



Ion distribution in the vicinity of phase-separated membrane

Yuji Higuchi^{a,*}, Hiroaki Ito^b, Naofumi Shimokawa^c, Jurij Reščič^d, Stefano Maset^e, Klemen Bohinc^{f,*}

^a Research Institute for Information Technology, Kyushu University, Fukuoka, 819-0395, Japan

^b Department of Physics, Graduate School of Science, Chiba University, Chiba 263-8522, Japan

^c School of Materials Science, Japan Advanced Institute of Science and Technology, Ishikawa 923-1292, Japan

^d Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, Ljubljana, Slovenia

^e Dipartimento di Matematica e Geoscienze, Università di Trieste, Via Weiss 2, Trieste, Italy

^f Faculty of Health Sciences, University of Ljubljana, Zdravstvena 5, SI-1000 Ljubljana, Slovenia

HIGHLIGHTS

- Phase-separated multicomponent phospholipid plane with one half consisting of charged lipids and the other half consisting of non-charged lipids in contact with an electrolyte solution composed of monovalent ions.
- The coarse-grained molecular dynamics simulations (MD), Monte Carlo simulations (MC) and modified Poisson-Boltzmann (PB) approach were used to consider the distribution of ions with finite size in the vicinity of the charge density discontinuity in the lipid layer.
- Results show that in the vicinity of the transition from the uncharged to the charged part a sigmoidal transition to an increased concentration of counterions is observed.

ARTICLE INFO

Keywords:

Ion distribution
Theory
Simulations
Lipids

ABSTRACT

The physical properties of multi-component phospholipid layers have attracted increasing attention as a result of their potential benefits in medicine. We consider a phase-separated multi-component phospholipid plane with one half consisting of charged lipids and the other half of non-charged lipids in contact with an electrolyte solution composed of monovalent ions. Coarse-grained molecular dynamics (MD) simulations were performed to simulate the phase transition between charged and non-charged lipids. In addition, the modified Poisson-Boltzmann (PB) approach was used to consider the distribution of ions with finite size in the vicinity of the charge discontinuity in the lipid layer. The passage between charged and non-charged parts of the plane was described by a sigmoid function obtained from MD simulations. In the vicinity of the lipid layer, in the transition from the uncharged to the charged part, a continuous transition to an increased concentration of counterions is visible.

1. Introduction

In industrial and biological systems, electrostatic interactions between charged objects in aqueous electrolyte solutions are of fundamental importance [1]. The accumulation of ions in the solution around a charged object leads to an electric double layer. The concept of the electric double layer has succeeded in explaining behaviors in classical systems of electrolyte solutions, such as the stability of colloidal aggregation and the electrochemical properties of electrodes. The Poisson-Boltzmann (PB) equation provides a theoretical description of the electric double layer under the mean-field assumptions, and in most

cases it is used to describe the electric potential in the direction normal to the charged surfaces. However, the charged objects in the real world often have heterogeneous structures even in the direction parallel to the surface. Examples are impurities on the electrode surfaces, Janus particles, and biological membranes, all of which are embedded in aqueous electrolyte solutions.

The biological membrane has a bilayer structure that consists mainly of lipid molecules. Lipids are amphiphilic molecules that consist of two different parts [2]. One part is hydrophobic and is referred to as a tail. The second part is hydrophilic and corresponds to a head group. Therefore, the generic structure for the membrane is a body of

* Corresponding authors.

Email addresses: higuchi.yuji.474@m.kyushu-u.ac.jp (Y. Higuchi), klemen.bohinc@zf.uni-lj.si (K. Bohinc).

low dielectric constant. Biological membranes contain charged lipids with charged head groups, which are in contact with the electrolyte solution as a body of high dielectric constant. The surface charges of the membrane can be fixed or mobile around some fixed position at the interface. In the simplest approximation to depict the biological membrane, the charged lipids are homogeneously mixed, and the surface charge density is constant. However, biological membranes undergo phase separation under certain conditions of temperature and form domains rich in specific lipid species to regulate biological functions [3,4]. Therefore, electrostatic interactions between ions and the electrically heterogeneous membrane are of special interest. As an experimental approach to investigating the impact of electrostatic interactions on phase separation in lipid membranes, the surface states of giant uni-lamellar vesicles composed of charged and electrically neutral phospholipids under various salt concentrations have been visualized by fluorescence microscopy[5–7]. These observations revealed that adding salt at physiological concentrations induces phase separation despite the electrostatic repulsion among charged lipids. However, the spatial distribution of ions remains experimentally inaccessible and is still poorly understood.

There were attempts to describe the electric double layer from a theoretical point of view. Helmholtz proposed a simple model where only electrostatic interactions between ions and charged objects were taken into account. Gouy and Chapman improved the theory by also including the entropic effect. Later this theory was named the PB theory. This theory can adequately describe solutions of monovalent ions close to charged objects with homogeneous charge distribution. An additional assumption of the theory is the dielectric continuum of the solution [8] which was improved by taking into account the solvent structure [9]. Different attempts were proposed to incorporate finite-size effects into PB theory [10]. Freise incorporated the finite size by a pressure-dependent potential, whereas Wicke and Eigen used a thermodynamic approach [11,12]. Later a lattice model was incorporated into the PB theory [10,13,14]. The functional approaches [15,16] were also improved. The Carnahan-Starling equation of the state [17,18] for hard spherical particles [16,19] was proposed. Usually the theoretical predictions are verified by Monte Carlo (MC) simulations [20].

Molecular simulations are useful for revealing both the structures and dynamics of lipid membranes at the molecular level [21]. While all-atomic molecular dynamics (MD) simulations can reveal the influence of the detailed chemical structures of lipid molecules such as the effect of charge in the hydrophilic head group on the hydration states [22–29], coarse-grained MD simulations can reveal the longer-time and larger-scale properties of phase separation [30]. By using a coarse-grained model with the Debye-Hückel electrostatic interaction potential, we have revealed the effect of charged head groups on phase separation processes in the presence of long-range electrostatic repulsion [7,31–34]. One of the widely known coarse-grained models is the MARTINI model [35–37], which includes coarse-grained ions and coarse-grained water molecules explicitly. Using the MARTINI model, phase separation processes have been studied at a molecular level [38–44]. Our recent study suggested dynamic lateral correlation between the lipid distribution in a membrane and ion distributions in a surrounding aqueous solution during phase separation processes [45]. For a deeper understanding of lateral ion distributions in the vicinity of the charged membrane, formulation of a theoretical description and a crosscheck with molecular simulations are needed.

In this work the modified PB equation for half a charged plane was solved. The finite size of ions was taken into account through the lattice model. The passage between the charged and non-charged parts of the planar surfaces is described by the sigmoid function. The parameters for the modified PB equation were obtained from coarse-grained MD simulations. The numerical results of the modified PB equation for counter- and co-ion profiles were compared with the MC simulations. Finally, the limiting cases of the modified PB equation were considered.

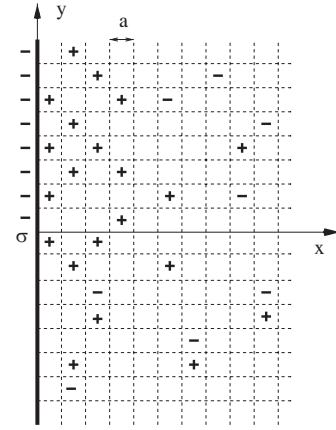


Fig. 1. Schematic presentation of infinitely large planar surface. Only half of the surface is negatively charged with surface charge density σ . The plane is in contact with an electrolyte solution composite of ions with finite size. The volume of each cell is a^3 .

2. Theory

We consider a planar surface in contact with an electrolyte solution. Half of the surface is negatively charged and the other part of the surface is neutral (Fig. 1). The total free energy F of the system is expressed as the sum of the energy F_{el} associated with the presence of an electrostatic field $\mathbf{E} = -\nabla\Phi$, [13]

$$F_{el} = \frac{k_B T}{8\pi l_B} \int_V d^3\mathbf{r} (\nabla\Psi)^2, \quad (1)$$

and translational entropy contributions of counterions and coions in the aqueous solution,

$$F_{en} = k_B T \int_V d^3\mathbf{r} \left[n_+ \ln(n_+ a^3) + n_- \ln(n_- a^3) + (a^{-3} - (n_+ + n_-)) \ln(1 - (n_+ + n_-) a^3) \right]. \quad (2)$$

$l_B = e^2/(4\pi\epsilon_0 k_B T)$ is the Bjerrum length, k_B is Boltzmann's constant, T is the absolute temperature, e is the elementary charge, ϵ_0 is the permittivity in free space and ϵ is the dielectric constant of the electrolyte solution. The scaled electrostatic potential $\Psi(\mathbf{r}) = e\Phi(\mathbf{r})/(k_B T)$ is a function of the position $\mathbf{r}$. The gradient ∇ applied to the electrostatic potential Φ gives the electric field strength $\mathbf{E}$. The integration is performed over the whole volume V of the system, $d^3\mathbf{r}$ being the infinitesimal volume element. The finite size of ions is introduced via a lattice model. The size of the lattice side is a .

In thermodynamic equilibrium the free energy $F(\Psi, n_+, n_-)$ attains a minimum with respect to the electrostatic potential Ψ and ion concentrations n_+ and n_- , subject to the Poisson equation $\nabla^2\Psi = -4\pi l_B \rho/e$, where ∇^2 denotes the Laplacian operator and $\rho = e(n_+ - n_-)$ is the local volume charge density. Demanding vanishing variation $\delta F(\Psi, n_+, n_-) = 0$ leads to Boltzmann distributions for the local ion concentrations. Insertion of these equilibrium distributions into the Poisson equation delivers the modified Poisson-Boltzmann equation [10,46]:

$$\nabla^2\Psi(x, y) = \frac{1}{l_D^2} \cdot \frac{n_s}{n_s - 2n_0} \cdot \frac{\sinh\Psi(x, y)}{1 + \frac{2n_0}{n_s - 2n_0} \cosh\Psi(x, y)} \quad (3)$$

and it is valid for $x \geq 0$ and $y \in \mathbb{R}$, i.e. in the domain $\Omega = [0, +\infty) \times \mathbb{R}$. Here Ψ is the unknown electrostatic potential in the region Ω , l_D is the Debye length $l_D = 1/\sqrt{8\pi l_B n_0}$, $n_s = 1/a^3$ is the number density of lattice

sites and n_0 is the bulk concentration of the monovalent ions. The volume of each cell is equal to a^3 . Equation (3) is equipped by four boundary conditions. The first boundary condition is at the charged surface $x = 0$ and it follows from the Gauss law

$$\vec{n} \cdot \nabla \Psi(0, y) = -4\pi l_B \frac{\sigma(y)}{e}, \quad y \in \mathbb{R}, \quad (4)$$

where $\vec{n} = (1, 0)$ is the normal vector at the surface pointing into the electrolyte, $\sigma(y)$ is the surface charge density and e is the positive elementary charge. The function σ is the Heaviside function given by

$$\sigma(y) = \begin{cases} \sigma_+ & \text{if } y \geq 0 \\ 0 & \text{if } y < 0. \end{cases} \quad (5)$$

Far from the charged surface, at $x = +\infty$, we have the second boundary condition

$$\Psi(+\infty, y) = 0, \quad y \in \mathbb{R}. \quad (6)$$

Far from the origin, at $y = +\infty$, we have the third boundary condition

$$\Psi(x, +\infty) = \psi(x), \quad x \geq 0, \quad (7)$$

where $\psi(x)$ is the potential when the plate is completely charged. Far from the origin, at $y = -\infty$, we have the fourth boundary condition

$$\Psi(x, -\infty) = 0, \quad y \in \mathbb{R}, \quad x \geq 0, \quad (8)$$

since 0 is the potential when the plate is not charged.

3. Numerical solution

We numerically compute the potential Ψ solution for the PB problem (3) with boundary conditions (4)-(6)-(7)-(8). This is done by using the functions of the Partial Differential Equation (PDE) Toolbox of MATLAB. Since the PDEs solved by this toolbox have a finite domain and the domain

$$\Omega = [0, +\infty) \times \mathbb{R}$$

of the PB problem is infinite, we need to restrict it to a square finite domain

$$\tilde{\Omega} = [0, 2L] \times [-L, L],$$

where $L > 0$ is a positive finite value. Now the first, second, third and fourth boundary conditions are on the edges $\{0\} \times [-L, L]$, $\{2L\} \times [-L, L]$, $[0, 2L] \times \{L\}$ and $[0, 2L] \times \{-L\}$, respectively.

Of course, it is fundamental to choose the right value for L . We decide to use $L = 10a$, where a is the size of the salt ions.

In the boundary condition (7) the potential $\psi(x)$ is numerically computed as the solution of a boundary value problem for an ordinary differential equation using the MATLAB function `bvp4c`.

4. MD simulation

To check the ion distribution on phase-separated charged membranes, the MARTINI molecular dynamics simulation is performed. In our previous study [45], the phase separation interface was not clearly visible in the phospholipid bilayers composed only of dipalmitoylphosphocholine (DPPC) and diarachidonoylphosphoglycerol (DAPG). The ion distribution parallel to the membrane surface was also blurred, and analytical results comparable to theory could not be obtained. Previous research using MARTINI MD simulations has shown that an increase in the calculation size to 10,000 phospholipid molecules [38] and the incorporation of 30 % cholesterol [39] are significant for clearly visualizing the phase separation interface. To realize the clear phase separation interface and conduct an ion distribution analysis comparable to theory, the calculation size in the current study is increased approximately

five times from 2,048 lipid molecules used in the previous study [45], and cholesterol molecules are added by 30 %. A lipid bilayer system is prepared, consisting of 3500 DPPC lipids, 3500DAPG lipids, and 3000 cholesterol (CHOL) molecules with NaCl salt at a concentration of 100 mM. The calculation size is larger than that used in our previous study [45], allowing for a more accurate analysis of the statistics of the ion distribution.

Three types of molecules are used: DPPC is a zwitterionic saturated phospholipid; DAPG is a negatively charged, monovalent, tetraunsaturated phospholipid; and CHOL is a neutral amphiphilic molecule. MARTINI coarse-grained models for lipids and cholesterol (version 2.0) [35–37] and polarizable water (version 2.2) [47,48] are used. For the neutral condition of the system, Na^+ as counterions for negatively charged lipids are added. The thickness of the water layer between two bilayers is set to 20 nm in the initial condition.

The CHARMM-GUI [49,50] is used to prepare the initial structures. MD simulations are performed with the GROMACS program [51] (version 2022.3) under an NpT condition (constant number of particles, constant pressure, and constant temperature). The simulations are performed under three-dimensional periodic boundary conditions. The temperature is set at 303.15 K by velocity rescaling [52] with a damping time of 1 ps, where the independent control for the membrane and solution is adopted. A Parrinello–Rahman barostat [53] with a damping time of 12 ps and compressibility of $3.0 \times 10^{-4} \text{ bar}^{-1}$ is used to control a pressure of 1 bar. The pressure is controlled independently in the other two directions, parallel and perpendicular to the lipid bilayer plane. The cut-off length is set to 1.1 nm for the Lennard–Jones potential. For electrostatic interactions, the reaction field electrostatics method is adopted with a Coulomb cut-off of 1.1 nm and a relative dielectric constant of 2.5.

5. MC simulation

A system containing charged and uncharged membranes in contact with the surrounding salt solution is treated using the canonical MC simulations. The charged part of the membrane consists of fixed discrete charged sites with 0.25 negative elementary charges each embedded in a hard sphere with a radius of 1 Å. Partial charges were used to approximate a uniform charge density model as in the theory.

Simulation box has a cuboid shape with dimensions $x = 207.18 \text{ Å}$, $y = 207.18 \text{ Å}$, and $z = 200 \text{ Å}$; this corresponds to the surface charge density of 0.215 C/m^2 . Periodic boundary conditions were applied in the $z = 0$ plane. There are 288 counterions present in the system to neutralize the lipid charges. Salt concentration was 0.126 M ; there were 650 positive and 650 negative ions present in the system; each ion was modeled as a charged hard sphere with a radius of 2 Å.

The temperature is set at 298 K. The Metropolis sampling scheme was used. The simulation run is divided into 20 blocks of 5000 attempted moves per particle, ensuring good sampling. Ion densities were calculated along the x -axis. All simulations were performed using the MOLSIM simulation package [54].

A case with a uniformly charged membrane is also studied. For this purpose the electrostatic energy between the membrane and small ions is calculated analytically [20]. Parameters were identical to those of the partially charged membrane.

6. Results and discussion

Fig. 2 shows the two-dimensional potential profile in the x - y plane obtained from the modified PB equation. The surface charge density of the charged half-plane is $\sigma_+/e = -0.1 \text{ nm}^{-2}$. The Bjerrum length is $l_B = 0.7 \text{ nm}$, the ion size is $a = 0.62 \text{ nm}$ and the bulk concentration of the ions is $n_0 = 0.075 \text{ nm}^{-3}$. The electrostatic potential Ψ is negative in the vicinity of the negatively charged surface, whereas Ψ in the vicinity of the neutral part of the surface reaches zero. Also, regardless of the coordinate y , the electrostatic potential far from the surface ($x \rightarrow \infty$)

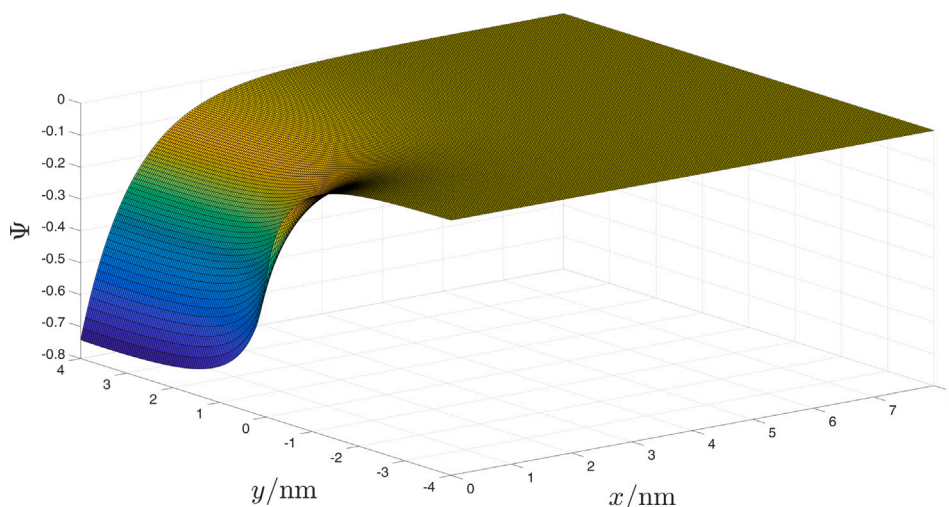


Fig. 2. Two-dimensional potential profile $\Psi(x, y)$ obtained from the theoretical model. The parameters are: surface charge density $\sigma_+/e = -0.1 \text{ nm}^{-2}$, bulk ion concentration $n_0 = 0.075 \text{ nm}^{-3}$ and ion size $a = 0.62 \text{ nm}$.

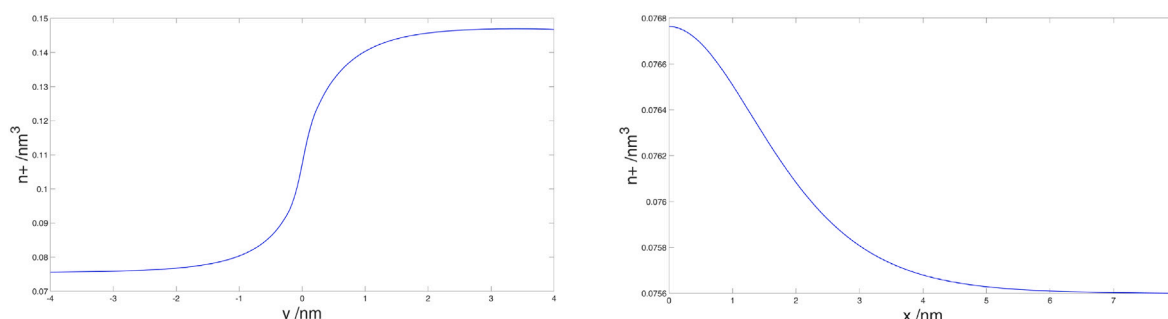


Fig. 3. The counterion concentration parallel ($x = 0$, left Fig.) and perpendicular ($y = 0$, right Fig.) to the charged surface. Parameters are: $l_B = 0.7 \text{ nm}$, $a = 0.62 \text{ nm}$ and $\sigma_+/e = -0.1 \text{ nm}^{-2}$.

is zero. In the surface vicinity, one can observe a continuous transition from -20 mV at large positive values of y to zero potential value at large negative values of y .

Fig. 3 shows the counterion profiles parallel and perpendicular to the charged surface for $l_B = 0.7 \text{ nm}$ and $a = 0.62 \text{ nm}$ and surface charge density $\sigma_+/e = -0.1 \text{ nm}^{-2}$. In the neutral part of the surface, counterion and coion concentrations are equal to the bulk concentration n_0 . Perpendicular to the charged part of the surface, the counterion concentration decays to the bulk value, whereas the coion concentration increases from very small concentrations at the charged surface to the bulk concentration far from the plane [8]. At the phase-separated lipid layer we have a sharp transition from charged to neutral surface which is taken into account in the theoretical model. The counterion distribution shows a continuous distribution from the bulk value n_0 at the neutral plane (negative y) to $0.15/\text{nm}^3$ at the negatively charged surface (positive y).

The phase separation process in the lipid bilayer membrane consisting of neutral DPPC and negatively charged DAPG lipids is investigated by a coarse-grained MD simulation with the MARTINI model. Snapshots are shown in Fig. 4. Starting from an initial state of homogeneously mixed DPPC and DAPG lipids, small domains are formed at $1.3 \mu\text{s}$. From $2.6 \mu\text{s}$ to $7.8 \mu\text{s}$, the domain coarsening undergoes over time and finally shows completely separated two-phases within the simulation cell. Because there is almost no change in the situation between $7.8 \mu\text{s}$ and $9.1 \mu\text{s}$, we consider the simulation results from $7.8 \mu\text{s}$ to $9.1 \mu\text{s}$ are in a steady state.

The concentration profiles of ions, lipid molecules, and cholesterol molecules are analyzed along the phase-separated y -axis direction (see the snapshots in Fig. 4), and the results at $9.1 \mu\text{s}$ are shown in Fig. 5. Here, we focus on the concentration profiles around the domain boundary. The concentration profiles of DAPG lipids and Na^+ ions are fitted with a sigmoid function, $A/(1 + \exp(-B(x - C))) + D$, around the domain boundary within the range of 30 nm in the y -axis direction. The fitted sigmoid functions are shown by the black lines in Fig. 5. In the graph, the y -coordinate of the domain boundary is shifted to be zero. DPPC lipids form the domain from -23 nm to 0 nm in the y -axis direction, and are almost absent from 0 to 20 nm . In contrast, DAPG lipids form the domain from 0 to 25 nm . Cholesterol molecules are present in the DPPC domain. Since DAPG lipids have a negative charge, a large amount of Na^+ is distributed on the DAPG domains. On the other hand, Cl^- is distributed slightly more on the DPPC domain than on the DAPG domain.

The phase-separated state observed in the coarse-grained MD simulation is similar to the situation assumed in the theory where half a plane is charged. To compare the simulation results with the present theoretical results, we further analyze the lateral concentration profiles across the domain boundary. The parameter C of the sigmoid function indicates the position of the domain boundary. The correlation coefficient between the C_{Na^+} and C_{DAPG} values obtained from the concentration profiles of Na^+ and DAPG, respectively, is 0.973 , indicating that there is a strong positive correlation between the distributions of DAPG lipids and Na^+ . The parameter B of the sigmoid function indicates the length

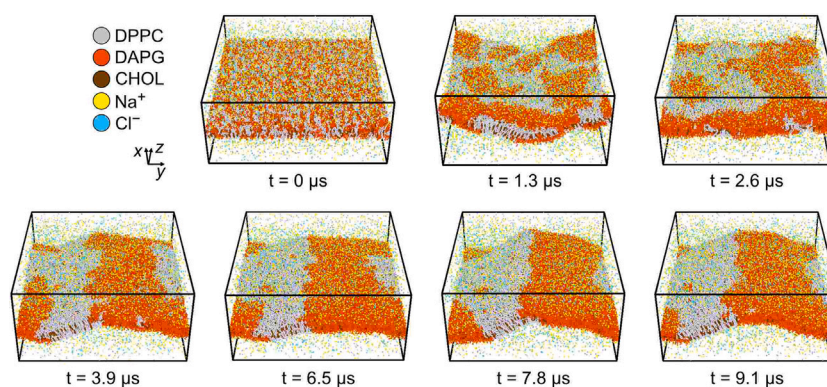


Fig. 4. MD simulation snapshots during the phase separation process of the lipid membrane consisting of 3500 DPPC lipids, 3500 DAPG lipids, and 3000 cholesterol molecules with a 100 mM NaCl salt using the MARTINI model.

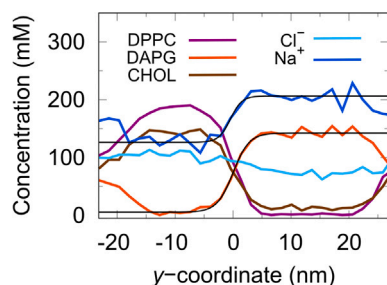


Fig. 5. Concentration profiles of DPPC lipids, DAPG lipids, cholesterol molecules, Na^+ , and Cl^- along y -axis at $9.1 \mu\text{s}$. Each concentration is calculated by averaging with respect to x - and z -axes. Black lines are sigmoidal fitting curves for DAPG and Na^+ .

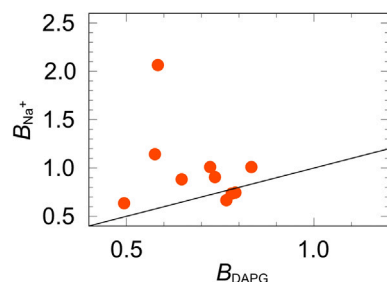


Fig. 6. Correlation between the parameters B_{Na^+} and B_{DAPG} , where the subscripts denote the fitting results for Na^+ and DAPG, respectively. The parameter indicates the thickness of the transient layer of the lateral concentration profile across the domain boundary. Black line indicates $B_{\text{Na}^+} = B_{\text{DAPG}}$ just for the eye guide.

scale of the concentration change along the y -axis, i.e., the thickness of the transient layer across the domain boundary. Fig. 6 shows the correlation between the parameters B_{Na^+} and B_{DAPG} , where the subscripts denote the species. As observed in the fitting curves in Fig. 5, it can be said that there is a positive correlation. The concentration change in Na^+ tends to be sharper than that of DAPG lipids.

Fig. 7 shows an MC snapshot where red beads correspond to the negatively charged part of the lipid layer. Yellow beads are counterions which are accumulated close to the charged part of the lipid layer. MC simulation results confirm MD simulation results. Namely, MC simulations deliver the average concentration of counterions in the vicinity of the charged layer of 150 mM, whereas MD simulations predict 200 mM. The concentration profiles of ions are fitted with a sigmoid function within the range of 10 nm in the y -axis direction. The parameters B of the sigmoid function are 1.56 and 1.76 in the MC simulation.

The concentration profiles for DAPG and Na^+ in the direction perpendicular to the membrane at $9.1 \mu\text{s}$ are also analyzed. Fig. 8(a), (b), and (c) show the concentration profiles at the y -coordinate of -6.6 nm corresponding to the DPPC domain from Fig. 5, that at the y -coordinate of 0.42 nm as the position of the domain boundary determined by the fitting, and that at the y -coordinate of 7.5 nm corresponding to the DAPG domain, respectively. In the DPPC domain (Fig. 8(a)), the concentration of Na^+ is almost constant in the aqueous solution. Note that there is a lipid bilayer membrane at x -coordinate of $\sim 13 \text{ nm}$. At the position of the domain boundary (Fig. 8(b)), the concentration of Na^+ is almost constant on one side (x -coordinate less than $\sim 13 \text{ nm}$) whereas Na^+ is localized at the other surface (x -coordinate of $\sim 18 \text{ nm}$) where a large amount of DAPG coexists. In the DAPG domain (Fig. 8(c)), the Na^+ concentration is high on the membrane surface. The ion concentration in the direction perpendicular to the membrane surface decreases within approximately 2 nm from the membrane surface and remains constant.

The simulation results, which show that the ion distribution perpendicular to the lipid membrane decreases at approximately 2 nm, are in good agreement with the theoretical results shown in Fig. 3(b). The fact that the ion distribution parallel to the lipid membrane is strongly influenced by the charge on the membrane surface (i.e., there is a strong correlation with the position of the domain boundary) in the simulation is also consistent with the theory. These results will serve as a reference for future theoretical and simulation studies of charged lipid membranes. One nontrivial issue is the change in ion distribution at the domain boundary. In the MD simulation, the interface is not discontinuous, and the influence of the interface fluctuations also affects the ion distribution. On the other hand, the theory and the MC simulation can uniquely determine the interface. It is very interesting to note that the theoretical ion distribution parallel to the membrane surface (Fig. 3(a)) reveals that the ion concentration does not change completely discontinuously across the domain boundary, but rather changes over a certain width. Indeed, the ion profile is well fitted with a sigmoid function in the MC simulation, and the ion distribution does not change discontinuously but has a range. Since the B parameter of the MC simulation is larger than that of the MD simulation, the thickness of the ion distribution at the interface is smaller in the MC simulation. This seems to be due to the fluctuation of the charged lipid phase separation interface in the MD simulation. On the other hand, the fact that there is a wide range of ion distribution at the interface in the MC simulation also suggests that the ion distribution is influenced by thermal fluctuations. In the MD simulation, the distribution width at the domain boundaries tends to be smaller for Na^+ ions than for DAPG lipids, which remains a challenging problem. We hope that the findings from our MD simulation and comparisons with the theory will stimulate further research.

To understand the behavior of molecular assemblies such as charged vesicles and macromolecular ions in solution, it is essential to characterize the electrostatic potential and ion distribution in the

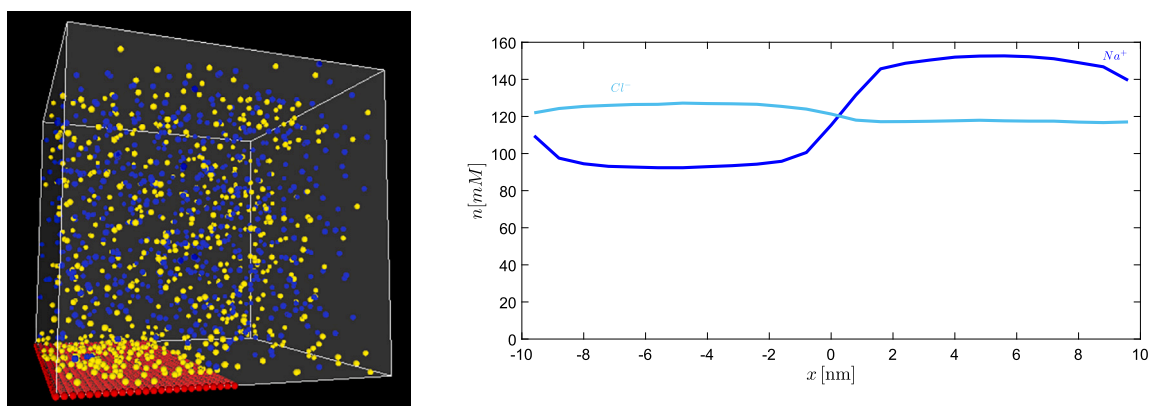


Fig. 7. MC simulation snapshot of the simulation cell: red beads correspond to the charged lipid head groups, yellow beads are Na^+ ions whereas blue beads are Cl^- . Na^+ , and Cl^- concentration profiles along y -axis. Each concentration is calculated by averaging with respect to x - and z -axes.

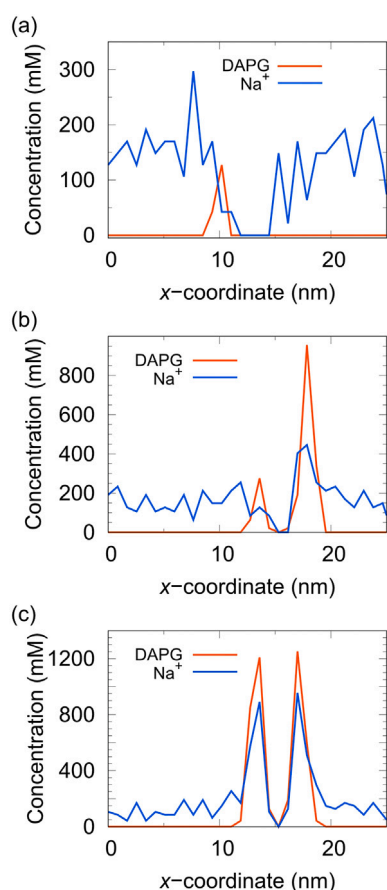


Fig. 8. Concentration profiles of DAPG lipid and Na^+ along x -axis at y -coordinates (a) -6.6 nm, (b) 0.42 nm, and (c) 7.5 nm at $9.1 \mu\text{s}$.

surrounding medium. Although studies on the electrostatic potential near charged surfaces in contact with electrolyte solutions have a long history, most of them have focused on surfaces with a uniform charge density. However, in phase-separated lipid membranes, particularly near the boundaries between phase-separated domains, the charge density cannot be regarded as uniform. The effects of such nonuniform surface charge densities on the ion distribution in solution remain poorly understood. One major reason is that it is experimentally difficult to observe ion distributions in real time, especially within the narrow regions around phase-separated domain boundaries. Therefore, predicting ion

distributions using numerical simulations and theoretical approaches is extremely important for guiding future experimental progress. By predicting ion distributions near surfaces with nonuniform charge densities, we expect to gain deeper insight into the interactions between such heterogeneous surfaces and ions in solution, as well as the stability of phase-separated structures in lipid membranes.

7. Conclusions

In this work, the behavior of ions at the phase-separated lipid head groups was studied. Namely, the physical properties of multi-component phospholipid layers attract increasing attention due to their potential benefit in biology and medicine. The problem was considered by coarse-grained MD simulation. The theoretical model based on Poisson-Boltzmann theory was applied and compared with MC simulations. Results show that close to the border between non-charged and charged lipid layer a continuous transition from the bulk ion concentration to an increased concentration close to the charged lipid layer of counterions is obtained. The transient profiles can be fitted by a sigmoid function, suggesting that there is a positive correlation between the lipid charge distribution and the cation distribution, and that there is likely a correlation between the widths of their distributions. The decay of the ion distribution perpendicular to the surface of the charged lipid layer was in good agreement between the theory and the MD simulation. Here we have investigated the phase separation of neutral and charged lipids, but the neutral lipid head group is actually a zwitterion with both positive and negative charges. In the future we are going to study realistic phase separated regions between charged and zwitterionic lipid layers and study how it impacts the structure of the electric double layer.

CRedit authorship contribution statement

Yuji Higuchi: Writing – original draft, Visualization, Software, Data curation. **Hiroaki Ito:** Writing – review & editing, Methodology, Conceptualization. **Naofumi Shimokawa:** Writing – review & editing, Methodology, Conceptualization. **Jurij Reščič:** Writing – original draft, Software, Conceptualization. **Stefano Maset:** Writing – review & editing, Software, Methodology, Data curation. **Klemen Bohinc:** Writing – original draft, Validation, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

K.B. and J.R. thank the Slovenian Research Agency for support through programs P3-0388 and P1-0201, respectively. This research was supported by JSPS and MESS Japan-Slovenia Research Cooperative Program Grant Number JPJSBP120215001. This research was also supported by JSPS KAKENHI Grant Numbers JP24K06972 (HI) and JP25K07244 (YH). The authors thank the Supercomputer Center at the Institute for Solid State Physics, the University of Tokyo, for the use of their facilities. The computation was partly carried out using the computer facilities at the Research Institute for Information Technology, Kyushu University, Japan. The University of Ljubljana interdisciplinary preparative project Nanostructurome 802-12/2024-5.

Data availability

Data will be made available on request.

References

- [1] D. F. Evans and H. Wennerström, *The colloidal domain, where physics, chemistry, and biology meet* (VCH Publishers, 1994), second ed.
- [2] D. Andelman, in *Structure and Dynamics of Membranes*, edited by R. Lipowsky, E. Sackmann (Elsevier, Amsterdam, 1995), vol. 1, section 12, pp. 603–642, second ed.
- [3] D. Lingwood and K. Simons, *science* 327, 46 (2010).
- [4] I. Levental, K. R. Levental, and F. A. Heberle, *Trends Cell Biol.* 30, 341 (2020).
- [5] N. Shimokawa, M. Hishida, H. Seto, and K. Yoshikawa, *Chem. Phys. Lett.* 496, 59 (2010).
- [6] M. C. Blosser, J. B. Starr, C. W. Turtle, J. Ashcraft, and S. L. Keller, *Biophysical journal* 104, 2629 (2013).
- [7] H. Himeno, H. Ito, Y. Higuchi, T. Hamada, N. Shimokawa, and M. Takagi, *Phys. Rev. E* 92, 062713 (2015).
- [8] K. Bohinc, G. Volpe Bossa, and S. May, *Adv. Coll. Int. Sci.* 249, 220 (2017).
- [9] A. Iglič, K. Gongadze, and K. Bohinc, *Bioelectrochemistry* 79, 223 (2010).
- [10] K. Bohinc, V. K. Iglič, and A. Iglič, *Electrochimica Acta* 46, 3033 (2001).
- [11] V. Freise, *Z. Elektrochem.* 56, 822 (1952).
- [12] E. W. M. Eigen, *J. Phys. Chem.* 58, 702 (1954).
- [13] I. Borukhov, D. Andelman, and H. Orland, *Phys. Rev. Lett.* 79, 435 (1997).
- [14] J.J. Bikerman, *Philos. Mag.* (1798-1977) 33, 384 (1942).
- [15] J. L. R. E. Trizac, *Phys. Rev. E* 60, 6530 (1999).
- [16] L. Lue, N. Zoeller, and D. Blankschtein, *Langmuir* 15, 3726 (1999).
- [17] N. F. Carnahan and K. E. Starling, *J. Chem. Phys.* 51, 635 (1969).
- [18] J.F. Dufreche, J.P. Simonin, and P. Turq, *J. Mol. Liq.* 79, 137 (1999).
- [19] B. Giera, N. Henson, E. M. Kober, M. S. Shell, and T. M. Squires, *Langmuir* 31, 3553 (2015).
- [20] B. Jönsson, H. Wennerström, and B. Halle, *J. Phys. Chem.* 84, 2179 (1980).
- [21] S. J. Marrink, V. Corradi, and P. C.T. Souza, H. I. Ingólfsson, D. P. Tieleman, and M. S.P. Sansom, *Chem. Rev.* 119, 6184 (2019).
- [22] F. Foglia, M. J. Lawrence, C. D. Lorenz, and S. E. McLain, *J. Chem. Phys.* 133, 145103 (2010).
- [23] T. Ishiyama, D. Terada, and A. Morita, *J. Phys. Chem. Lett.* 7, 216 (2016).
- [24] S. Abel, N. Galamba, E. Karakas, M. Marchi, W. H. Thompson, and D. Laage, *Langmuir* 32, 10610 (2016).
- [25] A. Srivastava and A. Debnath, *J. Chem. Phys.* 148, 094901 (2018).
- [26] E. Lee, A. Kundu, J. Jeon, and M. Cho, *J. Chem. Phys.* 151, 114705 (2019).
- [27] Y. Higuchi, Y. Asano, T. Kuwahara, and M. Hishida, *Langmuir* 37, 5329 (2021).
- [28] Md. K. Rahman, T. Yamada, N. L. Yamada, M. Hishida, Y. Higuchi, and H. Seto, *Struct. Dyn.* 10, 044701 (2023).
- [29] Md. K. Rahman, T. Yamada, N. L. Yamada, Y. Higuchi, and H. Seto, *J. Phys. Chem. B* 129, 3998 (2025).
- [30] I. R. Cooke, K. Kremer, and M. Deserno, *Phys. Rev. E* 72, 011506 (2005).
- [31] H. Ito, Y. Higuchi, and N. Shimokawa, *Phys. Rev. E* 94, 042611 (2016).
- [32] N. Shimokawa, H. Ito, and Y. Higuchi, *Phys. Rev. E* 100, 012407 (2019).
- [33] J. Guo, H. Ito, Y. Higuchi, K. Bohinc, N. Shimokawa, and M. Takagi, *Langmuir* 37, 9683 (2021).
- [34] H. Ito, N. Shimokawa, and Y. Higuchi, *J. Phys. Chem. B* 127, 8860 (2023).
- [35] S. J. Marrink, A. H. de Vries, and A. E. Mark, *J. Phys. Chem. B* 108, 750 (2004).
- [36] S. J. Marrink, H. J. Risselada, S. Yefimov, and D. P. Tieleman, and A. H. de Vries, *J. Phys. Chem. B* 111, 7812 (2007).
- [37] T. A. Wassenaar, H. I. Ingólfsson, R. A. Böckmann, and D. P. Tieleman, and S. J. Marrink, *J. Chem. Theory Comput.* 11, 2144 (2015).
- [38] G. A. Pantelopulos, T. Nagai, A. Bandara, A. Panahi, and J. E. Straub, *J. Chem. Phys.* 147, 095101 (2017).
- [39] G. A. Pantelopulos and J. E. Straub, *Biophys. J.* 115, 2167 (2018), ISSN 0006-3495.
- [40] J. Domański, S. J. Marrink, and L. V. Schäfer, *Biochim. Biophys. Acta (BBA) - Biomembranes* 1818, 984 (2012), ISSN 0005-2736, protein Folding in Membranes.
- [41] S. Thallmair, H. I. Ingólfsson, and S. J. Marrink, *J. Phys. Chem. Lett.* 9, 5527 (2018).
- [42] S. Baoukina, E. Mendez-Villuendas, W. F. D. Bennett, and D. P. Tieleman, *Faraday Discuss.* 161, 63 (2013).
- [43] Y. Liu, J. Barnoud, and S. J. Marrink, *Biophys. J.* 117, 1215 (2019), ISSN 0006-3495.
- [44] R. S. Davis, P. B. Sunil Kumar, M. M. Sperotto, and M. Laradji, *J. Phys. Chem. B* 117, 4072 (2013).
- [45] Y. Higuchi, K. Bohinc, J. Reščič, N. Shimokawa, and H. Ito, *Soft Matter* 19, 3640 (2023).
- [46] S. Lamperski and C.W. Outhwaite, *J. Electroanal. Chem.* 567, 263 (2004).
- [47] D. H. de Jong, G. Singh, W. F. D. Bennett, C. Arnarez, T. A. Wassenaar, L. V. Schäfer, X. Periole, D. P. Tieleman, and S. J. Marrink, *J. Chem. Theory Comput.* 9, 687 (2013).
- [48] S. O. Yesylevskyy, L. V. Schäfer, D. Sengupta, and S. J. Marrink, *PLOS Comput. Biol.* 6, 1 (2010).
- [49] S. Jo, T. Kim, V. G. Iyer, and W. Im, *J. Comput. Chem.* 29, 1859 (2008).
- [50] E. L. Wu, X. Cheng, S. Jo, H. Rui, and K. C. Song, E. M. Dávila-Contreras, Y. Qi, J. Lee, V. Monje-Galvan, and R. M. Venable, et al., *J. Comput. Chem.* 35, 1997 (2014).
- [51] M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess, and E. Lindahl, *SoftwareX* 1-2, 19 (2015), ISSN 2352-7110.
- [52] G. Bussi, D. Donadio, and M. Parrinello, *J. Chem. Phys.* 126, 014101 (2007).
- [53] M. Parrinello and A. Rahman, *J. Appl. Phys.* 52, 7182 (1981).
- [54] J. Reščič and P. Linse, *J. Comput. Chem.* 36, 1259 (2015).