

## Structure of the $L_\beta$ Phases in a Hydrated Phosphatidylcholine Multimembrane

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We report x-ray measurements on well aligned, freely suspended films of stacked bilayer membranes of the phospholipid dimyristoyl-phosphatidylcholine in a continuously variable humidity- and temperature-controlled environment. Remarkably, the most commonly observed  $L_\beta$  phase is, in fact, three distinct 2D phases where the phase transitions are driven by hydration forces and the phases are distinguished by the relation between the molecular tilt direction and the 2D bond direction.

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A great deal of interest in the precise nature of the phases and phase transitions of lyotropic liquid crystals composed of surfactants in solution has arisen in recent years.<sup>1-4</sup> In particular, the lamellar phases are of fundamental physical interest because they form weakly coupled layered structures which are inherently low dimensional. In one sense, these are similar to smectic thermotropic liquid crystals; however, in lyotropic systems, the thickness of the water layer which separates the lipid bilayers can be varied, thus tuning the interlayer coupling. These systems are also of fundamental biophysical interest since biomembranes are known to be composed of multicomponent lipid bilayers in which proteins and other biomolecules are embedded. Since the lamellar liquid-crystalline phases of water and single-component lipids exhibit a repeated bilayer-water structure, the pure mixtures serve as simple model biomembranes.

Since the pioneering work of Luzzati and co-workers,<sup>3</sup> many scattering experiments have been performed to determine the structures of the various phospholipid-water phases. The phase diagram for dimyristoyl-phosphatidylcholine (DMPC) has been refined since the early work, and the one proposed by Janiak, Small, and Shipley<sup>4</sup> was typical, prior to this work. In DMPC, near room temperature and at high water content (the biologically relevant regime), there are three major phases. The  $L_\alpha$  phase is a lamellar bilayer phase in which the polar head groups of the lipid are in contact with the interlayer water region and the melted hydrocarbon chains form the interior of the bilayer membrane. At lower temperature, the  $L_\beta$  or  $P_\beta$  phase forms. The  $L_\beta$  is a lamellar phase in which the hydrocarbon chains are frozen in the all-*trans* conformation and are ordered within the layers. The  $P_\beta$ , also known as the "rippled phase," has mostly frozen chains and greater in-plane order than the  $L_\alpha$ , but its most distinguishing feature is a long-wavelength (100–200 Å) in-plane modulation of the layers. The  $L_\alpha$  phase has been the most widely studied<sup>1-4</sup> of these phases, and much emphasis has also been placed on the determination of the structure of the  $P_\beta$

phase. Although it occupies a very large portion of the phase diagram, much less work has been done on the  $L_\beta$  phase which was thought to be well characterized.<sup>3,4</sup> We have studied  $L_\beta$  and shown that it actually consists of three distinct phases, each distinguished by the direction of the chain tilt with respect to the position of in-plane nearest neighbors. These phases are shown not to be 3D crystals, but rather 2D structures with no out-of-plane correlations and with in-plane correlations extending over  $\approx 200$  Å.

Previous scattering experiments were performed mostly on either powder samples or poorly aligned samples of fixed water concentration. In such partially ordered systems where the scattering consists of only a few broad reflections, it is difficult to discern much of the structural information. We have developed a new technique by which we can form well oriented, freely suspended films of lipids at controlled temperature  $T$  and relative humidity (RH). The good alignment of the layer normals characteristic of freely suspended films, giving a mosaic of  $\approx 0.1^\circ$ , allows us to disentangle the intralayer and interlayer correlations as well as to obtain unambiguous structural information. We have adopted the technique, originally used to form oriented samples of the lamellar phases of thermotropic liquid crystals,<sup>5</sup> of wiping a small amount of material across a hole in a plate, forming a film in which the layers are oriented parallel to the surface of the films. Because the water in hydrated DMPC samples easily evaporates, stable films are only possible in a humid environment. To accomplish this, we have incorporated into our film oven a computer-operated gas-handling system which enables us to control continuously the partial pressure of the water vapor in our oven.<sup>6</sup> This experimental arrangement allows us to investigate the phase diagram as a function of  $T$  and chemical potential of water ( $\mu$ ) in a continuous fashion.  $\mu$  is simply related to the partial pressures by  $\Delta\mu = (\mu - \mu_0) = RT \times \ln(P/P_0)$ , where  $\mu_0$  is the chemical potential of pure water per mole at temperature  $T$ ,  $P$  the partial water-vapor pressure over the film, and  $P_0$  the vapor pressure

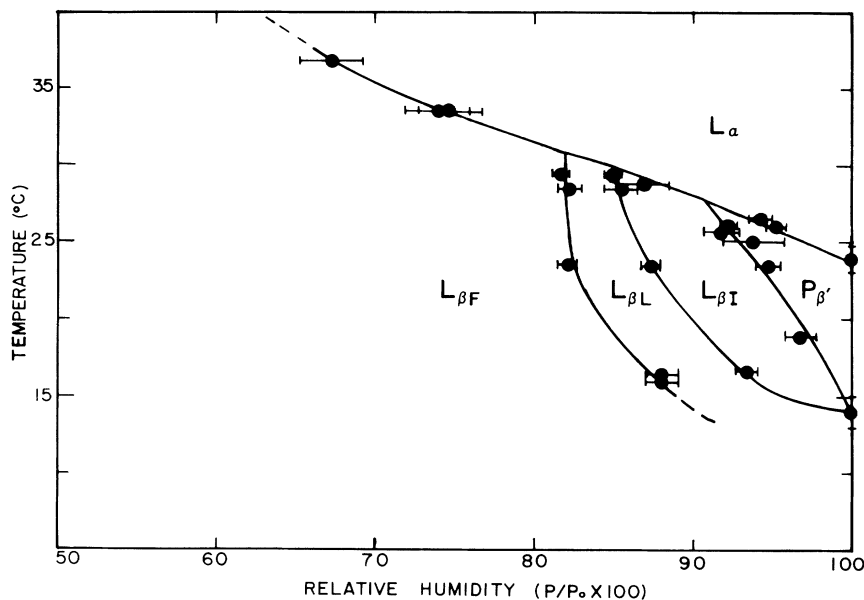


FIG. 1. Phase diagram of DMPC as a function of  $T$  and RH. This work has identified the three  $L_{\beta'}$  phases, previously not distinguished.

over pure water. The relative humidity is simply equal to 100 ( $P/P_0$ ). By doing our measurement as a function of two thermodynamic fields, we get one and only one phase in each region of the phase diagram (except exactly at the various phase boundaries). Previous ovens for freely suspended films<sup>7</sup> were designed to have the incident and scattered x rays nearly normal to the surface of the film (transmission geometry), probing the in-plane structure. Our oven also permits grazing incidence and scattering from the film (reflection geometry) which allows the direct measurement of the layer spacing.

The experiments were carried out with an 18-kW Rigaku RU300 rotating-anode x-ray generator, and selected data were taken at the Exxon beam line (X10A) at the National Synchrotron Light Source (NSLS). The Cu  $K\alpha$  line was selected by use of the (002) reflection of a vertically bent pyrolytic-graphite crystal focused at the sample. The angular width of the beam was determined by slits and gave us an arm zero width of  $0.07^\circ$  FWHM corresponding to  $0.005 \text{ \AA}^{-1}$ . Since our samples appeared to be powders with respect to orientation within the layers, the vertical resolution determined by slits was set wide, corresponding to an angular divergence of  $\approx 2^\circ$ . The spot size on the sample was 0.5 mm horizontally and 2 mm vertically. We define  $q_z$  as the reciprocal-space coordinate normal to the layers and  $q_r$  as the cylindrical in-plane coordinate. The resolution widths were (FWHM)  $\Delta q_z = 0.005 \text{ \AA}^{-1}$ ,  $\Delta q_r = 0.001 \text{ \AA}^{-1}$ , and out of plane  $\Delta q_z = 0.1 \text{ \AA}^{-1}$ . DMPC, > 99% pure, was obtained from Avanti Polar Lipids, Birmingham, Alabama.

Our experiments were carried out at constant  $T$  and  $\mu$  (or equivalently the RH) as opposed to a constant water

concentration. The phase diagram is shown in Fig. 1. The  $L_{\beta'}$ - $L_\alpha$ ,  $L_{\beta'}$ - $P_{\beta'}$ , and  $L_\alpha$ - $P_{\beta'}$  phase boundaries were determined by our observing a jump in layer thickness (measured in the reflection geometry) at the transitions, usually by the variation of the RH at various  $T$ . The various  $L_{\beta'}$  phases can only be distinguished by in-plane scans done in the transmission geometry on well aligned samples.

We have made a careful examination of the region classified as  $L_{\beta'}$  in hydrated DMPC and found that it consists of not one, but rather three phases with different structures, relating the molecular tilt direction to the bond direction. All of these phases have tilted distorted hexagonal 2D lattices with no in-plane correlations between bilayers. We define the angle  $\phi$  as the angle relating the tilt direction to the nearest-neighbor bond direction [Figs. 2(d)-2(f)]. Two of the phases, the low-RH  $L_{\beta F}$  and the high-RH  $L_{\beta I}$ , are analogous to the thermotropic phases smectic  $F$  and smectic  $I$  where the molecules are tilted between nearest neighbors ( $\phi = 0^\circ$ ) [Fig. 2(d)] and toward nearest neighbors ( $\phi = 30^\circ$ ) [Fig. 2(f)], respectively. The intermediate phase  $L_{\beta L}$  is a new phase, presently unique to this system.<sup>8</sup> In this phase, the tilt direction changes continuously with increasing RH from tilting between neighbors to tilting toward neighbors ( $0^\circ < \phi < 30^\circ$ ) [Fig. 2(e)].

Figures 2(a)-2(c) show constant-intensity contours in the  $(q_r, q_z)$  plane, and Figs. 2(d)-2(f) show the corresponding real-space structures of the hydrocarbon chains for the  $L_{\beta F}$ ,  $L_{\beta L}$ , and  $L_{\beta I}$  phases at  $T = 23.5^\circ\text{C}$ . The contour plots are composites of  $q_z$  scans at sixteen values of  $q_r$ . Since the sample is a powder within the planes, the scattering is independent of the in-plane orientation

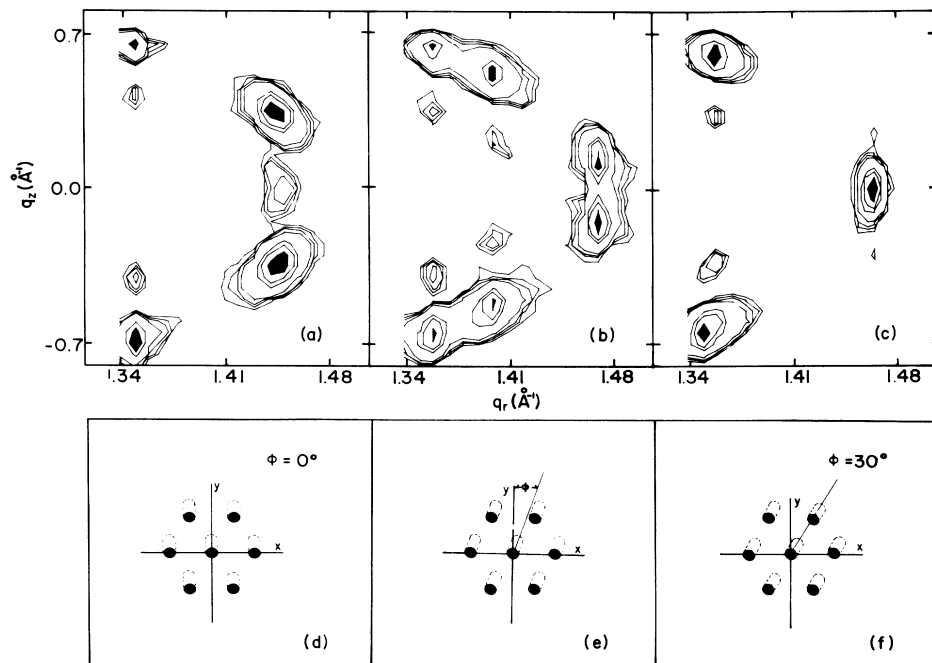


FIG. 2.  $q_r$  vs  $q_z$  contour plots at  $T = 23.5^\circ\text{C}$  in the phases (a)  $L_{\beta F}$ , RH = 80.4%; (b)  $L_{\beta L}$ , RH = 83.3%; and (c)  $L_{\beta I}$ , RH = 85.9%. The contour levels are 100, 113, 125, 150, 300, and 500 counts per 9 sec. The highest contour levels are filled in, to emphasize the fundamental form-factor peaks.  $\phi$  and  $\theta$  are determined from the positions of those peaks (stated in the text). Below each is a schematic [(d)–(f)] showing the tilt direction of the molecules relative to the bond direction (defining  $\phi$ ). Going from (a) to (b) the inner peaks split and the outer peak moves in; from (b) to (c) two peaks merge at  $q_z = 0$  and the other two pairs merge at finite  $q_z$ .

of the film ( $\chi$ ). These measurements were done on very thick films ( $\geq 1000$  layers), and so the 2D powder average can result from domains within single layers and/or different layers having different orientations. The highest-intensity contours for the strongest peaks are blackened to highlight the basic structure. In a single domain of a tilted hexagonal 2D lattice, the structure factor yields six rods of scattering. The scattering intensity would be peaked where they intersect the tilted pancake-shaped molecular form factor. Our contours result from a  $\chi$  powder of such a structure. The fact that there are distinct peaks in the  $(q_r, q_z)$  plane and not one arc of scattering proves that there is bond-orientational order. The data show that the tails form an angle  $\theta$  with respect to the layer normal, and their in-plane projections form an azimuthal angle  $\phi$  with respect to the direction shown. For the contours shown, in the  $L_{\beta F}$  phase, there are peaks at  $(q_r, q_z)$  coordinates  $(1.446, \pm 0.35)$  and  $(1.350, \pm 0.70)$ , where  $\theta = 27.4^\circ$ ; in the  $L_{\beta I}$  phase, peaks at  $(1.365, \pm 0.65)$  and  $(1.475, 0)$ , where  $\theta = 29.8^\circ$ ; and in the  $L_{\beta L}$  phase, peaks at  $(1.355, \pm 0.64)$ ,  $(1.399, \pm 0.52)$ , and  $(1.468, \pm 0.15)$ , where  $\theta = 27.4^\circ$  and  $\phi = 17.2^\circ$ . For  $\phi = 0^\circ$  or  $30^\circ$ ,  $\pm\phi$  are equivalent structures. In the  $L_{\beta L}$  phase, the reflection symmetry about the tilt direction is broken. As can be seen from the contour plots,  $q_z$  scans through the principal peaks are wide (FWHM =  $0.175 \text{ \AA}^{-1}$ ) and are accompanied

by subsidiary peaks. The widths, positions, and relative intensities of these peaks come from the molecular form factor of a pair of tails sitting end to end. This shows that in-plane correlations do not extend between bilayers.

The longitudinal widths of the peaks along  $q_r$  are wider than resolution and correspond to in-plane correlations lengths of  $\approx 200 \text{ \AA}$ . Further high-resolution studies are required to distinguish whether we have finite crystallites (2D crystals with a power-law line shape and finite-size effect) or a hexatic (Lorentzian line shape).

In Fig. 3, we show for  $T = 23.5^\circ\text{C}$ , the measured evolution of the tilt angle  $\theta$ , its azimuthal angle  $\phi$  (obtained from in-plane scans), and the layer spacing  $d$  (obtained from the fourth harmonic, in the reflection geometry) measured by the variation of the RH and plotted versus  $\Delta\mu$ . Given the uncertainties shown, we cannot conclude that there are discontinuities in either  $\theta$  or  $d$  at the transitions between the  $L_{\beta}$  phases.

As was demonstrated by Parsegian, Fuller, and Rand<sup>9</sup> in a series of beautiful experiments, the dominant force governing the interbilayer interactions in neutral phospholipids for small separation distances is the hydration force. This strong repulsive potential acts to prevent the approach of phospholipid bilayers embedded in water. A consequence of this interaction is that as the chemical potential of water is decreased, the bilayer thickness increases. Our data show that this change in membrane

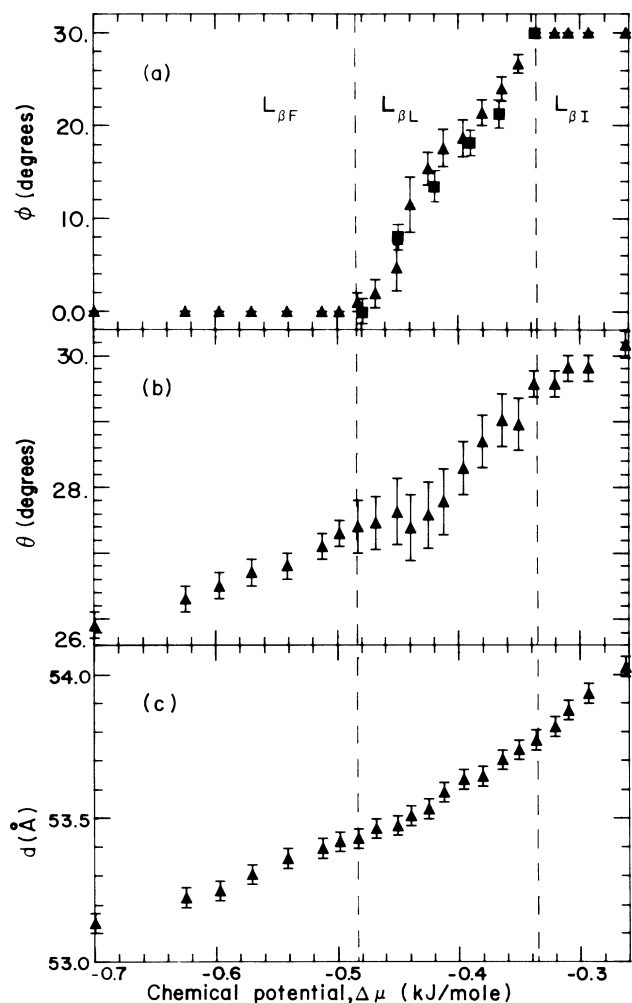


FIG. 3.  $\phi$ ,  $\theta$ , and  $d$  at  $T=23.5^\circ\text{C}$  plotted vs  $\Delta\mu$ . For  $\phi$ , the squares are data taken on hydrating, and the triangles, on drying showing that hysteresis is negligible.

thickness as a function of  $\mu$  in the  $L_{\beta'}$  region is accomplished through a tilt of the frozen hydrocarbon chains. This then results in an increase in the surface area per head group. We point out that the chains on the pseudohexagonal lattice are paired (with the tilt direction possible being determined by the average direction of pairing) and a dipole is associated with each: We suggest that the in-plane tilt-direction transitions may be driven by the interfacial dipole-dipole interaction (which varies with the average head-head distance) and the entropy associated with pairing points on a hexagonal lattice.<sup>10</sup>

The transitions between the three  $L_{\beta'}$  phases are novel in that the middle  $L_{\beta L}$  phase is of lower symmetry (point group  $C_1$ ) than the surrounding  $L_{\beta I}$  and  $L_{\beta F}$  which both have the same symmetry (point group  $C_m$ ). For finite  $\theta$ , the constraint of close packing of the tails results in two distinct phases with the same symmetry; thus, a direct

$L_{\beta F}$ - $L_{\beta I}$  transition has to be first order. However, this system can undergo a continuous transition because of the presence of the intermediate phase of lower symmetry, which can have continuous transitions to both phases. We propose  $\phi$  as the order parameter for the  $L_{\beta F}$ - $L_{\beta L}$  transition, and  $30^\circ - \phi$  for the  $L_{\beta I}$ - $L_{\beta L}$ . These transitions can be second order or they may have small first-order jumps (within our error bars).

In summary, we have found that the  $L_{\beta'}$  consists of three phases, two of which are similar in their tilt and bond structure to known thermotropic phases and one previously unobserved. These phases have bond-orientational order and finite in-plane correlations. They are not correlated across bilayers and appear to be truly 2D. It would be also interesting to obtain calorimetric data on these transitions since the latent heats of these types of transitions are known to be extremely small in thermotropics.

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<sup>1</sup>C. R. Safinya, D. Roux, G. S. Smith, S. K. Sinha, P. Dimon, N. A. Clark, and A. M. Bellocq, *Phys. Rev. Lett.* **57**, 2718 (1986); D. Roux and C. R. Safinya, *J. Phys. (Paris)* (to be published).

<sup>2</sup>D. R. Nelson and L. Peliti, *J. Phys. (Paris)* **48**, 1085 (1987); R. Lipowsky and S. Leibler, *Phys. Rev. Lett.* **56**, 2541 (1986); G. L. Kirk, S. M. Gruner, and D. L. Stein, *Biochemistry* **23**, 1093 (1984); M. B. Schneider and W. W. Webb, *J. Phys. (Paris)* **45**, 273 (1984).

<sup>3</sup>V. Luzzati, *Biol. Membr.* **1**, 71 (1968); A. Tardieu, V. Luzzati, and F. C. Reman, *J. Mol. Biol.* **75**, 711 (1973).

<sup>4</sup>M. J. Janiak, D. M. Small, and G. G. Shipley, *J. Biol. Chem.* **254**, 6068 (1979).

<sup>5</sup>C. Y. Young, R. Pindak, N. A. Clark, and R. B. Meyer, *Phys. Rev. Lett.* **40**, 773 (1978).

<sup>6</sup>G. S. Smith, C. R. Safinya, D. Roux, and N. A. Clark, *Mol. Cryst. Liq. Cryst.* **144**, 235 (1987); S. M. Gruner, *Rev. Sci. Instrum.* **52**, 134 (1981).

<sup>7</sup>E. B. Sirota, P. S. Pershan, L. B. Sorensen, and J. Collett, *Phys. Rev. Lett.* **55**, 2039 (1985), and *Phys. Rev. A* **36**, 2890 (1987); D. E. Moncton and R. Pindak, *Phys. Rev. Lett.* **43**, 702 (1979).

<sup>8</sup>In naming these phases, we maintain the lyotropic nomenclature (lamellar  $\alpha, \beta$ ), but also make use of the established thermotropic liquid-crystal nomenclature (i.e., smectics  $F, G, H$  and  $I, J, K$  extended to  $L, M, N$ ). (See references in Ref. 7.)

<sup>9</sup>V. A. Parsegian, N. Fuller, and R. P. Rand, *Proc. Natl. Acad. Sci.* **76**, 2750 (1979).

<sup>10</sup>E. B. Sirota, to be published.