Multiscale Membrane Models

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Chapter 2

Gangliosides Destabilize Lipid Phase Separation in Multicomponent Membranes

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Abstract

Gangliosides (GMs) form an important class of lipids found in the outer leaflet of the plasma membrane. Typically, they co-localize with cholesterol and sphingomyelin in ordered membrane domains. However, detailed understanding of the lateral organization of GM-rich membranes is still lacking. To gain molecular insight, we performed molecular dynamics simulations of GMs in model membranes composed of coexisting liquid-ordered and liquid-disordered domains. We found that GMs indeed have a preference to partition into the ordered domains. At higher concentrations (> 10 mol %), we observed a destabilizing effect of GMs on the phase coexistence. Further simulations with modified GMs show that the structure of the ganglioside head group affects the phase separation, whereas the nature of the tail determines the preferential location. Together, our findings provide a molecular basis to understand the lateral organization of GM-rich membranes.

Introduction

Biological membranes are both compositionally and structurally heterogeneous. The raft concept has introduced the existence of distinct nanodomains[4], which differ in chemical composition and in physical properties, creating an optimized environment for protein function. These nanodomains are presumably small and highly dynamic (sizes in the range from 10 to 200 nm and lifetimes of the order of ~10^{-3} s), and have been implicated to be important for many cellular processes, such as trafficking, signal transduction, and entry of pathogens[4-7]. Ordered nanodomains, extracted from plasma membranes, are enriched in saturated lipids such as sphingomyelin together with cholesterol, but typically also contain other lipids such as gangliosides (GMs).

GMs are anionic glycosphingolipids, commonly found in body fluids, tissues, and are in particular abundant in the nervous system, where they comprise up to 10% of the lipids. GMs are primarily found in the outer leaflet of the plasma membrane[14] and act as receptor or recognition sites for extracellular molecules or surfaces of neighboring cells. GMs consist of an oligosaccharide head group connected to a ceramide body. GM1 and GM3 are two of the most abundant GMs[55-56]. GM1 (monosialo-tetrahexosylganglioside) has a head composed of five sugar monomers (glucose, N-acetylgalactosamine, N-acetylneuraminic acid and two galactose sugars) and GM3 (monosialo-dihexosylganglioside) has a head containing three sugar monomers (galactose, N-acetylneuraminic acid, and glucose). GMs are important biological components and play an active role in a wide range of cellular processes[57-61]. Despite their importance, knowledge about the lateral organization of GMs is still incomplete. Fluorescence microscopy measurements show that GM1 co-localizes in the same domain with cholesterol in epithelial cells [62-63]. Furthermore, GM has significant influence on the formation and stabilization of laterally separated micro-domains, such as cholesterol-independent glycosynapses and cholesterol-dependent rafts or caveolae[64-65]. Crosslinking of GM1 is able to separate uniform membranes into coexistent liquid-ordered and disordered domains in membranes composed of sphingomyelin, cholesterol, and phosphatidylcholine (PC)[66]. Atomic force microscopy experiments show the aggregation of GMs into gel-phase domains in PC monolayers[67-69]. GMs can form aggregates even when the concentration is as low as 1%[70]. The size of GM aggregates depends on the saturation of the acyl chain and the length differences between PC acyl chains and the GM long-chain base[71]. An increase in the acyl chain unsaturation and decrease in its length enhances GM dispersion in the bilayer membrane[72]. The incorporation of Ca^{2+} can also promote GM aggregation inside liquid-ordered membrane domains[73].

Although GM aggregation and domain sorting have been investigated intensively by a large variety of experimental techniques, the influence of GMs on membrane organization and phase separation is not fully understood. A major complicating factor is the variety in GM chemical nature (e.g., type of oligosaccharide head group, tail length and saturation level) on the one hand, and the differences between in-vivo studies (where GMs are asymmetrically distributed, and part of a very complex lipid and protein environment) versus in-vitro studies on model membranes. To provide further insight into the lateral organizational principles of cell membranes, computational modeling offers an alternative approach[74-75]. Like the experiments, MD simulations also reveal a

wide scenario of GM behavior that is not always straightforward to interpret. Already more than a decade ago, atomistic simulations of a single GM1 in a DOPC membrane reveal that the anionic saccharide group of GM1 is able to form charge pairs with the choline part of DOPC lipids[76]. In a follow up study[77], two distinct orientations of the GM1 head group were observed, a protruding orientation in which the head group sticks out of the membrane, and an embedded one in which the head group adsorbs to the interface. In addition, a local condensing effect of GM1 on the DOPC membrane is found, although the DOPC tails become less ordered around the ganglioside. Similar conclusions were reached by simulations of single GM1 lipids in a DPPC membrane[78]. In a more recent atomistic simulation, a tendency toward self-aggregation of GM1 is observed in a GM1/SM/Chol mixed membrane, while GM1 remains more dispersed in a GM1/POPC membrane[79]. In another atomistic MD study, self-clustering of GM1 in POPC membranes was found at higher concentrations (30 mol%), together with the ability of the GM1 to induce positive curvature[65]. In order to study the effect of gangliosides on membrane phase behavior, longer simulations and larger systems are needed which require coarse-grain (CG) models. The parametrization of glycolipids, including GM, has been realized in the framework of the Martini CG force field, showing good agreement with atomistic simulations as well as experimental data[80-81]. Based on CG simulations, it is found that GM1 is able to affect or guide partitioning of proteins, such as WALP and LAT, between liquid-ordered and liquid-disordered domains[82], as well as affect the registration of such domains[83]. In CG simulations of more realistic plasma membrane models, GM1 and GM3 show a tendency to form transient clusters [1, 84], as also seen in CG simulations of model membranes[85-86], and to be a key component of the annular shell of membrane embedded proteins[87]. Asymmetrically incorporated GM1 can induce global curvature, as quantified in an experimental study on giant vesicles and supported by CG simulations[88-89]. Simulations in which tethers are pulled from a realistic membrane, however, show a depletion of gangliosides from the strongly curved regions[90].

Here, we use CG molecular dynamics simulations based on the Martini force field to investigate the effect of GMs on phase coexistence in a model multicomponent membrane. Our membrane setup is symmetric and consists of dipalmitoyl-phosphatidylcholine (DPPC) and dilinoleyl-phosphatidylcholine (DLiPC) lipids together with cholesterol. Such a ternary mixture separates into liquid-ordered and liquid-disordered domains and is believed to be a good model for membrane compartmentalization[29-30]. Various amounts (10%, 20% and 30%) of GM1 as well as

GM3 were incorporated into this ternary system at temperatures ranging from 280K to 325 K. Through analysis of the lipid-lipid contact fractions, membrane thickness, and order parameter, we found that GMs can induce mixing of the ordered and disordered phases. Modified GMs were also investigated to understand the origin of the mixing effect. We found that the polar saccharide head of GM is responsible for mixing the lipids, while the nature of the sphingosine tails determines the preferential lateral location of GM.

Method

System setup

We carried out molecular dynamics (MD) simulations using the Martini coarse-grained force field[91]. This force field has been widely applied to a number of biomolecular processes, in particular involving the lateral organizational principles of membranes[30, 92-93]. We simulated a symmetric lipid bilayer composed of DPPC, DLiPC and cholesterol with a molar ratio of 42:28:30. We incorporated 0%, 10%, 20%, or 30% GM molecules into the membrane. The simulations started either from a randomly mixed membrane, or from an already phase separated membrane to assess the convergence of the lipid mixing. The systems starting from the mixed phase were built with the tool insane[94], while the system starting with separated phase was built by incorporating a grid of GM into a pre-equilibrated membrane that was showing the coexistence of a liquid-ordered (Lo) and a liquid-disordered (Ld) phase (Figure 1). The grid arrangement evenly distributed the GM molecules in the membrane, so that GM can fully interact with both the lipid phases. We used GM1 and GM3 in our simulations as they are the most commonly found GMs in plasma membranes.

We simulated the membrane in aqueous environment and included sodium ions to keep the system electrically neutral. In systems simulated at 280 K, we replaced 10% of the water by antifreeze water to prevent the unwanted freezing of the simulated system. Compared with normal water, antifreeze water has no effect on key membrane properties, including the area per lipid, the transition temperature for formation of the gel phase, and the lateral self-diffusion constant of lipid molecules[91].

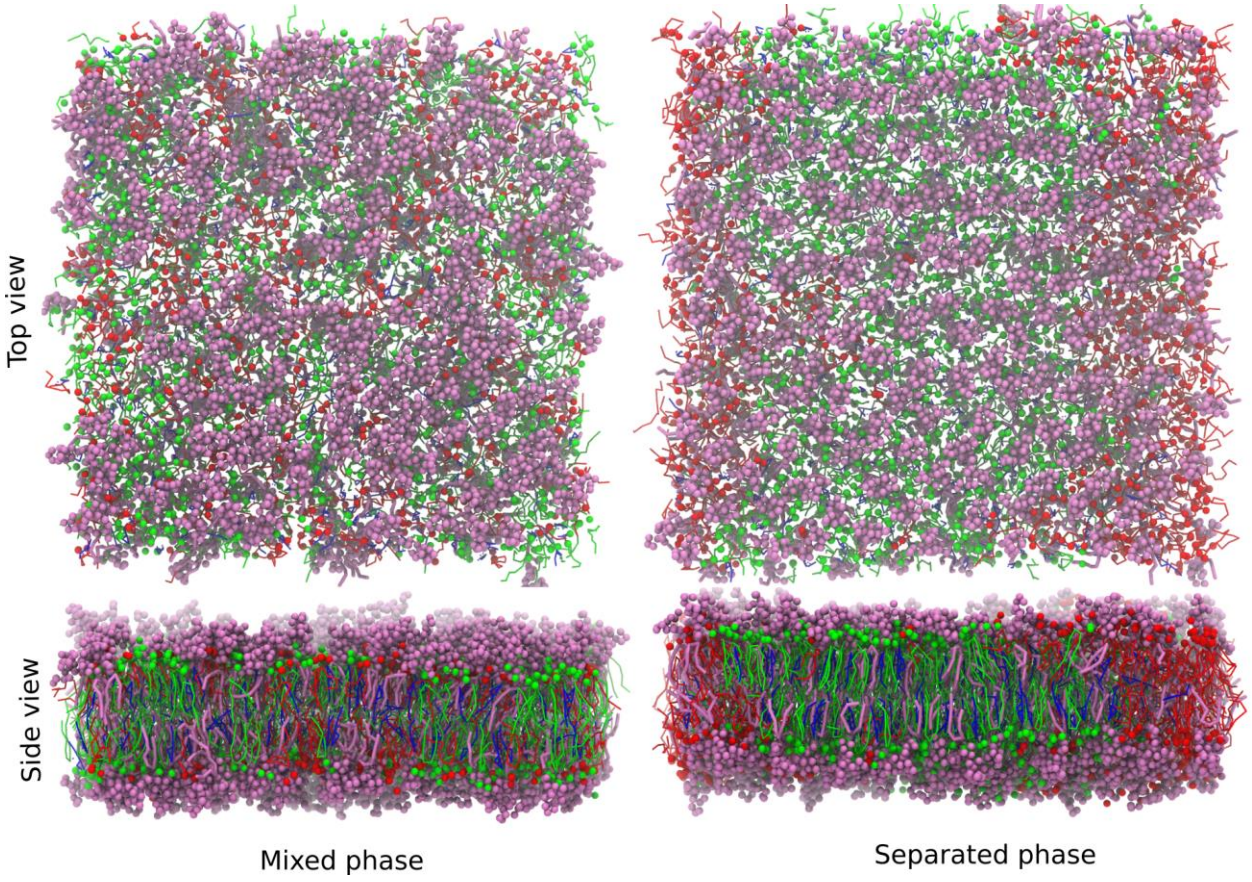

Figure 1. Systems setup. Snapshots of 20% GM1 incorporated membrane starting from mixed (left) and separated (right) phase. DPPC, DLiPC, cholesterol and GM1 are represented by green, red, blue and magenta, respectively. Water is not shown for clarity.

Simulation details

We carried out all the simulations using the GROMACS software suite (v 5.1.2) and the Martini 2.0 force field[91] in the NPT ensemble. For all the systems, we carried out an equilibration phase followed by a production run. While the protocol for the production run was identical for all systems, we used different equilibration protocols for the different starting configurations.

For the PC lipids, we used the refined parameters by Wassenaar et al.[94]. The cholesterol model was taken from Melo et al.[95], and the ganglioside models from Gu et al[81] (based on the original models of López et al.[80]). The Martini lipid types DPG1 and DPG3 were used as representatives for GM1 and GM3 in our simulations. The parameters for the different molecules are available on http://cgmartini.nl.

The Lennard-Jones and Coulomb potentials were shifted to zero at the cut-off 1.1 nm and long range electrostatics was treated using a reaction field as recommended for Martini simulations ("New-RF" settings)[42]. The neighbor list was updated with the Verlet neighbor search algorithm.

During the production simulations, the pressure was coupled with the Parrinello-Rahman algorithm at 1 bar using a semi-isotropic barostat, a compressibility of 3×10^{-4} bar^{-1}, and a time constant of 12 ps. The temperatures of the system were coupled at 280K, 295K, 310K and 335K using the v-rescale thermostat[96] with a time constant of 1 ps. All lipids are coupled in one thermostat group, while the water and ions are coupled in another group.

During the equilibration phase, the Berendsen barostat and thermostat were used. The protocols of equilibration were different for different starting configurations. For membranes starting from a randomly mixed state, we carried out successive equilibration runs with time steps of 2 fs, 10 fs and 15 fs for 4 ns, 20 ns and 30 ns, respectively. When starting from a phase separated membrane, there may be steric clashes between the membrane and the grid of GMs, requiring a more careful equilibration. Therefore, we used time steps increasing from 1 fs to 19 fs with a 2 fs interval. For each time step, a 10 ns simulation was performed with the use of a soft core potential[97]. In the Martini softcore interaction, $\alpha = 4$, $\sigma = 0.3$, $\lambda = 1$ and the r-power is 6.

Analysis method

The extent of phase separation in the membrane was quantified using the contact fraction f_{PC} between DPPC and DLiPC lipids. The contact fraction was computed as proposed in[98], and defined as:

$$f_{PC} = c_{DPPC-DLiPC}/(c_{DPPC-DLiPC} + c_{DLiPC-DLiPC}) \quad (1)$$

where c represents the number of contacts between the two lipid species. A distance threshold of 1.1 nm between the phosphate bead (PO4) of DPPC and DLiPC was applied, as proposed by Domanski[98]. Complete phase separation corresponds to $f_{PC} = 0$, while ideal mixing corresponds to $f_{PC} = 0.6$ (equaling the mole fraction of DPPC lipids with respect to total PC lipids). Values larger than 0.6 indicate an enhanced mixing of DLiPC with DPPC, while values smaller than 0.6 indicate more self-contacts and therefore more non-random mixing. The exact value of f_{PC} for which phase separation occurs is hard to define, given finite size effects due to the limited size of the simulated systems. A value of $f_{PC} = 0.2$ signals the clear phase separation.

Similarly, the GM lateral distribution was quantified with the GM-DLiPC contact fraction f_{GM} , defined as:

$$f_{GM} = c_{GM-DLiPC}/(c_{GM-DPPC} + c_{GM-DLiPC}) \quad (2)$$

where c represents the number of contacts between the two lipid species. The PO4 beads of the PC lipids and the center-of-mass of GM are used as reference to compute the GM-PC contact fraction. A distance threshold of 1.5 nm was used, which is estimated based on the radial distribution function (RDF) between GMs and both PC lipids (Figure S12). When DLiPC and GM are ideally mixed, the GM-DLiPC contact fraction is 0.4 (corresponding to the molar fraction of DLiPC with respect to all PC lipids). Above this value, GM forms more contacts with DLiPC lipids. Below it, GM forms more contacts with DPPC lipids.

We also computed other membrane organizational or structural parameters to quantify effects of GM on membrane. The lipid order parameter P was defined as

$$P = \langle 3\cos^2(\theta) - 1 \rangle /2 \quad (3)$$

where θ is the angle between bond vectors of the lipid CG beads and the bilayer normal (approximated as the Z unit vector of the simulation box), and the angle brackets represent the ensemble average over equivalent bonds for a given lipid type in a simulation frame. We averaged the order parameter over the last 5 μs of each simulation.

The order parameter landscape, partial density and membrane thickness landscape were also applied to measure and visualize the lipid distribution in the membrane. All landscapes were computed through a grid placed on the XY plane of membrane (10 × 10 cells) and the last 0.5 μs of the simulations were used to average the landscape. The order parameter in the order parameter landscape is computed through Equation 3, with the ensemble average done on all the lipid bonds within a grid cell. The density fraction in the density landscape is defined as the fraction of DPPC bead density over the total bead density of PC lipids. Membrane thickness landscape was computed based on the average distance of PO4 beads in both leaflets. All landscapes were calculated using g_thickness, g_ordercg and g_mydensity software[99] freely available from http://perso.ibcp.fr/luca.monticelli.

The membrane thickness of DPPC-rich and DLiPC-rich regions was computed using the FATSLIM software[100]. PO4 beads of the DPPC and DLiPC lipids were used as reference to compute the membrane thickness. We used the thickness per lipids outputted from FATSLIM to obtain a thickness per lipid type. Membrane thickness of DPPC-rich regions is computed based on the thickness of lipids with residue name DPPC and so does the thickness of DLiPC-rich regions is based on DLiPC lipid. Since the fluctuations of membrane may introduce noise when measuring

membrane thickness, the thickness is computed based on neighborhood-averaged coordinates to smooth the fluctuations. The specific explanation and software are freely available on website http://fatslim.github.io/.

Statistical errors are estimated from block averaging as implemented in the g_analyze tool of GROMACS[101], unless stated otherwise.

Results

Gangliosides destabilize phase separation

Using the Martini coarse-grained force field, we carried out MD simulations of a DPPC:DLiPC:cholesterol ternary mixture (molar ratio 42:28:30[29]) at 295K. As described previously[29], 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 phases form stripes that span across the periodic box (Figure 2a).

To observe the effect of gangliosides, we added GM1s to this ternary membrane, at molar concentrations of 10%, 20%, or 30%. We carried out simulations starting from a homogeneously mixed bilayer, i.e. all components are initially randomly distributed in the lateral plane of the membrane (see Methods). The GM1s preferentially partition to the Lo domain, as can be qualitatively assessed from the snapshots of the systems at the end of the simulation (Figure 2a and 2b). To quantify the lateral distribution of GM1s further, we calculated GM1-DLiPC contact fraction f_{GM} as a function of time. as shown in Figure 2c. If GM1 is ideally mixed in the membrane, f_{GM}=0.4 (which is the molar fraction of DLiPC with respect to all PC lipids), a greater value indicates that GM1 interacts more favorably with DLiPC (representative of the Ld domain), while a lower value indicates that GM1 interacts more favorably with the DPPC lipids enriched in the Lo domain. At all concentrations of GM1, the GM1-DLiPC contact fraction is less than 0.4 (Figure 2c). This indicates that GM1 interacts more with DPPC than with DLiPC, and therefore partitions into the Lo phase. However, the more GM1 we add into system, the higher is the GM1-DLiPC contact fraction. This implies that either the Lo phase becomes saturated with GM1s, or that the lipid phase separation itself becomes less pronounced.

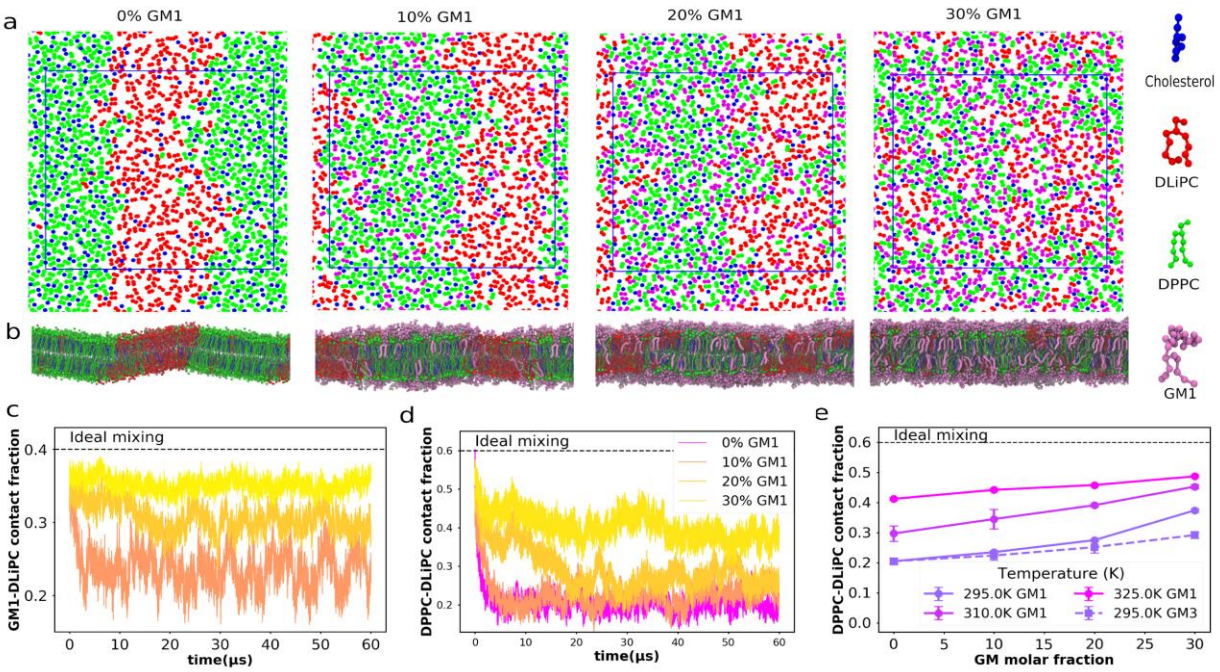

Figure 2. Sorting and domain mixing of GM1. (a) and (b) are the top view of the upper leaflet and side view of the bilayer membrane at 60 μs, respectively. DPPC, DLiPC, cholesterol and GM1 are represented by green, red, blue and magenta, respectively. Only the linker beads (DPPC, DLiPC, GM1) or head group bead (cholesterol) are depicted in the top view. Water is not shown for clarity. (c) Contact fraction between GM1 and DLiPC for system starting from mixed phase at 295K. The horizontal dashed line represents ideal mixing, above which GM1 prefers to interact with DLiPC lipids, below which GM1 prefer DPPC lipids. (d) is the DPPC-DLiPC contact fraction for system starting from mixed phase at 295K. 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). (c) and (d) share the same legend. (e) The DPPC-DLiPC contact fractions were sampled and averaged from the last 15 μs after the systems reach equilibrium. The DPPC-DLiPC contact fraction of GM1 and GM3 are shown with solid and dashed lines, respectively.

From visual inspection of the snapshots (Figure 2a and 2b), as well as the density maps (Figure S1 and S2), it appears that the phase separation is not lost, but clearly becomes destabilized upon addition of GM1s. We quantified the phase separation by computing the DPPC-DLiPC contact fraction f_{PC} , as shown in Figure 2d. The larger the value of DPPC-DLiPC contact fraction, the more mixed are the lipids, and therefore the weaker the phase separation. Complete phase separation corresponds to f_{PC} tending to 0; while ideal mixing corresponds to $f_{PC} = 0.6$ (being the molar fraction of DPPC lipids with respect to all PC lipids). In the absence of GM1, $f_{PC} = 0.2 \pm 0.02$, indicative of strong phase separation. Increasing the GM1 ratio increases the DPPC-DLiPC contact fraction until $f_{PC} = 0.39 \pm 0.025$ at 30% GM1. At high concentrations, GM1 is thus able to destabilize the phase separation in this system.

The membrane composition investigated shows a strong phase separation at 295K without GM. Such strong cohesion of the lipid phases may hide an effect of the GM that would be visible with a weaker, and probably biologically more relevant, phase separation. To explore this idea, we carried out simulations over a range of temperatures from 280K to 325K. As before, we computed the DPPC-DLiPC contact fraction for GM1 molar ratios from 0% to 30%. The results are shown in Figure 2e and Figure S3 and S4. As expected, in the absence of GM1, the DPPC-DLiPC contact fraction increases with the temperature indicating that the phase separation is less stable as the temperature increases. The DPPC-DLiPC contact fraction also increases with the ratio of GM1. Therefore, GM1 destabilizes the lipid phase separation regardless of how strong it initially is. This destabilization, however, appears stronger at lower temperatures where the phase separation initially is better defined.

To further investigate the destabilizing effect of gangliosides, we also studied the influence of GM3 on membrane phase separation. GM3 has a smaller oligosaccharide head group compared to GM1, lacking two sugar rings. We incorporated 10% to 30% GM3 into the same ternary membrane as we did for GM1 and got qualitatively similar results. The GM3-DLiPC contact fraction and DPPC-DLiPC contact fraction for GM3 incorporated systems are shown in Figure S5. In line with the results for GM1, the GM3-DLiPC contact fraction is below that of the ideal mixing line, indicating GM3 interacts more favorably with DPPC lipids and hence partitions into the Lo region. The addition of GM3 enhances the DPPC-DLiPC contact fraction and the contact fraction increases with GM3 ratio (Figure 2e). This indicates that GM3 also shows the ganglioside induced mixing effect, becoming stronger at higher GM3 ratio. Compared to GM1, however, the mixing effect of GM3 is weaker, since DPPC-DLiPC contact fraction of GM3 at a given ganglioside ratio is lower than that of GM1, as clear from Figure 2e.

Together, our results show that both GM1 and GM3 have a preference to reside in the Lo domain rather than the Ld domain, but can induce mixing of these domains at increasing concentrations. To assess the robustness of our predictions, three replica simulations were performed for GM1 incorporated systems at 295K (Figure S6), leading to quantitatively similar results. Furthermore, we verified that the starting configuration, a uniformly mixed membrane, did not result in any kinetic trap which would prevent reaching the thermodynamic equilibrium state of the system. To this end, we carried out additional simulations starting from a homogeneous grid of GMs covering an already phase separated membrane (see Methods), covering a temperature

range from 280 to 325K. At 295K, the DPPC-DLiPC and GM1-DLiPC contact fractions for the two initial configurations converge to the same value in less than 60μs at most GM1 concentrations (Figure S3 and S4). At higher temperatures (>295K), the contact fractions converge faster, in less than 20μs. Only in case of 280K at 30% GM1, convergence of f_{PC} is not reached within the 30μs sampled. Note that in case of GM3s, systems equilibrate faster (<10μs) which we attribute to their smaller head groups allowing faster diffusion compared to GM1. Overall, we conclude that, the results presented on membrane phase behavior and the lateral distribution of the gangliosides are not affected by the choice of starting structure, at least at temperatures above 280K.

Oligosaccharide ganglioside head group plays crucial role

A GM lipid can be divided into an oligosaccharide head group and a ceramide part. To test their respective influence on GM sorting and lipid mixing, we built modified lipids based on GM1, and we compared their behavior with the behavior of the original glycolipid. We carried out simulations at 295 K using these modified lipids with a glycolipid molar ratio of 20%.

To investigate the influence of the size of the oligosaccharide head, we replaced the oligosaccharide head by a linear sequence of 1 to 4 sugar rings (Figure 3, index 0 to 3). With values of GM1-DLiPC contact fraction between 0.3 and 0.35, the modified glycolipids distribute in the Lo phase in the same manner as the unmodified GM1 (index 7). They also have a similar effect on lipid mixing with values of f_{PC} ranging from 0.31 to 0.36 for 1 to 4 rings, comparable to $f_{PC} = 0.28$ of unmodified GM1 and significantly higher than $f_{PC} = 0.2$ for the membrane without GM1. We also removed completely the oligosaccharide head and replaced it by a PC head group, like the surrounding lipids (Figure 3, index 4). With a GM-DLiPC contact fraction of 0.16, this modified lipid distributes even more strongly in the Lo phase than GM1. Interestingly, the lipid mixing is not affected as shown by a DPPC-DLiPC contact fraction of 0.2, the same value as the membrane without GM. Therefore, it appears that the oligosaccharide head is needed for the solute to destabilize the lipid phase separation. Even a single sugar ring (index 0) already causes some lipid mixing.

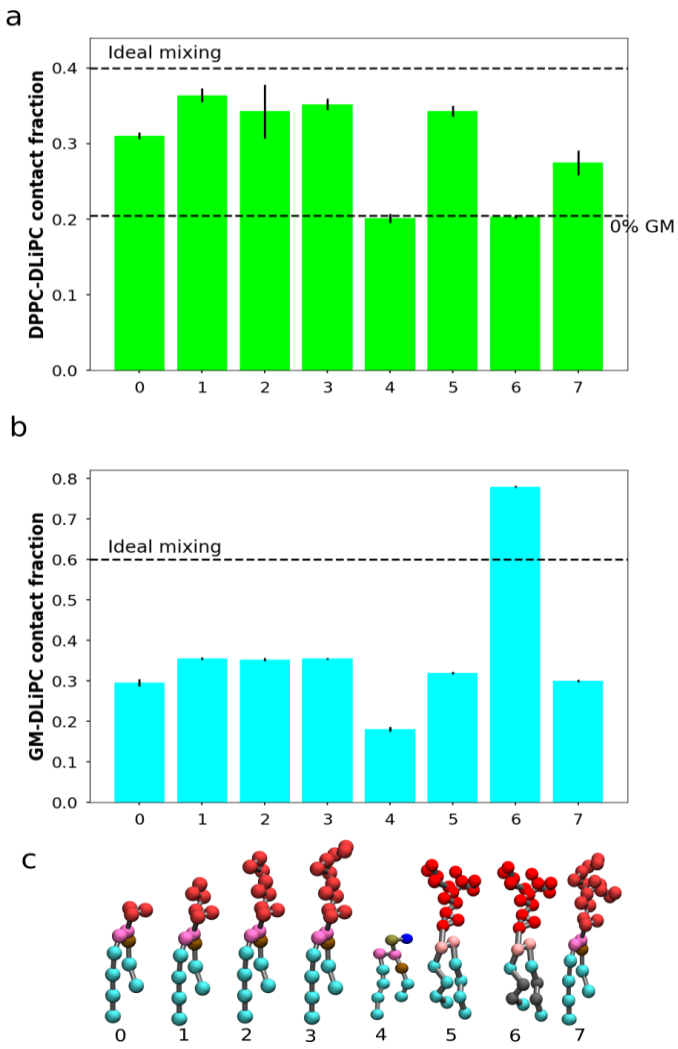

Figure 3. Influence of modified GM on membrane phase separation. Results obtained for 20% GM with different head groups or tails, at 295K. (a) and (b) are the contact fractions sampled and averaged for the last 10 μs. (c) Indices of modified GM1: one sugar GM1 (0), two sugar GM1 (1), three sugar GM1 (2), four sugar GM1 (3), PC head-GM1 tail (or sphingomyelin) (4), GM1 head-DPPC tail (5), GM1 head-DLiPC tail (6) and original GM1 (7). The sugar head of GM1 is composed of Glucose; head of two sugar GM1 composed of Glucose and Galactose; head of three sugar GM1 composed of Glucose, Galactose and N-acetylgalactosamine; head of four sugar GM1 composed of Glucose, N-acetylgalactosamine and two Galactose rings.

To investigate the influence of the ceramide part of GM1 on lipid mixing, we replaced it by a saturated di-palmitoyl pair of tails copied from DPPC (Figure 3, index 5), as well as by a poly-unsaturated di-linoleyl pair copied from DLiPC (Figure 3, index 6). In both cases, we kept the oligosaccharide head group of GM1. With a GM-DLiPC contact fraction of 0.32, the saturated glycolipid partitions in the Lo phase; instead, the poly-unsaturated glycolipid partitions in the Ld phase. The construct with GM1 head-DPPC tails clearly induce a mixing effect, while the GM1

head-DLiPC tail lipids have no such effect, as evidenced by DLiPC-DPPC contact fractions of 0.34 and 0.20 for the saturated and the poly-unsaturated lipid, respectively. This lack of effect of the modified unsaturated versions of the GM1 indicates that the oligisaccharide head group only has a destabilizing effect on the phase coexistence when they can partition into the Lo region.

The effect of tail unsaturation on the preferred localization of GM1 is easily understood, given the nature of the ordered and disordered domains enriched in saturated and unsaturated lipids, respectively. The underlying reason for the effect of the oligosaccharide head group, however, is less obvious. As shown in Figure S7, the GM1 head group is located at the membrane/water interface. In particular, the glucose and galactose moieties attached to the sphingosine backbone are deeply embedded, and are expected to exhibit a strong perturbing effect on the neighboring lipids. To quantify this, we analyzed the influence of GM on membrane conformational structure. We computed the average membrane thickness and order parameter of the PC lipids for the GM1 containing system at 295K, after the systems reached equilibrium. The results are shown in Figure 4 for different GM1 concentrations (2D thickness and order parameter landscapes are shown in Figure S8-11). With more GM1 added into the membrane, the order parameter of hydrophobic tail and membrane thickness of DPPC decrease. However, compared to effects on DPPC lipids, the GM1 effects on DLiPC lipids is weaker. To explore this in a more clear setup, we also investigated the effect of GM1 on thickness and order parameter of pure ordered (lipid ratio of DPPC, DLiPC and cholesterol is 61:1:37) and disordered phase mimicking membranes (lipid ratio of DPPC, DLiPC and cholesterol is 8:75:17). The results, shown in Table S1, point to a similar effect: upon addition of GM1 into the pure ordered phase system, the order parameter and membrane thickness of DPPC lipids decrease. Likewise, the GM1 effect on DLiPC lipids is limited in the pure disordered phase. At higher temperature, when the phase separation is less stable, the influence of GM1 on membrane thickness and order parameter becomes weaker (Table S1), but the trend of a decreasing difference between the conformations of DPPC and DLiPC lipids upon addition of GM1 remains. This trend is also observed in systems with an modified GM1 containing two sugar rings (Table S1), as well as for the GM3 system (Table S2). From this data we conclude that gangliosides cause a decrease in the difference between the conformational organization of DPPC and DLiPC lipids in ordered and disordered domains, respectively, as measured by the order parameter and membrane thickness (see also the 2D

thickness and order parameter analysis in Figure S8-11). It seems plausible that a closer structural resemblance between the Lo and Ld domains results in a larger degree of mixing between them.

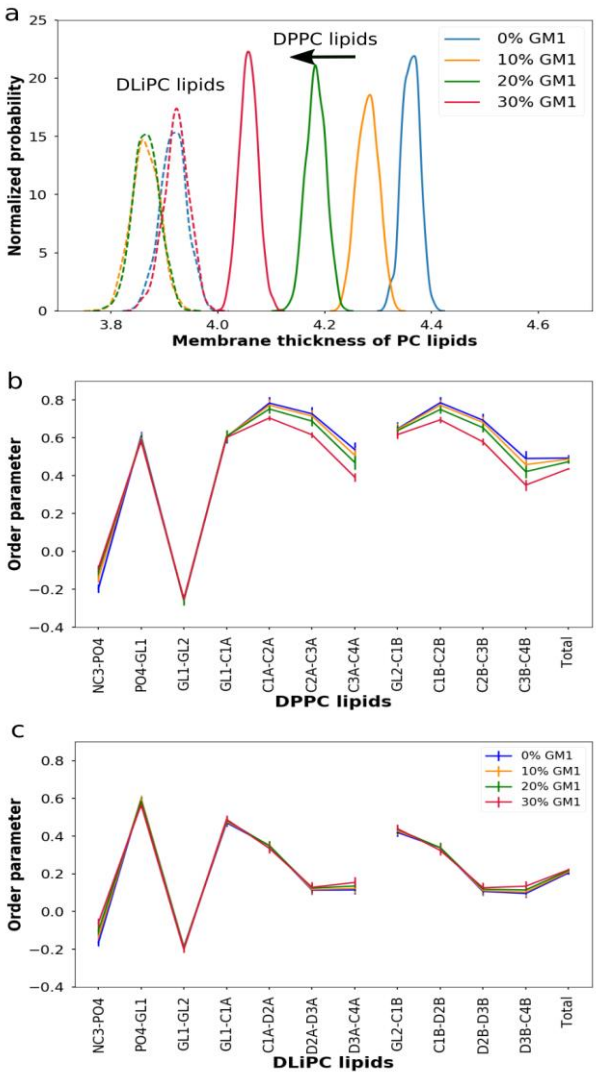

Figure 4. GM1 effect on order parameter and membrane thickness of PC lipids. (a) membrane thickness of DPPC-rich and DLiPC-rich regions are represented by solid and dashed lines. The arrow in the plot indicates the increasing of GM1 concentration for membrane thickness of DPPC-rich regions. Order parameter and membrane thickness were sampled from 55 μs to 60 μs of simulations at 295K in hybrid membrane system starting from mixed phase.

Discussion

This study aimed to determine whether and how GMs, like GM1 and GM3, have an effect on the lateral membrane organization. We found that GM has a mixing effect on co-existing liquid-ordered and disordered domains in model membranes, the mixing effect becoming stronger with more GM added into the system.

Experimental support for our findings comes from a number of atomic force microscopy (AFM) studies. For instance, it is found that the area of the ordered region decreases with the increase of GM1 concentration in SM/DOPC/GM1/cholesterol bilayers[102]. Another AFM study also proposed that GM1 can mix ordered and disordered phases at certain cholesterol concentration in SM/DOPC/GM1/cholesterol bilayers[103]. Based on our simulations, the mixing effect of GM can be attributed mainly to its disordering effect on the Lo domain. Together with a slight increase in order of the Ld domain, the domains resemble each other more closely and hence their coexistence is destabilized. Studies on the effect of GMs on membrane properties are controversial. GM is commonly believed to condense the lipid molecular area and increase the deuterium order parameter of the hydrocarbon chains[104-106]. However some studies propose otherwise. The disordering effect of bovine brain gangliosides on PC-12 cell membrane (which were commonly cultured in chemically defined SATO medium on a collagen-coated substratum) is found by a fluorescence microscopy study[107]. In an atomistic MD simulation study, a decrease in the order parameters of the DPPC hydrocarbon chains upon addition of GM1 was observed[78]. In our simulation, GM1 decreases the order parameter and membrane thickness of DPPC lipids both in the co-existing and pure ordered phase membranes (Table S1 and S3). The disordering effect is probably caused by the larger head group of GM1, containing five sugar rings partially embedded in the PC head region (Figure S7). Whereas the effective shape of a DPPC lipid is a cylinder inside a bilayer membrane, GM1 more closely resembles an inverted-cone shape. Therefore, GM is able to disturb the tight DPPC/cholesterol packing inside Lo region. The effect of GM1 on DLiPC lipids is more limited, likely because DLiPC lipids are more flexible to accommodate the large headgroup of the inverted-cone shaped GM1.

There are other factors that may also cause the mixing effect. As we showed (Figure S7), the first two sugar rings in the head of GM1 (i.e., those connected to the sphingosine backbone) are deeply embedded in the interfacial region of the membrane, in fact very similar to the location of disaccharides as observed in MD simulations[108]. Like the GMs, disaccharides were found to induce mixing of domains in the same MD study, a finding supported by experimental measurements[108]. Thus, the proposed mixing mechanism of disaccharides can also be applied here: more surface defects (shallow defects exposing the lipid glycerol moieties) are formed in a mixed membrane compared to a separated one. Hence, the bigger surface defect space can more

easily accommodate disaccharides, and likewise, the oligosaccharide GM1 head group. In this scenario, formation of defects suitable for sugar rings drives the domain mixing.

Finally, let us discuss some of the limitations of our study. The use of a CG model, necessary to reach the long time scales required for domain formation and destabilization, brings its own limitations as previously discussed in detail[38]. The reduced resolution of the lipids, and in particular of GM1 and GM3, is an obvious simplification that could affect the validity of our results. However, we recently reparameterized the CG model of gangliosides in an elaborate study involving close comparison to the behavior of gangliosides in all-atom simulations[81]. The overall behavior (e.g., orientation and embedding of the GM head, affinity to cluster) is well reproduced by the improved Martini model that is also used here. To make sure the effects observed here are not dependent on the details of the parameterization, we repeated some of the simulations (Table S4) with the previous GM model of Lopez et al.[109]. The results, in particular the ability of GMs to access the Lo phase and cause its destabilization, remain unaffected by the details of the model (Figure S13). Another limitation which is of particular relevance for the current study, is the strong phase separation between the Lo and Ld domains in the DPPC/DLiPC/Cholesterol mixture. A more realistic ternary mixture composed of mono-unsaturated lipids such as DOPC does not show phase separation with the Martini model unless the interaction parameters are specifically tuned[110]. Here, we tried to mimic a more realistic degree of phase segregation by enhancing the temperature. Indeed more mixing is observed, but the effect of GM was still noticeable. In future studies, it would be interesting to see how GMs affect the lateral organization in more realistic membranes, such as quaternary mixtures involving hybrid lipids (i.e., lipids with one saturated and one unsaturated tail)[111], and the recent multi-component and asymmetric plasma membrane models[1, 84].

Conclusion

To summarize, based on CG simulations of membranes composed of DPPC, DLiPC, GM and cholesterol, we found that GMs preferentially partition into the ordered phase. In doing so, through a reduction of the membrane thickness and order parameter difference between the ordered and disordered domains, GMs compromise the phase separation at concentrations exceeding 10 mol%. The head part of GM is responsible for the mixing effect, whereas the tail part determines the preferential location of GM. We expect our results to shed light on the mechanism and driving

forces of membrane phase behavior and domain perturbation in the presence of gangliosides, aiding the interpretation of often controversial experimental studies in this area.

Supplemental Information

TABLE S1. Effect of GM1 on membrane organization (starting from mixed phase)

Membrane	Tested lipids	Temperature (K)	Ratio of GM (%)	Membrane thickness (nm)	Statistical error	Order parameter	Statistical error
Hybrid system	DPPC	295	0	4.35	< 0.01	0.497	< 0.001
Hybrid system	DPPC	295	10	4.29	< 0.01	0.481	0.002
Hybrid system	DPPC	295	20	4.18	< 0.01	0.473	0.002
Hybrid system	DPPC	295	30	4.05	< 0.01	0.442	< 0.001
Hybrid system	DPPC	310	0	4.24	0.01	0.434	0.007
Hybrid system	DPPC	310	10	4.14	< 0.01	0.423	0.002
Hybrid system	DPPC	310	20	4.06	0.01	0.414	< 0.001
Hybrid system	DPPC	310	30	3.97	< 0.01	0.399	< 0.001
Hybrid system	DPPC	325	0	4.09	0.01	0.382	0.002
Hybrid system	DPPC	325	10	4.04	< 0.01	0.378	0.002
Hybrid system	DPPC	325	20	3.99	< 0.01	0.377	< 0.001
Hybrid system	DPPC	325	30	3.92	< 0.01	0.370	< 0.001
Hybrid system	DLiPC	295	0	3.91	< 0.01	0.205	< 0.001
Hybrid system	DLiPC	295	10	3.85	< 0.01	0.212	< 0.001
Hybrid system	DLiPC	295	20	3.87	0.02	0.215	0.002
Hybrid system	DLiPC	295	30	3.94	< 0.01	0.221	< 0.001
Hybrid system	DLiPC	310	0	3.92	0.02	0.197	< 0.001
Hybrid system	DLiPC	310	10	3.93	< 0.01	0.203	< 0.001
Hybrid system	DLiPC	310	20	3.92	< 0.01	0.207	< 0.001
Hybrid system	DLiPC	310	30	3.91	< 0.01	0.209	< 0.001
Hybrid system	DLiPC	325	0	3.94	< 0.01	0.189	< 0.001
Hybrid system	DLiPC	325	10	3.92	< 0.01	0.194	< 0.001
Hybrid system	DLiPC	325	20	3.90	< 0.01	0.196	< 0.001
Hybrid system	DLiPC	325	30	3.87	< 0.01	0.197	< 0.001
Ordered phase	DPPC	295	0	4.29	< 0.01	0.443	0.002
Ordered phase	DPPC	295	20	4.21	< 0.01	0.437	< 0.001
Disordered phase	DLiPC	295	0	3.71	< 0.01	0.200	< 0.001
Disordered phase	DLiPC	295	20	3.73	< 0.01	0.208	< 0.001
Ordered phase**	DPPC	295	10	4.24	< 0.01	0.439	0.002
Ordered phase**	DPPC	295	20	4.20	< 0.01	0.427	< 0.001
Ordered phase**	DPPC	295	30	4.15	< 0.01	0.420	< 0.001
Disordered phase**	DLiPC	295	30	3.76	< 0.01	0.264	0.002

*Hybrid system represents the membrane composed of coexisting ordered and disordered phase. The lipids

ratio of ordered phase is DPPC:DLiPC:cholesterol= 61:1:37; the lipids ratio of disordered phase is DPPC:DLiPC:cholesterol=8:75:17. All systems starting from mixed phase and data are averaged from the last 5 μs. **Ordered phase with two sugar ring GM1 incorporation.

TABLE S2. Effect of GM3 on membrane organization (starting from mixed phase)

Membrane	lipids	Temperature (K)	Ratio of GM (%)	Membrane thickness (nm)	Statistical error	Order parameter	Statistical error
Hybrid system	DPPC	295	10	4.27	0.04	0.489	0.002
Hybrid system	DPPC	295	20	4.17	0.03	0.480	< 0.001
Hybrid system	DPPC	295	30	4.09	0.01	0.466	0.002
Hybrid system	DLiPC	295	10	3.88	0.03	0.209	< 0.001
Hybrid system	DLiPC	295	20	3.90	0.06	0.213	0.001
Hybrid system	DLiPC	295	30	3.91	0.08	0.217	< 0.001

*Hybrid system represents the membrane composed of coexisting ordered and disordered phase. All systems starting from mixed phase and data are averaged from the last 5 μs.

TABLE S3. Effect of GM1 on membrane organization (starting from separated phase)

Membrane	lipids	Temperature(K)	Ratio of GM (%)	Membrane thickness (nm)	Statistical error	Order parameter	Statistical error
Hybrid system	DPPC	295	10	4.33	< 0.01	0.490	0.008
Hybrid system	DPPC	295	20	4.23	< 0.01	0.477	0.001
Hybrid system	DPPC	295	30	3.98	< 0.01	0.439	0.004
Hybrid system	DPPC	310	10	4.11	< 0.01	0.421	0.007
Hybrid system	DPPC	310	20	4.08	< 0.01	0.412	0.005
Hybrid system	DPPC	310	30	3.94	< 0.01	0.400	0.002
Hybrid system	DPPC	325	10	4.03	< 0.01	0.380	< 0.001
Hybrid system	DPPC	325	20	4.01	< 0.01	0.378	< 0.001
Hybrid system	DPPC	325	30	3.89	< 0.01	0.372	< 0.001
Hybrid system	DLiPC	295	10	3.94	< 0.01	0.209	< 0.001
Hybrid system	DLiPC	295	20	4.00	< 0.01	0.215	< 0.001
Hybrid system	DLiPC	295	30	4.03	< 0.01	0.219	< 0.001
Hybrid system	DLiPC	310	10	3.97	0.01	0.204	< 0.001
Hybrid system	DLiPC	310	20	3.99	0.001	0.208	< 0.001
Hybrid system	DLiPC	310	30	3.96	0.002	0.209	< 0.001
Hybrid system	DLiPC	325	10	3.94	< 0.01	0.193	< 0.001
Hybrid system	DLiPC	325	20	3.94	< 0.01	0.195	< 0.001
Hybrid system	DLiPC	325	30	3.91	< 0.01	0.197	< 0.001

*Hybrid system represents the membrane composed of coexisting order and disordered phase. All systems starting from separated phase and data are averaged from the last 5 μs.

TABLE S4. Effect of Lopez GM1 on membrane organization (starting from mixed phase)

Membrane	Lipids	Temperature (K)	Ratio of GM (%)	Membrane thickness (nm)	Statistical error	Order parameter	Statistical error
Hybrid system	DPPC	295	0	4.35	< 0.01	0.497	< 0.001
Hybrid system	DPPC	295	10	4.20	< 0.01	0.471	0.002
Hybrid system	DPPC	295	20	3.97	< 0.01	0.443	0.002
Hybrid system	DPPC	295	30	3.78	0.01	0.412	< 0.001
Hybrid system	DLiPC	295	0	3.92	<0.01	0.205	< 0.001
Hybrid system	DLiPC	295	10	3.81	< 0.01	0.203	< 0.001
Hybrid system	DLiPC	295	20	3.86	0.01	0.209	0.002
Hybrid system	DLiPC	295	30	3.73	< 0.01	0.199	0.002

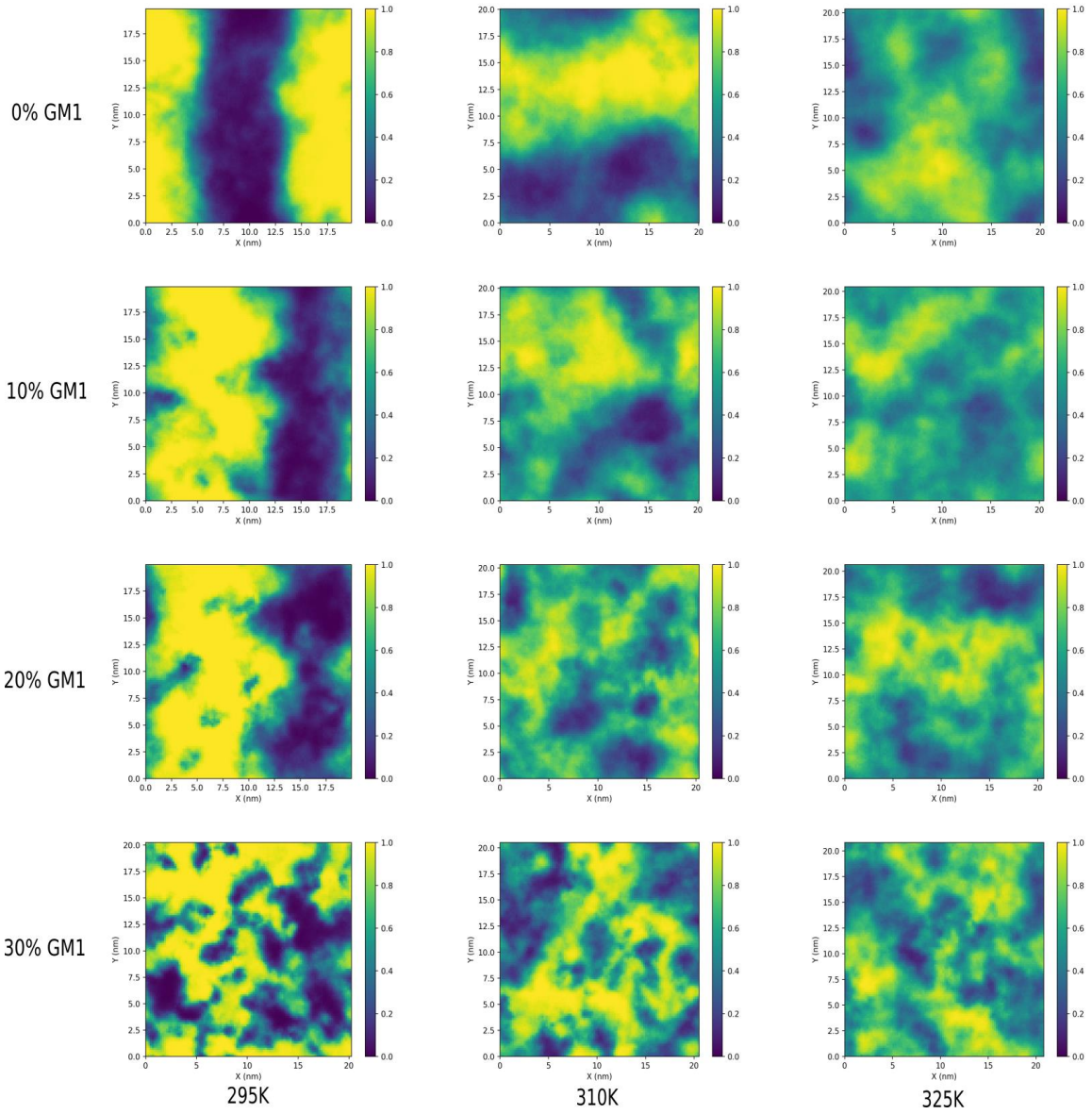

Figure S1. DPPC density landscape for system starting from mixed phase. Localization of DPPC lipids in upper leaflet are expressed as relative density fraction, defined as the fraction of DPPC density over the total density of PC lipids. Only tail and linker part of PC lipids are taken into consideration. Density landscapes are sampled from 59.5 μs to 60 μs of simulation at 295K; from 29.5 μs to 30 μs of simulation at 310K and 325K.

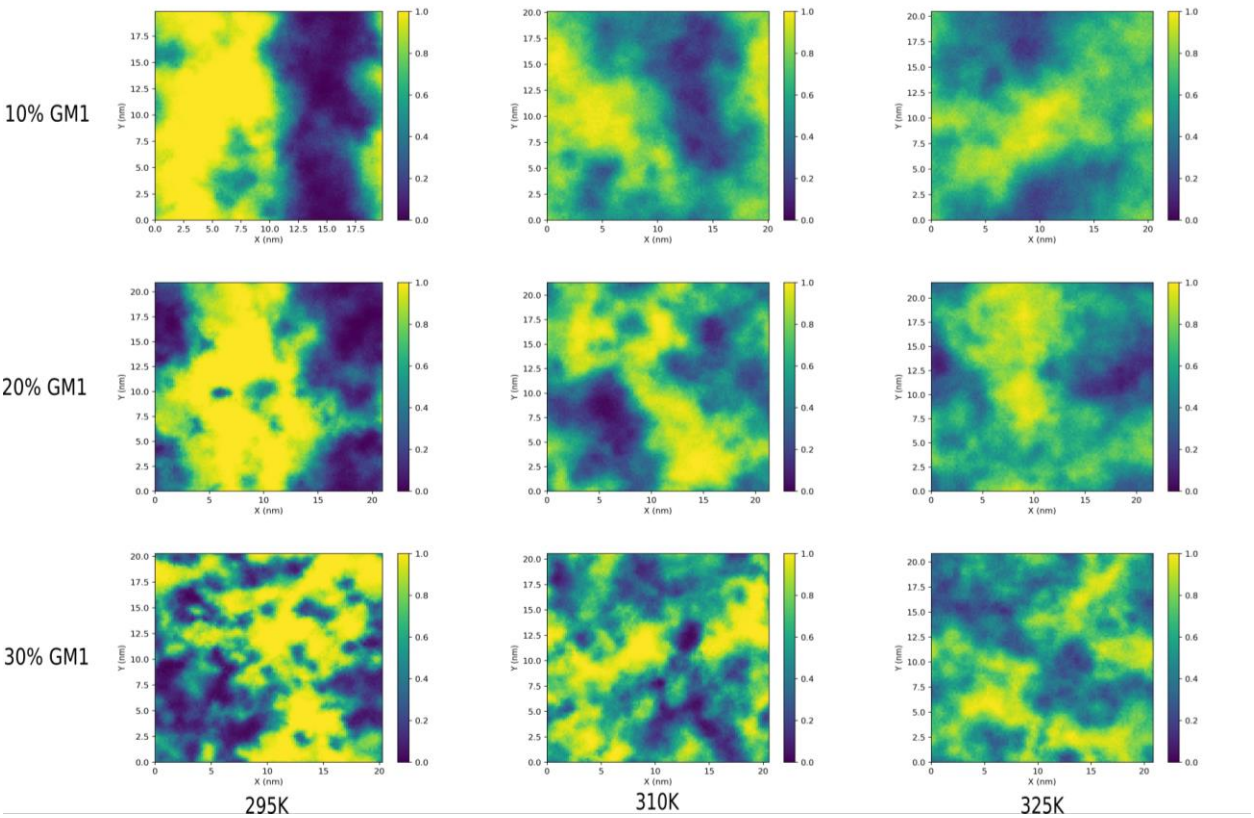

Figure S2. DPPC density landscape for system starting from separated phase. Localization of DPPC lipids in upper leaflet are expressed as relative density fraction, defined as the fraction of DPPC density over the total density of PC lipids. Only tail and linker part of PC lipids are taken into consideration. Density landscapes are sampled from 59.5 μs to 60 μs of simulation at 295K; from 29.5 μs to 30 μs of simulation at 310K and 325K.

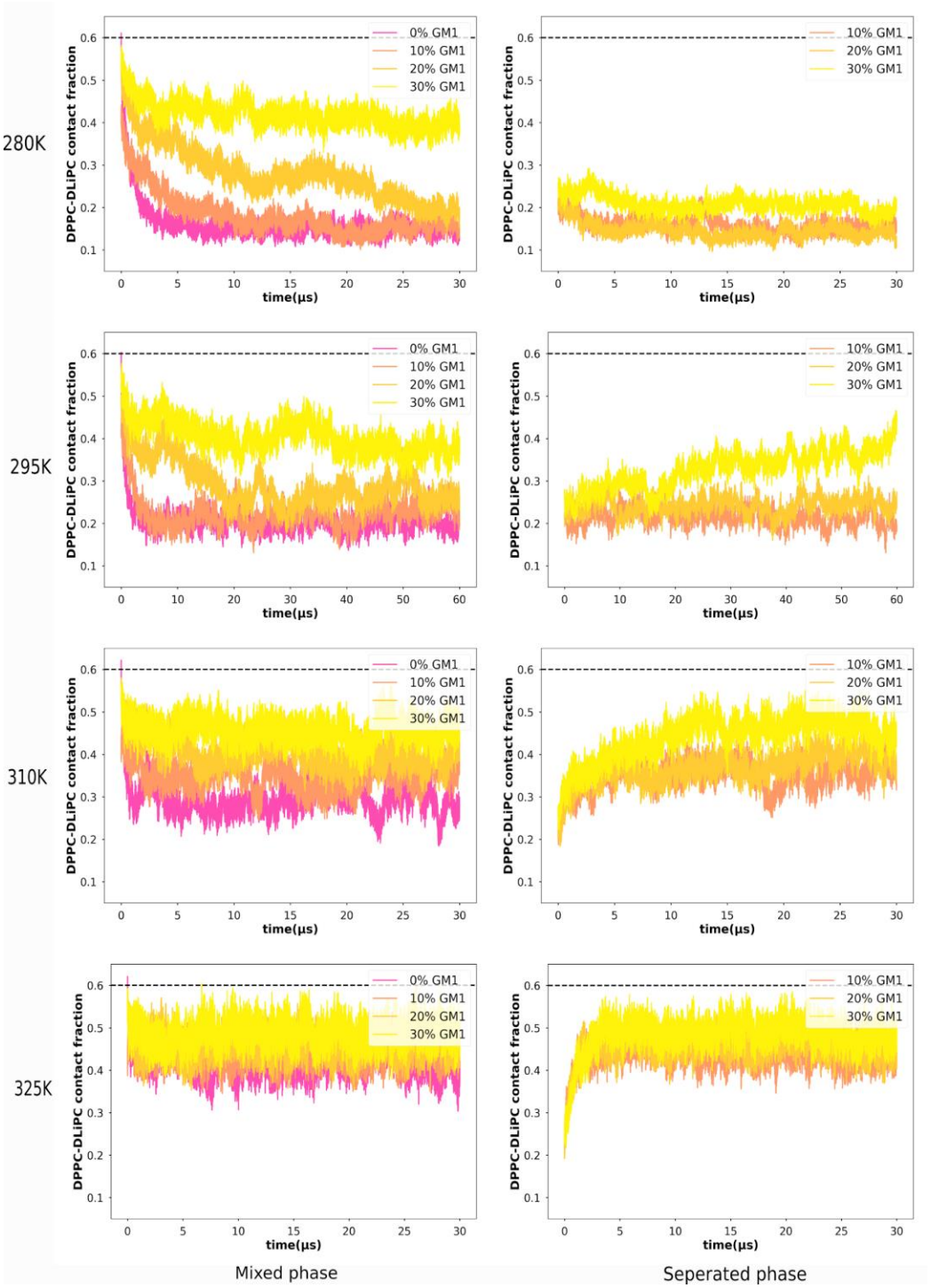

Figure S3. Contact fraction between DPPC and DLiPC for different temperatures. The horizontal dash lines in the Figure represent ideal mixing, above which DLiPC lipids prefer to contact with DPPC lipids (more mixed), below which DLiPC lipids prefer to contact with themselves (more separated).

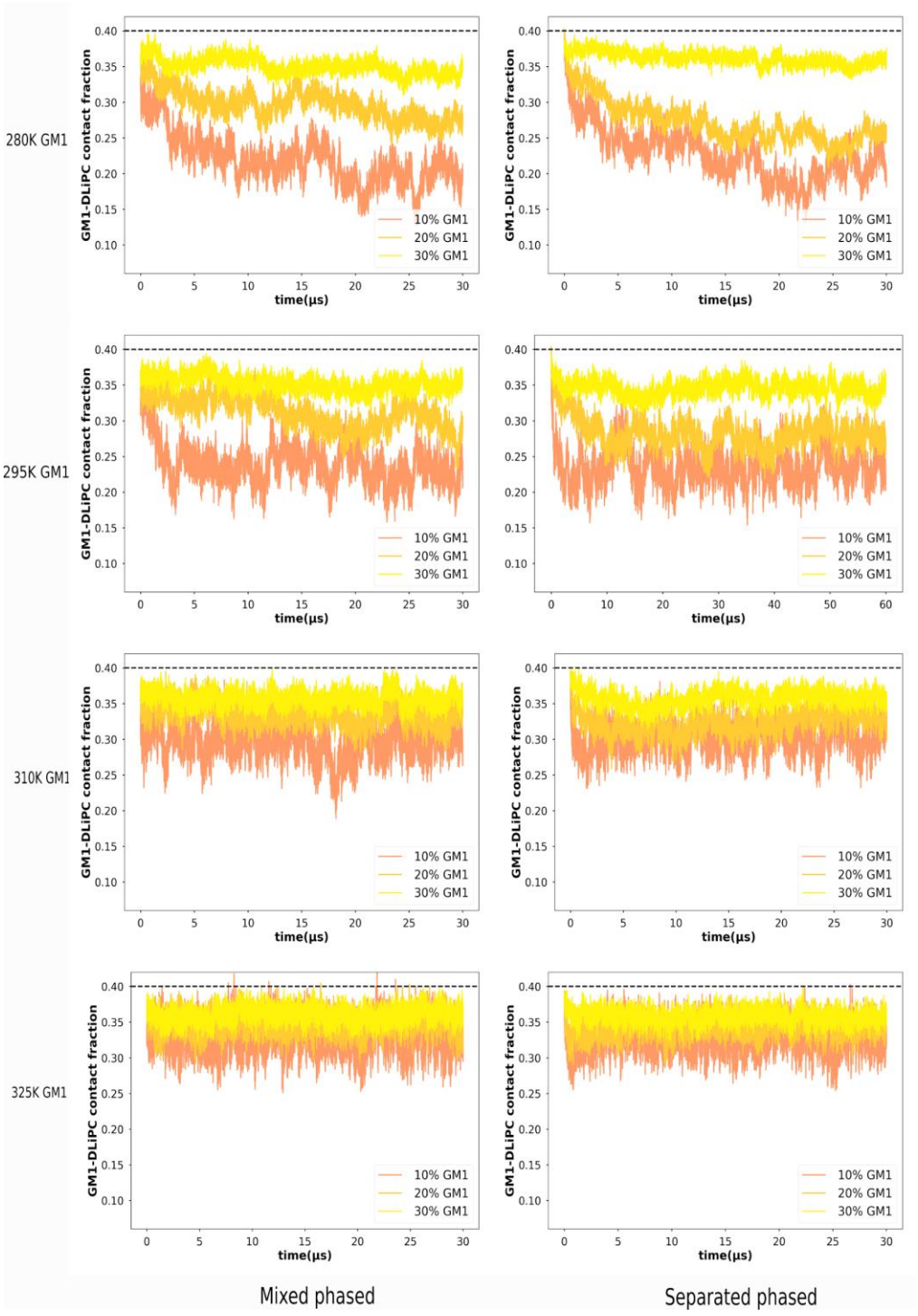

Figure S4. Contact fraction between GM1 and DLiPC for different temperatures. The horizontal dash lines in the Figure represent ideal mixing, above which DLiPC lipids prefer to contact with GM1 lipids, below which DLiPC lipids prefer to contact with themselves.

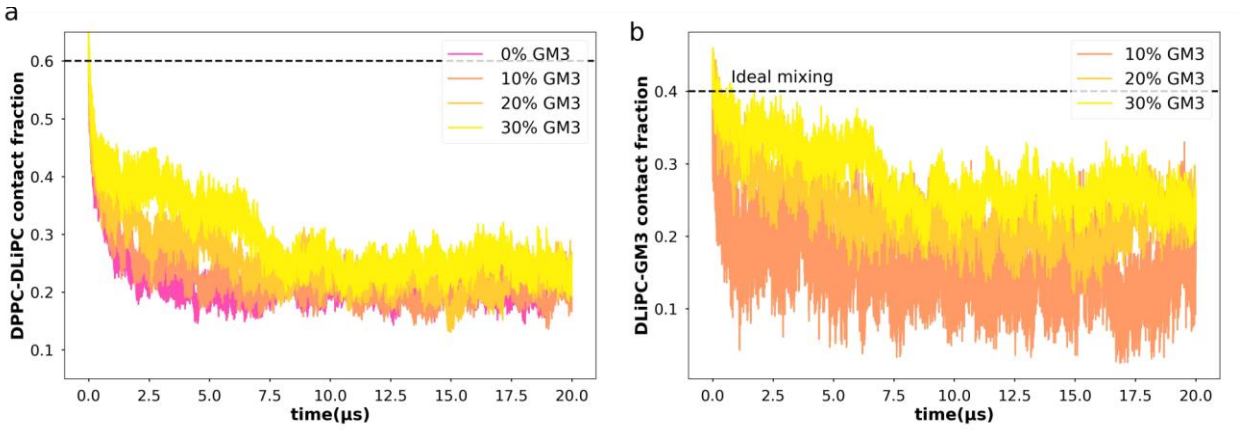

Figure S5. Contact fraction of GM3 membrane. (a) Contact fraction between DPPC and DLiPC for GM3 system at 295K. The horizontal dash line in the Figure represents ideal mixing, above which DLiPC lipids prefer to contact with DPPC lipids, below which DLiPC lipids prefer to contact with themselves. (b) Contact fraction between GM3 and DLiPC.

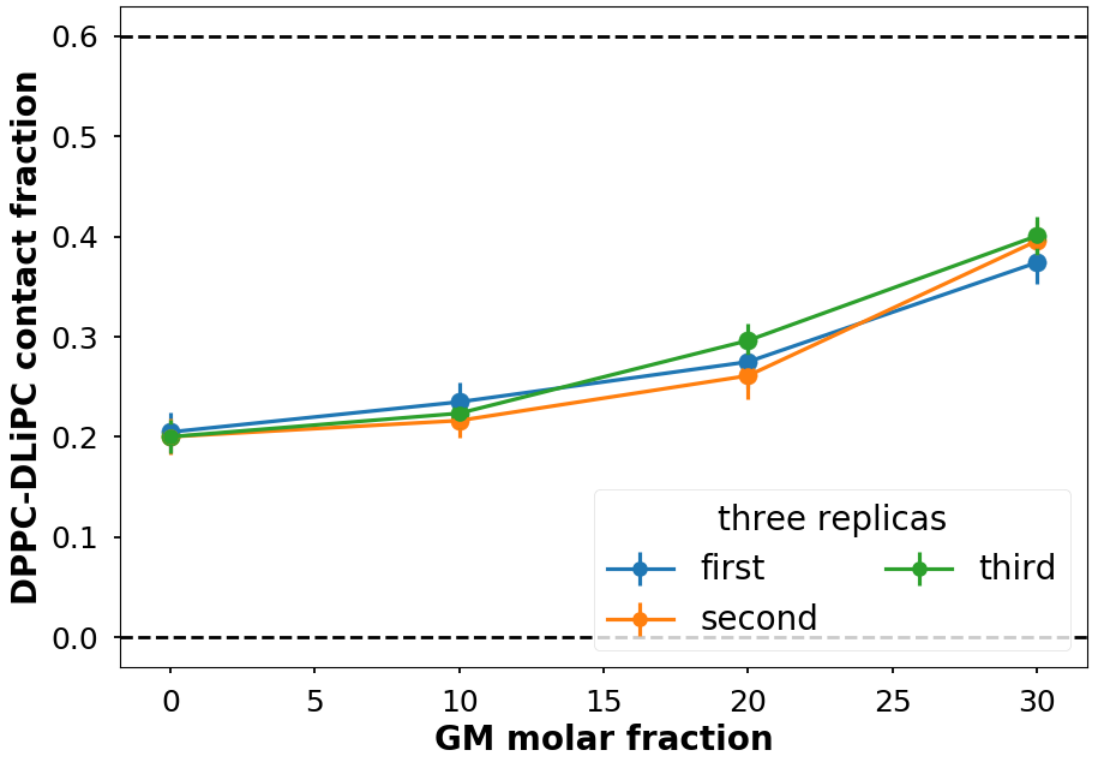

Figure S6. The DPPC-DLiPC contact fractions. The contact fractions were sampled and averaged for GM1 incorporated system at 295K after the system reached equilibrium. The three replicas of different GM1 ratios are shown. The error bars denote standard deviation.

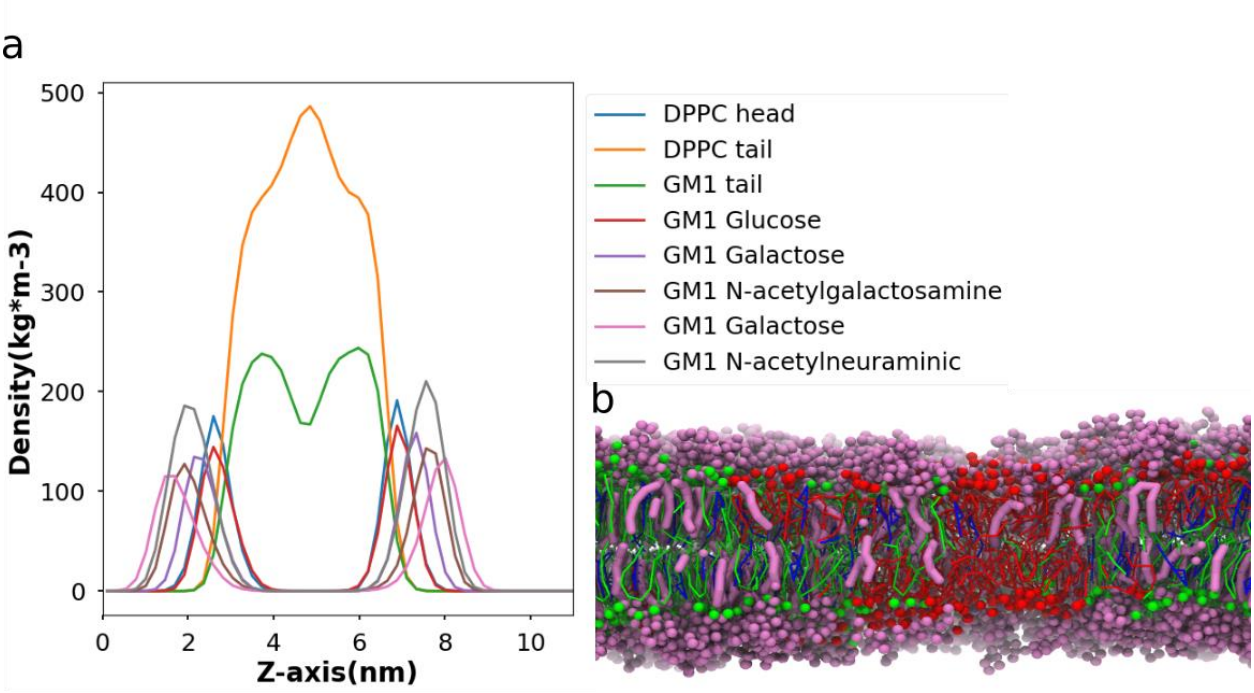

Figure S7. (a) Partial density of lipids for 20% GM1 membrane system at 295K. The partial density is computed and averaged over the last 2 μs of the simulation. (b) side view of corresponding membrane at 60 μs. DPPC, DLiPC, cholesterol and GM1 are represented by green, red, blue and magenta, respectively.

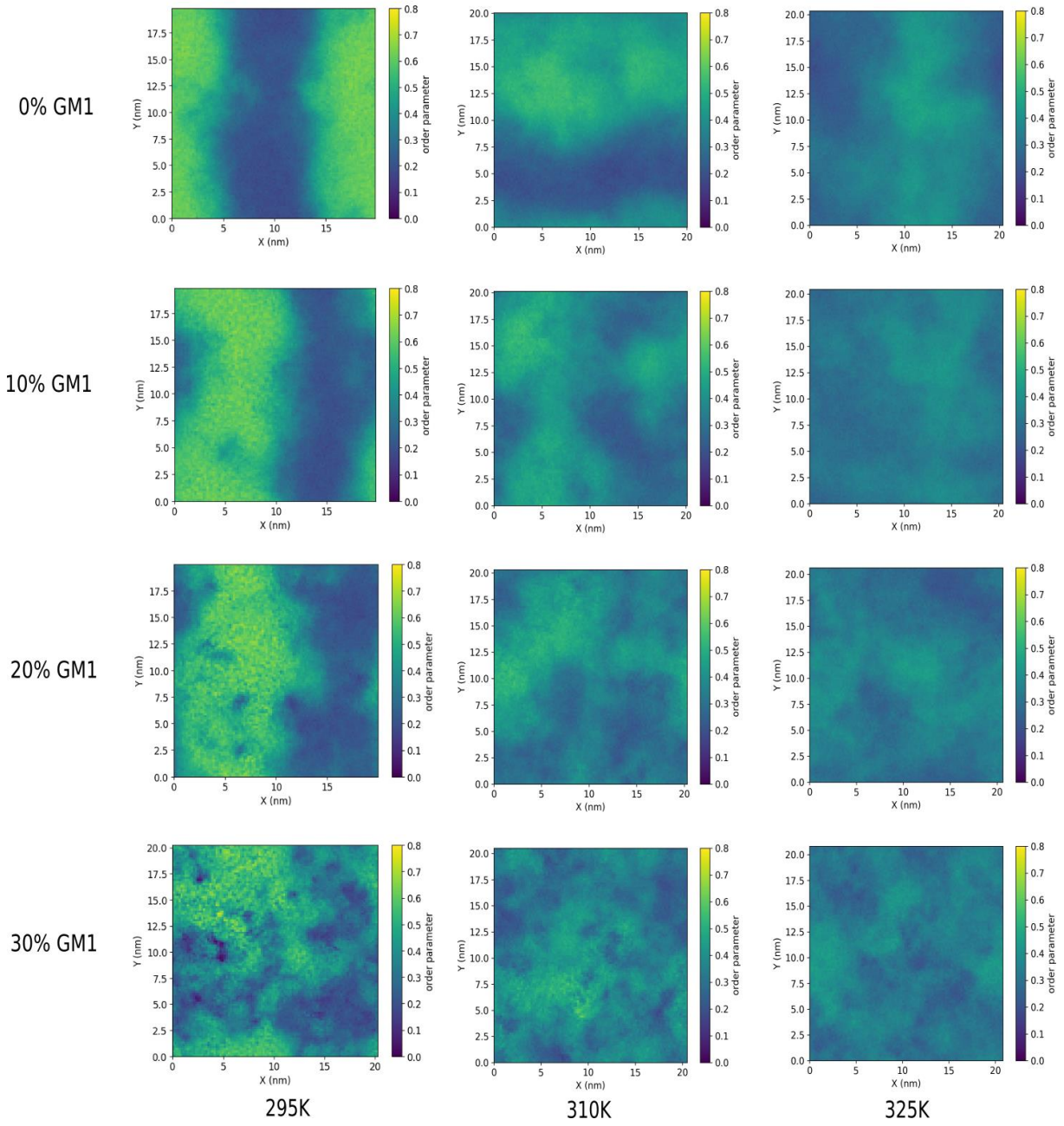

Figure S8. Order parameter landscape for system starting from mixed phase. The order parameter is computed based on the bonds in DPPC and DLiPC tails in the upper leaflet of the membrane. Order parameter landscapes are sampled from 59.5 μs to 60 μs of simulation at 295K; from 29.5 μs to 30 μs of simulation at 310K and 325K.

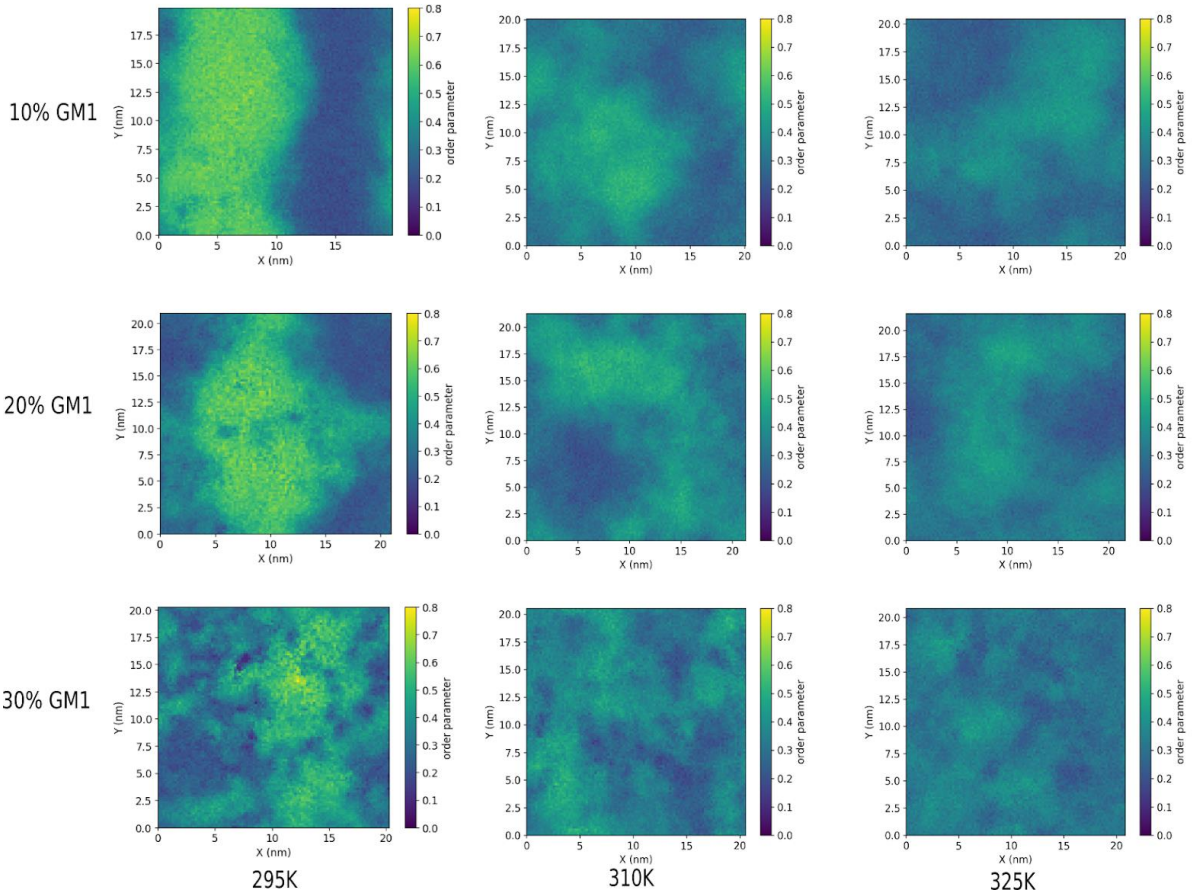

Figure S9. Order parameter landscape for system starting from separated phase. The order parameter is computed based on the bonds in DPPC and DLiPC tails in the upper leaflet of the membrane. Order parameter landscapes are sampled from 59.5 μs to 60 μs of simulation at 295K; from 29.5 μs to 30 μs of simulation at 310K and 325K.

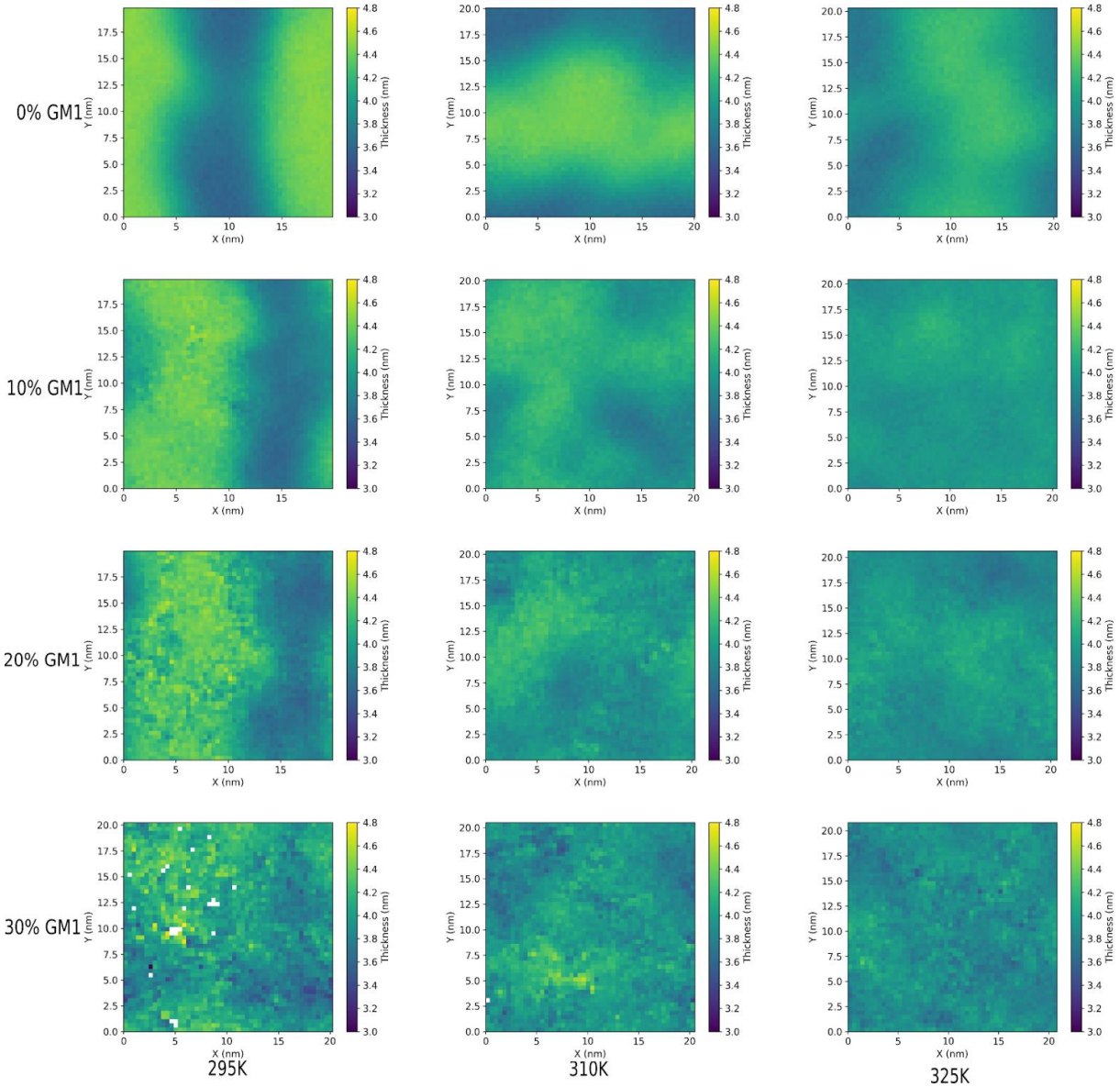

Figure S10. Membrane thickness landscape for system starting from mixed phase. Membrane thicknesses are computed as distance between the PO4 beads of PC lipids. Membrane thickness landscapes are sampled from 59.5 μs to 60 μs of simulation at 295K; from 29.5 μs to 30 μs of simulation at 310K and 325K. The white spots are caused by the presence of GM1 aggregates.

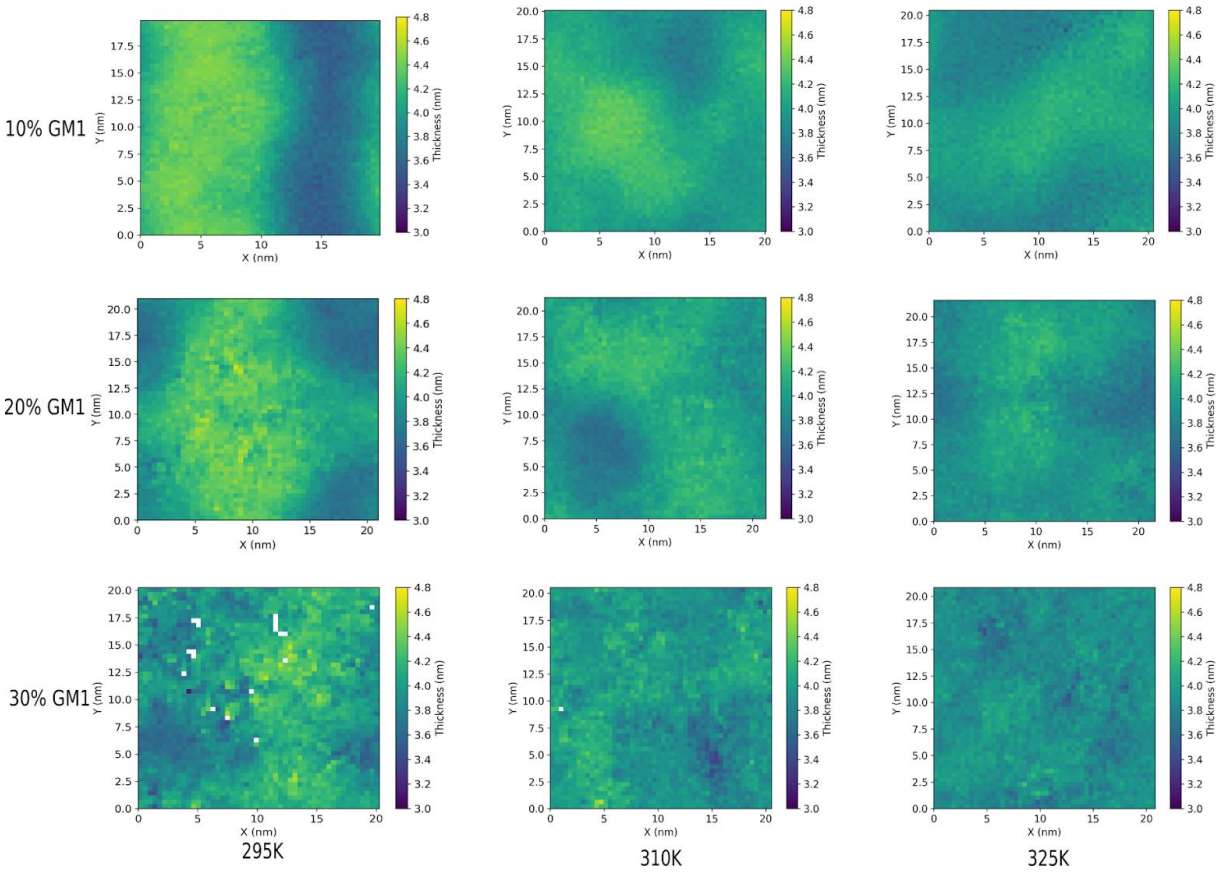

Figure S11. Membrane thickness landscape for system starting from separated phase. Membrane thicknesses are computed as distance between the PO4 beads of PC lipids. Membrane thickness landscapes are sampled from 59.5 μs to 60 μs of simulation at 295K; from 29.5 μs to 30 μs of simulation at 310K and 325K. The white spots are caused by the presence of GM1 aggregates.

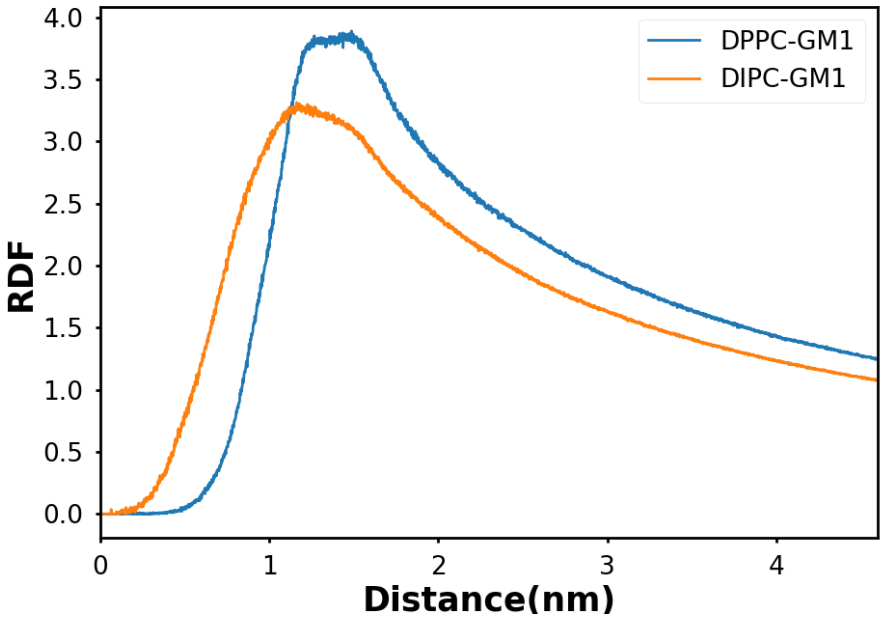

Figure S12. Radial distribution functions (RDF) between GM1 and PC lipids. RDF is computed and averaged over the last 5 μs in the simulations of GM1 in pure ordered phase or disordered phase.

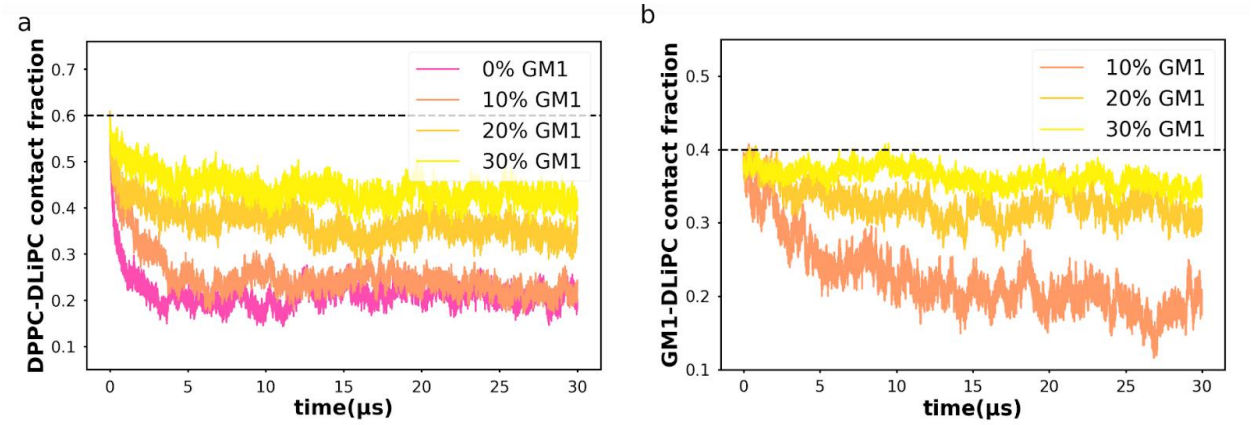

Figure S13. Contact fraction of Lopez GM1s membrane at 295K. (a) and (b) represent the contact fraction between DPPC and DLiPC and the contact fraction between GM1 and DLiPC, respectively.