Contribution of the Hydration Force to Vesicle Adhesion on Titanium Oxide

Supporting Information

Joshua A. Jackman†‡, Haw Zan Goh†‡, Zhilei Zhao†§‖, Nam-Joon Cho*†‡§

†School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue 639798, Singapore
‡Centre for Biomimetic Sensor Science, Nanyang Technological University, 50 Nanyang Drive 637553, Singapore
§School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive 637459, Singapore
‖School of Life Sciences, Peking University, 5 Yi He Yuan Road, Haidian, Beijing 100871, China

*Corresponding Author

E-mail: njcho@ntu.edu.sg
**Materials and Methods**

**Vesicle Preparation.** Negatively and positively charged vesicles were prepared with various mixtures of zwitterionic and charged lipids. 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC, zwitterionic), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (sodium salt) (POPS, negatively charged) and 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (chloride salt) (POEPC, positively charged) were used as-supplied from Avanti Polar Lipids (Alabaster, USA). Small unilamellar vesicles of desired compositions (POPC or POPC:POPS or POPC:POEPC mixtures) were prepared at a lipid concentration of 5 mg·mL$^{-1}$ by the extrusion method, as previously described$^1$. Vesicles were extruded by using a Mini Extruder (Avanti Polar Lipids) through polycarbonate membranes with 50 nm diameter pores, and then again with 30 nm diameter pores (at least 11 passes per membrane). The z-average diameter of extruded vesicles was ca. 55 nm with a polydispersity index below 0.1, as measured by dynamic light scattering. Vesicles were diluted before experiment, and were used within two days of preparation. An aqueous buffer solution with 200 mM NaCl and 10 mM Tris [pH 7] was used. All buffer solutions were prepared with 18.2 MΩ·cm MilliQ water (MilliPore, Oregon, USA).

**Quartz Crystal Microbalance-Dissipation (QCM-D).** The adsorption kinetics of vesicles onto titanium oxide-coated sensor chips were monitored by using a Q-Sense E4 instrument (Q-Sense AB, Gothenburg, Sweden). Prior to experiment, the substrates were cleaned with 1% w/w sodium dodecyl sulfate (SDS) solution, and then rinsed with water and ethanol, sequentially. After gentle drying with a stream of nitrogen air, the crystals were subjected to oxygen plasma treatment (Harrick Plasma, Ithaca, NY, USA) for one min immediately before experiment. Experimental data were collected at multiple odd overtones (n=3-11), and changes in frequency and energy dissipation were monitored as functions of time. For viscoelastic modeling,
measurement data \( n = 7, 9, 11 \) were fit to the Voigt-Voinova model in order to recover the effective Voigt thickness, shear modulus, and viscosity of the adlayer. A constant density of 775 kg·m\(^{-3}\) was used based on a previous estimate for an adsorbed vesicle layer on titanium oxide\(^2\).

**Atomic Force Microscopy (AFM).** Non-contact mode imaging (1 Hz scan rate) of the titanium oxide-coated QCM-D sensor chips was performed using an NX-Bio microscope (Park Systems, Suwon, South Korea) combined with an optical microscope (Eclipse Ti, Nikon, Japan). The tip probe was a PPP-NCHR cantilever (Nanosensors, Neuchatel, Switzerland), which has a nominal spring constant of 42 N·m\(^{-1}\), resonance frequency of 330 kHz, and a radius of curvature of ca. 7 nm.

**Continuum Model Calculations**

As previously described\(^3,4\), numerical calculations based on a continuum model that includes the DLVO forces and the non-DLVO hydration force were performed in order to estimate the total interaction energy that stabilizes intact vesicles on a titanium oxide substrate. We assumed the contact area between vesicles and the substrate can be represented as two planar surfaces. In this system, the total interaction energy is considered to be determined by the balance of the van der Waals force, double-layer electrostatic force, and hydration force. The van der Waals force is always attractive, the hydration force is always repulsive, and the double-layer electrostatic force can be either attractive or repulsive. Depending on experimental parameters, the combination of forces can yield an energy minimum that corresponds to a predicted separation distance between vesicles and the titanium oxide substrate.

**van der Waals Interaction Energy**

The van der Waals interaction energy as a function of separation distance was determined by using the Prieve and Russell approach\(^5\), as described by Eqs. (3)-(6) of Tero et al.\(^6\) (Fig. S2). X-
ray diffraction patterns of titanium oxide-coated QCM-D crystals used in the study indicate that the titanium oxide surface has an anatase crystalline structure, and accordingly we used material properties related to anatase titanium oxide in our calculations. Additionally, for comparative analysis of previous work by Tero et al., calculations were also performed by using material properties related to rutile titanium oxide (Fig. S6). Overall, the crystalline structure of the titanium oxide substrate did not significantly affect the predicted interaction between vesicles and the substrate. Within a certain range of the hydration force’s decay length, the crystalline structure affected the magnitude of the total interaction energy, but had marginal effect on the trend behavior as a function of decay length in general. A summary table of the parameters used in the modeling calculations is presented below:

<table>
<thead>
<tr>
<th>Medium</th>
<th>Dielectric Constant</th>
<th>Oscillator Parameter</th>
<th>Absorption Frequency (UV)</th>
<th>$A_{\nu=0}$ (J)</th>
<th>$A_{\nu&gt;0}$ (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatase TiO$_2$ (Ref. 8)</td>
<td>31</td>
<td>4.000</td>
<td>1.27×10$^{15}$</td>
<td>1.2568×10$^{-21}$</td>
<td>7.2433×10$^{-21}$</td>
</tr>
<tr>
<td>Rutile TiO$_2$ (Ref. 9)</td>
<td>104.8</td>
<td>5.162</td>
<td>1.20×10$^{15}$</td>
<td>-0.4368×10$^{-21}$</td>
<td>8.7071×10$^{-21}$</td>
</tr>
<tr>
<td>Lipid (Ref. 5)</td>
<td>2.04</td>
<td>1.041</td>
<td>2.64×10$^{15}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (Ref. 5)</td>
<td>77.6</td>
<td>0.762</td>
<td>3.17×10$^{15}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The inverse Debye length for our system is calculated by

$$\kappa^{-1} = \sqrt[2]{\frac{\varepsilon R_e^{-1} k_B T}{2N_A e^2 I}}$$

(1)

$I$ is the ionic strength of electrolyte in the units of mol/m$^3$, i.e., 150 mol/m$^3$ in the model calculations. The calculated inverse Debye length is 1.2805 nm$^{-1}$.

**Double-Layer Electrostatic Interaction Energy**

The double-layer electrostatic interaction energy as a function of separation distance was calculated based on the surface potential values of lipid vesicles and the titanium oxide substrate, as described by Eq. (9) of Nabika et al. (Fig. S3). The surface potential for titanium oxide at pH 7 was fixed at -5 mV$^{10}$. As shown in Table S1, the surface potential values for vesicles with
different lipid compositions was estimated based on previous zeta potential measurements on vesicles in solution\textsuperscript{11}.

**Hydration Interaction Energy**

The hydration interaction energy as a function of separation distance was calculated by an exponential decay function, as described by Eq. (11) of Nabika et al.\textsuperscript{4} (Fig. S4). The term $P_0$ is a parameter in the exponential decay function that is defined as the pressure needed to remove water completely from between the material substrate and adsorbed vesicle layer\textsuperscript{12}. Experimental data indicate that $P_0$ is particularly sensitive to ionic strength\textsuperscript{13}. As the ionic strength was fixed in our experiments, we assumed that $P_0$ is a constant in the model calculations ($P_0 = 1.15 \times 10^8$ N/m$^3$ based on Ref. 4). Another parameter in the exponential decay function is the decay length $\lambda_0$ and experimental data indicate that it is sensitive to both ionic strength and charge distribution\textsuperscript{13}. Hence, $\lambda_0$ is probably more sensitive to titanium oxide and vesicle surface properties, as compared to $P_0$. We therefore decided to vary $\lambda_0$ in the model calculations and its range (0.10 to 0.50 nm) was selected so that the hydration interaction energy was appreciable only within a 2 nm separation distance.

**Sensitivity Analysis**

Based on the continuum model calculations, it was observed that the relative contribution of the double-layer electrostatic force varies depending on the decay length of the hydration force. To quantitatively characterize the dependence on decay length, we calculated the range of decay length values wherein the overall system is sensitive to the electrostatic force. We considered cases only where an energy minima corresponding to an equilibrium separation distance occurred. Based on this condition, a quantity to express sensitivity, $S$, was defined based on the difference in the total interaction energy as a function of vesicle surface charge, namely related
to the 70:30 POPC:POEPC (most positively charged) and 70:30 POPC:POPS (most negatively charged) lipid compositions as follows:

$$S = \frac{|E_{70:30\ POPC:POEPC} - E_{70:30\ POPC:POPS}|}{E_{100\ POPC}}$$

where $E$ represents the total interaction energy at the equilibrium separation distance. We determined that the contribution of the double-layer electrostatic force is appreciable only within a certain range of decay length values. Outside of this range, the difference in total interaction energy that is caused by the range of vesicle surface charges is insignificant. If one considers a 30% change in the total interaction energy as a result of vesicle surface charge (within the tested range) to be the cut-off, then the range of decay length values wherein the overall system is sensitive to the electrostatic force is 0.17 to 0.28 nm (Fig. S5). Of note, when decay length is less than 0.13 nm, there is no equilibrium state so we did not consider decay length values of less than 0.13 nm in the sensitivity analysis.
**SI Figure Legend**

**Table S1. Summary of Vesicle Surface Potentials.** Zeta potential measurements were originally performed in 10 mM NaCl aqueous salt solution (Fig. 1 from Ref. 11). To a first approximation, the zeta potential values are assumed to be equal to the corresponding surface potential values and electrostatic shielding is also accounted for by Eq. (10) from Nabika et al. An ionic strength corresponding to 150 mM monovalent salt solution was used in the calculations because similar conditions were used in previous experimental works and the condition is also similar to the experimental work in the present study.

**Figure S1. Comparison of Electrostatic Interaction Energy in Different pH Regimes.** Two curves are presented corresponding to the electrostatic interaction energy for zwitterionic vesicles on titanium oxide in the acidic pH regime (pH 3), and 70:30 POPC:POEPC vesicles on titanium oxide in the neutral pH regime (pH 7). Although the electrostatic interaction energy between positively charged vesicles and titanium oxide at pH 7 is greater than that between zwitterionic vesicles and titanium oxide at pH 3, vesicle rupture only occurs for the latter case, not the former.

**Figure S2. van der Waals Interaction Energy as a Function of Separation Distance.** Two curves are presented corresponding to anatase and rutile titanium oxide, respectively.

**Figure S3. Double-Layer Electrostatic Interaction Energy as a Function of Separation Distance.**

**Figure S4. Hydration Interaction Energy as a Function of Separation Distance.** In the model calculations, different values corresponding to the decay length of the hydration force were tested, including (A) 0.10, (B) 0.25 and (C) 0.50 nm.
Figure S5. Sensitivity Analysis of Electrostatic Force Contribution to Total Interaction Energy.

Figure S6. Total Interaction Energy of Vesicle-Substrate Interactions on Titanium Oxide. Total interaction energy as a function of separation distance between vesicles and the rutile titanium oxide substrate from Tero et al.\textsuperscript{6} was estimated by the continuum model. In the model calculations, different values corresponding to the decay length of the hydration force were tested, including (A) 0.10, (B) 0.25 and (C) 0.50 nm.

Figure S7. Non-Contact Mode AFM Imaging on Titanium Oxide-Coated QCM-D Sensor Chip. Measurements were performed in order to determine the root-mean-square (RMS) surface roughness ($R_q$).
Table S1

<table>
<thead>
<tr>
<th>Lipid Composition (mol:mol %)</th>
<th>Surface Potential (10 mM) (mV)</th>
<th>Surface Potential (150 mM) (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70:30 POPC:POEPC</td>
<td>30.6</td>
<td>7.9</td>
</tr>
<tr>
<td>85:15 POPC:POEPC</td>
<td>14.8</td>
<td>3.8</td>
</tr>
<tr>
<td>100 POPC</td>
<td>-9.0</td>
<td>-2.3</td>
</tr>
<tr>
<td>85:15 POPC:POPS</td>
<td>-19.4</td>
<td>-5.0</td>
</tr>
<tr>
<td>70:30 POPC:POPS</td>
<td>-33.2</td>
<td>-8.6</td>
</tr>
</tbody>
</table>
Figure S1

Double Layer Electrostatic Energy (\(\mu\text{J/m}^2\)) vs. Distance (nm)

- pH=3  100 POPC
- pH=7  70:30 POPC:POEPC
Figure S2

van der Waals Interaction

Anatase TiO₂
Rutile TiO₂

Distance (nm)
Figure S3

Double Layer Electrostatic Interaction Energy ($\mu$J/m$^2$) vs. Distance (nm)

- 70:30 POPC:POEPC
- 85:15 POPC:POEPC
- 100% POPC
- 85:15 POPC:POPS
- 70:30 POPC:POPS
Figure S4

Hydration Interaction Energy ($\mu J/m^2$) vs. Distance (nm)

- $\lambda_0 = 0.10$ nm
- $\lambda_0 = 0.25$ nm
- $\lambda_0 = 0.50$ nm
Figure S5

![Graph showing sensitivity vs. decay length (nm)](image_url)
Figure S6

A

\[ \lambda_0 = 0.10 \text{ nm} \]

B

\[ \lambda_0 = 0.25 \text{ nm} \]

C

\[ \lambda_0 = 0.50 \text{ nm} \]
Figure S7
SI References

2. Reviakine, I.; Rossetti, F. F.; Morozov, A. N.; Textor, M., Investigating the properties of supported vesicular layers on titanium dioxide by quartz crystal microbalance with dissipation measurements. *Journal of Chemical Physics* 2005, 122, 204711.