Internal structures in membranes: ripples, hats, saddles, and egg cartons

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Membranes such as bilayers that form spontaneously in lipid–water systems can show surprising variety in their phase behavior, shapes, and textures. Many of these phases and textures seem to be the result of subtle effects of either competing interactions between the constituent molecules or the expression of internal degrees of freedom within these essentially two-dimensional systems. The richness of their behavior has made membranes fascinating model systems for both experimental and theoretical research. There have been recent developments in the study of such structures as 'ripple phases' and other textures, primarily in bilayer membranes.

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Abbreviations

cryo-TEM	cryo-transmission electron microscopy
DOPC	dioleoyl-phosphatidylcholine
EYPC	egg yolk phosphatidylcholine
Sm-A	smectic-*A*
Sm-C	smectic-C
STM	scanning tunneling microscopy
PEG	polyethyleneglycol

Introduction

Natural membranes are both essential building blocks and key functional elements of all cells. They form barriers between the inside and outside, and separate various compartments within plant and animal cells. These membranes are typically fluid-like and their constituents diffuse freely. Synthetic fluid membranes have been widely studied both as models of cell membranes as well as for basic studies of phase transitions and ordering in two-dimensional systems. The most abundant examples of natural and synthetic fluid membranes are bilayer membranes. These can form spontaneously when lipid or surfactant molecules, consisting of both a polar head group and one or more hydrocarbon tails, are dispersed in water at sufficient concentration. Each bilayer is a thin (approximately 50 Å thick) fluid film, consisting of two opposing monolayer films of lipid. The resulting structure may be regarded as a two-dimensional fluid film, consisting of a hydrocarbon core with polar exterior faces that make contact with water on either side.

More specifically, when pure, saturated lipids (i.e. with fully saturated hydrocarbon chains) are dispersed in water, they usually form a number of thermodynamically distinct condensed phases over a wide temperature range. On a coarse scale, these condensed phases are characterized by periodic stacks of nearly flat bilayer 'sheets' interspersed with thin layers of water. These lamellar phases are examples of lyotropic liquid crystals, in which phase changes can be caused by varying the density. These phases typically coexist with excess water at concentrations above about 20% water. At the highest temperatures, the stable phase is usually one that can be characterized as a two-dimensional fluid exhibiting broken translation symmetry only in the direction normal to the layers. Moreover, this phase is isotropic in the plane of the bilayers, that is, each bilayer is rotationally symmetric about an axis normal to the lamellae. This is the symmetry of a smectic-*A* (Sm-*A*) liquid crystal [1].

As the temperature is lowered, a series of typically first-order transitions is observed. The phases are distinguished either by density or by symmetry, in which case different degrees of molecular order of the hydrocarbon tails or polar head groups is evident. In addition to ordering of the lipids at a molecular scale, a number of 'internal structures' have been observed or predicted. These include regular, modulated phases, such as the 'ripple phase' of lecithin bilayers. Other modulated structures, including possible egg-carton-like or 'hats and saddles' structures [2,3•], have also been found. These structures are sometimes referred to as *superstructures*, as they are characterized by structure on length scales that are large compared with the molecular dimension. Here, I discuss some of the recent theoretical and experimental developments concerning these phases and structures, beginning with perhaps the best studied of these, the 'ripple phase.'

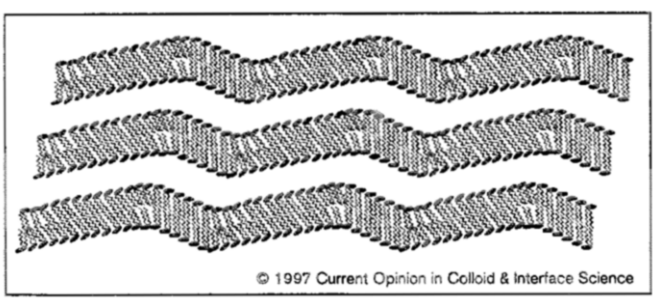
'Ripple phases'

Among the various lamellar phases of hydrated lipids are two common phases known as L_α and L_β [4]. The first of these is the high temperature phase described above, consisting of an array of isotropic fluid-like sheets. This Sm-*A* phase is often called the liquid crystal phase. At lower temperatures, a so-called *gel* phase (L_β) is observed, in which the lipid tails are frozen and form an ordered two-dimensional phase. This is, at the very least, an anisotropic phase, exhibiting six-fold bond orientational order [5], a so-called hexatic phase [6]. It may in some cases be a two-dimensional crystalline phase. The transition between the L_α and L_β phases is observed to be first-order [4]. In fact, there are actually two distinct gel phases, depending primarily on the size of the lipid head group: the L_β phase and the $L_{\beta'}$. The latter is typically associated with a large, bulky head group and is distinguished as a membrane phase by molecular tilt of

the hydrocarbon chains, as in the smectic-*C* (Sm-*C*) liquid crystal phase.

In addition to the L_α, L_β, and $L_{\beta'}$ phases, Tardieu *et al.* [7] reported a number of novel low temperature phases in a variety of pure and mixed lipid systems. These phases included an intermediate, modulated phase that was observed between the L_α and $L_{\beta'}$ phases. This phase, called $P_{\beta'}$, exhibits a periodic corrugation or modulation of the membrane shape (sketched in Fig. 1), in which the period is of the order 150–200 Å, this is often referred to as the 'ripple phase', and it has since been observed for a wide class of saturated lipids that form the tilted $L_{\beta'}$ phase. It has also been the subject of nearly constant experimental [5,8–16,17•,18•] and theoretical [19–27] activity over the past two decades, as it exhibits an equilibrium internal structure on an intermediate length scale between the molecular and macroscopic scales. This $P_{\beta'}$ phase has also posed a number of interesting, and in some cases still unresolved, questions regarding its structure and symmetry.

Figure 1



Schematic representation of the lamellar ripple phase in cross section. The individual lamellae extend out of the plane of the page in this figure. The bilayers are shown with filled solid head groups and double tails. Water occupies the regions in between lamellae. The ripples are asymmetric, and there is a phase shift in the ripple pattern from layer to layer producing a two-dimensional oblique lattice. The orientation of the lipid tails is not meant to be representative. In particular, as the membranes are two-dimensional, molecular tilt out of the plane is likely, even suggested by some experiments.

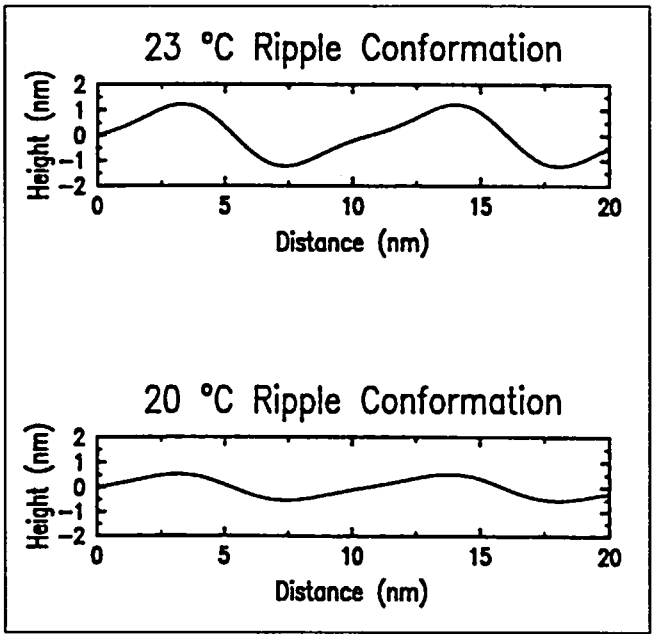
In a number of experiments that have been carried out over the years, several rather general observations have been made about the ripple phase: first, in lamellar systems, the $P_{\beta'}$ phase seems to occur in a narrow range of water content, from about 20 to 30% [4,5], although the ripple phase is also observed in single bilayers [28]; second, both the transition temperatures and ripple wavelengths increase systematically with lipid tail length [14]; and third, although the precise structure is not fully understood, and many ripple shapes have been suggested [7,18•,20–26,29], an asymmetric, rippled shape similar to the sketch in Figure 1 has now been firmly established [7,10–12,14,17•,18•,30,31•].

Theoretical attempts to model this $P_{\beta'}$ phase fall into two general categories: phenomenological models (often referred to as Landau theories) [20,22,24,26,32]; and microscopic statistical mechanics models [19,23,25]. An earlier review, especially of the latter, can be found in [33]. Among the first models proposed for the ripple phase were ones that suggested that the observed ripple was primarily the result of periodic variation of the bilayer thickness [20,22,24,32], as might be associated with a periodic array of domains. Indeed, a recent report [31•] has provided new evidence for a possible alternation of nearly fluid-like and solid-like regions in the ripple structure, as suggested by Falkovitz *et al.* [20]. After better characterizations of the ripple structure, and especially amplitude, became available, however, it was clear that such variations in the bilayer thickness alone could not account for the observed structure [11,13,17•,18•,31•].

Of particular note among recent experimental and theoretical contributions to the understanding of the ripple phase have been more detailed ripple shape characterizations [17•,18•,31•], as well as studies of the ripple symmetry and the role of molecular chirality [26,30,34•]. In [17•], scanning tunneling microscopy (STM) of freeze-fracture replicas was used to determine the ripple amplitude and shape (see Fig. 2). The amplitudes of the ripple can be as large as the full bilayer thickness. These experiments have also shown that the ripple amplitude and wavelength do not vary in a coupled manner, as predicted by some of the microscopic models based on fixed offset between neighboring molecules [25]. This observation is more easily accounted for by the continuum phenomenological models. One such model recently proposed a mechanism that can account for the observed asymmetry of the ripples, provided that the constituent lipids are chiral [26]. Two important recent experiments, however, have shown that racemic mixtures do not result in symmetric ripples [30], and that chiral domains do not form in these mixtures [34•]. Although this can not apparently be accounted for by the model of [26], the occurrence of asymmetric ripples in an achiral lipid system suggests a very intriguing possibility, namely, that a chiral superstructure forms, which spontaneously breaks the chiral symmetry of the system. If the molecular tilt has a component perpendicular to the ripple wavevector, as has been suggested by experiment [16] and can also be inferred from the observed ripple amplitude [17•,18•] and bilayer thickness [31•], then the asymmetric ripple structure is itself chiral. These observations may be related to apparent chiral symmetry breaking in the formation of lipid tubules (BN Thomas, personal communication).

These puzzles illustrate precisely why the ripple phase has been and continues to be such a fascinating model membrane system for both experimental and theoretical study. Although this phase may never occur in a biological membrane, it nevertheless provides interesting opportunities to study fundamental competing lipid interactions

Figure 2



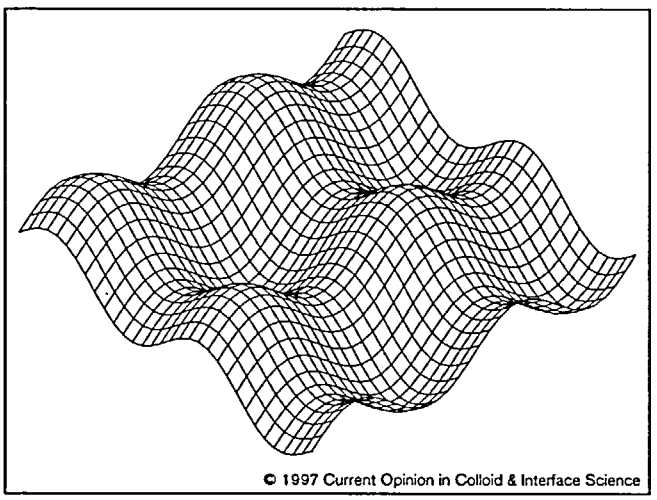
Average ripple surface shape in cross section, as determined from STM of freeze-fracture replicas at **(a)** 23°C and **(b)** 20°C. (From [18•].) The wavelengths are 10.7 and 10.6±1 nm, respectively. The amplitudes (peak to valley) are 2.4 and 1.1 nm. The asymmetry of the ripple is apparent in both cases. These experiments show only the shape of the membrane surface. Complementary structural information, including bilayer thickness in various regions of the ripple period can be found in [31•]. Reproduced with permission from [18•].

and their influence on membrane shape. Much has been learned experimentally since the discovery of the ripple phase, and theoretical models have provided significant insights along the way. As a result of the most recent experiments, however, challenges clearly still remain for existing and future models. In particular, these must account for both the shape asymmetry and possible chiral symmetry breaking. Also, these models must reconcile the overall ripple shape and its large amplitude with the growing evidence for clear structural variations of the bilayer, which may even signal distinct domains within a ripple period.

'Hats and saddles' and other membrane textures

As described above, the $P_{\beta'}$ phase is not believed to occur in cell membranes. However, there is evidence that another texture, or superstructure, may occur in biomembranes. This has been identified as a periodic, curved texture [35–38], which is seen in the membranes of *L*-form (wall-less) cells of *Streptomyces hygroscopicus* and in reconstituted bilayers formed in lipids extracted from cells. The modulated structure is believed to have an egg-carton-like shape of square symmetry, similar to Figure 3. A square-lattice structure of this kind has the interesting property that it appears the same from either side. One reason for postulating the existence of such superstructures is evidence of anomalous membrane roughness, which appears to be able to absorb significant membrane area [38,39•]. Studies of adhesion of membranes [38] have also been taken as indirect evidence of possible superstructures [2,40]. In this case, a disordered superstructure, or melted version of that in Figure 3 has been postulated.

Figure 3



A two-dimensional modulated structure of square-lattice symmetry. The sketch represents a sinusoidal variation in two orthogonal directions: Z=sin(qx)+sin(qy). Each unit cell of such a periodic landscape includes one hill ('hat'), one valley, and two passes ('saddle' points). Textures similar to this have been reported in lipid membranes [35–37]. Equilibrium modulated phases of the same symmetry have also been reported in liquid crystal films [53].

Recent experiments by the Berlin group [39•,41] have concerned the possible superstructure formation in lipid bilayers of egg yoke phosphatidylcholine (EYPC) [41] and dioleoyl-phosphatidylcholine (DOPC) [39•]. These experiments used cryo-transmission electron microscopy (cryo-TEM) on dilute vesicle dispersions, which did indeed show anomalous roughness, or grainy texture of EYPC membranes [41], but evidence for superstructure was not conclusive. The more recent work has turned to pure DOPC, whereas EYPC is a multi-component system. These experiments have reported the formation of disordered superstructures [39•].

Theoretical models for egg carton membrane shapes such as that in Figure 3 have proposed that cooperative saddle-shaped regions of membrane can result from bending elasticity of membranes [2]. These can be stabilized by high-order corrections to the elasticity of bilayers. These elementary, saddle-like deformations are suggested to form either ordered arrays (crystals), as in Figure 3, or disordered textures [3•]. The former are associated with the observed regular structures reported

in [35–37], whereas the latter are associated with the less ordered, grainy textures reported in [39•,41].

Other models based on orientational order within membranes have predicted egg-carton-like instabilities, or at least stable, anisotropically curved membrane regions such as saddles. The notion of orientational order in bilayers and its coupling to membrane shape has been the subject of a number of theoretical studies of membrane phase behavior and shape instabilities [26,27,42–47,48•–52•]. Helfrich and Prost [42] pointed out many of the key direct influences of molecular tilt order and chirality on membrane shape. Subsequent work showed indirect geometric influences of intrinsic membrane curvature on orientational order [43,44], and renormalization of chiral tilt couplings in membranes [45].

The coupling of orientational order to membrane shape has been implicated in the formation of various modulated structures by a number of recent papers [26,27,47,48•–52•]. In the series of papers by Chen *et al.* [27,47,51•], it was shown that both one- and two-dimensional modulated structures can be stabilized by the coupling of molecular orientation to membrane shape. In particular, a square-lattice, two-dimensional modulated phase exhibiting a structure similar to the 'hats and saddles' structure [2,3•] was predicted. This may help to explain the observation of modulated phases found in suspended films of thermotropic liquid crystal systems, such as 4-*n*-heptyloxybenzylidene-4-*n*-heptylaniline [53], in which both one- and two-dimensional modulated phases were reported as a function of film thickness and temperature. It was also shown [47,51•] that this phase can be expected to undergo continuous structural phase transitions to regular, distorted, and hexagonal structures, similar to those predicted for chiral thermotropic liquid crystal films in the Sm-*C* phase [54] when an electric field is applied perpendicular to the layers.

A number of recent papers have focused on the effects of generalized orientational order in membranes [48•–50•]. Fournier has shown that dilute, anisotropic 'inclusions' in a membrane can induce curvature of the membrane [48•]. This model would apply, for instance, to gemini surfactants, which have been found to lead to the formation of cylindrical micelles [55,56]. These are systems in which hydrocarbon tails are joined by a rod-like spacer head group. In such a system, it has been suggested that nematic-like order of the spacers can result in saddle-like curvature of a bilayer, provided that the alignment is opposite in the two halves of the bilayer. A similar effect has been shown for polymeric head groups, such as for polyethyleneglycol (PEG)-lipids [50•]. In contrast, Seifert *et al.* [49•] have shown that molecular tilt alone can drive a similar instability of flat bilayers, provided that the two halves of the bilayer are decoupled, that is, that the molecular tilt can differ across the bilayer.

Orientational order, especially in chiral systems, has also been implicated in the formation of lipid tubules [57,58]. These are cylindrical tubes of approximately one micron diameter that form spontaneously in lipid–water mixtures. In micrographs they appear as multilayer, barber pole-like structures that can extend for many microns in length. Synchrotron X-ray scattering studies have also recently been performed [59]. It has been suggested that a mechanism very similar to the orientational effect described by Helfrich and Prost [42] is responsible for the formation of a cylindrical shape [45,46,60]. If this is the mechanism, it can explain the apparent micron-size stripe texture seen in micrographs [61]. Very recent work has also shown that this mechanism can lead to the formation of a rippled texture or superstructure similar to that of the 'ripple phase' of phospholipids [52•], as has been reported in some experiments ([62]; BN Thomas, personal communication).

Discussion

The equilibrium phases of membranes and their relatively large-scale curved structures show both surprising variety in observed behavior and apparent subtlety in the mechanisms underlying this behavior. This richness appears in contrast to what seems to be such a simple basic structure: the often fluid-like bilayer, composed of small amphiphilic molecules. Even the bilayer structure itself may not be essential in order to understand some of the modulated structures that form, given the apparent similarity to the behavior of certain thermotropic liquid crystal systems [53].

Among the observed modulated phases and structures, the 'ripple phase' is now rather well characterized experimentally. Nevertheless, important structural details such as the precise form of possible molecular orientation and its variation within a ripple period remain unanswered by experiment. Although theories of this phase can individually account for many of the observed aspects, no single theory so far seems to be able to account for all of the existing experimental observations. The most recent experiments, particularly those concerning chiral symmetry [30,34•] and bilayer structure [31•], should provide important new input for future theoretical work.

Other, more complex membrane superstructures in pure lipid systems are only now being characterized in detail [39•]. These observations will no doubt lead to increased theoretical efforts as well. Recent efforts have focused on both fundamental bending elasticity [3•] and membrane curvature anisotropy and orientational order [48•–51•]. Equilibrium cylindrical vesicles reported in recent experiments [63] may also be caused by curvature anisotropy.

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