On ripples and rafts: Curvature induced nanoscale structures in lipid membranes

Friederike Schmid¹, Stefan Dolezel¹, Olaf Lenz², Sebastian Meinhardt¹

¹ Institut für Physik, Johannes Gutenberg-Universität Mainz, DE
² ICP, Universität Stuttgart, DE
E-mail: friederike.schmid@uni-mainz.de

Abstract.
We develop an elastic theory that predicts the spontaneous formation of nanoscale structures in lipid bilayers which locally phase separate between two phases with different spontaneous monolayer curvature. The theory rationalizes in a unified manner the observation of a variety of nanoscale structures in lipid membranes: Rippled states in one-component membranes, lipid rafts in multicomponent membranes. Furthermore, we report on recent observations of rippled states and rafts in simulations of a simple coarse-grained model for lipid bilayers, which are compatible with experimental observations and with our elastic model.

1. Introduction
Biomembranes are essential components of all living matter, and lipids are essential components of biomembranes. According to the venerable "fluid mosaic model" due to Singer and Nicolson [1], the basic structure of biomembranes is provided by a self-assembled lipid bilayer, which serves as a matrix to carry proteins which then perform various biological functions. In the last decades, it has become clear that biomembranes are not laterally homogeneous, but internally structured ("dynamically structured mosaic model" [2]), and this structure is believed to be important for many membrane-associated biological processes. In particular, the lipid raft hypothesis asserts that biomembranes are often filled with nanoscopic domains characterized by a different lipid composition – typically a higher cholesterol content – and higher local order [3–8]. There is ample indirect evidence for the existence of such domains, e.g., from diffusion experiments [9, 10], or from superresolution fluorescence images showing nanoscopic clusters of raft-associated proteins in membranes [10–12]. Nevertheless, the lipid raft hypothesis is still discussed controversially [8]. For example, it is argued that the observed clustering of proteins could be driven by proteins only.

The lipid raft concept is also intriguing from a theoretical point of view. Multicomponent lipid bilayers are known to phase separate in certain parameter regions into a “liquid disordered” (ld) and a “liquid ordered” (lo) phase [13–15], and this has also been observed in plasma membranes [16]. The hypothetical “raft state”, however, is not phase-separated, but rather believed to be a globally homogeneous state filled with nanodomains of sizes between 10 and 100 nanometers. To explain this, it was proposed that rafts might be nonequilibrium structures [17] which are prevented from merging by kinetic barriers [18]. Others have suggested that finite-
size equilibrium rafts might be stabilized by the cytoplasm [19], by special line-active lipids or proteins [20–23], or by elastic interactions arising from local composition differences in apposing monolayer leaflets [24] (the last mechanism requires that the membrane is under tension). Alternatively, it was argued that “rafts” might simply be a signature of critical fluctuations in the vicinity of critical points [25, 26].

Rafts are not the only nanoscale structures in lipid membranes. Many one-component lipid membranes exhibit a periodically modulated “ripple” phase ($P_{\beta'}$) in the transition region between the high-temperature fluid phase ($L_\alpha$) and the low-temperature gel phase. The characteristic wave length of the ripple phase (15 nm [27]) is of the same order than that typically attributed to lipid rafts (10-100 nm). Experimentally [28, 29] and in computer simulations [30–35], modulated phases are observed in lipid bilayers that exhibit a tilted gel state, and they are not observed in lipid bilayers with an untilted gel state $L_\beta$ [28–30, 36]. For example, we have introduced a simple generic lipid model [37–39] which reproduces the $L_\alpha$ phase and the $L_{\beta'}$ phase as well as the ripple phase [32], it the size of the head groups is chosen sufficiently large. However, if we reduce the head size such that the tilt in the gel phase vanishes, the ripple phase disappears as well [36].

These findings suggest that the ripple phase emerges as a response to internal stresses in the membrane, which are generated by a mismatch of head and tail group. At low temperatures, the lipids adjust to the mismatch by uniformly tilting in one direction. This partially relieves the stress, but the remaining stress profile is still substantial [40]. More efficient stress relaxation can be achieved if the monolayers curve around, such that lipids can splay. As domains with uniform monolayer curvature obviously cannot extend to infinity, this might explain the formation of gel stripes with finite width separated by small lines of fluid phase [32].

A similar mechanism could stabilize lipid rafts. Indeed, we have recently carried out simulations of a multicomponent version of the generic lipid membrane model mentioned above [39], and found a structure which bears all the characteristics of rafts. The membranes assume an in-plane structure which is akin to a two-dimensional microemulsion with nanoscale domains of ”liquid ordered” phase embedded in a sea of ”liquid disordered phase” [41]. We have also developed a simple elastic theory which rationalizes both rippled states and rafts in a unified manner.

This work is reviewed in the present conference proceedings paper. We will first present the theory sketched in Ref. [41] in some more detail. Then we will recapitulate some main results of the simulations, both of the ripple phase and of lipid rafts, that support the theory. We conclude with a brief summary.

2. Theory

Our starting point is an elastic theory for membranes that describes the lipid bilayer as a system of two coupled elastic monolayers [42–46]. The phase transition inside the membrane (liquid / gel or liquid ordered / liquid disordered, respectively), is described in a Ginzburg-Landau fashion by an internal order parameter $\Phi$, which is taken to be equal on both monolayer leaflets. Hence the bilayer is perfectly symmetric also in composition. This is clearly an oversimplification. In the raft case, it can be motivated by the experimental observation that globally phase separated liquid ordered domains are strongly correlated across bilayers and tend to oppose each other [47]. In the ripple case, the liquid domains typically are typically arranged in a staggered phase separated liquid ordered domains are strongly correlated across bilayers and tend to oppose each other [47]. In the ripple case, the liquid domains typically are typically arranged in a staggered order. This has consequences on the shape of the total membrane (it assumes a zigzag structure), which will be investigated in future work.

For perfectly symmetric membranes, the bilayer remains on average flat and the total free energy can be described by a combination of two terms, $\mathcal{F} = \mathcal{F}_\text{el} + \mathcal{F}_\Phi$, where $\mathcal{F}_\text{el}$ describes the elastic energy of the coupled monolayer system, and $\mathcal{F}_\Phi$ the Ginzburg-Landau energy associated with the order parameter $\Phi$. In Monge representation[48], the elastic energy can be written as
where \( u(r) \) denotes the local deviation from the mean monolayer thickness \( t_0 \), and the other parameters characterize the elastic properties of the membrane: \( k_c \) the (bilayer) bending stiffness, \( k_A \) the (bilayer) compression modulus, \( c_0 \) the spontaneous monolayer curvature, \( \zeta \) an associated curvature-related parameter [44, 45], and \( k_G \) the Gaussian curvature of monolayers. At fixed lipid number and membrane tension, Eq. (1) holds both for tensionless membranes and membranes under tension [49]. (However, elastic constants may depend on the tension [49, 50]). At fixed membrane area and variable lipid number, an additional term \( \mu l (\nabla u)^2 \) comes in for membranes under tension [51], which is associated with the chemical potential of the lipids, and can be incorporated in the parameter \( \zeta \) after partial integration. We have used Eq. (1) several times in the past to fit deformation profiles in the vicinity of inclusions, with good results down to molecular length scales [46, 52].

The Ginzburg-Landau associated with the order parameter \( \Phi \) is given by

\[
\mathcal{F}_\phi = \int d^2r \left\{ \frac{g}{2} (\nabla \Phi)^2 + f(\Phi) \right\},
\]

(2)

where \( f(\Phi) \) is a local free energy functional with two minima that account for the local tendency of phase separation. The elastic degrees of freedom \( u(r) \) are coupled with the order parameter \( \Phi \) through the fact that elastic parameters may vary spatially and depend on \( \Phi(r) \) [53]. For example, only recently [54] it was discussed how the monolayer curvature \( c_0 \) might depend on \( \Phi(r) \) for raft-forming lipids.

Here, we will assume for simplicity that all elastic parameter are constant except the monolayer curvature, \( c_0 \), which is taken to depend on \( \Phi \) in a linear fashion. In this case, the natural length unit in the system is the in-plane correlation length \( \xi = (k_c t_0^2/k_A)^{1/4} \), which is the length scale that one can construct from combining the bending rigidity \( k_c \) and the reduced compression modulus \( k_A/t_0^2 \) (a similar expression is found in membrane stacks [55, 56]). Micropipette experiments on a variety of lipid membranes [57] have shown that \( k_c \) is roughly proportional to \( k_A t_0^2 \), hence \( \xi \) should be of the order of the membrane thickness, \( t_0 \). Inserting actual numbers for the fluid phase of one-component bilayers made of dipalmitoyl phosphatidylcholine (DPPC, one of the most common lipids in natural biomembranes) from experiments, all-atom simulations, or coarse-grained simulations [46], one consistently obtains values around \( \xi \sim (0.9 - 1.4) nm \).

Rescaling all lengths in units of \( \xi \), the elastic energy, Eq. (1), can be rewritten as

\[
\mathcal{F}_\text{el} = k_c \int d^2\hat{r} \left\{ \frac{1}{2} (\Delta \hat{u})^2 + \frac{1}{2} \hat{u}^2 + 2k_c (c_0 + \frac{\zeta}{t_0} u) \Delta u + k_G \det(\partial_{ij} u) \right\},
\]

(3)

with the dimensionless characteristic membrane parameter \( b = 2\zeta \xi^2/t_0 \) (\( b \sim 0.7 \) in DPPC[41]) and \( \hat{c} = \xi c_0(\Phi)/\Phi = \text{const} \). The contribution of the Gaussian rigidity, \( k_G \), has been dropped, because it gives a constant at fixed topology according to the Gauss-Bonnet theorem. The Ginzburg-Landau energy is rescaled accordingly,

\[
\mathcal{F}_\phi = \int d^2\hat{r} \left\{ \frac{g}{2} (\nabla \Phi)^2 + \hat{f}(\Phi) \right\},
\]

(4)

with \( \hat{f}(\Phi) = \xi^2 f(\Phi) \).
2.1. Weak segregation limit and Landau-Brazovskii approximation
We first discuss the weak segregation case, where local deviations from a homogeneous reference state are small and \( \Phi \) remains close to zero. We minimize the elastic free energy, \( F_{el} \), with respect to the bilayer distortion \( \hat{\upsilon}(\hat{r}) \). This gives the Euler Lagrange equations

\[
\hat{\Delta}^4 \hat{\upsilon} + 2b \hat{\Delta} \hat{\upsilon} + \hat{\upsilon} + 2c \hat{\Delta} \Phi = 0 \quad (5)
\]

The solution is most conveniently calculated in Fourier space,

\[
\hat{\upsilon}_q = \frac{2c q^2}{q^4 - 2bq^2 + 1} \Phi_q, \quad (6)
\]

where the \( q \) are in-plane wavevectors. Inserting this expression in Eq. (4) and dropping boundary terms, one obtains

\[
F = \frac{1}{2} \sum_q |\Phi_q|^2 q^2 g_{st}(q^2) + \int d^2 \hat{r} \hat{f}(\Phi) \quad (7)
\]

with

\[
g_{st}(q^2) = g - 4kc \epsilon^2 \frac{q^2}{q^4 - 2bq^2 + 1}. \quad (8)
\]

Inspection of Eq. (7) shows that a homogeneous phase (\( \Phi \equiv \text{const.} \), i.e., \( \Phi_q = 0 \) for \( q \neq 0 \)) becomes unstable with respect to modulations with wavevector \( q^* \) if the stiffness parameter \( g \) becomes smaller than \( g < g^* = 2k_c c^2 / (1 - b) \). Close to the transition, modulations with \( q \approx q^* \) dominate, and we can expand \( q^2 g_{st}(q^2) \) about \( q^* \) up to second order in \( (q^2 - q^{*2}) \). Furthermore, we expand the local free energy density \( \hat{f}(\Phi) \) in powers of \( \Phi \) up to fourth order,

\[
\hat{f}(\Phi) = \frac{a}{2} \Phi^2 - \frac{\gamma}{6} \Phi^3 + \frac{\lambda}{24} \Phi^4. \quad (9)
\]

Putting everything together and transforming the stiffness contribution \( \sum_q |\Phi_q|^2 q^2 g_{st}(q^2) \) back to real space, our final free energy functional for \( \Phi \) can be written in the form

\[
F = \int d^2 \hat{r} \left\{ \frac{\Gamma}{8q_0^2} (\Delta + q_0^2)^2 \Phi^2 + \frac{\tau}{2} \Phi^2 - \frac{\gamma}{6} \Phi^3 + \frac{\lambda}{24} \Phi^4 \right\} \quad (10)
\]

with \( q_0^2 = (q^*)^2 (1 + (1 - b)(1 - g/g^*)) \), \( \Gamma/4q_0^2 = g^*/2(1 - b) \), and \( \tau = a - (g - g^*)(1 + q_0^2)/2 \). Eq. 10 is the free energy of the well-known Landau-Brazovskii model [58], which provides a general framework for the description of phase transitions driven by a short wavelength instability between a disordered phase and one or several ordered phases. In mean field approximation, the Brazovskii model predicts a continuous transition point between a homogeneous phase and several ordered phases (stripes, hexagonal). Fluctuations are known to destroy the order close to the multicritical point and stabilize a locally structured disordered phase via the so-called Brazovskii mechanism [58]. The order-disorder transition is shifted and becomes first order. Hence this model accounts for both disordered states with local structure and a characteristic wave length, such as raft states, and ordered modulated states such as ripple phases.

2.2. Strong segregation limit and raft domains
We can also approach the problem from the opposite side and consider membranes where the local tendency for phase separation is very strong, i.e., domains separated by narrow interfaces form throughout the system. Kuzmin et al. have pointed out in 2005 that curvature effects reduce the line tension between such domains [18]. Here, we will show that they may even
reduce the line tension to zero, such that globally phase separated states are no longer stable, and stabilize rafts of finite size.

We assume that the interfaces between the phase separated domains are sufficiently narrow that they can be treated as lines where the order parameter jumps from one value to another, with a bare line tension $\lambda_0$. The spontaneous curvature $c_0^i$ in the two phases differs and the difference is denoted $\delta c_0$. Within each domain, the elastic parameters are homogeneous, and the solution for $\hat{u}(\hat{r})$ that minimizes the elastic free energy $F_{el}$ must fulfill the homogeneous Euler-Lagrange equation,

$$\hat{\Delta}^4 \hat{u} + 2b\hat{\Delta} \hat{u} + \hat{u} = 0. \quad (11)$$

The solution depends on the boundary condition. Since the equation is fourth order, four sets of boundary conditions have to be given, e.g., equations for $\hat{u}(\hat{r})$ and the first three derivatives of $\hat{u}(\hat{r})$ along the boundary normals $n$. These boundary conditions must be chosen such that they minimize the total elastic energy. In particular, it is clear from Eq. (3) that $\hat{u}(\hat{r})$ and its normal derivative, $n \cdot \nabla \hat{u}$, must be continuous at the boundaries, otherwise the bending contribution $\int \frac{1}{2}(\Delta \hat{u})^2$ would diverge.

To derive the remaining boundary conditions, we carry out a variation of $F_{el}$ with free boundary conditions for all boundaries $C_i$ of domains $i$ with boundary normals $n_i$ (pointing outwards) and spontaneous curvatures $c_0^{(i)}$. This leads to the variational expression $\delta F_{el} = \delta F_{el,b} + \delta F_{el,s}$ with

$$\delta F_{el,b} \propto \int d^2\hat{r} \left[ \hat{\Delta}^4 \hat{u} + 2b\hat{\Delta} \hat{u} + \hat{u} \right] \delta \hat{u}$$

and

$$\delta F_{el,s} \propto \int_{C_i} dl \left\{ \delta(n_i \cdot \nabla \hat{u})[\hat{\Delta} \hat{u} + b\hat{\Delta} \hat{u} + 2\xi_c^{(i)}] - \delta \hat{u}[(n_i \cdot \nabla)\hat{\Delta} \hat{u} + b(n_i \cdot \nabla)\hat{u}] \right\}. \quad (13)$$

The functional $\delta F_{el}$ has to vanish for all possible variations $\delta \hat{u}(\hat{r})$ in the bulk and for all variations $\delta \hat{u}$ and $\delta(n \cdot \nabla \hat{u})$ at the boundaries. The bulk term $\delta F_{el,b}$ gives the Euler-Lagrange equations. The surface term, $\delta F_{el,s}$, is used to derive the appropriate boundary conditions. In our case, the membrane is completely filled with domains, hence every boundary integral element $dl$ appears twice with normals pointing in opposite directions. Since, furthermore, $\hat{u}$ and $n \cdot \nabla \hat{u}$ must be continuous at the boundaries, a number of terms drop out and we are left with the boundary conditions that $\nabla \hat{\Delta} \hat{u}$ and $(\hat{\Delta} \hat{u} + 2\xi c_0)$ must be continuous.

Inserting these boundary conditions and the Euler-Lagrange equations in $F_{el}$ (Eq. (3)), we obtain the following simple expression for the elastic energy,

$$F_{el} = k_c \delta c_0 \int_{C_i} dl \ n \cdot \nabla \hat{u},$$

where the line integral $\int dl$ runs once over all domain boundaries, $n$ is the normal vector, $\delta c_0$ is the curvature mismatch, i.e., the monolayer curvature difference in the inner and outer phase, and $\hat{u}$ is the solution ($u = \xi \hat{u}$) of the Euler-Lagrange equation with the appropriate boundary conditions.

The rest of the calculation is purely technical. In a one-dimensional stripe geometry, the general solution of the Euler Lagrange equations is given by an exponential

$$\hat{u}(\xi) = e^{\pm i\alpha \xi} \quad \text{with} \quad \alpha = \sqrt{\frac{1}{2} \left( \sqrt{1 + b} + i \sqrt{1 - b} \right)}. \quad (15)$$

Inserting the boundary conditions, one obtains the following solution for a single stripe domain of width $D$:

$$u(z) = \begin{cases} \frac{2\xi_c^2 \delta c_0}{\sqrt{1 - b^2}} \left\{ \Re[i e^{i\alpha D/2} \cos(\alpha z)] : 0 < z < D/2 \right\} \\
\Re[\sin(-\alpha D/2) e^{i\alpha z}] : z > D/2 \end{cases} \quad (16)$$
with \( u(-z) = u(z) \). The corresponding elastic free energy is \( F_{el} = 2L\lambda_{el} \) (\( L \) is the length of the stripe) with
\[
\lambda_{el}(D) = -\frac{\xi k_c \delta c^2}{\sqrt{1-b^2}} \mathbb{R}[\alpha(1-e^{i\alpha D})].
\]
We note that this expression is always negative. Relaxing the elastic energy at the boundaries goes along with a gain in free energy. The total energy of a stripe, which includes the bare tension \( \lambda_0 \), is given by \( F_{el} = 2L(\lambda_0 + \lambda_{el}) \), and it is reduced by the contribution of the elastic energy. In particular, in the limit \( D \to \infty \), one obtains the line tension
\[
\lambda = \lambda_0 + \lambda_{el}(\infty) \quad \text{with} \quad \lambda_{el}(\infty) = -\frac{\xi k_c \delta c^2}{\sqrt{2(1-b^2)}}.
\]
Hence the contribution due to the monolayer is found to reduce the line tension, in agreement with Kuzmin et al. [18]. At high curvature mismatch, the line tension may become negative. Even more interestingly, it exhibits an oscillatory behavior as a function of stripe width (Eq. 17), indicating that stripes of finite width are favored. The optimal width is of order \( 4\xi \), i.e., several nanometers, which is just the width of ripples and the width commonly attributed to rafts.

A similar exercise can be carried out for domains with spherical geometries. In that case, we look for radially symmetric solutions of the Euler Lagrange equations (11), which can be written in the form
\[
u(r) = \frac{\delta c_0}{\sqrt{1-b^2}} \frac{\pi D \xi}{2} \begin{cases} 
\mathbb{R}[\xi \alpha J_0(\alpha r) H_1^{(1)}(\alpha D/2)] & : r < R \\
\mathbb{R}[\xi \alpha J_1(\alpha D/2) H_0^{(1)}(\alpha r)] & : r > R
\end{cases},
\]
where \( J_n \) and \( H_n^{(1)} \) are the Bessel and Hankel functions of the first kind, respectively. These profiles satisfy the boundary conditions by virtue of the identity \( J_0(z)H_1^{(1)}(z) - J_1(z)H_0^{(1)}(z) = -2i/\pi z \). Inserting this into the free energy expressions, we obtain the following expression for the total line tension of disks of diameter \( D \):
\[
\lambda_{disk}(D) = \lambda_0 - \frac{\pi D k_c \delta c^2}{2\sqrt{1-b^2}} \mathbb{R}[\xi^2 J_1(\alpha D/2)H_1^{(1)}(\alpha D/2)].
\]
Again, it has a minimum at finite diameter around \( \sim 4\xi \).

Our result implies that domains of finite diameter become stable even before the asymptotic line tension \( (D \to \infty) \) vanishes: There should be a regime where the membrane is filled with small nanoscale domains, i.e., ripples or rafts. Since disk-shaped and stripe-shaped domains have similar line energies, the actual shape depends on the composition and on molecular details. We should also note that the raft domains interact with each other, hence the strong segregation theory actually predicts modulated phases with long-range order. This long-range order could be destroyed by thermal fluctuations as already discussed above in section 2.2.

2.3. Theory: Summary
To summarize, we propose a joint theoretical explanation for both ripples phases in one-component monolayers and lipid rafts in multicomponent monolayers, which is based on the coupled monolayer model. We assume that monolayers exhibit local phase separation into two phases with different order parameter (composition or other), and that the spontaneous curvature of the monolayer depends on the local order parameter. In the strong segregation limit where different phases are separated by narrow interfaces, we have shown that the line tension is reduced in the presence of a mismatch \( \Delta K_0 \) between the spontaneous curvatures of the two phases. This is because monolayers with a spontaneous curvature, which are forced into
being planar by the apposing monolayer, experience elastic stress. Some of that stress can be released at the domain boundaries. The resulting negative contribution to the line tension scales with $k_c (\delta c_0)^2$ and should be present wherever $\delta c_0$ is nonzero. A more detailed calculation shows that the elastic energy is minimized for spherical or stripe domains of a specific size, which is of the order of a few nanometers. This elastic mechanism can thus stabilize rafts of finite size for sufficiently large spontaneous curvature mismatch.

We have also considered the weak segregation limit, where the phase separation is incomplete, the interfaces are broad, and the free energy can be expanded in powers of the order parameter $\Phi$. We have shown that the expansion has a Landau-Brazovskii form [58] (Eq. (10), with a characteristic wave vector of the order $q_0 < 1/\xi$, where $\xi \sim 1 \text{nm}$ is the in-plane correlation length. The correlation length $\xi$ sets the order of magnitude and a lower limit for the characteristic wave length of phase separated structures in the membrane. The theory thus predicts local structure formation and with characteristic wave lengths of the order of a few nanometers. This is compatible with the experimental observations for ripple phases, and with experimental expectations for raft structures, should they exist in protein-free membranes. In the following section, we will show that both ripples and rafts can be observed in coarse-grained simulations, with features that support the theoretical picture.

3. Simulations
We will now describe the simulations of a generic simulation model for lipid bilayers, which reproduces both rippled states in one-component bilayers and rafts in multicomponent bilayers.

3.1. Simulation model
Lipids are represented by semiflexible linear chains of typically six tail beads attached to one slightly larger head bead [59–65]. Tail beads attract each other with truncated Lennard-Jones interactions, all other interactions are repulsive and of the Weeks-Chandler-Anderson type [66]. Lipids are immersed in a structureless fluid of so-called ”phantom” solvent particles [38], which do not interact with each other (hence the fluid is structureless), only with lipids. For details of the model, we refer to Ref. [39]. The single-component model roughly reproduces the main transition and elastic properties of DPPC bilayers in the fluid phase [46], if one maps the simulation units for length and energy to SI units according to $\sigma \sim 6 \text{\AA}$ and $\epsilon \sim 0.36 \cdot 10^{-20} \text{J}$ [67]. In the multicomponent extension [41], ”phospholipids” are mixed with ”cholesterol” molecules, which are shorter and stiffer and have a slight affinity to ”phospholipids” (motivated by [68]). The model was studied by Monte Carlo simulations at constant pressure and zero membrane tension. To this end, simulations were carried out in a parallelepiped with periodic boundary conditions and all parameters of the simulation box (side lengths and angles) were allowed to fluctuate. Model lipids self-assemble spontaneously to bilayer membranes. Typical run lengths were several million Monte Carlo sweeps.

Figure 1. Asymmetric ripple state ($P_{\beta'}$ phase) in a one-component (”phospholipid”) bilayer (20 000 lipids).
Figure 2. Left: Local chain length along the ripple direction in an asymmetric ripple state (one ripple). Right: Average chain length in the corresponding homogeneous states as a function of temperature. (At low temperature: Tilted gel phase $L_{\beta'}$; at high temperature: Fluid phase $L_{\alpha}$.) From Ref. [67].

3.2. One-component membranes and ripple structures

One-component model membranes exhibit the main phases observed experimentally, i.e., the fluid phase $L_{\alpha}$, the gel phase $L_{\beta'}$, and the ripple phase, $P_{\beta'}$. Fig. 1 shows an example of a ripple state, close to the transition to the fluid phase. One clearly recognizes how stripes with ordered lipids and curved monolayers alternate with more disordered regions. This can also be quantified, e.g., by looking at the average chain length as a function of the position in the membrane. Fig. 2 compares the profile of the average chain length in the direction perpendicular to a ripple structure (Fig. 2 a) with the average chain length in a small system undergoing a fluid-gel transition (Fig. 2 b). In the more ordered gel phase, the chains are stretched and the average chain length is roughly 10% larger than in the disordered fluid phase. The chain length profile across the ripple structure jumps between the gel value and the fluid value. We conclude that the ripple structure can indeed be described by a sequence of locally segregated fluid and gel domains, as required by the theory of Section 2. Integrating over the difference, one finds that about one fourth of all chains are in a fluid state. This is in rough semiquantitative agreement with experimental calorimetric measurements, which suggest that about 10% of all chains are melted [69].

In the homogeneous gel state, lipids are uniformly tilted with a tilt angle around 20 degrees. In the ripple state, the tilt angle continuously varies across the ripple structure. Fig. 3 shows the evolution across the ripple for three different ripple structures: The asymmetric ripple structure already shown in Fig. 1 (in Fig. 3 left), an intermediate structure with a single ripple defect which formed upon heating up a gel state (Fig. 3 right), and a symmetric ripple structure which is also observed occasionally both in simulations and in experiments (Fig. 3 c)). All structures are characterized by alternating gel and liquid domains in the monolayer leaflets, and in all structures, the average tilt angle varies linearly across the gel domains. Moreover, the slope of the tilt profiles is the same in all cases. $(d\theta/dx \sim 2.5/\sigma$ in simulation units $\sigma \sim 6\AA$). These findings underline that the ripple structures are stabilized by a propensity of lipids to splay, or in other words, by a propensity of monolayers to curve around.

3.3. Multicomponent membranes and rafts

We now turn to the discussion of multicomponent membranes. We have studied mixtures of two types of lipids, "phospholipids" and "cholesterol". These mixtures were first investigated at fixed composition (canonical ensemble) for relatively small system sizes. In particular, we have determined the chemical potential difference between the two species from the virtual acceptance
Figure 3. Configuration snapshots (left) and local tilt angle profiles along the ripple direction (right) in different ripple states. (a) Asymmetric ripple state. (b) Metastable "ripple" state with a single line defect, obtained from heating up a system from the gel state. (c) Symmetric ripple state. (from Ref. [32]).

Figure 4. Chemical potential difference between "Cholesterol" and "Phospholipid" molecules in a two-component bilayer system for a range of small system sizes as indicated (9 × 9 × 2 up to 18 × 18 × 2 lipids).

rate of identity switches of single molecules. (The identity switches were not performed). Fig. 4 shows the result for different system sizes as a function of cholesterol concentration for the temperature $k_B T = 1.4 \epsilon$. A similar graph with a somewhat worse statistics was presented in Ref. [41]. One clearly sees the signature of a phase transition between two different phases with a transition at cholesterol concentration around 30%. In a macroscopically stable system, the chemical potential difference must decay as a function of cholesterol concentration. Based on a Maxwell equal-area construction, one would hence expect phase separation in large systems.
between two phases with cholesterol concentrations around 5% and 40%.

However, the simulations of large systems tell a different story. These simulations were done in a semi-grandcanonical ensemble with constant total number of lipids, but variable composition. In case of global phase separation, the system should evolve towards one of two homogeneous phases, either an ordered phase with high cholesterol concentration, or a disordered phase with low cholesterol concentration. Instead, one obtains small ordered domains immersed in a disordered matrix: Lipid rafts (Fig. 5). Looking at the lateral structure of the membranes, we can establish that there is indeed a characteristic length scale in the system. To this end, we have identified "raft" and "non-raft" regions through a prescription based on a combination of the local density, the local composition, and the local nematic order parameter of the chains. This generated binary two-dimensional maps with black and white domains for each monolayer. The maps were then Fourier transformed. The result, radially averaged, is shown in Fig. 6 for different chemical potential differences $\mu$. In the "unstable" region around $\mu = 8.5k_B T$, it is large, indicating strong fluctuations, and clearly features a peak at nonzero wavevector $q$. For higher $\mu$, i.e., lower cholesterol concentrations, it becomes smaller and flat. Interestingly, a peak is also observed in the cholesterol-rich regime at lower $\mu = 6.2, 6.9k_B T$. This peak reflects the existence of ripple-like defects in the cholesterol-rich phase (Meinhardt et al, to be published).

We conclude that rafts of finite size with a well-defined characteristic length scale can be observed in binary mixtures. A detailed analysis of their internal structure including the stress distribution and the spontaneous curvature is under way.

One comment is in place here. Typical "raft mixtures" used for studying rafts in model membranes have at least three components. This is because three components seem necessary to bring about global lateral phase separation [14]. Here we report raft-like structures in simulations of a coarse-grained model for binary mixtures, but as in experiments, their systems do not show global phase separation between fluid states. Likewise, there is also experimental evidence that nanoscopic domains may already be present in binary mixtures – in particular mixtures of saturated lipids (lipids with high main transition temperature) and cholesterol. Studies based
Figure 6. Radially averaged structure factor of raft conformations from semi-grandcanonical simulations of large bilayers (20,000 lipids) for different values of the chemical potential difference $\mu$.

...on local techniques such as ESR, NMR, or diffusivity measurements have indicated the existence of immiscible liquid phases [14, 70, 71], whereas in fluorescence microscopy, one only observes one homogeneous phase [14]. This suggests that these two-component membranes phase separate on the nanoscale, while remaining homogeneous on the global scale, and that they thus feature many of the intriguing properties attributed to rafts. Shortly after we published our paper [41], this picture was supported by an experimental neutron scattering study of lipid cholesterol mixtures [72, 73].

4. Summary

In sum, we have presented a simple theory that accounts in a unified manner for both ripple phases and raft states in membranes. The prerequisites for the formation of such modulated phases is (i) local phase separation (e.g., in the ripple case, between a liquid and a gel phase, or in the raft case, between a liquid disordered and a liquid ordered phase) and (ii) curvature stress in at least one of the two phases (typically the ordered one), resulting, e.g., from a size mismatch between head group and tails. In order to reproduce rippled states or rafts, coarse-grained simulation models must meet these criteria. This is often not the case. For example, in the standard version of the popular MARTINI model [74], simulations of saturated phospholipids tend to give an untilted low-temperature gel state, and it takes very advanced sampling methods to also recover the tilted gel state [75]. Moreover, coarse-grained phospholipids and cholesterol interacting with MARTINI force fields show no sign of nonrandom mixing [76]. Therefore, it will probably be very difficult, if not impossible, to find rippled structures or nanoscale equilibrium rafts with this model.

The theory presented here is still very simplified. In future work, we will have to account for the possibility that the rafts on apposing monolayers are not rigorously coupled, and that the lipid composition on the two leaflets might be different. Moreover, the effect of fluctuations must be studied in detail in order to obtain the phase diagram, i.e., to identify parameter regions where one expects ordered modulated phases, and parameter regions where the final structure is a two dimensional microemulsion.
Our theory is generally applicable to two systems that undergo local phase separation between phases with different spontaneous monolayer curvature. However, the actual coupling between the membrane composition, the order parameter, and the spontaneous curvature depends on the system under consideration. In symmetric binary membranes, it seems plausible that the order parameter is simply proportional to the difference of the concentrations of the two components. In ternary systems, the relation is more complicated, which further enriches the phase behavior. For example, binary mixtures of phospholipids and cholesterol do not demix globally above the main transition, whereas adding a third lipid component may induce lipid-lipid phase separation [14]. A possible explanation is that the third component relieves curvature stress, thus making phase separation possible. Elucidating such effects will also be an interesting direction for future research.

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