Multiscale Membrane Models

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DOI:
10.33612/diss.136221782

Chapter 5

Applying the virtual site hybrid scheme to accelerate phase separation in ternary membranes

Abstract

Understanding the lateral organization in plasma membranes remains an open problem and is of great interest to many researchers. Model membranes consisting of coexisting domains are commonly used as simplified models of plasma membranes. The coarse-grain (CG) Martini force field has successfully captured spontaneous separation of ternary membranes into a liquid-disordered and a liquid-ordered domain. With all-atom (AA) models, however, phase separation is much harder to achieve due to the slow underlying kinetics. To remedy this problem, here, we apply the virtual site (VS) hybrid method on a ternary membrane composed of DPPC, DLiPC and cholesterol and investigate the phase separation. The VS scheme couples the two membrane leaflets at CG and AA resolution. The hypothesis is that the rapid phase separation reached by the CG leaflet can accelerate and guide this process in the AA leaflet.

Method

Simulation setup

We have applied the VS hybrid scheme as described in Chpater 4 to a membrane system as illustrated in Figure 1. The VS hybrid scheme is a multiscale scheme that can concurrently couple AA and CG force fields. Here we combine the atomistic in-house GROMOS force field (described and tested in [200]) and the CG Martini force field[26] in a single membrane simulation. We kept the resolution interfaces at the apolar region in the center of the bilayer, and restrained water penetration through the membranes. The details of this setup can be found in [200].

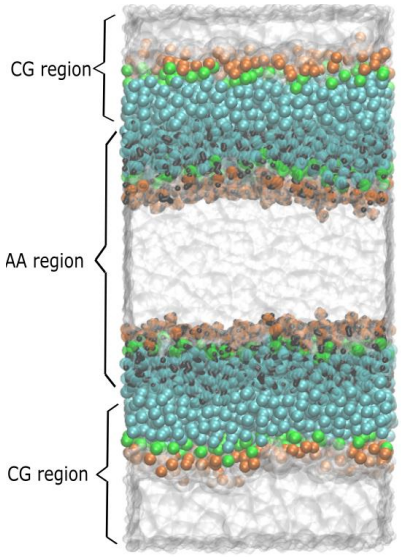

Figure 1. Virtual site hybrid scheme of a membrane. Lipids' tail, linker, and head parts are represented by cyan, green, and orange color, respectively. The water is rendered by the white transparent surface.

The membrane consists of a DPPC:DLiPC:cholesterol ternary mixture (molar ratio 42:28:30 and about 1500 lipids in total), which forms co-existing Lo and Ld domains as shown previously[195]. The ternary membrane is simulated in a box of $15\text{nm} \times 15\text{nm} \times 15\text{nm}$. Forcefield details are described in Chapter 4. The simulations were carried out using the GROMACS (2016) software[33], using an integration time step of 4 fs. The Verlet cut-off scheme was used for the non-bonded interactions and the allowed energy error due to the Verlet buffer is 0.005 kJ/mol/ps per atom. The potential modifiers were used to shift the complete LJ and Coulomb potential value to zero at the cut-off. Electrostatic interactions were treated using PME with a 0.12 nm Fourier grid spacing. The temperature was maintained at 295 K by integrating the equations of motion with the leapfrog stochastic dynamics integrator[181] with inverse friction constants of 1 ps to CG (or VS) particles and 0.1 ps to AA particles. AA lipids, AA water, CG lipids, CG water and VS beads were coupled separately to a thermostat. The pressure was kept at 1 bar independently in the lateral and normal directions with the Berendsen barostat[34] in a semi-isotropic pressure bath ($\boldsymbol{\tau}_p$= 0.5 ps). The compressibility was 4.6×10^{-6} bar^{-1}. The AA bonds were constrained with the LINCS algorithm, and simple point charge (SPC) water[182] was constrained using SETTLE[183]. A flat bottom potential was used on the polar headgroup of cholesterol (CG or VS ROH beads) to restrain flip-flop as suggested in[194].

The fully AA membranes simulated using CHARMM force field were built with the CHARMM-GUI web server. The bilayer systems were simulated using the equilibration and production protocol provided by CHARMM-GUI. The simulations last for more than 40 ns.

Results

Recalibration of the VS hybrid scheme for lipid bilayer systems

We have applied the VS hybrid scheme on the ternary mixture (DPPC, DLiPC and cholesterol) at 295K to investigate the phase separation. However, even though the cholesterol flip-flop is limited (see Methods), coupling the two different resolutions still resulted in a strong interdigitation between the two leaflets, shown in Figure 2a. The cholesterol from the AA leaflet is found embedded deeply into the CG leaflet. It appears that the attraction between the AA cholesterol and CG leaflet is too strong. The underlying reason can be found in the different CG bead densities of the two cholesterol models. In Figure 3, the bond distance distributions of the CG virtual sites of cholesterol in the AA leaflets are computed and compared with their counterparts of the CG leaflets using the indicated mapping. Note, the bonds far from the resolution interface are not taken into consideration. As expected, apart from the C1 and C2 bonds, the rest of bond distributions in cholesterol (especially the ring structure) disagree between the two resolutions. The modified VS (CG) bond distances of the AA model causes the wrong local density of interaction sites and entails the risk of compromising the trends of free enthalpy and partitioning behavior against the standard CG model[137]. This could explain the strong interdigitation between CG and AA leaflets in the ternary membrane .

To solve the interdigitation problem, we applied a scaling factor of 0.6 on the epsilon of the LJ potential between the subset of the VS beads in AA cholesterol (R3-R5 beads) and the C4 beads of DLiPC lipids in the CG leaflet. As shown in Figure 2b, the interdigitation is now much weaker, although there are still one or two cholesterols translocated along the perpendicular direction of the membrane toward the CG leaflet. This result confirms that the strong attraction between AA cholesterol and the CG leaflet is the reason for excessive interdigitation in VS hybrid ternary membrane, and also that the scaling factor of 0.6 is not enough. Besides, this approach only decreases the attractions between cholesterol and DLiPC, while the attractions between

cholesterol and DPPC are kept unchanged. This setup artificially increases the cholesterol partition into the ordered phase.

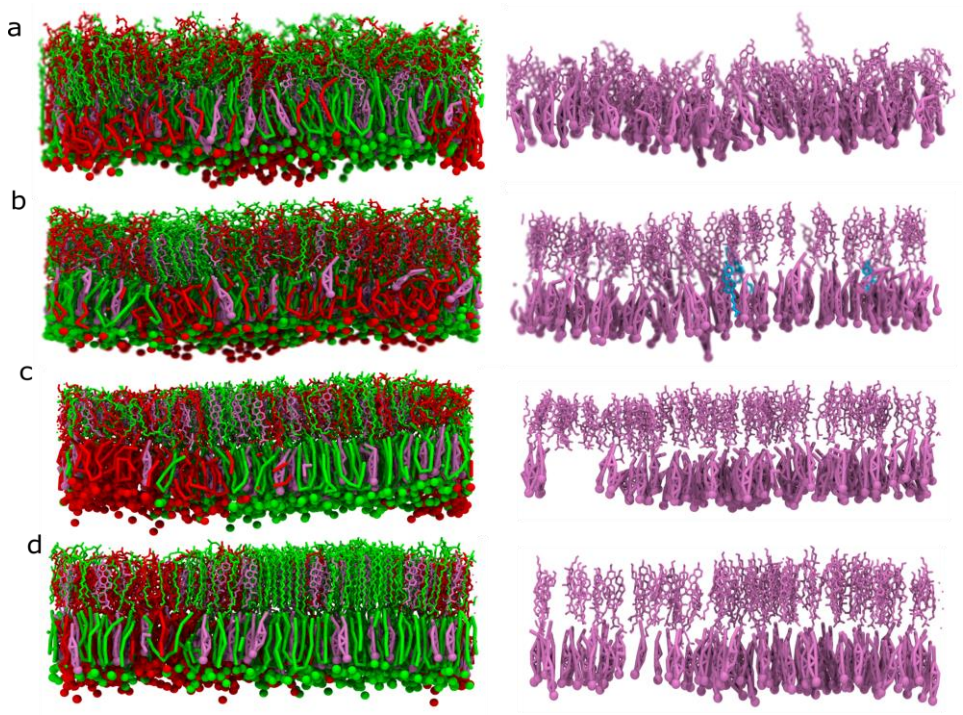

Figure 2. Snapshots of ternary membrane in VS hybrid setup. (a) and (b) represent the VS hybrid system and the scaled VS hybrid system (the attractions between cholesterol (R3-R5 beads) in AA leaflet and the DLiPC lipids in the other CG leaflet are scaled down by a factor of 0.6), respectively. (c) and (d) represent the VS hybrid systems starting from half phase separated phase and already phase separated phase after about 6 μs, respectively. The C6 of the LJ potential between the cholesterols (R3-R5 beads) and the other leaflet is set to 0. The right column only show the cholesterols of the ternary membranes in the left column and cholesterol in the wrong position is rendered in cyan in (b). DPPC, DLiPC and cholesterol are represented by green, red, and magenta, respectively. Thin and thick lines represent the AA and CG resolutions, respectively. Water is not shown for clarity.

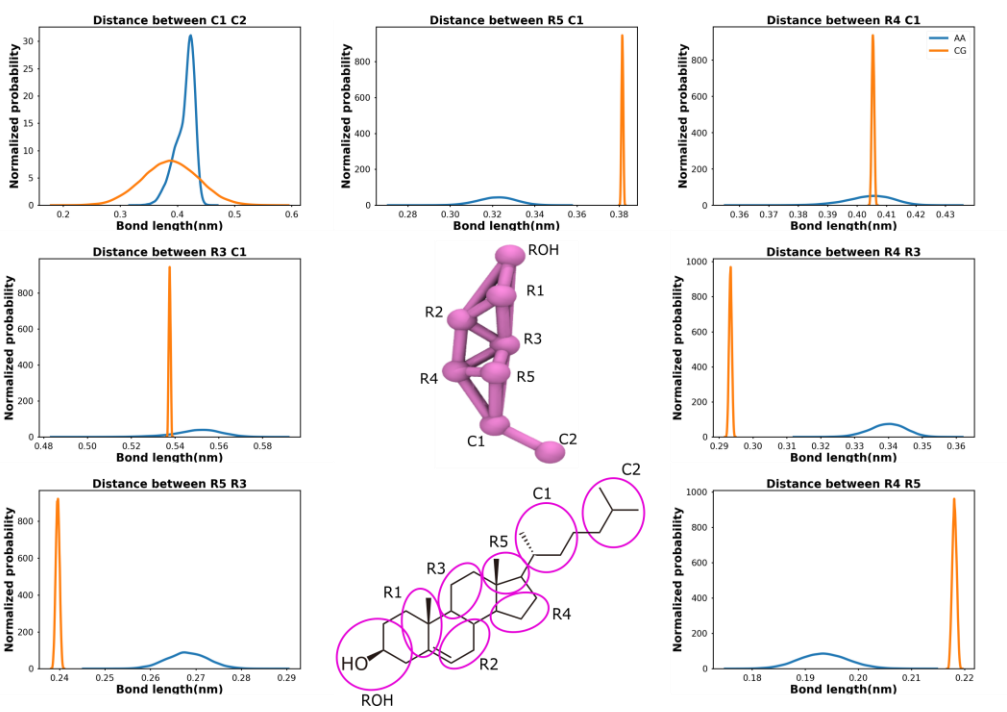

Figure 3. Cholesterol bond distributions. The distances between VS in AA resolution are computed and compared with the CG counterparts. Only the bonds close to the resolution interface are taken into consideration. A CG cholesterol structure and corresponding mapping scheme are shown in the middle.

Shown in Figure 3, for some cases, the CG bonds are longer than the AA (VS) bonds, while for the others, the trend goes opposite. Thus, there is no uniform scaling factor of the attractions between the cholesterol and the other leaflet. To avoid rebuilding either the AA or CG cholesterol model, we next tried an approach in which the C6 of the LJ potential between the cholesterols (R3-R5 beads) and the other leaflet is set to 0, while keeping the C12 unchanged. Thus, the flip-flop and vertical translocation of cholesterol is further suppressed while the interactions neither in AA nor in CG resolution are affected. To validate this modification, we tested the VS hybrid scheme on pure DLiPC, DPPC/CHOL and DLiPC/CHOL membranes at 295K and compared the membrane properties against their corresponding references. As shown in Table 1, the area per lipid, and membrane thickness agree well between AA, CG and VS hybrid models for DLiPC and DPPC/CHOL membranes. In case of the DLiPC/CHOL membrane, the properties of the VS hybrid membrane agree with the AA simulation, however, the difference between AA and CG resolutions is bigger. A similar picture emerges from comparison of the density profiles and the deuterium order parameter (Figure 4). Since the properties of the hybrid membrane agree better with the AA simulation (Table 1), further analysis will focus on the AA components in VS GROMOS/Martini

hybrid model. In addition, we compare the DLiPC and DLiPC/CHOL membrane properties with other fully AA simulations in Table 2. We found that the area per lipid of DLiPC/CHOL membranes simulated with in-house GROMOS force field (applied in our hybrid scheme and described in [200]) agrees with the membrane simulated with other force fields. However, in DLiPC membranes, the difference between the area per lipid of the bilayers simulated with different force fields is significant. This may be caused by the differences in the force fields. Or maybe the simulations of membranes are not fully converged after tens of nanoseconds simulation. As shown in Figure S1, the area per lipid looks converged after 40ns, but the numbers can still deviate from the averaged value after hundreds of nanoseconds. It is reported that, for DPPC membrane, the equilibration of the area per lipid requires up to 25 ns and even 100 ns simulation is not sufficient to sample all the states statistically[180]. This could also be true for DLiPC.

Table 1. Structural properties of different membranes at 295K

Resolution	Lipids	Lipid ratios	Area per lipid (nm^2)	Membrane thickness (nm)
AA	DLiPC	-	0.736±0.008	3.46±0.03
CG	DLiPC	-	0.735±0.010	3.58±0.05
VS hybrid	DLiPC	-	0.717±0.005	3.62±0.05
AA	DPPC/CHOL	61/37	0.430±0.008	4.56±0.02
CG	DPPC/CHOL	61/37	0.421±0.001	4.48±0.02
VS hybrid*	DPPC/CHOL	61/37	0.418±0.003	4.45±0.02
AA	DLiPC/CHOL	41/9	0.590±0.004	3.84±0.02
CG	DLiPC/CHOL	41/9	0.634±0.003	3.61±0.05
VS hybrid*	DLiPC/CHOL	41/9	0.595±0.002	3.87±0.02

* The C6 of the LJ potential between the cholesterols (R1-R5 beads) and the other leaflet is set to zero, while keeping the C12 unchanged.

** All the simulations last for more than 50 ns.

Table 2. Structural properties of fully AA membranes simulated using different force fields

Force field	Lipids	Lipid ratios	Temperature (K)	Area per lipid (nm^2)
In-house GROMOS*	DLiPC	-	295	0.737±0.004
CHARMM 36	DLiPC	-	295	0.691±0.004
In-house GROMOS*	DLiPC	-	303	0.751±0.004
CHARMM36**	DLiPC	-	303	0.707 ± 0.02
GROMOS 43A1-S3***	DLiPC	-	303	0.650±0.005
In-house GROMOS*	DLiPC/CHOL	41/9	295	0.590±0.004
CHARMM 36	DLiPC/CHOL	41/9	295	0.599±0.004
In-house GROMOS*	DLiPC/CHOL	1/1	303	0.454±0.005
GROMOS 43A1-S3**	DLiPC/CHOL	1/1	303	0.443±0.002

* The in-house GROMOS force field is described and tested in [200].

**The membrane properties are obtained from[201]

***The membrane properties are obtained from[202]

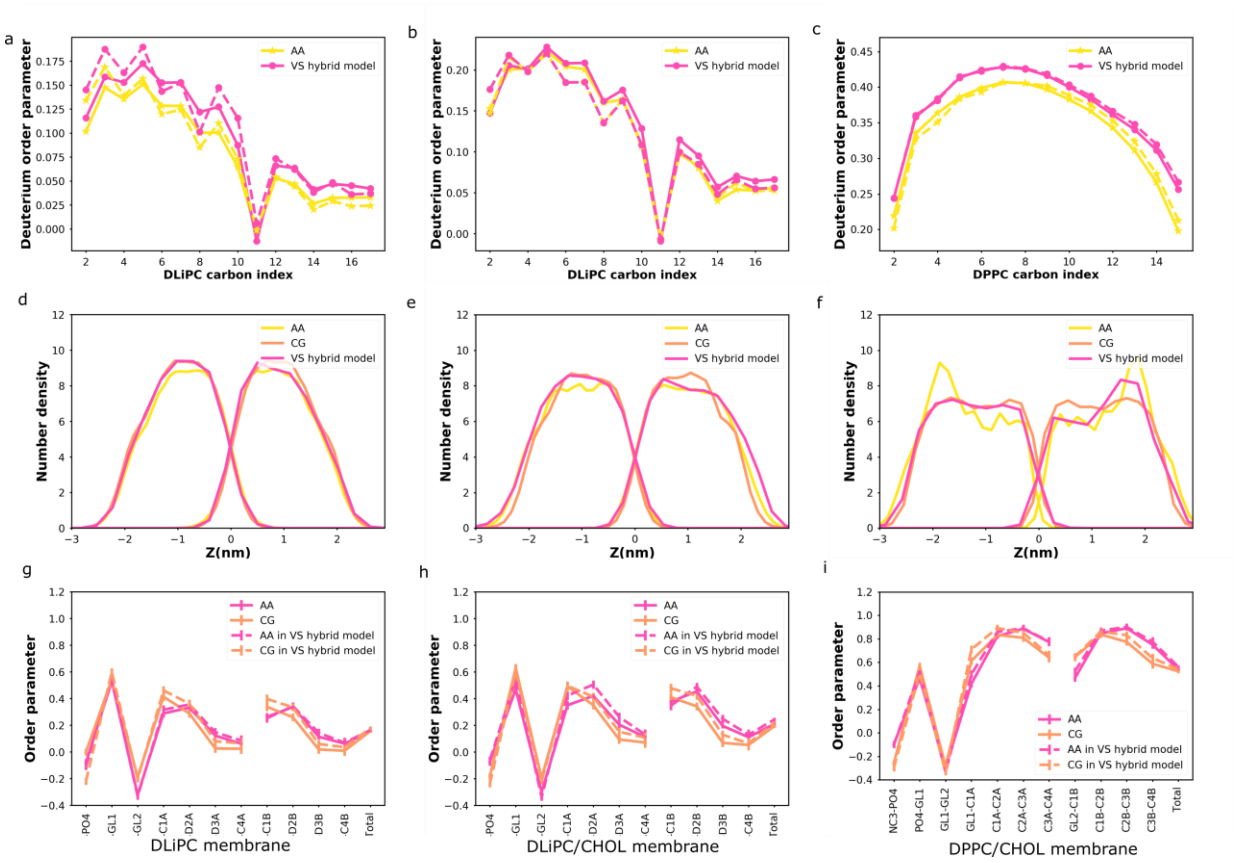

Figure 4. Membrane properties in different resolutions. (a-c) show the deuterium order parameter of pure AA simulation and AA components in VS hybrid simulation. Solid and dashed lines represent two tails of the PC lipids, respectively. (d-f) represent the partial number density of VS and CG beads in membranes. The scaled Martini potential was applied in the VS hybrid model. (g-i) illustrate the CG (VS) order parameter of pure CG and AA simulation, as well as CG and AA components in VS hybrid simulation.

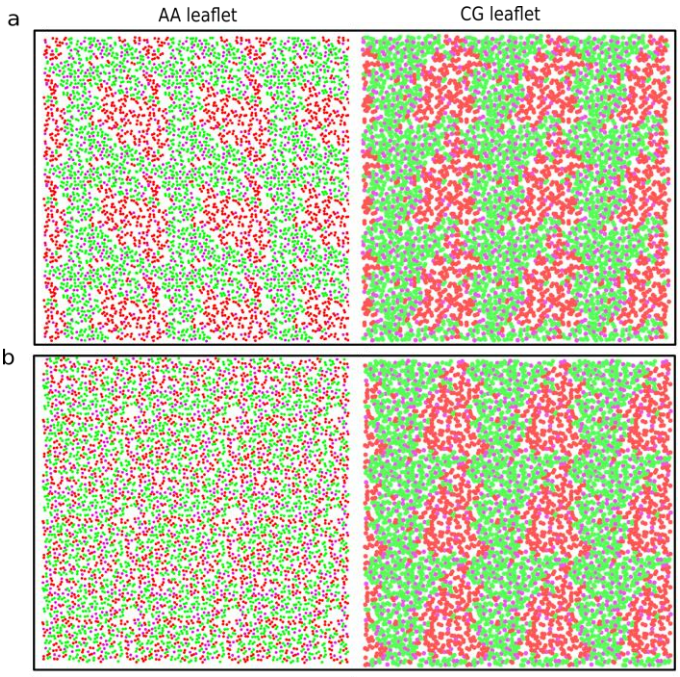

Figure 5. Ternary membrane setup. (a) and (b) represent the starting structures of the two VS hybrid systems: already phase-separated and half phase-separated systems, respectively. The snapshots in the left and right represent the two leaflets in one membrane with different resolutions. DPPC, DLiPC and cholesterol are represented by green, red, and magenta, respectively. Only the linker beads (DPPC and DLiPC) or head group bead (cholesterol) are depicted in the top view. To better illustrate the ordered/disordered regions in the membrane, the center box and 8 periodic images in xy plane are shown in each leaflet. Water is not shown for clarity.

Phase separation in VS hybrid membrane

To test whether our optimized VS hybrid approach can speed up domain formation at the AA level, we first created a suitable starting configuration. To this end, we carried out simulations of a DPPC:DLiPC:cholesterol ternary mixture (molar ratio 42:28:30) at 295K using the Martini CG force field. As described previously[195], at this state point the membrane phase separates to form a DPPC and cholesterol-rich liquid-ordered (Lo) phase, and a DLiPC rich liquid-disordered (Ld) phase. The last frame of the CG simulation is shown in Figure 5a. Using the last frame of this simulation, we built two different starting configurations for the GROMOS/Martini VS hybrid systems. In one of the starting configurations, the AA leaflet is also phase separated (obtained via backmapping of the CG structure, shown in Figure 5a left), and in the other configuration the AA leaflet is homogeneously mixed (Figure 5b left). In both setups, the CG leaflet is phase separated, as shown in Figure 5a right and Figure 5b right. From these starting points, two simulations of about 6 μs each were performed. The flip-flop and vertical translocation of cholesterol is

suppressed, shown in Figure 2c and 2d. To monitor the extent of phase separation, we computed the DPPC-DLiPC contact fraction for each of the two leaflets (both resolutions), as shown in Figure 6. A relatively high contact fraction denotes mixing, whereas low contact fraction hallmarks the phase separated state. As can be seen from the temporal evolution of the contact fraction, in case of the already phase-separated membrane, both leaflets keep the phase separated state. In the half phase-separated membrane, the CG leaflet also keeps the phase separation and the mixed AA leaflet starts to phase separate. We can see from the snapshot in Figure 6 that the phase separation level of AA leaflets in half phase separated membrane is similar to that observed by Hakobyan and Heuer[196], where a membrane with the same lipid composition was simulated at 300K for 9 μs using a united atom force field. We can already sample this level of phase separation after 2 μs, suggesting our method is more efficient. We can also compare the phase separation level with the ternary membrane (composed of DPPC, DOPC and cholesterol with a molar ratio of 7:7:6) simulated using Slipids at 290K by Gu et al.[197]. Here we use a more quantitative measure, namely the enrichment value of DPPC lipids in the DPPC environment as defined in Gu et al.[197]. From our data, we obtain an enrichment value of 1.13 ± 0.02 considering the AA leaflet in half phase separated membrane. This value is close to the value of 1.10 ± 0.02 reported in[197], hence the phase separation level is similar. However, the Slipids simulations are not fully equilibrated after 10 μs[197]. Our simulations have not reached equilibrium after ∼6 μs, either. We therefore need to further extend the simulation to see whether full phase separation will eventually occur. To rigorously prove our hybrid model is faster than a pure AA simulation, we need to compare with a fully AA simulation using the same state condition and simulation settings as our hybrid model in the future.

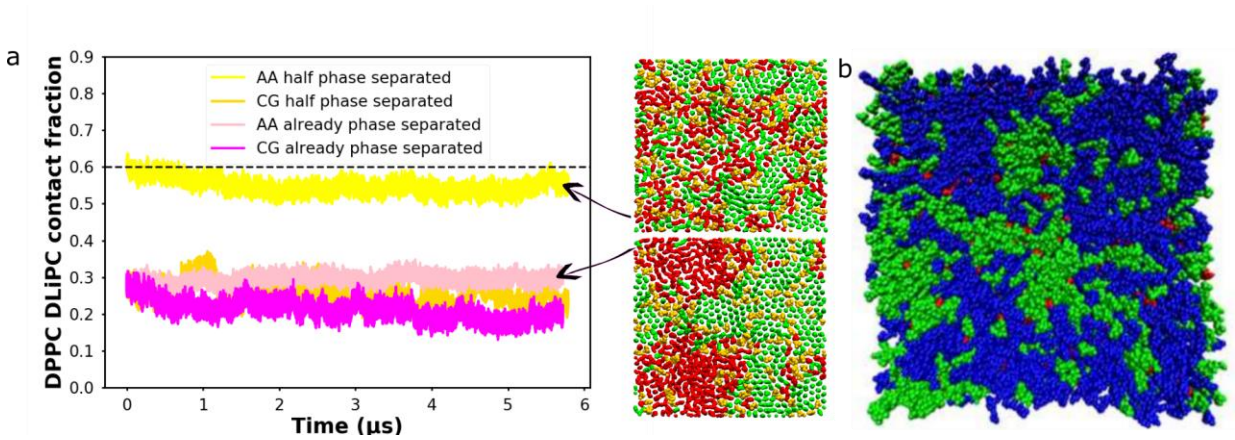

Figure 6. Phase separation of ternary membrane. a) DPPC-DLiPC contact fraction. The thin line and thick lines represent the AA and CG resolutions, respectively. The horizontal dashed line represents ideal mixing, above which DLiPC lipids prefer to contact DPPC lipids (more mixed), below which DLiPC lipids prefer contacts with themselves (more separated). The last frames of AA leaflets in half phase separated (top) and already phase separated (bottom) systems are illustrated on the right. Only lipids tails are shown and the color scheme is the same as Figure 1. b) the last frame of the complete AA ternary membrane simulation by Hakobyan and Heuer after 9 μs. The membrane is composed of DPPC, DLiPC and cholesterol, which are represented by green, blue and red color, respectively. This image is reproduced from Figure 4 in Hakobyan and Heuer[196] . Reprinted (adapted) with permission from [196] Copyright 2013 American Chemical Society.

Supplemental Information

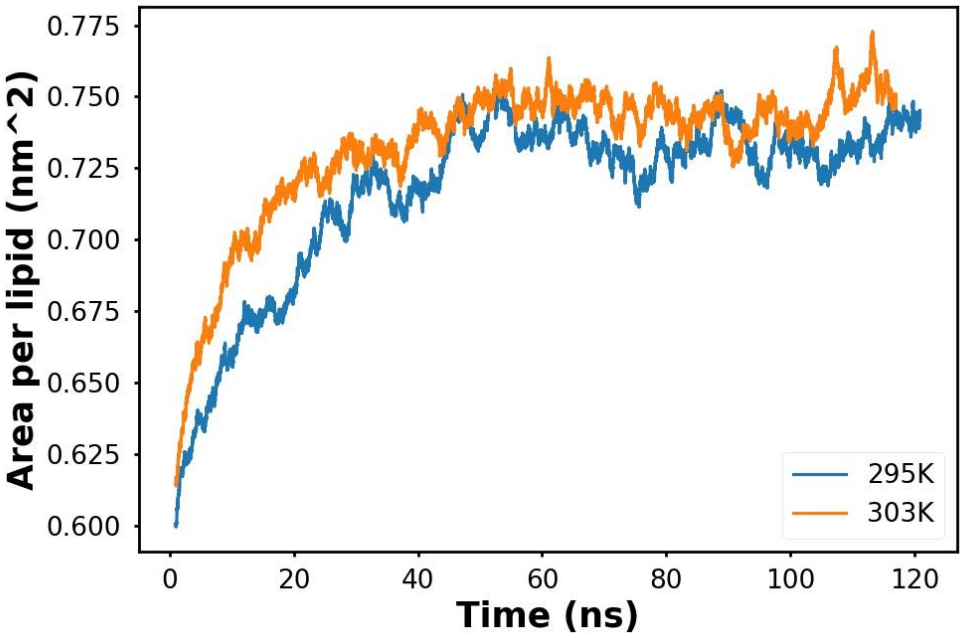

Figure S1 Area per lipid as a function of time. The systems are composed of 200 DLiPC lipids and simulated with the in-house GROMOS force field.