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Line active molecules promote inhomogeneous structures in membranes: Theory, simulations and experiments

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ABSTRACT

We review recent theoretical efforts that predict how line-active molecules can promote lateral heterogeneities (or domains) in model membranes. This fundamental understanding may be relevant to membrane composition in living cells, where it is thought that small domains, called lipid rafts, are necessary for the cells to be functional. The theoretical work reviewed here ranges in scale from coarse grained continuum models to nearly atomistic models. The effect of line active molecules on domain sizes and shapes in the phase separated regime or on fluctuation length scales and lifetimes in the single phase, mixed regime, of the membrane is discussed. Recent experimental studies on model membranes that include line active molecules are also presented together with some comparisons with the theoretical predictions.

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1. Introduction

The lateral organization of multi-component model membranes has been the focus of numerous investigations for two decades. Under some conditions, these components can self-assemble into domains of finite sizes (much smaller than the overall size of the membrane). However, finite size domains are not consistent with typical equilibrium phase behavior of mixtures in which the components either uniformly mix or macroscopically phase separate (domains of the order of the membrane size). Understanding how such finite domains arise is a key challenge to a physical understanding of the "lipid raft" hypothesis in real cells which postulates that nanoscale lateral heterogeneities of cholesterol and sphingolipids in the plasma membrane are necessary for the cells to be functional [1,2]. Examples of functions whose postulated mechanism utilizes lipid rafts include protein membrane sorting and cell signaling [1,3,4].

So far, the evidence for rafts in biological cells comes from indirect observations; the rafts are too small to be resolved with the most powerful microscopes. In several studies, cell membrane domains enriched in cholesterol were discovered to resist detergent extraction (see [1,2,5] and references therein). In others, Förster Resonance Energy Transfer studies of fluorescent proteins revealed that they tend to aggregate in small domains; an effect attributed to their favorable partitioning in rafts (see [6] and references therein). These two indirect

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observations are perhaps the most common, but other techniques have also been used [2]. From the compilation of various experimental results on plasma membranes, rafts are now believed to have sizes of the order of 10–100 nm [6].

The study of model membranes provides useful information regarding the interactions among membrane constituents since they are not subject to other types of interactions present in real cells (i.e. coupling with the cytoskeleton and/or active processes). One useful approach measures the properties of self-assembled giant unilamellar vesicles (GUVs) or suspended bilayers whose lipid composition can either be controlled [7–13] or extracted from cell membranes [7,14,15].

In parallel to those experimental studies, several theoretical models for the formation and stability of small (of the order of estimated raft sizes) domains have been proposed. One class of models is based on line active molecules or "linactants". The term linactant (2D analogs of surfactants) was first proposed in a recent study [16] that demonstrated how specifically designed non-lipid molecules can reduce the line tension between domains in monolayers. The linactant molecule has a lower free-energy when it resides at an interface compared to its free energy in either of the bulk phases. Hence, with linactants, a membrane can adopt equilibrium, locally phase separated, conformations with finite size domains that are stable despite their much larger total interfacial length. This short review focuses on related models applied to lipid mixtures. We highlight studies that indicate how lipid linactants can promote inhomogeneous structures in mixed membranes. Due to the simplicity of these theories, a comparison with experimental data obtained on model membranes (rather than real cells) is more appropriate. Other types of mechanisms for lateral heterogeneity have also been proposed. Some are based on the coupling between the membrane curvature and the lipid composition [17–20], the interleaflet couplings [21,22,20], the height mismatch between domains [23,24,19] and electrostatic interactions [25,26]. In biological cells, the interactions between lipids and proteins [27–29] can also favor small domains as do cell–cell adhesion [23]. Of course, the role of line active molecules on the generic stability of nanodomains does not exclude any of these mechanisms which may have equally important roles. We direct readers interested in these mechanisms to the appropriate references and to the review paper by Komura and Andelman that appears in this special issue.

The paper is organized as follows. In Section 2, we outline the basic features of model membrane phase diagrams and highlight the relevance to lipid rafts. Section 3 is the main part of the paper. It reviews recent theoretical models that include line active molecules that can provide a mechanism for lateral heterogeneities in membranes. Section 3.1 focuses on coarse-grained theoretical views of lipid membranes where the natural amphiphile is a hybrid lipid while Section 3.2 focuses on more microscopic models based on molecular dynamics simulations that incorporate hybrid lipids and other line active molecules. Section 3.3 discusses the role of line active lipids in increasing the probability of small scale fluctuations of the lipids in the uniformly mixed phase of the membrane, even near the critical point, where normally large scale fluctuations are most probable. As we will show, composition fluctuations are also predicted to have longer lifetimes in the presence of linactants. A review of experimental studies that focus on model membranes that comprise linactants is presented in Section 4 and Section 5 concludes the paper with final remarks.

2. Phase diagram of model membranes

The role that linactants play in the lateral organization of model membranes is usually understood in terms of the phase behavior of the membrane. In its simplest description, the membrane is treated as a binary mixture in 2D. The lipids that tend to phase separate at low temperatures are classified as A and B and they have unfavorable nearest-neighbor interactions (in principle, A and B can both describe more than one type of lipids). In such a two-component mixture, the mean-field membrane free-energy per molecule can be written as (for example, see Refs. [30,31]),

$$\frac{F}{k_b} = T[\phi \log\phi + (1-\phi)\log(1-\phi)] + 2J\phi(1-\phi), \quad (1)$$

where the first term is the entropy of mixing of the two components, ϕ is the fraction of class A lipid molecules ($(1-\phi)$ is the fraction of class B), $J(>0)$ is the energy cost associated with nearest-neighbor A and B pairs, k_B is Boltzmann's constant and T is the temperature. Fig. 1 shows the mixing temperature, $T_m(\phi)$, of the membrane as a function of the composition that results from this binary mixture model. For $T > T_m(\phi)$ the membrane is in the single phase; the system is uniform on average and lipid components A and B are mixed. Lowering the temperature in the region $T < T_m(\phi)$ gives rise to coexisting regions enriched (depleted) in $A(B)$ and depleted (enriched) in $B(A)$; the system phase separates. In this simplified picture, the maximum mixing temperature defines a critical point, $T_c = T_m(\phi = 1/2)$.

The phase diagram of real model membranes is much richer than the simple binary mixture diagram shown in Fig. 1 since they usually contain saturated lipids (characterized by hydrocarbon chains that have no double bonds, see Fig. 2A), unsaturated lipids (where both chains have double bonds, see Fig. 2A) and cholesterol. Moreover, the lipid molecules have chain conformational degrees of freedom (and possibly, orientational degrees of freedom in addition to the local composition degree of freedom) that contribute to the phase behavior. In fact, the phase separated regime can be subdivided into numerous phases that display different degrees of order. For a review of the typical phases observed in model membranes, see Refs. [32,33]. In summary, the liquid-disordered phase, L_d or L_α, is usually found at high temperatures and/or large cholesterol fraction. In that phase, the lipid chains are disordered and the lipid translational motion is rapid (characterized by a large diffusion coefficient, $D \approx 1\ \mu m^2/s$). In decreasing order of fluidity, the liquid-ordered, L_o, phase follows. There, the lipid chains are ordered (straight), but lipid diffusion is still rapid ($D \approx 1\ \mu m^2/s$). Next is the gel phase (the gel phase can often be found by reducing the temperature and/or decreasing the cholesterol content), L_β, where the chains are ordered and lipid diffusion is slow ($D \approx 10^{-3}\ \mu m^2/s$). The nomenclature for these various phases is the one proposed in Refs. [32,33], but there are papers that use a different one. Note that crystalline phases have been observed in monolayers made of cholesterol and ceramides [34], but the crystal order was maintained over short distances only ($\approx$10 nm). Recall that long-range order is strictly forbidden in 2D by the Mermin–Wagner theorem [35] (also see Section 6.1 in Ref. [30]). Each of these phases can be observed in specific composition and temperature ranges. In particular, the liquid ordered phase is often called the lipid raft phase since it requires (and it is enriched in) cholesterol and saturated lipids which dominate the composition of detergent

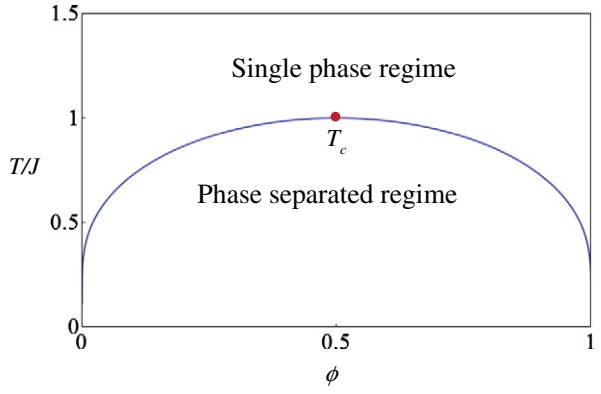

Fig. 1. The mixing temperature, $T_m(\phi)$ for a binary mixture as a function of the composition, ϕ. T_c indicates the position of the critical point.

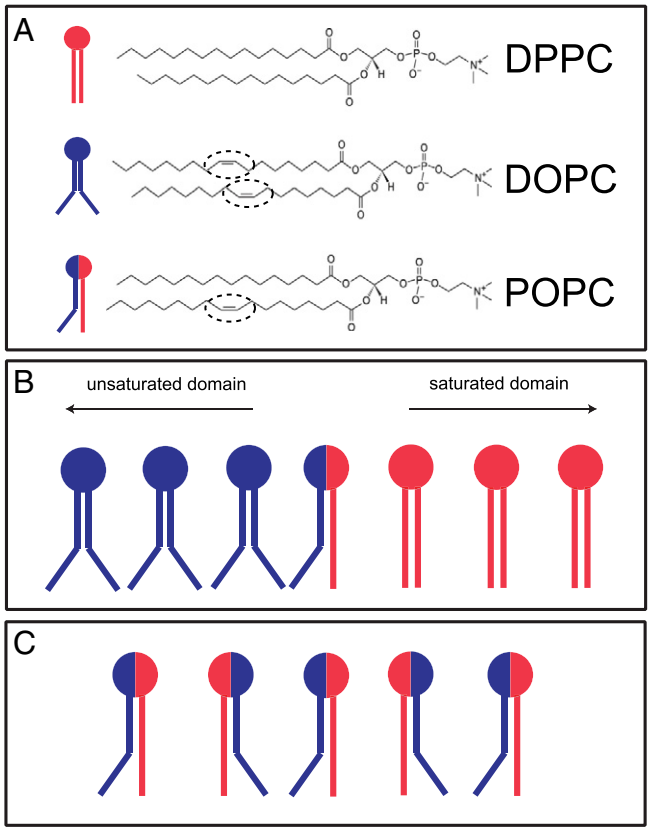

Fig. 2. A. Example of saturated, unsaturated, and hybrid lipids. They are, respectively, DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), and POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine). Dashed ellipses highlight the presence of double bonds along the hydrocarbon chains. B. The packing incompatibility between bent chains of the unsaturated lipids and the straight chains of the saturated lipids can be reduced by inserting a hybrid lipid at the interface between domains. The hybrid lipid must have the correct orientation (with its straight chain facing the saturated lipids and its bent chain facing the unsaturated lipids) to decrease the line tension. C. Illustration of domains rich in hybrid where the orientation of the hybrid lipids changes sign over a molecular length scale.

resistant membrane domains associated with rafts. Note that this classification of the phase behavior in terms of L_d, L_o and L_β is based on the local order of the lipids in the various phases and does not provide any information about domain sizes and shapes. A recent comprehensive model that accurately predicts the bulk phase diagram of saturated, unsaturated lipids and cholesterol can be found in Ref. [36].

For ternary model membranes containing cholesterol, the measured phase boundary between the liquid disordered (L_d) region and the liquid disordered/liquid ordered (L_d/L_o) coexistence region of the phase diagram contains a critical point. Hence, *locally*, the phase diagram can be qualitatively understood in terms of a binary mixture model such as the one described above (Eq. (1) and Fig. 1) if the class *A* lipid molecules represent the unsaturated lipid fraction and the class *B* lipid molecules include both saturated lipids and their associated cholesterol. In this case, the composition range that goes from $\phi = 0$ to 1 in Fig. 1 should not be taken literally. Rather, it represents an effective parameter that accounts for the saturated lipid and cholesterol composition. Of course, this type of effective binary mixture model can only describe a *single* phase transition. The complete phase diagram of ternary model membranes includes regions of L_d/L_o, L_d/L_β, L_o/L_β and $L_d/L_o/L_\beta$ coexistence as well as pure L_d, L_o and L_β phases. Describing all the transition between all these phases goes beyond the binary model. This can be done by more complex models but then physical insight into the role of line active molecules in lowering the line tension would be lost in the details.

Note that the phase transition of binary mixtures of saturated and unsaturated lipids (and no cholesterol) has been studied experimentally [37] and shows that the membrane is in the L_o phase at high enough temperatures and displays L_o/L_β phase coexistence when the temperature is lowered. Recent experiments [38] also reported the effect of adding line active molecules on the phase behavior of such binary model membranes that exhibit L_o/L_β phase coexistence. However, such a transition, which is analogous to a solid–liquid transition, does not have a critical point.

We now turn to the role of line active molecules in the phase separated and single phase regime, in the language of Fig. 1. Note that when the chains are not oriented and the lipids are macroscopically mixed (i.e., in the single phase), line active molecules can have an effect on composition fluctuations. These are characterized by a non-zero value of the local composition that is different from the membrane average composition (order parameter) and that exists only for a short period of time. This is in contrast to stable domains that arise in the phase-separated region of the phase diagram where the local composition modulations fluctuate about a well-defined (and non-zero) order parameter that is time independent. The fluctuation "domains" are characterized by typical length and time scales as we explain below. For a review of fluctuations in model membranes, refer to Ref. [39].

3. Theoretical membrane models with linactants

3.1. Continuum models with "hybrid" lipids

This subsection reviews various theories based on a naturally occurring linactant [16] that is a major component of cell membranes that we call a hybrid lipid. The line active character of hybrid lipids was first proposed by Brewster et al. [40]. Fully saturated lipids are characterized by hydrocarbon chains that have no double bonds while for fully unsaturated lipids, both chains have double bonds (see Fig. 2A). The presence of one or more double bonds creates permanent bends in the chain that are responsible for the packing incompatibility between saturated and unsaturated lipids. Hence, real membranes made of certain types of fully saturated and unsaturated lipids (with and without cholesterol) phase separate close to room temperature [41,42] (i.e. the energy related to the chain incompatibility, k_BJ in Eq. (1), is of the order of k_BT). It was therefore hypothesized [40] that hybrid lipids, where one chain is saturated and the other unsaturated, can assemble at the boundary between domains of saturated and unsaturated lipids, reduce the packing incompatibility and hence diminish the line tension associated with the interface (see Fig. 2A and B). If the line tension is reduced to extremely small values, this can allow finite size domains to be stable even in equilibrium.

Neglecting hybrid–hybrid interactions, Brewster et al. developed a Landau–Ginzburg [43] type of continuum free-energy appropriate for a membrane with a dilute amount of hybrid lipids. In the model, the reduction of the line tension was due to a three-body interaction between saturated, unsaturated and hybrid lipids. The effects of the hybrids on the miscibility critical point (defined by the critical temperature, T_c, and the critical composition of saturated lipids, ϕ_c) were predicted. For temperatures below but close to T_c, an interface separating two bulk phases of saturated and unsaturated lipids has an associated line tension, γ, which was predicted by the model to be,

$$\gamma \propto \left(\frac{T_c - T}{T}\right)^{3/2} \sqrt{1 - \frac{J\phi_h}{4T}}, \tag{2}$$

where ϕ_h is the fraction of hybrid in the membrane and where $T_c \propto J$. As usual, as $T \to T_c$, the difference in free energy between the two coexisting phases decreases and the line tension tends to zero. The expression further shows that the line tension can be decreased by increasing the amount of hybrid lipids. However, since ϕ_h was assumed to be small, the reduction of the line tension is not significant unless the system is deep into the phase separated region ($T \ll T_c$); a regime which is less relevant experimentally since T_c is close to room temperature and regimes where $T \ll T_c$ may imply temperatures below the

freezing point of water. Close to T_c, a large translational entropic cost is associated with the local assembly of hybrids at the interface. As a result the hybrids tend to distribute uniformly in the membrane (i.e. they do not significantly accumulate at the interface) and their effect on the line tension is small.

Unsaturated lipids are minor components of biological membranes where hybrid lipids are rather abundant. Hence, it was proposed that biological membranes, as opposed to model membranes, may be better described as mixtures of saturated lipids, hybrid lipids, and cholesterol, where three-body interactions between saturated lipids, hybrids, and unsaturated lipids are absent. In such membranes, hybrid lipids are the major species in the liquid disordered domains and their saturated chains are disordered; in this conformation, the hybrids effectively behave as unsaturated lipids. In contrast, the saturated chains of the hybrids at the interface between domains are orientationally ordered due to their interactions with the liquid ordered domain; in this conformational state, hybrids behave as linactants. Yamamoto et al. [44,45] developed a liquid crystal model for saturated/hybrid/cholesterol mixture that predicts how hybrids can reduce the line tension in such membranes. There, the three-body interaction involves saturated lipids, interfacial chain-ordered hybrids, and bulk chain-disordered hybrids (in the L_d phase). Since the hybrid lipids are already at the interface in such membranes, they can reduce the line tension between domains with no associated translational entropic cost. Hence, the theory predicted that zero line tension can be achieved at higher temperatures. However, this temperature is still lower than physiologically relevant temperatures (although higher than those predicted by Brewster et al. [40]). This is due to the entropic cost that originates from the chain conformation and the hybrid orientation; both of these degrees of freedom must lie in a narrow range (compared to all possible chain conformation and lipid orientations) to reduce the line tension.

Expanding on the role of hybrid lipids, Hirose et al. [22] proposed a phenomenological continuum model to describe a membrane self-assembled from saturated and hybrid lipids. The theoretical free-energy was constructed from symmetry arguments and non-local interactions were described using a small gradient expansion (valid for long-wavelength modulations). They extended their previous theory for coupled bilayers [21] by implicitly including an effective interaction related to the compositional degrees of freedom that accounts for the effect of the hybrid lipid orientation. Further, they analyzed under which conditions the domains/modulations in the two leaves of the bilayer are in registry. The phase diagram of the membrane was reported as a function of the phenomenological constants that enter the model. For the case of decoupled leaflets, they showed that, by increasing the coupling between the composition and orientation degrees of freedom, a membrane in the phase separated regime (at equilibrium) could cross over to a structured-disordered phase (the system is mixed on average, but local modulations can be observed for a finite lifetime) and then to a fully disordered phase with no compositional modulations. Note that modulations in the mixed phase are typically characterized by a structure factor that has a peak at a non-zero wavenumber.

The works of Brewster et al. [40] (describing a membrane made of saturated/unsaturated and small amounts of hybrids) and of Hirose et al. [22] (describing a membrane made of saturated and hybrids) are similar in the sense that they do not account for the chain order explicitly. Within the same framework, these theories were then extended by Palmieri et al. [46] who proposed a ternary mixture model for membranes made of saturated/unsaturated and hybrid lipids and that include hybrid–hybrid interactions so that it is applicable to the entire composition range and not only for small hybrid fractions. The proposed continuum model was inspired by a lattice model which is a special case of that of Matsen and Sullivan [47] for microemulsions (numerous lattice models for microemulsions followed the pioneer work of Alexander [48] and Widom [49] and others are reviewed in [46]). It includes the hybrid orientation degrees of freedom explicitly and extends previous models in the following ways: 1) it contains a single parameter, J (which is fully specified by the demixing temperature); 2) the composition dependence of the system is fully taken into account; and 3) it allows for spatial variations at all length scales (including the smallest one which is defined by a molecular size). This latter point was shown to be important since it permits the theory to describe the reorientation of the hybrids, which, from an energetic point of view, tends to occur over a molecular distance (see Fig. 2C). It was shown that this effect could further stabilize the interface at large hybrid fractions.

The model was used to predict composition fluctuations in the macroscopically uniform phase (mixed regime). The critical temperature and characteristic length scale of composition fluctuations (defined by the correlation length, ξ) were predicted to depend on the interactions and hybrid lipid volume fraction as,

$$T_c = 4J(1-\phi_h) \tag{3}$$

and,

$$\left(\frac{\xi}{a}\right)^2 = \left(\frac{T-T_c}{T_c}\right)^{-1}\left[\frac{1-3\phi_h/2}{4(1-\phi_h)}\right], \tag{4}$$

where a is a molecular size. Note that the last two equations are valid for $\phi_h < 2/3$ and for equal fractions of saturated and unsaturated lipids. These results show that with increasing hybrid fraction, the miscibility transition temperature uniformly decreases; the hybrids tend to demix the membrane. The characteristic fluctuation domain sizes decrease with increasing hybrid fractions and $\phi_h < 2/3$. At that point ($\phi_h = 2/3$, called a Lifshitz point), small length scale fluctuations can be observed close to the critical temperature in a regime where large correlation lengths would be expected with no hybrids. For membranes with hybrid fractions exceeding the Lifshitz point, the model predicted modulated "stripe-like" fluctuations (characterized by stripes rich in saturated and unsaturated lipids separated by stripes of correlated orientation of the hybrids) that have some similarities with those reported by Hirose et al. [22] in their structured-disordered phase. One difference is that the former model predicts that the length scale that characterizes the modulations (distance between stripes) decreases with increasing hybrid fractions and tends to molecular scale, a, as $\phi_h \to 1$ (in a membrane that contains only hybrids). The theory of Hirose et al. cannot describe nanoscale stripe-like patterns since they used an extra level of coarse-graining for the hybrid lipid orientation (it is averaged over a region that is large compared to a). For real membranes containing cholesterol, ϕ_h should be interpreted as an "effective" parameter that is not identical, but that is correlated with the hybrid lipid contents (recall our discussion in Section 2 where binary mixture and real model membrane phase diagrams are compared). This implies that some of the predictions (i.e. the appearance of a Lifshitz point) may occur at lower hybrid fractions.

3.2. Near atomistic model

The effect of hybrid lipids and other line active molecules on the lateral heterogeneities in membranes was also studied using nearly atomistic approaches such as coarse-grained molecular dynamics studies. All of the molecular dynamics studies reviewed below are based on the MARTINI [50] force-field; a model commonly used in biomolecular simulations where four heavy atoms (and their associated hydrogen atoms) are mapped to one coarse-grained interaction site.

In simulations related to the model of Brewster et al. [40], Schäfer and Marrink considered a membrane made of cholesterol, saturated (DPPC), unsaturated (DLiPC) and a small amount of hybrids (2% mol fraction of POPC) [51]. In their computation, a small but measurable aggregation of the hybrids at the interface between domains rich in saturated and unsaturated lipids was observed. The simulated membrane contained 2% mol fraction of hybrids and the calculation predicted a 30% line tension reduction compared to the case with no hybrids at

295 K. The miscibility temperature for their system was not reported so their results cannot be quantitatively compared with the prediction made by Brewster et al. described in Eq. (2).

The role of the degree of unsaturation of the unsaturated hydrocarbon chains was also studied by Rosetti and Pastorino [52] from simulations of membranes that comprise cholesterol/saturated/unsaturated lipids and cholesterol/saturated/hybrid lipids. In both cases, the number of double bonds in the unsaturated hydrocarbon chains (both chains for the unsaturated, the single chain for the hybrid lipids) varied from 1 to 4. In agreement with some of the predictions made from the continuum models [40,46], the system with hybrids was found to be in the mixed state (single phase) while the one with unsaturated lipids was in the phase separated regime. Moreover, a preferred orientation of the hybrid lipids when they lie at an interface was clearly observed (the saturated tail tends to point toward the saturated domain). Their results showed that increasing the number of double bonds along the hydrocarbon chains resulted in an increased line tension between domains of saturated/unsaturated lipids and increased the orientational correlations of the hybrids at the interface of saturated/hybrid domains. This last point suggests an increase in the line activity of hybrids in such systems.

The line activity of non-lipid molecules in model membranes comprising saturated and unsaturated lipids has recently been studied combining experimental methods with simulations [53]. The candidate line active molecules studied were: vitamin A, benzyl alcohol and Triton X-100 (a popular detergent to isolate lipid rafts). These three chosen molecules had the following effect on the phase behavior of the membrane: vitamin A increased the tendency to mix while benzyl alcohol and Triton-X 100 increased the tendency to separate saturated and unsaturated lipids, suggesting that vitamin A may act as a linactant in biological membranes.

Our focus here is on linactant based models to explain lateral membrane heterogeneity and not on other mechanisms that may be present in biological cells. Nevertheless, recent molecular dynamics simulations of membrane-embedded proteins suggested that some proteins can themselves be line active [54,55]. This latter study was inspired by recent experiments that are described in Section 4 and suggested that the N-Ras protein, which is connected to the membrane via lipid tails that act as anchors, tends to accumulate at the boundary between liquid ordered and liquid disordered domains, thereby demonstrating its line active character. The line activity of other real cell components such as molecular complexes made of ganglioside lipids and transmembrane peptide was also demonstrated through molecular dynamics simulations [54].

Analogous to model membranes, monolayer of polymer blends can also be used as models to study phase separation in 2D. This approach was taken by Bernardini et al. [56] who considered binary polyalkyl–methacrylate mixtures and showed, using a self-consistent field theory that includes near atomistic details, that the phase-separation is induced by the ester side-chain length mismatch; this parallels the phase packing incompatibility between saturated and unsaturated lipids in biological membranes. When the simulations added a line active diblock copolymer (analogous to the hybrid lipid) to the blend, the results showed that the linactants accumulated exclusively at the interface and significantly reduced the line tension.

3.3. Single phase: stability

When a membrane is in the single phase, the composition (and other degrees of freedom such as the hybrid orientation or the number of gauche bonds within a given chain) locally fluctuates about their uniform average values. Such fluctuations can have large length scales and long lifetimes when the system is close enough to a critical point [39]. These typically large fluctuation length scales can be modified by linactive molecules. As shown in Ref. [46] and in Eq. (4), the amplitude of small scale fluctuations is increased (even near the critical point where such fluctuations typically have small amplitudes) by the presence of the linactive hybrids. The effects of hybrid lipids on fluctuation dynamics were first studied by Hirose et al. [22] who reported the dynamic structure factors of composition fluctuation using their phenomenological model for membranes with saturated lipids and hybrids. Further the continuum theory proposed in Ref. [46] (that explicitly accounts for the interactions associated with the orientation of the hybrid lipids) was extended to describe the fluctuation dynamics. The results predicted a 2–3 orders of magnitude increase in fluctuation lifetimes for large hybrid lipid fractions ($\phi_h \to 2/3$) and nanoscale fluctuations ($\approx$10 nm) [57]. This latter prediction depends on a dimensionless parameter given by the ratio of the time scale for a lipid to diffuse over one molecular size to the hybrid reorientation time scale. The largest increase in fluctuation lifetime was predicted to occur when the latter time scale is longer than the former. Recent measurements (using saturated lipid probes) suggest that this dimensionless parameter is of order unity [58], although more experiments are required to better characterize the local lipid dynamics in different types of environments.

The role of linactants on the fluctuation lifetimes complements other mechanisms focused on the coupling between composition fluctuations and hydrodynamics of the membrane and the bulk water [59–62]. In particular, the predictions of Inaura and Fujitani [60] and Haataja [61] were verified experimentally by Honerkamp–Smith et al. [63] who showed how coupling composition fluctuations with hydrodynamic modes results in shorter fluctuation lifetimes when the correlation length is larger than a characteristic hydrodynamic length. On the other hand, the linactant and hydrodynamics based mechanisms act at very different length scales. Ref. [63] reported the effects of the coupling with hydrodynamics for composition fluctuations of length scales > 1 μm while the linactant effect predicted in Ref. [57] is important at much smaller length scales ($\approx$10 nm).

Note that molecular dynamics studies also predict dynamical slowing down for systems with linactive molecules (see for example, the slowing down induced by linactive transmembrane helices on the lipid diffusion reported in Ref. [54]).

4. Experiments

Model membranes comprising line active molecules have also been the focus of numerous experimental studies. Since cholesterol is one major component of cell membranes, many experimental groups have studied its role on the phase behavior of saturated lipid monolayers [64] and bilayers [9,65]. Ref. [64] proposed that cholesterol may form complexes with saturated lipid of sufficiently long hydrocarbon chains in monolayers. Others have suggested that the specific mole ratios of cholesterol and phospholipids in model membranes are not due to the formation of complexes but are rather a consequence of the “umbrella effect” where a saturated phospholipid can shield an integer number of cholesterol molecules from water in the bulk [66,67]. Nevertheless, in monolayers at high cholesterol fraction, small domains rich in cholesterol phase separate from domains depleted in cholesterol. The small domain sizes that are observed suggest that cholesterol may act as a linactant. The results were somewhat different in bilayers [9,65] where cholesterol had no line activity. There, cholesterol was shown to lower the miscibility temperature, a typical feature of linactants [40,46]. However, in these studies, the observed domains where metastable and, with time, coalesced to large domains of the order of the vesicle size.

Specifically designed non-lipid molecules were shown to reduce the line tension between domains in Langmuir monolayers in Ref. [16]. As stated in the Introduction, the authors were the first to propose the term “linactant”. More specifically, 2D binary mixtures were prepared at an air–water interface. These mixtures were made of hydrocarbon and fluorocarbon molecules that tend to phase separate at room temperature. Interfacial line tension was measured with different techniques [68]. By adding a small amount of linactant (a molecule that contains both a hydrocarbon and a fluorocarbon part), the line tension

was reduced to 80% of its initial value. In this system, this small decrease in line tension was enough to induce much smaller length scale modulations whereas macroscopic phase separation was observed without the linactants.

Experimental studies of model membranes that include hybrid lipids have also recently been carried out. For example, Heberle et al. [11] combined Förster Resonance Energy Transfer (FRET) and electron spin resonance (ESR) measurements to characterize domain sizes in membranes with varying compositions. Using this technique they were able to detect domains below the limits of optical resolution. Their results suggest that bilayers made of specific types of saturated and hybrid lipids (and cholesterol) contain small raft-like domains of the order of 10 nm (of the order of the Förster distance for energy transfer).

Following this work, similar studies were performed on membranes where unsaturated lipids were added to the mixture of saturated and hybrid lipids and cholesterol. Konyakhina et al. [12] and Goh et al. [69] used various optical techniques and focused on bilayers with a fixed composition of saturated lipids and cholesterol while systematically tuning the composition of hybrid and unsaturated lipids. These studies revealed that, within optical resolution, a membrane containing a large fraction of hybrids is in the single (mixed) phase. As the fraction of hybrid is reduced, the structure exhibits various types of modulated phases until it reaches a macroscopically phase separated regime as the hybrids are almost completely substituted by unsaturated lipids. The observed heterogeneous structures displayed micron scale modulation wavelength that depends on the composition. In the experiments, the modulations occurred in a narrow composition range, suggesting that real cells may be able to induce lateral organization in their membranes by slightly perturbing their composition.

Prior to the simulation studies of membrane-embedded proteins [55] mentioned in Section 3.2, Weise et al. [70] performed two-photon fluorescence microscopy and tapping-mode atomic force microscopy measurements on model membranes containing lipidated peptides and lipidated N-Ras protein. Their results demonstrated the favorable partitioning of the lipidated peptide or protein into the liquid disordered phase of the membrane. Perhaps more interestingly, the high spatial resolution of their AFM measurements was able to demonstrate a significant accumulation of the N-Ras protein at the boundaries between liquid ordered and liquid disordered domains; demonstrating the line activity of the protein. This result was reproduced by the simulations of de Jong et al. [55].

Phase separation studies of membrane stacks made of saturated, unsaturated and hybrid lipids in the presence of calcium ions were performed by Szekely et al. [38]. When phase separation occurs, the calcium ions are absorbed by the saturated lipid domains, the membranes become charged and the system swells considerably. As a result, the spacing between the membranes, probed by X-ray scattering, is indicative of the composition of the lipids in the membrane domains since regions containing a significant amount of unsaturated lipid would not adsorb calcium, thus diminishing the electrostatically-induced swelling. The results showed that these distances changed in a manner that suggested the mixing of saturated and unsaturated lipids occurs as the fraction of hybrid lipids was increased; in agreement with some of the theoretical and computational predictions [40,46,52].

Recently [71], small-angle neutron neutron scattering, a probe free technique, has been used to study the domain sizes in sub-micron (60 nm) diameter unilamellar vesicles, with bilayers comprising saturated/unsaturated and hybrid lipids (and cholesterol). As in Refs. [12] and [69], the ratio of hybrid/unsaturated lipids was varied and small domains were observed at relatively large hybrid fraction. The study reported domain sizes as small as 10 nm although here, the decrease in line tension was attributed to the thickness mismatch between ordered and disordered domains in contrast to the mechanism based on the orientation of the hybrids at the interface proposed in Refs. [22,40,44,45,46]. Note that the same group also performed similar experiments with a short chain saturated lipid (about 30% shorter than the other saturated/unsaturated lipids in their model membrane) replacing the hybrid and found similar results for the domain sizes [72]. Their claim is that the saturated (non-hybrid) short-chain lipid also acts to reduce the line tension between domains of saturated and unsaturated lipids. Whether this reduction results from a "hybrid-like" mechanism, where identical chains assume different conformations when they interact with different phases, or a completely different one remains to be determined.

Vesicles assembled from lipids (including hybrids) with different head groups have also been recently studied [73–75]. These lipid mixtures were made of hybrid phospholipid and different types of ceramides (that have smaller [73,74] or larger [75] headgroups). Perhaps the most striking effect reported in these studies is that, in certain composition ranges, the membrane not only displays lateral heterogeneity, but also undergoes dramatic morphological changes such as tubular structures. Identifying the mechanism that drives such morphological alterations and determining whether the line activity of the hybrid plays a role, remain to be investigated.

5. Discussion

In this paper, we reviewed several theoretical models that provide a mechanism for the lateral organization of lipids in model membranes. Many of the features of coarse-grained continuum models are also observed in near-atomistic molecular dynamics simulations or self-consistent theories. Comparison of the theoretical model with experiments is more challenging. The reason is that many of the unique features that distinguish different mechanisms (based on line active molecules or other effects) are manifested at the nanoscale; in a regime which has not been well characterized experimentally. In other words, the morphologies of the lateral heterogeneities characterized in the experiments are usually reported for micron size length scales. Nevertheless, some of the trends that are observed experimentally are consistent with the models (the mixing temperature generally decreases with increasing fraction of linactants). On the other hand, at such length scales, mechanisms that include longer range interactions (for example, electrostatics dipoles [77,78]) should not be neglected. With some exceptions (i.e. the AFM studies of Weise et al. [70] mentioned in Section 4), nanoscale domains have been indirectly inferred from scattering or FRET experiments, but

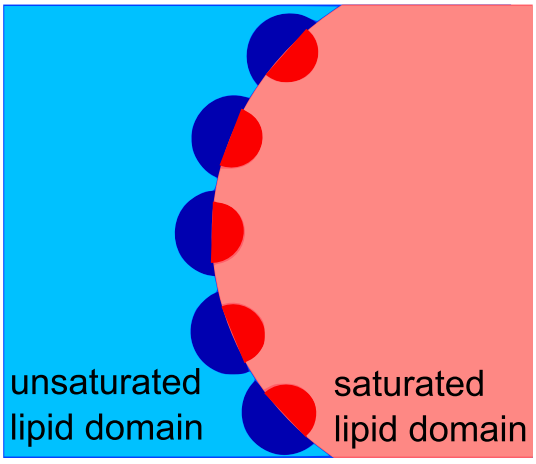

Fig. 3. Top-down view of a section of a membrane with a curved interface separating domains rich in saturated lipids (light red) and rich in unsaturated lipids (light blue). The spontaneous curvature originates from the fact that the area projected on the membrane of the unsaturated tail of a hybrid lipid (blue semi-circle) is larger than that of the saturated tail (red semi-circle) [76]. Only the hybrids at the interface are shown.

those report a scalar parameter that characterizes the average domain length scale and does not provide any information on the domain shape.

As an example, if one considers the hybrid based model proposed in Ref. [46], nanoscale lamellar or uniform domains (that have no preference to bend toward either the saturated or the unsaturated domains) are predicted to occur. Compact vs. stripe-like domains may be favored by hybrids that also carry an in-plane spontaneous curvature [79] as proposed in Ref. [76]. This energetic contribution originates from the fact that the area projected on the plane defined by the membrane of the unsaturated tail of a hybrid lipid is larger than that of the saturated tail (see Fig. 3). Thus, such effects could stabilize nanoscale micellar domains rich in saturated lipids. On the other hand, such nanoscale morphological differences may be hard to distinguish with the experimental tools currently favored in the study of lateral organization in membranes. For example, lamellar and hexagonal phases cannot be distinguished from scattering experiments that report structure factors as a function only of the magnitude of the wavevector.

As a final remark, we would like to point out that, for equilibrium modulations to arise in membranes from a linactant based mechanism alone, zero line tension must be achieved. On the other hand, real systems contain many other types of interactions that may compete with the line tension (i.e. membrane curvature and electrostatics as described by Seul and Andelman [78]) and stabilize finite size domains [80,81]. In such cases, linactants are still important since they can reduce the line tension to small enough (but still positive) values so that these other interactions can compete with it and hence produce equilibrium modulated phases.

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