Electric Field Effect on Phospholipid Monolayers at an Aqueous–Organic Liquid–Liquid Interface

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ABSTRACT: The electric potential difference across cell membranes, known as the membrane potential, plays an important role in the activation of many biological processes. To investigate the effect of the membrane potential on the molecular ordering of lipids within a biomimetic membrane, a self-assembled monolayer of 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) lipids at an electrified 1,2-dichloroethane/water interface is studied with X-ray reflectivity and interfacial tension. Measurements over a range of electric potential differences, −150 to +130 mV, that encompass the range of typical biomembrane potentials demonstrate a nearly constant and stable structure whose lipid interfacial density is comparable to that found in other biomimetic membrane systems. Measurements at higher positive potentials, up to 330 mV, illustrate a monotonic decrease in the lipid interfacial density and accompanying variations in the interfacial configuration of the lipid. Molecular dynamics simulations, designed to mimic the experimental conditions, show that the measured changes in lipid configuration are due primarily to the variation in area per lipid with increasing applied electric field. Rotation of the SOPC dipole moment by the torque from the applied electric field appears to be negligible, except at the highest measured potentials. The simulations confirm in atomistic detail the measured potential-dependent characteristics of SOPC monolayers. Our hybrid study sheds light on phospholipid monolayer stability under different membrane potentials, which is important for understanding membrane processes. This study also illustrates the use of X-ray surface scattering to probe the ordering of surfactant monolayers at an electrified aqueous–organic liquid–liquid interface.

1. INTRODUCTION

It is well-known that the membrane potential influences the organization and functionality of cell membranes. For instance, membrane potentials on the order of 100 mV play a critical role in ATP synthesis.4,5 Other examples include the opening and closing of A-type voltage-gated K+ channels which occur at membrane potentials between −100 and +100 mV4 and the destabilization of cholesterol–phospholipid complexes.5 Direct effects on the integrity of the biomembrane, such as reversible permeabilization and electroporation, occur at higher membrane potentials.5,6

Lipid monolayers at the interface between immiscible aqueous and organic electrolyte solutions have been used as a model system for investigating electric field effects on membrane processes.7–15 The lipids are dissolved in an organic electrolyte phase and self-assemble at the aqueous–organic liquid–liquid interface. The applied electric potential difference between aqueous and organic electrolyte solutions, \( \Delta \phi^{w-o} \), expresses the difference between the Galvani electric potentials far from the interface in the bulk aqueous phase, \( \phi^{\text{water}} \), and in the bulk organic phase, \( \phi^{\text{organic}} \). Experiments on interfacial layers of phosphatidylcholine (PC) lipids have demonstrated low and nearly constant values of interfacial tension for a range of negative values of \( \Delta \phi^{w-o} \), thus suggesting a nearly constant adsorption of lipids over this range of potentials.8,13–16 Increasing \( \Delta \phi^{w-o} \) to positive values led to an increase in interfacial tension. Several authors have suggested that interfacial PC lipids become positively charged by proton or cation binding at an appropriate positive value of \( \Delta \phi^{w-o} \) and are subsequently desorbed from the interface into the bulk organic phase, thereby raising the interfacial tension.13,14,16,17

Complementary to these electrochemical studies of lipid monolayers at the aqueous–organic interface are electrochemical, fluorescence, and AFM force–distance studies of lipid monolayers at the mercury–aqueous liquid–liquid interface.18–20 These studies have provided evidence for a series of conformational transitions of phospholipid layers that include pore formation and other structural transitions. At this interface, monolayers of dioleoylphosphatidylcholine (DOPC) are measured to be stable and unchanging over a range of potential differences that correspond to \( \Delta \phi^{w-m} = \phi^{\text{water}} - \phi^{\text{mercury}} \) varying from −200 to +600 mV (note that the negative
of these potentials is usually quoted in the mercury–water literature.\textsuperscript{20} Transitions occur at higher potential differences, starting at $\Delta \phi^{w-o} \approx 600$ mV, and are believed to be correlated with the penetration of ions from the aqueous phase through the lipid layer to rest against the mercury surface.\textsuperscript{31} The range of $\Delta \phi^{w-o}$ for which unchanging, stable monolayers are observed at the mercury–aqueous interface spans the entire range of potential differences that are presented in this manuscript for which changes are observed at the aqueous–organic interface. The chemical environments for lipids at the two interfaces are quite different. The lipids are insoluble in mercury, and stable monolayers at the mercury–aqueous interface are believed to have the phospholipid tails adjacent to but not mixed with or penetrating into the mercury phase. In contrast, lipid monolayers at the aqueous–organic interface are Gibbs monolayers that self-assemble from lipids dissolved in the organic phase.

Molecular dynamics (MD) simulations have also been used to investigate the role of membrane potential on many essential biological processes. These include the study of the potential distribution throughout the membrane,\textsuperscript{22} ion conduction and water transport through membranes,\textsuperscript{23–26} ion channel gating,\textsuperscript{27} conformational changes induced in membrane proteins,\textsuperscript{28} and peptide insertion into membranes.\textsuperscript{29,30} MD simulations of large membrane potentials of $\sim 1–3$ V reoriented the headgroup dipole moments in dioleoylphosphatidylcholine (DOPC) bilayers to induce electroporation.\textsuperscript{31,32} Related simulations have shown that the effect of the transmembrane potential on the dipole moment of the PC headgroup contributes to the dipole moment of the PC headgroup.

These experimental and computational studies highlight two different aspects of phospholipid behavior at electrified interfaces that can alter phospholipid ordering at the interface: electric-potential-driven chemical binding or reactions and the torque imposed on the lipid dipole moment by the interfacial electric field. To further investigate these and other aspects of lipid ordering at an aqueous–organic electrified interface on the molecular length scale, we present a study that combines X-ray reflectivity measurements and molecular dynamics (MD) simulations of PC lipid layers at the electrified liquid–liquid interface. X-ray reflectivity probes the electron density profile perpendicular to the interface, which can be interpreted in terms of molecular ordering on the sub-nanometer length scale.\textsuperscript{34} This technique has been used numerous times to investigate the ordering of lipid monolayers and lipid–protein interactions at the liquid–vapor interface, though not under conditions that allow for application of a controlled electric potential.\textsuperscript{35–43} More recently, X-ray reflectivity has been applied to the study of surfactant and lipid ordering at oil–water interfaces, though the role of electrostatic interactions was not explored.\textsuperscript{23,44–48} In these studies, X-ray reflectivity was used to characterize the thickness and electron density of the headgroup and tailgroup regions of the surfactant layers, which can be related to headgroup and tailgroup packing. This can lead to conclusions, for example, as to whether the tailgroups are all-trans and close-packed or disordered and loosely packed. Depending upon the system studied, conclusions can also be drawn about the penetration or adsorption of solvent molecules or electrolytes into the interfacial region occupied by the surfactant layer. X-ray reflectivity measurements are not likely to be sensitive to the orientation of a surfactant headgroup within a monolayer, because the orientation will not usually have a significant effect on the electron density; nevertheless, X-ray reflectivity measurements of proteins bound to lipid monolayers have determined the extent of penetration of protein molecules into the lipid layer as well as the orientation of the protein.\textsuperscript{38,39}

In the present work, X-ray reflectivity and electrochemistry studies of 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) lipid monolayers are carried out on the liquid–liquid interface between aqueous (pH 7.2) and organic electrolyte solutions. Aqueous solutions of 100 mM NaCl in contact with PC lipid headgroups were used to model physiological conditions of the outer leaflet of the plasma membrane of eukaryotic cells.\textsuperscript{33} Unsaturated lipids were chosen for study because of their prevalence in natural biomembranes\textsuperscript{50} and their relevance for planned studies of electrostatic effects on lipid–protein interactions. In addition, it is known that these unsaturated lipids form homogeneous monolayers, in contrast to monolayers of saturated lipids that often exhibit domains.\textsuperscript{38,51} The absence of domains simplifies the interpretation of X-ray reflectivity measurements which average over the in-plane footprint of the X-ray beam on the interface.\textsuperscript{36}

The applied electric potential difference $\Delta \phi^{w-o}$ between the bulk electrolyte solutions was varied from $-150$ to $330$ mV. Electrochemical cyclic voltammetry and interfacial tension measurements are consistent with results from prior studies on DPPC, L-R-lecithin, and egg-yolk phosphatidylcholine lipids.\textsuperscript{8,13,14} X-ray reflectivity measurements probe the variation in lipid monolayer thickness and electron density profile with applied electric potential difference $\Delta \phi^{w-o}$. These measurements demonstrate that stable and reproducible SOPC monolayers are formed with interfacial densities that can be varied from the high densities typical of unsaturated lipids in biomembranes\textsuperscript{52} ($\sim 70$ Å$^2$ per lipid molecule) to densities lower by about a factor of 2. Importantly, stable high density monolayers are observed with essentially unchanged structure over the range from $-150$ to $+130$ mV that is often relevant to physiological conditions. These data do not confirm a recent report of a 2 Å thinning of a tethered bilayer of POPC lipids studied by neutron reflectivity when the transblayer voltage was varied between 0 and 100 mV.\textsuperscript{53}

MD simulations were performed for controlled variations of lipid interfacial density and electric field amplitude. Variations in monolayer thickness and electron density of the headgroup and tailgroup agree with the results of X-ray reflectivity. MD simulations also reveal that variations in the lipid configuration, such as the angular orientation of the lipid, are determined primarily by the lipid interfacial density and not by the electric field torque on the lipid dipole moment. The results of our X-ray reflectivity measurements and MD simulations, which characterize the ordering on the molecular length scale of a PC lipid layer subject to an electric field, are qualitatively consistent with the previously reported chemical-reaction-driven adsorption/desorption process of interfacial lipid molecules.\textsuperscript{13,14,17}

2. MATERIALS AND METHODS

2.1. Materials. The unsaturated phospholipid 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC, C$_{44}$H$_{86}$NO$_5$P, CAS® #56421-10-4) was used as received from Avanti Lipids. The aqueous electrolytes, NaCl (Alfa Aesar Puratronic, 99.9999%, metals basis) and LiCl (certified ACS, crystal from Fisher Scientific Company), were roasted at 450 °C for 45 min to remove organic contaminants and water. Water from a Nanopure UV Barnstead system was used to prepare aqueous
alkali chloride solutions. HEPES buffer solution (1 M) was purchased from Fisher Scientific. 1,2-Dichloroethane (DCE, CHROMASOLV, for HPLC, 99.8%) was purchased from Sigma-Aldrich and purified by passing it six times through a column of activated basic alumina. DCE was further filtered through a 0.2 μm Millipore filter (OMNIPOR membrane filter, PTFE) using a stainless steel pressure filter holder (Sartorius-Stedim Biotech 16249), to remove residual alumina. The organic electrolytes tetrabutylammonium tetrakis(4-chlorophenyl)borate (TDATPBCl) and tetrabutylammonium chloride (TDACl, >97%) were used as received from Aldrich. 1-Hexadecanethiol (99%) was purchased from Sigma-Aldrich and used as received.

A concentrated SOPC stock solution was prepared by dissolving about 25 mg of SOPC into 250 mL of DCE. It was stored in a refrigerator prior to further use within 1 month. A concentrated TDATPBCl stock solution was prepared from stored in a refrigerator prior to further use within 1 month. A concentrated TDATPBCl stock solution was prepared from DCE and then saturated with water by placing it in contact with a few mL of pure water and rocking the solution for about 12 h.54 This solution was then filtered through a 0.2 μm Millipore filter, as described above. Organic solutions used in the experiments were prepared by diluting the concentrated TDATPBCl and SOPC stock solutions with pure DCE. The saturated TDACl solution placed in contact with one of the reference electrodes is prepared by soaking 100 mg of TDACl in a gently shaken flask containing 100 mL of 10 mM LiCl aqueous solution. Sodium chloride was dissolved in water and mixed with 1 M HEPES aqueous buffer solution to produce a pH 7.2 aqueous solution with 100 mM NaCl and 20 mM HEPES. This solution was saturated with DCE by placing it in contact with a few mL of purified DCE, rocking the solution for about 12 h,54 and then filtering it through a Millipore filter, as described above.

2.2. Liquid–Liquid Electrochemical Sample Cell. Figure 1 is a schematic of the four-electrode electrochemical cell used for all reported measurements. The electric current and potential difference across the electrochemical cell can be measured and precisely controlled, and the interfacial tension measured concurrently with X-ray reflectivity measurements.

The galvanic cell is represented by the following scheme:

\[
\text{Ag/AgCl(Re2) | LiCl (1 M) | SOPC 1 mM, DCE | DCE | DCE} \]

where \( \parallel \) represents the interface of interest and \( X \) is varied from 0 to 12.5. The aqueous and organic electrolyte solutions are electrically conductive; therefore, the external potential across the interface can be controlled electrochemically to investigate the lipid monolayer response to electric potential differences between the adjacent bulk solutions. Two platinum meshes (platinum gauze, #2 mesh woven from 0.1 mm diameter wire, 99.9% metals basis, purchased from Alfa Aesar) are used as counter electrodes. The meshes are large, ∼25 cm², to ensure that the electric field is uniform across the plane of the interface. The mesh in the top aqueous phase is positioned ∼1 cm from the interface; the mesh in the bottom organic phase is ∼1.5 cm from the interface. Ag/AgCl reference electrodes placed in Luggin capillaries located within a few mm of the interface are used to monitor the electric potential difference across the interface. The reference electrode in the Luggin capillary connected to the DCE phase is immersed in a 10 mM LiCl and saturated TDACl conductive aqueous solution. The applied electric potential difference is controlled and recorded by a Solartron 1287 (Solartron Instruments, England). The electric potential difference across the galvanic cell, as measured by the Solartron 1287, is denoted \( \Delta \phi_{\text{gall}} \). We adopt the rational potential scale (70, 71) that approximates the electric potential difference \( \Delta \phi_{\text{water}} - \phi_{\text{DCE}} \) between the aqueous and organic bulk phases (far from interfaces and electrodes) as \( \Delta \phi_{\text{water}} - \phi_{\text{DCE}} = \Delta \phi_{\text{gall}} - \Delta \phi_{\text{xic}} \), where the potential of zero charge, \( \Delta \phi_{\text{xic}} = 0.167 \, \text{V} \), is determined from the apex of the electrocapillary curve measured for a sample without SOPC lipids.

A glass tube sealed with a float glass bottom extends downward from the cap and is immersed in the water phase to provide a path for the laser beam used for quasi-elastic light scattering (QELS) measurements of interfacial tension. A 1 cm diameter hole in the middle of each Pt mesh allows for passage of this laser beam. Comparative X-ray reflectivity measurements with and without this hole were similar, indicating that the presence of the hole had a negligible effect on the interfacial structure. Similarly, tension measurements from sample cells with Pt meshes that had smaller holes (∼3 mm in diameter) were identical.

X-ray reflectivity measurements require a very flat interface. Pinning the interface to the cell wall and adjusting the volume of the lower DCE phase accomplishes this. The interface can be pinned by the top edge of a hydrophobic strip when the cell
glass is hydrophilic. The cell was soaked in concentrated (98%) sulfuric acid solution of ammonium persulfate (18 g/L of H₂SO₄) for 5 h to render it hydrophilic. In previous experiments that studied the interfacial distribution of just the glass is hydrophilic. The cell was soaked in concentrated (98%) sulfuric acid solution of ammonium persulfate (18 g/L of H₂SO₄) for 5 h to render it hydrophilic. In previous experiments that studied the interfacial distribution of just the glass is hydrophilic. The cell was soaked in concentrated (98%) sulfuric acid solution of ammonium persulfate (18 g/L of H₂SO₄) for 5 h to render it hydrophilic. In previous experiments that studied the interfacial distribution of just the glass is hydrophilic. The cell was soaked in concentrated (98%) sulfuric acid solution of ammonium persulfate (18 g/L of H₂SO₄) for 5 h to render it hydrophilic. In previous experiments that studied the interfacial distribution of just the glass is hydrophilic. 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simulations, the $x$–$y$ simulation cell parameters and thus the lipid density are kept constant at values that match experimental values. Fluctuations of the simulation box size occur only in the $z$ direction and the total height $Z$ of the box was determined at the end of the simulation. A uniform electric field was applied in the $z$-direction to every charge in the simulation cell.²³

2.5.2. Systems. Simulated systems consist of 50 NaCl, 50 BTPPATPBCl, 200 SOPC, 20496 water, and 8588 DCE molecules arranged within five layers in the following order: (1) aqueous solution (200 mM NaCl), (2) SOPC monolayer (100 SOPC lipids), (3) DCE solution (200 mM BTPPA+/TPBCl⁻ (bis-triethylphosphorylidene/tetakis(4-chlorophenyl)borate)), (4) SOPC monolayer (100 SOPC lipids), (5) aqueous solution (200 mM NaCl). We present results for only the top SOPC monolayer; the bottom monolayer prevents mixing of the solvents and improves system stability. In the absence of an applied electric field, the bottom half of the unit cell is practically a mirror image of the top. This is no longer true when an electric field is applied in the direction perpendicular to the lipid monolayer. In addition, significant monolayer curvature out of the $x$–$y$ plane is inhibited by the application of a small force (0.2 kcal/mol/Å) on the C2 carbon of each lipid (as shown later in Figure 9).

The choice of systems with different areas per lipid and applied electric fields (see Table 1) was guided by experimental conditions. The organic cation BTPPA⁺ was used in place of the experimental cation TDA⁺ to reduce simulation time. Under conditions of positive electric potential difference, $\Delta \phi_{\text{w-o}} > 0$, organic cations are depleted from the region adjacent to the lipid monolayer. Therefore, organic cations only influence the potential drop in the DCE phase and use of a slightly different cation in the organic solvent should not significantly affect our results.

### 3. RESULTS AND ANALYSIS

#### 3.1. Cyclic Voltammetry

Figure 3 shows the cyclic voltammograms for liquid–liquid interfaces between DCE and aqueous electrolyte solutions with and without SOPC lipids dissolved in the DCE phase. The high current at the positive and negative edges of the potential windows is due to transport of supporting electrolytes across the aqueous--organic interface. Excess current is observed for $\Delta \phi_{\text{w-o}} > 0.1$ V when SOPC is in the DCE phase. Similar observations of excess current using other PC lipids have been attributed to lipids leaving the interface and redissolving in the bulk DCE phase.¹⁵ Currents are much lower during X-ray reflectivity and interfacial tension measurements because the voltage ramping rate is lower, 1 mV s⁻¹, and the measurements take place after a period of relaxation to a steady state. The maximum electric potential differences for X-ray reflectivity measurements were chosen to limit the maximum currents to 0.13 $\mu$A cm⁻² to reduce the effect of ion transport on these measurements. Typical values of relaxed currents in the central part of the potential window were 0.026 $\mu$A cm⁻².

#### 3.2. Interfacial Tension Measurements

Figure 4 shows the interfacial tension $\gamma$ as a function of electric potential difference $\Delta \phi_{\text{w-o}}$. The change in interfacial tension is expressed as $\gamma = \gamma_{\text{w-o}} - \gamma_{\text{w-o}}$, where $\gamma_{\text{w-o}}$ is the interfacial tension of the aqueous phase with SOPC and $\gamma_{\text{w-o}}$ is the interfacial tension of the DCE phase.

![Figure 4](image)

Figure 4. Electrocapillary curves (interfacial tension $\gamma$ as a function of electric potential difference $\Delta \phi_{\text{w-o}}$) measured at $T \approx 23$ °C for interfaces between a DCE solution of $X$ mM SOPC and 5 mM TDATPBCl and an aqueous solution of 0.1 M NaCl and 20 mM HEPES, with SOPC concentrations (in mM, top to bottom) of $X = 0$ (●, black), 1.6 (●, blue), 2 (◇, orange), 2.4 (◇, blue), 3 (▲, green), 3.4 (▽, black), 5 (▲, purple), 10 (△, red), 12.5 (■, black).

### Table 1. Simulation Systems

<table>
<thead>
<tr>
<th>area per lipid ($\text{Å}^2$)</th>
<th>unit cell ($X \times Y \times Z$) ($\text{Å}^3$)</th>
<th>electric field (kcal mol⁻¹ Å⁻¹ e⁻¹)</th>
<th>electric potential difference (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72.25 (85 × 85 × 288)</td>
<td>0.01839</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>72.25 (85 × 85 × 288)</td>
<td>0.00000</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>81.00 (90 × 90 × 260)</td>
<td>0.03095</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>90.25 (95 × 95 × 233)</td>
<td>0.03947</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>121.00 (110 × 110 × 175)</td>
<td>0.07200</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>

*The simulation electric field has units of 1 kcal mol⁻¹ Å⁻¹ e⁻¹ $\approx 4.2 \times 10^8$ V m⁻¹. Determination of the electric potential difference will be discussed in section 3.5.*
−(∂y/∂Δϕw−o)ec where T is the thermodynamic temperature, P is the external pressure, and μi is the chemical potential of species i. As described previously, a hyperbolic cosine describes the data well enough to determine the potential of zero charge, Δϕw−o, for which Qw(Δϕw−o) = 0.59.

The other curves in Figure 4 show that the interfacial tension γ for a fixed concentration of SOPC is nearly constant below ~0.15 V but increases above 0.15 V. This variation in γ is reversible. Other authors have reported qualitatively similar variations in interfacial tension for other lipids at the electrified liquid–liquid interface. The regions of increasing tension correspond to a stable monolayer of PC lipids in their zwitterionic form, and the region of increasing tension represents cationic PC lipids whose phosphate group has been neutralized by either protons or other aqueous cations. It has been further suggested that the cationic form of PC lipids can transfer protons or other cations from the aqueous phase into the organic phase, thereby reducing the interfacial concentration of lipids and raising the interfacial tension.76,77

The surface excess or adsorption of SOPC at the interface can be calculated from the variation of γ with sample concentration, according to (∂y/∂ ln c)Δϕw−o,ec = −RTΓ, where Γ is the Gibbs surface excess of SOPC, c is the mole fraction of SOPC, R is the gas constant, and the concentrations of all other components are held constant.78 In spite of the presence of multiple components in our system, a full thermodynamic analysis shows that the variation of tension with lipid concentration leads to the standard Gibbs equation for lipid surface excess.14 The linear relation between γ and log(C/M), where C is the molar concentration shown in Figure 5, indicates a constant adsorption Γ over a range of bulk concentration for a fixed value of Δϕw−o. The deviation from linearity that occurs upon increasing log(C/M) above a certain value indicates that the critical micelle concentration (CMC) has been exceeded, though the interface remains saturated with SOPC lipids. Linearity between γ and log(C/M) holds for all values of Δϕw−o for the 3 μM (log(C/M) = −5.52) SOPC sample that will be studied with X-ray reflectivity. The other SOPC concentration studied by X-ray reflectivity, 12.5 μM (log(C/M) = −4.9), is above the CMC.

Table 2 lists the slopes of the lines drawn in Figure 5 and the associated interfacial area per SOPC molecule for the 3 μM SOPC samples. Essentially the same values are valid for the 12.5 μM SOPC samples. Interfacial areas of 70–74 Å² per SOPC molecule are measured for Δϕw−o ≤ 0.2 V. These are in good agreement with values of 70.1 and 66.5 Å² reported for DOPC and DPPC bilayer vesicles, respectively, and the value of 71 Å for DOPC monolayers at the electrified liquid–liquid interface.76,78

Table 2. Inverse Interfacial Density (Area per Molecule) of SOPC

<table>
<thead>
<tr>
<th>Δϕw−o (V)</th>
<th>slope (mN m⁻¹)</th>
<th>area per SOPC (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−0.17</td>
<td>5.56 ± 0.12</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>−0.07</td>
<td>5.56 ± 0.12</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>0.03</td>
<td>5.56 ± 0.12</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>0.13</td>
<td>5.87 ± 0.18</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>0.18</td>
<td>5.87 ± 0.18</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>0.23</td>
<td>5.12 ± 0.29</td>
<td>80 ± 4</td>
</tr>
<tr>
<td>0.28</td>
<td>4.83 ± 0.23</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>0.33</td>
<td>3.33 ± 0.27</td>
<td>123 ± 10</td>
</tr>
</tbody>
</table>

*Calculated from the data in Figure 5 for 3 μM SOPC samples.

SOPC samples. The monolayer is stable at 3 μM SOPC. Essentially the same values are valid for the 12.5 μM SOPC samples. Interfacial areas of 70–74 Å² per SOPC molecule are measured for Δϕw−o ≤ 0.2 V. These are in good agreement with values of 70.1 and 66.5 Å² reported for DOPC and DPPC bilayer vesicles, respectively, and the value of 71 Å for DOPC monolayers at the electrified liquid–liquid interface.76,78

Table 2 also demonstrates a reduction in interfacial SOPC density for increasing Δϕw−o > 0.2 V. This reduction is consistent with the additional current observed in the cyclic voltammogram in Figure 3 for Δϕw−o > 0.15 V if, as suggested previously, the neutral SOPC lipids are charged either by protonation or complexation with other cations prior to desorbing from the interface.13,15,76 A small reduction in the area per SOPC is observed at Δϕw−o = 0.13 and 0.18 V, which is consistent with the minimum in interfacial tension observed in Figure 4 for this range of Δϕw−o.

3.3. X-ray Reflectivity Data. Figure 6 illustrates X-ray reflectivity data R(Qz) normalized to the Fresnel reflectivity Rf(Qz) calculated for a flat, structureless interface14 from samples with 3 and 12.5 μM SOPC in the DCE phase. X-ray reflectivity probes the electron density variation with depth (ρ(z)), which has been averaged over the in-plane (x−y) region of the X-ray footprint on the sample interface. The coordinate axis z is perpendicular to the interface. The data exhibit Kiessig fringes in the variation of reflectivity with Qz, which are analogous to interference fringes generated by reflections from the top and bottom of thin films. The presence of these fringes indicates that the lipid layer has a well-defined electron density profile perpendicular to the interface. Reflectivity measurements for −150 mV ≤ Δϕw−o ≤ +130 mV are nearly indistinguishable, indicating that the electron density profile of the SOPC layer is essentially unaffected over this biologically relevant range of electric potential difference. Larger positive values of Δϕw−o lead to successive variations in the measured reflectivity, as indicated in Figure 6 by the monotonic shift in reflectivity minima and maxima. As discussed below, the
these differences in $R(Q_z)/R_0(Q_z)$ for the two different concentrations of SOPC are primarily the result of the smaller interfacial tension of the larger concentration (12.5 μM SOPC) sample, whose larger interfacial roughness influences the X-ray reflectivity. The data shown in Figure 6 are the result of measurements that span 8 orders of magnitude for each curve, from $R(Q_z) = 1$ to $R(Q_z) ≈ 10^{-8}$. Since $R(Q_z)/R_0(Q_z) \sim \exp(-\sigma Q_z^2)$, where $\sigma$ is the interfacial roughness, the measurable range of $Q_z$ is limited by the roughness, which varies with interfacial tension, as described in section 3.4. For example, the smaller range of $Q_z$ for the reflectivity measured for $\Delta \phi^{w-o} = -0.12$ V in Figure 6b is due to the reduction in reflectivity at larger $Q_z$ that occurs for the large interfacial roughness of ~12 Å. The data sets for the 12.5 μM sample over the range $-0.12$ V $\leq \Delta \phi^{w-o} \leq 0.23$ V, which were restricted to a smaller range of $Q_z$, provide limited spatial resolution in the analysis of the SOPC layer structure and will not be used for quantitative analysis of the electron density profile. Extending the range of $Q_z$ beyond that shown was generally not possible because the additional time required for the measurement would have produced radiation damage.

Careful monitoring and reproduction of the data in Figure 6 ensured that radiation damage of the samples was negligible. Several methods were used, including constant checks that data at a given value of $Q_z$ for the same sample were repeatable, data reproducibility between different samples, and repeatability of data measured under conditions of different incident flux on the interface. Although X-ray reflectivity measurements from the liquid–liquid interface are more time-consuming than those from the liquid–vapor interface, the X-ray absorption of the top liquid phase greatly reduces the incident flux on the interface and, consequently, the likelihood of radiation damage.

3.4. X-ray Data Analysis. X-ray reflectivity data are fit to determine the electron density profile and, consequently, the arrangement of SOPC lipid molecules at the interface. A one-slab model that describes the interface as a single slab of thickness $L$ and homogeneous electron density sandwiched between the bulk aqueous and DCE electrolyte solutions could not fit the data in Figure 6. A two-slab model provided a good fit to all the data. As described below, this model provides a sensible correspondence between each slab and the SOPC headgroups or tailgroups.

It is well-known that the applied electric potential difference $\Delta \phi^{w-o}$ establishes an interfacial ion distribution consisting of supporting electrolytes. Therefore, it is to be expected that in addition to the monolayer of lipid molecules at the interface there is a distribution of ions on either side of the lipid layer, which decays from a value near the lipid layer to the bulk ion concentration. Approximately matched X-ray contrast of the ions and solvents produced an ion distribution that has a negligible effect on the interfacial electron density profile. This was confirmed by X-ray reflectivity measurements from samples containing only the electrolyte solutions, i.e., without lipids. These X-ray reflectivity data, measured for $0 \leq \Delta \phi^{w-o} \leq 0.33$ V, could be fit with a simple interface consisting of a step function intrinsic electron density profile roughened by capillary waves, thus demonstrating the essentially negligible effect of the chosen supporting electrolytes on the electron density probed by X-ray reflectivity measurements. The negligible effect of the supporting electrolytes allowed us to fit the X-ray reflectivity data with an interfacial model that consisted of only the lipid monolayer.

A two-slab model of the electron density profile $\langle \rho(z) \rangle$ contains the sum of three error functions (erf), each of which models the variation of electron density through an internal interface (water/headgroup, headgroup/tailgroup, and tailgroup/DCE) within the DCE/SOPC-monolayer/water interfacial structure.
Four parameters are fit to the X-ray reflectivity data: electron densities $\rho_h$ and $\rho_l$ of slabs that include the SOPC headgroups and tailgroups plus intercalated solvents, total thickness $L = L_h + L_o$ of the SOPC monolayer, and thickness $L_t$ of the lipid tailgroup slab. Large error bars on the thickness parameters result from correlations between fitting parameters; however, the resultant electron density profile is accurately determined as described in section 4.3. The headgroup slab thickness $L_h$ is calculated from $L$ and $L_t$. A lower limit $L_{min} = 950$ Å is imposed when fitting the SOPC tailgroup layer thickness, which does not affect the fitted values but only influences the "--" error bar. Similarly, $L - L_t = L_o$ is restricted to lie between 5 and 12 Å. A conservative estimate for $L_{min}$ is given by the volume occupied by the SOPC tailgroup $V_l$ (ignoring possible solvent intercalated into the tailgroup region) divided by the area per lipid $A_l$ listed in Table 2. The value of 950 Å in the expression for $L_{min}$ is determined by the known value of the POPC tailgroup volume $V_r$ which has two fewer carbon atoms in each tail than SOPC. The interfacial roughness $\sigma$ is calculated from capillary wave theory, where the "++" error is due to measured errors on the interfacial tension and the "--" error is due to the possibility of a small interfacial bending rigidity. $\kappa = 1.5 k_BT$, as described in section 4.2. Fitting parameters for $\Delta \phi^{\pi-o}$ < 0.18 V (−0.12 V, −0.07 V, −0.02 V, 0.03 V, 0.08 V, 0.13 V) are essentially the same, so the values of $\Delta \phi^{\pi-o} = -0.12 V$ are given as representative values. Samples with 12.5 μM SOPC and $\Delta \phi^{\pi-o} < 0.28 V$ have an interfacial roughness larger than 8 Å, which produces X-ray reflectivity measurements with limited spatial resolution and, consequently, large errors on the fit parameters. Fitting parameters for these values of $\Delta \phi^{\pi-o}$ are not shown; nevertheless, analysis of these samples yielded parameters that agreed within error bars with those for the 3 μM SOPC sample at the same values of $\Delta \phi^{\pi-o}$.

The electron densities in eq 2 represent the average electron density in the interface region of SOPC headgroups, $\rho_h$, and tailgroups, $\rho_d$ and of the bulk aqueous (water), $\rho_w$ and DCE, $\rho_{DCE}$ phases. The thicknesses of the slabs that correspond to the SOPC headgroup and tailgroup regions are represented, respectively, by $L_h$ and $L_t$. The interfacial roughness $\sigma$ represents thermal fluctuations of the interface.84

X-ray reflectivity is calculated from $\langle \rho(z) \rangle$ by use of the Parratt algorithm for which $\langle \rho(z) \rangle$ is divided into 300 layers along the z-axis (with a layer spacing of 0.2 Å in the interfacial region) to model the continuously varying density.81 The calculated reflectivity is used to fit the data in Figure 6 by nonlinear least-squares fitting of the parameters $\rho_h$, $\rho_l$, $\rho_o$ and the total thickness $L = L_h + L_o$. In addition, a small offset in $Q_{r}$ on the order of $1 \times 10^{-3}$ Å⁻¹ or less, is fit to the data to account for a slight misalignment of the reflectometer. The other three parameters in eq 2, $\rho_w$, $\rho_{DCE}$ and $\sigma$, are calculated.

The bulk electron densities $\rho_h$ and $\rho_{DCE}$ are calculated using $\rho_i = \sum_{n} \rho_i(N_i - V_i)$, where $\rho_i$ represents the electron density of the pure solvent (water or DCE), $N_i$ is the number density of ion $i$ of the supporting electrolytes, and $N_i$ and $V_i$ are the number of electrons and volume of ion $i$. The electrolytes associate into TDA⁺, TPBCl⁻, Na⁺, and Cl⁻ ions, which do not cross the interface to any appreciable extent for the values of $\Delta \phi^{\pi-o}$ studied, as illustrated by the cyclic voltammogram in Figure 3. The volumes of Na⁺ and Cl⁻, respectively, 3.82 and 24.82 Å³, are calculated from their spherical diameters: 1.94 Å for Na⁺ and 3.60 Å for Cl⁻.82 The volumes of TDA⁺ and TPBCl⁻, respectively 1219.02 and 531.32 Å³, are calculated as solvent excluded volumes within the software Gaussian.83 This yields the bulk electron densities of the DCE and aqueous phases to be 0.3795 and 0.3344 e⁻ Å⁻³, only slightly different from the values of 0.3804 e⁻ Å⁻³ for pure DCE and 0.3335 e⁻ Å⁻³ for pure water (at 23 °C). The critical wave vector for total reflection, $Q_{c} = 0.00795$ Å⁻¹, determined by these densities is consistent with the X-ray reflectivity data.84

The interfacial roughness $\sigma$ is calculated from the measured interfacial tension $\gamma$ and capillary wave theory, $\sigma^2 = (k_BT/2\pi\rho) \ln(q_{min}/q_{max})$, where $T$ is the temperature, $q_{min} = 2/r = 2(\pi/\lambda)$ is the cutoff for the smallest wavelength capillary waves that uses $r$ as the effective cross-sectional radius, $A$ is the area per lipid, and $q_{max}$ is the smallest capillary wave vector that can be resolved by the X-ray measurements, given by $q_{max} = (2\pi/\lambda)\Delta x / \sin(\Delta x) = 5.6 / 10^{-4}$ is the vertical angular acceptance of the detector, where $l = 1.8$ mm is the electronic slit size and $d = 3.19$ m is the distance from the sample center to the CCD detector).84 Table 3 lists the calculated values of $\sigma$ and fitting parameters for the best fits of the X-ray reflectivity data shown in Figure 6.

### Table 3. Parameters for Two-Slab Model Fitting to X-ray Reflectivity Measurements

<table>
<thead>
<tr>
<th>SOPC concentration (μM)</th>
<th>$\Delta \phi^{\pi-o}$ (V)</th>
<th>$L_h$ (Å)</th>
<th>$\rho_h$ (e⁻ Å⁻³)</th>
<th>$L_l$ (Å)</th>
<th>$\rho_l$ (e⁻ Å⁻³)</th>
<th>$\sigma$ (Å)</th>
<th>$L_t$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>−0.12</td>
<td>6.1±0.4</td>
<td>0.411±0.007</td>
<td>16±3</td>
<td>12.5</td>
<td>0.321±0.009</td>
<td>7.4±0.07/−0.58</td>
</tr>
<tr>
<td>3</td>
<td>0.08</td>
<td>6.5±0.5</td>
<td>0.415±0.005</td>
<td>16±3</td>
<td>13.2</td>
<td>0.323±0.001</td>
<td>7.8±0.1/−0.6</td>
</tr>
<tr>
<td>3</td>
<td>0.23</td>
<td>8.8±0.4</td>
<td>0.402±0.009</td>
<td>16±3</td>
<td>11.2</td>
<td>0.339±0.002</td>
<td>7.1±0.05/−0.36</td>
</tr>
<tr>
<td>3</td>
<td>0.28</td>
<td>8.6±0.3</td>
<td>0.395±0.020</td>
<td>16±3</td>
<td>10.5</td>
<td>0.341±0.009</td>
<td>6.6±0.005/−0.37</td>
</tr>
<tr>
<td>12.5</td>
<td>0.28</td>
<td>9.1±0.5</td>
<td>0.408±0.034</td>
<td>15.2±3</td>
<td>10.5</td>
<td>0.334±0.001</td>
<td>7.7±0.07/−0.48</td>
</tr>
<tr>
<td>12.5</td>
<td>0.33</td>
<td>8.7±0.7</td>
<td>0.410±0.029</td>
<td>15.1±3</td>
<td>7.85</td>
<td>0.337±0.002</td>
<td>6.8±0.005/−0.39</td>
</tr>
</tbody>
</table>

The quantum mechanical calculation parameters in eq 2, \(\rho_h\) and \(\rho_l\), are calculated using the SOPC headgroup fitting parameters; however, the resultant electron density profile is accurately determined as described in section 4.3. The headgroup slab thickness \(L_h\) is calculated from \(L\) and \(L_t\). A lower limit \(L_{min} = 950\) Å is imposed when fitting the SOPC tailgroup layer thickness, which does not affect the fitted values but only influences the "--" error bar. Similarly, \(L - L_t = L_o\) is restricted to lie between 5 and 12 Å. A conservative estimate for \(L_{min}\) is given by the volume occupied by the SOPC tailgroup \(V_l\) (ignoring possible solvent intercalated into the tailgroup region) divided by the area per lipid \(A_l\) listed in Table 2. The value of 950 Å in the expression for \(L_{min}\) is determined by the known value of the POPC tailgroup volume, which has two fewer carbon atoms in each tail than SOPC. The interfacial roughness \(\sigma\) is calculated from capillary wave theory, where the "++" error is due to measured errors on the interfacial tension and the "--" error is due to the possibility of a small interfacial bending rigidity, \(\kappa = 1.5 k_BT\), as described in section 4.2. Fitting parameters for \(\Delta \phi^{\pi-o} < 0.18 V\) (−0.12 V, −0.07 V, −0.02 V, 0.03 V, 0.08 V, 0.13 V) are essentially the same, so the values of \(\Delta \phi^{\pi-o} = -0.12 V\) are given as representative values. Samples with 12.5 μM SOPC and \(\Delta \phi^{\pi-o} < 0.28 V\) have an interfacial roughness larger than 8 Å, which produces X-ray reflectivity measurements with limited spatial resolution and, consequently, large errors on the fit parameters. Fitting parameters for these values of \(\Delta \phi^{\pi-o}\) are not shown; nevertheless, analysis of these samples yielded parameters that agreed within error bars with those for the 3 μM SOPC sample at the same values of \(\Delta \phi^{\pi-o}\).
constant as \( z \) varies from the interface to the bulk, in the absence of lipids.

The simulated SOPC lipid monolayer is further characterized by calculating its thickness \( L_s \) and orientation angles \( \alpha \) of the SOPC headgroup and \( \beta \) of the entire SOPC molecule, where the angles are illustrated in Figure 9.87 The headgroup tilt angle \( \alpha \) is measured between the \( z \)-axis and a line connecting P to N atoms in the headgroup. The molecule tilt angle \( \beta \) is measured between the first principle axis of the lipid molecule and the \( z \)-axis. Average values of these parameters are calculated by averaging over all 100 lipids in the monolayer and then further averaging over the 500 simulation frames that span the last 4 ns of each simulation trajectory. The thickness \( L_s \) is the difference between the maximum and minimum \( z \) atomic coordinates of each lipid. Table 4 shows that the membrane thins with increasingly positive electric fields.

Figure 8. Simulated electric potential difference profiles. The simulation cell consists of water/DCE/water electrolyte solutions with a lipid monolayer at each interface. The simulated electric potential difference between the water and DCE phases (across the SOPC monolayer at \( z \approx 100 \) Å) in the absence of an applied electric field is shown by \( \Delta \phi_s \). The electric potential difference in the presence of electric fields is shown for one value of \( \Delta \phi_s \).

4. DISCUSSION

4.1. Electron Density Profile. X-ray reflectivity measurements of the SOPC monolayer were analyzed in terms of an intrinsic electron density profile roughened by thermal capillary waves, where the two-slab model given in eq 2 with the roughness \( \sigma \) set to zero describes the intrinsic electron density profile. The full experimental electron density profile probed by X-ray reflectivity is given by the two-slab model with the best-fit parameters listed in Table 3, including nonzero roughness \( \sigma \) whose effect is to smear the profile along the \( z \)-axis. Neither the full nor the intrinsic electron density profiles can be compared directly to the profile from MD simulations shown in Figure 10 because the small cross-sectional dimensions of the simulation...
such close-packed chains have a cross-sectional area.

This procedure allows for the comparison of equally roughened

roughening them using values of $\sigma$. The simulated pro-

Figure 10.

Electron density profiles calculated from MD simulations for different values of $\Delta \phi^\text{w-o}$. cell (e.g., $85 \times 85 \text{Å}^2$) limit the capillary wave roughness to a small but nonzero value, 3.2 Å, whereas the experimental value of roughness is close to 7 Å.

Figure 11 illustrates a comparison between the full experimental electron density profiles and MD simulated electron density profiles that have been modified by fitting the simulated profiles with the two-slab model and then roughening them using values of $\sigma$ from experiment (Table 3). This procedure allows for the comparison of equally roughened profiles from experiment and simulation. Importantly, this comparison uses electron density profiles that are determined directly from experiment. The two sets of profiles are qualitatively similar, though there are some quantitative differences. The simulated values of total monolayer thickness are slightly larger than experimental values, though the trend toward thinning with increasingly positive electric potential difference is observed in both sets of profiles. The simulated electron density value is smaller in the tailgroup minimum by about 0.01 e$^-\text{Å}^{-3}$ and larger in the headgroup maximum by a similar amount. Similar discrepancies between experimental and simulated values are present in the electron densities of the bulk DCE and water phases.

4.1.1. Tailgroup Region. Close-packed saturated alkyl chains have electron densities that vary from 0.325 to 0.343 e$^-\text{Å}^{-3}$ for alkane rotator phases to crystalline bulk phases of long chain alkanols.88 Such close-packed chains have a cross-sectional area of $\sim 19 \text{Å}^2$ that is much smaller than half the 70 Å$^2$ per lipid or greater interfacial area that is occupied by the disordered monolayer of two-tailed SOPC. The unsaturated oleoyl tail of SOPC frustrates the chain packing, forcing it to occupy a larger interfacial area than close-packed chains. Therefore, even though values of the intrinsic electron density $\rho_i$ of the tailgroup slab, determined by X-ray reflectivity, vary from 0.32 to 0.34 e$^-\text{Å}^{-3}$ (Table 3), they cannot represent close-packed

$\Delta \phi^\text{w-o}$

<table>
<thead>
<tr>
<th>$\Delta \phi^\text{w-o}$ (V)</th>
<th>area per lipid (Å$^2$)</th>
<th>$L_s$ (Å)</th>
<th>$L_{SOPC}$ (Å)</th>
<th>$\alpha$ (deg)</th>
<th>$\beta$ (deg)</th>
<th>$N_{H2O}$</th>
<th>$N_{DCE}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.12</td>
<td>72.25</td>
<td>23.9 ± 0.2</td>
<td>24.9 ± 0.2</td>
<td>66.1 ± 1.6</td>
<td>16.5 ± 1.1</td>
<td>11.1 ± 0.3</td>
<td>4.0 ± 0</td>
</tr>
<tr>
<td>0</td>
<td>72.25</td>
<td>23.7 ± 0.2</td>
<td>24.8 ± 0.2</td>
<td>65.7 ± 1.8</td>
<td>17.4 ± 1.2</td>
<td>11.0 ± 0.1</td>
<td>4.0 ± 0</td>
</tr>
<tr>
<td>0.23</td>
<td>81.00</td>
<td>23.1 ± 0.2</td>
<td>24.7 ± 0.3</td>
<td>66.5 ± 1.5</td>
<td>20.5 ± 1.4</td>
<td>13.1 ± 0.5</td>
<td>4.7 ± 0.5</td>
</tr>
<tr>
<td>0</td>
<td>81.00</td>
<td>23.3 ± 0.1</td>
<td>24.7 ± 0.2</td>
<td>65.6 ± 1.4</td>
<td>19.5 ± 1.1</td>
<td>13.1 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>0.28</td>
<td>90.25</td>
<td>22.3 ± 0.2</td>
<td>24.7 ± 0.4</td>
<td>68.3 ± 1.4</td>
<td>25.5 ± 2.0</td>
<td>14.6 ± 0.6</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>0</td>
<td>90.25</td>
<td>23.1 ± 0.1</td>
<td>24.8 ± 0.2</td>
<td>65.7 ± 1.7</td>
<td>21.3 ± 0.9</td>
<td>15.5 ± 0.6</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>0.33</td>
<td>121.0</td>
<td>21.3 ± 0.2</td>
<td>24.7 ± 0.4</td>
<td>73.4 ± 2.4</td>
<td>30.3 ± 2.4</td>
<td>19.5 ± 0.9</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>0</td>
<td>121.0</td>
<td>21.8 ± 0.2</td>
<td>24.9 ± 0.3</td>
<td>68.7 ± 2.0</td>
<td>28.9 ± 1.0</td>
<td>21.5 ± 0.6</td>
<td>7.8 ± 0.4</td>
</tr>
</tbody>
</table>

Table 4. Simulation Results

$\alpha$, $\beta$, $N_{H2O}$, and $N_{DCE}$ represent the simulated thickness of the lipid monolayer, the calculated value $L_{SOPC} = L_s/\cos \beta$ is the total length of SOPC molecules calculated from the simulated $L_s$ and $\beta$, where the angles $\alpha$ and $\beta$ denote the tilt angles of the SOPC headgroup and the entire SOPC molecule measured from the z-axis (Figure 9), $N_{H2O}$ is the number of water molecules per lipid within the headgroup region, and $N_{DCE}$ is the number of DCE molecules per lipid in the tailgroup region. The multiple simulations for $\Delta \phi^\text{w-o} = 0$ V for different areas per lipid will be discussed in section 4.4.
tailgroups. Instead, this electron density is the combination of loosely packed, disordered tailgroups, with an average electron density less than 0.32 \( \text{e}^{-} \cdot \text{Å}^{-3} \), and DCE molecules, whose bulk phase electron density is 0.38 \( \text{e}^{-} \cdot \text{Å}^{-3} \), intercalating into the tailgroups. A simple estimate of the number \( n \) of DCE molecules per lipid that intercalate into the tailgroup, based upon the number of electrons and volume of the tailgroups and DCE molecules, is provided by 
\[
\rho_{\text{tail}} = \frac{274 + 50n}{960 + 131n},
\]
where 274 is the number of electrons in the two-chained tailgroup \((C_{34}H_{70})\), 50 is the number of electrons in a DCE molecule, 960 Å\(^3\) is an estimate for the volume occupied by the tailgroup chains,\(^8\) and 129 Å\(^3\) is the volume of a DCE molecule determined from the bulk density of DCE. Varying the value of \( \rho_{\text{tail}} \) over the measured values of 0.32–0.34 \( \text{e}^{-} \cdot \text{Å}^{-3} \) suggests that 4–10 DCE molecules per lipid intercalate into the tailgroup region. This is consistent with the 4–8 DCE molecules observed in the tailgroup region by MD simulations (\( N_{\text{DCE}} \) in Table 4). As \( \Delta \phi_{w-o} \) increases, the area per lipid increases, thus allowing for greater intercalation of the DCE and an increase in the tailgroup electron density. Figure 12 shows that the trend in electron density with \( \Delta \phi_{w-o} \) is consistent with MD simulations.

The disordered nature of SOPC tailgroups is consistent with the 15–16 Å thickness of the tailgroup slab determined by X-ray reflectivity (Table 3). An all-trans alkyl chain with 17 carbon atoms will have a length of 21.7 Å \((=(17 - 1 + (9/8)) \times 1.265 \text{ Å})\),\(^8\) significantly longer than the measured thickness of the tailgroup slab.

4.1.2. Headgroup Region. The thickness of the SOPC headgroup region, 6.1–9.1 Å (Table 3), is consistent with literature values that vary from 7 to 12 Å for PC lipid headgroup thickness in monolayers on liquid surfaces.\(^9\) Similarly, the measured headgroup electron density of 0.399–0.415 \( \text{e}^{-} \cdot \text{Å}^{-3} \) lies within the range of values previously observed.\(^9\) Note that measurements of the volume of the PC headgroup of \( \sim 320 \text{ Å}^3 \) yield a bare headgroup electron density of 0.53 \( \text{e}^{-} \cdot \text{Å}^{-3} \).\(^8\) Substantial hydration of the headgroup is required to reduce its bare electron density to the measured values. A simple estimate, based upon the number of electrons and volume of the headgroups and water molecules, suggests that 15–20 water molecules per lipid hydrate the headgroup region, respectively, when the headgroup electron density varies from 0.415 to 0.399 \( \text{e}^{-} \cdot \text{Å}^{-3} \) (Table 3). This is fairly consistent with the 11–21 water molecules in the headgroup layer observed by MD simulations (Table 4). Both experiments and simulations are consistent with an increase in headgroup water with area per lipid. However, closer observation of the simulations reveals that only five of the water molecules are hydrogen-bonded to the headgroup (Figure 13), a number that remains constant for all areas per lipid. Both non-H-bonded and H-bonded water molecules are present at all areas per lipid; however, as the area per lipid increases, additional nonbonded water molecules move into the headgroup region to fill the available space (Figure 14).

4.2. Bending Rigidity. The fluctuation spectrum of lamellar lipid bilayer phases is known to be influenced by the bending rigidity (or modulus) \( \kappa \).\(^9\) Similarly, the roughness \( \sigma \) of an interface is influenced by \( \kappa \), which preferentially suppresses short wavelength thermal fluctuations of the interface, as

![Figure 12. Comparison of the intrinsic electron density of the interfacial tailgroup slab determined by fitting X-ray reflectivity measurements (Table 3) and MD simulations.](image)

![Figure 13. MD simulation snapshot of hydrogen-bonded water molecules in the SOPC headgroup region.](image)

![Figure 14. Top view snapshots of SOPC monolayer simulations showing only H-bonded water in red and SOPC molecules in gray for just the top monolayer in the simulation cell. These snapshots illustrate the space available for non-H-bonded water molecules and DCE molecules to intercalate into the monolayer.](image)
observed from the expression for the energy \( u(\vec{q}_{xy}) \) per capillary mode with wave vector \( \vec{q}_{xy} \):

\[
u(\vec{q}_{xy}) \approx \frac{\hbar^2(q_{xy})^2}{2\Lambda_{tot}^2} \left( \Delta \rho_{tot} + \gamma |\vec{q}_{xy}|^2 + \kappa |\vec{q}_{xy}|^4 + \ldots \right)
\]

(3)

where \( h(\vec{q}_{xy}) \) is the Fourier transform of the surface height function, \( \Lambda_{tot} \) is the total surface area, \( \Delta \rho_{tot} \) is the mass density difference between the two phases, and \( g \) is the gravitational acceleration. The surface bending rigidity \( \kappa \) is the amplitude of the \( |\vec{q}_{xy}|^4 \) term, which is large at short wavelength (i.e., large \( |\vec{q}_{xy}| \)) capillary waves. The expression in eq 3 leads to a revised expression for the interfacial roughness \( \sigma^2 \):

\[
s^2 \approx \frac{k_B T}{2\gamma} \ln \left( \frac{\gamma/\kappa}{q_{min}} \right)^{1/2}
\]

(4)

where the value of \( q_{min} \) was given in section 3.4. X-ray reflectivity measurements probe the spatially averaged (over the \( x-y \) plane of the interface) fluctuation spectrum to yield the interfacial roughness \( \sigma \) but cannot separate the effect of interfacial tension \( \gamma \) and surface bending rigidity \( \kappa \) on the roughness. Our QELS measurements also probe the thermal fluctuation spectrum but do so at much longer capillary wavelengths (8.5 x 10^{-5} m for measurements of the third order peak scattered from the grating), thus allowing for a measurement of \( \gamma \) that is unaffected by small values of \( \kappa \). The analysis of X-ray reflectivity presented in section 3.4 used the value of roughness \( s^2 \approx (k_B T/2\gamma) \ln(q_{max}/q_{min}) \) calculated from the QELS measurement of \( \gamma \). Reanalysis of X-ray reflectivity data with the smaller roughness given by eq 4 can place an upper limit on values of \( \kappa \) that are consistent with the data. Since the surface bending rigidity will have a greater effect on interfaces with low interfacial tension, reanalysis of the 12.5 \( \mu \)M SOPC sample provides the highest accuracy determination of \( \kappa \) within our set of data. In addition, \( \kappa \) is maximized at the highest interfacial density of lipids,\(^{94}\) therefore, reanalysis of the X-ray reflectivity data from the 12.5 \( \mu \)M SOPC sample at \( \Delta \phi^{W-O} = -0.12 \) V is used. These data provide an upper limit of \( \kappa \leq 1.5 k_B T \). Under these conditions, the interfacial roughness \( \sigma \) decreases from a value of 12 Å when \( \kappa = 0 \) to 10.5 Å when \( \kappa = 1.5 k_B T \).

4.3. Total Monolayer Thickness. The values of monolayer thickness \( L \) determined from X-ray measurements (listed in Table 3) and values of \( L \) from MD simulations (listed in Table 4) are very similar, \( \sim 22-25 \) Å, depending on the interfacial area per lipid. These values are comparable to the thickness of SOPC or mixed SOPC:SOPS monolayers previously studied at the water—vapor interface.\(^{38,39,43}\) The relatively large standard deviations on the X-ray fitting parameters and the possibility of a bending rigidity complicate the detailed comparison of these values, in particular, their variation with \( \Delta \phi^{W-O} \). As shown in Table 3, the fitted values of \( L \) have one standard deviation errors up to \( \pm 3 \) Å; nevertheless, the electron density profiles that are produced by sets of fit parameters that vary within one standard deviation of the best-fit parameters are visually indistinguishable from the best-fit electron density profiles shown in Figure 11. This shows that the electron density profile is accurately determined by X-ray measurements but that correlations among the fitting parameters yield unphysically large variations. This complication is exacerbated by the inability to directly measure the bending rigidity described in the previous section. Varying the bending rigidity over its range of possible values (0–1.5 \( k_B T \)) yields an additional variation of \( \sim 3 \) Å in the fitting parameter \( L \); however, this does not change the electron density profile. These complications can be circumvented by directly analyzing the electron density profiles.

Figure 15 shows the 10–90 thickness of the SOPC monolayer, calculated by analysis of the electron density profiles in Figure 11a to determine the largest distance between positions \( z \) where the density is less than the DCE bulk phase density by 10 and 90% of the density difference between the two bulk phases. Although this measure of monolayer thickness includes the effect of thermal roughness and is substantially larger than the intrinsic monolayer thickness of \( \sim 22-25 \) Å listed in Tables 3 and 4, its advantage is that it allows for a direct comparison of the results of X-ray reflectivity measurements and MD simulations without the complication of correlations in the X-ray fitting parameters. Figure 15 demonstrates that the 10–90 thicknesses are up to 5 Å larger for the simulations, as is confirmed by visual observation of the two panels in Figure 11. Also observable from both experiment and simulation is the thinning of the monolayer at about the same values of \( \Delta \phi^{W-O} \), although a larger effect is observed in the simulations.

4.4. Role of the Electric Field. Interfacial tension and X-ray reflectivity measurements of the SOPC monolayer are essentially constant over a biologically relevant range of electric potential differences, \( -150 \) mV \( \leq \Delta \phi^{W-O} \leq +130 \) mV, indicating that the structure of the SOPC layer is unchanged over this range of \( \Delta \phi^{W-O} \). MD simulations also show a similar structure at two values of electric potential within this range, \( -120 \) mV and 0.0 V, calculated for the \( \sim 72 \) Å² area per lipid determined by our experiments. Recent neutron reflectivity measurements of a bilayer of POPC lipids that contained one leaflet (monolayer) tethered to a solid support and the other leaflet physisorbed on top reported a 2 Å thinning of the physisorbed leaflet when the transbilayer voltage was changed from 0 to 100 mV.\(^{53}\) The neutron measurements displayed a peak in \( R(Q) / R_0(Q) \) but could not observe a full oscillation because of the smaller measured range in \( Q \) (\( \leq 0.15 \) Å⁻¹). Our X-ray reflectivity data shown in Figure 6a and the variations in the measured electron density profile shown in Figure 11a are indistinguishable over the range of potential from \( -150 \) to +130 mV and are not consistent with a change of 2 Å in, for example, the 10–90 thickness of the monolayer, within a statistical accuracy of at least four standard deviations. Fitting parameter variations that led to a change of 2 Å in the 10–90 thickness of the monolayer over this range of potentials would produce a poor fit to the X-ray reflectivity data.
Measurements at higher positive potentials, from 180 to 330 mV, illustrate a monotonic increase in the interfacial area per lipid and accompanying variations in the interfacial ordering of the lipid. MD simulations of the headgroup angle $\alpha$ show little change until it increases at the highest potential differences of 280 mV (90.25 Å$^2$) and 330 mV (121 Å$^2$), yet the molecule angle $\beta$ has changed substantially by 230 mV (81 Å$^2$) and has an overall larger increase than $\alpha$ up to potentials of 330 mV (121 Å$^2$); see Table 4. If SOPC rotated as a rigid body, then increased values of $\phi$ should lead to similar increases in $\alpha$. It seems that the headgroup interactions, possibly with the aqueous phase or with neighboring headgroups, have a stabilizing effect on the headgroup orientation. Nevertheless, constancy of the length along the first principle axis of the SOPC, shown by values of $L_{\text{OPC}} = L_s/\cos \beta$ listed in Table 4, indicates that the variation in membrane thickness is due primarily to nearly rigid-body rotation of the entire SOPC molecule and only secondarily to changes in the molecular configuration.

It is interesting to ask if the changes in the structure of the lipid monolayer with electric potential difference are due to rotation of the headgroup dipole by the electric field or to some other mechanism. The approximate energy of a headgroup dipole in an electric field, $U = -\vec{p} \cdot \vec{E}$, is calculated to be $\sim 0.1 k_B T$ for a headgroup dipole moment of 10 D and electric field amplitudes of $\sim 10^3$ V m$^{-1}$, which are similar to those used in the simulations (Table 1). Although the effective headgroup dipole moment can be varied by complexing with water or other lipids, the conclusion of small dipole energies should apply to our single-component monolayers. In addition, if electric dipole interactions were the dominant source of the observed changes, then we would have expected changes in the headgroup angle $\alpha$ to lead changes in $\beta$ as a result of the torque on the headgroup dipole. Instead, MD simulations show the opposite; changes in $\alpha$ lag those in $\beta$.

Alternatively, the changes in lipid monolayer structure could be due to the larger area per lipid that is due to something else besides the electric field torque on the headgroup. Several authors have suggested that the increased interfacial tension observed at higher $\Delta \phi^{w-o}$ as well as electrochemical observations of increased current in the cyclic voltammogram at similar values of $\Delta \phi^{w-o}$ are the result of a decreased interfacial density of lipids. They proposed a chemical mechanism in which interfacial PC lipids become positively charged by proton or cation binding at an appropriate positive value of $\Delta \phi^{w-o}$ and are subsequently desorbed from the interface into the bulk organic phase. Although our X-ray experiments and MD simulations did not directly test this chemical mechanism, our MD simulations show that most of the observed structural effects are due to variations in lipid interfacial density and not to the applied electric field. Note that the MD simulations did not try to mimic this chemical mechanism but fixed the number of lipid molecules in the layer. Nevertheless, the MD simulations can independently vary the area per lipid and the applied electric field. Simulations at $\Delta \phi^{w-o} = 0$ for different interfacial areas per lipid produced similar values for the monolayer thickness and tilt angles as did the corresponding simulations at the same areas per lipid but at values of $\Delta \phi^{w-o}$ that mimic the experiments (Table 4). Only at the two highest values of area per lipid, 90 and 121 Å$^2$, are differences observed in the monolayer structure between simulations at 0 V and the two corresponding highest values of potential difference, 280 and 330 mV. It appears that there is some influence of the electric field interaction with the lipid dipole on lipid ordering at the highest values of $\Delta \phi^{w-o}$, but the primary determinant of lipid ordering is the interfacial area per lipid.

5. Conclusions

The effect of membrane potential on the molecular ordering of lipids within a biomimetic membrane—a self-assembled monolayer of SOPC lipids at a liquid—liquid interface between two immiscible electrolyte solutions—was studied with X-ray reflectivity and interferential tension measurements, as well as MD simulations. Interfacial tension and X-ray reflectivity measurements demonstrate that stable and reproducible SOPC monolayers are formed with interfacial densities that can be varied from values typical of unsaturated lipids in biomimetic systems ($\sim 70$ Å$^2$ per lipid molecule) to densities lower by about a factor of 2. Measurements of the high density SOPC monolayers are nearly indistinguishable over a biologically relevant range of electric potential differences, $-150$ mV $\leq \Delta \phi^{w-o} \leq +130$ mV, indicating that the molecular order within the SOPC layer is unchanged over this range of $\Delta \phi^{w-o}$. Measurements at higher positive potentials, up to 330 mV, illustrate a monotonic increase in the interfacial area per lipid and accompanying variations in the interfacial ordering of the lipid.

X-ray reflectivity measurements determined values of the thickness and electron densities of the two interfacial regions containing SOPC headgroups and tailgroups. These values demonstrated that solvent must intercalate into the SOPC layer, specifically water hydration of the headgroup and DCE solvation of the tailgroup. Upon accounting for this intercalation, the structure of the SOPC layer is consistent with values in the literature for layers of unsaturated lipids in other geometries, such as bilayer vesicles or Langmuir monolayers. In addition, the predictions of MD simulations for the number of hydration water molecules and intercalated DCE molecules are in quantitative agreement with the measured electron densities. Ion penetration into the lipid headgroup region is small in the MD simulations and not observable in the X-ray reflectivity measurements. The variation in total monolayer thickness with electric potential difference $\Delta \phi^{w-o}$ determined by X-ray reflectivity is consistent with the MD simulations, with small quantitative differences between them. Finally, a combination of X-ray reflectivity and quasi-elastic light scattering measurements placed a small upper limit on the interfacial bending rigidity, $\kappa \leq 1.5 k_B T$, of the monolayer.

The close correspondence between the structure of the monolayer predicted by MD simulations and measured by X-ray reflectivity over the experimental values of $\Delta \phi^{w-o}$ provides a justification to use the simulation results to address whether the change in monolayer structure with $\Delta \phi^{w-o}$ is due to the torque on the dipole moment of the PC lipid headgroup or to some other influence of $\Delta \phi^{w-o}$ that leads to an increase in interfacial area per lipid. Simulations show that the lipid molecular ordering is determined primarily by changes in interfacial area per lipid, and only secondarily to the electrostatic interaction of the dipole moment with the electric field. These results provide indirect support for a previously published proposal that protonation or cation binding to the lipid, which takes place at an appropriate positive value of the potential difference $\Delta \phi^{w-o}$, leads to a loss of lipid from the

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interface and, consequently, to an increase in area per lipid at higher values of $\Delta_g^{\text{w-o}}$.

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**Notes**

The authors declare no competing financial interest.

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