

## New Phases of Phospholipids and Implications to the Membrane Fusion Problem

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*Membrane fusion is involved in many biological processes, such as cellular transportation, signal transduction, and viral infection. A key element of the study of membrane fusion is to understand the behavior of the primary structural component of the membrane, the lipid bilayer, in order to elucidate fusion proteins' regulatory role. In this study, we use x-ray diffraction at beamline X21 to study how the lipid composition affects bilayer fusion.*

When two lipid bilayers fuse, they must undergo molecular rearrangements at the point of merging. The role of the fusion proteins that strictly control membrane fusion is therefore most likely to modify the

relative free energy of different lipid configurations, and to exert mechanical work that shifts the system over the energy barriers along the fusion pathway. In order to understand the role of fusion proteins and how lipid structure transitions occur, researchers studied the phase transition of pure lipid membranes between the lamellar ( $L$ ) phase (planar lipid layers) and the inverted hexagonal ( $H_{II}$ ) phase (hexagonally stacked lipid tubes), based on the idea that lipid must undergo a similar rearrangement, as in fusion.

However, previous investigations on the system of dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylethanolamine (DOPE) did not reveal intermediate phases between the  $L$  and  $H_{II}$  phases. Recently, we found a rhombohedral phase ( $R$ ) in diphytanoylphosphatidylcholine (DPhPC) between its  $L$  and  $H_{II}$  phases using substrate-supported samples. Here we report the observation of the rhombohedral phase, as well as a previously not observed distorted hexagonal phase in DOPC-DOPE mixtures.

The experiments were performed on model membranes that are made of hundreds of lipid bilayers prepared on silicon substrate. We observed the phase behavior of the lipid while varying the composition of the sample as well as its temperature and water content (**Figure 1**). The samples in general showed all three phases that were observed in DPhPC. However, depending on the sample composition, these three phases appeared at the different parts of the phase diagram.

It has been shown that the rhombohedral phase of DPhPC contains a lattice of stalk structure that was thought to be an intermediate structure during the fusion process. The existence of this phase in DOPC/DOPE mixtures once again confirms the stalk hypothesis for the  $L$ - $H_{II}$  transition. Furthermore, the  $R$  phase exists only for a certain range of lipid composition (i.e. for a certain range of spontaneous curvature). This implies that the free energy barrier in the fusion pathway is directly determined by the spontaneous curvature of the lipid bilayer.

The samples with mixed DOPC and DOPE also showed (**Figure 2**) a distorted hexagonal phase ( $D$ ). Though the detailed structure of this phase is yet to be determined, because of the symmetry of the structure and the fact that the  $D$  and  $H_{II}$  phases are neighbors in the phase



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diagram, the *D* phase very likely contains distorted lipid tubes. This implies that, under stress, lipids may partially demix to adjust its local spontaneous curvature in order to achieve energy minimum. Therefore demixing, or lipid sorting, may be a mechanism to promote membrane fusion.

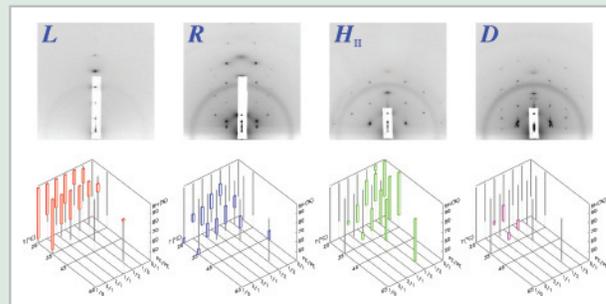


Figure 1. The phases diagrams of the structure of DOPC/DOPE multilayers as functions the composition, the temperature of the samples and the environmental relative humidity.

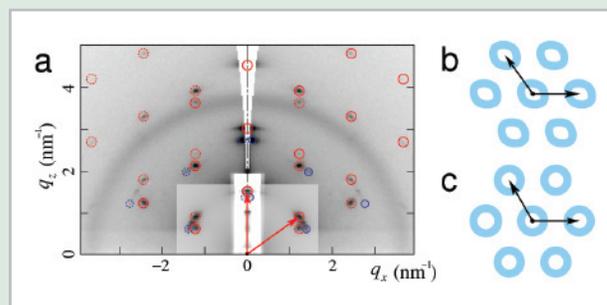


Figure 2. The diffraction pattern (a) from the distorted hexagonal phase of DOPC/DOPE suggests that the structure of this phase (model b) may contain distorted lipid tubes, much like those in the  $H_{II}$  phase (c).