

Chapter 4

Structure of the Metastable Symmetric Ripple Phase

4.1 Introduction

As discussed in chapter-I, metastable ripples are sometimes **formed while** cooling lipids like DMPC or DPPC from the L_α phase [1]. While the basic structural features of the stable asymmetric ripple has now been well established, even the shape of the metastable ripples is still a topic of debate. In this chapter we discuss the electron density map of the symmetric ripples that we have calculated from our x-ray data. We first discuss the earlier work on this phase. In section-4.2 we describe the freeze fracture experiments and in section-4.3 we describe the x-ray experiments on this phase. In section-4.4 we describe our results and present the electron density maps of this phase. Finally in section-4.5 we summarize our results and speculate about the possible origin of the increased water layer thickness seen in this phase.

The type of ripples that we now know to be metastable and symmetric, were first seen in freeze-fracture experiments [2, 3, 4], where they show up as undulations with wavelength of about 270 Å, typically about 1.8 times the wavelength of the coexisting symmetric ripples. Because of the long wavelength, they are sometimes called A-ripples and the shorter wavelength **asymmetric** ripples are called $\Lambda/2$ ripples. From careful analysis of the defect structures present in freeze-fractured samples, the A-ripples were shown to have a symmetric shape with a plane of reflection normal

to the ripple wave-vector [5]. Later, x-ray experiments also detected ripples with wavelength about 1.8 times that of the stable asymmetric ripple. The angle γ was found to be $\frac{\pi}{2}$ and layer spacing was found to be about 80 Å which is **higher** than the layer spacing of about 70 Å seen in the stable symmetric ripples (the values are for DPPC [1]).

4.2 Freeze fracture electron microscopy of metastable ripples

Sackmann, Ruppel and Gebhardt [6] have developed a method to reconstruct the height profiles of ripples from freeze fracture electron micrographs. The sample is prepared by depositing a layer of platinum at an angle of about 45° (followed by a stabilizing layer of carbon deposited at 90°) onto a freshly freeze-fractured surface. The method is based on the analysis of the platinum layer thickness which can be determined by electron beam absorption. This thickness is a direct function of the orientation of the local tangent to the membrane surface and thus contains information about the surface profile.

Using this technique, experiments were done on vesicles of DMPC. The A ripples were found to have grooves along the maxima (figure-4.1). Thus according to this picture, a A ripple has a mirror plane perpendicular to the ripple direction: passing through the groove. No such groove was found in the case of the $\Lambda/2$ ripples. An analysis of the lengths of the arms and the amplitudes of the ripples and of the grooves show that the grooves could be a result of a structure where every second saw-tooth of an asymmetric ripple structure is rotated by 180°. In this case, two minor arms lie next to each other and form the groove. **Thus** the ripple is in the form of a series of 'M's.

Zasadzinski and Schneider [7] also analyzed freeze fractured samples of DMPC in the $P_{\beta'}$ phase and arrived at the same conclusion regarding the symmetry of the two kinds of ripples. From the height analysis of A ripples of wavelength 220 Å, they

conclude that the amplitude of the A ripples is at least 90 Å, the depth of the groove is 20 Å and the total width of the groove is 40 Å.

Because the method of three dimensional reconstruction is not very accurate, the existence of the groove is not well established. Defect analysis of freeze fractured samples on the other hand, is a much more direct method of getting information about the symmetry of the ripples, though it does not yield unambiguous results about the details of the shape (for example, the existence of the groove). Such an analysis was done by Ruppel and Sackmann [5], again on DMPC samples. In the model discussed above, A ripples have a two-fold rotation axis. **Translations** by multiples of a wavelength are allowed along the rippling direction and continuous translations are allowed in a direction perpendicular to both the ripple direction and the mean layer normal. It can be shown that in such a system (plane group: pm), stable point defects are allowed whereas line defects are not stable [8]. The point defects (**disclinations**) are analogous to two-dimensional defects in nematic liquid crystals (see [9] and figure-4.2). As in any system with a two fold axis, half-defects are stable. An analysis of the freeze-fractured surface shows that in the regions where the A ripples are present, the defect structure can be reduced to sums of half disclinations. This shows that the A ripples have a two fold rotation axis as described before.

In a system where no two-fold rotation axis is present, like in the case of the $\Lambda/2$ ripples, only integer strength defects are allowed. These defects are analogous to strength +1 and -1 defects in the c-director field of Smectic-C liquid crystals (see [9] and figure-4.2). An analysis of the $\Lambda/2$ ripple domains shows that this is indeed the case though the strength one defects decompose into two half strength defects bound by a finite line-defect. This may happen because the elastic distortions associated with the line-defects, which have a rather wide core is smaller than that associated with a point-defect with a smaller core. Thus the defect analysis of $\Lambda/2$ ripples shows that they do not have a two fold rotation axis in the plane that contains

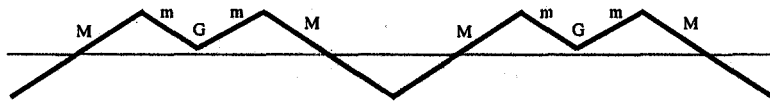


Figure 4.1: The ripple profile for the symmetric ripple as predicted from freeze fracture experiments. The 'M's are the major arms and the 'm's are the minor arms. The grooves are marked 'G'.

the ripple direction and the layer normal (plane group pl or $p2$). This result is in agreement with x-ray diffraction [10, 11] and recent freeze fracture [12] experiments.

4.3 X-ray studies of metastable ripples

The A ripples (referred to as the secondary ripple in x-ray literature) are sometimes observed in x-ray experiments. Typically they are much fainter than the $\Lambda/2$ (primary) ripples [1, 13]. The x-ray data from multi-domain samples is difficult to analyze because of the coexistence of the two kinds of ripples. Yao et. al. [1] indexed their diffraction pattern from samples of DPPC cooled from the L_α phase in terms of two lattices; one corresponding to the A ripples with wavelength (A) of 266 Å, bilayer separation (d) of 82 Å and $\gamma = 90^\circ$; the other corresponding to $\Lambda/2$ ripples with $A = 136.4$ Å, $d=70.9$ Å and $\gamma = 95^\circ$. This indexing is consistent with the freeze fracture results.

Rappolt and Rapp [14] argued that the coexistence seen in freeze fracture should not bias the indexing of reflections in x-ray experiments and went on to show that their data (DPPC) can be indexed on a single lattice with $\lambda_r=262$ Å, $d=86.3$ Å and $\gamma=107^\circ$. They phase the reflections by a pattern recognition method and get a structure which is very different from the structure of the $\Lambda/2$ ripples and is also different from the structure of the A ripples inferred from freeze fracture experiments discussed above. The bilayers have both thickness and height modulations in a manner such that the water is present in form of pockets in-between the bilayers. The bilayer thickness varies between 39 Å and 44 Å; therefore the chains are thought to be

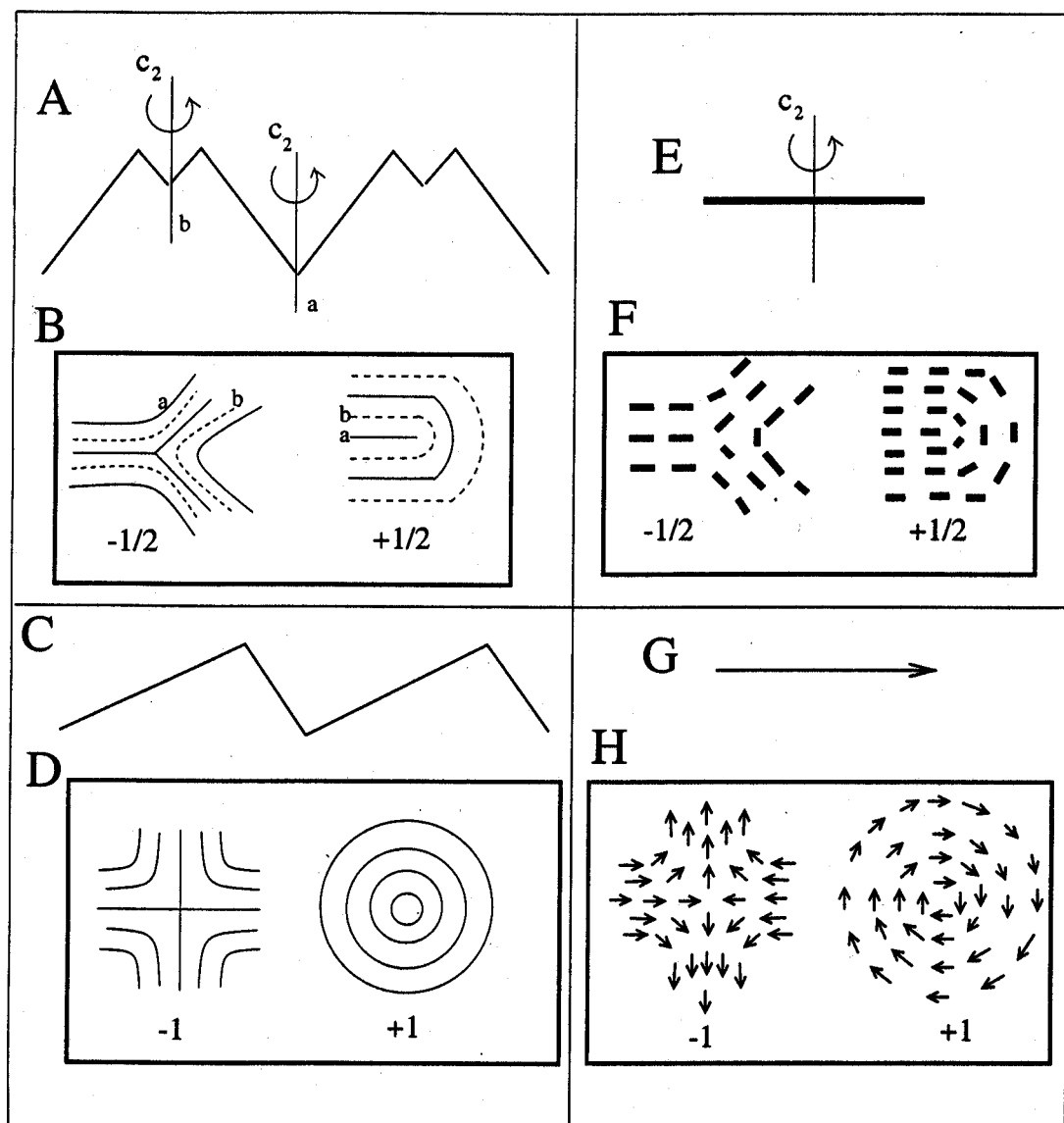


Figure 4.2: Schematic diagram of strength $1/2$ and strength 1 defects in two dimensional systems. A: the profile for A ripples with two 2-fold (C_2) rotation axis a and b. The symmetry is same as that of an apolar vector or a nematic director. B: half strength defects seen in freeze fracture of the A ripples. The solid lines correspond to a and the dashed lines to b. C: profile for $\Lambda/2$ ripples - there is no 2-fold rotation axis and therefore the symmetry is that of a vector. D: strength one defects expected in freeze fracture of $\Lambda/2$ ripples. E: Nematic director, which has the same symmetry as A ripples. F: director configuration in half strength defects in nematics. G: a vector, which has the same symqetry as the $\lambda/2$ ripples. An example is the c-vector in Sm, liquid crystals. H: the c-vector configuration in strength one defects.

all-trans and tilted, with the tilt angle varying from 30° to 40° .

Mason et. al. [15] also index their x-ray data from the metastable phase on a single lattice: $\lambda_r=330 \text{ \AA}$, $d=73 \text{ \AA}$ and $\gamma=126^\circ$. They further show that the data of Yao et. al. [1] can also be fitted using this single lattice model, with $\lambda_r=350 \text{ \AA}$, $d=71 \text{ \AA}$ and $\gamma=129^\circ$. The major problem with this sort of indexing is that it would imply that an altogether different sort of phase with a wavelength of $\approx 250 \text{ \AA}$ is observed in freeze fracture.

4.4 Electron density map of metastable ripples in DPPC

We have calculated electron density maps of the metastable ripples for oriented Samples of DPPC. The experiments were performed at the CHESS synchrotron facility (Cornell high-energy synchrotron source, Ithaca, USA) using incident radiation of wavelength $1.3808 \text{ \AA}$. The data was collected using a CCD camera. The sample was prepared using the method described in chapter II. The humidity was maintained at 100%. The A ripples are formed while cooling the sample from the L_α phase. From the diffraction patterns it can be seen that the angle γ is always $\pi/2$ for the Λ ripples. Thus the indexing in references [14] and [15] are clearly incorrect. In all such pictures, there is coexistence of the A phase (with $\gamma=\pi/2$) and the $\Lambda/2$ phase (with $\gamma \neq \pi/2$). A typical diffraction pattern is shown in figure-4.3. This data corresponds a temperature of 39.2°C and shows $\lambda_r=254.5 \text{ \AA}$, $d=79.5 \text{ \AA}$ and $\gamma=90^\circ$. These results are in good agreement with freeze fracture results [5, 7] and with the results of Yao et. al. [1].

The data reduction procedure for calculating the electron density maps is described in chapter-11. Since $\gamma=90^\circ$ implies an overlap of reflections with Miller indices $+\mathbf{k}$ and $-\mathbf{k}$, an assumption that the $+\mathbf{k}$ and the $-\mathbf{k}$ reflections have the same intensity is necessary. Therefore, the intensity recorded for each satellite reflection has been

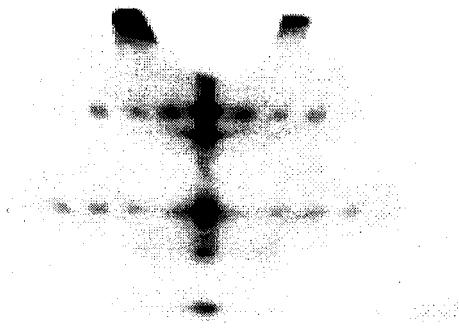


Figure 4.3: **A typical diffraction pattern for the metastable ripples at 39.2 °C and 100 % RH.** A small part of the main beam that has escaped the beam stop is seen near the top. Some of the main reflections are over exposed in this picture - this is necessary to get the satellite reflections. The over exposure is corrected for by comparing with an image where exposure time is less. The (1,0) and (2,0) reflections from the asymmetric ripple (seen as weaker spots immediately below the corresponding main reflection from the symmetric ripple) is also present. The angle γ is clearly 90° .

equally distributed between the $+k$ and $-k$ reflections. The geometrical corrections are applied as before. The corrected intensities are fitted using the modeling and least squares fitting procedure described in chapter-11. To phase the reflections, different initial models for the ripple profile $C(x, z)$ have been tried, but none of them give a completely satisfactory electron density map. The different possible forms for the ripple profile $C(x, z)$ shall be discussed below. For the trans-bilayer profile $T_\psi(x, z)$, model-I where the head-groups and the methyl end group are modeled as delta functions (see chapter-11) was used.

4.4.1 Sawtooth shape (0-groove model)

The simplest choice for $C(x, z)$ is the saw-tooth shaped model that was used to phase the asymmetric ripples (see chapter-11). In the limit where the length of the longer arm is equal to half the wavelength ($\lambda_1 = \lambda_r/2$), the saw-tooth goes to a symmetric triangular shape. The electron density map for the saw-tooth model is given in figure-4.4 and the corresponding observed and calculated structure factors are given

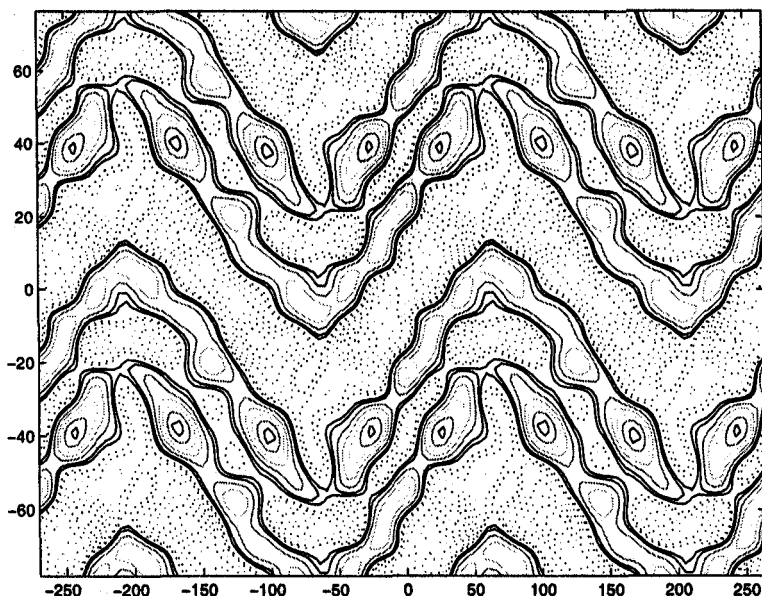


Figure 4.4: **Electron Density Map of the ripple phase of DPPC at temperature = 39.2°C and RH=100% using the 0-groove model.** The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 50 Å, whereas that of the water region is about 30 Å.

in table-4.1. The phases are determined by the least squares fitting method described in chapter-I1 but since convergence is poor, the maps are also checked for their goodness using some criteria expected to be satisfied by the electron density distribution. These criteria are: the bilayers are continuous and distinct, bilayers do not cross or touch and the head-group region has a fairly uniform thickness. Maps corresponding to a lower least square deviation are sometimes rejected if the above criterion are not fulfilled. The converged values of the fitted parameters are given in table-4.4.

As can be seen from the figure, the ripple shape has a plane of reflection normal to the ripple wave-vector. The peak-to-peak amplitude of the ripples is about 60 Å, the bilayer thickness varies from about 50 Å to 53 Å and the water layer thickness is about 30 Å. The thickness of the head-group region in one of the monolayers of the bilayer is very small near the peaks; this probably indicates that the initial model chosen differs considerably in the peak region from the real structure.

Table 4.1: Observed and calculated structure factors for **DPPC** at temperature = **39.2°C** and **RH=100%** (0-groove model).

h	k	$ F_o $	$ F_c $	h	k	$ F_o $	$ F_c $
1	0	100.0	-28.3	2	-5	46.2	-32.7
1	1	78.1	91.9	3	0	43.6	12.9
1	-1	78.1	-88.3	3	1	5.2	-2.8
1	2	67.4	-78.6	3	-1	5.2	3.8
1	-2	67.4	-80.3	3	2	36.7	-15.7
1	3	96.7	25.5	3	-2	36.7	-14.7
1	-3	96.7	-32.6	3	3	8.9	2.4
1	4	47.5	8.1	3	-3	8.9	-6.9
1	-4	47.5	4.4	3	4	10.3	36.6
2	0	114.8	34.7	3	-4	10.3	28.4
2	1	5.2	-29.9	3	5	28.7	-54.3
2	-1	5.2	31.7	3	-5	28.7	53.3
2	2	68.1	-58.8	3	6	44.1	31.3
2	-2	68.1	-46.3	3	-6	44.1	41.0
2	3	90.4	125.0	3	7	48.1	0.6
2	-3	90.4	-112.6	3	-7	48.1	8.9
2	4	107.7	-94.7	4	0	29.4	9.4
2	-4	107.7	-99.1	5	0	56.4	-16.9
2	5	46.2	17.6	6	0	24.8	-2.0

4.4.2 Triangular ripple with groove at both extrema (2-groove model)

From the electron density map obtained with the saw-tooth model, it is clear that this shape is only an approximation to the real shape especially near the peaks. Therefore, keeping in mind the freeze fracture result of Sackmann et. al. [6], the initial ripple profile was taken to be a triangular shape with grooves (with variable depths) at the peaks and troughs. Though the freeze fracture shows grooves only at the peaks and not at the troughs, a model with grooves at both the extrema was used since the one-groove model is non-centrosymmetric and therefore difficult to phase. It was expected that some features of the structure may be obtained even with the centrosymmetric model, which could be used as input for the non-centrosymmetric model discussed later.

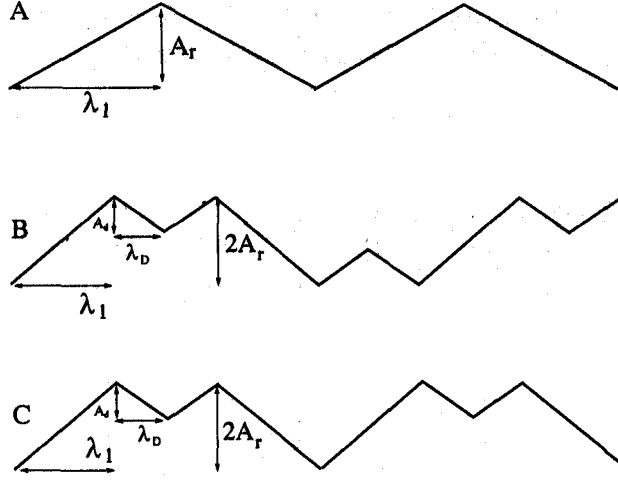


Figure 4.5: Schematic diagram of the model ripple profile for the (A) 0-groove, (B) 2-groove and (C) 1-groove models.

$C(x, z)$ in this case is:

$$C(x, z) = \delta(z - u(x))$$

$u(x)$ is given by $u(x) = m(x + c)$, where

$m = \frac{A_r}{\lambda_D}$	$c = 2(\lambda_1 + \lambda_D)$	when $-2(\lambda_1 + \lambda_D) < x < -(\lambda_1 + 2\lambda_D)$
$m = -\frac{A_d}{\lambda_D}$	$c = (\lambda_1 + 2\lambda_D - A_r \frac{\lambda_D}{A_d})$	when $-(\lambda_1 + 2\lambda_D) < x < -(\lambda_1 + \lambda_D)$
$m = \frac{A_d}{\lambda_D}$	$c = \lambda_1 + A_r \frac{\lambda_D}{A_d}$	when $-(\lambda_1 + \lambda_D) < x < -\lambda_1$
$m = -\frac{A_r}{\lambda_1}$	$c = 0$	when $-\lambda_1 < x < \lambda_1$
$m = \frac{A_d}{\lambda_D}$	$c = -(\lambda_1 + A_d \frac{\lambda_D}{A_d})$	when $\lambda_1 < x < \lambda_1 + \lambda_D$
$m = -\frac{A_d}{\lambda_D}$	$c = -(\lambda_1 + 2\lambda_D - A_r \frac{\lambda_D}{A_d})$	when $\lambda_1 + \lambda_D < x < \lambda_1 + 2\lambda_D$
$m = \frac{A_r}{\lambda_1}$	$c = -2(\lambda_1 + \lambda_D)$	when $\lambda_1 + 2\lambda_D < x < 2(\lambda_1 + \lambda_D)$

Here A_r is the ripple amplitude, A_d is the groove depth, λ_D is half the width of the groove and λ_1 is $(\lambda_r/4 - \lambda_D)$, A_r is the wavelength of the ripple (see figure 4.5). This model gives the best fit for $A_d=0$. This is a special case where the top and the bottom of the triangular shape is flat. The electron density maps calculated with this model is shown in figure-4.6. The corresponding observed and calculated structure factors are given in table-4.2. The converged values of the fitted parameters are given in table-4.4.

The ripple shape clearly has two 2-fold rotation axis in the plane containing the rippling direction and the layer normal (plane of the paper). In figure-4.6 the thickness of the bilayer varies from about 32 Å for the region near the peak to about 56 Å for

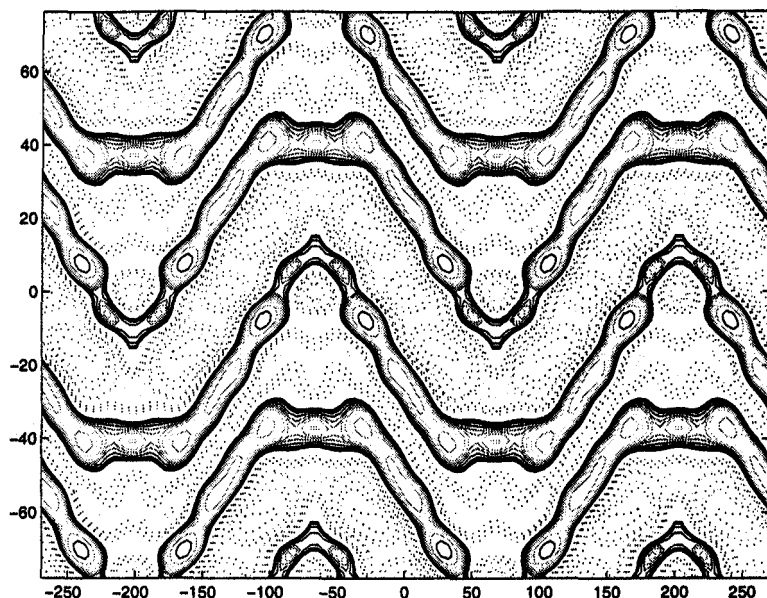


Figure 4.6: Electron Density Map of the ripple phase of DPPC at 39.2°C and 100% RH for the 2-groove model. The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 50 Å, whereas that of the water region is about 30 Å.

Table 4.2: Observed and calculated structure factors for DPPC at 39.2°C and 100% RH. (2-groove model)

h	k	$ F_o $	$ F_c $	h	k	$ F_o $	$ F_c $
1	0	100.0	-34.3	2	-5	46.2	16.5
1	1	78.1	-107.5	3	0	43.6	11.1
1	-1	78.1	94.8	3	1	5.2	-2.8
1	2	67.4	-71.1	3	-1	5.2	9.7
1	-2	67.4	-72.0	3	2	36.7	-19.8
1	3	96.7	-31.2	3	-2	36.7	-21.6
1	-3	96.7	19.2	3	3	8.9	7.9
1	4	47.5	8.0	3	-3	8.9	-1.5
1	-4	47.5	10.1	3	4	10.3	38.6
2	0	114.8	60.4	3	-4	10.3	37.1
2	1	5.2	11.9	3	5	28.7	53.2
2	-1	5.2	-28.9	3	-5	28.7	-49.3
2	2	68.1	-68.7	3	6	44.1	32.7
2	-2	68.1	-65.4	3	-6	44.1	30.6
2	3	90.4	-129.1	3	7	48.1	5.7
2	-3	90.4	110.3	3	-7	48.1	-1.2
2	4	107.7	-82.2	4	0	29.4	8.6
2	-4	107.7	-75.6	5	0	56.4	4.0
2	5	46.2	-31.2	6	0	24.8	1.8

rest of the bilayer. This indicates that most of the chains are fully stretched and have zero mean tilt. The water layer thickness is about **39 Å** and the peak to peak amplitude of the ripple is about 50 Å.

Both the centrosymmetric shapes considered so far yield electron density maps where the top and bottom monolayers of the bilayers seem to have different shapes. In case of the 2-groove model, wherever the top monolayer is flat, the bottom monolayer has a sharp maximum and vice versa. This sort of shape where the two monolayers of the bilayer have different profiles, is highly unlikely. Therefore we conclude that though the gross features like the amplitude, layer thickness etc. can be reliably read off these electron density maps, the map is not good enough to detect the finer features. Since centrosymmetric structures fail to give a good electron density map of the feature at the top, the structure is probably non-centrosymmetric.

4.4.3 Non-centrosymmetric profile (1-groove model)

The ripple profile suggested by freeze fracture experiments [6, 7] indicates the presence of a groove only at one of the extrema of the triangular shape. This shape, with one groove per wavelength, is non-centrosymmetric and hence the phase factor is no longer constrained to be ± 1 . Since now Φ can be any angle, it is no longer possible to follow a multidimensional fitting procedure to obtain the phases because the model does not always converge to any reasonable point.

$C(x, z)$ in this case is:

$$C(x, z) = \delta(z - u(x))$$

where $u(x)$ is given by $u(x) = m(x + c)$,

$$\begin{array}{llll} m = \frac{A_r}{\lambda_D} & c = 2(\lambda_1 + \lambda_D) & \text{when} & -2(\lambda_1 + \lambda_D) < x < -(\lambda_1 + 2\lambda_D) \\ m = -\frac{A_d}{\lambda_D} & c = (\lambda_1 + 2\lambda_D - A_r \frac{\lambda_D}{A_d}) & \text{when} & -(\lambda_1 + 2\lambda_D) < x < -(\lambda_1 + \lambda_D) \\ m = \frac{A_d}{\lambda_D} & c = \lambda_1 + A_r \frac{\lambda_D}{A_d} & \text{when} & -(\lambda_1 + \lambda_D) < x < -\lambda_1 \\ m = -\frac{A_r}{\lambda_1} & c = 0 & \text{when} & -\lambda_1 < x < \lambda_1 + \lambda_D \\ m = \frac{A_r}{\lambda_1} & c = -2(\lambda_1 + \lambda_D) & \text{when} & \lambda_1 + \lambda_D < x < 2\lambda_1 \end{array}$$

Here A_r is the ripple amplitude, A_d is the groove depth, λ_D is half the width of the

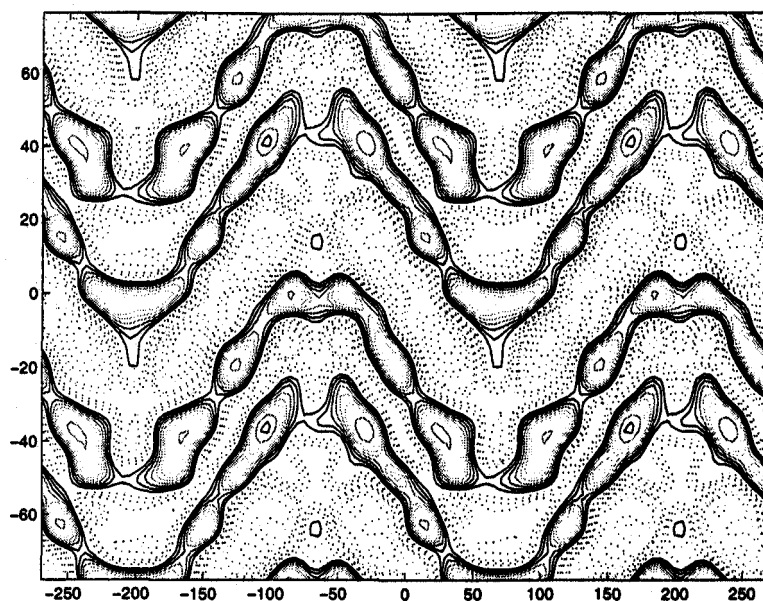


Figure 4.7: **Electron Density Map of the ripple phase of DPPC at 39.2°C and 100% RH using the l-groove model.** The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 50 Å, whereas that of the water region is about 30 Å.

groove and λ_1 is $(\lambda_r/4 - \lambda_D)$, A_r is the wavelength of the ripple (see figure-4.5). All the ripple parameters like A_r , the bilayer thickness L , the angle ψ and the ratio of head-group to terminal methyl group electron density R_{hm} are fixed at the values measured from the electron density maps obtained with the centro-symmetric models. The depth of the groove is allowed to vary and a one dimensional least squares fit is performed. This is followed by iterative least square fits of each of the variables taken one at a time till the variations from one fit to the next are negligible. The maps are checked for continuity and other criterion for goodness **as** discussed earlier. The values of A_r and A_d reported in freeze fracture experiments [7] have also been tried **as** inputs but the electron density maps corresponding to values close to these are found to be unphysical. The best electron density map that could be obtained is given in figure-4.7 and the corresponding calculated structure factors and phases are given in table-4.3. The converged values of the fitted parameters are given in table-4.4.

The overall shape of the final structure is similar to the structure obtained using the

Table 4.3: **Observed and calculated structure factors and phases Φ_c for DPPC at 39.2°C and 100% RH: $-\pi < \Phi_c < \pi$ (l-groove model)**

h	k	$ F_o $	$ F_c $	Φ_c	h	k	$ F_o $	$ F_c $	Φ_c
1	0	100.0	34.5	3.0	2	-5	46.2	18.0	0.4
1	1	78.1	107.7	3.1	3	0	43.6	11.6	-0.3
1	-1	78.1	94.9	0.0	3	1	5.2	3.6	-2.5
1	2	67.4	71.1	3.1	3	-1	5.2	10.0	-0.2
1	-2	67.4	72.1	3.1	3	2	36.7	21.7	2.7
1	3	96.7	31.2	3.1	3	-2	36.7	22.8	2.8
1	-3	96.7	19.5	0.2	3	3	8.9	8.6	-0.4
1	4	47.5	10.2	-0.7	3	-3	8.9	1.7	-2.8
1	-4	47.5	10.1	0.0	3	4	10.3	38.7	-0.1
2	0	114.8	61.3	-0.2	3	-4	10.3	37.5	-0.1
2	1	5.2	12.9	-0.4	3	5	28.7	53.2	-0.1
2	-1	5.2	31.4	2.7	3	-5	28.7	49.3	3.1
2	2	68.1	68.7	3.1	3	6	44.1	33.8	0.2
2	-2	68.1	65.6	3.1	3	-6	44.1	31.0	0.2
2	3	90.4	130.0	3.0	3	7	48.1	6.7	-0.6
2	-3	90.4	110.7	-0.1	3	-7	48.1	2.0	2.2
2	4	107.7	83.3	3.0	4	0	29.4	10.5	-0.6
2	-4	107.7	75.9	3.1	5	0	56.4	8.2	-1.0
2	5	46.2	31.3	3.1	6	0	24.8	2.2	0.5

centrosymmetric models. The **peak-to-peak** amplitude is about 48 Å and the layer thickness varies from about 46 Å to about 55 Å. The finer features are again poorly resolved. A shallow depression is seen at the peak but no corresponding elevation is evident at the trough. This non-centrosymmetric structure is to be expected, given the constraints on the starting model. Even with this non-centrosymmetric model, the electron density map is not good enough to make any meaningful comment on the details of the structure of the ripples near the peaks.

The final converged values of the various parameters, the crystallographic R factor and the least square deviation (C) are given in table-4.4. The converged values of the parameters are important only because they are used to calculate the structure factors and hence the phases of the reflections. The phases of the reflections are not very sensitive to small changes in these parameters and these parameters are not used directly to calculate the electron density maps. Therefore, the values of these

Table 4.4: The converged values of the parameters using the three models:

	0-groove	2-groove	1-groove
λ_1	131.5	57.4	124.6
A_r	67.0	18.0	30.0
A_d		0.0	17.0
ψ	0.0	0.0	0.0
R_H/R_M	1.2	1.5	1.2
L	49.0	49.0	49.0
R	0.5	0.5	0.5
Σ	309034	252169	46510

measured from the electron density maps are different from the converged values given in table-4.4.

4.4.4 Patterson Map

A plot of the fourier transform of the intensities (rather than the structure factors) is called the Patterson map. The Patterson map represents the convolution of the electron density function with its centrosymmetric image. Patterson map peaks occur at positions that correspond to interatomic vectors within the real space unit cell. Therefore, if there are N atoms in an unit cell, there are $N(N - 1)$ peaks in the Patterson map. The use of Patterson map to determine a complicated structure like the present case is limited but a comparison of the Patterson maps obtained using the observed intensities and the intensities calculated from each of the above models may give some clue about the relative accuracy of each of the three models.

The Patterson map calculated with the observed structure factors is given in figure-4.8. The central peak corresponds to the self correlation of the atoms and is the dominant feature. There are a few relatively weaker peaks and a weak “X”-pattern is seen centered on the central maximum. We are unable to understand these features of the map though a comparison with the Patterson maps obtained using the calculated structure factors show that the 0-groove and the 1-groove models capture some of these features.

For comparison, the Patterson map from the asymmetric ripple phase (data of Wack and Webb [16]) is shown in figure-4.12. The central peak is the dominant feature here- it consists a thick tilted "X" with unequal arms. This represents the correlation within each arm of the saw-tooth.

4.5 Conclusion

From the electron density maps presented here, it is clear that the metastable ripples have a two fold rotation axis in the plane containing the layer normal and the ripple wave-vector. The amplitude is about 50 Å and is in agreement with freeze fracture results of an amplitude of **atleast** 45 Å. The thickness of the water layer is about 30 Å, about 10 Å more than in the asymmetric ripples. A layer thickness of about 50 Å indicates that the chains are mostly all-trans at least away from the peaks. The shape of the ripples near the peaks is poorly resolved. Though our calculations indicate that the structure is non-centrosymmetric with probably a groove at the peak, depth of this groove is likely to be much less than the values estimated from freeze fracture experiments [5, 6, 7].

The interesting question here is what determines the thickness of the water layers and why this thickness should be greater in the symmetric ripples than in the asymmetric ripples. The long range interactions possible in these systems are the electrostatic interaction and the Helfric interaction [17] arising from thermal fluctuation of the bilayers. Since the chains are mostly all-trans and rigid, the Helfric interaction should be negligible. The only possible source of coulomb interactions is through the dipolar head groups. Since the configuration of the head-groups in the two kinds of ripples are not known, the details of dipolar interactions can not be further commented upon. An indirect way of checking this **hypothesis** would be to look at lipid-water systems with added salt so that the electrostatic interaction is screened. Another interesting

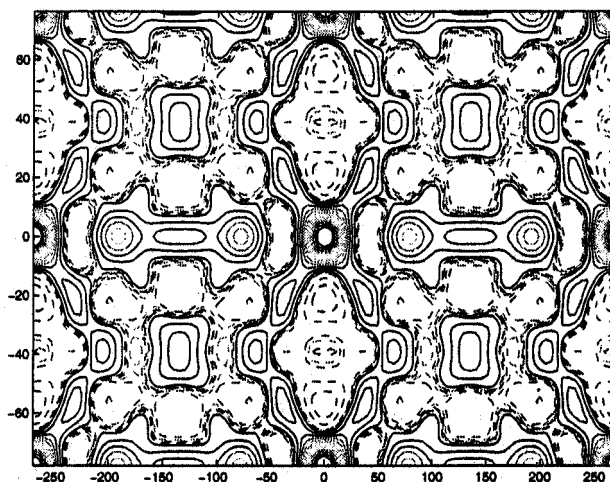


Figure 4.8: Patterson map of data set given in table I. The positive (negative) contours are represented by solid (dashed) lines.

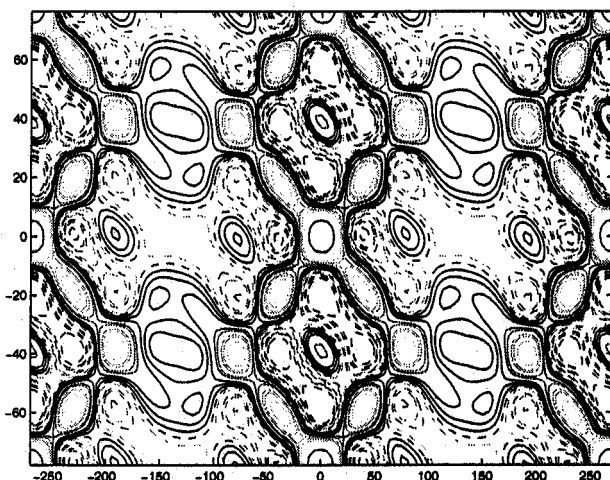


Figure 4.9: Patterson map using intensities calculated from the O-groove model. The positive (negative) contours are represented by solid (dashed) lines.

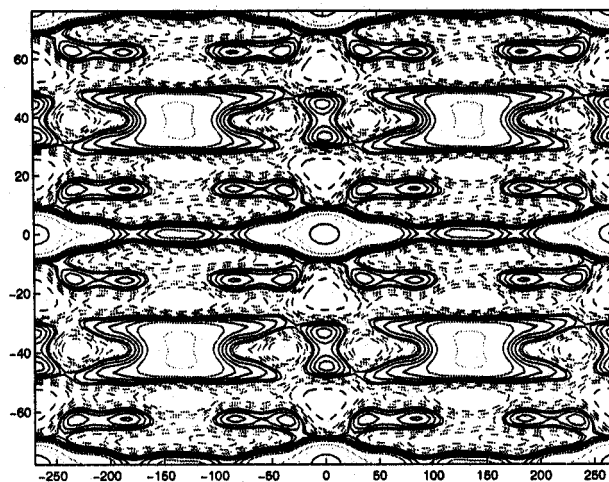


Figure 4.10: Patterson map using intensities calculated from the 2-groove model. The positive (negative) contours are represented by solid (dashed) lines.

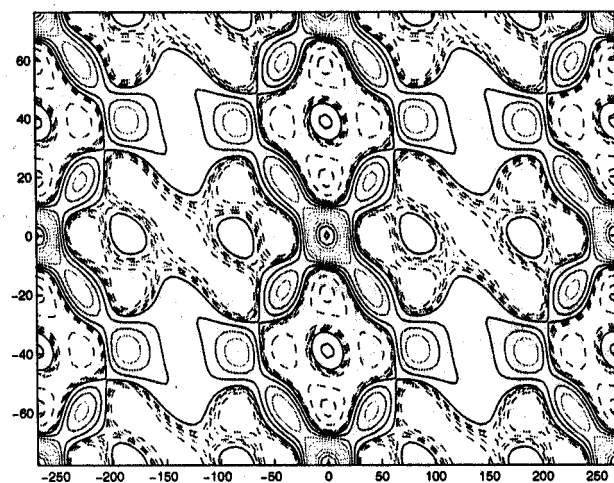


Figure 4.11: Patterson map using intensities calculated from the 1-groove model. The positive (negative) contours are represented by solid (dashed) lines.

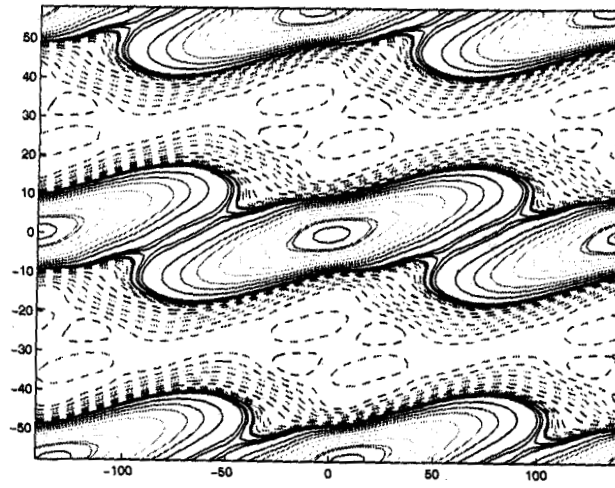


Figure 4.12: Patterson map using observed intensities for the asymmetric ripple (data from [16]). The positive (negative) contours are represented by solid (dashed) lines.

possibility is to introduce divalent cation that are known to increase the layer spacing [18] and check if the ripples go from asymmetric to symmetric when they swell.

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Chapter 5

A Landau Theory of the Ripple Phase: Asymmetric ripples in Achiral Membranes

5.1 Introduction

As discussed in earlier chapters, recent freeze fracture and x-ray experiments have shown that the height modulated bilayers in the $P_{\beta'}$ (ripple) [1, 2, 3, 4] phase of phospholipids lack a plane of reflection normal to the ripple wave-vector and hence are asymmetric [5, 6]. On cooling the sample from the L_{α} phase, metastable symmetric ripples are sometimes seen [7, 8, 9, 10]. These metastable ripples have a wavelength about twice that of the stable asymmetric ripples. The shape of these symmetric ripples has not yet been determined unambiguously. Hentschel and Rustichelli [11] have reported yet another kind of ripple where the height modulation is symmetric but the wavelength is comparable to that of the asymmetric stable ripple phase.

In this chapter we first describe the various theories that have been proposed for the ripple phase. In section-5.2 we discuss the **Lubensky-MacKintosh** [12, 13] model in detail. In section-5.3 we discuss theories that predict asymmetric modulations in achiral systems. In section-5.4 we discuss our extension of **Lubensky-MacKintosh** theory which, in contrast to the results of the Lubensky-MacKintosh model, admits the experimentally observed *asymmetric* ripple phase in *achiral* bilayers.

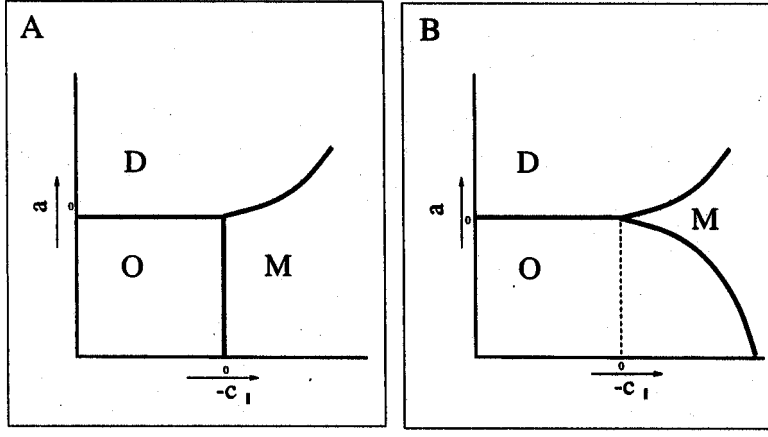


Figure 5.1: Schematic phase-diagrams for the Lifshitz free energy density (eq.:5.1). (A) for a vector order parameter and (B) for a scalar order parameter. D: disordered phase, O: ordered phase and M: modulated phase.

Before describing the various theories of the ripple phase, it would be relevant to discuss the Landau theory with a Lifshitz point used to describe modulated phases in general.

5.1.1 Lifshitz free energy for modulated phases

Modulated phases can usually be described by a Lifshitz free energy density of the form [14]:

$$f_{Lif} = \frac{1}{2}a\phi^2 + \frac{1}{4}b\phi^4 + \frac{1}{2}c_{\parallel}(\nabla_{\parallel}\phi)^2 + \frac{1}{2}c_{\perp}(\nabla_{\perp}\phi)^2 + \frac{1}{2}\alpha(\nabla^2\phi)^2 \quad (5.1)$$

The field ϕ can be a scalar or a n-component vector. When a , $c_{\parallel}$ and $c_{\perp}$ are positive, a disordered phase ($\phi = 0$) is the lowest energy state. As a changes sign, the system goes over to an ordered state ($\phi \neq 0$). When both $c_{\parallel}$ and $c_{\perp}$ are positive, the ordered phase is spatially uniform. If, however, $c_{\parallel}$ (or $c_{\perp}$) is negative, the system can lower its energy by creating spatially modulated structures with wave-vectors of magnitude $|c_{\parallel}|/2\alpha$ (or $|c_{\perp}|/2\alpha$). The last term in eq.-5.1 is the stabilizing term and therefore the coefficient α has to be always positive. The phase boundaries between the disordered phase and the two ordered phases are second order lines. The phase boundary between the ordered uniform and the ordered modulated phase is second order when the order parameter is a vector and is first order when the order parameter is a scalar. The three phase boundary lines (separating the ordered phase,

the disordered phase and the modulated phase from each other) meet at the point $a = 0, c_{\parallel} = 0$ (or $a = 0, c_{\perp} = 0$). This point is called the Lifshitz point (see figure-5.1). The ripple phase, being a modulated phase should also be describable in terms of a Lifshitz like free energy.

5.1.2 Previous theories of the Ripple phase

The theories proposed for the ripple phase fall into two main categories (see appendix A in [15] for a review). (1) Microscopic theories which are based on properties of individual molecules forming the bilayer. (2) Continuum theories in which the bilayers are treated as a continuous membrane. The Landau theories of the ripple phase fall in this category. There are a few other approaches, for example those based on interactions between adjacent bilayers.

Before discussing the theories of the ripple phase, we shall briefly describe the models that have been proposed, based on experimental work, for the structure of this phase. The first model was that of Tardieu et. al. [3] (also see chapter-I). In the Tardieu model the chains of the lipid molecules are assumed to be stiff and parallel. The height modulation of the bilayer is produced by relative sliding movements of the stiff close-packed chains. Helfrich [16] proposed spontaneous curvature as the specific mechanism which may lower the total elastic energy of the rippled bilayers.

Larsson [17] proposed a structure consisting of alternating regions of vertical and tilted chains, for this reason the bilayer is saw-tooth shaped ('folded' in the language used by Larsson). The mechanism proposed by Larsson for the formation of such a structure was the following. It has been experimentally seen [4] that as the sample is heated from the $L_{\beta'}$ phase, at the pre-transition (ie. the $L_{\beta'}$ to $P_{\beta'}$ transition), the chain packing goes from orthorhombic to hexagonal, indicating an increase in the effective chain area (and hence an increase in intra-chain disorder). This would force a simultaneous increase in the head-group area. Larsson postulated that only

a portion of the total chain population becomes disordered at the pre-transition. In that case, a change in the head-group area can be avoided by having alternate regions of tilted and untilted chains. The tilted chains are less disordered and are taken to be in the all-trans conformation. The tilt is due to a vertical shift by one zig-zag unit (corresponding to two carbons on the chain) of adjacent chains. The tilted molecules sit in the short arm of the saw-tooth. The disordered chains have a larger cross-sectional area and therefore need not tilt in order to accommodate the head-groups. The untilted chains sit in the long arm of the saw-tooth.

Based on their freeze fracture experiments, Sackmann *et. al.* [8, 9] proposed a possible structure for both the stable asymmetric and the metastable symmetric ripples. The structure of the stable asymmetric phase is somewhat similar to that proposed by Larsson. The metastable symmetric phase is pictured as being formed by joining a one-wavelength long piece of the ripple with its own mirror image, thus giving the resultant ripples a "M" shape. Here, the driving mechanism of ripple formation is a mismatch between the requirements of the packing of the rigid zig-zag chains and the packing of the head-groups. The difference between this picture and that of Larsson is that here the long and the short arms of the saw-tooth have chains tilted at 28° (shift by 2 carbon atoms) and 46° (shift by 4 carbon atoms) instead of 0° (no shift) and 28° (shift by 2 carbon atoms) respectively.

Recently Sun *et. al.* [5], based on their electron density map for the ripple phase obtained from data of ref. [18], have proposed a model where the ripple has a saw-tooth shape; the smaller arm of the saw-tooth has molten L_α like chains and the longer arm has stretched $L_{\beta'}$ like chains. The drawbacks of this model were discussed in chapter-III.

We have reanalyzed the data of ref.- [18] and have shown that all the features of the electron density maps can be explained in terms of a mean tilt of the chains in the

rippling direction (see chapter-11). In our model, the chains are predominantly *all-trans* and parallel to each other. Like in the Tardieu model, the height modulation arises from relative shifting of the chains. In the shorter arm, they make an angle of about 30° with respect to the local layer normal whereas they are almost parallel to the local layer normal in the longer arm. Thus the shorter arm has a $L_{\beta'}$ like structure and the longer arm has a L_{β} like structure (see chapter-I). Thus our results are in agreement with the model proposed by Larsson.

We now describe the various theories that have been proposed for the ripple phase and discuss their relative merits with reference to the above mentioned structure of $P_{\beta'}$ phase that we have inferred from the electron density maps.

Microscopic theories:

Based on the idea that the inter-chain interactions drive the formation of the ripple phase, Hawthorn and Keeler [19] numerically calculated the van der Waals energy of a spatially modulating layer and showed that in one regime the free energy is lower than that of a flat layer.

In many of the microscopic theories, the packing constraint imposed by the **head**-groups is an important feature. Pearce and Scott [20] studied a model based on the microscopic mechanism of the competition between the van der Waals attraction between the hydrocarbon chains and the packing hindrance imposed by the head groups. In their description, interactions between the nearest and the next nearest neighbor is taken into account. The Hamiltonian of this system can be reduced to that of the nearest neighbor Ising model (ANNI) under certain approximations. This model exhibits a rippled phase between a high temperature disordered phase and a low temperature ordered phase.

Carlson and Sethna [15] proposed a model where the height modulation of the

bilayer arises due to periodic defect walls.. In this model, each lipid molecule is pictured as a head-group attached to a single chain (which represents the average of the two chains). The local packing properties of a one dimensional monolayer of such molecules are described in terms of pair interactions between nearest neighbors. The optimal separation h between the head-groups is taken to be larger than the equilibrium separation r_0 between two free chains. This competition leads to three distinct phases that correspond to a L_α like phase with zero tilt, a uniformly tilted $L_{\beta'}$ like phase and a modulated $P_{\beta'}$ like phase. The minimum energy configuration depends on the size mismatch and the relative strengths of the chain and head group pair interactions.

The van der Waals interaction between neighboring chains is described in terms of a potential $U(r)$, where r is the distance between adjacent chains. $U(r)$ has a minimum at $r = r_0$. The most important feature of $U(r)$ is that it should be soft. As long as $U(r)$ is soft enough, the specific shape of $U(r)$ is not important.

The coupling between the head-groups and the chains is a function of the chain tilt θ alone and is taken to be a harmonic potential: $\frac{1}{2}W\sin^2(\theta)$. r and θ are related by $r = h\cos(\theta)$. The free energy, $U(\bar{r}) + \frac{1}{2}W\sin^2(\bar{\theta})$ ($\bar{r}$ and $\bar{\theta}$ are the course-grained versions of the molecular variables r and θ) has three distinct solutions. (1) When $U(r)$ has one global minimum at $\bar{\theta} = 0$, $\bar{r} = h$ and this corresponds to a high temperature, non-tilted L_α like phase. (2) When $U(r)$ has a pair of global minima at $\bar{\theta} = \pm\theta_1$, a uniformly tilted phase like the $L_{\beta'}$ phase is obtained. (3) Solutions corresponding to a modulated $P_{\beta'}$ like phase is also possible. To see this, the zero-force equation for the n^{th} molecule is numerically solved to get the tilt θ_n of the n^{th} molecule. $U(r)$ is taken to be a soft Lennard-Jones potential given by $U(r)=G[(\frac{r_0}{r})^{12} - 2(\frac{r_0}{r})^6]$. The softness of the potential $U(r)$ allows for a solution where the tilt slowly oscillates in space about a zero mean and then suddenly changes direction and then starts to oscillate slowly again, thus giving rise to periodic defect walls. Carlson and Sethna argue that

such walls would result in a height modulation of the bilayers. These three phases are found in the parametric space spanned by $\frac{W}{G}$ and $\frac{h}{r_0}$. They also present a continuum version of their model, which essentially leads to similar results. In this model, the tilt is constrained to be in the ripple direction; but this need not be the case. Also, this model predicts a periodic array of defects in a flat bilayer. It is conjectured that such an array would give rise to a height modulation but the specific mechanism for this is not clear.

Recently, Misbah et. al. [21] have proposed a theory based on electrostatic considerations. This theory is based on the interaction between two sheets of dipoles (head-groups). In this model, a membrane modulation reduces the dipolar energy. At a critical intermembrane distance, the ripple-wave vector goes from zero to a non-zero value. This model is able to account for the experimentally observed asymmetric ripples, which lack a plane of reflection normal to the rippling direction. The main drawback of this theory is that it does not take care of the fact that tilt of the chains is essential for the formation of the ripple phase.

Landau theories of the ripple phase:

Almost all the phenomenological theories of the ripple phase [12, 13, 22, 23, 24, 25, 26] use free energies similar to eq.-5.1, but with different order parameters. Goldstein and Leibler [23] considered a one dimensional modulation of a scalar order parameter given by the bilayer thickness. Using a Lifshitz free energy similar to eq.-5.1, they were able to obtain a modulated structure with a periodic modulation in the bilayer thickness. But from x-ray diffraction data it is clear that the ripple is a periodic height modulation rather than a thickness modulation [4].

Falkovitz et. al. [24] postulate that chain melting is the driving force behind the transition to the ripple phase. In the Falkovitz model as well as in another model proposed by Marder et. al. [25], the order parameter is the bilayer thickness; the

free energy expression has a term favoring either rigid or molten chains, a term proportional to the square of the curvature and a coupling term. These models yield a structure where roughly half of the hydrocarbon chains are melted in the modulated phase. This is inconsistent with the fact that experimentally, the latent heat of the pre-transition is roughly one-tenth of that of the main-transition (the $P_{\beta'}$ to L_{α} transition), suggesting that only a few of the chains are molten (or disordered) in the ripple phase.

Empirically, the rippling occurs only in those lipids where the chains have a tilt in the lower temperature gel phase. Therefore many theories propose a periodic variation in the chain tilt to explain the rippling. The theory of Doniach [26] again considers a one dimensional modulation in a scalar order parameter now given by the angle between the layer normal and the long axis of the chains. The free energy again has a form similar to eq.-5.1 but the stabilizing term is $(\nabla\phi)^4$ instead of $(\nabla^2\phi)^2$. There is a competition between a preferred tilt and a preferred bilayer curvature and this leads to a modulated structure. This theory predicts the existence of two different kinds of ripples one of which is symmetric and the other asymmetric, depending on the values of the Landau coefficients. In this paper the conditions for the appearance of these two types of ripples is discussed but the phase diagram is not calculated. The main draw back of this theory is that the order parameter is taken to be a scalar given by the magnitude of the tilt, whereas in reality, the direction of the tilt can also vary.

Of all the phenomenological theories proposed so far, the Landau theory of Chen, Lubensky and MacKintosh [12, 13] is probably the most appealing. Here the order parameter is a vector, given by the projection of the chains on the layer plane. Again a Lifshitz free energy similar to eq.-5.1 is used. In this theory, as in many of the earlier theories, the inter bilayer interactions are ignored. This is justified because ripples have been observed in unilamellar vesicles [27]. For achiral systems, this theory predicts the existence of two ripple phases both of which are symmetric. They also

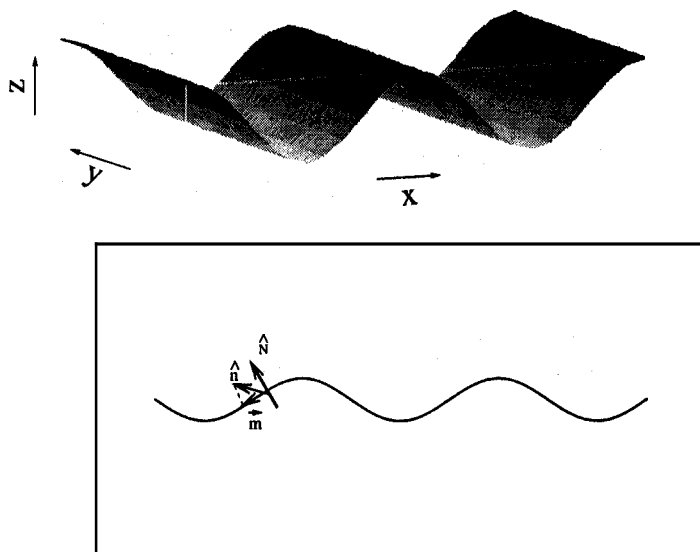


Figure 5.2: Schematic diagram of a rippled bilayer

predict a two dimensionally modulated square lattice phase. According to this theory, asymmetric ripples are allowed only in chiral systems. Thus this theory fails to explain the experimental observation of asymmetric ripples in racemic mixtures [6, 28].

Seifert et. al. [22] have proposed a Landau theory where the order parameter is (as in Lubensky-MacKintosh theory), the projection of the hydrocarbon chains onto the layer plane but here the tilt of the chains in the two leaves of the bilayer need not be coupled. In this model, though the free energy does not explicitly have any chiral terms, the ripple profile can still be asymmetric. The major drawback of this theory is that the predicted shape for the ripple profile is an arc of a circle which is very different from what is seen experimentally.

5.2 The Chen-Lubensky-MacKintosh theory

In this theory [12, 13], the projection of the chains onto a plane tangential to the surface of the bilayer is the vector order parameter (see figure-5.2) and is denoted by $\mathbf{m}$.

The Landau-Ginzberg free energy per unit area of the bilayer is:

$$f_{LM} = \frac{1}{2}a|\mathbf{m}|^2 + \frac{1}{4}b|\mathbf{m}|^4 + \frac{1}{2}\tilde{c}_{\parallel}(\nabla \cdot \mathbf{m})^2 + \frac{1}{2}c_{\perp}(\nabla \times \mathbf{m})^2 + \frac{1}{2}\alpha(\nabla^2 \mathbf{m})^2 \quad (5.2)$$

The first two terms are the usual Landau terms; the next two terms are the splay and the bend elastic terms; the last term is required for stability, in case one of the two elastic terms becomes negative.

This is the free energy density for the variations in $\mathbf{m}$ on a flat membrane. In a real membrane, the variations in the tilt can couple to the height variable. Therefore, the curvature energy terms:

$$f_c = \frac{1}{2}\kappa(\nabla^2 h)^2 - \gamma(\nabla^2 h)(\nabla \cdot \mathbf{m})$$

have to be added to eq.-5.2. Here ‘ h ’ is the height variable in Monge gauge (height measured with respect to the $x - y$ plane), κ is the bending rigidity and γ determines the strength of coupling between the curvature and the gradients in the tilt. A minimization with respect to the height variable ‘ h ’ gives the following expression for the height profile that determines the equilibrium membrane shape

$$\nabla^2 h = \frac{\gamma}{\kappa}(\nabla \cdot \mathbf{m}) \quad (5.3)$$

Using eq.-5.3, the variable h can be eliminated from the total free energy density. This leads to the same expression for the free energy density as eq.-5.2 but with the coefficient of the splay term renormalized to : $(c_{\parallel} = \tilde{c}_{\parallel} - \gamma^2/\kappa)$. thus the effective free energy density reads:

$$f_{LM} = \frac{1}{2}a|\mathbf{m}|^2 + \frac{1}{4}b|\mathbf{m}|^4 + \frac{1}{2}c_{\parallel}(\nabla \cdot \mathbf{m})^2 + \frac{1}{2}c_{\perp}(\nabla \times \mathbf{m})^2 + \frac{1}{2}\alpha(\nabla^2 \mathbf{m})^2 \quad (5.4)$$

If this renormalized splay elastic coefficient $c_{\parallel}$ becomes negative, the bilayers have a spontaneous local curvature and can have periodic modulations. To determine the mean-field phase diagram, a single wave length modulation is assumed. Under this assumption, all the possible ripple phases can be described by the ansatz:

$$m_x = m_0^L + m_1^L \cos(qx)$$

$$m_y = m_0^T + m_1^T \sin(qx)$$

The modulation is assumed to be in the x-direction. It can be shown that a phase with $m_0^L \neq 0$ is not possible. Thus in this theory, there cannot be an average tilt in a direction parallel to the modulation direction. Three distinct ripple phases are possible, depending on which of the coefficients m_1^L , m_0^T and m_1^T are non-zero.

- when $m_1^L \neq 0$, $m_0^T \neq 0$ and $m_1^T = 0$, the ripple is symmetric and the phase is called $P_{\beta'}^{(1)}$ phase.
- when $m_1^L \neq 0$, $m_0^T = 0$ and $m_1^T = 0$, the ripple is still symmetric and the phase is called $P_{\beta'}^{(2)}$ phase.
- when $m_1^L \neq 0$, $m_0^T = 0$ and $m_1^T \neq 0$, the ripple is again symmetric but the winding number is one and not zero like in the other two phases described above. This phase is called $P_{\beta'}^{(3)}$ phase.

These phases differ from each other in having different spatial variation of m (see [12, 13] for a pictorial representation). Apart from these, a square lattice phase described by

$$m_x = m_1 \cos(qx)$$

$$m_y = m_1 \cos(qy)$$

is also considered. The free energy density of the various phases described above is calculated and then integrated over one wavelength to get the average free energy density. The average free energy density is then minimized with respect to the amplitudes of the components of m and the wave-vector q to get the equilibrium values of these coefficients in terms of the Landau parameters. These values are put back into the free energy expression and the free energy for the various phases are equated to determine the phase boundaries. A schematic phase diagram for this model is shown in figure-5.3.

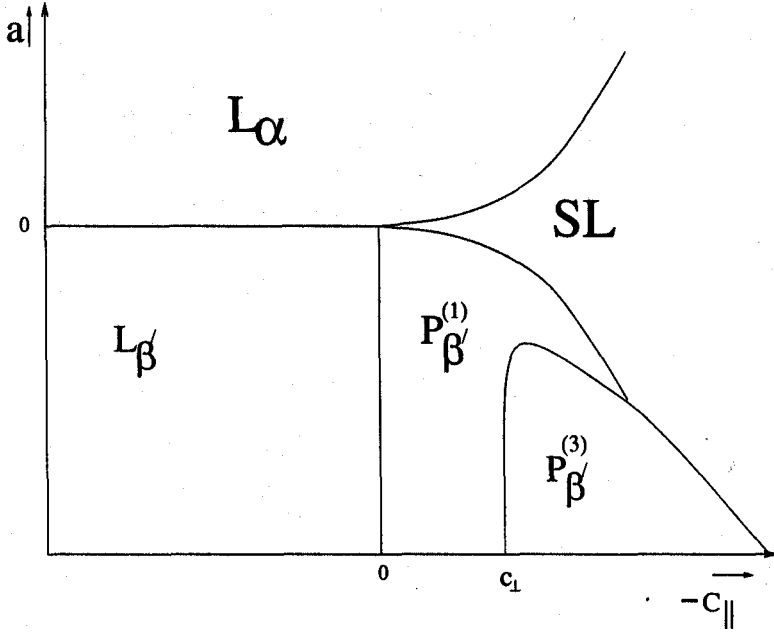


Figure 5.3: A schematic phase-diagram for the Landau-MacKintosh model for achiral membranes. SL: square lattice phase, $P_{\beta}^{(1)}$ and $P_{\beta}^{(3)}$ are modulated phases where the modulations is symmetric. See [12, 13] for details.

If the system is chiral, the free energy can have additional symmetry allowed terms. The lowest order chiral term is $\lambda_b |\mathbf{m}|^2 (\mathbf{V} \times \mathbf{m})$. Apart from this the chiral coupling of the tilt to the membrane shape, $\lambda_{HP} \epsilon_{ki} (\nabla_i \nabla_j h) m_j m_k$ also has to be added. This is the Helfrich-Prost [29] term that accounts for the tendency of the molecules of a chiral two-dimensional membrane embedded in a three dimensional space to twist. As in the achiral case, the effective free energy in terms of the tilt $\mathbf{m}$ can be found by a functional minimization of the free energy with respect to h . This gives:

$$f_{LM} = \frac{1}{2} a |\mathbf{m}|^2 + \frac{1}{4} b |\mathbf{m}|^4 + \frac{1}{2} c_{||} (\nabla \cdot \mathbf{m})^2 + \frac{1}{2} c_{\perp} (\nabla \times \mathbf{m})^2 + \frac{1}{2} \alpha (\nabla^2 \mathbf{m})^2 + \lambda'_b |\mathbf{m}|^2 (\nabla \times \mathbf{m}) \quad (5.5)$$

here

$$\lambda'_b = \lambda_b + \frac{\lambda_{HP}}{2\kappa}$$

The height is now given by

$$\nabla^4 h = \frac{\gamma}{\kappa} \nabla^2 (\nabla \cdot \mathbf{m}) - \frac{\lambda_{HP}}{\kappa} \epsilon_{ki} [\nabla_i \nabla_j (m_j) (m_k)]$$

the resulting profiles can be expressed as a Fourier series:

$$h(x) = a_1 \sin(qx) + a_2 \sin(2qx) + b_2 \cos(2qx) + a_3 \sin(3qx) + b_3 \cos(3qx) + \dots \quad (5.6)$$

It can be shown that in the P_{β}^3 phase both a_1 and a_2 are non-zero thus giving an asymmetric height profile. Thus in the **Lubensky-MacKintosh** theory, an asymmetric ripple profile is expected only for chiral systems. Therefore, a clear prediction of this model is the existence of only symmetric ripples in an achiral system.

To test this prediction, Katsaras and Raghunathan looked at the x-ray diffraction from racemic mixture (achiral) of DMPC and found that the ripples were asymmetric [28]. This result could still have been accounted for by this theory if the two enantiomers in the racemic mixture had separated and formed chiral domains within the bilayer. This possibility has been ruled out by later experiments [30]. It is therefore clear that some modification to the **Lubensky-MacKintosh** theory is required to account for the occurrence of asymmetric ripples in achiral systems.

5.3 Previous theories that predict asymmetric modulations in achiral systems

5.3.1 Doniach's theory:

In Doniach's theory [26] a competition between a preferred layer curvature imposed by the head-group interactions and a preferred tilt imposed by the chain-chain interactions gives rise to the rippled structure. The magnitude of the tilt angle θ is the order parameter. The free energy density is written as:

$$f_{don} = \frac{1}{a} \left\{ \lambda \left[\left(\frac{ad\theta}{dx} \right)^2 - \theta_0'^2 \right] + \gamma (\theta^2 - \theta_0^2)^2 \right\} \quad (5.7)$$

where a is a length scale of the order of inter-chain distance. The first term sets the preferred curvature to θ_0' and the second term sets the preferred tilt to θ_0 .

In the language we have been using so far, the above free energy would be of the form:

$$f_{don} = \frac{1}{2}a\theta^2 + \frac{1}{4}b\theta^4 + \frac{1}{2}c(\nabla_x\theta)^2 + \frac{1}{4}\beta(\nabla_x\theta)^4 \quad (5.8)$$

This is similar to eq.5.1 except that the stabilizing term now is $(\nabla_x\theta)^4$ instead of

$(\nabla_x^2 \theta)^2$. Also, in eq.-5.8, all the coefficients are not independent of each other.

The bilayer height profile is calculated for different values of the coefficients. Two regimes are identified depending on the value of the dimensionless quantity $\eta = (\frac{\theta'_0}{\theta_0})^2 (\frac{\lambda}{3\gamma})^{\frac{1}{2}}$. When $\eta < 1$, the tilt oscillates in space between θ_{max} and θ_{min} but the average remains non-zero. When $\eta > 1$, the tilt oscillates about a zero mean. The tangent of the angle $\theta(x)$ at point x gives the slope of the membrane at x . This is integrated with respect to x to get the height profile $y(x)$. The first case ($\eta < 1$) corresponds to an asymmetric height profile and the second case ($\eta > 1$) to symmetric height profile. The main drawback of this theory is that here the tilt order parameter is a scalar whereas in reality, not only the magnitude but also the direction of the tilt can vary.

5.3.2 The "rippled commensurate phase" of Jacobs et. al.

Jacobs et. al. [31] studied a one dimensional model with a real scalar order parameter (ϕ) that depends on a single spatial coordinate and showed that the inclusion of the higher order term $(\nabla_x \phi)^4$ in the free energy density induces a phase where the order parameter oscillates about a non-zero mean value.

The free energy here is:

$$f_{jac} = \frac{1}{2}a\phi^2 + \frac{1}{4}b\phi^4 + \frac{1}{2}c(\nabla_x \phi)^2 + \frac{1}{2}\alpha(\nabla_x^2 \phi)^2 + \frac{1}{4}\beta(\nabla_x \phi)^4 \quad (5.9)$$

where the last term is the new higher order term. Both the $(\nabla_x^2 \phi)^2$ term and the $(\nabla_x \phi)^4$ term are stabilizing terms. This reduces to the Lifshitz free energy (eq.5.1) in the $\beta = 0$ limit and the Doniach model (eq.5.8) in the $a = 0$ limit.

For the case $\beta = 0$ in eq.-5.9, the order parameter in the modulated phase has a zero spatial average and can therefore be Fourier expanded as $\phi(x) = \sum_{n=1}^N \phi_n \sin(2n - 1)qx$. This phase is a symmetrically modulated phase. In the uniformly ordered

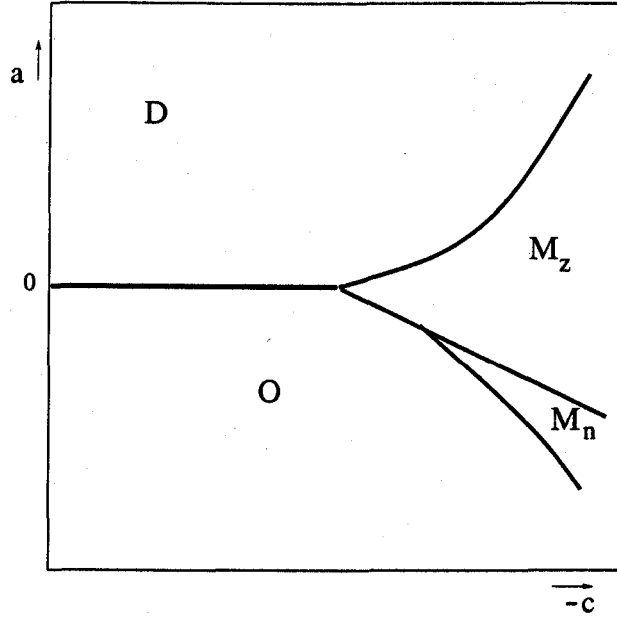


Figure 5.4: A schematic phase diagram for the Jacobs model. D: disordered phase, O: ordered phase, M_z : modulated phase with order parameter oscillations around a zero mean, M_n : modulated phase with order parameter oscillations around a non-zero mean.

phase, (for both $\beta = 0$ and $\beta \neq 0$) the order parameter has a value $\phi = \pm\phi_c \neq 0$. They also show that the ordered phase is unstable to oscillations of ϕ about ϕ_c for $a > \frac{c^2}{8\alpha}$. However, when $\beta = 0$, this line falls in the region of stability of the symmetrically modulated phase and hence a phase where ϕ oscillates about a non-zero mean is not obtained.

When β is increased from zero, the symmetrically-modulated to uniformly-ordered phase transition line goes up in the $a - c$ plane. Eventually it crosses the $a = -\frac{c^2}{8\alpha}$ line, above which ϕ oscillates about 0. Hence if β is large enough, there is now a region in the $a - c$ plane, where the order parameter oscillates around a non-zero mean. A schematic phase diagram is given in figure-5.4.

5.3.3 Analytical calculations of Benguigui

Benguigui [32] obtained the $a - c$ phase diagram analytically for the Jacobs model assuming a sinusoidal variation in the order parameter ϕ . He used this model to explain the Smectic- A_R liquid crystalline phase. In this model, the order parameter

is the polarization vector which has both x and z dependence. In the present discussion, for the sake of clarity, we suppress the z dependence: this does not affect the arguments or conclusions.

An ansatz $\phi = \phi_0 + \phi_1 \sin(qx)$ is put in eq. 5.9 and the $a - c$ phase diagram is constructed. The possible phases are: Smectic- A_1 ($\phi_0=0, \phi_1=0$), Smectic- A_2 ($\phi_0 \neq 0, \phi_1=0$), Smectic- $\tilde{A}$ ($\phi_0=0, \phi_1 \neq 0$) and Smectic- A_R ($\phi_0 \neq 0, \phi_1 \neq 0$). In case of phase transitions in phospholipid membranes with the order parameter given by the magnitude of the tilt of the hydrocarbon chains, the corresponding phases would be: untilted L_α phase ($\phi_0=0, \phi_1=0$), uniformly tilted $L_{\beta'}$ phase ($\phi_0 \neq 0, \phi_1=0$), symmetric ripple phase with no mean tilt ($\phi_0=0, \phi_1 \neq 0$) and asymmetric ripple phase where the tilt oscillates about a non-zero mean ($\phi_0 \neq 0, \phi_1 \neq 0$). The coordinates of the triple point where the uniformly tilted, symmetrically oscillating and asymmetrically oscillating phases coexist is determined to be ¹ :

$$a_{trip} = -\frac{10ba}{4P}, \quad c_{trip} = -2\alpha\sqrt{\frac{5b}{\beta}}. \quad (5.10)$$

These expressions are only approximate since in the derivation, the higher order terms were dropped. The actual coordinates of the triple point on the numerically calculated phase diagram are therefore different (see [31] and figure-5.4 and -5.6), though eq.-5.10 indicates correctly the trend of the dependence of a_{trip} and c_{trip} on the other Landau coefficients.

5.4 A Landau theory for achiral bilayers

We now extend the Chen-Lubensky-MacKintosh model by including the higher order term introduced by Jacobs et. al. and an anisotropy of the bending modulus of the bilayer. With these modifications, we are able to account for asymmetric ripples in achiral bilayers.

¹

¹There is a minor mistake in the expression in ref. [32] that has been corrected here.

The order parameter here (as in the **Lubensky-MacKintosh** model [12, 13]) is the projection $\mathbf{m}$ of the chains on to the local tangent plane. One has to deal with a vector order parameter that can be a function of either one or two spatial coordinates. The mean bilayer plane is taken to be the $x - y$ plane and the ripple is taken along the x -direction (see figure-5.2). The bend elastic term $(\nabla \times \mathbf{m})^2$ is not included in this free energy. The inclusion of this term would simply lead to some parts of the phase diagram now occupied by the asymmetric ripple being taken over by a winding phase [12, 13] (with a non-zero $\nabla \times \mathbf{m}$). If the fact that in the ripple phase the chains are packed parallel is taken into account, the winding phase, where the chains are not parallel to one another, is expected to be energetically unfavorable. In this case the free energy density is:

$$f = \frac{1}{2}a|\mathbf{m}|^2 + \frac{1}{4}b|\mathbf{m}|^4 + \frac{1}{2}c(\nabla \cdot \mathbf{m})^2 + \frac{1}{2}\alpha(\nabla^2 \mathbf{m})^2 + \frac{1}{4}\beta(\nabla \cdot \mathbf{m})^4 \quad (5.11)$$

Here the coefficient c of the $(\nabla \cdot \mathbf{m})^2$ term is the renormalized (see the discussion of **Lubensky-MacKintosh** theory) splay coefficient. We assume the following ansatz for $\mathbf{m}$:

$$\begin{aligned} m_x &= m_{0x} + m_{1x}\cos(qx) + m_{2x}\cos(2qx) \\ m_y &= m_{0y} + m_{1y}\cos(qx) \end{aligned} \quad (5.12)$$

The ripple is always in the x -direction. Depending on the value of the coefficients m_{0x}, m_{1x}, m_{0y} and m_{1y} , various kinds of ripples are obtained. It can be seen from our numerical results that $m_{2x} \neq 0$ if and only if $m_{0x} \neq 0$. Following types of ripple phases are allowed:

- A symmetric ripple phase with no mean tilt: the tilt oscillates sinusoidally about a zero mean. This will be referred to as the P_β phase. This corresponds to $m_{0x} = 0, m_{0y} = 0, m_{1x} \neq 0, m_{1y} = 0, m_{2x} = 0$.
- A symmetric ripple phase where the tilt oscillates sinusoidally about a non-zero tilt in a direction perpendicular to the ripple direction. This will be referred to

as the $P_{\beta'}^t$ phase (this is same as $P_{\beta'}^{(1)}$ phase in [12, 13]). This corresponds to $m_{0x} = 0, m_{0y} \neq 0, m_{1x} \neq 0, m_{1y} = 0, m_{2x} = 0$.

- An asymmetric ripple phase where the chains oscillate about a non-zero mean tilt in the ripple direction. We have checked that in this case the modulation is unstable with respect to a second harmonic and hence this implies an asymmetric ripple profile (ie. $m_{2x} \neq 0$). This phase will be referred to as the $P_{\beta'}^l$ phase. This corresponds to $m_{0x} \neq 0, m_{0y} = 0, m_{1x} \neq 0, m_{1y} = 0, m_{2x} \neq 0$.
- A square lattice phase characterized by a 2-dimensional ripple. This is same as the square lattice phase in the Chen-Lubensky-MacKintosh model. This is called the $P_{\beta'}^{2D}$ phase. Here the ansatz for $\mathbf{m}$ is

$$m_x = m_1 \cos(qx)$$

$$m_y = m_1 \cos(qy)$$

The L_α phase is characterized by $m_{0x} = 0, m_{0y} = 0, m_{1x} = 0, m_{1y} = 0, m_{2x} = 0$ and the $L_{\beta'}$ phase by $m_{0x} \neq 0, m_{0y} = 0, m_{1x} = 0, m_{1y} = 0, m_{2x} = 0$.

The phase transitions from the flat phases and also between the various kinds of ripples take place as the values of a and c are varied and hence the $a - c$ phase diagram is plotted here. In case of a vector order parameter $\mathbf{m}$, it is not possible to calculate the phase boundaries analytically as was done in reference [32] since with this free energy expression, it would involve solving cubic equations. Therefore, the phase diagrams presented here are numerically calculated.

The following procedure is adopted to calculate the phase diagrams. The ansatz for $\mathbf{m}$ corresponding to one of the ripple profiles described above is put in eq.-5.11 and the free energy density is integrated over a wavelength and then divided by the wavelength to obtain the average free energy density. The free energy density is then numerically minimized ² with respect to the wave-number q and the coefficients of the

²The simplex method (amoeba) described in ref. [33] is used to do the minimization

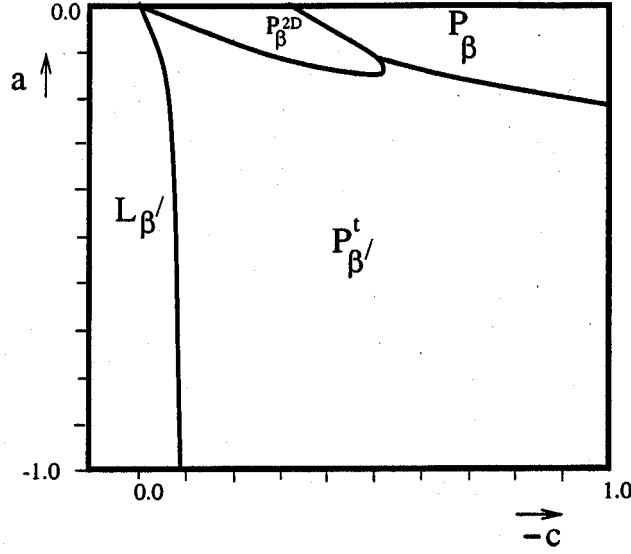


Figure 5.5: The phase diagram for a 2-dimensional system with free energy given by equation 5.11. Here $b=1.0$, $\alpha=1.0$ and $\beta=4.00$

components of $\mathbf{m}$: $m_{0x}, m_{1x}, m_{0y}, m_{1y}$ etc. This is done for fixed values of the coefficients b , a , p , a and c . a and c are varied over a range and the above procedure is repeated. This gives the value of the free energy for a particular kind of ripple, at each value of a and c . This is done for all the different ripples described above. The energy for each kind of ripple is then compared and the minimum energy configuration at each point on the a - c plane is identified.

When the tilt is restricted to be in the direction of the ripple, ie. in the *one dimensional limit* (same as [31]), the asymmetric $\mathbf{p}_x$ phase is obtained in parts of the $a - c$ plane and the results of ref. [31] are recovered. When this constraint is removed and $\mathbf{m}$ is allowed to have a component perpendicular to the ripple, ie. in the *two dimensional* case, the $P_{\beta'}^l$ phase goes over to the $P_{\beta'}^t$ phase. In other words, it costs less energy to have spatial tilt oscillations in a direction perpendicular to the direction of average tilt. Therefore in the two dimensional case, $\mathbf{m}_{0x}$ is always equal to zero in the modulated phase. This case is similar to the case discussed in ref.- [12], except that the term $(\nabla \cdot \mathbf{m})^4$ destabilizes the two dimensional square lattice phase in favor of the one-dimensional symmetric ripple with no mean tilt (figure-5.5).

From the above discussion we conclude that if due to some reason the tilt is constrained to be in the rippling direction, an asymmetric ripple is possible even in an achiral systems. An anisotropy in the elastic constants is a mechanism by which the tilt can be constrained. In a layered system where the molecules are tilted with respect to the layer normal, a curvature in the direction of the tilt and a curvature orthogonal to the tilt may have different energies. In thermotropic systems, it has been shown that near the Smectic-A to Smectic-C transition, a curvature of the layer transverse to the direction of $\mathbf{m}$ is energetically more favorable than a tilt in the direction of curvature [34]. But deep inside the Smectic-C phase, this need not necessarily be true. In the case of lipid bilayers, the L_α phase is the analogue of the Smectic-A phase and the $L_{\beta'}$ phase is the analogue of the Smectic-C phase. We are interested in transitions from a tilted phase ($L_{\beta'}$) to a modulated phase. Therefore the anisotropy in the elastic constants here need not necessarily favor a transverse tilt. In fact, if the one constant approximation imposed in ref. [34] is lifted and higher order terms are included, it can be shown that under certain conditions, a bilayer curvature in the direction of the tilt is favored [35].

5.4.1 The anisotropy in the elastic constant

In a system where the molecules are tilted, symmetry considerations allow the elasticity of the $\mathbf{m}$ -field to be anisotropic. Therefore the anisotropy terms given by

$$\frac{\xi}{2}[(\mathbf{m} \cdot \nabla)\mathbf{m}]^2 + \frac{\zeta}{2}[(\mathbf{m} \times \nabla)\mathbf{m}]^2$$

are added to eq.-5.11. When there is a nonzero mean tilt, these terms distinguish between gradients of $\mathbf{m}$ along $\mathbf{m}$ and gradients of $\mathbf{m}$ orthogonal to $\mathbf{m}$.

The full free energy reads:

$$f = \frac{1}{2}a|\mathbf{m}|^2 + \frac{1}{4}b|\mathbf{m}|^4 + \frac{1}{2}c(\nabla \cdot \mathbf{m})^2 + \frac{1}{2}\alpha(\nabla^2 \mathbf{m})^2 + \frac{1}{4}\beta(\nabla \cdot \mathbf{m})^4 + \frac{\xi}{2}[(\mathbf{m} \cdot \nabla)\mathbf{m}]^2 + \frac{\zeta}{2}[(\mathbf{m} \times \nabla)\mathbf{m}]^2 \quad (5.13)$$

As before, the height profile is related to the tilt via equation 5.3.

The phase diagram

The phase diagram is calculated as before and is shown in figure-5.6 (for $\xi < \zeta$) and in figure-5.7 (for $\xi > \zeta$). When $\xi < \zeta$ it is easier to bend the bilayer in the direction of the tilt. In the region of the parameter space shown, $|\mathbf{m}| \leq 0.5$. We find two major differences from the phase diagram obtained from the **Lubensky-MacKintosh** model. Firstly, the square lattice phase is not stable in the present model. This is expected since because of the anisotropy in the elastic constants, modulations in one direction is favored over modulations in the other directions. This is consistent with the fact that such a phase has never been experimentally observed. Secondly, the $P_{\beta'}^l$ phase is now stable in part of the parameter space. In this phase m_{2x} is always non-zero and hence, as can be seen from eq.-5.3, the ripple profile is asymmetric. However the asymmetry is rather small; m_{2x} is typically two orders of magnitude smaller than m_{1x} . This is about an order of magnitude smaller than that observed experimentally. As discussed before, (eq.-5.10), the coordinates of the $P_{\beta'}^l$ - $P_{\beta'}^t$ - $L_{\beta'}$ triple point depend on the ratio α/β of the two stabilizing terms. As this ratio is decreased, the triple point moves towards the origin. Note that $L_{\beta'}$ phase is now stable till $(c_{||} + \xi m_o^2)$ or $(c_{||} + \zeta m_o^2)$ becomes negative.

When $\zeta < \xi$, the $P_{\beta'}^l$ phase disappears from the phase diagram (figure-5.7). This is similar to the $\xi = \zeta = 0$ case except that the square lattice phase is now absent.

5.4.2 A different ansatz

Experimentally, the ripple profile is usually saw-tooth shaped. It is therefore better approximated by the following ansatz for the height $h(x)$ profile:

$$h(x) = \eta \tanh(\nu x) - \mu x \quad (5.14)$$

here η and ν are constants to be determined by the minimization of the free energy. μ is related to the wavelength by the fact that the height $h(x)$ has to go to zero at

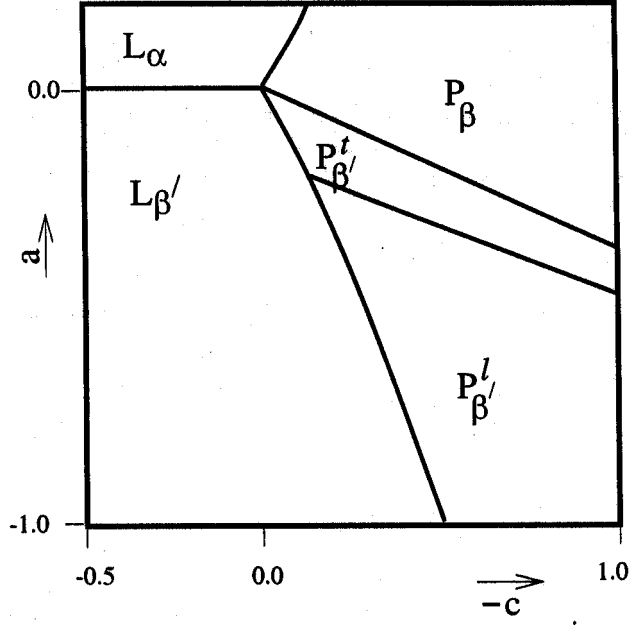


Figure 5.6: Numerically calculated mean-field phase diagram for an achiral membrane, free energy is given by eq.-5.13 with $b=1.0$, $\alpha=0.01$, $\beta=4.0$, $\xi=0.1$ and $\zeta=0.7$.

$\pm\pi/q$ where q is the wave-number. Figure-5.8 shows a possible height profile drawn using this ansatz.

The tilt $\mathbf{m}$ is then given by (see eq.-5.3)

$$m(x) = \eta \operatorname{sech}^2(\nu x) + \rho x$$

p is a constant of integration. This ansatz for $\mathbf{m}$ is put in eq.-5.13 (ie. the free energy expression), the free energy density is integrated over a wavelength and divided by the wavelength to get the average free energy density and then minimization is done with respect to η, ν, p and q .

This ansatz was found to give typically about 20 % lower values of the free energy compared to the Fourier ansatz (eq.-5.12) at all points in the parameter space corresponding to the different ripple phases. The height profile was found to be always weakly asymmetric, consistent with the weak asymmetry seen in the $P_{\beta'}^l$ phase using the Fourier ansatz. In this phase $|\mathbf{m}_o| \gg 0$, whereas in the other ripple phases $|\mathbf{m}_o| \approx 0$. Further, in the $P_{\beta'}^l$ phase the longer arm of the asymmetric ripple has a

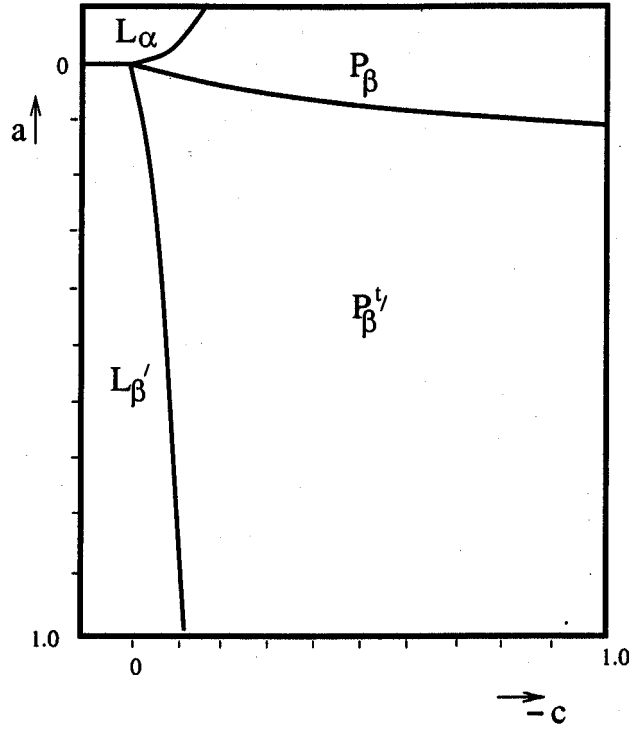


Figure 5.7: Numerically calculated mean-field phase diagram for an achiral membrane, free energy is given by eq.-5.13 with $b=1.0$, $\alpha=0.01$, $\beta=4.0$, $\xi=0.7$ and $\zeta=0.1$.

lower value of m . We have checked that the phase diagram obtained with this ansatz is qualitatively similar to that deduced using the Fourier ansatz, though the present ansatz cannot differentiate between the $P_{\beta'}^t$ and P_{β} phases.

A physical picture

In order to understand the origin of the asymmetric ripples, we first consider the stability to height modulations of a bilayer where the equilibrium mean value of the chain tilt is non zero and is given by $\mathbf{m}_o$. With $\xi < \zeta$ in eq.-5.13, variations in m along $\mathbf{m}_o$ are favored over those perpendicular to $\mathbf{m}_o$. At sufficiently small values of the coefficient of $(\mathbf{V} \cdot \mathbf{m})^2$, the system becomes unstable to the formation of ripples with a wave-vector along $\mathbf{m}_o$ (recall that a non-zero $\mathbf{V} \cdot \mathbf{m}$ implies a non-zero curvature in height: eq.-5.3). Let $\mathbf{m} = \mathbf{m}_o + \delta\mathbf{m}$ (where $\delta\mathbf{m}$ is a small variation in $\mathbf{m}$ due to the rippling). The term proportional to $|m_o|^2(\mathbf{m}_o \cdot \delta\mathbf{m})$ (coming from the term $|\mathbf{m}|^4$ in the free energy) ensures that it is favorable to have $\delta\mathbf{m}$ in the direction opposite to $\mathbf{m}_o$. That is, it costs less energy to decrease the tilt than to increase it.

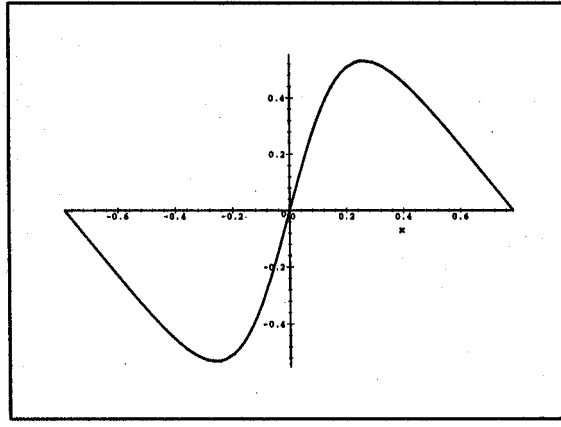


Figure 5.8: A height profile drawn using equation 5.14 for $\eta = 0.5$, $\nu = 5.0$, $\mu = 1.0$ and $q = 4.0$.

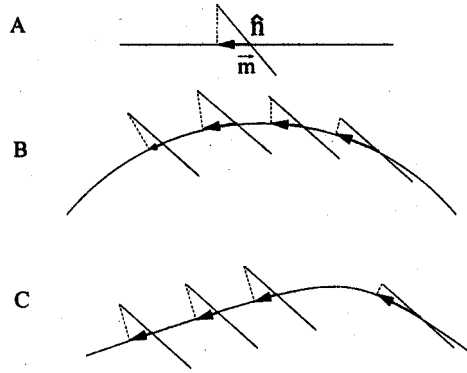


Figure 5.9: a: $\mathbf{m}$ for a flat bilayer, b: $\mathbf{m}$ for a bilayer with uniform curvature corresponding to a symmetric ripple profile. Though all the chains make the same angle with respect to the z -axis, the angle with respect to the bilayer normal increases as one goes from left to right. C: The length of left side of the symmetric profile has been increased to maximize the region of favorable tilt.

Now the system can lower its energy by taking advantage of the symmetry-allowed bias discussed above. If the ripple shape is asymmetric and in the longer arm of the ripple $\delta \mathbf{m} < 0$, the energy can be lowered. Thus the presence of a mean tilt along the ripple wave-vector favors asymmetric ripples (figure-5.9) with an asymmetric height profile

It is clear from the above discussion that the effective bending modulus of the bilayer must be smaller in the direction of the **mean** tilt in order to see asymmetric ripples. We may now speculate on the origin of this kind of anisotropy in the bending modulus.

It is well known that the tilt of the hydrocarbon chains with respect to the bilayer normal is achieved by relative sliding of the chains along their long axes. If the chains are constrained to be parallel, as seems to be the case in lipid bilayers, the curvature of the bilayer is produced by gradients in this sliding displacement. Thus the molecular tilt as well as the bilayer curvature result from the same basic molecular mechanism. When the chains are tilted, the overlap between two adjacent chains along the tilting direction decreases, whereas in the perpendicular direction the overlap remains the same as in the untilted phase. Therefore, the net restoring force between the chains can be expected to be lower in the tilt direction, making it easier for the bilayer to bend in this direction.

5.5 Conclusion

In this chapter we have discussed the various theories that have so far been proposed to explain the ripple phase of phospholipids and have explained the drawbacks of each theory. We have extended the Landau theory of Chen-Lubensky-MacKintosh to account for asymmetric ripples in achiral systems. In contrast to the results of the **Lubensky-MacKintosh** model, our model admits the experimentally observed *asymmetric* ripple phase in *achiral* bilayers. **Further**, we show that the two-dimensionally modulated square lattice phase predicted by the **Lubensky-MacKintosh** model is not stable. Our model thus removes some discrepancies between the results of the **Lubensky-MacKintosh** model and experimental observations.

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