PATTERN FORMATION IN NONEQUILIBRIUM LIPID MEMBRANES: FROM MEMBRANE UNDULATIONS TO LIPID RAFTS

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Received 14 December 2009
Revised 25 February 2010

Lipid membranes, particularly under nonequilibrium conditions, have recently been investigated ever more vigorously because of their relevance in the biological context. We survey our recent approaches to the theoretical study of lipid bilayers that are perturbed in different ways. Self-organization phenomena involving curvature and/or composition spatiotemporal organization are investigated in membrane systems subjected to externally induced chemical reactions, transversal mass transport and insertion of proteins. The outcomes of these studies are expected to be applicable to different curvature and lateral organization phenomena in synthetic lipid bilayers and also in plasmatic cell membranes.

Keywords: Lipid membrane; nonequilibrium; pattern formation.
1. Introduction

Amphiphilic molecules such as lipids spontaneously self-assemble and aggregate with their hydrophilic portions facing water to form bilayer membranes. These bilayers typically exhibit an in-plane fluid-like nature, and are highly flexible surfaces, so they can display a large variety of lateral lipid organizations and shape conformations.1–3

Although artificial (synthetic) lipid bilayers are widely used in a number of nanotechnological applications ranging from solar energy transduction and biosensors to drug development,4 the ultimate motivation for the study of lipid bilayers lies in their likeness to cell membranes. Biological membranes are multicomponent lipid bilayers formed by hundreds of different lipid species and inserted proteins. They surround the cell and its inner organelles, and control the traffic of different compounds across them. In addition to their role as a physical barrier, they are also actively involved in countless cellular functions. Most of these functions require the collective and concerted combination of the individual properties and functionalities of the different membrane constituents. In this context, a cell membrane can be thought of as an extremely precise self-organizing material where lipids and proteins organize at many space and time scales to provide a particular response to a specific external perturbation.

Such self-organizing phenomena may involve different properties of a membrane. For example, cellular membranes bend and curve in striking ways to perform various functions such as movement, division and vesicle trafficking.5,6 Membrane curvature is known to be controlled by the constituent lipids and proteins, but the curvature itself is known to provide mechanisms to spatially organize these membrane components. Moreover, during the past decade a great deal of evidence has revealed that the functionality of the cell membrane is related to its lateral heterogeneity: lipids in the membrane are hierarchically organized at different length scales ranging from the nanometric to the micrometric.7 To provide a more precise description, Simons and Ikonen8 proposed the concept of rafts to refer to lateral domains rich in saturated lipids and cholesterol, dispersed throughout a phase rich in unsaturated lipids. Such structures are estimated to occur in sizes ranging from tens to hundreds of nanometers,9,10 and are endowed with membrane protein sorting and signal transduction functions.11,12 Furthermore, they have recently been unveiled as dynamic and scale-dependent structures whose size and stability may dynamically change under specific signals or stimuli, contributing to the diversification of cellular responses.13–15

It is clear that the complexity of the cell membrane is responsible for the arsenal of possible pathways of the cellular signal–response machinery. Synthetic lipid bilayers of two or three components forming giant vesicles or planar membranes are much simpler systems that do not display most of the self-organizing capabilities observed in biological membranes. However, due to their simplicity, they are easier to manipulate in controlled experiments and are often used to study the behavior of
cell membrane scenarios of reduced complexity. Conformational behavior,\textsuperscript{1–3} shape fluctuations,\textsuperscript{16,17} fusion and fission,\textsuperscript{18,19} and phase segregation\textsuperscript{20,21} are some of the issues that have been extensively investigated using lipid bilayers. Among many other techniques, these studies have relied on the fabrication of synthetic giant vesicles\textsuperscript{22} and planar lipid membranes\textsuperscript{23} as well as on micropipet aspiration.\textsuperscript{16} Understanding the behavior of such simplified systems continues to yield new insights into their biological function.

Parallel to the experimental advances, theoretical modeling of multicomponent and flexible membranes has also come a long way.\textsuperscript{24,25} The use of models and computer simulations has become an important and necessary tool to interpret experimental observations, understand membrane behavior, and inspire further experiments. Coarse-grained modeling schemes are generally used since atomistic approaches are not able to cover the length and time scales of membrane collective behavior. Instead, a small number of averaged properties is chosen, and based on them, coarse-grained schemes are formulated.

The formulation of coarse-grained models to describe membrane behavior is a non-trivial agenda in the first place. As we will see in this review, a certain level of modeling can be accomplished by directly adapting well-known schemes based on an appropriate construction of the free energy of the system as a function of continuous variables that characterize its compositional and conformational configurations.\textsuperscript{26} From the energy description, the temporal evolution of the characterizing fields is obtained by applying the constitutive relations from linear nonequilibrium thermodynamics and mass balance equations. The resulting kinetic equations are analogous to the usual Cahn–Hilliard formulation,\textsuperscript{27} and can be supplemented with additional kinetic terms to account for different nonequilibrium and perturbation contributions. On the other hand, it is sometimes difficult to simply “guess” the appropriate phenomenology. In these cases, it may be possible to start from a simple microscopic model that can guide the phenomenology. One such modeling path relies on a discrete Ising-like model\textsuperscript{28} where the atomic description is greatly simplified. Each molecule is modeled as a “spin” residing on a lattice site, with prescribed particle–particle interaction parameters. Dynamical information can be extracted from the microscopic model directly by implementing a kinetic Monte Carlo (MC) algorithm to cause the system to evolve. However, this microscopic approach to the dynamics is limited by the fact that it is not suitable to describe processes at macroscopic length and time scales. Rather, the utility of the microscopic model lies in the fact that coarse-graining can then provide the basis for a mesoscopic phenomenology, from which dynamical information at the scales of interest can more easily be extracted.

Our interest in this review goes beyond an equilibrium description of the membrane behavior to stress modeling approaches oriented toward unveiling self-organizing dynamic responses to externally controlled perturbations. We start in Sec. 2 by reviewing a continuum free-energy-based model for a deformable reactive bilayer composed of two differently shaped lipids. As a result of the competition
between thermodynamic phase segregation and a nonequilibrium mixing reaction, the model leads to stationary finite-sized domains of composition and curvature.\textsuperscript{29} We also show how the mechanical influence of the reactive process on the membrane may lead to the formation of spatiotemporal structures.\textsuperscript{30} In Sec. 3 we address the problem of phase separation of a two-component bilayer system that demixes due to the inclusion of a third component that interacts preferentially with one of the original two components.\textsuperscript{31} To formulate this setting, we find it useful to be guided by a simple Ising-like kinetic model that we then explicitly coarse grain. This scenario is interesting in the context of raft phenomena in cell membranes, and for ternary model membranes where the presence of cholesterol has been observed to cause the lipids to separate even under conditions that do not lead to separation in the absence of cholesterol. In Sec. 4 we carry the three-component scenario further to study a nonequilibrium model for a ternary phase-separating mixture in a membrane system where the component that promotes phase separation (cholesterol) is continuously recycled. The nonequilibrium recycling process halts the segregation of components and leads to the formation of finite-sized domains.\textsuperscript{32} Despite its simplicity, the model captures the length and time scales of the raft phenomenology in cell membranes when realistic model parameters are chosen.\textsuperscript{32,33} Finally, Sec. 5 reviews the effect of inserted proteins on the dynamic organization of lipid domains. Whereas the insertion of static proteins in a membrane promotes and stabilizes the formation of nanoscale lipid domains,\textsuperscript{34} the inclusion of mobile (diffusing) proteins provides an additional active effect on the lipid lateral organization that may be used to diversify cell membrane functionality.

2. Nonequilibrium Curvature Patterns in Reactive Membranes

The simplest continuum formulation starts with a nonequilibrium kinetic model of a two-component deformable layer. The two intrinsically immiscible components are assumed to have different shapes. It is a nonequilibrium model because there is an externally activated chemical process that leads to the interconversion of the two components. For example, in Ref. 35 light is used to switch the morphology of fluid vesicles by photoaquation of ferricyanide taking place in the aqueous medium inside or outside giant vesicles. In Ref. 36 it is shown that the enzyme endophilin-I changes inverted-cone-shaped lysosphosphatidic acid into cone-shaped phosphatidic acid, thus affecting the formation of free vesicles pinched off from the plasma membrane (fission). Additional experiments\textsuperscript{37,38} also point to the importance of such chemical processes. We show how such a process acting on a membrane that is described through a curvature-composition coupling leads to rich pattern formation phenomenology.

Our membrane is composed of two species: lipid A, which is assumed to be cone-shaped, and lipid B, which has an inverted cone shape (see Fig. 1). In the simplest bilayer scenario, the outer layer of the membrane is composed of A and B lipids,
whereas the inner layer is composed of a single component without any curvature effect. Motion of the lipids between the inner and outer layers is proscribed.

The membrane is modeled as a two-dimensional surface with an order parameter \( \phi(r) \), \( r = (x, y) \), to describe the concentration difference \( (c_A(r) - c_B(r)) \), and a local extrinsic curvature \( H \). The rigidity of the membrane leads to an elastic energy contribution \( \frac{\kappa}{2} \int (H - H_{sp}(\phi))^2 \, d\mathbf{r} \), where \( \kappa \) is the bending rigidity modulus, and the spontaneous (equilibrium) curvature \( H_{sp}(\phi) \) reflects the shape asymmetry between the two lipid components. For simplicity, we adopt a linear dependence on \( \phi \), \( H_{sp} = \phi H_0 \), where \( H_0 \) is simply a parameter in this linear form. A schematic with \( H_0 > 0 \) is shown in Fig. 1. In the Monge parametrization\(^{39}\) a deformable surface is described by \( (x, y, h(x, y)) \), where \( h(x, y) \) is the displacement (height) field for the local separation from the flat conformation. This representation is valid for surfaces that are nearly flat with only gradual variations of \( h \), and allows the approximation \( H \approx \nabla^2 h \). If not nearly flat, further nonlinear contributions in \( h \) must be included.

As a function of these variables, the proposed energy functional reads
\[
\mathcal{F} = \int \left[ -\frac{\alpha}{2} \phi^2 + \frac{\beta}{4} \phi^4 + \frac{\gamma}{2} |\nabla \phi|^2 + \frac{\kappa}{2} (\nabla^2 h - \phi H_0)^2 \right] \, d\mathbf{r}.
\]

The first three terms correspond to the typical Ginzburg–Landau expansion responsible for phase separation \((\alpha, \beta, \gamma > 0)\), with an equilibrium concentration difference \( \phi_{eq} = \pm \sqrt{\alpha/\beta} \), and a typical interface width \( \zeta = \sqrt{\gamma/\alpha} \) connecting phases that are primarily curved “upward” to those that are primarily curved “downward.” For self-assembled free membranes, the surface tension contribution \( (\frac{\sigma}{2} |\nabla h|^2) \) in the free energy can be neglected, and we have not included it in Eq. (1).

The kinetics of \( \phi \) is obtained by invoking balance equations for the chemical potential according to a standard conserved scheme,\(^{40}\) augmented by the reaction

Fig. 1. The two membrane components A and B have opposite cone shapes. (a) A reaction interconverts the species, with \( k_+ \) and \( k_- \) being the interconversion rate coefficients. (b) Schematic representation of the composition-curvature coupling in an unstable membrane.
contribution,

\[ \frac{\partial \phi}{\partial t} = D \nabla^2 \left[ \frac{\partial F}{\partial \phi} \right] - \Gamma (\phi - \phi_0). \]  

(2)

Here \( D \) is the diffusion coefficient assumed to be equal for the two lipids, \( \Gamma = k_+ + k_- \), \( \phi_0 = (k_- - k_+)/\left(k_+ + k_-\right) \), \( k_+ \) and \( k_- \) are the forward and backward reaction rate constants, respectively, and \( \phi(x, y) = \phi_0 \) corresponds the homogeneous (no patterns) stationary solution of Eq. (2). Considering a permeable membrane (i.e. ignoring hydrodynamic interactions), we adopt the following relaxational equation for the evolution of the height field,

\[ \frac{\partial h}{\partial t} = -\Lambda \frac{\delta F}{\delta h} + \Gamma (\phi - \phi_0) \xi, \]  

(3)

where \( \Lambda \) is a mobility parameter proportional to the inverse of the typical relaxation time \( \tau_h \).

The last term in Eq. (3) is based on the following hypothesis. The reaction that interconverts A and B can be understood as an isomerization-like chemical transformation involving a strong modification of the shape of the membrane constituents. This process implicates the displacement of parts of these molecules that could have a mechanical effect on the local membrane shape. For example, active proteins are known to act as force centers when inserted in lipid bilayers,\(^{41}\) and other experimental studies\(^{42,43}\) also provide evidence that reactive processes may locally modify the membrane shape in red blood cells. If additionally, the process is strongly energetically activated by an external energy source, it might exert a local force on the membrane. A simplifying approximation is to assume that the forward and backward reactions exert opposite forces on the membrane. These forces are assumed to act locally for a negligible period of time (the time needed to complete the reaction is much shorter than any other time scale of the system), in the same direction as the preferred curvature of the reaction product component, that is, positive (outwards) for \( A \rightarrow B \), and negative (inwards) for \( B \rightarrow A \). This is modeled in a generic way\(^{44}\) by the last term in the membrane height kinetic equation, where \( \xi \) is a parameter accounting for the strength of the effect of the reaction on the shape of the membrane. Here, we take the parameter \( \xi \) to have the same sign as \( H_0 \).

The kinetic equations can be adimensionalized by measuring the energy in units of \( k_B T \) where \( T \) is the temperature and \( k_B \) is Boltzmann’s constant, time in units of \( \tau_h \), and length in units of \( \sqrt{D \tau_h} \). The adimensional equations are:

\[ \frac{\partial \phi}{\partial t} = (\kappa H_0^2 - \alpha) \nabla^2 \phi + 3\beta \phi^2 \nabla^2 \phi + 6\beta \phi |\nabla \phi|^2 - 2\gamma \nabla^4 \phi - \kappa H_0 \nabla^4 h - \Gamma (\phi - \phi_0), \]  

(4a)

\[ \frac{\partial h}{\partial t} = -\kappa \nabla^4 h + \kappa H_0 \nabla^2 \phi + \Gamma (\phi - \phi_0) \xi. \]  

(4b)
In this scaled form, one refers to “simulation length units” (s.l.u.) for distances and “simulation time units” (s.t.u.) for times. The diffusion coefficient is unity in units of (s.l.u.)²/(s.t.u.).

In the absence of a reaction (Γ = 0), Eqs. (4a) and (4b) describe the spinodal decomposition of two inherently immiscible components into distinct regions or phases of different chemical composition. Due to the composition/curvature coupling \( H_0 \), these regions form complementary patterns. As phase segregation progresses, membrane regions with positive (negative) \( \phi \) deform in such a way that the curvature becomes positive (negative), as shown in Fig. 1(b). In the absence of reaction, this coarsening process does not end until there is complete segregation into two large domains at thermal equilibrium.

A nonequilibrium reaction that converts one species into the other amounts to a large-scale mixing mechanism that counteracts the short-scale-induced phase separation. At the large-\( \Gamma \) extreme, the inherent immiscibility is overcome by the mixing effect of the \( A \rightleftharpoons B \) interconversion. In this regime, at thermal equilibrium the stationary uniform state corresponding to \( \phi = \phi_0 \) and arbitrary \( h = \vec{h} \) is the most stable state. There is an intermediate \( \Gamma \) regime where segregation does occur, but the segregated structures grow only until mixing and ordering effects compensate, resulting in stationary patterns of finite size. These types of nonequilibrium patterns are also found in other systems such as polymer blends\(^{45,46} \) as well as in monomolecular adsorption on metal surfaces\(^{47,48} \), and have to be distinguished from typical Turing patterns\(^{49} \) that arise from differences in the diffusivities of different chemical species. Even though they emerge from a mathematical instability of the same universality class, the physical source of patterns in the two cases is entirely different. In the model presented here, the domains result from the competition between a local thermodynamic affinity of equal species and a nonequilibrium reaction mixing effect.

One can find the parameter boundaries between different behaviors of the fields by testing the linear stability of small perturbations added to a known solution. Specifically, for example, one can test the stability of the uniform solution \( \phi = \phi_0 \) and arbitrary \( h = \vec{h} \) of the kinetic equations (4a) and (4b), by adding a small perturbation of a given wave vector \( q \) to this solution and following the linear stability analysis described in the appendix. To place subsequent analytic results in context, we anticipate the numerical results presented later and exhibit in Fig. 2 some different views of a typical phase diagram resulting from this analysis. Regime III is the stable regime where the mixing due to the interconversion is sufficiently strong to produce a homogeneous phase. Here “sufficiently strong \( \Gamma \)” depends on the values of the other parameters. Beyond this stable regime, there are two instability regimes. One, called region II in the diagrams, is the regime leading to time-independent nonequilibrium patterns of finite sizes. The curve bounding region II obtained from the linear stability analysis described in the appendix occurs at

\[
\Gamma_{II}^0 = \frac{(\alpha - 3\beta\phi_0^2)^2}{4\gamma(1 + H_0\xi)}.
\]
This patterned regime involves a finite range of unstable modes so that there are lower and upper limits to the associated wave vectors. This means that the patterns have finite sizes delimited by the inverses of these two wave vectors. On the other hand, in region I of the phase diagram, one observes dynamical (i.e. time-dependent) patterns. The boundaries between regions I and III obtained from the analysis occur at

\[ \Gamma_0^I = \frac{(\alpha - 3\beta\phi_0^2 - \kappa H_0^2)^2}{4(\gamma + \kappa)}, \quad \xi_0 = \frac{\kappa q_0^2}{\Gamma H_0}(H_0^2 + q_0^2), \]  

with dynamical patterns observed when \( \Gamma < \Gamma_0^I \) and \( \xi > \xi_0 \). We note that regime I disappears entirely if \( \xi = 0 \), that is, if one disregards the mechanical force on the membrane caused by the A \( \rightarrow \) B interconversion. Also, the only effect of curvature is to reduce the growth rate of the patterns; it has no effect on the final patterns. In other words, the effect of curvature is exclusively related to the kinetics of the phase separation process but not to the final stationary (albeit time dependent) state of the membrane.

Numerical integration of Eqs. (4a) and (4b) has been performed in two dimensions using an explicit Euler scheme in a square lattice with periodic boundary conditions. Small random perturbations around \( \phi = \phi_0 \) and \( h = 0 \) are implemented as initial conditions. The coordinate step \( \Delta x \) was chosen equal to 1, and the time step was usually \( \Delta t = 10^{-4} \) to assure good numerical accuracy (length and time in dimensionless simulation units). The representative numerical results exhibited below correspond to highly immiscible components (a situation called a “deep quench,” achieved in our case by choosing \( \alpha = 1 \) and \( \beta = 1 \), leading to an equilibrium value of \( \phi_{eq} = \pm 1 \)), and an interface thickness of the order of the space discretization (\( \zeta = 1 \), which leads to \( \gamma = 1 \)). The bending rigidity modulus is taken...
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Fig. 3. Height field (vertical axis) for an unstable membrane in region II of the phase diagram for \( \alpha = \beta = \gamma = 1 \), \( H_0 = 0.2 \), \( \Gamma = 0.14 \), \( \kappa = 10 \), and \( \xi = 0 \) at \( t = 2000 \) obtained from numerical simulations based on Eqs. (4a) and (4b). Some exaggeration along the vertical direction has been applied to display the membrane height. The \( \phi \)-fields (not shown) mirror the \( h \)-fields. Panel (a): \( \Gamma = 0.05 \), \( \phi_0 = 0 \). Panel (b): \( \Gamma = 0.2 \), \( \phi_0 = 0 \). Panel (c): \( \Gamma = 0.2 \), \( \phi_0 = 0.1 \) (off-critical quench).

equal to 10 (in units of \( k_B T \)), and we consider two constituent lipids of very different shapes by setting \( H_0 = 0.2 \). All our numerical results are consistent with the predictions of the linear stability analysis and the amplitude equations mentioned in the appendix.

In Fig. 3 we show simulation results for three different situations when \( \xi = 0 \). These results are also representative of the behavior in phase regime II when \( \xi \neq 0 \). Panel (a) corresponds to small \( \Gamma = 0.05 \) and a critical quench, defined as the case of equal amounts of the two components (\( \phi_0 = 0 \)). We see the development of a laberynthine pattern (since \( \phi_0 \) is now no longer the equilibrium state) that is still evolving at \( t = 2000 \). The coarsening process progressively slows down later on. The \( \phi \)-field profiles of these domains (not shown) reveal regions where \( \phi_{eq} \) is +1 or −1 connected by abrupt boundaries that indicate the short spatial range over which the equilibrium order parameter changes from one value to the other. This is a signature of the fact that the phase separation process is dominant for this value of \( \Gamma \). The second and third panels correspond to large \( \Gamma = 0.2 \) (the marginal condition for the selected parameters is \( \Gamma_0^\text{II} = 0.25 \)) showing laberynthine (critical quench in (b)) and quasi–hexatic droplet-like (off-critical quench, \( \phi_0 = 0.1 \), in (c)) patterns. A spatial Fourier transform of this stationary pattern shows a clear hexagonal structure. These domains are already stationary at times longer than \( t = 2000 \), and can indeed be considered as nonequilibrium stable phases of the system, involving both composition and curvature modulations. Their \( \phi \)- and \( h \)-field profiles have a smooth harmonic shape due to the strength of the reactive process. In both cases, since we are close to the phase transition boundary, small local deviations from the stationary values are obtained. We especially monitor the variations of the height field and find that \( \langle |\nabla h| \rangle < 0.05 \) is satisfied, so that the model has a real physical correspondence.

In order to assess the kinetic ordering process more quantitatively, we have also monitored the domain size \( L(t) \), which can be computed from the composition correlation functions \( \langle \phi(r', t)\phi(r' + r, t) \rangle \) or from the height correlation functions...
\[ \langle h(r', t) h(r' + r, t) \rangle. \] Similar studies have been carried out in the absence of a reaction, where it is found that in general curvature slows down the coarsening segregation process.\textsuperscript{51,52} In the presence of reaction, the final size \( L_f \) of the nonequilibrium stationary domains is finite and determined by the reaction parameter \( \Gamma \) (and is independent of the curvature). Our results\textsuperscript{29,30} reproduce the limiting behaviors discussed in the literature, \( L_f \sim \Gamma^{-1/3} \) for large \( \Gamma \) and \( L_f \sim \Gamma^{-1/4} \) for small \( \Gamma \).

Representative numerical results for \( \xi > 0 \) again show good agreement with the predictions of the linear stability and amplitude equation analyses. Now we set the bending rigidity modulus \( \kappa \) equal to 0\textsuperscript{5} (in units of \( k_B T \)), which is much lower than the value \( \kappa = 10 \) chosen for our earlier simulations (this choice is discussed later).

We focus on numerical results for regime I in the phase diagram. The nature of the spatiotemporal patterns is determined by the value of \( \phi_0 \) and by the associated relative magnitudes of the amplitudes of waves traveling in different directions. Thus, for a critical quench (\( \phi_0 = 0 \)), the system typically displays some transients with domains that travel in different directions until they organize into a coherent train of traveling stripes. The post-transient spatiotemporal behavior is described in Fig. 4. We plot the height fields and one-dimensional cross-sections showing its

![Fig. 4. Height field (vertical axis) for an unstable membrane in region I of the phase diagram for \( \alpha = \beta = \gamma = 1, H_0 = 0.2, \Gamma = 0.14, \) and \( \kappa = 0.5 \). Panel (a): \( \phi_0 = 0, \xi = 5 \). Upper: \( t = 15000 \), where the structures are robust. The stripes travel from left to right at constant velocity. Lower: \( h \)-profile (frontal axis) vs time (along sheet axis) from \( t = 15,000 \) to \( t = 15,500 \). Panel (b): \( \phi_0 = -0.14, \xi = 4 \). Upper and lower panels have the same specifications as in (a), but \( t = 22,500 \) (upper) and \( t \) goes from \( t = 22,500 \) to 23,000 (lower). Bud-like curvature domains oscillate. Panel (c): \( \phi_0 = -0.14, \xi = 3 \). Upper: the droplet-like structures move from the upper-left corner to the lower-right one at constant velocity while they oscillate. The upper panel snapshot is at \( t = 18,000 \). The temporal profile in the lower panel goes from \( t = 18,000 \) to 18,500. Some exaggeration along the vertical directions has been applied.](image-url)
temporal evolution. Numerical profiles at a given post-transient stage reveal the mechanism that leads to the motion of the generated spatial structures. In Fig. 5, we observe that \( \phi \)- and \( h \)-profiles are slightly displaced, the field \( \phi \) being ahead in the direction of propagation.

Off-critical quenches \((\phi_0 = -0.14)\) display different spatial and temporal behaviors. As before, we first observe transients, but now involving concentration droplets and bud-like surface deformations. The fields settle into an oscillating pattern of buds rich in the minority species and of a geometry that depends on \( \xi \) (see panels (b) and (c) of Fig. 4). For \( \xi = 5 \), a spatial Fourier transform of the pattern indicates clear square symmetry. For \( \xi = 3 \), the domains are rather hexagonal and they oscillate as well as move in one direction.

The ideas presented in this section are of course inspired by phenomena observed in real cellular systems. The limit on the predictive power of these models is mainly determined by the identification of the correct time, length and energy scales accessible to the experiments. We can return dimensions to the kinetic equations (4a) and (4b) by choosing typical values for the model parameters. For lipids in a liquid-disordered phase membrane, the diffusion coefficient is known to be in the range \( 10^{-8} - 10^{-7} \) cm\(^2\)/s.\(^{53}\) The height mobility parameter can be estimated considering solvent hydrodynamic effects\(^3\) as \( \Lambda = (4\mu q)^{-1} \), where \( q \) is taken as the typical unstable mode \((q \approx 0.5)\) found for our set of parameters (see Fig. 4 in Ref. 29). The kinematic viscosity parameter \( \mu \) is 1 cP for water at 20°C. Given these, s.l.u. = 0.2, 2 \( \mu \)m (for \( D = 10^{-7}, 10^{-8} \) cm\(^2\)/s, respectively) and s.t.u. = 4 \( \times \) 10\(^{-3}, 4 \) s (for \( D = 10^{-7}, 10^{-8} \) cm\(^2\)/s, respectively). The choice of the bending rigidity modulus \( \kappa \) requires special notice. The elastic properties, and thus the bending rigidity, of lipid bilayers are strongly determined by the size, shape and molecular
elasticity of their constituents. Membranes composed of phospholipids (which have two hydrophobic chains) are characterized by a bending rigidity of the order of tens of $k_B T$ \(^{50}\), and the value we chose for the simulations shown in Fig. 3 is in this regime. Cell membranes containing large molar fractions of cholesterol display even higher rigidities.\(^{54}\) For such large values of $\kappa$ the typical sizes of the predicted dynamical patterns emerging from a wave instability are of the order of millimeters, much larger than the size of experimentally accessible giant vesicles. Moreover, for $\kappa \gtrsim 10$, wave-like unstable modes are observed to be suppressed at long times by the other existing Turing-like unstable modes, so that only stationary structures are asymptotically generated in this parameter region. However, single- and/or short-chain lipid surfactants are known to form much more flexible bilayers, with bending rigidities of the order of $k_B T$ or even less.\(^{55}\) Furthermore, recent theoretical models\(^{56}\) show how bilayers composed of surfactants of rather distinct shapes (as in our case) may exhibit smaller rigidities than one-component membranes. For the simulations shown in Fig. 4, we chose $\kappa = 0.5$ in units of $k_B T$, which leads to pattern sizes in the range of 2–20 $\mu$m. This size may be accessible in experiments on giant vesicles and planar bilayers. For this purpose, we suggest azobenzene compounds as suitable candidates to test our predictions. The shape of these amphiphilic compounds can be strongly modified by means of photoisomerization reactions and have been shown to develop complex spatiotemporal behaviors in Langmuir monolayers.\(^{57}\) In the context of bilayers, recent experiments on azobenzene-containing vesicles revealed photoinduced changes in the membrane shape at the micrometer scale.\(^{58}\) Thus, experimental work on these synthetic vesicles could be specifically designed to confirm the results of our model. For example, the selection of the wavelength and intensity of the applied light may determine both the fraction of the two isomers ($\phi_0$) and the strength of the reactive process ($\Gamma$), which can in turn modify/control the membrane spatiotemporal behavior. Additionally, the analysis of shape fluctuations of these irradiated vesicles could be performed by means of micropipet experiments and compared to the fluctuation spectrum derived from our model.\(^{29}\)

Our quantitative assignment of $\xi$ has been made with the energetic feasibility of a number of external sources in mind. To see this, note that the characteristic time for a single reactive event is of order $1/\Gamma$. During this time interval, the change in the height of the membrane due to the contribution of the reactive term is of order $\xi$ (see Eq. (3); $\phi - \phi_0$ is of $O(1)$). Moreover, the local energetic “cost” of such a height increase due to the reactive term is of order $2\kappa \xi^2/(\Delta x)^2$ (see Eq. (1)). In most of our simulations, we have used $\xi = 5$ (in s.l.u.), which, for $\kappa = 0.5$, corresponds to an energy cost of order 25 (in units of $k_B T$). If the energy source is light, as suggested for the above mentioned photosensitive azobenzene vesicle, the power required to produce $25k_B T$ within a time interval of order $\Gamma^{-1}$ for $\Gamma = 0.14$ as used in our simulations is approximately $0.3 \times 10^{-6}$ mW/cm\(^2\), which is much lower than the power produced by typical commercial light sources used, for example, in photosensitive Langmuir monolayer experiments.\(^{57}\) In the biological context, energy
is provided in units of the energy involved in the conversion of ATP to ADP. The dephosphorylation of an ATP releases about 50 kJ/mol or $10^{-19}$ J/ATP, which at room temperature is of order $20k_BT$. While some of the energy provided by these external sources (ATP, light, etc.) would be used for processes other than the purely elastic motion of the membrane, including dissipation into the thermal surroundings and the movement of other masses, this order of magnitude estimate shows that the proposed mechanism is feasible in both synthetic model and biological living membranes. In the latter context, regulation of the lipid membrane conformation is critical for many cellular functions, and in most cases, processes affecting the shape of the individual membrane lipids play a central role. As an example, a reaction that interconverts two differently shaped membrane phospholipids enables the fission of vesicles in nervous cells, crucial for the synaptic function.36,37

3. Phase Stability of Three-Component Lipid Membranes: Importance of Cholesterol

In the previous section, we have shown that multi-component lipid bilayers may display a rich phenomenology in terms of dynamic patterning involving curvature and composition modulations. However, this phenomenology as discussed so far does not yet incorporate the important observation of raft formation. The raft hypothesis is based on the idea that lipids in plasma membranes are distributed inhomogeneously, forming small domains rich in cholesterol and saturated lipids. These domains are known as rafts and are embedded in a medium preferentially containing unsaturated lipids.8 Such structures have been implicated in many biological processes, and their investigation has attracted enormous attention in recent years.9,11–15

Although some aspects of the raft phenomenology remain currently controversial, it is well accepted that the preferential packing of cholesterol and saturated lipids to form a liquid ordered (lo) phase, segregated from a liquid disordered (ld) medium rich in unsaturated lipids, underlies raft formation.21,59,60 Consequently, a more realistic description of patterning in cell membranes must account for the emergence of lo/ld phase separation due to the presence of cholesterol.

We have recently contributed to this issue by addressing the role that cholesterol plays in phase separation of lipid mixtures.31 To that end, we studied two-dimensional (2D) ternary mixtures where two of the components (A and B) that are by themselves miscible above a critical temperature may nevertheless undergo phase separation above that temperature due to the inclusion of a third molecular species (C) that displays preferential affinity for one of the other two components (say A). In the context of model membranes, A, B and C stand for saturated lipids, unsaturated lipids, and cholesterol, respectively.

The formulation of a coarse-grained phenomenology for the ternary scenario is not a priori obvious, and so we start with a simple Ising-like model that we subsequently coarse-grain. Our discrete (on-lattice) system consists of a 2D triangular...
Fig. 6. Schematic representation of the coarse-graining procedure presented in Sec. 3. At the left the two interconnected lattices used to describe the ternary mixtures are plotted: A/B (saturated/unsaturated lipids) fully occupy the triangular lattice (black and grey circles, respectively); C (cholesterol) resides on the complementary hexagonal lattice (black crosses). In the continuous approximation (right) any single point, e.g. \( r \), contains information about “many” discrete points (see text).

One of the reasons that justifies these lattice choices is related to the area per molecule occupied by each species: common membrane lipids occupy about 0.6–0.8 nm\(^2\)/molec, whereas cholesterol molecules occupy about 0.35–0.4 nm\(^2\)/molec.\(^{65} \) The combination of the two proposed lattices follows the observed 2/1 ratio for the lipid/cholesterol area per molecule. The species A, B, and C are modeled as spins. We consider a set of \( N^2 \) spins \( \{S_i\} \) (representing A and B) which are located on the sites \( \{i\} \) of the triangular lattice, and \( 2N^2 \) spins \( \{\hat{S}_\alpha\} \) (representing C) on the sites \( \{\alpha\} \) of the hexagonal lattice. We next introduce the following two-state Ising Hamiltonian:

\[
\frac{\mathcal{H}}{k_B T} = -J_0 \sum_{\langle ij \rangle} S_i S_j - G_0 \sum_{\langle \alpha \rangle} S_i \hat{S}_\alpha, \tag{7}
\]

where only nearest-neighbor interactions are considered within the A/B lattice (denoted by \( \langle ij \rangle \)) and between the two lattices (denoted by \( \langle \alpha \rangle \)). \( J_0 > 0 \) corresponds to the strength of the exchange interaction between A and B lipids, and \( G_0 \) to the interaction with the lattice containing the cholesterol C. The spins \( S_i \) take on the values +1 or -1 denoting the presence of an A or B particle at site \( i \), respectively. The spin \( \hat{S}_\alpha \) is equal to 1 if a C particle occupies the site \( \alpha \) in the hexagonal lattice, and 0 otherwise. Note that \( G_0 > 0 \) corresponds to a preferential affinity between A and C components (saturated lipids and cholesterol, respectively).

We note that the Hamiltonian in Eq. (7) does not imply any particular spin dynamics toward equilibrium. And yet, it is an important starting point for studying these dynamics. On the one hand, we can apply a MC algorithm based on this
Hamiltonian and observe the evolution of the system at the microscopic level. While we do this below, we note the tremendous CPU cost to achieve the spatiotemporal scales associated with phase separation and raft formation. On the other hand, we can use this Hamiltonian as a starting point for a coarse-graining approach. We do this below as well, and display a comparison of the two approaches. We note that the coarse-grained phenomenology here is sufficiently complex to require this sort of starting point for guidance.

We start with the microscopic approach. In order to reproduce the phase separation dynamics, we implemented both kinetic and nonkinetic MC algorithms adapted to deal with the three component problem (details can be found in our earlier work\textsuperscript{31}). Our simulations start from a disordered initial configuration, where $N_A$ and $N_B$ particles are randomly placed in the triangular lattice ($N_A + N_B = N^2$) and $N_C$ particles in the hexagonal lattice, and we define $\varphi \equiv N_A/N^2$ and $\psi \equiv N_C/2N^2$ as the molar fractions of A and C with respect to their own lattices. For the pure A/B binary mixture at the so-called critical concentration ($\varphi = 0.5$), Onsager theory provides the critical value for the parameter $J_0 = 0.2747$, for which phase separation is achieved.\textsuperscript{66} However, if the mixture is at an off-critical concentration (for which the transition point parameter is larger) and/or the third component is added, no exact critical values are known for $J_0$ and $G_0$.

Figure 7 shows an illustrative example of the main point of this section, namely, how cholesterol drives phase separation of an A/B lipid mixture that is by itself miscible. The parameters here are $\varphi = 0.5$, $J_0 = 0.2$, $\psi = 0.5$, and $G_0 = 0.5$ (note that $J_0 < J_c$ for the A/B lipid mixture). General trends of the values of $G_0$ necessary to promote phase separation can be obtained qualitatively by performing MC simulations with other values of $J_0$ and $\psi$. Thus, decreasing the value of $J_0$ leads to the requirement of a higher $G_0$ to demix the system. Intuitively, it is clear that for smaller $J_0$, it will be more difficult for the cholesterol to separate A from B lipids since the repulsion of these components is weakened. The concentration of cholesterol is obviously another important variable. One would expect that when $\psi$ is small, that is, when there is a small amount of C present, $G_0$ must be large to cause demixing. Conversely, it is clear that if there are more C’s, a weaker pull is sufficient to separate the A’s from the B’s. However, this behavior turns out to be non-monotonic.\textsuperscript{31} When the amount of C is large, one arrives at a situation where too many C’s are present and the A’s are “satisfied” wherever they are because there are always C’s nearby. The separating effect of the C species thus decreases and a larger $G_0$ is then required to achieve phase separation.\textsuperscript{31}

Kinetic MC simulations describe the dynamics toward equilibrium, but provide only qualitative trends because of the tremendous CPU cost. As noted earlier, a quantitative characterization of the critical values for phase separation can be obtained using a continuum approach. Moreover, as also noted earlier, the continuous approach allows us to extend the spatiotemporal range of our simulations with respect to that of the MC simulations. We very briefly describe the steps to obtain a set of continuum equations in the spirit of our starting point in Sec. 2, but
Fig. 7. Panels corresponding to the lipid (A/B) configurations in numerical simulations of the discrete (left) and continuous (right) models. The color code for saturated/unsaturated lipids is black/white. In both approaches, the cholesterol distribution (not shown) follows the pattern displayed by the saturated lipids. From top to bottom, the panels display snapshots of typical temporal evolutions of the phase separation process. Simulation parameters for the discrete model are \( \phi = 0.5, \psi = 0.5, J_0 = 0.2, \) and \( G_0 = 0.5. \) The parameters of the continuous model are \( \phi_0 = 0, c_0 = 0.5, \) \( G = 1.5, \) and \( J = 0.4. \) In both cases, if cholesterol were absent the phase separation would not occur. We point out that the spatiotemporal scales of these simulations are very different (see text).

now consistent with our microscopic starting point — we refer the reader to our earlier work\(^{31}\) for details of the coarse-graining procedure. The approach is based on Landau theory\(^{26}\) to construct a free energy functional from which we can in turn obtain kinetic equations for the fields of interest. To that end, the system is partitioned into cells labeled by an index \( k. \) Each cell is sufficiently large to contain many sites of both intercalated lattices, but sufficiently small so that the system contains many cells. Average field variables \( \phi(r_k) \) and \( c(r_k) \) are introduced for each cell by defining

\[
\phi(r_k) = \sum_{i \in k} S_i = \frac{N^A_k - N^B_k}{N^A_B}, \quad c(r_k) = \sum_{\alpha \in k} \hat{S}_\alpha = \frac{N^C_k}{N^C_\emptyset}.
\]

Here, each sum is over the spin variables inside cell \( k, \) and \( r_k \) denotes the center of cell \( k. \) The cell contains \( N^A_k \) and \( N^B_k \) spins of species A and B respectively, and \( N^A_B = N^A_k + N^B_k, \) the total number of sites of the triangular lattice inside the cell.
Similarly, \( N^k_C \) denotes the number of C’s in the cell, and \( N^k_C \) is the total number of hexagonal lattice sites in the cell, those occupied by C’s as well as the empty sites. The field variables are then the averaged cell order parameters. They can vary from cell to cell, \( \phi \) denoting the local differential composition of A and B lipids (\( \phi > 0 \) indicating predominance of A), and \( c \) corresponding to the local fraction of cholesterol with respect to the maximum allowed concentration of this component in the mixture. The expression of the system’s energy is written as a function of the two compositional fields, and is supplemented with the entropic contributions. This expression is then expanded to fourth order about \( \phi = 0 \) and a mean value \( c = c_0 \). This leads to an expression for the local free energy \( F[\phi(\mathbf{r}_k), c(\mathbf{r}_k)] \). The total free energy of the system is the sum of the local free energy functional over all cells. The sum is routinely replaced by an integral under the assumption that the cell-to-cell variations are small so that \( \phi(\mathbf{r}) \) and \( c(\mathbf{r}) \) are smooth functions,

\[
F = \int d\mathbf{r} \left[ F(\phi, c) + \frac{\gamma}{2} |\nabla \phi| \right]^2. \tag{9}
\]

As in Sec. 2, Eq. (1), a contribution appears in the integrand that represents the effect of the surface tension and the associated energy cost of the interface of A-rich and B-rich phases. Finally, the kinetic equations can be obtained from this functional by invoking balance equations in terms of chemical potential (conserved scheme). The procedure leads to a pair of coupled kinetic equation

\[
\frac{\partial \phi}{\partial t} = D \nabla^2 \left[ \left( 1 - 2J \right) \phi + \frac{1}{3} \phi^3 - Gc - \gamma \nabla^2 \phi \right],
\]
\[
\frac{\partial c}{\partial t} = D \nabla^2 \left[ -G\phi + \eta_1 + 2\eta_2(c - c_0) + 3\eta_3(c - c_0)^2 + 4\eta_4(c - c_0)^3 \right], \tag{10}
\]

where \( D \) is the common diffusion coefficient of the bilayer components. Here \( J = J_0 z, G = G_0 z \) (\( z \) being the coordination number of the lattice), and the coefficients \( \eta_j \) are functions of \( c_0 \).

\[
\eta_1 = -2 \ln(1 - c_0) + 2 \ln c_0, \quad \eta_2 = \frac{1}{(1 - c_0)} + \frac{1}{c_0},
\]
\[
\eta_3 = \frac{1}{3(1 - c_0)^2} - \frac{1}{3c_0^2}, \quad \eta_4 = \frac{1}{6(1 - c_0)^3} + \frac{1}{6c_0^3}. \tag{11}
\]

The line tension parameter \( \gamma \) can be estimated from Cahn–Hilliard theory as \( \gamma \approx J_0 \zeta^2 \), where \( \zeta \) is the characteristic interfacial width described in Sec. 2.

Numerical simulations of the proposed kinetic equations are performed in a two-dimensional square lattice of \( N \times N \) sites with periodic boundary conditions

\[\text{\footnotesize*}\text{The expressions for the coefficients } \eta_i \text{ provided in this review are different from those in Eq. (12) of our earlier work. In that work, the inclusion of the entropic terms in Eq. (10) does not take into account that cholesterol occupies half of the area of a lipid molecule. With this in mind, a factor of 2 in the cholesterol entropic terms should be included. This results in a factor of 2 in the expressions for the coefficients } \eta_i \text{ in this review. Moreover, in our earlier work, there is a typo in the expression of } \eta_4 \text{ the sign } - \text{ must be replaced by a } + \text{ as done herein.}\]
using the discretization described in Sec. 2. The right panels of Fig. 7 show an example of simulation results for the continuum model in a lattice of size $N = 128$. In agreement with the MC simulations, phase separation of the lipid mixture is driven by the presence of cholesterol. As a matter of fact, all the trends observed in the MC simulations, including the nonmonotonic behavior of phase separation as a function of the cholesterol concentration, are also obtained in the numerical simulations of the continuous model.

A comparison of the left panels of Fig. 7 (obtained from a kinetic MC simulation of the microscopic spin-like version of the model) and the right panels (obtained from the coarse-grained kinetic equations) requires a careful examination of the spatial and temporal scaling. We recall that energy, length and time variables in the coarse-grained kinetic model are provided in units of $k_B T$, simulation length units (s.l.u.), and simulation time units (s.t.u.), respectively. The discretization mesh size $\Delta x = 1$ in s.l.u. is taken to correspond to the interfacial width $\zeta \approx 5 \text{nm}$ in physical units. The simulation time units s.t.u. can be extracted from the value of the diffusion coefficient that is chosen in our simulations to be $D = 1$ in units of $(\text{s.l.u.})^2/\text{s.t.u.}$. Since for a generic lipid or cholesterol molecule in a bilayer the diffusivity is of the order of $\mu m^2/\text{s}$, this implies that a s.t.u. is of order $10^{-5}$ s.

The right panels of Fig. 7 show an example of results at two different times along the simulation for the continuum model in a lattice of size $N = 128$ (that is, in a $0.64 \times 0.64 \mu m^2$ membrane) run up to time $0.5$ s (lower panel). According to these scales, mean-field simulations indeed cover the typical length and time scales in raft phenomena and in general in macroscopic phase separation processes with modest computational resources. As a comparison, the MC simulations displayed in the left panels of Fig. 7 correspond to a $80 \times 80 \mu m^2$ membrane (considering that a lipid molecule has a linear cross-sectional size of $0.8 \text{nm}$), and the number of iterations needed to achieve the same evolution times as in the continuum simulations requires CPU usages thousands of times longer than for the mean-field approach.

In addition to the previous advantages, the continuum model provides tractable kinetic equations that provide quantitative analytical predictions via the linear stability analysis described in the appendix. We find that the homogeneous state first ceases to be stable when

$$G^2 = G_c^2 = 2\eta_2[(1 - 2J) + \phi_0^2],$$

and there is phase separation when $G > G_c$. This is the central result of the linearization analysis. Several points are noteworthy about this result. First, if there is no C lattice interaction ($G = 0$) then the condition for phase separation is the familiar mean-field condition on $J$ for A and B obtained from the model of Sec. 2. Written in terms of the parameters used in this section, if $G = 0$ phase separation occurs when $J > J_{cAB}^A$, where

$$J_{cAB}^A = \frac{1}{2}(1 + \phi_0^2).$$
Second, note that alternatively we can rewrite Eq. (12) as
\[ \Delta J = J^{ABC}_c - J^{AB}_c = -c_0(1 - c_0)G^2, \] (14)
where \( J^{ABC}_c \) is the critical coupling parameter between A and B components when a third component C is present. This form is obtained by making the substitution (13) in (12) and then explicitly using the form given in Eq. (11) for \( \eta_2 \). Since \( 0 \leq c_0 \leq 1 \), it follows that \( \Delta J \leq 0 \). That is, the presence of a third component decreases the critical coupling for phase separation between A and B. Therefore, this analysis confirms the results of the MC simulations that show that the presence of C can cause phase separation of the mixture even when \( J \) is too small to cause it in its absence. Accordingly, increasing \( J \) for a given set of concentrations lowers \( G_c \) since it is now easier to separate the A and B components. Also, we find that the minimal interaction \( J \) required for phase separation occurs when \( \phi_0 = 0 \) and \( c_0 = 1/2 \).

Other points concerning the linearization analysis are also noteworthy. On the one hand, increasing \( \phi_0 \) for a given \( c_0 \) and a given \( J \) requires a stronger \( G \) to cause phase separation. On the other hand, the linearization analysis confirms the non-monotonic dependence of \( G_c \) on the average amount of C present. The important bottom line is that the C’s can indeed effectively induce the phase separation of A and B below the critical value of their direct interaction. It does so most effectively at an intermediate concentration of C’s as compared to a very low or very high concentration.

In this section, we studied the equilibrium behavior of a ternary mixture of saturated and unsaturated lipids and cholesterol. Many experiments on vesicles composed of these types of mixtures have exhibited the lo/ld coexistence captured by our model (see, for example, the work of S. L. Keller and S. L. Veatch on sphingomyelin/DOPC/cholesterol giant vesicles\(^{21}\)). Although we recognize that our approach omits a number of complications that exist in real (synthetic and biological) systems, it provides a simple and manageable tool to describe the dynamics toward equilibrium of phase-separating ternary lipid mixtures, a tool that may be applicable to raft formation in cell membranes. However, biological membranes are subjected to nonequilibrium conditions (examples of which were considered in earlier sections of this review for binary lipid mixtures). In the next two sections, we augment our ternary mixture model with such nonequilibrium model components.


The study of rafts is rather complicated because they develop at very small scales, in the range of tens to a few hundreds of nanometers.\(^{67}\) Most of our current knowledge concerning lipid raft organization in living cells originally came from fluorescence
and electron microscopy. More recently, advances in microscopy and spectroscopy have shown their potential to reach scales below 100–200nm (see the work of D. Lingwood and K. Simons for an exhaustive review on this issue). Despite these advances, the raft field is now at a technical impasse because the experimental methods to study biomembranes of characteristic raft length and time scales are still being developed. In the meantime, theoretical approaches have become a powerful tool to propose plausible mechanisms to explain the raft phenomenology. In this context, the ability of cholesterol to promote ordered liquid phases provides the thermodynamic driving force to support the raft hypothesis. In phase separating synthetic bilayers, however, segregating domains continue to grow until they reach a length scale of the order of the system size at equilibrium. Thus, thermodynamic arguments alone cannot explain the far smaller nanometric size of rafts. Moreover, structural and dynamic properties of raft organization are believed to be dynamically regulated by the cell state and the specific signals or stimulus that may modify this state. These considerations point to the need to deal not only with the thermodynamics of the lipid mixture but also with the nonequilibrium contributions affecting the cell membrane such as, for example, transport of its components across the bilayer. Among many others, recent experiments have shown that raft organization is extremely sensitive to cholesterol homeostasis. Mammalian cells synthesize and transport cholesterol to the plasma membrane, and at the same time, cholesterol is continuously released to external circulation.

The continuum (coarse-grained) scheme developed in the previous section provides a kinetic description of a membrane composed of saturated and unsaturated lipids and cholesterol when relaxing to equilibrium. That scheme also points to ways of including nonequilibrium scenarios resulting in stationary actively-maintained states of the membrane. To study the particular case of cholesterol recycling, we start with the kinetic equations (10) and choose the particular mean value $c_0 = 0.5$.

We next supplement the kinetic equation for the $c$ field with a new kinetic exchange term $\rho_{\text{ex}}(c)$.

\[
\frac{\partial \phi}{\partial t} = D \nabla^2 \left[ (1 - 2J)\phi + \frac{1}{3} \phi^3 - Gc - \gamma \nabla^2 \phi \right],
\]

\[
\frac{\partial c}{\partial t} = D \nabla^2 \left[ - G\phi + \frac{8}{3} \left( c - \frac{1}{2} \right) + \frac{32}{3} \left( c - \frac{1}{2} \right)^3 \right].
\] (15)

We consider a homogeneous intake flux of cholesterol from the cytoplasmatic medium with frequency $\rho_{\text{in}}$, and a release of cholesterol to external circulation at a rate proportional to its local concentration, $\rho_{\text{out}}c$. This results in $\rho_{\text{ex}}(c) = -\rho(c - \tau)$, where $\tau = \frac{\rho_{\text{in}}}{\rho_{\text{out}}}$ is the averaged cholesterol fraction in the membrane, and $\rho = \rho_{\text{out}}$ is assumed to be the characteristic recycling frequency. The conservation of the total amount of cholesterol $\tau$, as assumed here, is the simplest way to introduce a unique time scale $\rho^{-1}$ for the recycling process. With this restriction, we are thus able to characterize the recycling process by this single parameter.
Finally, in order to provide a more dynamic description, we add Gaussian white noise terms to Eqs. (15) to represent thermal fluctuations.

Our cell membrane is an inherently miscible binary lipid mixture that undergoes phase separation due to the inclusion of cholesterol, that is, a membrane with $J < J_{c}^{AB}$ of Eq. (13) and $G > G_{c,eq}$ of Eq. (12). We set the free energy parameters according to experiments with different lipid systems. A reasonable estimation for our model (with generic saturated and unsaturated lipids) leads to $J \in (0.1, 0.4)$ and $G \in (1.5, 3)$, in $k_{B}T$ energy units. The choices $J = 0.25$ and $G = 2.5$ are used in this section. For the average membrane composition, we have chosen a 2 : 3 molar ratio of saturated/unsaturated lipids ($\phi = -0.2$) and $c = 0.214$ for cholesterol. We have ascertained that this is a plausible cell membrane composition.

A first attempt to study the new nonequilibrium scenario is provided by the linear stability analysis. In the absence of recycling ($\rho = 0$), we start with the results of the previous section. A range of unstable modes is observed for moderate recycling rates. It is instructive to explicitly exhibit the wavenumber of the mode that first becomes unstable, which for $q \ll 1$ is estimated to be

$$q^2 \approx \frac{2\rho(J_{c}^{AB} - J)}{(G^2 - G_{c}^2)}. \quad (16)$$

We see that the mechanism leading to nonequilibrium pattern size selection implicit in Eq. (16) is the competition between the short-scale ordering effect of phase separation [terms $(J_{c}^{AB} - J)$ and $(G^2 - G_{c}^2)$], and the large-scale mixing effect of the cholesterol trafficking ($\rho$). According to this prediction, in the presence of cholesterol recycling the domain growth is halted at a finite and characteristic size that decreases if the flux rate is increased. Weak thermodynamic phase separation combined with sufficiently rapid cholesterol recycling may lead to stationary domains at the nanometric scale. Figure 8 shows detailed results for a variety of parameter values. When the recycling rate is higher than a critical value $\rho_{c}$, all positive wavenumber modes become stable, which means that the nonequilibrium recycling process is so fast that the system is kinetically maintained in a miscible state. The critical value $\rho_{c}$ is found to be given by

$$\rho_{c} = \frac{(G_{c} - G)^2}{\gamma}. \quad (17)$$

Simulations are performed in lattices of $N = 256$ for up to $10^8$ iterations, which corresponds to a system size of $1.28 \times 1.28 \mu m^2$ simulated for 2.5 s. To illustrate pattern evolution, in Fig. 8(b) some pattern snapshots of the numerical simulations are presented for different recycling rates. As a general behavior, we observe how the system is segregated into coarsening domains, and that this coarsening process is halted at smaller structures as the recycling frequency is increased.

To quantify the effect of the recycling rate on the stationary domains, simulations for different values of $\rho$ are performed. With each simulation, we capture the temporal evolution of the characteristic length $R$ of the patterns, computed as the smallest distance at which the spatial correlation function vanishes. The
Fig. 8. (a) Growth rate $w(q)$ for different parameter values. For all curves $J = 0.25$, $\gamma = 0.25$, $D = 1$, $\varphi = -0.2$ and $\varphi = 0.214$. When $\rho = 0$ and $G = 0 < G_c = 2.394$, the system is miscible. When $G$ is increased above its critical value ($G = 2.5 > G_c = 2.394$, $\rho = 0$), equilibrium phase separation is predicted. When a moderate recycling rate is applied ($G = 2.5$, $\rho = 0.01$), unstable modes appear at $q \in (q_-, q_+)$ leading to domains of finite sizes. Faster recycling increases the value of the minimum unstable mode $q_-$, and thus smaller domains are expected. If $\rho > \rho_c = 0.045$, unstable modes become stable and the miscibility of the mixture is recovered. (b) Temporal evolution of the simulation patterns in a $256 \times 256$ system for different recycling rates. The other parameters are the same as in panel (a). Each snapshot corresponds to a grayscale representation of the parameter $\phi$. Darker regions correspond to higher values of this variable. The snapshots for $\rho > \rho_c$ follow the same distribution (not shown). The domains in the last snapshots do not statistically grow if simulations are prolonged.
results are plotted in Fig. 9(a), in which we observe a stabilization of the pattern to a stationary length. Only the cases with very slow recycling still display domain growth at late times, which means that the stationary state has not yet been achieved for these cases. The area and the roughness of the emerging domains have also been studied.\textsuperscript{33} An analysis of distribution of areas and of the associated roughness histograms yields more detailed information about the structure of the stationary domains, and are presented in Fig. 9(b). Note that the average domain area diminishes, as does its dispersion, when the exchange process is speeded up. The stationary mean domain size, $L_{st}$, is calculated as the square root of the mean domain area computed for each area distribution. As expected, its value depends on the exchange rate. In particular, larger $\rho$ favors smaller domains. A plot of the inverse of the stationary sizes $1/L_{st}$ is presented in Fig. 9(c) as a function of $\rho^{1/2}$. Note that the prediction in Eq. (16) concerning the dependence on $\rho$ is clearly
fulfilled by our numerical simulations. Note also that increasing $\rho$ results in rougher domains (see the insets in Fig. 9(b)).

Finally, the stability of the domains is analyzed by means of the normalized temporal correlation functions computed at the stationary state (see details in our earlier work\textsuperscript{33}) plotted in Fig. 9(d) for different values of $\rho$. Clearly, higher recycling rates cause the domains to become less stable. In summary, for a given set of parameters, the effect of increasing the recycling frequency favors the generation of monodisperse small, irregular and unstable domains, whereas slow recycling leads to polydisperse large, rounded stable structures.

Modifying the thermodynamic conditions results in a change in the “distance” of the state of the cell membrane from the phase boundary. We analyze such changes by varying the interaction energy $G$ while keeping the remaining parameters fixed.\textsuperscript{33} This analysis reveals that an increment of the interaction energy $G$ above $G_c$ (that is, working with a deeper mixture quench) results in larger, more circular, more stable domains. Conversely, when the separating mixture is closer to the phase boundary ($G$ approaching $G_c$ from above), the mixing effect due to the exchange process leads to domains that are smaller and more irregular and unstable. These results agree with the prediction of the linear stability analysis in Eq. (16).

Experimental research on model (synthetic) bilayers could be designed to test the predictions of our model. Cholesterol transport across the membrane could be mediated by cyclodextrins, tubular oligosaccharides that have been proven to be excellent vehicles for the rapid delivery and extraction of membrane sterols.\textsuperscript{72} Actually, recent experiments with supported membranes treated with cholesterol-loaded cyclodextrin have been conducted, and the resulting formation of ordered lipid phases has been monitored using atomic force microscopy.\textsuperscript{73} However, these experiments describe relaxation to equilibrium, whereas our proposal invokes a stationary nonequilibrium scenario where cholesterol is continuously inserted and released. This could be achieved by keeping different particular fractions of loaded/nonloaded cyclodextrins at the two sides of the membrane. In turn, this would imply recycling and homogenizing techniques that are beyond the scope of this review and the knowledge of its authors.

Beyond academic experimental setups performed on an ad hoc basis for the validation of our model results, the main purpose of this proposal is to provide a plausible mechanism to explain raft phenomena in living membranes. Our approach describes the cell membrane as a lipid mixture that phase-separates due to the presence of cholesterol that is, in turn, dynamically recycled or exchanged with the membrane environment. The model outcome shows that fast recycling processes may lead to nanoscale pattern formation, so that raft formation in cell membranes may fit the scenario described by our model. However, some caution must be exercised when comparing the spatial and temporal scales of the numerical examples provided in this paper so far. For instance, the case with $G = 2.5$ in Fig. 8(c) leads to nonequilibrium domains of $\approx 62.5\,nm$ for $\rho = 40\,s^{-1}$ and $\approx 38.5\,nm$ for $\rho = 400\,s^{-1}$.
Both size values are in good agreement with the typical raft characteristic lengths, but the estimation of the values for the recycling frequencies are much larger than the biological values (of the order of the s\(^{-1}\)).\(^{72}\) Despite this discrepancy, we also showed that similar small domain sizes could be attained for smaller recycling frequencies if the mixture were placed close enough to the phase boundary (for example, by lowering the interaction parameter \(G\) closer to its critical value \(G_c\)). Actually, this is the accepted situation for lipid mixtures in cell membranes.\(^{13}\) Weak thermodynamic phase separation combined with sufficiently rapid cholesterol recycling may lead to stationary domains at the nanometric scale. Therefore, our model may fit raft formation phenomena in the limit of close proximity to the phase boundary.

Our model takes into account dynamic aspects of lipid domains that may dynamically change under specific signals or stimuli,\(^{13–15}\) and results in a hierarchical picture of an active lipid organization at different length scales that are exploited for distinct functions.\(^{74,75}\) The existence of small, transient lipid-ordered domains may induce short-lifetime protein interactions necessary to facilitate specific biochemical reactions in the membrane. Larger and stabilized rafts, resulting from the coalescence of small and temporary domains, may be required for protein trafficking, endocytosis and signaling.

Other models based on similar nonequilibrium ingredients for raft formation can be found in the literature. A nice proposal has been presented by M. S. Turner et al.,\(^{76}\) whose approach follows a purely temporal aggregation scheme without spatial resolution, so in this sense is rather different from ours but complementary. We must note that work contains a more realistic implementation of the cholesterol recycling process. In our model, we considered the simplest choice for this phenomenon, although recent experiments indicate that cholesterol membrane exchange involves the fusion and secretion of small liposomes with specific lipidic composition\(^{69}\) rather than the incorporation or release of single cholesterol molecules. In the work of Turner et al.,\(^{76}\) the exchange of membrane pieces has been considered.

In order to incorporate a higher complexity of the cell membrane, curvature effects such as those described in Sec. 2 could be straightforwardly incorporated in our model equations. One would expect that regions of different local curvature will accompany segregating domains, and the only important effect might be related to a slow-down of the thermodynamic separation action as found in Sec. 2. This effect would favor the formation of smaller domains for the same recycling frequencies.

In a similar context, a remarkable proposal by Foret and Sens\(^{77}\) suggests that lipid trafficking across the membrane is mediated by the formation of protein-coated vesicles. In that proposal, proteins (instead of cholesterol) are dynamically incorporated in the membrane, and then aggregate and curve the membrane forming coated vesicles that are eventually secreted to the medium. They show how stationary coats of finite-sized area emerge from the competition between coat growth and the transport of proteins.
5. Effect of Proteins in Lateral Membrane Lipid Organization

Although plasma cell membranes are mainly composed of lipids, they are also highly crowded systems in which (integral) proteins can be inserted, representing near 30–50% in membrane weight. The diversity of integral proteins inserted in the plasmatic cell membrane is huge. As an example, transmembrane proteins span the entire membrane, and the function of some of them is to attach the cytoskeleton network to the membrane in order to provide mechanical support, cell shape and eventually membrane movement necessary in migration, engulfment and division processes. These proteins may have short-range interactions with surrounding lipids that may be due to a variety of effects. Coulombic interactions with charged lipids or between charged groups of proteins and lipids are an option. A more general effect can be caused by lateral packing preferences. Amino acid side chains protruding from the transmembrane part of the protein accommodate better when surrounded by liquid-disordered lipids that have sufficient flexibility to adapt their acyl tails to the rough protein boundary. Instead, liquid-ordered lipids and, particularly, cholesterol molecules display a large lateral nonconformability with integral proteins, and are usually excluded from their boundary. Another effect is the hydrophobic mismatch, understood as the energy penalty due to the fact that the hydrophobic span of the inserted protein and that of the lipid membrane do not coincide. Therefore, it is thought that transmembrane proteins try to be surrounded by the appropriate lipid phase that better matches their hydrophobic region.

In any case, it is clear that proteins should have an important influence on the phase behavior of the membrane lipid mixture. In order to study this impact, we make use of the continuum approach based on the kinetic \((\phi, c)\) description developed in Sec. 3 for a ternary mixture of unsaturated and saturated lipids and cholesterol. The numerical simulations of the kinetic equations are performed in a square lattice of \(N \times N\) sites. We decorate the bilayer system with a number \(N_P\) of protein particles that are considered to have a much larger cross-sectional area than lipids and fully occupy a lattice site of area \(\zeta^2 = 25 \, \text{nm}^2\) (as in Secs. 3 and 4). In order to account for the protein–lipid interaction, the free energy functional is supplemented with a term \(+\lambda c\) at all sites neighboring a protein. In this review, we consider that transmembrane proteins have a preference for the ld phase since its lipid components are more flexible and can be bent and stretched more easily to adapt to the rough protein boundary and to minimize the hydrophobic energy penalty. This implies a positive value for the \(\lambda\) parameter. Similar results are obtained if a preference for the lo phase (\(\lambda\) negative) is considered.

The system is dynamically evolved in parallel for the compositional fields and protein inclusions by assuming a sort of adiabatic approach that considers that lipids diffuse much more rapidly than protein particles. Therefore, all the results presented here are valid for protein diffusivities \((D_P)\) much smaller than lipid
diffusivities \( D \approx 1 \mu m^2/s \). The kinetic scheme is as follows. Each protein particle freely diffuses following a kinetic MC scheme.\(^8\) One particle is randomly selected and jumps to one of its four neighbouring locations. Each protein diffusion event is taken to imply a time gap of \( (8N_P D')^{-1} \) in s.t.u. units,\(^b\) where \( D' \) is the ratio of protein and lipid diffusivities, \( D_P/D \). After a protein jump, the \( \phi \) and \( c \) scalar fields are allowed to evolve for the same time span, and the process is repeated by selecting a new MC protein move. A similar numerical approach has been used to describe phase separation of polymer blends in the presence of solid filled particles.\(^8\)

We have performed numerical simulations with the same lipid interaction parameters \( J = 0.25, G = 2.5 \) and membrane composition \( (\phi = -0.2, \tau = 0.214) \) used in Sec. 4. The effect of cholesterol transport through the membrane is ignored here, but \( N_P = 1000 \) proteins are randomly inserted in the membrane lattice system of size \( N = 256 \) \((0.64 \times 0.64 \mu m^2)\), with a protein–lipid interaction \( \lambda = 1 \) in \( k_B T \) energy units. The reference results correspond to simulated proteins 100 times slower than lipids \( (D' = 0.01) \). We observe the formation of segregating domains, and a clear preference of protein particles to be surrounded by the low-cholesterol phase. The phase separation process, however, is arrested in the late stage, and dynamic lipid domains of a stationary size are obtained (see left panel in Fig. 10(a)). Therefore, a similar outcome as in Sec. 4 is observed, but here the mixing effect is not due to the transversal lipid transport but to the mixing effect of the free protein diffusion motion and its preference for a given lipid phase. Lipid domains grow following the thermodynamic action, but proteins freely move and nucleate the low-cholesterol phase, eventually breaking the growing domains. Competition between these two effects leads to finite-sized domains.

To quantify the effect of the protein diffusion rate, simulations for different values of \( D' \) are performed. As an example, a long-time pattern for the case of \( D' = 0.025 \) is shown in the right panel of Fig. 10(a). As in Sec. 4, the temporal evolution of the characteristic length \( R \) of the patterns is captured. The results are plotted in Fig. 10(b) and a stabilization of the pattern to a stationary length is observed. As expected, larger (smaller) \( D' \) favors smaller (larger) domains.

The effects of protein density and protein–lipid interaction are analyzed by simulating a membrane with \( N_P = 500 \) and \( \lambda = 1 \), respectively. The smaller amount of diffusing proteins and the weaker interaction with lipids leads to larger lipid domains (see Fig. 10(b)). Note that in the limits of immobile proteins or in the absence of proteins or in the absence of lipid preference, the nonstop phase separation process to equilibrium is recovered.

\(^b\)For a freely diffusing protein the mean square displacement is \( L = \sqrt{2D_P t} \), so for the jump of one protein, we estimate \( L \approx 1 \) s.l.u. Taking into account that there are \( 4N_P \) possible protein jumps, the mean time gap after a protein jump reads \( \tau = [1\text{ (s.l.u.)}^2/2 D_P 4N_P] \) where \( D_P \) is the protein diffusivity. Assigning the ratio of protein and lipid diffusivities \( D' = D_P/D \), and taking into account that the lipid diffusion coefficient is taken equal to 1 in simulation units \( (D = 1 \text{ (s.l.u.)}^2/1 \text{ (s.t.u.)}) \), we obtain a time gap \( \tau = (8N_P D')^{-1} \) (in s.t.u. units) between protein jumps.
Fig. 10. (a) Simulation patterns of protein-decorated membranes in the stationary state for different protein diffusivities, $D' = 0.01$ (left) and $D' = 0.025$ (right). Each snapshot corresponds to a grayscale representation of the parameter $\phi$. Darker regions correspond to higher values of this variable. The snapshots for $c$ follow the same distribution (not shown). Black sites correspond to protein particles. (b) Log-log temporal evolution of characteristic length, $R$, for different protein diffusivities, protein particles and protein–lipid interactions. In all panels, simulations are run for $256 \times 256$ membranes with the following values for the model parameters: $\Phi = -0.2$, $\Psi = 0.214$, $D = 1$, $\gamma = 0.25$, $J = 0.25$ and $G = 2.5$.

Finally, we discuss the applicability of our model to biologically relevant scenarios. While we have proposed plausible values for the membrane composition, interaction among membrane components and diffusivities that lead to a lateral lipid organization structured in dynamic nanometric domains that may correspond to the conjectured functional rafts in the plasma membrane, the correspondence with a biological environment must be analyzed with caution. We have modeled the proteins as freely diffusive in the membrane plane, independently of the lipid substratum. In other words, the lipid mixture evolves to satisfy the preference of proteins to be surrounded by a particular lipid phase, but the motion of the proteins
is completely random. This picture addresses, for example, transmembrane proteins bound to the cytoskeleton and anchored to the membrane as ‘pickets’ whose motion is determined by the random dynamics of the (external) cytoskeleton structure. It is the randomness of the protein motion, together with the affinity for a specific lipid phase, that causes the arresting of the phase-separating process. This description would not be appropriate when simulating other kinds of proteins, for example, the GPI-anchored proteins, a very common category of integral proteins that are not generally subjected to any externally-driven motion. In the case of these proteins, other mechanisms of lipid organization must be invoked, since they are known to nucleate lo phases and also to interact among themselves in different ways. This scenario is beyond the scope of this review.

Another assumption of our approach is the description of the proteins as discrete particles, namely, as “macroscopic” entities with respect to the lipid and cholesterol components described as continuum fields. This implies that a protein particle has a much larger cross-sectional size than the lipidic components. Integrins, Glycophorins and Band-3 are common transmembrane proteins and they have a cross-sectional radius\(^c\) of about 1.3 nm, 2 nm and 50 nm, respectively. These values correspond to 8, 20 and 500 times the typical area per lipid in a fluid phase, so that the results reported in this section could be applicable. However, other transmembrane or integral proteins occupy an area similar to that occupied by the lipid components in the membrane, so that the combination of discrete and field descriptions would not be applicable. Instead, lipids, cholesterol and proteins should each be treated as single particles, and an Ising approach and MC algorithm would be more appropriate. The MC simulation of a membrane system composed of saturated and unsaturated lipids, cholesterol and proteins has been recently performed by one of us,\(^{34}\) following a recent paper of Yethiraj and Weisshaar.\(^{86}\) In these approaches, for simplicity proteins are considered as static and neutral obstacles of a size comparable to the lipid and cholesterol molecules. Even under these assumptions, the effect on the lipid mixture phase stability is dramatic. Membrane proteins act as obstacles reducing the number of the lipid–lipid interactions that are responsible for promoting phase separation, and therefore keep the lipid mixture in a rather mixed state. In this situation, (neutral) proteins act as surfactants between different lipid phases, stabilizing small lipid domains that may be identified with nanometric lipid rafts. Note that such microscopic insight is not available when using a continuum description of the membrane composition (even if proteins are considered as discrete particles). Additionally, the kinetic analysis of the lipid phase stability has shown that the insertion of proteins results, in some cases, in a strong stabilization of the lipid composition fluctuations up to several tens of milliseconds.\(^{34}\) Protein–lipid interactions and protein mobility are currently being addressed in this context.

\(^c\)See for example the “Orientation of Proteins in Membranes” (OPM) data base at http://www.opm.phar.umich.edu/
6. Summary and Conclusions

Concepts of physics, chemistry and biology are involved in the study of the structural and dynamic properties of lipid bilayers. On the one hand, the physics of deformable surfaces is required for the study of the mechanics of membranes that modify their shape by means of local or global curvature changes. On the other hand, the chemical affinity between the different lipid components has to be invoked to understand lateral segregation processes in a bilayer system. In the biological context, both the capacity for changing their curvature and the ability to spatially organize their lipid mixture are fundamental in the development of the rich spectrum of biological events that take place in cellular membranes. Cell membranes deform during processes such as movement, division, vesicle trafficking, etc. Many signal transduction cellular functions are regulated by the formation of lateral lipid aggregates in the cell membrane. Beyond equilibrium considerations, dynamic and nonequilibrium aspects must be considered to understand most of these phenomena. For this reason, the study of self-organization phenomena in bilayer systems subjected to nonequilibrium conditions has attracted enormous attention in recent years.

We have presented a brief account of different theoretical approaches that integrate the aspects listed above. We have proposed a combination of discrete and continuum simulation and modeling schemes to deal with different membrane scenarios. Phase separation of differently shaped active lipids lead to the formation of spatiotemporal structures. Inclusion of cholesterol in a mixture of low-melting and high-melting temperature lipids may induce their segregation in the membrane. In this situation, the cholesterol transport across the membrane results in the formation of lipid domains whose size, shape and stability are determined by the competition between thermodynamic and nonequilibrium forces. Insertion of protein inclusions strongly influences the phase stability of the lipid mixture, providing alternative mechanisms for the control of lipid aggregates in the membrane. In all the studied scenarios, the correspondence of the results to synthetic bilayers and biological membranes has been discussed.

We would like to finish this review by encouraging the experimentalists to consider the proposals presented here in their future research. We believe that our results may help in the understanding of the behavior not only of synthetic bilayers but also of biological membranes in different nonequilibrium scenarios. Experimental work on synthetic giant vesicles and on planar model membranes can be specifically designed to confirm the results of the proposed models and to test our predictions.

Acknowledgments

Partial support was provided by SEID through projects FIS200603525 and FIS2009-11104, and by DURSI through project 2009-SGR1055. Partial support was provided by the Office of Basic Energy Sciences at the U.S. Department of Energy under
Appendix A. Some Formal Details of the Linear Stability Analysis

In this appendix, we briefly discuss some of the formal aspects of our theory. We start with the ubiquitous linear stability analysis that provide the first point at which unpatterned or uniform solutions for the fields that describe the configuration of the system become unstable against the formation of patterns. This analysis always consists of adding small plane-wave perturbations of wave vector $q$ to the uniform solutions, linearizing the kinetic equations in these perturbations, and determining the eigenvalues $\omega_q$ of the Jacobian associated with the linearization matrix. When the real parts of the eigenvalues are negative, the system remains stable and no phase separation is observed (i.e. all modes except for $q = 0$ have negative growth rates $\omega_q < 0$). The first appearance of a positive real part of an eigenvalue indicates a positive linear growth rates of the perturbation, pointing to an instability of the homogeneous solutions and the appearance of structures of size proportional to the inverse of that wave vector. In the case of the kinetic equations (4a) and (4b), the first mode to become unstable starting from the uniform solutions $\phi = \phi_0$ and arbitrary $h = \bar{h}$ depends on the values of the other parameters.

As discussed in Sec. 2, in one parameter regime the first unstable mode occurs at $q^2 = \alpha - 3\beta \phi_0^2 = \sqrt{\frac{\Gamma_{II}^f (1 + H_0 \xi)}{\gamma}}$, leading to a time-independent patterned structure of domains whose size is proportional to the inverse of this wavevector. As $\Gamma$ is further increased, a finite range of modes becomes unstable. Now the patterned regime involves all of the unstable modes, so that there are lower and upper limits to the sizes of the associated stationary patterns. On the other hand, if the reaction parameter is sufficiently small and the reaction–shape coupling parameter is sufficiently large, the linear stability analysis leads to wave bifurcation solutions, that is, one observes a dynamical patterned structure. In this regime, the eigenvalues $\omega_q$ of the linear stability analysis whose real part become positive also contain a nonzero imaginary part, as appropriate for spatio-temporal pattern formation. The first unstable dynamical mode has wave vector $q^2 = \alpha - 3\beta \phi_0^2 - \kappa H_0^2 = \sqrt{\frac{\Gamma_{II}^f}{\gamma + \kappa}}$. Further light can be shed on the expected pattern formation process by performing a weakly nonlinear analysis using the amplitude equation technique. This analysis allows us to compute a solution for our problem near the bifurcation threshold, find the universality class of the pattern formation mechanism, and explain some properties such as spatio-temporal arrangements of the patterns found in

Grant No. DE-FG02-04ER46179, and by the National Science Foundation under Grants No. PHY-0354937 and PHY-0855471.
the numerical simulations. The details of this calculation can be found in our earlier work.\textsuperscript{29,30} In particular, establishing the universality class allows us to borrow results from other models that belong to the same class.

References

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