Ion Distribution near Phase Separated Charged Phospholipid Membranes

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1. Introduction

Phospholipids, the main components of cell membranes, spontaneously form bilayer structures in aqueous solution. Phospholipid bilayers as a model for biological membranes have been studied to elucidate their structures and physical properties. In multicomponent lipid bilayers, heterogeneous lipid distributions and phase separation arise depending on the lipid composition and temperature [1]. This membrane heterogeneity has attracted broad interest because it resembles lipid raft domains observed in biological membranes [2], which are thought to play pivotal roles in intracellular signal transduction and membrane trafficking.

Charged lipids are abundant in biological membranes [3] and participate in a wide range of biological functions, including protein adsorption [4] and ion channel activity [5]. Experimental investigations of phospholipid membranes containing charged lipids have demonstrated that long-range electrostatic interactions influence phase separation behavior [6, 7, 8, 9, 10]. For example, Shimokawa et al. used fluorescence microscopy to show that the phase diagram of charged–neutral lipid mixtures shifts with salt concentration [9]. Himeno et al. observed large-scale deformation of charged lipid liposomes accompanying phase separation [10]. Collectively, these experimental studies establish that the ionic environment in solution plays a critical role in phase separation involving charged lipids. However, the spatial distribution of ions near the heterogeneous membrane is difficult to observe experimentally, and the molecular-scale picture of ion distributions remains incompletely understood.

Molecular simulation is a powerful tool for resolving membrane structure and dynamics at the molecular level [11]. All-atom molecular dynamics (MD) simulations have shed light on how headgroup charges influence hydration states and membrane physicochemical properties [12, 13]. However, all-atom simulations long enough to capture phase separation are computationally prohibitive. Coarse-

grained MD simulations can instead target longer-timescale, larger-scale phenomena such as phase separation [14] and bilayer deformation [15]. Using a coarse-grained model with a Debye–Hückel electrostatic potential, our group has previously elucidated characteristic deformation processes of phospholipid membranes driven by long-range electrostatic interactions [10, 16, 17, 18]. However, in our own earlier coarse-grained simulations using a Debye–Hückel potential, ions were not treated explicitly, making it difficult to directly observe the relationship between ion distributions and phase separation at the molecular scale. The MARTINI model [19] explicitly includes coarse-grained ions, lipid molecules, and water molecules, enabling simultaneous treatment of charged membranes and ions. Previous MARTINI studies have revealed the Ca^{2+}-mediated fusion of charged lipid membranes [20] and elucidated phase separation dynamics and lipid molecular orientation at the molecular level [21, 22]. Nevertheless, the correlation between lipid phase separation and ion distributions in the surrounding aqueous solution has remained unresolved.

The ion distribution perpendicular to charged lipid membranes is classically described by Poisson–Boltzmann (PB) theory [23], which has also been applied to phase-separated membranes [9]. By contrast, the lateral ion distribution parallel to the membrane—particularly near the boundary between charged and uncharged lipid domains—has been a difficult problem to elucidate. Clarifying the spatiotemporal correlation between lipid phase separation and ion distributions at the molecular scale therefore represents the central challenge addressed in this report.

Here we summarize two studies that take up this challenge. The first one employed coarse-grained MD simulations with the MARTINI model to investigate the spatiotemporal correlation between phase separation and ion distributions in charged phospholipid bilayers [24]. The second one extended this work to a larger system and compared the results with Monte Carlo (MC) simulations and modified PB theory to quantitatively characterize how ion distributions change near the phase-separation interface [25].

2. Models and Methods

Coarse-grained MD simulations were performed using the MARTINI model (version 2.0) [19], which explicitly incorporates ions, lipid molecules, and water molecules and is well suited to simulating long-timescale, large-scale phenomena such as phase separation. A polarizable water model (version 2.2) [26] was adopted for the solvent. The lipid components were the zwitterionic saturated phospholipid DPPC (dipalmitoylphosphocholine), the negatively charged monounsaturated phospholipid DOPG (dioleoylphosphoglycerol), and the negatively charged, tetraunsaturated phospholipid DAPG (diarachidonoylphosphoglycerol). NaCl was chosen as a representative monovalent salt, and simulations were carried out at several salt concentrations.

In Section 3, each lipid bilayer system consisted of 2,048 lipid molecules. In Section 4, the system size was expanded approximately fivefold relative to the earlier work—to 3,500 DPPC, 3,500 DAPG, and 3,000 cholesterol molecules—to obtain a sharper phase-separation interface and improved statistical precision. This inclusion of 30% cholesterol was motivated by findings from prior MARTINI MD studies [21].

Initial structures were built with CHARMM-GUI [27]. MD calculations were performed using GROMACS [28] under isothermal–isobaric (NpT) conditions at 303 K and 1 bar.

The degree of phase separation was quantified by the local order parameter ϕ_i, defined as the fraction of neighboring lipids of the same

species around each lipid molecule. Ion distributions in the aqueous phase near the membrane were analyzed both perpendicular and parallel to the bilayer plane.

3. Correlation between Phase Separation and Ion Distribution

Coarse-grained MD simulations with the MARTINI model revealed the spatiotemporal correlation between phase separation dynamics and ion distributions in charged phospholipid bilayers [24]. In a DPPC:DAPG = 1:1 mixture at 100 mM NaCl, phase separation began within 0.1 µs, and DPPC-rich and DAPG-rich domains became fully segregated after approximately 1.0 µs. Figure 1 shows a representative snapshot at 2.0 µs with a NaCl concentration of 100 mM (left) and the corresponding cation density map just above the membrane surface (right). The cation concentration is elevated above the negatively charged lipid regions, demonstrating a clear spatial correlation between charged lipid distribution and cation localization.

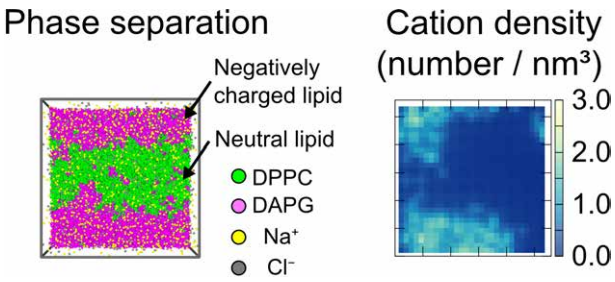

Figure 1: Phase separation and cation distribution in the charged–neutral lipid mixture at 2.0 µs with a NaCl concentration of 100 mM. Left: top-view snapshot of the membrane. Right: cation density (number/nm^3) near the membrane surface.

Two-dimensional mapping of phase separation and ion distribution showed that the Na^+ density profile closely tracked the DAPG distribution throughout the phase separation process. A clear correlation persisted up to 0.5 nm above the surface. Beyond 0.5–1.0 nm, the correlation decayed rapidly. Quantitative analysis using the time correlation function confirmed that cations follow the motion of negatively charged lipids on a microsecond timescale. Analysis of the perpendicular ion distribution further showed that the peak Na^+ concentration above DAPG increased with the local phase-separation order parameter ϕ_i, i.e., with the local area fraction of charged lipids.

This behavior can be understood from the large difference in diffusivity: lipid molecules diffuse on the membrane with a coefficient of approximately 10^0 µm^2/s [29], roughly three orders of magnitude slower than ions in aqueous solution ($\sim 10^3$ µm^2/s). Since the collective diffusion of phase-separated domains is even slower, ions can equilibrate their distribution on the timescale of phase separation. This rapid ionic redistribution screens the electrostatic repulsion between charged lipids and is expected to facilitate domain formation. Analysis of the Coulomb energy as a function of time further supported this picture: the energy decreased more substantially in the phase-separated DPPC:DAPG = 1:1 system than in the DPPC:DOPG = 1:1 system without phase separation, indicating that the screening of negatively charged headgroups by Na^+ is particularly pronounced when phase separation occurs.

Comparison of the simulation results of the ion distribution just above the charged lipids with classical PB theory [23] revealed that the classical theory significantly overestimates the cation concentration near the membrane surface. This discrepancy arises because classical PB theory treats ions as point charges, thereby neglecting the steric repulsion between ions crowded near the membrane surface. Applying a modified PB theory that incorporates the finite ion size through a lattice model [30, 31] showed that accounting for the hydrated sodium ion diameter ($\sim$0.6 nm) brought

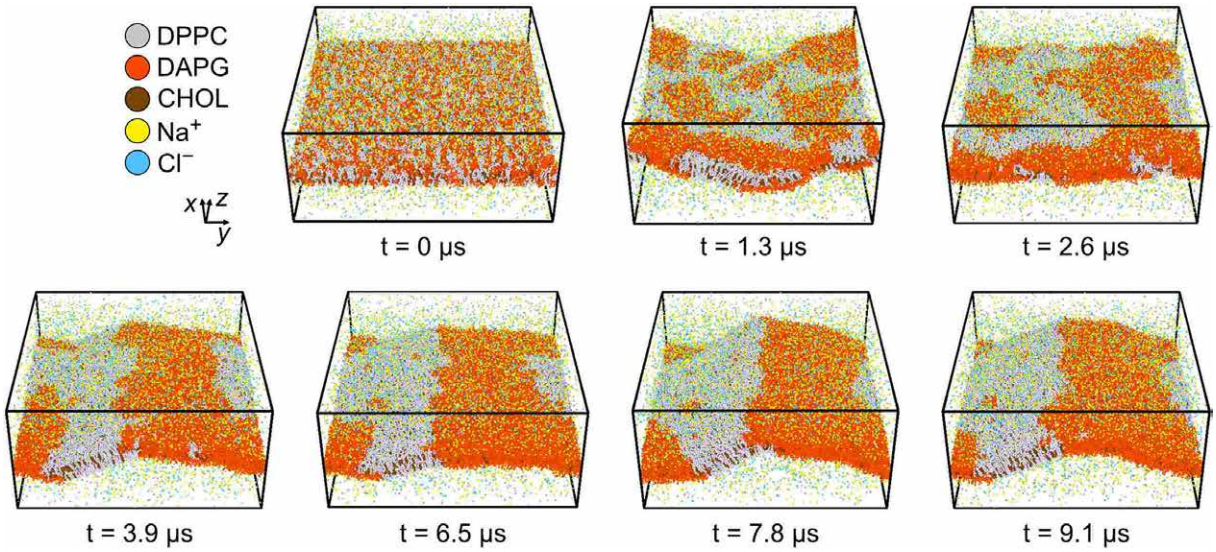

Figure 2: Time evolution of the phase separation process in a lipid bilayer membrane composed of 3,500 DPPC, 3,500 DAPG, and 3,000 cholesterol molecules at a NaCl concentration of 100 mM, obtained from coarse-grained MD simulations using the MARTINI model. Each panel shows a snapshot at the indicated simulation time. Reprinted from Higuchi Y., Ito H., Shimokawa N., Reščič J., Maset S., Bohinc K., *J. Mol. Liq.*, **444**, 129168 (2026), `https://doi.org/10.1016/j.molliq.2025.129168`, under CC BY 4.0.

the theoretical predictions into quantitative agreement with the simulation results. This ion size was independently supported by analysis of the radial distribution function of water molecules around Na^+ obtained from all-atom MD simulations based on the third-order density functional tight-binding (DFTB3) method [32]: the first hydration shell has a radius of 0.29 nm (diameter 0.58 nm), consistent with the value used in the modified PB model.

4. Ion Distribution near the Phase-Separation Interface

Although the previous study [24] characterized the ion distribution on the membrane surface, the ion distribution at the phase-separation interface was not resolved. The relatively small system size caused the lipid distribution at the interface to be blurred. To overcome this limitation, we performed larger-scale coarse-grained MD simulations [25]. The system was expanded approximately fivefold to ∼10,000 molecules (3,500 DPPC, 3,500 DAPG, and 3,000 cholesterol), and 30% cholesterol was included to produce a sharper, well-defined phase-separation interface. Figure 2 shows simulation snapshots during the phase separation. Starting from a homogeneously mixed initial configuration, small domains formed by 1.3 μs, grew progressively from 2.6 to 7.8 μs, and eventually produced two separated phases within the simulation cell. The system reached a steady state between 7.8 and 9.1 μs, and the results from this period were used for analysis.

The concentration profiles of DAPG and Na^+ across the phase-separation interface were fitted with sigmoid functions (Figure 3). The correlation coefficient between the sigmoid center positions of Na^+ and DAPG was 0.973, providing quantitative evidence for the strong colocalization of cations with the charged lipid domain. A positive correlation was also observed for the sigmoid width parameter, which characterizes the thickness of the transition

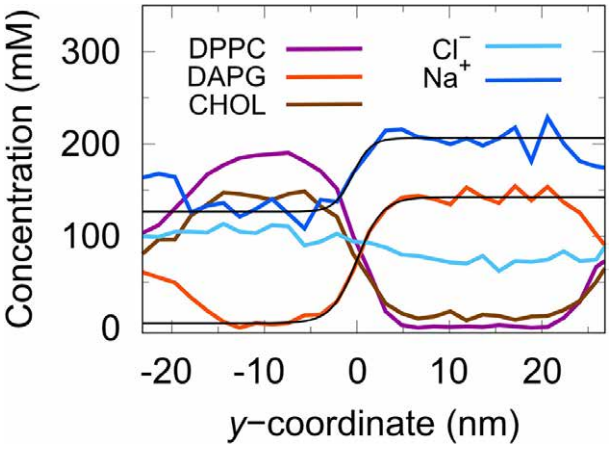

Figure 3: Lateral concentration profiles of DPPC, DAPG, cholesterol, Na^+, and Cl^- as a function of the y-coordinate at 9.1 µs, obtained by averaging over the x- and z-directions. The sigmoid fits to the DAPG and Na^+ profiles are shown as black lines. Reprinted from Higuchi Y., Ito H., Shimokawa N., Reščič J., Maset S., Bohinc K., *J. Mol. Liq.*, **444**, 129168 (2026), `https://doi.org/10.1016/j.molliq.2025.129168`, under CC BY 4.0.

zone across the interface. The width of the Na^+ concentration change tended to be narrower than that of the DAPG lipids, suggesting that fluctuations of the lipid distribution at the interface are larger than those of the ion distribution. Analysis of the perpendicular concentration profiles further showed that Na^+ concentration is high near the surface of the DAPG domain and converges to the bulk value within approximately 2 nm of the membrane.

As a more idealized model, the ion distribution at a planar surface that is half charged and half neutral was also investigated using MC simulations and modified PB theory. Both approaches yielded a continuous, sigmoid-shaped transition in counterion concentration from the uncharged to the charged region. This sigmoid-shaped transition is consistent with the MD results. Interestingly, the ion distribution can be well represented by a sigmoid function, even though the boundary between the charged and neutral surfaces is ideally sharp and discontinuous. The mean counterion concentration near the charged domain was approximately 150 mM in the MC simulations and 200 mM in the MD simulations, showing reasonable overall agreement.

Regarding the width of the ion concentration transition at the interface, the MC simulations predict a sharper change than the MD simulations. This is because the interface in the MC model is defined by discrete, fixed charges and has no positional fluctuation, whereas the phase-separation interface in the MD simulations undergoes continuous thermal motion, which broadens the effective width of the ion distribution. On the other hand, MD simulations indicated that, at domain boundaries, the distribution width for Na^+ ions tends to be narrower than that for DAPG lipids, and the reason remains an unresolved challenge.

The decay length of the perpendicular ion distribution (∼2 nm) agreed well between the MD simulations and the theoretical calculations, validating the theoretical model. Together, these results demonstrate the overall consistency of the three approaches and establish a quantitative foundation for understanding the electric double-layer structure at phase-separation interfaces in charged lipid membranes.

5. Summary and Outlook

We have summarized a series of studies on the correlation between phase separation and ion distributions in charged phospholipid bilayers. Coarse-grained MD simulations demonstrated that cations follow the dynamics of charged lipids on a microsecond timescale during phase separation, and that incorporating ion-size effects through modified PB theory is essential for a quantitative understanding of the ion distribution [24]. Analysis of a larger system and comparison with MC simulations and theory

showed that the lateral change in ion concentration near the phase-separation interface can be described by a sigmoid function, and quantified the correlation between charged lipid and cation distributions [25]. These findings substantially advance the molecular-level understanding of the interplay between charged lipid membranes and electrolyte solutions.

Beyond ion distributions, the behavior of water molecules near the membrane is also an important subject. Recent studies have begun to reveal that the charge state of phospholipid membranes significantly affects water dynamics near the interface [13, 33, 34]. Specifically, neutron scattering experiments and all-atom MD simulations have shown that the orientational order of water molecules and the hydration structure at the membrane interface are strongly sensitive to lipid charge [33, 34]. Since water–lipid interactions influence everything from membrane hydration to the stability of supramolecular assemblies, an integrated understanding of ionic environments and water dynamics represents an important future challenge.

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