

# Structure of the ripple phase in chiral and racemic dimyristoylphosphatidylcholine multibilayers

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We present electron density maps of the ripple phase of chiral and racemic dimyristoylphosphatidylcholine. The structures of the two systems are found to be identical within experimental errors, thus unambiguously showing that the chirality of the lipid molecules does not influence the structure of this phase. [S1063-651X(99)06602-7]

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## I. INTRODUCTION

Lipid water mixtures show a variety of phases as a function of temperature and humidity, including a number of diverse lamellar phases [1–3]. These lamellar phases are of interest because they serve as model systems for biological membranes. Further, phase transitions in these systems are also a topic of current interest [2]. In the high-temperature  $L_\alpha$  phase, which has the symmetry of a smectic-A liquid crystal, the hydrocarbon chains are molten and exhibit no in-plane ordering. In the low-temperature  $L_{\beta'}$  phase, the chains are in the all-*trans* conformation and are ordered on a two-dimensional lattice [1,4]. In addition to these two lamellar phases, some phospholipids also exhibit an intermediate ripple or  $P_{\beta'}$  phase, characterized by periodic one-dimensional height modulation of the bilayer [1–3,5–7]. In this phase, the conformation of the chains has not yet been unambiguously determined, but from x-ray data it is clear that most of the hydrocarbon chains are ordered in the same manner as in the  $L_{\beta'}$  phase.

A complete theory of the ripple phase that explains all the experimental observations is yet to be formulated. Recently, Lubensky and MacKintosh [8,9] have proposed a Landau theory that describes phase transitions between the  $L_\alpha$ ,  $P_{\beta'}$ , and  $L_{\beta'}$  phases in chiral and achiral bilayers. In the case of achiral bilayers, this model predicts the existence of two distinct symmetric ripple phases in addition to a square lattice phase. When the system is chiral, one of these two phases becomes asymmetric [10]. Thus, according to this phenomenological model, achiral bilayers can exhibit only symmetric ripples, while both symmetric and asymmetric ripples can occur in chiral bilayers.

In order to test the predictions put forth by Lubensky and MacKintosh [8] concerning the influence of molecular chirality on the structure of the ripple phase, Katsaras and Raghunathan [11] carried out x-ray diffraction experiments on aligned films of chiral (*l*) and racemic (*dl*) dimyristoylphosphatidylcholine (DMPC) bilayers. They found that under similar experimental conditions, the diffraction patterns from the two systems were practically indistinguishable. Moreover, both the systems were found to have an oblique unit cell, indicating the presence of asymmetric ripples.

The observation of asymmetric ripples in the racemate can still be reconciled with the predictions of the Lubensky-

MacKintosh model if the *d* and *l* enantiomers within each layer phase separate into chiral domains. To investigate this possibility, Katsaras *et al.* performed calorimetric measurements on mixtures of *l*-dipalmitoylphosphatidylcholine having perdeuterated hydrocarbon chains (*l*-DPPC- $d_{62}$ ) and *d*-DPPC [12]. They found the data consistent with a binary system whose components exhibit complete mutual solid solubility. This suggests that the asymmetric ripples seen in racemic DMPC multibilayers are not the result of the formation of chiral domains due to phase separation.

The structural features that can be obtained directly from

TABLE I. The observed and calculated structure factors at 24 °C and 98% RH.

<b>h</b>	<b>k</b>	<i>l</i> -DMPC		<i>dl</i> -DMPC	
		$ \mathbf{F}_o $	$\mathbf{F}_c$	$ \mathbf{F}_o $	$\mathbf{F}_c$
1	0	100.0	-80.2	100.0	-117.1
1	-1	42.5	-38.0	59.2	-32.9
2	0	29.7	-19.8	65.0	-43.3
2	-1	43.6	-40.5	68.0	-55.2
2	-2	9.3	-15.4	29.0	-38.5
2	2	15.7	-14.6	21.7	-3.2
2	3	10.6	7.9	10.6	0.3
3	0	21.2	-2.9	32.9	11.5
3	-1	42.4	33.9	53.3	39.3
3	-2	29.2	32.0	35.0	38.3
3	2	9.5	17.1	9.0	16.0
3	3	16.5	-15.9	12.3	-0.5
3	4	16.3	10.3	13.3	2.0
3	5	12.4	-4.2		
4	0	29.9	27.9	31.1	22.5
4	-1	25.5	-31.2	33.1	-37.4
4	-2	55.5	-79.5	56.8	-57.5
4	-3	27.7	-33.9	27.9	-35.8
5	0	8.5	0.6	9.6	4.6
5	-1	5.9	0.8	12.0	-0.3
5	-2	7.8	-10.1	10.8	-29.4
6	0	4.4	1.2	6.4	-5.6
6	-1	5.9	-5.4		
7	0			5.5	-2.2
9	0			4.5	-1.3

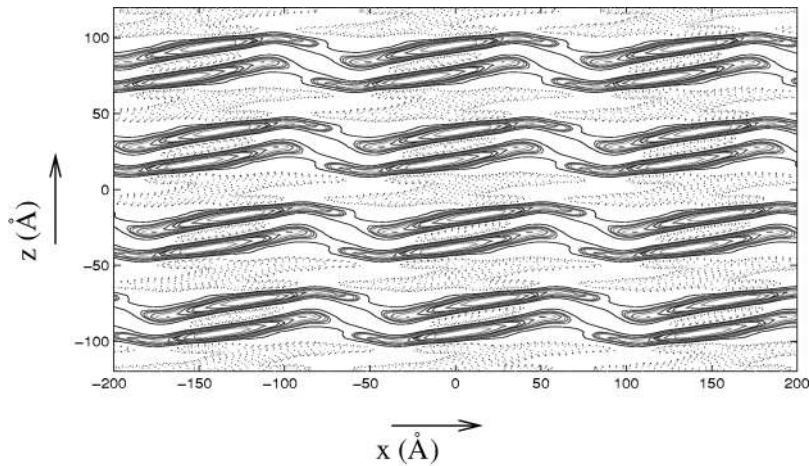


FIG. 1. Electron density map of the ripple phase of *dl*-DMPC at 24 °C and 98% RH. The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head groups. Note that the thickness of the bilayers is about 40 Å, whereas that of the water region is about 15 Å.

a diffraction pattern are the following: the wavelength of the ripples ( $\lambda$ ), the average separation between the bilayers ( $d$ ), and the angle ( $\gamma$ ) between the two axes of the unit cell. The values of these three parameters are found to be comparable in chiral and racemic DMPC bilayers under similar experimental conditions [11]. In order to check whether the detailed shapes of the ripples in these two systems are also similar, we have now calculated the electron density profiles of the ripple phase of *l* and *dl*-DMPC. In this Brief Report we present the results of these calculations. All the structural features of the ripple phase in the two systems are found to be identical within experimental errors. This confirms the conclusion of Ref. [11] that molecular chirality does not play any significant role in determining the structure of the ripple phase.

## II. EXPERIMENT

The sample was deposited on a curved glass surface from a concentrated lipid/methanol solution to obtain a stack of bilayers oriented parallel to the surface. However, the ripple direction does not get fixed by this procedure. The result is a sample that is aligned along the layer normal but is a powder in the plane of the bilayer. The temperature was controlled to

within  $\pm 0.5$  °C and the relative humidity was maintained at  $98 \pm 2\%$ . Further experimental details are to be found in Ref. [11].

## III. DATA ANALYSIS

We have applied geometrical corrections to the observed intensities relevant to our system, taking into account the fact that the sample is a powder in two dimensions and oriented in one. We have ignored absorption corrections as the thickness of the sample was not accurately known. However, by assuming reasonable values of the sample thickness, we have confirmed that the electron density profiles are not significantly affected by these corrections [13].

In order to calculate the electron density map, the phase of each Bragg reflection must be determined. As the structure possesses a center of symmetry, the phase can only take values of either 0 or  $\pi$ . To determine the phases of the reflections we have adopted the procedure developed by Sun *et al.* [14]. The electron density function  $\rho(x, z)$  is described as the convolution of a ripple contour function  $C(x, z)$  and the transbilayer electron density profile  $T_\psi(x, z)$ . Here  $\hat{x}$  is the direction of the ripple wave vector and  $\hat{z}$  is the direction

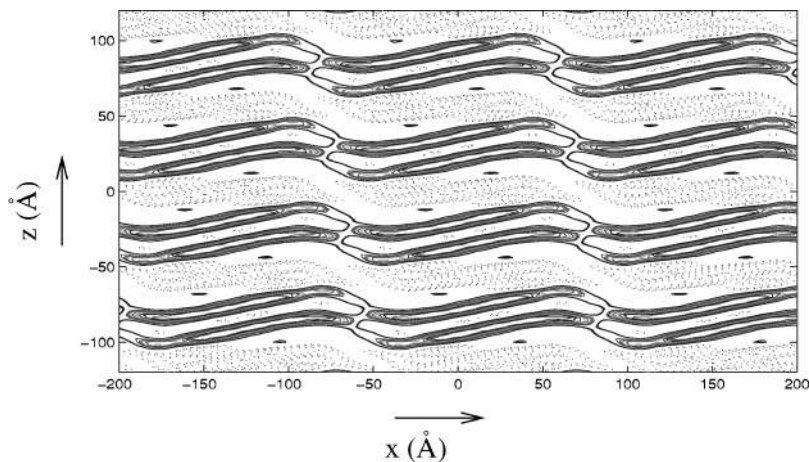


FIG. 2. Electron density map of the ripple phase of *l*-DMPC at 24 °C and 98% RH.

TABLE II. The structural parameters of the ripple phase.

		$\gamma(^{\circ})$	$\lambda(\text{\AA})$	$d$	$\lambda_1(\text{\AA})$	$A(\text{\AA})$
<i>l</i> -DMPC	24 °C	99±1	142±2	56±1	97±2	18±1
	21 °C	99	145	56	98	18
<i>dl</i> -DMPC	24 °C	98	141	56	100	19
	21 °C	98	140	56	99	18

of the average layer normal.  $C(x,z) = \delta[z - u(x)]$ , where  $u(x)$  describes the ripple profile and is taken to have the form of a sawtooth with peak-to-peak amplitude  $A$ .  $\lambda_1$  is the projection of the longer arm of the sawtooth on the  $x$  axis.  $T_{\psi}(x,z)$  gives the electron density at any point  $(x,z)$  along a straight line, which makes an angle  $\psi$  with the  $z$  axis. The electron density in the methylene region of the bilayer is close to that of water and is taken as zero.  $T_{\psi}(x,z)$  is modeled as consisting of two  $\delta$  functions with a positive coefficient of magnitude  $\rho_H$ , corresponding to the headgroup regions separated by a distance  $L$ , and a central  $\delta$  function with negative coefficient of magnitude  $\rho_M$ , corresponding to the methyl region. The six adjustable parameters in the model are  $A$ ,  $\lambda_1$ ,  $\psi$ ,  $\rho_H/\rho_M$ ,  $L$ , and a common normalizing factor. Using this model for the electron density profile, the expected structure factors at the observed  $(h,k)$  values are calculated. The calculated structure factors are compared with the observed ones and a  $\chi^2$  value is obtained, which is subsequently minimized by varying the adjustable parameters in the model. The phase of each of the Bragg reflections is obtained from the structure factors calculated from the converged model. These calculated phases are combined with the observed magnitudes of the structure factors and inverse Fourier transformed to get the electron density function.

#### IV. RESULTS AND DISCUSSION

The observed and calculated structure factors ( $\mathbf{F}_o$  and  $\mathbf{F}_c$ , respectively) for *l* and *dl*-DMPC at 24 °C and 98% RH are

given in Table I. Almost all the phases of the corresponding reflections in the two cases are the same. We have calculated the electron density profiles of the ripple phase of *l* and *dl*-DMPC bilayers at various temperatures and a relative humidity of 98±2%. The profiles at 24 °C are presented in Figs. 1 and 2. The structural parameters of the ripple phase at two different temperatures are given in Table II. As one can observe, the ripples formed by chiral and racemic DMPC bilayers do not differ appreciably. The peak-to-peak amplitude in both cases is approximately 18 Å, and the ratio of the length of the major arm to that of the minor arm is found to be 2.1 and 2.4 for *l*-DMPC and *dl*-DMPC bilayers, respectively. Furthermore, the thickness of the bilayer in the two arms is different in both cases. This can be explained in terms of an average tilt of the hydrocarbon chains along the ripple wave vector [13]. The minor differences in the two figures arise most probably because we have not been able to apply absorption corrections. Thus at all temperatures studied, the structure of the ripple phase in chiral and racemic DMPC bilayers is practically identical.

#### V. CONCLUSION

In this Brief Report we have presented electron density profiles of chiral and racemic DMPC bilayers in the ripple phase. The maps show asymmetric ripples in both the systems. Moreover, all the structural features in the two cases are found to be practically identical under similar experimental conditions. The present study thus unambiguously confirms our earlier conclusion that the structure of the  $P_{\beta'}$  phase does not depend on the chirality of the lipid molecules constituting the bilayer [11].

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