# Combined effects of headgroup charge and tail unsaturation of lipids on lateral organization and diffusion of lipids in model biomembranes

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Lateral organization and dynamics of lipids in plasma membranes are crucial for several cellular processes such as signal transduction across the membrane and still remain elusive. In this paper, using coarse-grained molecular dynamics simulation, we theoretically study the combined effects of headgroup charge and tail unsaturation of lipids on the lateral organization and diffusion of lipids in ternary lipid bilayers. In neutral ternary lipid bilayers composed of saturated lipids, unsaturated lipids, and cholesterols, under the conditions of given temperature and components, the main factor for the phase separation is the unsaturation of unsaturated lipids and the bilayers can be separated into liquid-ordered domains enriched in saturated lipids and cholesterols and liquid-disordered domains enriched in unsaturated lipids. Once the headgroup charge is introduced, the electrostatic repulsion between the negatively charged lipid headgroups will increase the distance between the charged lipids. We find that the lateral organization and diffusion of the lipids in the (partially) charged ternary lipid bilayers are determined by the competition between the headgroup charge and the unsaturation of the unsaturated lipids. In the bilayers containing unsaturated lipids with lower unsaturation, the headgroup charge plays a crucial role in the lateral organization and diffusion of lipids. The headgroup charge may make the lipid domains unstable and even can suppress phase separation of the lipids in some systems. However, in the bilayers containing highly unsaturated lipids, the lateral organization and diffusion of lipids are mainly dominated by the unsaturation of the unsaturated lipids. This work may provide some theoretical insights into understanding the formation of nanosized domains and lateral diffusion of lipids in plasma membranes.

**Keywords:** biomembrane, phase separation, molecular dynamics simulation, coarse grain

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## 1. Introduction

The plasma membrane is not only a barrier segregating the cell from its external environment, but also plays important roles in some biological processes such as signal transduction and material transportation across the cell membrane.[1–6] The plasma membrane is a highly heterogeneous and dynamic lipid bilayer and composed of various kinds of lipids and proteins. It is widely accepted that there are some domains which contain different lipids and proteins and exhibit different physicochemical properties in the plasma membrane.[7–11] However, due to the complex dynamical and structural properties of the plasma membrane, the microscopic mechanism of the formation of the lipid domains (especially the nanosized domains such as lipid rafts) and the lateral dynamics of the lipids is not well understood, yet.[12–16]

Many factors may influence the lateral organization and diffusion of lipids and proteins in plasma membranes. For example, membrane curvature can significantly influence the lateral organization of biomembrane.[17–19] Another widely-studied factor which affects the domain formation in the plasma membrane is the unsaturation of the lipid tails.[20–22] For example, in the ternary model membrane composed of saturated lipids, unsaturated lipids, and cholesterols, the saturated lipids and cholesterols are aggregated to form liquid-ordered (Lo) domains while the unsaturated lipids tend to form liquid-disordered (Ld) domains under certain temperature and component situations.[3,22–25] Additionally, in some lipid bilayers containing hybrid lipid which has one saturated tail as well as one unsaturated tail, the hybrid lipids were found to be able to reduce the order and compactness of the Lo domains and the domain size.[26,27] All of these studies showed that the unsaturation of the lipid tails is crucial for the formation of the domains in the plasma membranes. Furthermore, the unsaturation of the lipids also influences the lateral diffusion of the lipids and the unsaturated lipids in the Ld domains laterally diffuse faster than the saturated lipids in the Lo domains in some ternary lipid bilayers.[20,28]

In real plasma membrane (especially in the inner leaflet of plasma membrane), there are many lipids with charged headgroups.[10,29] However, in previous relevant work, most efforts were focused on the phase separation of neutral membranes while the effect of charge on the phase separation of

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lipid bilayers has been barely considered.[11,24,30,31] In recent years, some attention was paid to the effect of headgroup charge of lipids on the domain formation in the model membranes. For example, in some recent experimental studies, it was found that some charged lipids may enhance the phase separation of lipid bilayers while other charged lipids may suppress the phase separation, and the charged lipids may also influence the distribution of cholesterols.[32,33] However, in some other systems, the charged lipids were believed to only have a minimal effect on the phase behavior of the lipid bilayers.[34] In addition, to neutralize the negatively charged lipid bilayers, some cations should be added into the system, and the lateral organization of the lipids in lipid bilayers or vesicles can also be influenced by the addition of the cations.[35,36] In summary, although some progress has been made, the influence of electrostatic interactions on the formation of lipid domains and the lateral diffusion of lipids is far from being completely or systematically understood. In particular, some theoretical efforts should be further devoted to understanding the combined effects of the electrostatic interaction and the unsaturation of lipid tails on the lateral organization and diffusion of lipids in model and/or natural membranes.

In recent years, the coarse-grained molecular simulation has become a powerful and indispensable tool to study the microscopic structures of biomembranes and interactions between biomembrane and nanoparticles.[10,23,37–46] In this paper, we investigate the phase separation and lateral diffusion of the lipids in either neutral or negatively charged ternary lipid bilayers composed of saturated lipids, unsaturated lipids with different unsaturation, and cholesterols using coarse-grained molecular dynamics (MD) simulation. We mainly focus on the combined influences of headgroup charge and tail unsaturation of lipids on the lateral organization and diffusion of lipids. The results show that the headgroup charge has a significant influence on both phase separation and lateral diffusion of lipids in the lipid bilayers. The phase separation of the lipid bilayers containing charged lipids is mainly dominated by the competition between the electrostatic repulsion between the charged lipids and the unsaturation of the unsaturated lipids.

## 2. Model and methods

### 2.1. Molecular dynamics simulations with Martini force field

In this paper, all the systems are simulated using Martini coarse-grained force field which is one of the most successful models for biomolecular simulations.[47–49] In particular, Martini includes many kinds of coarse-grained lipids and has gained great successes in large-scale biomembrane simulations.[10,23,37] Martini force field adopts a four-to-one mapping scheme, namely, four heavy atoms are mapped to one interaction bead on average.[49] This coarse-grained force field defines four bead types: polar (P), apolar (C), nonpolar (N), and charged (Q).

In the simulations, the nonbonded interaction parameters are given as follows: the Coulomb interaction is shifted to zero between 0 nm and 1.2 nm and the Lennard-Jones interaction is shifted to zero between 0.9 nm and 1.2 nm.[10,38,39] The ensemble of constant particle number, pressure, and temperature (NpT) is used in the simulations. The temperature is maintained at 305 K using velocity-rescaling coupling scheme with a relaxation time $\tau_T = 1$ ps.[50] The bilayer plane ($xy$) and the normal direction ($z$) are separately coupled to a pressure bath of 1 bar (1 bar $= 10^5$ Pa) using Parrinello–Rahman approach with a relaxation time $\tau_p = 12$ ps.[51,52] The compressibility of the systems is set as $5 \times 10^{-6}$ bar$^{-1}$ in both lateral and normal directions to ensure that the lipid bilayer is tensionless. For all the systems, after energy minimization and 20-ns equilibrating simulations, the production simulations are run for 10 μs. All the MD simulations are performed using the package of GROMACS 5.0 with an integration time step of 25 fs.

### 2.2. System setup

We mainly examine some neutral or charged ternary lipid bilayers composed of saturated lipids, unsaturated lipids with different unsaturation, and cholesterols (CHOL) to reveal the combined effects of headgroup charge and tail unsaturation of lipids on the lateral structures and dynamics of the bilayers. All the systems are summarized in Table 1.

**Table 1.** Overview of the simulation systems and the charged and unsaturated lipids in each lipid bilayer. The molar ratio of saturated lipid:unsaturated lipid:cholesterol is 4:3:3 in all systems.

| No. | System | Charged lipids | Unsaturated lipid (unsaturation degree) |
|---|---|---|---|
| I | DPPE/DIPE/CHOL | – | DIPE (2) |
| II | DPPS/DIPS/CHOL | DPPS, DIPS | DIPS (2) |
| III | DPPE/DIPS/CHOL | DIPS | DIPS (2) |
| IV | DPPS/DIPE/CHOL | DPPS | DIPE (2) |
| V | DPPE/DFPE/CHOL | – | DFPE (3) |
| VI | DPPS/DFPS/CHOL | DPPS, DFPS | DFPS (3) |
| VII | DPPE/DFPS/CHOL | DFPS | DFPS (3) |
| VIII | DPPS/DFPE/CHOL | DPPS | DFPE (3) |

Here, to reveal the microscopic mechanism of lateral organization of plasma membranes, we mainly consider two kinds of the abundant lipids in plasma membranes with different headgroups: phosphatidylethanolamine (PE) and phosphatidylserine (PS). At the coarse-grained level, the structures of PS and phosphatidylglycerol (PG) are similar. Therefore, our results are also comparable to the relevant experimental results of PG-containing membranes.[32,34] The molecular structures of coarse-grained lipids of PE and PS in Martini force field used in this work are shown in Fig. 1. PE is zwitterionic and can be considered as neutral lipid while PS is anionic and can be considered as negatively charged lipid. For the unsaturated lipids, each tail of dilinoleoyl-PE (DIPE) or dilinoleoyl-PS (DIPS) contains two unsaturated bonds while each tail of dioctadecatrienoyl-PE (DFPE) or dioctadecatrienoyl-PS (DFPS) contains three unsaturated bonds. For convenience, we define the number of unsaturated bonds per tail as the unsaturation degree of the unsaturated lipids, namely, the unsaturation degrees of DIPE (or DIPS) and DFPE (or DFPS) are 2 and 3, respectively.

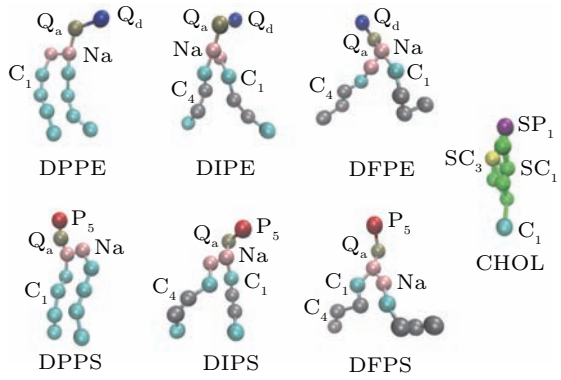



**Fig. 1.** (color online) The molecular structures of coarse-grained lipids used in this work. The ethanolamine groups of PE and serine groups of PS are represented by positively-charged beads $Q_d$ and polar beads $P_5$, respectively. The phosphate groups and glycerol groups of all lipids are represented by negatively-charged beads $Q_a$ and nonpolar beads Na, respectively. In the fatty acid tails of the lipids, the units linked with saturated bonds are represented by apolar beads $C_1$ and the units linked with unsaturated bonds are represented by apolar beads $C_4$. Cholesterol is mapped with five beads and three virtual sites.[47,48,53,54]

All the ternary lipid bilayers are built using the script of INSANE developed by Martini developers.[53] Each lipid bilayer consists of 288 saturated lipids, 216 unsaturated lipids, and 216 cholesterols and thus the molar ratio of saturated lipids, unsaturated lipids, and cholesterols is 4:3:3. In addition, each system includes about 31900 coarse-grained water beads to solvate the lipid bilayer. To maintain electroneutrality of the simulation systems, some sodium ions ($Na^+$) are added into the solvent of the systems containing negatively-charged lipids and no additional salt is added. The initial size of the simulation box is 15 nm × 15 nm × 25 nm and periodic boundary conditions are used in all three dimensions. In all simulations, mimicking the effect of cytoskeleton network in the real plasma membranes, we apply weak position restraints to some lipids in $z$ direction to suppress large-scale bilayer undulation.[10] All of the bilayers are simulated from the same initial mixed state.

### 2.3. Data analysis

In order to examine the structures of the lipid bilayers during the simulations, we use the visualization software VMD to obtain the snapshots of the systems.[55] To quantitatively determine the lipid domain size, we calculate the number of aggregated lipids in lipid domains by the membrane analysis tool g_aggregate.[56] Here, we suppose that lipids are aggregated if the distance between their headgroups is less than 0.95 nm. We mainly analyze the time evolution of the number of saturated lipids in the biggest Lo domains ($N_s^{Lo}$) in every system. Additionally, to examine the packing state of the lipids in the lipid bilayers, we calculate the order parameters of the lipids using the tool g_ordercg.[57] The lipid order parameter is defined as

$$P = \frac{1}{2}(3\langle\cos^2\theta\rangle - 1), \tag{1}$$

where $\theta$ is the angle between the membrane normal and the bond vectors. According to this definition, the order parameter will be 1 if all the molecules are perfectly aligned with the membrane normal, $-0.5$ if all of them lie in the membrane plane , and 0 if the $\theta$ angle is distributed randomly. We also analyze the area per lipid (APL) using the grid-based analysis tool GridMAT-MD to reveal the lateral organization and dynamics of the lipids.[58]

Finally, in order to reveal the lateral diffusion of the lipids, we calculate the lateral mean square displacements (MSD) and the lateral diffusion coefficients of the saturated and the unsaturated lipids using the analysis tool g_msd.[59,60] We calculate the MSD of lipids in the upper and lower leaflets separately through analyzing the last 6 μs of the simulations and the overall center of mass motion is removed. The fitting of diffusion coefficient starts at 4.6 μs and ends at 9.4 μs.

## 3. Results and discussion

In this section, we will first focus our concentration on examining the effect of the charge on the phase separation of the lipid bilayers. We now consider the four different lipid bilayers composed of DPPE/DIPE/CHOL, DPPS/DIPS/CHOL, DPPE/DIPS/CHOL, and DPPS/DIPE/CHOL, respectively. In these four systems, the unsaturated lipids (DIPE and DIPS) have the same unsaturation degree of 2. Figure 2 shows the final structures of the 10-μs simulations of these lipid bilayers and the corresponding time evolutions of the number of saturated lipids in the biggest Lo domain. We find that both the DPPE/DIPE/CHOL and the DPPS/DIPS/CHOL lipid bilayers are separated into large Lo domains enriched in saturated lipids (DPPE or DPPS) and cholesterols and Ld domains enriched in unsaturated lipids (DIPE or DIPS). Additionally, the curves of the number of saturated lipids in the biggest Lo domain indicate that both the lipid bilayers complete the phase separation within the initial 1 μs and the domains are

relatively stable for the rest of the simulation time. The large average values of the number of the saturated lipids in the biggest Lo domain also imply a relatively strong segregation between Lo and Ld domains in these two systems. However, in the lipid bilayers composed of DPPS/DIPE/CHOL and DPPE/DIPS/CHOL, we do not find strong phase separation. The relatively small values of the number of saturated lipids in the biggest Lo domain further indicate that the domain sizes are smaller in these two lipid bilayers than in the other two lipid bilayers shown in Figs. 2(a) and 2(b).

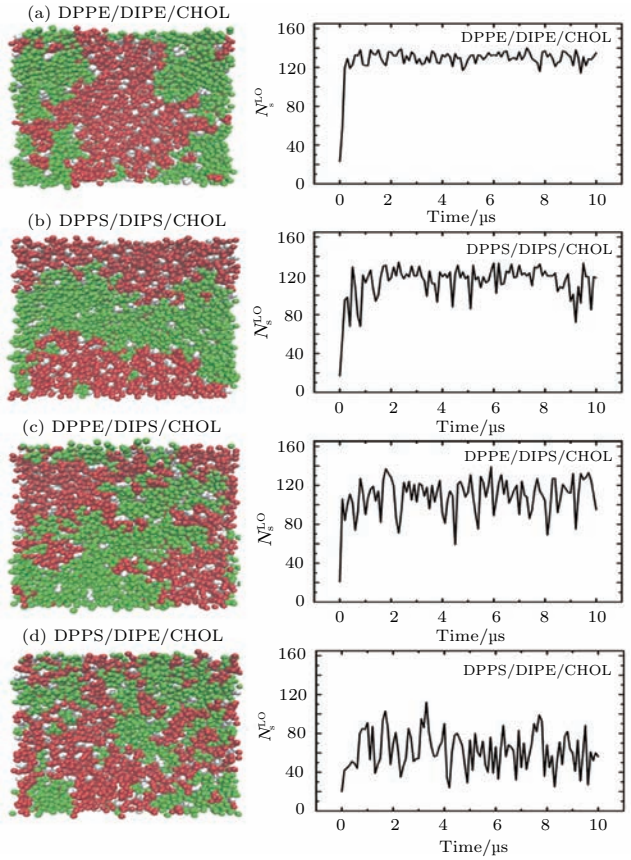



**Fig. 2.** (color online) Left: the final structures of the 10-μs simulations of the lipid bilayers composed of (a) DPPE/DIPE/CHOL, (b) DPPS/DIPS/CHOL, (c) DPPE/DIPS/CHOL, and (d) DPPS/DIPE/CHOL. Here, the saturated lipid, the unsaturated lipid, and cholesterol are shown in red, green, and white, respectively. Right: the time evolutions of the number of saturated lipids in the biggest Lo domain ($N_s^{Lo}$) in the corresponding left lipid bilayers.

For the neutral ternary lipid bilayer of DPPE/DIPE/CHOL, the main factor determining the phase separation of the bilayer is the unsaturation of the unsaturated lipid (DIPE) just as demonstrated in relevant previous studies.[23,24] For the negatively charged lipid bilayer composed of DPPS/DIPS/CHOL, because both the headgroups of the saturated and the unsaturated lipids are negatively charged, there is an electrostatic repulsion between any two lipids no matter whether they are saturated or unsaturated and the electric field in the bilayer plane is nearly uniform. The uniformity of electric field erases the effect of the headgroup charge on lateral organization of lipids and keeps the unsaturation of the unsaturated lipids as a dominant factor for the phase separation of the lipid bilayer containing both charged saturated and unsaturated lipids.

For the lipid bilayer composed of DPPE/DIPS/CHOL or DPPS/DIPE/CHOL, although the saturated lipids and unsaturated lipids tend to be segregated from each other to form Lo and Ld domains due to the difference of the saturation of the lipid tails, the electrostatic interaction between the negatively charged lipid headgroups produces a repulsion between the charged lipids (DIPS or DPPS) and thus these lipids prefer to separate from each other with a relatively large distance. On the contrary, there is no repulsion between the charged and neutral lipids. The contacts between the charged lipids (DIPS or DPPS) and the neutral lipids (DPPE or DIPE) do not obviously increase the overall energy of the system even though some conformational entropy of lipids will be lost due to the contacts between saturated and unsaturated lipids. Therefore, in these two systems, the electrostatic repulsion between the charged lipids plays an important role in the lateral distribution of the lipids and restrains the phase separation of the saturated and unsaturated lipids. This inhibition of phase separation in these two systems qualitatively agrees with the results of some relevant experiments.[33,35] However, there are still some differences between the structures of the lipid bilayers composed of DPPE/DIPS/CHOL and DPPS/DIPE/CHOL. In the lipid bilayer of DPPE/DIPS/CHOL, on the one hand, the distance between DIPS lipids in the Ld domains is not very small due to the loose and disordered packing of the lipids and thus the electrostatic repulsion between DIPS lipids is not very strong; on the other hand, the ordered packing of DPPE lipids can reduce the loss of conformational entropy of lipids. Therefore, in this lipid bilayer, although the phase separation is not so strong as that in the lipid bilayer of DPPE/DIPE/CHOL or DPPS/DIPS/CHOL and the boundary of the domains is rather rough, the sizes of the lipid domains are comparable to the domain sizes in the DPPE/DIPE/CHOL or DPPS/DIPS/CHOL bilayer. In contrast, in the lipid bilayer of DPPS/DIPE/CHOL, if the saturated DPPS lipids are aggregated to form a large Lo domain, the distance between DPPS lipids will be very small and the the electrostatic repulsion between DPPS lipids will be very strong due to the tight and ordered packing of the lipids. Therefore, the saturated and unsaturated lipids prefer to form a nearly homogeneous bilayer where the negatively charged lipids (DPPS) and the neutral lipids (DIPE) are mixed as shown in Fig. 2(d).

To confirm the above discussions on the lateral distribution of lipids in the lipid bilayers of DPPE/DIPS/CHOL and DPPS/DIPE/CHOL, we calculate the areas per lipid (APL) in these two systems and show them in Table 2. We find that, in the lipid bilayer of DPPE/DIPS/CHOL, the APL of DPPE is much smaller than the APL of DIPS. Thus, the DPPE may be in the Lo state while DIPS in the Ld state and they tend

to be separated into large Lo and Ld domains. However, due to the repulsion between the charged DIPS lipids, the contacts between saturated and unsaturated lipids are increased and the phase separation is not very strong. In the DPPS/DIPE/CHOL lipid bilayer, both DPPS and DIPE have relatively large APLs. Therefore, the electrostatic repulsion obviously increases the distance between the saturated DPPS lipids. The saturated DPPS lipids are not packed so tightly as DPPE lipids in the DPPE/DIPS/CHOL bilayer and no large Lo domains can be formed in this lipid bilayer.

**Table 2.** The areas per lipid of the saturated lipids ($APL_s$) and the unsaturated lipids ($APL_u$) in the lipid bilayers composed of DPPE/DIPS/CHOL and DPPS/DIPE/CHOL.

| System | $APL_s$/nm$^2$ | $APL_u$/nm$^2$ |
|---|---|---|
| DPPE/DIPS/CHOL | DPPE 0.45 | DIPS 0.67 |
| DPPS/DIPE/CHOL | DPPS 0.52 | DIPE 0.59 |

Based on the above discussions on the lateral organization of the lipid bilayers composed of DPPS/DIPE/CHOL and DPPE/DIPS/CHOL, we can reasonably suppose that the effect of headgroup charge of lipids is an important factor for the formation of the nanosized lipid domains in the plasma membrane which contains thousands of kinds of neutral and charged lipids.[29]

Furthermore, if we take a careful look at the curves of the number of the saturated lipids in the biggest Lo domain in Fig. 2, we can find that the fluctuation of the number of saturated lipids in the biggest Lo domain is greater in the (partially) charged lipid bilayers than in the neutral bilayer, which implies that the introduction of the headgroup charge may influence the dynamical property of the lipids and make the domains unstable.

**Table 3.** The diffusion coefficients of the saturated lipids ($D_s$) and unsaturated lipids ($D_u$) in the bilayers containing the unsaturated lipids with unsaturation degree 2.

| System | $D_s/10^{-7}$ (cm$^2$/s) | $D_u/10^{-7}$ (cm$^2$/s) |
|---|---|---|
| DPPE/DIPE/CHOL | DPPE 1.23±0.08 | DIPE 1.81±0.04 |
| DPPS/DIPS/CHOL | DPPS 1.72±0.16 | DIPS 2.65±0.13 |
| DPPE/DIPS/CHOL | DPPE 1.95±0.16 | DIPS 2.38±0.17 |
| DPPS/DIPE/CHOL | DPPS 2.35±0.23 | DIPE 3.34±0.14 |

To determine the stability of lipid domains and the lateral dynamics of the lipids in the lipid bilayers, we calculate the MSD and the lateral diffusion coefficients of both the saturated and unsaturated lipids and the results are shown in Fig. 3 and Table 3, respectively. The diffusion coefficients in Table 3 are comparable to the experimental data.[61] Both the MSD and the lateral diffusion coefficients indicate that both the saturated and unsaturated lipids diffuse faster in the (partially) charged lipid bilayers than in the neutral lipid bilayer. Particularly, from Fig. 3, we find that the lateral diffusion of saturated lipids is influenced by the headgroup charge more obviously than that of the unsaturated lipids. This is mainly because the electrostatic repulsion between the charged lipids can significantly change the packing of the saturated lipids whereas the packing of the unsaturated lipids is not affected greatly by the electrostatic repulsion. Furthermore, the much larger APL of DPPS compared to APL of DPPE also indicates great influence of headgroup charge on the lateral distribution of saturated lipids (DPPS) (Table 2). This result further confirms that the introduction of the headgroup charge can enhance the lateral mobility of the lipids and reduce the stability of the lipid domains.

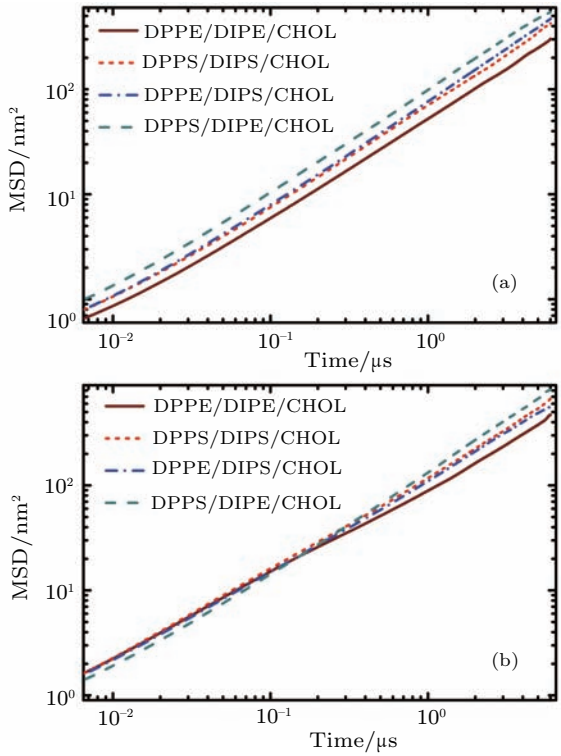



**Fig. 3.** (color online) The mean square displacements (MSD) of (a) saturated lipids and (b) unsaturated lipids in the lipid bilayers composed of DPPE/DIPE/CHOL, DPPS/DIPS/CHOL, DPPE/DIPS/CHOL, and DPPS/DIPE/CHOL.

Subsequently, we examine the effect of the unsaturation degree of the lipids on the lateral organization of the lipids in the lipid bilayers. We increase the unsaturation degree of unsaturated lipids from two to three and consider four lipid bilayers composed of DPPE/DFPE/CHOL, DPPS/DFPS/CHOL, DPPE/DFPS/CHOL, and DPPS/DFPE/CHOL, respectively. The simulation results of these four systems are shown in Fig. 4. We find that, in all four systems, the unsaturation of the unsaturated lipids plays an absolutely dominant role in the phase separation of the lipid bilayers and thus all four lipid bilayers are separated into large Lo and Ld domains. The curves of the number of saturated lipids in the biggest Lo domain also show that the saturated and unsaturated lipids are strongly separated and the lipid domains are stable. The effect of the headgroup charge is completely suppressed by the effect of unsaturation of the lipids in the lipid bilayers shown in Fig. 4.

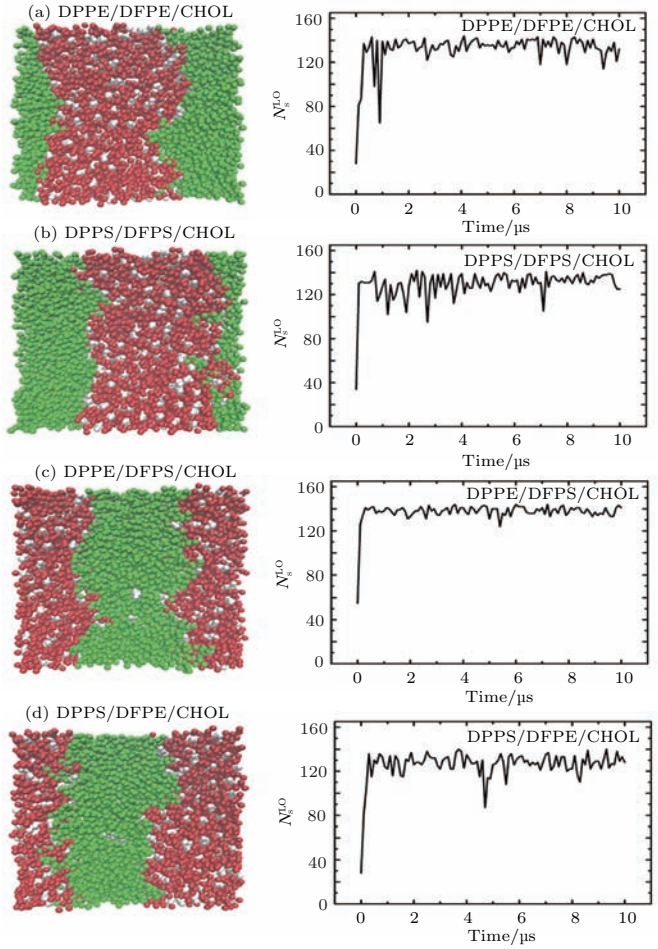



**Fig. 4.** (color online) The final structures of the 10-μs simulations of the lipid bilayers composed of (a) DPPE/DFPE/CHOL, (b) DPPS/DFPS/CHOL, (c) DPPE/DFPS/CHOL, and(d) DPPS/DFPE/CHOL, and the corresponding time evolutions of the number of the saturated lipids in the biggest Lo domain ($N_s^{Lo}$) in the bilayers.

We also calculate the MSD and lateral diffusion coefficients of the lipids as shown in Fig. 5 and Table 4, respectively. Although lateral diffusion of the saturated lipids is faster in the lipid bilayers containing charged lipids than that in the neutral lipid bilayer of DPPE/DFPE/CHOL, the saturated lipids diffuse much slower in these four lipid bilayers than in the DIPE- or DIPS-containing lipid bilayers (Table 3). However, for the unsaturated lipids, DFPS and DFPE diffuse much faster than DIPS and DIPE except that DFPE lipids in the DPPS/DFPE/CHOL bilayer diffuse slower than DIPE lipids in the nearly homogenous bilayer of DPPS/DIPE/CHOL. This is because, in these strongly separated systems, the saturated lipids (DPPE and DPPS) are in Lo state and they are packed more orderly and tightly than in the DIPE- or DIPS-containing lipid bilayers whereas the unsaturated lipids (DFPS or DFPE) are packed more loosely than the unsaturated lipids (DIPS or DIPE) in the bilayers shown in Fig. 2. Here, the lipid bilayer of DPPS/DFPE/CHOL is an exception. In this lipid bilayer, due to the electrostatic repulsion between DPPS lipids, the distance between DPPS lipids is increased while the distance between DFPE lipids is compressed and the packing of DFPE may become tighter (the APL of DFPE (0.672 $nm^2$) in DPPS/DFPE/CHOL bilayer is smaller than the APL of DFPE (0.686 $nm^2$) in DPPE/DFPE/CHOL bilayer). Therefore, the DFPE lipids in this system diffuse slower than the unsaturated lipids (DFPE or DFPS) in the other three systems shown in Fig. 4. However, the packing density or APL is not the unique factor determining the lateral diffusion coefficients of lipids. Here, the lateral diffusion coefficients of lipids are averaged in whole membrane plane. In the nearly homogenous bilayer of DPPS/DIPE/CHOL, although APL of DIPE is relatively small, DIPE can nearly diffuse in the whole membrane. In contrast, in the bilayer of DPPS/DFPE/CHOL, DFPE lipids are confined in the Ld domain but cannot diffuse into Lo domain. Consequently, the mean value of lateral diffusion coefficient of DIPE in bilayer of DPPS/DIPE/CHOL is larger than that of DFPE in bilayer of DPPS/DFPE/CHOL as shown in Tables 3 and 4.

**Table 4.** The diffusion coefficients of the saturated lipids ($D_s$) and unsaturated lipids ($D_u$) in the bilayers containing unsaturated lipids with unsaturation degree 3.

| System | $D_s/10^{-7}$ cm$^2\cdot$s$^{-1}$ | $D_u/10^{-7}$ cm$^2\cdot$s$^{-1}$ |
|---|---|---|
| DPPE/DFPE/CHOL | DPPE 0.38 ± 0.03 | DFPE 3.72 ± 0.28 |
| DPPS/DFPS/CHOL | DPPS 0.48 ± 0.04 | DFPS 3.93 ± 0.56 |
| DPPE/DFPS/CHOL | DPPE 0.44 ± 0.06 | DFPS 4.46 ± 0.34 |
| DPPS/DFPE/CHOL | DPPS 0.44 ± 0.04 | DFPE 3.24 ± 0.49 |

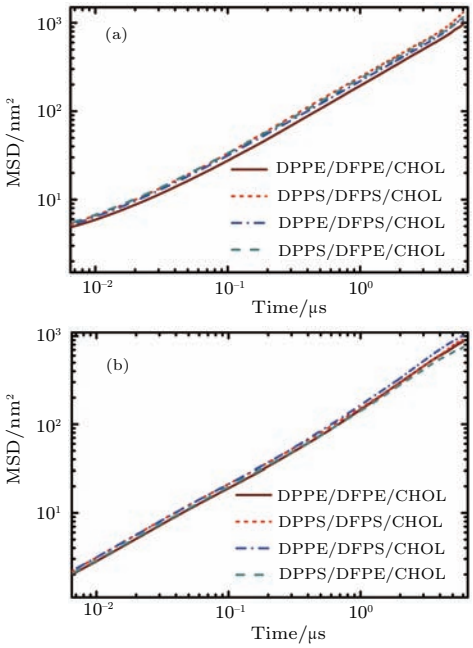



**Fig. 5.** (color online) The mean square displacements (MSD) of (a) saturated lipids and (b) unsaturated lipids in the lipid bilayers composed of DPPE/DFPE/CHOL, DPPS/DFPS/CHOL, DPPE/DFPS/CHOL, and DPPS/DFPE/CHOL.

Furthermore, comparing Fig. 5 with Fig. 3, we also find that, the influence of headgroup charge on the lateral diffusion of lipids in DFPS- or DFPE-containing systems is not so obvious as that in the DIPS- or DIPE-containing systems, which implies that the unsaturation of the lipids is also a dominant

factor determining the lateral diffusion of lipids in the bilayers shown in Fig. 4.

**Table 5.** The order parameters of the saturated lipids ($OP_s$) and the unsaturated lipids ($OP_u$) in the lipid bilayers.

| System | $OP_s$ | $OP_u$ |
|---|---|---|
| DPPE/DIPE/CHOL | DPPE 0.44 | DIPE 0.20 |
| DPPS/DIPS/CHOL | DPPS 0.47 | DIPS 0.22 |
| DPPE/DIPS/CHOL | DPPE 0.41 | DIPS 0.21 |
| DPPS/DIPE/CHOL | DPPS 0.45 | DIPE 0.22 |
| DPPE/DFPE/CHOL | DPPE 0.50 | DFPE 0.19 |
| DPPS/DFPS/CHOL | DPPS 0.52 | DFPS 0.20 |
| DPPE/DFPS/CHOL | DPPE 0.49 | DFPS 0.19 |
| DPPS/DFPE/CHOL | DPPS 0.52 | DFPE 0.19 |

To further examine the packing state of the lipids in the lipid bilayers, we calculate the order parameters of the lipids and summarize them in Table 5. We find that, the order parameters of the saturated lipids (DPPE and DPPS) in the DFPE- or DFPS-containing lipid bilayers are larger than that in the DIPE- or DIPS-containing lipid bilayers while the order parameters of the DFPE and DFPS are smaller than that of DIPE and DIPS. For clarity, figure 6 shows the lateral views of the final structures of lipid bilayers of DPPS/DIPE/CHOL and DPPS/DFPE/CHOL. We can intuitively find that, just as reflected by the order parameters, the saturated lipids are packed more tightly and orderly while the unsaturated lipids are packed more loosely and disorderly in the lipid bilayers shown in Fig.4 than in the lipid bilayers shown in Fig. 2. Therefore, the saturated lipids diffuse slower while the unsaturated lipids diffuse faster in the DFPE- or DFPS-containing lipid bilayers.

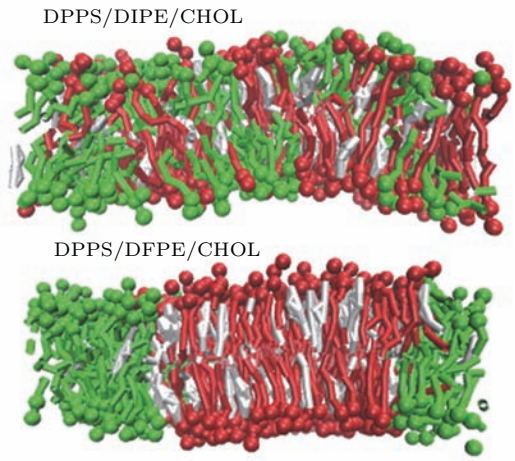



**Fig. 6.** (color online) The lateral views of the lipid bilayers composed of DPPS/DIPE/CHOL and DPPS/DFPE/CHOL.

## 4. Conclusion

In this work, using coarse-grained MD simulation, we systematically study the combined effects of headgroup charge and tail unsaturation of lipids on the lateral organization and dynamics of lipids in ternary lipid bilayers. In the neutral ternary lipid bilayer composed of saturated lipids, unsaturated lipids, and cholesterols, as revealed in previous work, the lipid bilayer will be separated into Lo and Ld domains. However, there are plenty of lipids with negatively charged headgroups especially in the inner leaflet of plasma membrane. The electronegative headgroups introduce an electrostatic repulsion between the charged lipids and the repulsion will increase the distance between the charged lipids. Consequently, the phase separation of the lipids may be influenced by the charged lipids and the lateral organization of the lipids will be determined by the combined effects of headgroup charge and unsaturation of lipids. In the charged lipid bilayers containing unsaturated lipids with lower unsaturation degree, if the neutral and charged lipids coexist, the phase separation of the lipids may be suppressed by the electrostatic repulsion between the charged lipids and no strong phase separation will occur, which indicates that the headgroup charge may be crucial for the formation of nanosized lipid domains in the plasma membranes. Particularly, if the saturated lipids are charged and the unsaturated lipids are neutral, the electrostatic repulsion obviously increases the distance between the saturated lipids and destroys the tight and ordered packing of the saturated lipids. Thus, the bilayer will become nearly homogeneous. If the saturated and unsaturated lipids are both charged, because the electric field is nearly uniform, the unsaturation will determine the lateral organization of the lipids and large domains will be formed. With the increase of the unsaturation degree of the unsaturated lipids, the lateral organization of the lipids will be mainly dominated by the effect of unsaturation of the unsaturated lipids and strong phase separation will take place in both neutral and (partially) charged lipid bilayers.

Furthermore, we also examine the lateral diffusion of the lipids in the lipid bilayers. We find that the introduction of headgroup charge of lipids can enhance the lateral diffusion of the charged lipids because of the larger distance induced by the electrostatic repulsion between them. However, the lateral diffusion of the unsaturated lipids is mainly determined by the unsaturation of the lipids. With the increase of the unsaturation degree of the unsaturated lipids, the packing of the lipids may become looser and the lateral diffusion of the unsaturated lipids will consequently be increased.

Our findings may provide some theoretical insights into combined effects of headgroup charge and unsaturation of the lipids on the lateral organization and diffusion of the lipids in plasma membranes. In particular, our results may be helpful for understanding the formation of nanosized lipid rafts in plasma membrane.

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