Physical mechanisms of micro- and nanodomain formation in multicomponent lipid membranes

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Abstract
This article summarizes a variety of physical mechanisms proposed in the literature, which can generate micro- and nanodomains in multicomponent lipid bilayers and biomembranes. It mainly focuses on lipid-driven mechanisms that do not involve direct protein-protein interactions. Specifically, it considers (i) equilibrium mechanisms based on lipid-lipid phase separation such as critical cluster formation close to critical points, and multiple domain formation in curved geometries, (ii) equilibrium mechanisms that stabilize two-dimensional microemulsions, such as the effect of linactants and the effect of curvature-composition coupling in bilayers and monolayers, and (iii) non-equilibrium mechanisms induced by the interaction of a biomembrane with the cellular environment, such as membrane recycling and the pinning effects of the cytoplasm. Theoretical predictions are discussed together with simulations and experiments. The presentation is guided by the theory of phase transitions and critical phenomena, and the appendix summarizes the mathematical background in a concise way within the framework of the Ginzburg-Landau theory.

Keywords: Membrane, Cholesterol, Lipid domains, Lipid phase separation, Lipid sorting, Curvature, Two-dimensional microemulsions, Membrane recycling, Cytoplasm

1. Introduction
Ever since Simons and Ikonen first coined the term "rafts" to describe certain lateral inhomogeneities in lipid membranes [1], the question whether rafts exist in vivo and why they form has been the subject of a lively and often controversial debate in the biophysics community [2–17]. Even the meaning of the word "raft" has long remained vague, until in 2006 the participants of a Keystone Symposium on Lipid Rafts and Cell function have formulated a "consensus definition" [18]: "Membrane rafts are small (10-200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions." This description now serves as a reference for the identification of raft-like structures, even though not all structures that have been associated with rafts fulfill all criteria. For example, "rafts" do not always contain sphingolipids [19, 20].

The raft concept is supported by increasing experimental evidence – e.g. from tracking of lipid diffusion [21], or from superresolution microscopy [22, 23] – that lipid membranes are heterogeneous on a nanometer scale. Nano- and microdomains in membranes have been observed in a large range of organisms, including prokaryotic cells [19, 20], single-cell organisms [24], and plant cells [25]. Motivated by the observation that sphingolipids (sphingomyelins, glycosphingolipids, ceramides) – which participate in cellular signalling – tend to enrich in raft domains [28, 30], it has been speculated that rafts may serve a biological purpose in cell-cell recognition and signal transduction [28, 31]. On the other hand, raft-like structures were also observed in prokaryotic membranes which do not contain sphingolipids [19].

One class of lipids which seems to be very prominently involved in raft formation is the sterol class [32, 33]. With few exceptions [34], higher sterols such as cholesterol and ergosterol are typically enriched in rafts. Recently, LaRocca et al. performed a systematic substitution study on prokaryotic membranes, and reported that domain formation was suppressed if cholesterol was depleted or substituted with the wrong sterol [20]. Since the cholesterol molecule with its rigid structure has an ordering effect on the acyl chains of the surrounding lipids [35–37], the apparently dominant role of cholesterol suggests an interpretation of rafts in terms of a local nucleation of ordered cholesterol-rich "liquid ordered" (lo) domains in a "liquid disordered" (ld) sea of cholesterol-poor phase [33]. "Inverse domains" have also been observed [38], where highly disordered domains with a high content of polyunsaturated fatty acids segregate from a cholesterol-rich environment.

The idea that lipid-cholesterol bilayers may demix into "liquid ordered" and "liquid disordered" states had been put forward already in 1987 by Ipsen et al. [39] based on experimental data by Vist et al. [40] (see Sec. 2). In contrast to equilibrium phase separated domains, however, lipid rafts are small and transient structures. The question
why such domains should form has intrigued scientists for some time. In vivo, membranes are filled with proteins and lipid domains are typically correlated with protein clusters. Therefore, it has been argued that the observed membrane heterogeneities could be driven by protein-protein interactions alone. If "raft proteins" associate with "raft lipids" such as cholesterol, it seems conceivable that a protein cluster could nucleate a liquid ordered lo lipid domain in its vicinity. On the other hand, recent experiments by Sevcsik et al. have shown that immobilized lipid anchored raft proteins do not seem to have a measurable effect on the membrane environment. This suggests that the formation of lipid domains is primarily driven by the lipids and not by the membrane proteins.

Indeed, nanostructures and microstructures are also observed in pure model lipid bilayers. One prominent example in one-component bilayers is the modulated "ripple phase" \( P'_\alpha \), which generically emerges in the transition region between the fluid phase \( L_\alpha \) and a tilted gel phase \( L'_\beta \) (see Sec. 2). Here and throughout, the term "modulated" refers to periodic or quasi-periodic patterns – in this case striped patterns with periodicities of the order of 10 nm. In multicomponent lipid bilayers, experimental evidence for the existence of nanoscopic cholesterol-rich domains has been provided by Förster resonance electron transfer (FRET) and electron spin resonance (ESR) experiments, neutron scattering methods, interferometric scattering microscopy, and atomic force microscopy. Micron-size domain patterns were observed in multicomponent giant unilamellar vesicles. We will discuss these observations in more detail further below.

On the side of theoretical membrane science, a number of mechanisms have been proposed that can generate micro- and nanostructures in lipid bilayers. The purpose of the present paper is to give an overview over these mechanisms and to explain and discuss some prominent examples. The review mainly focusses on lipid driven mechanisms that do not involve direct protein-protein interactions. Since virtually all of these mechanisms are based on the phase behavior of membranes in one way or another, the discussion will be guided by the theory of phase transitions and critical phenomena. To make it accessible for a general audience while still giving mathematical background, the mathematical aspects of the discussion are presented separately in the appendix.

This overview is far from complete. For further information on different aspects of the topic, the reader is referred to other recent reviews, e.g. by Fan et al. (focussing on nonequilibrium mechanisms), Palmieri et al. (focussing on mechanisms based on line active molecules), Lipowsky (focussing on membrane shapes and membrane remodelling), and Komura and Andelman (focussing on phase separation, phase separation dynamics and on micromulsions). In particular, the present article only touches on the issue of multiscale computer simulations of heterogeneous lipid bilayers, which is a challenge in itself and has been discussed in several recent review articles.

The remaining article is organized as follows: In the next section, Sec. 2 we briefly discuss the phase behavior of lipid membranes, focussing on lipid-lipid phase separation. Sec. 3 considers mechanisms of domain formation in pure membranes which are based on incomplete phase separation. This includes the formation of critical clusters just above a critical demixing point (Sec. 3.1) as well as the appearance of multidomain structures on curved vesicles that emerge in order to minimize the total bending energy (Sec. 3.2). In Sec. 4 we review physical mechanisms that generate equilibrium two-dimensional microemulsions, e.g., due to line active agents (Sec. 4.1), or due to lipid curvature induced elastic interactions (Sec. 4.2 and 4.3). Sec. 5 reviews domain-stabilizing mechanisms that rely on an interaction between the membrane and its environment, such as membrane recycling (Sec. 5.1), the presence of pinning sites (Sec. 5.2), or other interactions that influence the phase separation kinetics (Sec. 5.3). We summarize and conclude in Sec. 6. Finally, Appendix A provides a unified mathematical description of the domain-forming mechanisms discussed in the main text within the framework of the Ginzburg-Landau theory.

The following abbreviations will be used in the text:
- AFM (atomic force microscopy)
- NMR (nuclear magnetic resonance)
- FRET (Förster resonance electron transfer)
- ESR (electron spin resonance)
- GUV (giant unilamellar vesicle)
- GMVP (giant plasma membrane vesicle)
- DPPC (dipalmitoylphosphatidylcholine)
- DMPC (dimiristoylphosphatidylcholine)
- DSPC (1,2-Distearoyl-sn-glycero-3-phosphocholine)
- DOPC (1,2-Dioleoyl-sn-glycerol-3-phosphocholine)
- POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine)
- SM (sphingomyelin), Chol (Cholesterol)
- ld (liquid disordered), lo (liquid ordered)

2. Lipid phase behavior and lipid-lipid phase separation

The basis of lipid-driven micro- or nanodomain formation is the observation that lipid bilayers can undergo phase transitions and in particular, that lipids in multicomponent bilayers can phase separate. Already one component lipid bilayers exhibit a complex phase behavior, which is similar for different classes of lipids. At high temperatures, they are in the "fluid" state \( (L_\alpha) \), which is characterized by a low in-plane shear viscosity and a high number of chain defects in the hydrocarbon chains. At lower temperature, the bilayers assume a "gel" state, where the shear viscosity is higher and the hydrocarbon chains have very few chain defects. Depending on the details of the lipid structure, in particular the effective size and the interactions of the polar head groups, the gel state exists in different variants:
Straight chains ($L_\beta$ phase), collectively tilted chains ($L_{\alpha'}$ phase), or even interdigitated chains ($L_{\beta''}$ phase). The fluid and the gel regions are separated by the so-called "main" or "melting" transition, which typically occurs at temperatures around room temperature or higher. If the low temperature state is a tilted gel state, a $P_{3\beta}$ ripple phase interferes in the transition region, which corresponds to the modulated nanostructured structure mentioned in the Introduction. The general structure of lipid phase diagrams seems to be quite generic and is also reproduced by coarse-grained simulations of very simplified model "lipid" molecules [33, 51, 52, 83]. The most relevant phase in the biomembrane context is the $L_{\alpha}$ phase. With few exceptions [77], living organism tend to tune the lipid composition of their membranes such that they remain fluid.

In multicomponent lipid membranes, the lipids may not only order, but also demix. The first experimental phase diagram of a multicomponent lipid bilayer, a DPPC/Chol mixture, was published in 1990 by Vist and Davis [40]. It was derived based on deuterium NMR spectroscopy and differential scanning calorimetry, and it included a demixed two-phase region between two coexisting fluid phases, which were subsequently termed ld (liquid disordered), and lo (liquid ordered) phase [78] based on a theoretical proposal by Ipsen et al. [39]. This terminology is now widely used in descriptions of rafts. Veatch and Keller later investigated the same binary system by fluorescence microscopy on mixed GUVs and saw no indication of fluid-fluid coexistence [79, 80]. Around the same time, Filippov et al. studied binary mixtures of DMPC and cholesterol by NMR diffusion experiments and rationalized their findings by postulating the existence of small "coexisting" lo domains in the mixtures, which rapidly exchange lipids with an ld environment [81, 82]. This suggests an explanation for the seemingly contradictory reports on the phase behavior of binary lipid-cholesterol mixtures [79]: Phase separation between lo and ld phase is typically observed with methods that are sensitive to local ordering and rearrangements, whereas the same systems appear homogeneous in microscopic studies that detect phase separation on the scale of micrometers. Hence these systems are presumably filled with very small domains. Indeed, recent neutron diffraction experiments by Rheinstätter and coworkers provided evidence for the existence of highly ordered nanoscopic lipid domains in the DPPC/Chol mixtures [53, 54, 55, 56]. Similar structures were observed by us in coarse-grained computer simulations of binary mixtures [52, 54]. Inverted nanoscale domains (ld in lo) were reported by Kim et al. from atomic force microscope (AFM) imaging of DPPC/Chol monolayers [55].

These nanodomains are clearly interesting in the raft context, but they are not phase separated in a thermodynamic sense. The fact that they have been observed does not explain why such tiny structures form, and this is our key question here which we will discuss in the following sections.

In binary lipid membranes, fluid-fluid phase separation in a thermodynamic sense does not seem to occur. However, it can be observed in mixtures of more than two components. In 2001, Dietrich et al. [86] reported the formation of large phase separated fluid domains (several tens of microns) in GUVs containing ternary mixtures of a phospholipid, cholesterol, and sphingomyelin. Phase diagrams for a many other ternary systems were later studied in detail by several groups using a variety of experimental methods [79, 80, 79, 80, 79, 80]. Veatch and Keller [79] observed that fluid-fluid ld-lo separation is a frequent phenomenon in mixtures of cholesterol, a lipid with high melting temperature $T_m$, and a lipid with low $T_m$. Feigenson et al. noted that both macroscopic lo-ld phase separation and nanodomain formation can be found in such systems, depending on the choice of lipids, and introduced the categories of "Type I" and "Type II" mixtures to distinguish between two types of phase behavior: The phase diagrams of type II mixtures feature a region of macroscopic ld-lo phase separation. This demixed region is missing in type I mixtures and replaced by a region with prominent nanoscopic domain formation [92, 93]. To study the transition between type I and type II behavior, Feigenson and coworkers carried out systematic studies of four-component mixtures with compositions that "interpolate" between typical type I and type II mixtures, and found that ordered modulated structures emerge at intermediate compositions [62, 64].

Nowadays, ternary mixtures of saturated and unsaturated phospholipids (which have a high and low melting temperature, respectively) and cholesterol serve as standard model systems for systematic studies of raft formation in membranes [95, 96]. Large scale atomistic and coarse-grained computer simulations have been carried out to study fluid-fluid phase separation in such systems [97, 98]. However, lipid-lipid phase separation is not restricted to model systems; it has also been observed in membranes of natural lipids that were extracted from brush border membranes [86], and even in membranes that were directly extracted from living cells [59, 100]. Hence it seems to be a generic phenomenon in multicomponent membranes, and it seems reasonable to associate it with raft formation.

Nevertheless, the question remains why rafts are so small, and – possibly related – why small nanoscopic domains are observed in model membranes. In a classical phase separation scenario, the interface between domains is penalized by a line tension, and the equilibrium state is one that minimizes the length of phase boundaries, i.e., the system partitions into only two regions ld and lo. In the following sections, we will present a variety of mechanisms that interfere with large scale phase separation and may lead to the formation of small domains.

3. Domain formation due to incomplete phase separation

We first discuss isolated multicomponent membranes that exhibit macroscopic phase separation (i.e., "type II") mixtures in the categorization of Feigenson [93]. Two
AFM pictures of critical clusters in supported multicomponent bilayers published along with the corresponding radially averaged correlation functions (from Connell et al. [54]).

From the theory of critical phenomena, one expects several quantities to show a peculiar power law behavior close to a critical point (see Appendix A.1): As one approaches the critical point at fixed critical composition, the correlation length should diverge as $\xi \propto |T - T_c|^{-1}$, the line tension should vanish according to $\lambda \propto |T - T_c|$, and the width of the miscibility gap at $T < T_c$ along the tie lines should vanish according to a power law with $\Delta c \sim (T - T_c)^{1/8}$. These theoretical predictions have been verified by Keller and coworkers [60, 61] using fluorescence microscopy on model multicomponent membranes and the expected power law behavior was confirmed within the experimental error. The same group also studied the dynamics of thermal fluctuations close to $T_c$ [101] and the dynamics of phase separation and coarsening below $T_c$ [102] and again found good agreement with theoretical predictions. Connell et al. showed by AFM studies of supported bilayers that the critical behavior persists up to temperatures where the correlation length is only of the order of a few nanometers [54].

Hence, critical phenomena are one plausible explanation for the observation of nanoscale raft-like structures in lipid membranes. However, they can only be observed in membranes with close-to-critical lipid compositions. This raises the question why living organisms should go through the effort to maintain such highly specific compositions in their membranes. One possible answer is that it may be of advantage to keep cell components close to phase transitions [103]. In the vicinity of phase transitions, systems respond very sensitively to external stimuli, and this provides efficient control mechanisms which may be useful in the complex interaction network of a cell. Machta et al. [104] proposed that nature may also take advantage of the relatively long-range fluctuation-driven forces between membrane inclusions (so-called Casimir forces) that emerge close to critical points. Such forces can possibly be exploited to manipulate the lateral organization of membrane proteins.

3.1. Domains close to a critical point

Keller, Veatch and coworkers proposed a strikingly simple possible explanation why small domains are observed in membranes [58, 59]: They argued that these domains could simply be fingerprints of critical behavior in the vicinity of critical demixing points: Even though membranes made of natural lipid mixtures have been shown to phase separate at low temperatures, one would expect that body temperatures are typically well above the miscibility gap. Indeed, Veatch et al. studied giant plasma membrane vesicles (GMPVs) that were extracted directly from the living rat basophil leukemia cells [59], and found that they undergo a demixing transition at temperatures around $T_c \sim 20^\circ C$, well below the body temperature of rats (which is similar to that of humans). The transition temperatures for different GMPVs where widely spread over a range of 5-10 $^\circ C$. Interestingly, the lipid compositions were nevertheless always found to be near-critical. This suggests that the small domains in real membranes may simply be critical clusters, as occur naturally in the vicinity of critical point (see Appendix A.1 for a mathematical description).

Critical clusters are characterized by a fractal structure and a broad size distribution. The average domain size is set by the correlation length, which diverges at the critical point. At critical compositions, the correlation length at temperatures of 10 degrees above the demixing temperature $T_c$ can still be of the order of 10 nm [54]. Fig. 1 shows classes of mechanisms have been proposed that may generate small domains in such systems: Critical fluctuations and lipid sorting due to background curvature.

3.2. Multiple domain formation in curved geometries

Below the demixing point, phase separation is often coupled to conformational changes in the membrane [67, 68]. One prominent example is domain-induced budding [54, 62, 105, 112], where phase separated membrane domains bulge out of a membrane in order to reduce the contour length of the domain boundary. This effect was first proposed theoretically in 1992 by Lipowsky [105] and later confirmed experimentally by Baumgart and coworkers [56, 109] by experiments on multicomponent vesicles. Depending on the membrane tension, the size of the domains, their bending stiffness, and other elastic properties, one observes transitions between buds, dimples, and flat domains [110, 112]. Domain-induced budding effect does...
not stabilize multidomain formation *per se*, but it does facilitate it by reducing the total costs of domain nucleation. Furthermore, the dimpled domains repel each other through curvature-mediated interactions [62, 110], which slows down the aggregation.

A related effect which does have the potential to induce multi-domain formation in curved geometries is curvature-driven lipid sorting. It is based on the fact that the coexisting phases in multicomponent bilayers often have very different bending rigidities, e.g., membranes in the *ld* phase are stiffer than membranes in the *ld* phase. Pipette experiments have shown that different lipids partition to areas of different curvature [113], and that *ld* phases may nucleate in regions of high local curvature [114]. In closed vesicles, it can hence be energetically favorable if the vesicle partitions into flatter and more curved regions in order to better accommodate the *lo* domains. Lipowsky and coworkers have analyzed this situation theoretically and by computer simulations of an elastic membrane model. They showed that the interplay of line tension and elastic energy may stabilize conformations with multiple domains [115, 116], provided the coexisting phases have different bending rigidity, different Gaussian curvature moduli, or different spontaneous curvature. Risselada et al. [117] verified this prediction with coarse-grained molecular simulations and observed that the domain distribution on nanovesicles may change under mechanical compression. Feigenson and coworkers [64, 118, 119] used computer simulations of an elastic model to specifically study the multidomain formation on vesicles that are constrained to almost spherical shapes. In some sense, this corresponds to a situation where a frail rigid sheet (the *lo* domain) is tightly wrapped around a rigid sphere: The *lo* domain breaks up into many domains that can be much smaller than the vesicle diameter. Gueguen et al. [120] analyzed this problem analytically and established an analogy to the theory of microemulsions.

The domain patterns observed in the simulations of Feigenson and coworkers [64, 118, 119] were very similar to the experimentally observed patterns on multicomponent GUV's [63, 64] (Examples are shown in Fig. 2). However, to obtain this agreement, the authors had to tune the bending rigidities to values that are about one order of magnitude higher than typical experimental values and the line tensions had to be chosen about two orders of magnitude lower than typical values reported for phase separating lipid mixtures [118]. A subsequent careful analysis showed that the simulations are strongly affected by grid renormalization effects [119]. Nevertheless, the results suggest that in the experimental systems, the curvature-driven lipid sorting mechanism must be supplemented by another mechanism that reduces the line tension and promotes the formation of small domains. Such mechanisms are discussed in the next section.

4. Two-dimensional microemulsions

The domain formation mechanisms discussed in the previous section rely on the existence of true fluid-fluid phase separation. They cannot explain the observations of equilibrium nanoclusters in model membranes that do not exhibit a miscibility gap (see Sec. 2). There also exist a number of mechanisms that stabilize small domains at the level of the equilibrium phase diagram. Lipid mixtures that show such a behavior typically have a well-defined characteristic wave length, and are called “microemulsions” in the language of the theory of phase transitions [121]. This section is devoted to describing mechanisms that can produce such microemulsions in two dimensions. A mathematical framework for the description of these structures is provided in Appendix A.2.

The experiments on model membranes suggest that several types of domains with different characteristic length scales may exist and possibly coexist in such membranes. On the one hand, large modulated micron-size patterns have been observed in GUVs by fluorescence microscopy [56, 57, 62–64]. An example is shown in Fig. 2. On the other hand, as already reviewed in Sec. 2, increasing experimental evidence points to the existence of nanosize clusters in certain multicomponent mixtures, e.g., in binary phospholipid-cholesterol mixtures or in those multicomponent mixtures that Feigenson [63] classified as ”type I” mixtures. Fig. 3 shows a recent example of an in-plane neutron diffraction pattern for a mixture of DPPC and deuterated cholesterol, which clearly shows a series of weak Bragg peaks superimposed to a broad fluid-like spectrum [52]. This is attributed to the existence of small ordered cholesterol-rich *lo* clusters in a sea of disordered *ld* phase. Recent all-atom simulations by Sodt et al. [122] indicate that even in phase-separating mixtures, the *lo* phase itself may have a nanoscale substructure.

One factor which has the potential of stabilizing finite size domains in phase separating systems is electrostatics [123, 124]. Modulated structures may emerge due to the

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**Figure 2**: Modulated patterns on GUVs of four-component lipid mixtures composed DSPC/(DOPC/POPC)/Chol with varying (DOPC/POPC) fractions. (A) Almost pure POPC (0.03:0.27); (B-E) intermediate compositions (0.05:0.25), (0.06:0.33), (0.1:0.29), (0.05:0.25); (F) pure DOPC (0.3:0). Scale bar corresponds to 10 µm. Reprinted from Biophys. J. 101, T. M. Konyakhina et al., *Modulated phases in four-component DSPC/DOPC/POPC/Chol giant unilamellar vesicles*, L06–L10 63. Copyright (2013) with permission from Elsevier.
competition of line tension and and electrostatic dipolar interactions between the head groups. This mechanism is known to be effective in monolayers at the air-water interface, where it leads to the formation of complex micron-size domain patterns. In contrast to monolayers, however, bilayers are fully immersed in water, where the strength of electrostatic interactions is significantly weakened due to the high dielectric permittivity. Moreover, the interactions are largely screened at physiological salt concentrations. Hence electrostatic effects should be much weaker than in monolayer systems. Amazon and Feigenson recently studied the effect of electrostatic dipolar interactions on domain formation in vesicles by coarse-grained simulations and concluded that electrostatic dipolar interactions might suppress phase separation and generate microemulsion structures in multicomponent membranes, but only if the dipoles are forced to reside inside the membrane. Since the apolar membrane interior tends to repel polar and charged monomers, this does probably not happen very frequently, and electrostatic interactions can presumably be neglected in most cases.

In the following, we will mainly focus on the mechanisms sketched in Fig. 4: Mechanisms that stabilize microemulsions due to line active membrane components, or due to a coupling between the local lipid composition and the local bilayer or monolayer curvature.

4.1. Linactants

Adding surfactants is an efficient way of stabilizing microemulsions. Safran, Andelman and coworkers have argued that the heterogeneities observed in biomembranes might to a large extent be attributed to the presence of line active molecules. An extensive recent review with many references can be found in [66], here we describe only some basic aspects of the mechanism. Line active molecules (linactants) aggregate to domain boundaries, thereby reducing the line tension, until they may eventually destroy the demixing transition and stabilize ordered or disordered modulated structures. A simple generic mechanism is outlined in Appendix A.3.1 and yields the phase diagram shown in Fig. 5. This calculation is very much simplified and does not account for important effects such as, e.g., the fact that domain boundaries eventually become saturated with linactants. Nevertheless, it qualitatively reproduces the basic structure of domains that are much larger than the Debye length in the surrounding medium, which is around 1 nm at physiological conditions. Both studies used similar approximations (most notably, the linear Poisson-Boltzmann approximations), but they differ in their Ansatz where to place the dipoles. Liu et al. considered dipoles that are buried inside the membrane, whereas Travesset assumed that the dipoles are located at the membrane surface. Hence we can conclude that electrostatic interactions between dipolar membrane components can disrupt phase separation and generate microemulsion structures in multicomponent membranes, but only if the dipoles are forced to reside inside the membrane. Since the apolar membrane interior tends to repel polar and charged monomers, this does probably not happen very frequently, and electrostatic interactions can presumably be neglected in most cases.

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The light lines denote second order phase transition, the dark lines observed in membrane systems. In particular, they may partly account for the heterogeneities that have been

coworkers [125, 126] argued that this linactant scenario two phase region, but stays finite.

croemulsion and the modulated state, at a so-called "Lifshitz multicritical point" (see Appendix A.2). The char-

gemulticritical point temperature of the pure system and $\alpha, \nu$ are phenomenological constants.

Figure 5: Generic mean field phase diagram for ternary mixtures with linactants in the plane of the temperature-like parameter $t$ vs. bulk linactant concentration $\rho_l$ (i.e., linactant concentration far from domain boundaries), as calculated in Appendix A.3.1. The relation between $t$, the temperature $T$, and the surfactant concentration $\rho_s$ is roughly linear, $T = T_c + \alpha(\rho_s - \nu \rho_l)$ where $T_c$ is the demixing temperature of the pure system and $\alpha, \nu$ are phenomenological constants.

The light lines denote second order phase transition, the dark lines first order phase transition, the circle indicates the position of a Lif-

shitz multicritical point (see text). The thin dashed line corresponds to the Lifshitz line, which marks the onset of disordered structure formation in the homogeneous phases [122].

the expected mean-field phase behavior of systems containing linactant molecules. Here the term "mean-field" refers to the approximation that long range thermal fluct-

tuations, e.g., fluctuations of the domain boundaries, are neglected.

At low linactant concentrations, the main effect of lin-

actants is to reduce the line tension. The system still un-

dergoes a demixing transition, but the transition point is shifted to lower temperatures. If the linactant concen-

tration reaches a certain threshold value, the line tension vanishes, demixing transition is suppressed and an ordered modulated phase forms instead. Beyond this threshold, the homogeneous phase also acquires local structure with a well-defined characteristic wave length, which is of the order of the underlying molecular correlation length, i.e., in the range of a few nanometers. This is the structure called "microemulsion". The crossover from a fully ho-

mogeneous state to the microemulsion state is marked by the so-called "Lifshitz line". The demixing line meets the order-disorder line, i.e., the transition line between the micro-

emulsion and the modulated state, at a so-called "Lif-

shitz multicritical point" (see Appendix A.2). The character-

istic wave length diverges at the Lifshitz point.

As discussed in Appendix A.3.1, the mean-field sce-

nario is modified in the presence of thermal fluctuations. The Lifshitz point disappears, the order-disorder transition shifts to lower temperatures and becomes first or-

der, and in return, the disordered "microemulsion" widens significantly. The characteristic wave length of the microstructures no longer diverges at the transition to the two phase region, but stays finite.

Safran and coworkers [66, 127, 131] and Andelman and coworkers [125, 126] argued that this linactant scenario may partly account for the heterogeneities that have been observed in membrane systems. In particular, they sug-

gested that hybrid lipids with one saturated and one unsaturated tail may serve as linactants that accumulate at ld-lo boundaries and reduce the line tensions. This hypoth-

esis is consistent with a number of experimental findings [63, 64, 132]. Here we specifically discuss the work on four-

component mixtures by Feigenson and coworkers [63, 64] which lead to the structures shown in Fig. 2.

These structures were obtained within a systematic study of four-component mixtures with compositions chosen such that they interpolate between the phase separating "type II" mixture DSPC/DOPC/Chol (Fig. 2F) and the "type I" mixture DSPC/POPC/Chol, which does not phase separate [63]. If one starts with DSPC/DOPC/Chol and gradually substitutes the fully unsaturated lipid DOPC with the hybrid lipid POPC, phase separation gives way to the modulated structures shown in Fig. 2B-E. This suggests that hybrid lipids act as linactants and reduce the line tension, thus facilitating one of the domain forming mechanisms that can generate large micron size structures (such as the lipid sorting mechanism described in Sec. 3) or the Leibler-Andelman mechanism to be described in Sec. 4.2. Indeed, molecular simulations of a similar mix-

ture have indicated that the substitution of fully saturated lipids by hybrid lipids leads to a decrease of the line tension between coexisting phases [64].

Intriguingly, experimental studies of a four-component mixture consisting of purely nonhybrid lipids revealed a scenario which is very similar to that shown in Fig. 2 [133]. In that work, DOPC was not substituted with the hybrid lipid POPC, but with the fully saturated lipid DLPC. Thus lipids do not need to be hybrid lipids to reduce the line tension. Nevertheless, they may still act as linactants, if they accumulate at domain boundaries. According to the gen-

eral description of linactants outlined in Appendix A.3.1, all molecules and particles [134] that are attracted to do-

main boundaries have linactant properties.

According to the scenario displayed in Fig. 5 linac-

tants at high concentrations might altogether suppress phase separation, which suggests that the linactant mech-

anism might be responsible for the lack of global phase separation in the type I mixtures DSPC/POPC/Chol or DSPC/DLPC/Chol. However, this would imply that the pure DSPC/Chol mixture, without linactant, should phase separate into a lo and ld phase, which is not the case (see Sec. 2). Furthermore, neutron scattering studies on small four-component vesicles made of DSPC/(DOPC/POPC)/Chol mixtures with low DOPC content (still type I mixtures) have indicated that the nanodomain size regime is mainly controlled by the bi-

layer thickness mismatch between the nanodomains and the surrounding phase [50], which suggests that linactant effects are not dominant in the type I regime. Hence the situation seems to be more complicated than suggested by the simple linactant scenario. We will now discuss other mechanisms that can prevent phase separation and stabil-

ize nanoscale clusters.
4.2. Bilayer curvature coupling: The Leibler-Andelman mechanism

Already in 1987, Leibler and Andelman [136] proposed a mechanism that may stabilize modulated structures and microemulsions with typical sizes in the range of 100 nm to micrometers in mixed membranes. The basic idea is sketched in Fig. 4b). If the local lipid compositions on two apposing monolayer leaflets differ from each other, the bilayer acquires “spontaneous curvature”, i.e., a tendency to bend in a certain direction [137]. An unconstrained bilayer would simply curve around and close up to form a vesicle [138]. By applying lateral tension, the membrane can be forced to remain planar on average. Now, if the lipids in each monolayer have a tendency to demix, the membrane can accommodate to the curvature stress by developing lateral structure, such that regions with different spontaneous bilayer curvature alternate with each other. For strong demixing force, this mechanism generates a variety of competing ordered mesophases with striped or hexagonal domain patterns [121, 136, 139, 140]. However, membranes may develop fluctuating lateral heterogeneities with a well-defined characteristic size even in the mixed regime. This was noted by Liu et al. [122] and more recently by Schick [141], and Schick and coworkers analyzed the possible implications for raft formations in some detail [112, 142]. The characteristic wave length of the mesostructures is of order 100 nm to µm or higher for realistic membrane parameters (see Appendix A.3.2) and scale as \(1/\sqrt{\Gamma}\) with the membrane tension \(\Gamma\) [123, 141].

Shlomovitz et al. [142, 143] pointed out that the Leibler-Andelman mechanism induces a Lifshitz point scenario which is very similar to that expected for liactants (Fig. 5), if one replaces the liactant concentration \(c\) by the strength of the curvature-composition coupling difference \(\tilde{C}\). The underlying mathematical description is outlined in Appendix A.3.2. It results in the mean-field phase diagram shown in Fig. 6 (redrawn from Ref. [135], see also [143], which is very similar to Fig. 5). At weak curvature coupling, the lipids phase separate and the two phases partition to the two sides of the monolayer. For strong coupling, a modulated structure develops. The two regimes are separated by a Lifshitz multicritical point. As discussed earlier and in Appendix A.2, the order/disorder transition between the disordered fluid and the ordered microdomains becomes first order in the presence of thermal fluctuations, and the Lifshitz point is destroyed. Simulations by Shlomovitz et al. [143] and Sadeghi et al. [145] indicate that it is replaced by a tricritical point: If one increases the curvature coupling, the demixing transition first becomes first order before being replace by the order/disorder transition.

The Leibler-Andelman mechanism requires the lipid composition on the two monolayers to be different, hence it seems to rely on the assumption that the lipid domains on opposing monolayers are anticorrelated or at least decoupled [146]. Experiments and simulations rather suggest that lipid domains across bilayers tend to be in registry [147–149]. Collins and Keller [150] studied asymmetric lipid bilayers, with lipid compositions chosen such that only one side is phase separating, by fluorescence microscopy. They found that domain formation on the phase separating side may induce domain formation on the other side. Conversely, they also reported that the coupling to the non phase separating leaflet may suppress domain formation on the phase separating leaflet. Recent neutron scattering experiments by Heberle et al. [151] showed a similar coupling on the subnanometer scale: A disordered fluid leaflet can partially fluidize an ordered apposing leaflet. Hence the domain structures on the two sides of bilayers seem to be rather strongly coupled.

Nevertheless, the Leibler-Andelman mechanism may still be effective in bilayers with positive domain coupling for several reasons. First, the energy gain associated with bilayer curvature driven microdomain formation may compensate the energy costs of staggered domain arrangements even in membranes with positive domain coupling [152]. Second, Williamson and Olmsted [152] have recently shown by computer simulations that bilayers may be kinetically trapped in a configuration with anticorrelated (staggered) domains even if the thermodynamically favored state is one where domains are in registry. Third, real biomembranes are typically asymmetric. Even domains that are in perfect registry usually have different lipid compositions on both sides and hence the membrane can be expected to develop spontaneous bilayer curvature, which will be different for different phases. This is sufficient to put the Leibler-Andelman mechanism to action. Shlomovitz and Schick [142] studied specifically the case where only one side of a bilayer has a driving force towards phase separation, and argued that this is sufficient to bring about domain formation on both sides through a phase coupling of domains across the bilayer.
The Leibler-Andelman mechanism is one of the candidates that may explain the observation of modulated micron or submicron structures in multicomponent GUVs [56, 57, 62, 64]. In Sec. 3.2, we have already discussed an alternative mechanism, the lipid sorting mechanism, which may account for multidomain formation in close-to-spherical vesicles. On the other hand, Baumgart et al. [56] and Rozovsky et al. [57] have observed micron size structures in vesicles that are clearly not spherical. The relation between bilayer curvature induced structure formation and lipid sorting induced structure formation has been discussed theoretically by Hu et al. [116] and by Gueguen et al. [120]. Apart from the fact that the lipid sorting mechanism requires background curvature and does not work for planar membranes, the two mechanisms are quite similar. In particular, the theory of lipid sorting induced structure formation can formally be mapped onto a theory of micromesostructures on curved geometries [124].

4.3. Monolayer curvature coupling

The mechanisms discussed so far do not explain the observation of nanoscale clusters in binary lipid mixtures, e.g., DPPC/Chol mixtures, which were discussed in Sec. 2. These mixtures do not phase separate on a global scale, which rules out an interpretation in terms of critical clusters, and they clearly do not contain linear-antit components. Comparable clusters are also observed in planar tensionless membranes in computer simulations [52, 84, 153], which rules out interpretations in terms of the lipid sorting mechanism and the Leibler-Andelman mechanism. Fig. 7 shows configuration snapshots of such structures which were obtained from semi-grandcanonical Monte Carlo simulations of a coarse-grained model for binary lipid bilayers [84, 154, 155]. In these simulations, the overall number of molecules was kept constant, but the composition was allowed to fluctuate, and all molecules could at any time switch their identity from "lipid" to "cholesterol". This rules out the possibility that the clusters simply result from incomplete phase separation.

An analysis of the domain patterns showed that they have a characteristic length scale of around 25 nm, which is not only the order of magnitude that has been discussed for rafts, but also comparable to the periodic wave length of the modulated ripple phase $P_{\beta'}$ discussed in Sec. 2 (a typical configuration is shown in Fig. 7, bottom), and to the wavelength of a soft peristaltic mode which we have observed in the spectrum of the $L_{\beta'}$ gel phase in one-component membranes. These observations lead us to conjecture a connection between the nanodomains in mixed membranes and the ripple structure in one-component membranes, and to propose a domain forming mechanism which is driven by a coupling of the local lipid structure to the monolayer curvature. The fact that internal curvature stress in membranes should be taken into account in theories for rafts had also been stressed by Bagatolli and Mouritsen in a recent review [157].

The basic picture behind the monolayer curvature coupling mechanism is sketched in Fig. 8). We describe bilayers in terms of two coupled elastic monolayers. This idea goes back to Dan et al. [158], and we have recently verified the validity of the approach at the example of copolymeric bilayers [159]. Furthermore, we assume that the lipid composition couples to the spontaneous curvature of monolayers – i.e., monolayers have a tendency to bend inwards or outwards depending on their local composition. Since monolayers are coupled to each other, this creates internal elastic stress, which can be partially relieved at domain boundaries [17, 84]. Kuzmin et al. [160] showed already in 2005 that a mismatch of spontaneous monolayer curvature in coexisting lipid phases can lead to a reduction of the line
tension. If the curvature stress is large, the system reacts by keeping domain sizes small.

In practice, determining monolayer curvatures from experiments or simulations is a difficult task. In simulations, they are typically calculated from the first moment of the pressure profile [161] and the results are plagued by large statistical errors. Barragán Vidal et al. [162] have recently proposed an alternative method which allows to extract directly the strength of curvature coupling, and applied it to mixtures of DPPC with various other lipids, but unfortunately not yet to cholesterol. On the experimental side, Kollmitzer et al. [163] recently estimated values for the spontaneous curvature of various lipid mixtures from neutron scattering data on inverted hexagonal lipid phases based on simple mixing rules. Their results suggest that the concentration of cholesterol should indeed have a strong influence on the spontaneous curvature of lipid-cholesterol mixtures, as assumed by the monolayer curvature coupling model.

The mathematical background of the theory is briefly summarized in Appendix A.3.2. In the symmetric case (two equivalent monolayers and critical lipid composition), the theory yields the mean-field phase diagram shown in Fig. 8. It has obvious similarities to the phase diagrams discussed previously, Figs. 5 and 6. The phase behavior is again controlled by the strength of curvature coupling, \( \tilde{C} \). At low coupling, the system phase separates, and at high coupling, nanodomains form. However, in contrast to Figs. 5 and 6, the two regimes are not connected by a Lifshitz point. The characteristic wave length remains finite for all values of the renormalized curvature coupling \( \tilde{C} \) down to the Lifshitz line. It depends on the elastic parameters of the membrane and is of the order of the membrane thickness, i.e., a few nanometers.

The same theory can also be used to describe the formation of the ripple structure \( P_{\theta} \) (in a very simplified manner), since it does not explicitly require that the two coexisting phases have different lipid compositions. They may also simply have different order, i.e., gel and fluid order. Provided that the gel and fluid state have largely different spontaneous monolayer curvature, our theory would hence also predict the existence of modulated structures or phases in the vicinity of the main transition – in agreement with the experimental observation that the ripple phase seems to be a generic phenomenon in one-component membranes with a tilted (i.e. elastically stressed) gel phase \( L_{\theta} \) [42, 133].

Reigada and Mikhailov [164] have recently carried out a linear stability analysis and dynamic Ginzburg-Landau simulations of a monolayer curvature coupling model and calculated a phase diagram in a different plane of parameters than Fig. 8. They found lamellar, hexagonal and phase separated structures. Interestingly, they observed that even phase separating systems may initially be trapped in a nanostructured state after a quench from the disordered phase. Hence it may be possible to observe kinetically stabilized nanoscale domains even in parameter regions where they are not thermodynamically stable.

Since the monolayer curvature coupling mechanism relies on a coupling between lipid composition and curvature coupling, it is bound to compete with the Leibler-Andelman mechanism described in Sec. 4.2 Whenever monolayer curvature coupling is possible, bilayer curvature coupling becomes possible as well. We have recently used the unified mathematical description outlined in Appendix A.3.2 to study the interplay of the two mechanisms [133]. Two selected phase diagrams are shown in Fig. 9. Whereas the disordered micro- and nanostructures can easily coexist (as long as the curvature coupling parameter \( \tilde{C} \) is higher than the respective Lifshitz lines), the ordered structures are found to exclude each other, and microstructured modulated phases are separated from nanostructured modulated phases by first order phase transitions.

Interestingly, the theoretical phase diagram of Fig. 9j) features the same sequence of morphologies from nanodomains \( \mu D \) via microdomains to phase separation than the experimental four-component model membrane systems studied by Feigenson et al. (Fig. 2). One should note that

![Figure 9: Examples of complex phase diagrams resulting from the competition of the Leibler-Andelman mechanism and the monolayer curvature coupling mechanism. (a) Phase diagram in the plane of the temperature-like parameter \( t \) vs. dimensionless curvature coupling parameter \( \tilde{C} \) for weakly positively coupled bilayers. (b) Phase diagram in the plane of the temperature-like parameter \( t \) vs. dimensionless membrane tension parameter \( \Gamma \) at fixed curvature coupling parameter, which was chosen such that the stable phase at low tension is the microdomain phase. The light solid lines denote second order phase transitions, the dark lines first order transitions; the thin blue dashed line the Lifshitz line with respect to microdomains, the thin red dotted line the Lifshitz line with respect to nanodomains. The thick pink line denotes a line below which nanodomains may form within the \( \mu D \) regime close to microdomain boundaries. Redrawn from [162] with slightly different parameters, see Appendix A.3.2.](image)
the dimensionless coupling parameter \( \tilde{C} \) in fact depends on a combination of elastic parameters, \( \tilde{C} \propto \tilde{c} \frac{\kappa_{c}}{g} \), where \( \tilde{c} \) is the "bare" coupling between lipid composition and monolayer curvature, \( \kappa_{c} \) is the bending rigidity of the membrane, and \( g \) is proportional to the free energy penalty on domain boundaries, i.e., the "local line tension" (see Appendix A.3.2). Variations of any of these parameters can trigger a morphological transition. A sequence of discontinuous transitions such as those observed in the experimental system of Fig. 2 could be brought about if the gradual substitution of DOPC with POPC had at least one of the following effects: Either a gradual increase of the (bare) coupling between lipid composition and spontaneous monolayer curvature, or a gradual increase of the average bending rigidity of the bilayer, or a gradual decrease of the "local line tension" at domain boundaries. At least the line tension seems to show this dependence (see also Sec. 4.3). Hence the theoretical scenario of Fig. 9(b) might account for the discontinuous transitions between nano- and microscale patterns and global phase separation observed in four-component membranes.

For other coupled microemulsions, the combination of ordering mechanisms often results in complex structures that combine elements of both underlying patterns (e.g. Hirose et al. [125, 126]). Here, we find that different curvature coupling induced patterns tend to suppress each other. This is because the competing structures are associated with different correlations between domains across the bilayers. In the microdomain phase stabilized by the Leibler-Andelman mechanism, they are staggered, and in the nanodomain phase stabilized by the monolayer coupling mechanism, they are in registry. Therefore, the two structures cannot easily be combined. Nevertheless, membranes in a microstructured phase may still feature nanodomains at the boundaries of the microdomains (below the thick pink line in Fig. 9b) [132]. This opens up a possibility how microstructures – which can be manipulated by varying the membrane tension – can be used to control the lateral organization of nanodomains [132].

5. Domain formation due to dynamical interactions with the membrane environment

So far we have considered isolated membranes, without accounting for their interactions with the environment. Real biomembranes are embedded in a cellular context that cannot be neglected. A number of mechanisms have been proposed that inhibit large scale demixing in biomembranes below an equilibrium demixing point through interactions with the cellular environment. They mostly belong to one of two categories: Mechanisms related to the perpetual lipid turnover in the membrane, and mechanisms based on the coupling of the membrane with the cytoskeleton. Examples of these two mechanisms are sketched in Fig. 10.

![Figure 10](image)

5.1. Membrane recycling

We will first discuss the first category, i.e., domain formation due to membrane recycling. In living cells, membranes constantly exchange lipids and other membrane components with their environment, e.g., through vesicle budding and fusion. The fact that vesicle traffic may explain membrane patchiness was first pointed out in 1999 by Gheber and Edidin [168], and this has triggered a series of theoretical work on this issue.

The most intensely discussed mechanism is the FRET mechanism, which describes membrane recycling in terms of a dynamical lipid exchange with an external reservoir. In a seminal paper of 2005 [169], Foret proposed to model membrane recycling by a dynamic Ginzburg Landau theory that accounts for diffusive in-plane phase separation and includes an additional sink/source term describing lipid exchange (see Eq. (A.9) in Appendix A.4). He studied the phase separation dynamics in this model by computer simulations and showed that the initial domain growth is indeed arrested due to the sink term. Estimating the final size of the domains from the characteristic time at which the domain growth stops, he obtained an upper limit \( t^{*} \) which scales as \( t^{*} \propto \tau_{r}^{1/3} \) with the inverse rate \( \tau_{r} \) of lipid exchange.

As shown in the appendix (see also [170]), the FRET model can be mapped on an established model for equilibrium copolymeric microemulsions, the Ohta-Kawasaki model [171]. In the Ohta-Kawasaki model, global phase separation is suppressed by a Coulomb-type long-range interaction term. In the FRET model, an effective long-range interaction is mediated through the lipid exchange with an omnipresent lipid reservoir. The scaling of the characteristic domain size for the Ohta-Kawasaki model is well-known [171] and by analogy, one would expect the characteristic domain size to scale as \( t^{*} \propto \tau_{r}^{1/4} \) in the FRET model (see Appendix A.4). This prediction has not yet been tested explicitly in the literature, but it
seems to be compatible with all published data referenced below.

Most later studies of membrane recycling effects were based on the Foret model. Gomez et al.\cite{172} applied the Foret model to cholesterol turnover and carried out a linear stability analysis as well as further computer simulations. According to the linear theory, the most unstable wavelength in the initial stage of phase separation scales as $l^* \propto \tau_r^{-1/2}$ with the inverse turnover rate $\tau_r$. Motivated by this result, they fit their simulation results for the final domain sizes $R_f$ by a linear law $R_f^{-1} = a + b\tau_r^{-1/2}$. However, their data seem equally compatible with a fit to $R_f = c\tau_r^{-1/4}$.

Das et al.\cite{173} tested the Foret model against experimental data obtained by FRET ( Förster resonance energy transfer) imaging of living human cancer cells. The lipid recycling times were tuned using an ATP inhibition method. They showed that the experimental results could be fitted quantitatively with simulation predictions of the Foret model. Sornbundit et al.\cite{174} applied the Foret model to a system of two coupled monolayers, where phase separation occurs on one side only, and show that this leads to domain formation on both sides. Garcke et al.\cite{170} presented a detailed mathematical analysis of the Foret model and pointed out the relation to the Onsager-Kawasaki functional.

Almost in parallel to Foret, in 2005, Turner et al.\cite{175} developed a closely related model which was based on a master equation for concentrations $c_n$ of clusters containing $n$ "monomers" (raft proteins or other raft components) to describe cluster growth in the presence of lipid turnover. The model accounted for thermodynamically driven fusion of domains and included an additional term describing the addition or removal of clusters, which takes the role of the source/sink term in the Foret model. Using computer simulations, Turner et al. showed that this model also produces finite size domains with characteristic domain sizes $n^*$ that increases according to $n^* \propto 1/\sqrt{j}$ with the lipid exchange rate $j$. With the identifications $n^* \propto l^2$ and $j = 1/\tau_r$, this result is in very good agreement with the prediction $l^* \propto \tau_r^{1/4}$ of the Foret model. The Turner model was later refined by Foret\cite{166}, who explicitly accounted for the energy dissipation due to lipid turnover and identified three steady-state regimes depending on the choice of parameters: Macroscopic phase separation, Formation of finite domains, and a "disordered state" with isolated monomers.

Fan et al. in 2008\cite{177} proposed an alternative Ginzburg-Landau model of membrane recycling, where effect of lipid turnover is incorporated at the level of a non-local active noise (see Appendix A.3). In this model, the lipid redistribution is restricted to a finite spatial range. In contrast to the Foret model, it cannot be mapped to an equivalent equilibrium model, hence it describes a truly nonequilibrium system. In a later publication\cite{176}, Fan et al. compared the morphologies obtained with this "stochastic recycling" model with morphologies obtained from other models, i.e., critical clusters, the Foret mechanism, and arrested phase separation due to pinning sites (see next section). Examples of configurations are reproduced in Fig. 11. The Foret mechanism is the only one that produces nearly regular arrays of clearly separated domains. In the stochastic recycling of Fan et al., the clusters are much less ordered and less well-defined. Fan et al.\cite{176} suggested that this insight could be used to distinguish between different membrane recycling mechanisms in experiments.

### 5.2. Cytoskeleton coupling

The second important group of externally driven domain stabilization mechanisms comprises mechanism that depend on the coupling between a plasma membrane and the cytoskeleton\cite{14}. An example is sketched in Fig. 10b). Since one important effect of the cytoplasm is to impose disorder on the membrane by immobilizing certain membrane inclusions (e.g., anchor proteins) which then serve as pinning sites, most mechanisms in this category can also be categorized as mechanisms based on quenched disorder.

Yethiraj and Weisshaar were the first to point out that global phase separation can be suppressed and domains can be forced to break up if the membrane is filled randomly with immobile inclusions\cite{178}. They demonstrated this by Monte Carlo simulations of a simple lattice model, where randomly placed particles were added that acted as pinning sites for domain boundaries. We will refer to such impurities as "interface coupled" pinning sites. A similar situation was later studied by Fan et al.\cite{176} using a dynamic Ginzburg-Landau model (see Appendix A.4). Immobile interface-coupled inclusions reduce the line tension, which shifts the critical temperature to lower values\cite{178}. Several researchers have considered an alternative scenario where the pinning sites have an affinity to one of the coexisting phases\cite{177,179,183}. We will denote such impurities as "bulk coupled" pinning sites, see Fig. 10b) for an illustration. Bulk coupled pinning sites tend to nucleate domains of this phase, which suppresses global phase separation.

These observations can be understood within the theory of critical phenomena: Vink and coworkers\cite{179} pointed out that membranes with bulk coupled random pinning sites can be associated with the so-called random field Ising model (RFIM) \cite[184, 185]{184, 185}, which is one of the archetype models for studies of phase transitions in disordered systems. The RFIM is derived from the Ising model (IM), which consists of an array of interacting "magnetic spins" and exhibits a continuous ordering transition characterized by the same critical properties than the demixing transition (it belongs to the same "universality class"). In the RFIM, disorder is introduced by exposing the spins to random, but fixed "magnetic fields"\cite{184}. It has been shown that the presence of such fields, however weak, fully suppresses the phase transition\cite{183}. Vink argued that a membrane filled with randomly distributed, bulk
coupled immobile obstacles should belong to the RFIM universality class, hence the demixing transition in such membranes should be fully suppressed as well. He and coworkers tested this hypothesis by simulations of various membrane models with different levels of coarse-graining, ranging from lattice models to realistic chain models [179–181]. They consistently found that these system no longer phase separate in the presence of the obstacles. Phase separation was restored if the obstacles did not favor particular phases, or if they were arranged on a regular lattice [179].

By a similar analogy, membranes with interface coupled pinning sites can be associated with the class of random bond Ising models (RBIM), which is another archetype for disordered systems where the disorder is introduced at the level of the interaction between spins. In RBIM models, the strength of disorder must exceed a critical threshold before the ordering transition is fully suppressed. Weak disorder only moves the transition point to lower temperatures [180]. This is consistent with the findings of Yethiraj and Weisshaar [178]. Hence we conclude that, somewhat unexpectedly, bulk-coupled random pinning sites that have a special affinity for one of the co-existing phases suppress the demixing transition more effectively than interface-coupled random pinning sites that attract domain boundaries.

In the studies discussed so far, the pinning sites were distributed randomly on the membrane. Other groups have modelled the cytoskeleton network more realistically by introducing an underlying mesh to which the pinning sites are attached [182, 187, 188]. The mesh thus organizes the pinning sites and compartmentalizes the membrane by erecting “fences” of pinning sites. In this context, an additional dynamic coupling mechanism between cytoskeleton and membrane may also becomes important [182, 188]. The experimentally observed fact that the diffusivity of lipids is reduced in the vicinity of the cytoskeleton [188, 190]. Based on analytical arguments and Ginzburg-Landau simulations, Fan et al. [182] predicted that both this diffusion-based mechanism and the standard bulk coupling mechanism stabilize domain structures that are strongly correlated with the underlying cytoskeleton structure. Machta et al. [187] studied the bulk coupling case by lattice simulations and confirmed the predictions of Fan et al. [182]. They also considered the effect of bulk coupling on lipid diffusivity and concluded that bulk coupling to a cytoskeleton can account for diffusion confinement in membranes close to the critical demixing point of the bare membrane, or if the density of pinning sites is very large. Honigmann et al. [188] recently combined fluorescence correlation spectroscopy and superresolution stimulated emission depletion (STED) imaging for an extensive experimental study of supported bilayers coupled to an actin network. They showed that, indeed, the actin network disrupted the formation of phase separated domains over the whole range of considered temperatures. However, when carrying out simulations of the Machta model with simulation parameters matched to the experiments, they found that the standard bulk coupling mechanism alone is not sufficient to explain the experimental findings. The discrepancy could be resolved by postulating an additional, curvature-based coupling between the membrane and the actin [188].

Sikder et al. [191] identified yet another mechanism through which a cytoskeletal meshwork in close proximity to a membrane can suppress phase separation even far below the critical region: In their model, the actin filaments do not directly influence the lipid membrane, but they locally stop the motion of membrane-bound mobile proteins. If these proteins have an affinity to one membrane phase, this also generates small and relatively stable domains. All these mechanisms explain domain formation by an interaction of a membrane with an immobile cytoskeleton network below a demixing point. In living cells at physiological temperatures, membranes are more likely maintained above the demixing point (see the discussion in Sec. 3.1), and the cytoskeleton is not immobile, but subject to constant remodeling [192].

Recent theories of
cytoskeleton-induced domain formation are beginning to take this into account.[167, 193] Gomez et al.[167] proposed a simple model where bulk-coupled pinning sites occasionally detach from the cytoskeleton due to the repolymerization of the actin meshwork and reattaches elsewhere. They show that in the presence of other larger mobile bulk-coupled membrane inclusions, transient domains may form even above the lipid demixing point. A similar mechanism of protein nanocluster formation was studied by Gowrishankar et al.[193] using a more detailed and realistic model for the dynamically evolving actin network and including hydrodynamic interactions.

The possible role of the cytoskeleton for domain formation has also been highlighted by recent experimental work of Kraft et al.[194] on sphingolipid domains in plasma membranes of fibroblasts. They found the these domains (which are — as a side note — not enriched with cholesterol[34]) seem to depend on the coupling to the cytoskeleton and disappear if the cytoskeleton is disrupted.

5.3. Other nonequilibrium mechanisms

Other nonequilibrium processes are conceivable that should also lead to domain formation in membranes.

For example, Chen and Chen[195] have considered the effect of membrane inclusions (e.g., protein channels) that actively switch between two different states. They studied a situation where domain-bound inclusions switch stochastically between one inert state and one state where they locally deform the membrane and attract each other. The curvature-induced and direct interactions trigger protein aggregation, which is however arrested due to the stochastic switching. Mathematically, this mechanism is similar to the Föret mechanism[169] described in Sec. 5.1 and the phenomenology is also similar: One observes relatively ordered arrays of domains whose size decreases as a function of switching rate.

Ngamsaad et al.[196] suggested a rather different mechanism that can arrest phase separation in plasma membranes at least on intermediate time scales. They considered the phase separation on a bilayer with strongly asymmetric demixing dynamics on each monolayer. The dynamics on one leaflet, representing the outer leaflet of a plasma membrane, was assumed to be fast and accelerated by hydrodynamics, whereas the dynamics on the other, inner leaflet was taken to be slow and purely diffusive, i.e., hydrodynamic interactions were taken to be screened due to the presence of the cytoplasm. In this case, the domain growth on the outer leaflet is arrested by the coupling to the smaller domain pattern on the inner leaflet. The mechanism has similarity to the pinning effects described in Sec. 5.2. Unless some real pinning takes place as well, however, further coarsening takes place on large time scales which is governed by the slow dynamics on the inner leaflet.

In general, the fact that phase transition kinetics can be controlled from the outside in membranes offers rich opportunities for generating complex transient domain structures. In another example due to Shimobayashi et al.[197], the transition from macro- to microphase separation was studied experimentally for ternary demixed vesicles that were exposed to a solution containing glycolipid micelles. As the glycolipids were gradually incorporated in the membrane, this triggered a transition to a modulated state via an intriguing intermediate pattern of ring like structures on the vesicle.

6. Conclusions and Outlook

Summarizing, we have reviewed a large variety of mechanisms that can contribute to heterogeneities and domain formation in multicomponent membranes, some operating already at equilibrium and some in the nonequilibrium context of a living organism. Given the fact that biomembranes are part of the highly dynamic and sophisticated system of the cell, it would seem much more surprising if they turned out to be homogeneous than if they were heterogeneous.

The overview shows that lipids presumably contribute substantially to such heterogeneities, since micro- and nanostructuring seems to be a generic, omnipresent phenomenon in multicomponent lipid membranes. Thus the question "whether" or "why" rafts exist may not be well-posed. The more relevant question is probably: How does nature take advantage of the existence of rafts, which seem to be imposed by the (bio)physics of membranes and hard to avoid? Nature must find ways to control raft formation. Since most domain forming mechanisms are related to phase transitions, they can be manipulated by controlling the phase behavior. Control parameters are not only provided by the lipid composition, the temperature and the composition of the surrounding medium, but also by mechanical factors such as the hydrostatic pressure[135, 142] and the membrane tension[117, 135, 142, 198, 199], and by geometric factors such as the membrane curvature (see Sec. 5.2). Furthermore, we have seen that domain forming mechanisms interact with each other, suppress each other or promote each other. This provides nature with a rich toolbox that can be exploited to pre-structure and organize membranes already at the level of their physical properties, in order to optimize them for their role in the cells.

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Appendix A. Ginzburg-Landau theory as a theoretical framework for the description of membrane domains

In the following, we provide a unified mathematical description of the phenomena discussed in the main text, using the framework of the Ginzburg-Landau theory, which is suitable to describe generic phenomena related to phase transitions\textsuperscript{200}. Ginzburg-Landau approaches are commonly used in theoretical descriptions of order/disorder phenomena in lipid membranes, see, e.g., Ref.\textsuperscript{68} for a recent review. The basic idea of the Ginzburg-Landau approach is to (i) define an “order parameter” that describes the phenomenon of interest and is typically a density of an extensive quantity, (ii) make an Ansatz for a free energy functional by expanding a local free energy density in terms of powers of the order parameter and its spatial derivatives, taking into account the symmetries of the system, and finally (iii) minimize this free energy functional with respect to the order parameter to calculate (mean-field) phase diagrams, or use it as input in a statistical field theories that allow to assess the effect of fluctuations on the phase behavior. We will now give a brief overview how this approach can be used to describe domain formation in membranes.

Appendix A.1. Phase separation and critical fluctuations

In the framework of the Ginzburg-Landau theory, fluid-fluid phase separation on a planar membrane can be described by a single scalar order parameter $\Phi(\mathbf{r})$ describing the local composition in the two phases, and the Ginzburg-Landau free energy functional takes the simple form

$$F[\Phi] = \int d^2r \left\{ \frac{t}{2} \Phi^2 + \frac{1}{4} \Phi^4 + \frac{g}{2} (\nabla \Phi)^2 + h \Phi \right\}. \quad (A.1)$$

Here the integral $\int d^2r$ runs over the membrane plane, the cubic term in the expansion has been removed by appropriately shifting the origin of $\Phi$, $\Phi$ has been rescaled such that the fourth order term has the fixed coefficient $1/4$, and higher order terms have been neglected. The coefficients $t, g, h$ are phenomenological parameters, which depend on the physical control parameters temperature, pressure, and chemical potentials. For example, the parameter $t$ can loosely be associated with a shifted and rescaled temperature, the parameter $h$ controls the composition and takes the role of a chemical potential difference, and the parameter $g$ controls the line tension.

Minimizing $F$ with respect to $\Phi(\mathbf{r})$, one finds that all compositions $\Phi$ are possible for $t > 0$, but a miscibility gap opens up for $t < 0$, where two phases with compositions $\nabla \Phi = \pm \sqrt{-t}$ coexist with each other. The system has a critical point at $t = 0$ which belongs to the Ising universality class. Close to the critical point, the correlation length diverges according to $\xi \propto |t|^{1/4}$ in mean field approximation. Right above the critical point, the system therefore contains large correlated “domains”, which have no sharp boundaries and a broad distribution of domain sizes. Right below the critical point, interfaces between coexisting phases become increasingly fuzzy (the interfacial width is set by $2\xi$) and the line tension vanishes according to $\lambda \propto |t|^2$. This qualitatively explains the properties of critical clusters described in Sec.\textsuperscript{44}. Quantitatively, the exponents of the power laws deviate from the mean-field prediction due to the effect of thermal fluctuations. In reality, the order parameter at coexistence, the correlation length, and the line tension scale as $\xi \sim |t|^{1/4}$, $\lambda \sim |t|^{-1}$ in two dimensional Ising-like systems. These exponents have indeed been measured within the error in multicomponent membranes\textsuperscript{60,61}. We note that at the level of these power laws, the exact interpretation of the order parameter $\Phi$ does not matter. Most quantities that depend linearly on local compositions and drop to zero at the critical point will show the same scaling.

Appendix A.2. Modulated structures and Microemulsions: Generic theory

To describe microemulsions and modulated phases within the Ginzburg-Landau framework, the gradient contribution $(\nabla \Phi)^2$ in Eq.\textsuperscript{A.1} must be generalized. A typical Ansatz includes terms with higher order spatial derivatives of $\Phi$, which are however still quadratic in $\Phi$\textsuperscript{121}. The generic form of the free energy functional is hence most conveniently written in Fourier representation

$$F[\Phi] = \int d^2r \left\{ \frac{t}{2} \Phi^2 + \frac{1}{4} \Phi^4 + \frac{g}{2} (\nabla \Phi)^2 + h \Phi \right\} + \sum_q \frac{1}{2} G(q) |\hat{\Phi}_q|^2,$$

where $q$ are the Fourier wave vectors, $\hat{\Phi}_q$ corresponds to the Fourier transform of $\Phi(\mathbf{r})$, $A$ is the membrane area, and $G(q)$ subsumes all gradient terms. For example, Eq.\textsuperscript{A.1} corresponds to the case $G(q) \equiv g q^2$.

To investigate domain formation in the system defined by Eq.\textsuperscript{A.2}, we must analyze the quantity $\Gamma(q) = t + G(q)$, which corresponds to the inverse structure factor in a reference homogeneous disordered (mixed) state. This homogeneous state is unstable towards order parameter modulations if the minimum value of $\Gamma$ is negative. Let $q^*$ denote the wave vector that minimizes $\Gamma(q)$. At $t = -G(q)|_{q^*}$, mean field theory thus predicts a continuous phase transition from a mixed and homogeneous state, either to a demixed state if $q^* = 0$, or to a modulated state with the well-defined characteristic wave vector $q^*$ if $q^* \neq 0$. This latter transition belongs to the so-called Brazovskii universality class, and it has the remarkable feature that fluctuations not only shift the transition point to lower $t$, but also change it into a first order transition\textsuperscript{201,202}. Above the transition, the system is thus globally disordered, but it is still ordered on local scales and filled with domains with the characteristic length scale $2\pi/q^*$. Such a local order characterizes a microemulsion. It sets in at the so-called Lifshitz line where $\Gamma(q) = 0$, first develops a minimum at a nonzero wave vector $q$\textsuperscript{121}. 15
Specific choices of $\tilde{G}(q)$ have received particular interest in the literature. The simplest and most prominent choice is $\tilde{G}(q) = g \frac{q^2 + kq^4}{1 + kq^4}$ with $k > 0$. For $g > 0$, this system exhibits a regular Ising-type demixing transition at $t = 0$, and for $g < 0$, it undergoes a Brazovskii transition to a modulated phase with a characteristic wave vector $q^* = \sqrt{-g/2k}$ at $t = g^2/4k$. The Brazovskii and the Ising line meet at $t = g = 0$ in a multicritical point, the so-called Lifshitz point [203, 204], which is also the end point of a free energy functionals of the form (A.2).

Microemulsions, i.e., mechanisms that generate effective microscopic fluctuations, were first introduced by [143, 145]. This form was originally proposed by Ohta and Kawasaki [171] 205]. Note that the contribution $G_L = a/q^2$ no longer describes a short range interaction in real space, it corresponds to a long-range Coulomb-type interaction term of the form

$$a \int d^2r \int d^2r' G_L(r, r') \delta \Phi(r) \delta \Phi(r')$$

in the free energy functional, where we have used the notation $\delta \Phi(r) = \Phi(r) - \langle \Phi \rangle$ and $G_L$ is the solution of $\Delta G_L(r, r') = -2\pi \delta(r-r')$ in two dimensions. This non-local term inhibits macroscopic phase separation and stabilizes structures in mesophases with a characteristic wave vector of $(a/g)^{1/4}$ [171].

### Appendix A.3. Line active molecules

The effect of line active additives can be described by adding an additional density field $\rho_t$ representing the density of linactants, to Eq. (A.1). The resulting coupled functional (at $h = 0$) reads

$$F[\Phi, \rho_t] = \int d^2r \left\{ \frac{1}{2} \frac{\Phi^2}{4} + \frac{g}{2} (\nabla \Phi)^2 + \rho_t \ln (\rho_t) - \rho_t \right\} ,$$

where $\rho_t(r)$ depends on $\Phi$ and subsumes the local interactions between the surfactants and the order parameter field. Since the linactants are attracted to the domain boundaries, we can assume the simple form $\rho_t = \rho_l + \alpha \Phi (\nabla \Phi)^2$, where $\rho_l$ is the chemical potential of linactants, and $\alpha$ describes the strength of the attraction. Minimizing the free energy functional (A.4) with respect to $\rho_l$ and omitting terms of order $(\nabla \Phi)^2$, we recover a functional of the form (A.1), except that $g$ is replaced by $g_{eff} = g(1 - \alpha \rho_l / \rho_\infty)$, where $\rho_\infty = \exp(\mu_l)$ is the linactant density in the bulk, far from the domain boundaries.

Thus the linactants effectively reduce the line tension. For sufficiently high coupling constant $\alpha$, the line tension becomes negative at a critical linactant density, and the system turns into a microemulsion 	extit{via} a Lifshitz point. The corresponding phase diagram (based on Eq. (A.3) with an additional stabilizing term $\frac{1}{2} (\Delta \Phi)^2$) is shown in Fig. 5. This phase diagram was produced with the parameters $g = 1$ and $\alpha = 1$ and a single-mode approximation was used to calculate the first order boundary between the modulated and the phase separated region [153]. We note that adding surfactants typically also reduces the driving force for phase separation, hence $t$ should shift to higher values with increasing $\rho_l / \rho_\infty$. In the main text, we propose to use the simple Ansatz $\rho_{lin} = \rho_0 + \nu \rho_l / \rho_\infty$, which corresponds to the leading order of $\rho_l / \rho_\infty$.

### Appendix A.3.2. Curvature induced mechanisms

Following our recent work [155], we discuss the bilayer and monolayer curvature coupling mechanisms within a single unified framework. The starting point is a description of the membrane in terms of two coupled elastic sheets, representing the monolayers. This type of description was originally proposed by Dan et al. [158, 204, 207] and has been used successfully by others and us to describe membrane deformations due to inclusions and membrane fluctuations [199, 208, 211]. For nearly planar membranes, the monolayer positions can be parametrized by functions $z_{1,2}(r)$ and the coupled elastic energy in quadratic approximation can be written in the form

$$F_{el} = \int d^2r \left\{ \frac{k_4}{8 \eta_0} (z_1 - z_2)^2 + \frac{k_c}{4} ((\Delta z_1)^2 + (\Delta z_2)^2) + k_c (c_1 (\nabla z_1)^2 + c_2 (\nabla z_2)^2) \right\}$$

where $c_1, c_2$ are the elastic moduli. This form was used to calculate the first order boundary between the modulated and the phase separated region [153].

### Appendix A.3.3. Line active mechanisms

We turn to the description of mechanisms that stabilize microemulsions, i.e., mechanisms that generate effective free energy functionals of the form (A.2).
This expression contains all terms up to second order of $z_{1,2}$ and second order derivatives that are allowed by symmetry, and the parameters have the following interpretation: $t_0$ - mean monolayer thickness, $k_A$ - area compressibility, $k_c$ - bending energy, $c_i$ - spontaneous curvature on monolayer $i$, $\Gamma$ - membrane tension \cite{[194, 212, 214]}, and $\zeta$ - an additional curvature-related parameter. The values of the parameters can be adjusted to values known from experiment or simulations \cite{[208, 209]}. We assume that every monolayer carries lipids that have a tendency to phase separate, hence monolayers $i$ carry an order parameter $\varphi_i$ with a free energy functional derived from Eq. (A.1).

\[
F_{\varphi}[\varphi_1, \varphi_2] = F_0(\varphi_1) + F_0(\varphi_2) - \int d^2 r \frac{s}{2} \varphi_1 \varphi_2 \tag{A.6}
\]

with $F_0(\varphi) = \int d^2 r \left\{ \frac{g}{4} (\nabla \varphi)^2 + \frac{r}{4} \varphi^2 + \frac{1}{8} \varphi^4 \right\}$.

The parameter $s$ describes the coupling across the leaflets, i.e., domains tend to be in registry for $s > 0$ and they tend to be staggered for $s < 0$. The curvature coupling is implemented via $c_i(r) = c_0 + \tilde{c} \varphi_i(r)$, i.e., the spontaneous curvature of a monolayer is taken to depend on the local composition of the monolayer. The bilayer and monolayer coupling mechanisms are then obtained by minimizing the total free energy, $F = F_{el} + F_{\varphi}$, with respect to a fluctuating membrane height $h(r) = \frac{1}{2}(z_1 + z_2)$ or with respect to a fluctuating membrane thickness parameter $u(r) = \frac{1}{2}(z_1 - z_2)$ while confining the other parameter to a constant. This yields in both cases a free energy functional of the form (A.2) for a single scalar order parameter which is a combination of the $\varphi_{1,2}$.

In the Leibler-Andelman (bilayer curvature coupling) mechanism, the thickness is kept constant and the free energy is minimized with respect to height fluctuations. This results in a free energy functional $F(\Psi)$ for the local parameter difference on the two leaflets, $\Psi = \frac{1}{2} (\varphi_1 - \varphi_2)$, which has the form (A.2) with $t = r + s$ and

\[
\tilde{G}_{\Psi}(q) = g - 4k_c \tilde{c}^2 \frac{(\xi_q)^2}{1 + (\xi_q)^2}, \tag{A.7}
\]

where $\xi_q = \sqrt{k_c / q}$ \cite{[123, 141]}. Expanding $G$ in powers of $q$, one obtains $G = g_{st} + kq^2$ with $g_{st} = g - 4k_c \tilde{c}^2 \xi_0^4$ and $k = 4k_c \tilde{c}^2 \xi_0^4$, which leads to the Lifshitz point scenario described in Appendix A.2. If one decreases $t$, the system phase separates for low curvature coupling $\tilde{c}$ and/or large membrane tension $\Gamma$ and forms a modulated structure at high $\tilde{c}$ and/or low $\Gamma$. The two regimes are connected by a Lifshitz point where the characteristic wave length of domains diverges. Fig. 4 shows a typical example of such a phase diagram \cite{[133]}. Similar phase diagrams have been published by Shlomovitz et al. \cite{[142, 143]}. We note that the characteristic length scale $\ell$ also becomes large if the membrane tension $\Gamma$ is small, since it scales with $\xi_\ell$. (More precisely, the characteristic wave length of the modulations is given by $q^* = \sqrt{\frac{k_c}{4\tilde{c}}} \sqrt{2\ell / \sqrt{Ag}}$. With typical experimental values for the bending rigidity in the range of $5 - 20 \cdot 10^{-20}$ \cite{[215]} for and for the membrane tension in the range of $\Gamma \sim 10^{-5}$ N/m in plasma membranes \cite{[216, 217]}, one finds that $\xi_\ell$ is of the order ~100 nm.

In the monolayer curvature coupling mechanism, the height is kept constant and the free energy is minimized with respect to thickness fluctuations. One obtains a free energy functional $F(\Psi)$ for the local average of the order parameters on the two leaflets, $\Phi = \frac{1}{2} (\varphi_1 + \varphi_2)$, which has the form (A.2) with $t = r - s$ and

\[
\tilde{G}_{\Phi}(q) = g_q q^2 - 4k_c \tilde{c}^2 \frac{(\xi_0 q)^4}{1 - (\xi_0 q)^2} + A(\xi_0 q)^2. \tag{A.8}
\]

The characteristic length scale $\xi_q = (g_q k_c / k_A)^{1/4}$ and the dimensionless parameter $\Lambda = 2 - 4\zeta \sqrt{k_c / k_A}$ are intrinsic material constants that cannot directly be controlled externally. Assuming that the bending rigidity $k_c$ is roughly proportional to $k_A$ of $\tilde{c}$, one can expect $\xi_\ell$ to be of the order of the membrane thickness. Indeed, if one inserts the elastic parameters of DPPC bilayers, one obtains $\xi_\ell \approx 1$ nm, and the parameter $\Lambda$ in this case is $\Lambda \approx 0.7$ \cite{[84, 135]}.

Analyzing the functional $F(\Psi)$ in more detail, one finds again that the system behaves like a micromulsion for high curvature coupling and turns into a regular phase separating system for low curvature coupling. However, the transition from one regime to the other does not occur via a Lifshitz point, and the characteristic wave vector of the modulations remains finite and roughly constant for all values of $\tilde{c}$, $q^* \sim 1 / \xi_\ell$. Hence the typical size of domains, $2\pi / q^*$, is always of the order of a few nanometers. Fig. 5 shows a typical phase diagram for this situation.

If one releases the constraints on the thickness or the height, one can study the interplay of the two coupling mechanisms \cite{[133]}. The phase diagrams shown in Figs. 5 are all calculated using a single-mode approximation and further approximations described in Ref. \cite{[133]}.
The first term describes the diffusion of lipids with mobility $M$ in the free energy landscape given by a Ginzburg-Landau functional, the second term is a source/sink term that accounts for a constant exchange of lipids with an external lipid reservoir (the Foret mechanism [167]) with an exchange rate $\tau_r$, and the last term subsumes thermal and active noise. The Ginzburg-Landau functional proposed by Fan et al. is derived from (A.3) except that it accounts for the possible presence of immobilized inclusions $i$ acting as pinning sites for domain boundaries. It has the form

$$
\mathcal{F}[\Phi] = \int d^2r \left\{ \frac{\sigma^2}{2} \Phi^2 + \frac{1}{4} \Phi^4 + \frac{q}{2} (1 - \alpha \rho_p(r)) (\nabla \Phi)^2 \right\},
$$

where $\rho_p(r) = \frac{\pi}{\sigma^2} \sum_{i=1}^{N} \exp\left(-\frac{|r - r_i|^2}{2\sigma^2}\right)$ is the smeared density of pinning sites and $\alpha$ the coupling parameter (see also in Eq. (A.3)). Finally, the noise is Gaussian distributed with mean $\langle \eta \rangle = 0$ and correlator

$$
\langle \eta(r, \tau) \eta(r', \tau') \rangle = -\frac{\hbar^2}{2\pi} \Delta K_0 \left(\frac{|r - r'|}{l}\right) \delta(\tau - \tau'),
$$

where $K_0(x)$ denotes the zeroth order modified Bessel function of the second kind. This noise term accounts for "stochastic recycling" and is constructed such that it conserves the order parameter on large scales, but breaks the continuity equation on length scales smaller than $l$. Hence, it provides an alternative description of lipid turnover, since lipids are allowed to enter and leave the membrane on local scales while the average global composition remains constant. In the limit $l \to 0$, the noise term reproduces spatially uncorrelated thermal noise. If $l$ is chosen much larger than the equilibrium correlation length $\xi$, the noise is incompatible with the fluctuation-dissipation theorem and this creates a true nonequilibrium situation.

Since the model (A.9) contains elements of several domain forming mechanisms, they can be compared by choosing the parameters appropriately. For example, if one chooses (I) $\alpha = 0$, $l < \xi$, $\tau_r^{-1} = 0$, one obtains a dynamical description for a phase separating system at equilibrium, and can study critical clusters in the limit $t \to 0$. Starting from this system as the reference system, one can study (II) domain pinning due to immobilized pinning sites by choosing $\alpha > 0$ at $t < 0$, (III) stochastic recycling by choosing $l \gg \xi$ at $t > 0$ and (IV) $t < 0$, and (V) the Foret mechanism of lipid turnover by choosing $\tau_r^{-1} \neq 0$ at $t < 0$. Examples of resulting morphologies are shown in Fig. 11 (taken from 170). Remarkably, most mechanisms produce rather disordered, fractal domains, except for the Foret mechanism, which generates a large well-ordered spatially periodic array of domains. This finding may seem surprising at first, but it can be rationalized if one realizes that the Foret mechanism can be mapped onto the Ohta-Kawasaki model described in Sec. Appendix A.2 (Eq. (A.3)). Indeed, the lipid reservoir term in Eq. (A.9) can easily be reproduced by adding a contribution of the form $\lambda \alpha$ in the free energy functional (A.10) with prefactor $\alpha = 1/M \tau_r$. Thus the Foret mechanism effectively produces a microemulsion with a characteristic wave vector that scales as $q^* \sim \tau_r^{-1/4}$ with the rate $\tau_r$ of lipid exchange.

The dynamical model (A.9) can be extended to describe other and more complex situations. For example, the free energy functional in (A.9) can be chosen at will and supplemented with all the terms that were discussed in the previous section. Eq. (A.10) describes a situation where pinning sites couple to domain boundaries, but the pinning term can easily be designed such that it describes pinning sites that attract one phase – the coupling term then takes the simple form $\int d^2r \alpha \rho_p \Phi$ [167]. A realistic model for membrane dynamics should also include hydrodynamic interactions. This can also be done, e.g., following the theoretical approaches that are reviewed in Ref. 68.

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