Communication

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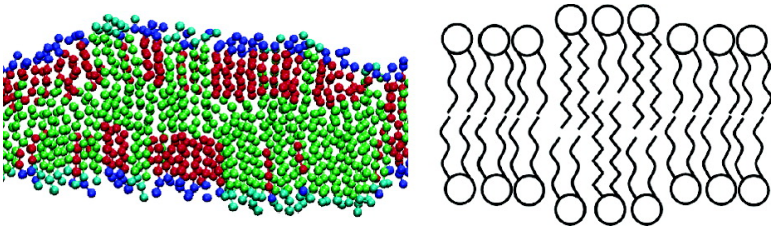

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Complementary Matching in Domain Formation within Lipid Bilayers

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The importance of lipid bilayers in biological processes is being more and more recognized as fundamental to biological function.[1] Structures within the lipid bilayers have been observed for a while now and have been shown to influence the function of membrane proteins, for example.[2−4] The most simple systems that possess domains within bilayers are binary lipid systems in which one lipid type has a bulk liquid phase and the other lipid type has a bulk gel phase at the given temperature.[5−7] A coexistence of liquid and gel domains can occur in such binary systems. The molecular scale structure of these coexisting systems is not fully understood. Recently, transbilayer complementarity has been demonstrated by nearest-neighbor recognition (NNR) experiments.[8] This method uses thiolate−disulfide interchange reactions to effectively take snapshots of the nearest-neighbor organization in membranes.[9] For a binary lipid system with the two lipid types differing only in the tail length, the NNR method demonstrated a complementary organization across the bilayer.[8] Where a long lipid is in one monolayer, a short lipid exists underneath in the bottom monolayer (Figure 1A).

In this paper, the dynamics and structure of such mixed bilayers are studied by molecular dynamics simulations that use a coarse grained model of the lipid molecules.[10,11] The lipid is treated as a bead-spring molecule. The model is based on coarse-grained polymer models and related to minimal models used to treat protein folding.[12,13] The coarse-graining enables the treatment of long time dynamics such as that in membrane fusion.[14] The present model works best for neutral lipids such as saturated phosphatidylcholine lipids. Each tail is composed of N_T hydrophobic beads of type T, and the head is composed of three hydrophilic beads of type H. The solvent is represented by a single bead, which is equivalent to the head type. The details of the model have been described elsewhere, including the calculation of the liquid−gel phase transition for lipids with different tail lengths.[15] A single tail bead corresponds to about 3 C atoms in a lipid tail.

Simulations of two binary lipid mixtures in a single bilayer have been performed. The two types differ only in their tail length and will be referred to as the short (S) and long (L) lipid types. Each system is composed of 12096 total lipids and about 300000 solvent particles. System I has $N_T = 4$ and 8 beads per tail in a 2:1 mixture. System II has $N_T = 4$ and 6 in a 1:1 mixture. Since each tail bead corresponds to about 3 C atoms, the $N_T = 8$ is long with respect to typical bilayers, but the large length difference makes the effects more pronounced. The second system is one of the experimentally studied systems (DLPC:DSPC)[5] and demonstrates that complementarity occurs in these simulated systems as well. Each system is initially constructed by random placement of the two lipid types in a bilayer and equilibration at a temperature above the melting temperatures of both lipid types. This equilibration removes any memory of the initial state. To study the domain formation dynamics, the system was cooled to a temperature between the melting temperatures of the two lipid types. Specifically, we simulated at the temperature $T = 1.05\ T_m$, where T_m is the melting temperature for the $N_T = 4$ lipids.[15] The simulations were run in the constant pressure and temperature ensemble for about 30 million time steps corresponding to 0.7 ms.

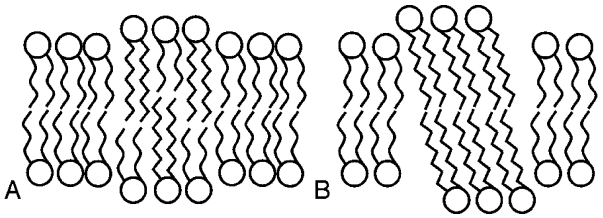

Figure 1. Schematics of possible gel domain structures. (A) Complementary structure of a domain in which a long tail lipid in the top monolayer has a short tail lipid beneath it and vice versa. (B) Fully phase separated structure with only long tail lipids in the gel domain with tilt. Both cases are surrounded by the small lipid liquid phase.

The lipid dynamics has been visualized in movies, which are available in the Supporting Information. The movie shows multiple, distinct gel domains form and grow as the simulation progresses. The lipids within a domain move as a collective body performing Brownian dynamics in the background fluid composed primarily of the short lipid. Figure 2A shows the state of the final configuration for system I. Only the tails of the lipids in the top monolayer are shown for visual clarity. The gel domains are visible in Figure 2A by the clustering of the green tails of the L lipids. Also, Figure 2A shows there are S lipids within the cluster of L lipids. The movie shows that the S lipids are dynamically part of the gel domain; i.e., S lipids move as a unit with the domain. Between these domains are regions primarily composed of S type lipids in a liquid phase. For these S lipids in the liquid phase the diffusion is always faster than the L lipids. As the domains form and grow, the diffusion rate for both types decreases.[7] Since the diffusion rate is a sum of lipids in the liquid and gel phases, the rate decreases as the gel domains grow increasing the smaller contribution of the gel phase. In addition, the path of the S lipids in the liquid phase is constrained by the gel domains reducing their diffusion rate.

The domains are composed of both lipid types in a complementary match between the two monolayers.[8] Figure 2B shows a slice through the system, which is marked by the two black lines in Figure 2A. The slice shows the variation in the bilayer thickness as a function of position. Where the long lipids have clustered to form the gel domains, the bilayer is thick. The remaining region primarily composed of the short lipids in the liquid state is thin. Figure 2C shows a magnification of a gel domain. This thin slice shows that the lipids in the top and bottom monolayers form a complementary match of the lipid types: where an L lipid is on the top monolayer, an S lipid occurs on the bottom monolayer, and vice versa as seen in the recent NNR experiments.[8]

Figure 3 shows quantitatively the 2D radial distribution functions for a chain of type α in one layer and type β in the other layer. In both systems, there is a peak at $r = 0$ in the S:L distribution demonstrating that it is more likely that the opposite chain type is below the given chain. The oscillations for system I in the S:L curve imply that in a layer there is a switching between S and L

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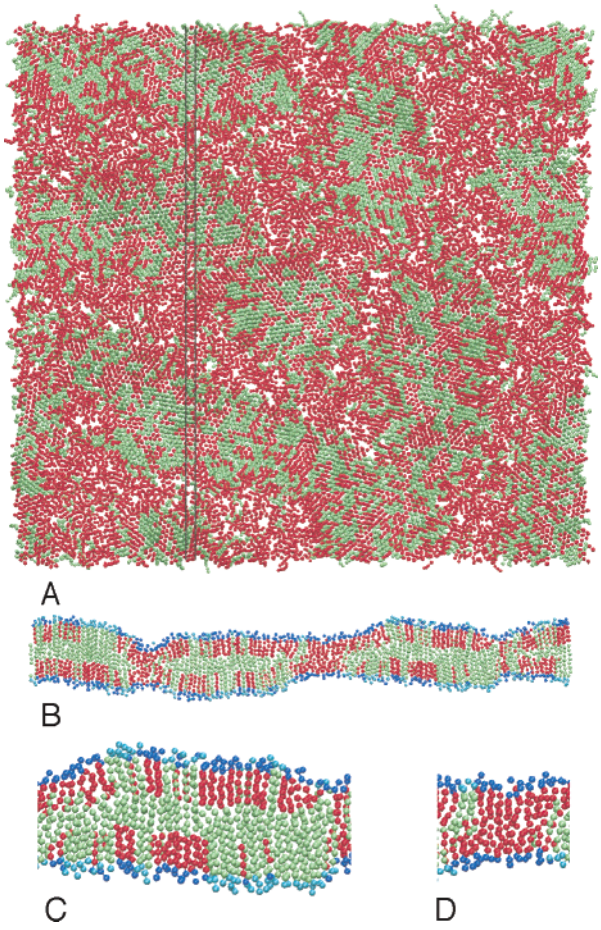

Figure 2. Images of domain formation for system I. (A) Only the tail beads of the top monolayer. (B) Transmembrane slice marked by the two black lines in image A. The subsequent images show magnified views of this slice. (C) Part of a domain, exhibiting the complementary matching of the lipid types between monolayers. (D) The liquid region is primarily composed of the short lipid. Colors: for $N_T = 8$ lipids, head is cyan and tail is green; for $N_T = 4$ lipids, head is blue and tail is red. The image uses smaller spheres (0.7 diameter) than actual size for clarity.

types which confirms the picture given in Figure 2A. System II, with the shorter L lipid and thus weaker tail−tail interactions, does not exhibit such longer ranged correlations.

In single lipid simulations at the temperature T, the $N_T = 4$ lipid bilayer has a thickness $t = 8.0\sigma$, where σ is the Lennard−Jones particle diameter.[13] For the $N_T = 8$ lipid bilayer $t = 14.4\sigma$. Thus, a mixed L−S bilayer (system I) should have $t = 11.2\sigma$. However, the thickness of the gel domains in the binary system is larger, about 13σ, which is between the values given above. The reason that $t > 11.2\sigma$ is that the lipids do not tilt in the gel domains of system I. The combined lengths of the long and short lipids in the gel domain without tilt yield a thickness closer to that of a gel domain containing only long lipids that tilt. This example provides a caution that one should take in interpreting experimental thickness data (especially AFM) without other sufficient geometrical detail of the molecular orientation.

One of the issues in a system with two coexisting phases is how the boundary structure ameliorates the cost of the boundary mismatch (cf. Figure 1). With the gel and liquid phases having different thicknesses, there would be a hydrophobic mismatch at

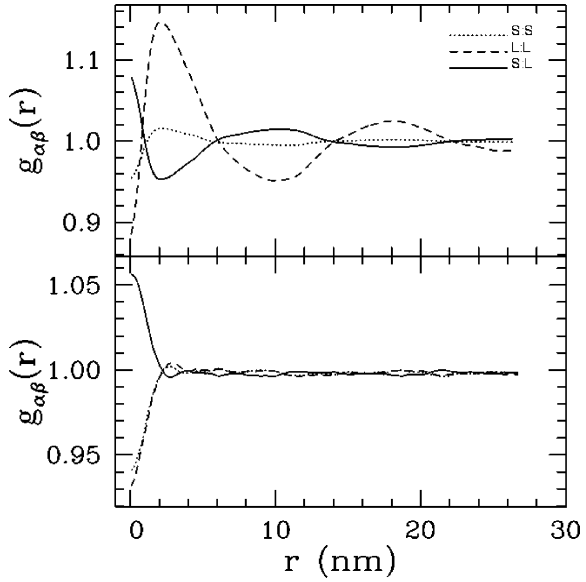

Figure 3. Radial distribution functions between chain types in different leaflets of the bilayer. Top figure is for $N_T = 4{:}8$ mixture (System I) and bottom is for $N_T = 4{:}6$ mixture (System II). Both cases have at $r = 0$ a peak in the S:L data showing that it is more likely to occur.

the boundary, unless the boundary structure adapts. Figure 2B shows that in the present case the boundary structure adapts to yield a smooth headgroup position that limits the water contact with lipid tails. At the boundaries, the surface of the bilayer is typically more curved allowing the transition in bilayer thickness to occur smoothly. On the molecular scale, examination of individual lipid conformations shows that the L lipids near the boundary adopt the structure of the liquid phase thereby reducing the bilayer thickness.

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Supporting Information Available: Movies of the domain formation are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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