. An attractive van der Waals force was understood to modulate the vesicle rupturing process, with electrostatic forces serving to either enhance or oppose the vesicle–substrate interaction. From this initial work on ionic strength conditions, attention was drawn to understanding the effects of osmotic pressure on the adsorption kinetics of zwitterionic vesicles onto solid supports by using label-free measurement tools such as the QCM-D technique.

Reimhult, Höök, and Kasemo<sup>27</sup> studied vesicle–substrate interactions on gold and silicon oxide, and employed zwitterionic vesicles with 150 mM NaCl salt solution inside of the vesicles and a varying ionic strength of NaCl in the bulk solution that ranged from 115 to 300 mM. On gold, the vesicles remained intact after adsorption, and the effect of the changes in osmotic pressure on the corresponding kinetics was minor. On silicon oxide, as expected, vesicle rupture and formation of a planar bilayer were observed after reaching a critical coverage of adsorbed vesicles. Importantly, these findings established that the time scale of the vesicle adsorption and rupturing processes decreased appreciably with increasingly positive osmotic pressure. Moreover, it was shown that the kinetic effects occurred primarily due to osmotic compression, and not solely due to osmotic stress which can be related to either compression or expansion of a vesicle.

Building on this work, Seantier et al.<sup>34</sup> examined the influence of buffer composition on the adsorption kinetics of zwitterionic vesicles onto silicon oxide. In this case, the ionic strength inside and outside of the vesicles was equivalent and hence there was no osmotic pressure before adsorption. In the presence of 150 mM NaCl salt, vesicles initially adsorbed until reaching a critical coverage and then ruptured to form a planar bilayer. In the absence of NaCl salt, vesicles also adsorbed on silicon oxide, but the rupturing process did not require a critical coverage and occurred presumably through a different pathway. A related study by Boudard et al.<sup>30</sup> further characterized the effects of NaCl concentration and identified the NaCl concentration ranges in which the various pathways of planar bilayer formation occur. Collectively, these results established a correlation between ionic strength and the kinetics of vesicle adsorption and rupturing on silicon oxide. In relation to the subject under consideration, these findings suggest that the effects of osmotic pressure can be at least be partially understood through how concomitant changes in ionic strength influence vesicle–substrate and vesicle–vesicle interactions.

The adsorption of vesicles onto solid supports has also been proposed to induce or modulate an osmotic stress. This behavior has been previously suggested by theory based on the change in the internal volume associated with deformation of an adsorbed, single vesicle.<sup>35</sup> However, the effects of vesicle deformation on the rupturing process are not well understood experimentally. Recently, Hain et al.<sup>36</sup> investigated how osmotic effects influence the kinetics of the adsorption and rupture of zwitterionic vesicles on silicon oxide. The osmotic pressure was induced by the adsorption process during which a vesicle deforms, resulting in a decrease in the internal volume while the number of ions inside of the vesicle remains the same. The authors observed that impermeable solutes inside of vesicles have a limited effect on vesicle rupturing by marginally slowing down the process due to a negative osmotic pressure buildup. By contrast, permeable solutes, and/or methods to increase appreciably the permeability of vesicles, increase the rate of



**Figure 1.** Positive osmotic pressure is a promoter of vesicle rupture on silicon oxide. The adsorption of lipid vesicles onto silicon oxide was investigated as a function of external NaCl concentration at (A) 125 mM, (B) 150 mM, (C) 175 mM, and (D) 250 mM. QCM-D monitoring was employed to capture the adsorption kinetics in terms of changes in resonance frequency (blue) and energy dissipation (red). After establishing a baseline signal, vesicles were added at 5 min and, in all cases, resulted in adsorption and rupture to form a planar lipid bilayer.

vesicle rupturing because solutes can cross the membrane and reduce the negative osmotic pressure buildup associated with vesicle deformation. Hence, it was demonstrated that the kinetics of vesicle rupture to form a planar bilayer is related to the deformation of adsorbed vesicles by way of osmotic effects.

Taken together, the previous results<sup>27,30,34,36</sup> suggest that osmotic pressure may play a complex role in modulating the formation of planar bilayers on solid supports. And yet, it is still not fully clear how the magnitude and direction of the osmotic gradient may contribute to vesicle adsorption kinetics alongside the other forces which constitute the adhesion energy. Depending on the direction of the osmotic gradient, theory predicts that osmotic pressure may either favor or oppose the volume changes associated with deformation of adsorbed vesicles on solid supports.<sup>32,37</sup> To contribute to this area, we have performed QCM-D measurements that expand upon previously reported studies.<sup>27,30,34,36</sup> The key new ingredient of our work is that we have used positive and negative osmotic pressures across a much wider range (the external NaCl concentration was varied from 25 to 1000 mM while the NaCl concentration inside of the vesicles remained at 125 mM unless otherwise noted). With this extension, we have found new kinetic regimes compared to those observed earlier.<sup>27,30,34,36</sup> The experimental results have been complemented by theoretical analysis clarifying the physics behind the process, and our findings support adhesion energy-based models that describe the adsorption and spontaneous rupture of vesicles on solid supports.

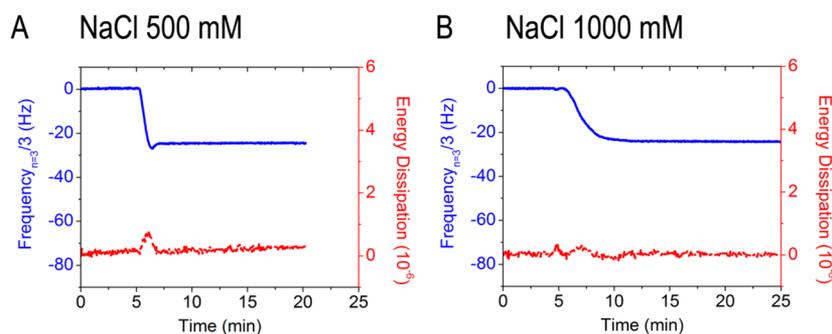
## MATERIALS AND METHODS

**Vesicle Preparation.** Small unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Avanti Polar Lipids, Alabaster, AL) were prepared by the extrusion method.<sup>38</sup> After mixing to the desired molar ratio 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<sup>-1</sup>. Following a minimum of five hours in a vacuum desiccator to remove

any residual chloroform, the lipid film was hydrated in Tris buffer (10 mM Tris and 125 mM NaCl, pH 7.7), and then vortexed for 5 min to form multilamellar vesicles. Afterward, the vesicles were subsequently sized by a miniextruder (Avanti Polar Lipids) through a series of track-etched polycarbonate membranes (a minimum of 19 times per membrane) with progressively smaller pore diameters of 100, 50, and finally 30 nm.<sup>38</sup> The resulting small, unilamellar vesicles were diluted immediately before experiment in buffer solution at the appropriate ionic strength, and were generally used within 24 h of preparation. Based on the permeability coefficients of Na<sup>+</sup> and Cl<sup>-</sup> ions, it is unlikely that equilibration of the osmotic stress would occur during the experimental time period.<sup>27,39</sup> All solutions were prepared in 18.2 MΩ·cm Milli-Q water (MilliPore, Billerica, MA).

**Dynamic Light Scattering.** A 90Plus particle size analyzer (Brookhaven Instruments) with a 658.0 nm monochromatic laser was employed to measure the size distribution of extruded vesicles. In order to minimize the reflection effect, the scattering angle was set at 90°. The data was collected by digital autocorrelator software, and all autocorrelation functions were analyzed by CONTIN and non-negatively constrained least-squares algorithms to check for multimodal distributions. The average diameter of vesicles used in the study was determined to be ca. 55 nm with polydispersity less than 0.1, which is in agreement with literature values.<sup>40,41</sup>

**Quartz Crystal Microbalance-Dissipation (QCM-D).** Adsorption kinetics and the properties of the adsorbed layer were monitored *in situ* using a Q-Sense E4 (Q-Sense AB, Gothenburg, Sweden), as described elsewhere.<sup>42</sup> The E4 system allows for the simultaneous measurement of resonance frequency and energy dissipation changes for four individually mounted quartz crystals. AT-cut crystals (Q-Sense) with 14 mm diameter and 50 nm thermally evaporated silicon oxide coats were used for all QCM-D experiments. Prior to experiment, each sensor crystal was treated with oxygen plasma at 80 W for 5 min (March Plasmod Plasma Etcher, March Instruments, California). 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 the frequency and dissipation changes at 5, 15, 25, 35, 45, and 55 MHz. The data presented in the main text was recorded at the third overtone (15 MHz) and normalized based on the overtone number. QCM-D data analysis was performed using the Sauerbrey and Voigt models, as



**Figure 2.** Osmotic pressure can induce single vesicle rupturing. The adsorption of lipid vesicles onto silicon oxide was investigated as a function of external NaCl concentration at (A) 500 mM and (B) 1000 mM. QCM-D monitoring was employed to capture the adsorption kinetics in terms of changes in resonance frequency (blue) and energy dissipation (red). After establishing a baseline signal, vesicles were added at 5 min and resulted in rupture immediately after adsorption.

described elsewhere.<sup>43</sup> In principle, the adsorption of rigid films should follow overtone-independent kinetic profiles while the adsorption of soft, viscoelastic films should follow overtone-dependent kinetic profiles.<sup>44</sup> For Voigt modeling of viscoelastic adlayers that is described in the Supporting Information, the QCM-D signals at the fifth, seventh, ninth, and eleventh overtones were employed. The temperature of the measurement cell was 25.0 °C with an in-cell Peltier element ensuring thermal fluctuations no larger than  $\pm 0.5$  °C.

## RESULTS

**Osmotic Pressure is a Promoter of Vesicle Rupture on Silicon Oxide.** To confirm the formation of a planar bilayer on silicon oxide, the adsorption of 55-nm diameter POPC lipid vesicles in 125 mM NaCl external solution was observed by QCM-D monitoring (Figure 1A). Since the NaCl concentration inside of the vesicle was identical to that of the external solution, there was no osmotic pressure exerted on the vesicle in this case. Upon vesicle adsorption, there was a change in resonance frequency of  $-52$  Hz before reaching the critical coverage of vesicles, which is identified by a minimum in the resonance frequency. Adsorbed vesicles then ruptured, resulting in the formation of a planar bilayer that corresponded to changes in resonance frequency and energy dissipation of  $-24$  Hz and  $0.1 \times 10^{-6}$ , respectively. This line of evidence suggests that osmotic pressure is not necessary to promote the rupture of adsorbed vesicles on solid supports. To show how osmotic pressure is expected (according to earlier studies<sup>27,30,34,36</sup>) to influence vesicle adsorption kinetics, the external NaCl concentration was increased while the encapsulated solvent inside of the vesicles remained at 125 mM NaCl. In turn, the resonance frequency at which the critical coverage was reached decreased (Figure 1B-D). In 250 mM NaCl external solution, the critical coverage was reached at  $-35$  Hz, indicating that significantly less vesicle mass is required to initiate rupturing at higher positive osmotic pressures. Within this regime of external NaCl concentration (125–250 mM), planar bilayers formed in all cases, as indicated by changes in resonance frequency and energy dissipation of  $-24$  Hz and less than  $0.1 \times 10^{-6}$ , respectively.

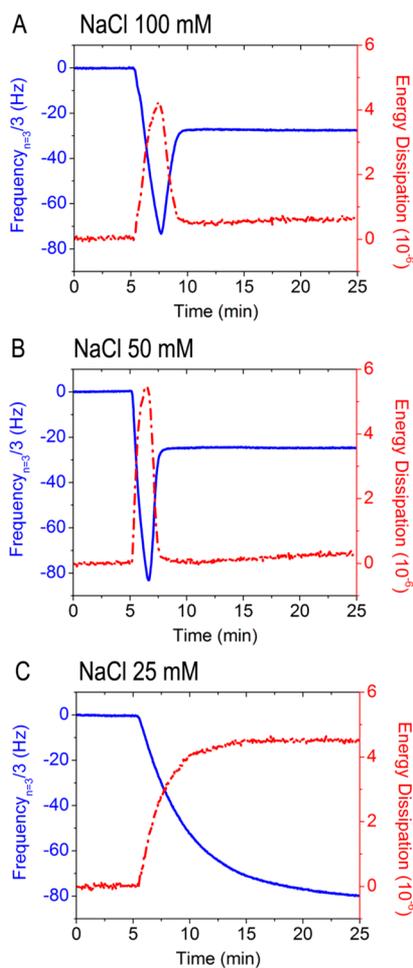
As expected in this regime,<sup>27,30,34,36</sup> vesicles adsorb until reaching a critical coverage (i.e., the surface concentration of vesicles approximately corresponding to a minimum in the resonance frequency) and then rupture to form a planar bilayer. In relation to the differences in mass load of vesicles at the critical coverage, the time to reach the critical coverage was longer in the presence of greater positive osmotic pressure (cf. Figures 1A and D; note also that the effect is weak and that,

with a further increase in osmotic pressure, the time becomes shorter as shown below in Figures 2A-B). This effect may be due to vesicles changing shape in order to equilibrate the ion concentrations inside and outside of the vesicles.<sup>45</sup> In this equilibration process, the vesicle volume would be expected to decrease and, in turn, the longest axis of the vesicle would increase.<sup>46</sup> Hence, the effective hydrodynamic radius of the vesicle would be larger, which would slow down the vesicle's diffusion in bulk solution.

As described in the Supporting Information, a relationship between the time at which rupture occurs and the mass load of vesicles at the critical coverage can be established if one also considers the size of adsorbed vesicles. However, in this model, there are three variables which are dependent on osmotic pressure, and their relative contributions can hardly be determined. For this reason, we restrict our kinetic analysis to qualitative comparisons. Overall, the adsorption profiles support that the vesicles follow diffusion-limited kinetics and that variations in the critical coverage are likely related to the effects of osmotic pressure on vesicle shape, as discussed further below. Collectively, these results suggest that positive osmotic pressure can have a promoting effect on vesicle rupture.

**Osmotic Pressure Can Induce Single Vesicle Rupturing.** We next further increased the external NaCl concentration to amplify the effect of osmotic pressure on vesicle adsorption kinetics. At 500 mM NaCl concentration, the critical coverage was reached quickly and corresponded to changes in resonance frequency and energy dissipation of  $-25$  Hz and  $0.5 \times 10^{-6}$ , respectively (Figure 2A). After formation of the planar bilayer, the final changes in resonance frequency and energy dissipation were  $-24$  Hz and  $0.2 \times 10^{-6}$ , respectively. These negligible differences between the QCM-D measurement values at the critical coverage and upon formation of a planar bilayer suggest that vesicle-vesicle interactions play only a minor role in the rupturing process. Indeed, at 1000 mM NaCl concentration, vesicles ruptured immediately to form a planar bilayer and there is no minimum observed in either the QCM-D resonance frequency or energy dissipation signal (Figure 2B). Since the rupture of single, adsorbed vesicles is the primary channel of bilayer formation in this regime, we do not interpret the kinetics in terms of critical coverage. Based on these observations, we conclude that, at very high positive osmotic pressures, single vesicles can rupture upon adsorption (this regime was not observed earlier<sup>27,30,34,36</sup>). As such, osmotic pressure can affect not only the kinetics of planar bilayer formation, but also the pathway by which vesicles rupture.

**Negative Osmotic Pressure Stabilizes Adsorbed Vesicles.** We next investigated the effects of changing the direction of the osmotic pressure gradient. Under negative osmotic pressure conditions, vesicle adsorption kinetics in 100 mM NaCl external solution (with encapsulated solvent inside of the vesicle remaining at 125 mM NaCl concentration) indicated that vesicles adsorb intact onto the substrate until reaching a critical coverage and then rupture (Figure 3A).



**Figure 3.** Negative osmotic pressure stabilizes adsorbed vesicles. The adsorption of lipid vesicles onto silicon oxide was investigated as a function of external NaCl concentration at (A) 100 mM, (B) 50 mM, and (C) 25 mM. QCM-D monitoring was employed to capture the adsorption kinetics in terms of changes in resonance frequency (blue) and energy dissipation (red). After establishing a baseline signal, vesicles were added at 5 min and resulted in either adsorption and rupture after reaching a critical vesicle coverage, or intact vesicle adsorption.

Similar to the data obtained under mildly positive osmotic pressure conditions (up to 150 mM NaCl external solution), the critical coverage was reached quickly and corresponded to a change in resonance frequency of  $-74$  Hz. Further decreasing the external ionic strength to 50 mM NaCl solution also resulted in comparable adsorption kinetics, albeit with the critical coverage in this case corresponding to a change in resonance frequency of  $-83$  Hz (Figure 3B). Again, these findings are consistent with previous observations<sup>27,30,34,36</sup> that positive osmotic pressures increase vesicle spreading while negative osmotic pressures hinder spreading.

Upon closer inspection, the time corresponding to the critical coverage was reached more quickly in low external NaCl concentrations, as compared to high external NaCl concentrations. These kinetic are caused probably by the negative osmotic pressure, namely because such conditions do not cause deformation of the vesicles. Therefore, there would be no increase in the effective hydrodynamic radius of vesicles with a negative osmotic pressure. By contrast, vesicles with a positive osmotic pressure would deform and have a greater effective hydrodynamic radius. As a result, vesicles with a negative osmotic pressure would presumably be smaller and diffuse more quickly in the bulk solution. This effect is consistent with the observed adsorption kinetics.

We also performed an additional experiment to measure the adsorption kinetics of vesicles in 25 mM NaCl external solution (Figure 3C). In this condition, we did not observe formation of a planar bilayer but rather the monotonic adsorption of lipid vesicles onto silicon oxide (this regime was not observed earlier<sup>27,30,34,36</sup>). Final changes in resonance frequency and energy dissipation corresponded to  $-82$  Hz and  $4.5 \times 10^{-6}$ , respectively. Voigt modeling was performed to estimate the contrast ratio of the adsorbed vesicles (Figure S4). We assumed the adsorbed vesicles constitute a uniform adlayer and calculated that the 55 nm diameter vesicles deform upon adsorption, resulting in a soft film that is at least 12 nm thick. This height range is consistent with a previous atomic force microscopy study reported by Schönherr et al.,<sup>47</sup> which investigated the adsorption of similarly sized vesicles on glass under low surface coverage conditions. In such conditions, the authors showed that vesicles adsorb and remain intact on glass, albeit while deforming and flattening to heights ranging between 5 and 22 nm.<sup>47</sup>

## DISCUSSION

The experimental findings indicate that osmotic pressure can affect both the thermodynamics and kinetics of planar bilayer formation. Three different types of self-assembly pathways were observed, including (i) adsorption of intact vesicles, (ii) adsorption and rupture after reaching a critical vesicle coverage, and (iii) rupture just after adsorption. Positive osmotic pressures promoted formation of a bilayer while negative osmotic pressures hindered this process. In general, bilayer formation occurs via vesicle rupture. As already noted in the Introduction, the latter process is related to vesicle deformation<sup>48</sup> and may be induced by the vesicle–substrate interaction alone or in combination with other interactions.<sup>21</sup> Osmotic pressure is known to influence the shape of vesicles and accordingly may influence the rupture rate. If osmotic pressure is formed by ions (e.g., by  $\text{Na}^+$  and  $\text{Cl}^-$  as in our case), it or, more precisely, ionic strength can also influence the rupture rate via its effect on the vesicle–substrate interaction. Below, we discuss the likely role of these two factors in the processes under consideration.

### Influence of Osmotic Pressure on the Vesicle Shape.

Physically, it is clear that positive osmotic pressure results in deformation of vesicles. With increasing positive osmotic pressure, the deformation becomes more appreciable and, just after adsorption, a vesicle occupies a larger area due to spreading. Hence, deformation facilitates rupture irrespective of its mechanism. Positive osmotic pressure increases vesicle spreading, which is consistent with the observed adsorption kinetics including the transformation in self-assembly pathway to single vesicle rupture at highly positive osmotic pressures.

Likewise, negative osmotic pressure promotes a more spherical shape of adsorbed vesicles that does not induce further vesicle deformation beyond what is caused by the vesicle–substrate interaction alone.

The qualitative analysis above is in line with the results of our experiments. To generalize the analysis, it is instructive to consider the effect of positive osmotic pressure on vesicle rupture. In this case, the rupture kinetics can be qualitatively interpreted in terms of critical coverage by analyzing the shape of adsorbed vesicles. In vesicles, the energy scale associated with a macroscopic stretching of the lipid bilayer is several orders of magnitude greater than that involved in bilayer bending.<sup>49</sup> Practically, this means that, during vesicle adsorption, the forces related to bending can be balanced by the forces related to stretching provided that the stretching energy is much smaller than the bending energy. For this reason, it is often assumed that stretching is negligible and the focus is on bending.<sup>32</sup> In this approximation, the free energy of an adsorbed vesicle can be represented as a sum of three terms

$$E = E_b + E_c + E_p \quad (2)$$

corresponding to bending energy, vesicle–substrate contact energy, and osmotic pressure, respectively. The simplest expressions for the former two energies are

$$E_b = \left(\frac{\kappa}{2}\right) \int dA (C_1 + C_2)^2 \quad (3)$$

$$E_c = \pi R^2 W \quad (4)$$

where  $\kappa$  is the bending rigidity,  $C_1$  and  $C_2$  are the principal curvatures,  $A$  is the bilayer area,  $R$  is the radius of the contact area, and  $W$  is the contact energy per unit area. The last term in eq 2 is customarily represented as  $E_p = \Delta PV$ , where  $V$  is the vesicle volume, and  $\Delta P = P_{\text{ex}} - P_{\text{in}}$  is the osmotic pressure that is treated usually as a Lagrange multiplier.<sup>35</sup> During adsorption,  $V$  and  $\Delta P$  both change accordingly. For this reason, the use of  $\Delta P$  as a Lagrange multiplier is not convenient to describe the situation directly. Rather, in our analysis, we calculate  $E_p$  more explicitly. Let us consider first that a vesicle is in solution, and its shape is spherical so that the volume is  $V_o = (4/3) \pi R_o^3$ , where  $R_o$  is the radius. The number of  $\text{Na}^+$  and  $\text{Cl}^-$  ions trapped by a vesicle during its fabrication is  $n_o = 2c_o V_o$ , where  $c_o$  is the corresponding  $\text{Na}^+$  or  $\text{Cl}^-$  concentration. If, during an experiment, the exterior  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations are  $c_*$  and the vesicle volume is  $V$ , the osmotic pressure defined by eq 1 can be represented as

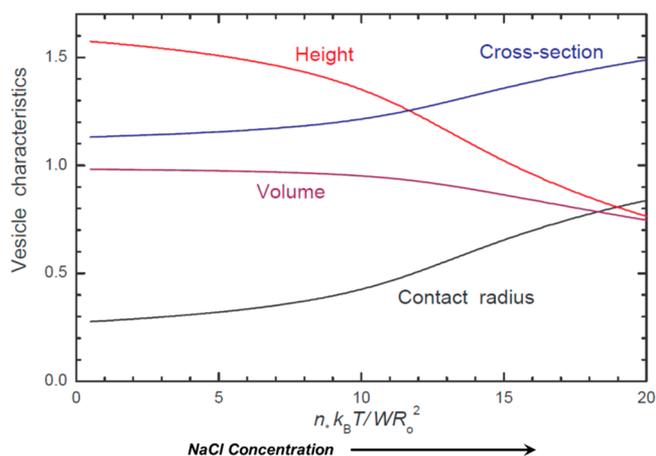
$$\Delta P(V) = \left(\frac{n_*}{V_o} - \frac{n_o}{V}\right) k_B T$$

where  $n_* = 2c_* V_o$ . The corresponding contribution to the free energy is given by

$$\begin{aligned} E_p &= \int_{V_o}^V \Delta P(V') dV' \\ &= [n_*(V - V_o)/V_o - n_o \ln(V/V_o)] k_B T \end{aligned} \quad (5)$$

To calculate the vesicle shape, the vesicle energy defined by eqs 2–5 should be minimized provided that the vesicle bilayer area is constant, i.e., equal to  $4\pi R_o^2$ . The results of calculations depend on three dimensionless parameters,  $\kappa/WR_o^2$ ,  $n_o k_B T/WR_o^2$ , and  $n_* k_B T/WR_o^2$  (note that the latter two parameters are proportional to the corresponding NaCl concentration in

solution). To reproduce a physically reasonable shape of vesicles, we take into account that the experimentally studied vesicles are small. Such vesicles cannot be appreciably deformed without rupture. In our calculations, we use  $n_* k_B T/WR_o^2$  as a governing parameter (this parameter is proportional to the NaCl concentration in solution). The values of the other two parameters,  $\kappa/WR_o^2 = 1$  and  $n_o k_B T/WR_o^2 = 2$ , were chosen so that the deformation of the vesicle shape is modest except for the situation when  $n_* k_B T/WR_o^2 \gg n_o k_B T/WR_o^2$  (this condition corresponds to high NaCl concentration in solution). With these specifications, our numerical calculations (Figure 4)



**Figure 4.** Adsorbed vesicles undergo greater deformation at high osmotic pressure. Normalized vesicle characteristics including the contact radius,  $R/R_o$ , height,  $h/R_o$ , maximum vesicle cross section along the surface,  $s/(\pi R_o^2)$ , and volume,  $V/V_o$ , as a function of  $n_* k_B T/WR_o^2$  for  $\kappa/WR_o^2 = 1$  and  $n_o k_B T/WR_o^2 = 2$ .

predict that, by increasing  $n_* k_B T/WR_o^2$  from 0.5 to 20, the contact radius increases, the vesicle height (maximum size perpendicular to the substrate) decreases, the maximum vesicle cross section (along the substrate) increases, and the vesicle volume decreases. The product of the latter cross section and vesicle surface concentration defines vesicle coverage.

The experimental findings are consistent with this model and show that, with an increase in the external NaCl concentration, (i) the QCM-D resonance frequency shift corresponding to the onset of vesicle rupture decreases, and (ii) eventually vesicle rupture starts just after vesicle adsorption. Our model helps to understand both of these observations. Concerning observation (i), our model also demonstrates that the vesicle volume becomes smaller with increasing NaCl concentration in solution. Due to this factor, the amount of water trapped by vesicles becomes smaller as well, and accordingly the contribution of each vesicle to the QCM-D frequency shift slightly decreases. This is an additional reason why the QCM-D frequency shift corresponding to the onset of vesicle rupture decreases with increasing NaCl concentration in solution. As such, our model provides a physically reasonable rationale to explain the effects of osmotic pressure on vesicle adsorption kinetics.

There is also an interesting comparison between our findings and those previously reported,<sup>30,34</sup> which focused on the effects of NaCl concentration under isosmotic conditions whereby the ionic strength inside and outside of the vesicles was equivalent. In those works, it was determined that formation of a planar bilayer on silicon oxide followed one-step adsorption kinetics

under low NaCl concentrations and two-step adsorption kinetics under high NaCl concentrations. Initially, these data appear to conflict with our model which indicates greater deformation of adsorbed vesicles with increasing NaCl concentration. However, there is in fact agreement because the deformation of adsorbed vesicles under initially isosmotic conditions would result in the formation of a negative osmotic pressure that counters the rupturing process. The resulting membrane tension induced by the negative osmotic pressure would be greater in magnitude as a function of ionic strength so higher ionic strengths would require a greater degree of vesicle-vesicle interactions to complement vesicle-substrate interactions. Hence, these past experimental findings and our model are consistent.

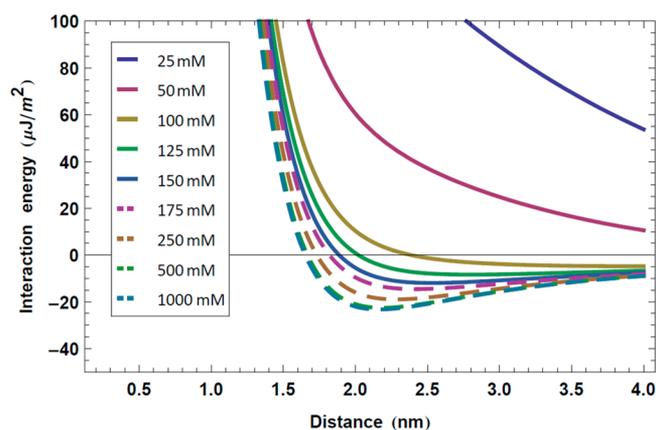
**Influence of Ionic Strength on the Vesicle-Substrate Interaction.** The kinetics of vesicle adsorption to solid supports crucially depend on the vesicle-substrate interaction. Despite its importance, understanding of this interaction is still poor.<sup>50</sup> The corresponding interface is often believed to contain a few layers of water. Physically, it is clear that if the substrate and/or vesicles are charged, then the vesicle-substrate interaction may in this case depend on the ionic strength due to its role in the screening of the charges. Concerning our system, we note that zwitterionic vesicles have a negative surface charge under near-neutral pH conditions, as determined by zeta potential measurements of vesicles in solution.<sup>51</sup> At 100 mM NaCl salt concentration, the zeta potential of zwitterionic vesicles in solution is approximately  $-7$  mV (Table S1). If one assumes the zeta potential is equivalent to the surface potential to a first approximation,<sup>52</sup> then the surface charge density of vesicles is on the order of  $-0.01$  C/m<sup>2</sup> by the Grahame equation for a 1:1 electrolyte solution.<sup>33</sup> Likewise, the surface potential of a silicon oxide substrate in 100 mM monovalent salt at near-neutral pH is on the order of  $-0.1$  C/m<sup>2</sup> based on charge titration experiments.<sup>53</sup> While the interaction of two negatively charged surfaces would be presumably repulsive based on the double-layer electrostatic force, vesicle-substrate interactions are governed by several different types of forces and there are models available to describe this behavior.

Vesicle-substrate interactions are generally characterized by employing DLVO-type models<sup>54,55</sup> that include the van der Waals, double-layer electrostatic, and hydration forces. Molecular dynamics simulations are another method of investigation.<sup>56,57</sup> Due to the complexity of the problem, both approaches include many explicit or implicit assumptions and approximations, and model analyses must be considered appropriately. The advantage of the DLVO-based approach is in conceptual and mathematical simplicity. Bearing this point in mind, we have tried to interpret our results by employing one of the versions<sup>54,55</sup> of the extended-DLVO theories. We are interested in the vesicle-silicon oxide interaction in the situation where the separation distance between the vesicle and silicon oxide substrate is much smaller than the vesicle size. In this limit, we focus on the interaction in the contact area, replace a vesicle by a planar bilayer, and calculate the interaction energy per unit surface. The distributions of charge on the silicon oxide and vesicle surfaces are considered to be uniform and fixed as in the case when a vesicle is in the bulk solution. For silicon oxide, this situation corresponds to reality because the charges are immobile. In a vesicle, the lipids are highly mobile, the charges are mobile as well, and accordingly the charge in a vesicle during adsorption can easily be redistributed. This effect is negligible provided that

$$|eU| \ll k_B T \quad (6)$$

where  $e$  is the electron charge and  $U$  is the difference in the vesicle surface potential for the situations when a vesicle is in the solution and near the substrate, respectively. In the case under consideration, the surface charge concentrations are relatively small, and criterion 6 is fulfilled provided the ionic strength of NaCl is not low (see below).

The total interaction energy determined as a function of separation distance (for the details, see the Supporting Information) is shown in Figure 5. At ionic strengths above



**Figure 5.** Effects of ionic strength on the vesicle-substrate interaction. The total interaction energy between a vesicle and silicon oxide was investigated as a function of separation distance by an extended-DLVO model under varying ionic strength conditions. The total interaction energy was assumed to result from three interfacial forces, namely, the van der Waals, double-layer electrostatic, and hydration forces. At high ionic strengths, an equilibrium separation distance was identified corresponding to an energy minimum. At low ionic strengths, no such energy minima were predicted based on the model.

150 mM NaCl, an energy minimum corresponding to attractive vesicle-substrate interactions was identified at separation distances between 2.0 and 2.5 nm, which is large compared to neutron reflectometry studies<sup>58</sup> that indicate the separation distance is about 1 nm. The corrugation of the potential profile along the surface at this distance is also weak, and one could expect fairly rapid diffusion of adsorbed vesicles. This is, however, not confirmed by the experiments (see Discussion<sup>59</sup>). Notably, at ionic strengths of 500 mM NaCl or greater, there is a large screening effect and the total interaction energy plots converge. This behavior is consistent with the insensitivity of the van der Waals force to ionic strength in this regime (Figure S1) and the nearly negligible role of the double-layer electrostatic force in this case (Figure S2). Hence, we may conclude that the observed vesicle adsorption kinetics are due to differences in osmotic pressure, and not due to changes in the vesicle-substrate interaction. At ionic strengths between 100 and 150 mM NaCl, there remains long-range attraction between vesicles and the substrate but no energy minimum at distances expected for vesicle adsorption, presumably due to reduced screening of the double-layer electrostatic force.

With a further decrease in the ionic strength (e.g., at 50 and 25 mM external NaCl concentrations), the model predicts repulsive interactions between vesicles and the substrate (Figure 5). However, the experimental results in this regime indicate that vesicle adsorption is possible. To test whether the results are caused primarily by the low ionic strength condition

or the negative osmotic pressure gradient, we also measured the adsorption kinetics of vesicles onto silicon oxide in isosmotic conditions of 25 mM NaCl. In this case, the vesicles adsorbed until reaching a critical coverage and then formed a planar bilayer (Figure S5). Based on this result, we rule out that low ionic strength prevents bilayer formation in this case. Therefore, the negative osmotic pressure gradient is responsible for the adsorbed vesicles remaining intact on silicon oxide. Moreover, the formation of a planar bilayer on silicon oxide under the low ionic strength condition of 15 mM NaCl isosmotic solution has also been observed previously.<sup>52</sup> One of the reasons the theory does not predict attractive interactions in this limiting case is that criterion 6 is not fulfilled for ionic strengths of 50 mM NaCl or less. In principle, the charge redistribution in a vesicle can be taken into account in calculations, but this is beyond the scope of our work. Importantly, the overall trends predicted by the extended-DLVO model, namely that the vesicle–substrate interaction is more attractive under higher ionic strength conditions, support qualitatively the experimental findings of this work.

**Additional Remark.** After submission of this work for publication, some results related to the subject under consideration were published by Zhu et al.<sup>60</sup> Specifically, the effects of NaCl- and sucrose-induced osmotic stress on vesicle adsorption kinetics onto silicon oxide were studied experimentally. The key finding of their work was that vesicle adsorption kinetics are influenced mainly by vesicle–substrate interactions, not osmotic stress. Within the scope of their study, formation of a planar bilayer was always observed by the same channel, namely vesicle adsorption until reaching a critical coverage. Neither single vesicle rupture nor adsorption of an intact vesicle layer was observed. In part, the different results between the two studies can be explained by two factors. The vesicles in our study are appreciably smaller than the vesicles in the former study. Additionally, the range of osmotic pressures in our study is much greater than that of the former study, thereby allowing us to access kinetic regimes that were not observed previously. At the same time, the results of the two studies are consistent if one focuses on the results obtained under similar ionic strength conditions. Within the ionic strength range of the former study (150–250 mM NaCl), our extended–DLVO model calculations predict some changes in the strength of the vesicle–substrate interaction. The trend in the predicted changes is in agreement with the experimental results of the former study, and supports that the effects of osmotic pressure can have a varying contribution relative to other factors.

## CONCLUSION

We have experimentally demonstrated that, by changing the magnitude and direction of the osmotic pressure across a wide range, all three different types of the kinetics of vesicle adsorption and rupture, from adsorption of intact vesicles to rupture of vesicles just after their adsorption, can be observed in one system. Positive osmotic pressure promotes the rupture of adsorbed vesicles on silicon oxide, while negative osmotic pressure acts to stabilize adsorbed vesicles. Theoretical analysis of pressure-induced deformation of adsorbed vesicles helps to rationalize these observations. Since the osmotic pressure was adjusted by varying the NaCl concentration in bulk solution, we also considered the effects of ionic strength on interfacial forces. DLVO-type analysis of the vesicle–substrate interaction qualitatively supports our observations. In summary, the

findings in this work improve our understanding of how osmotic pressure can influence the adhesion of vesicles to solid supports, and may help to optimize fabrication of solid-supported model membrane systems. In particular, we may conclude that osmotic pressure is an important factor to control the adsorption kinetics of vesicles onto solid supports, and must be considered alongside related parameters such as vesicle size/shape and ionic strength.

## ASSOCIATED CONTENT

### Supporting Information

More detailed information is provided about the diffusion-limited kinetics of vesicle adsorption, and a model of the interaction between a planar lipid bilayer and an oxide film (Table S1 and Figures S1–S3), along with additional QCM-D experimental results and Voigt-Voinova model analysis (Figures S4 and S5). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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