I. INTRODUCTION

A multicomponent system which is homogeneous at high temperatures can be rendered thermodynamically unstable by a quench below a critical temperature. When this happens, the mixed state is no longer stable and the system separates into domains enriched in the different components that grow in size as time evolves. Understanding the process of phase separation in multicomponent systems is important both for its fundamental aspects and for particular applications in a broad variety of physical and biological problems.

More specifically, the study of phase separation in lipid mixtures that form bilayers is particularly relevant in relation to one of the emergent issues in biophysics: the raft concept for cell membranes. 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. Such structures have been implicated in many biological processes, and their investigation has attracted enormous attention in recent years. Although some aspects of the raft phenomenology remain currently controversial, it is well accepted that the preferential packing of cholesterol and saturated lipids underlies the thermodynamic driving force for raft formation. This has spurred numerous experimental investigations of ternary bilayers of saturated and unsaturated lipids and cholesterol as simplified model membranes, and in all cases, the relevant role of cholesterol in the phase separation of otherwise miscible mixtures of lipids has been noted. In spite of these experimental contributions, only a few recent theoretical studies exist for ternary mixtures. Interestingly, a recent study has investigated the role played by a solid additive (nanoparticles) in the phase-separation process of a binary mixture. Here, instead, we contribute to this issue by addressing the study of a two-dimensional (2D) lipid ternary mixture where two of the components (A and B), being miscible down to a critical temperature, may undergo phase separation due to the inclusion of a third molecular species (C). Preferential affinity of C for one of the other two components (say, A) could “advance” their demixing at temperatures higher than the original critical temperature. In relation to model membranes, A, B, and C stand, respectively, for saturated lipids, unsaturated lipids, and cholesterol. As a distinction with respect to other work, we do not aim to obtain a phase diagram for a specific ternary system. Instead, our approach is more phenomenological and is based on a generic thermodynamic description of lipids in membranes. From this perspective, we develop kinetic equations based on continuum compositional fields to describe the phase separation dynamics in these systems. Apart from obtaining expressions for critical values of the phase separation...
model parameters, these equations may eventually serve as a first approximation to more complex modeling schemes to characterize segregation dynamics in model membranes or in raft formation phenomena.

Our starting point is based on the simplest discrete (on-lattice) scheme of an interacting many-particle system: the Ising model. In this approach, the atomic description is circumvented and each molecule is considered as a particle residing on a lattice site. The model is then constructed as a function of the particle-particle interaction parameters. Here we discuss a simple adaptation of this discrete formalism using a lattice system appropriate to the description of lipid/cholesterol membranes. The AB mixture is described by a 2D triangular grid, whereas the effect of the third component, C, is introduced by energetically coupling this lattice to its complementary hexagonal counterpart where C is allowed to reside. A kinetic Monte Carlo (MC) algorithm is applied to cause the system to evolve, showing the main features of the segregation process.

MC methods based on Ising formulations are conventional computational tools for inferring equilibrium and dynamical properties of phase-separating systems. They are simple and easy to implement in a computer algorithm, but exhibit several limitations for the study of phase separation. Probably the two main shortcomings lie in the fact that Ising-based MC schemes are hardly tractable by analytical means, and that they are not suitable to describe processes at macroscopic length and time scales. These problems can be overcome by using continuum formulations that are generally based on an appropriate construction of the free energy of the system as a function of continuous variables that characterize its compositional configuration. In this case, numerical simulations of the dynamics of phase separation at macroscopic length and time scales can be carried out with better performances than discrete simulations. Additionally, different levels of complexity of the system can easily be introduced by supplementing the kinetic equations with different terms. For the particular case of lipid membranes, one could think of the inclusion of curvature effects, the consideration of chemical or mass gradients applied to the system, hydrodynamic effects, etc. These and other ingredients may eventually be invoked to characterize the pattern formation and the raft phenomenology.

Landau theory is one of the most ubiquitous continuum approaches in condensed systems. Its crucial hypothesis is that in the vicinity of the critical point, one may expand the free energy as a power series of a compositional continuous field. Although Landau theory was initially conceived as a phenomenological description, mean-field treatments of discrete Ising Hamiltonians also result in a power series expansion, providing a conceptual connection between the Landau coefficients and microscopic interaction parameters. Here, we perform such a mean-field approximation for our particular interconnected-lattice Hamiltonian following standard recipes (see, for example, Goldenfeld). From the Ginzburg-Landau free energy functional, the kinetic equations satisfying a conserved scheme are finally obtained satisfying a conserved scheme. Analytical expressions for critical parameters of the problem (for example, the minimum amount of C necessary to phase separate the binary AB mixture for a given set of interaction parameters) are obtained from the linear stability analysis of the kinetic equations. Finally, numerical simulations are provided to support these results, and the correspondence with experimental and biological data is presented.

This paper is organized as follows. In Sec. II we present the MC lattice approach by defining the lattice system, the Ising Hamiltonian description, and the kinetic MC protocols. Some illustrative simulations are also provided. In Sec. III the mean-field procedure that leads to a continuum Ginzburg-Landau free energy is outlined. In Sec. IV the kinetic equations for the continuum description are presented, and the analytical treatment and numerical exploration of these equations are performed. Correspondence of the model results with experimental and biological facts are shown in Sec. V. We conclude with a brief summary in Sec. VI.

II. LATTICE APPROACH
A. Ising-lattice system description

The lattice description of our 2D system is sketched in Fig. 1. The binary mixture composed of A and B fully occupies a 2D triangular lattice with \( N^2 \) sites. The binary system is perturbed by a third component, C, which is allowed to reside intercalated in the complementary honeycomb lattice with \( 2N^2 \) sites. We note here that a 2D triangular lattice is often used in computer simulations to model lipids in membranes, and the combination with a complementary hexagonal lattice has been also used elsewhere to study the effect of cholesterol on the lipid mixture. There are several reasons for this choice, the most important being related to the area/molecule occupied for each species. Common membrane lipids occupy about 0.65–0.8 nm\(^2\)/molecule, whereas cholesterol molecules fill up about 0.35–0.4 nm\(^2\)/molecule. The combination of the two proposed lattices follows the observed 2/1 ratio for the lipid/cholesterol area per molecule.

Consider a set of \( N^2 \) spins \( \{\hat{S}_i\} \) which are fixed on the sites \( \{i\} \) of the triangular lattice, and \( 2N^2 \) spins \( \{\hat{S}_\alpha\} \) on the sites \( \{\alpha\} \) of the hexagonal lattice. According to these spin variables, our two-state Ising Hamiltonian has the form

\[
\hat{H} = J \sum_{i,j \in s} \hat{S}_i \hat{S}_j + J' \sum_{i,j \in h} \hat{S}_i \hat{S}_j
\]

where \( s \) and \( h \) denote the triangular and hexagonal lattices, respectively. The interaction parameter \( J \) is the energy cost of having two spins in the same state, whereas \( J' \) is the energy cost of having a spin in the same state as its nearest neighbor in the other lattice. The corresponding kinetic equations are obtained by applying the Landau-Ginzburg mean-field approximation to the Ising Hamiltonian, which leads to a set of coupled differential equations for the order parameters of the system.
\[
\mathcal{H} = -J_0 \sum_{\langle ij \rangle} S_i S_j - G_0 \sum_{\langle \alpha \omega \rangle} S_\alpha \hat{S}_\omega,
\]
where only nearest-neighbor interactions are considered in the A-B lattice (denoted by \(\langle ij \rangle\)) and between the two lattices (denoted by \(\langle \alpha \omega \rangle\)). \(k_B\) stands for the Boltzmann constant and \(T\) is the temperature. \(J_0 > 0\) corresponds to the strength of the exchange interaction between A and B, and \(G_0\) accounts for the interaction with the lattice containing 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. In our approach, we consider that all compounds are in the same phase state, that is, that there are no phase transitions due to internal degrees of freedom.

### B. Monte Carlo spin dynamics

The Hamiltonian in Eq. (1) contains the information concerning the equilibrium configuration of the system, but it does not imply any particular spin dynamics toward equilibrium. In order to reproduce real phase separation dynamics, the most frequently used method was introduced by Kawasaki\(^\text{26}\) and only allows the exchange of adjacent particles. The adaptation of the standard algorithm to the three component problem is implemented here as follows. First a list is made with all possible adjacent pairs \(\langle ij \rangle\) and \(\langle \alpha \beta \rangle\) with \(S_i \neq S_j\) or \(\hat{S}_\alpha \neq \hat{S}_\beta\). Each pair corresponds to a possible event \(k\) with a probability \(p_k = (1/P) \exp(-\Delta E/2k_B T)\), where \(\Delta E\) is the energy involved in the exchange \(k\). \(P\) is computed as the sum \(\Sigma_k \exp(-\Delta E/2k_B T)\) over all moves, \(k_{\max}\), in the event list. The exchange move \(m\) is chosen if

\[
\sum_{k=1}^{m-1} p_k < \nu \leq \sum_{k=m}^{k_{\max}} p_k,
\]

where \(\nu \in [0, 1]\) is a uniform random number. The hop is performed and the event list is updated according to the new configuration. Notice that the event list contains exchange moves involving pairs on both lattices. Since the C lattice has double the number of sites and half the coordination of the AB lattice, all probabilities \(p_k\) corresponding to C moves are multiplied by a factor of 4. This assures equal mobilities for A, B, and C species. Finally, reliable temporal behavior is obtained if at each MC step the time is increased by

\[
\Delta t = -\frac{\ln(\eta)}{P},
\]

where \(\eta\) is a uniform random number between 0 and 1.\(^{27,28}\) It is easy to check that the probabilities proposed in our method satisfy detailed balance, so that ergodicity is preserved. We refer to this algorithm as kinetic MC simulations.

Results from kinetic MC simulation are shown in Sec. II C. For the pure AB binary mixture at a critical concentration \(\phi = 0.5\), see Sec. II C for definition, Onsager theory provides the critical value for the parameter \(J_0, J_c = 0.2747\).\(^{29}\) However, if the mixture is at an off-critical concentration and/or the third component is added, no exact critical values are known for \(J_0\) and \(G_0\). In these situations and especially close to the phase boundary, kinetic MC simulations become increasingly long and may lead to misleading conclusions about the phase stability of the mixture. We circumvent this problem by using a nonkinetic MC algorithm based on a completely rough dynamics of attempted moves whereby (any) two spins of a given lattice may exchange places. The energy variation \(\Delta E\) that would occur if the spins were exchanged is computed and determines an exchange probability proportional to \(p = \exp(-\Delta E/2k_B T)\). The acceptance of the move is based on the Metropolis algorithm:\(^{30}\) if \(p \geq 1\), the move is accepted; if \(p < 1\), a random number \(\xi\), uniformly distributed between 0 and 1, is generated, and the move is accepted if \(p > \xi\). In our situation this process is alternatively applied to the two lattices at each MC step. This method does not reproduce the realistic kinetics of the system since diffusion in a real system occurs by exchange of nearest neighbor particles. However, the proposed dynamics ensures the fastest equilibration and thus helps to determine which side of the phase boundary corresponds to the simulation conditions. In the context of this work, we refer to this algorithm as nonkinetic MC simulations.

### C. Monte Carlo simulations

All our kinetic MC simulations were performed on \(N^2\) and \(2N^2\) lattices of size \(N = 100\) and run up to \(2 \times 10^6\) MC steps. Periodic boundary conditions are applied to both lattices. The disordered initial configuration is obtained by randomly placing \(N_A\) and \(N_B\) particles in the triangular lattice and \(N_C\) particles in the hexagonal lattice, with \(N_A + N_B = N^2\), and we define \(\phi = N_A/N^2\) and \(\psi = N_C/2N^2\) as the molar fractions of A and C with respect to their own lattices. For each kinetic simulation, we run the corresponding nonkinetic counterpart both in a small, \(N=100\), and on a large system, \(N=1000\), in both cases up to \(2 \times 10^6\) MC steps. The small nonkinetic simulations are used in our panel figures, whereas the large ones (not shown) are only used to confirm the phase stability of the system.

The situation we want to study in this paper is the one where C species interacting with a binary mixture, that is, by itself miscible, can promote its phase separation. An example is provided in Fig. 2 for a system with \(\phi = 0.5\), \(\psi = 0.5\), \(J_0 = 0.2\), and two possible values of \(G_0\): 0.2 and 0.5. In the first case (bottom panels), the system remains mixed, whereas for a sufficiently large interaction parameter \(G_0 = 0.5\), the system clearly demixes (upper and middle panels). Note that \(J_0\) is below the critical value of 0.2747 for the AB binary system in the triangular lattice.

General trends on the values of \(G_0\) necessary to promote phase separation can be qualitatively obtained by performing MC simulations with other values of \(J_0\) and \(\psi\) to compare with those used in the reference case in Fig. 2. For example, in Fig. 3 we show that decreasing the value of \(J_0\) (\(J_0 = 0.05\) in this picture) requires a higher \(G_0\) parameter to demix the system. In Fig. 3 the case with \(G_0 = 0.5\) that leads to phase separation in the reference case in Fig. 2 now displays a uniform phase (bottom panels), and \(G_0\) has to be increased in
order to phase separate the mixture \((G_0 = 1\) in the upper and middle panels). Intuitively it is clear that for smaller \(J_0\), it will be more difficult for the C component to separate A from B, since the repulsion of these components has now been considerably weakened.

The concentration of the C component is obviously another important variable. First, one would expect that for a small amount of C in the system, that is, when \(\psi\) is small, a very high interaction \(G_0\) is required to cause demixing, with this value decreasing as \(\psi\) increases. It is clear that if there are more C’s, a weaker pull is sufficient to separate the A’s that have an affinity to them from the B’s. This is checked in Fig. 4, where \(\psi = 0.2\), a lower C concentration than in the reference case in Fig. 2. In this case \(G_0 = 0.5\) is not enough to demix (lower panels), and a larger interaction parameter is necessary to cause phase separation \((G_0 = 1\) in the upper and middle panels). However, this behavior is nonmonotonic. When the amount of C is too 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. A larger \(G_0\) is then required for the occurrence of phase separation, as shown in Fig. 5 with \(\psi = 0.8\). Again, \(G_0 = 0.5\) that induced demixing for \(\psi = 0.5\) in the reference case in Fig. 2 does not do so here (lower panels), and \(G_0\) has to be raised in order to segregate the system \((G_0 = 1\) in upper and middle panels).

In all the cases studied, only qualitative trends can be obtained. However, a quantitative characterization of the critical values for phase separation can be obtained on the basis of the continuum approach presented in the next section.

III. GINZBURG-LANDAU FREE ENERGY FUNCTIONAL IN MEAN-FIELD APPROXIMATION

Ginzburg and Landau in the 1930s proposed a phenomenological mean-field approach to phase transitions that has been highly instructive in the understanding of the universal features of these systems. In this approach one deals with a free energy functional written in powers of the order parameter near the critical point. From the behavior of this functional, one can determine the location and nature of the phase transition and also the evolution of the system to the equilibrium state from a nearby initial condition. From the Ising-like Hamiltonian in a mean-field approximation. One imagines the system 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

\[
\phi(r_k) = \sum_{i\in k} S_i = \frac{N_A^k - N_B^k}{N_{AB}^k},
\]
The cell, and phase-separating system correspond to the lowest panels. The spins can be organized, lattice in the cell. Similarly, but they can vary from cell to cell. There are degeneracies the field variables are thus average cell order parameters, sites in the cell, those occupied by C’s as well as empty sites. The field variables are thus average cell order parameters, but they can vary from cell to cell. There are degeneracies $g^k_{AB}$ and $g^k_{C_0}$ associated with each cell because of the ways in which the spins can be organized,

$$g^k_{AB} = \frac{N^k_{AB}}{N^k_A!N^k_B!} \quad g^k_{C_0} = \frac{N^k_{C_0}}{N^k_C!(N^k_{C_0} - N^k_B)!}.$$ (5)

An added assumption is that spins only interact with one another within each cell, but that different cells do not interact, so that the energy of the entire system is simply the sum of the cell energies,

$$E = \sum_k E_k,$$ (6)

where the cell energy is determined by the Hamiltonian,

$$\frac{E_k}{k_B T} = -J_0 \sum_{(ij) \in k} S_i S_j - G_0 \sum_{(ia) \in k} S_i \hat{S}_a.$$ (7)

Under the mean-field approach, one assumes that the total effect on each spin caused by the neighbors in that cell (on the same lattice, or on the complementary lattice, as appropriate for the first or second term in the energy) is simply the mean-field variable defined in Eq. (4) multiplied by the number of neighbors, that is, for example,

$$\sum_{(ij) \in k} S_i S_j = J_0 \sum_{i \in k} S_i \phi(r_i) = J \phi^2(r_k).$$ (8)

The first step thus decouples the sums, which permits us to do the second sum and arrive at the simple expression involving only the mean fields in the cell. Here $z$ is the number of spins with which each spin interacts, i.e., the appropriate coordination number. For simplicity, we absorb this number into the interaction parameter and define $J=zJ_0$, and similarly for $G$. The result is the final cell energy,

$$\frac{E_k}{k_B T} = -J \phi^2(r_k) - G \phi(r_k) c(r_k).$$ (9)

The free energy functional $F[\phi(r_k), c(r_k)] = -k_B T \ln Z_k$ is obtained from the cell-dependent partition function $Z_k = g_k \exp(-E_k/k_B T)$, where $g_k = g^k_{AB}g^k_{C_0}$. The factorials in the degeneracy factors are expanded using Stirling’s formula and all variables are expressed in terms of the field variables introduced in Eq. (4),

$$\frac{F[\phi(r_k), c(r_k)]}{k_B T} = -J \phi^2 - G \phi c + \frac{1 - \phi}{2} \ln \left( \frac{1 - \phi}{2} \right)$$

$$+ \frac{1 + \phi}{2} \ln \left( \frac{1 + \phi}{2} \right)$$

$$+ c \ln c + (1 - c) \ln (1 - c).$$ (10)
Clearly, the terms beyond the first two energetic terms on the right are simply the entropic contributions to the free energy.

The Landau form of the free energy functional is finally obtained by expanding the entropic contributions up to fourth order. In implementing this expansion, we must choose the points about which to expand, and this is done with the physical context and symmetry of the problem in mind. The entropy for the \( \phi \) variable has a maximum at \( \phi = 0 \) and is symmetric about that point. As it is clear from the study of two-component systems, whatever the mean value of \( \phi \) in our system, this symmetry is essential in the determination of the critical point for phase separation. The expansion of \( \phi \) is therefore carried out about \( \phi = 0 \). On the other hand, although the entropy for the \( c \) variable has a maximum at \( c = 0.5 \) and is also symmetric about that point, this symmetry is not essential in the determination of the critical point and it is therefore more appropriate to carry out this expansion about a mean value \( c_0 \), whatever this value may be.

As a function of \( \phi \), this functional either has a single minimum associated with a mixed AB system, or two minima associated with a phase-separated system. It is the detailed behavior around this transition that is best captured by the expansion about the particular value \( \phi = 0 \).

The total free energy of the system is the sum over all cells of this free energy functional, which is routinely replaced by an integral under the assumption that the cell-to-cell variations are very slow,

\[
F[\phi(r), c(r)] = \int dr \left[ F(\phi(r), c(r)) + \frac{1}{2} \nabla \phi(r)]^2 \right].
\]

Note that one additional contribution beyond the energy and entropy terms has been added to the integrand. This final piece, which contains the 2D differential gradient operator \( \nabla \), is the well-known effect of the surface tension and the associated energy cost when there is spatial separation of species leading to an interface or, for that matter, any spatial inhomogeneity, \( \gamma \) being the so-called line tension parameter. There is only such a contribution for the A and B species.

The C lattice interacts only energetically with the AB lattice and is otherwise independent of it, and there is no surface tension between C-rich regions and empty regions.

IV. KINETIC EQUATIONS: ANALYSIS AND SIMULATIONS

A. Kinetic equations

The next step in the analytic procedure is to obtain the kinetic equations from the functional equation [Eq. (13)]. The compositional field variables must obey the balance equations,

\[
\frac{\partial \phi}{\partial t} = -\nabla \cdot J_\phi, \quad \frac{\partial c}{\partial t} = -\nabla \cdot J_c.
\]

Here \( J_\phi \) and \( J_c \) are the compositional internal fluxes that can be written in terms of the chemical potentials \( \mu_\phi, \mu_c \) via the constitutive relations

\[
J_\phi = -D_\phi \nabla \mu_\phi, \quad J_c = -D_c \nabla \mu_c,
\]

where \( D_\phi \) and \( D_c \) correspond to the molecular diffusivities. For simplicity, we take \( D_\phi = D_c = D \). The chemical potentials are generally written as the functional derivatives of the free energy with respect to the corresponding order parameters, leading to a pair of coupled modified Cahn-Hilliard equations,

\[
\frac{\partial \phi}{\partial t} = D \nabla^2 \frac{\partial F[\phi, c]}{\partial \phi} + \frac{\partial c}{\partial t} = D \nabla^2 \frac{\partial F[\phi, c]}{\partial c}.
\]

The resulting kinetic equations are

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

\[
\frac{\partial c}{\partial t} = D \nabla^2 \left[ -G \phi + \eta_1 + 2 \eta_2 (c - c_0) + 3 \eta_3 (c - c_0)^2 \right.
\]

\[
+ 4 \eta_4 (c - c_0)^3 \right].
\]

B. Linear stability analysis

Some results can be anticipated via the linear stability analysis of the model equations [Eq. (17)]. The one-dimensional linear stability of the uniform average field solutions \( [\phi(x)=\phi_0, c(x)=c_0] \) is tested by adding small perturbations \( \delta \phi \exp[i \omega q t + i q x] \) and \( \delta c \exp[i \omega q t + i q x] \), and linearizing Eq. (17). This procedure determines the \( 2 \times 2 \) linearization matrix \( \mathcal{L} \),

\[
\mathcal{L}_{11} = -D q^2 [1 - 2J + \phi_0^2 + \gamma q^2],
\]

\[
\mathcal{L}_{22} = -2 D q^2 \eta_2,
\]

\[
\mathcal{L}_{12} = \mathcal{L}_{21} = D q^2 G.
\]

The largest eigenvalue of the Jacobian associated with the matrix \( \mathcal{L} \) corresponds to the linear growth rate \( \omega(q) \) of the perturbations. Solving the eigenvalue problem, we obtain an explicit expression for \( \omega(q) \) which is in itself not particularly interesting other than to note that it vanishes when \( q \).
Phase separation in lipid membranes


FIG. 6. Upper panel: AB-C critical interaction parameter $G_c$ as a function of the A-B interaction parameter $J$. The A-B system is phase separated above the curves and mixed below. Note that $J$ in all cases is below the critical value $J_c$ that would be required for phase separation if there were no C present. Increasing AB-C coupling is needed for phase separation as $J$ becomes weaker, and the optimal situation for phase separation occurs when $c_0=1/2$. The curve for $c_0=0.2$ shown in the figure is identical to the figure for $c_0=0.8$, that is, the behavior is symmetric about 1/2. We also illustrate the effect of modifying the proportion of A and B present in the system. For $J=0.15$, the pure lipid mixture is placed at the cross symbol. After adding cholesterol and fixing $G=1.8$, the mixture is placed at the black spot symbol. Lower panel: $G_c$ as a function of the fraction $c_0$ of C occupancy. There is A-B phase separation in the region above the curves, while the system is mixed below the curves. Note again that $J$ in all cases is below the critical value $J_c$, in the absence of C. Also, $G_c$ for demixing increases with decreasing $J$ and with a deviation on either side of $c_0$ from the “optimal” value of 1/2.

=0. Thus, the first unstable wavevector is $q=0$, consistent with a separation of the system into macroscopic phases. The occurrence of an instability is diagnosed by the parameter values at which the derivative $\left(\delta \omega(q)/\delta q^2\right)_{q=0}$ crosses from being negative [no instability because $\delta \omega(q)$ then decreases with $q$] to being positive. This does indeed occur when

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

(18)

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, that is, $J > J_c^{AB} = 1/2 (1 + \phi_0)$. Second, note that alternatively we can rewrite Eq. (18) as

$$\Delta J = J^{AB} - J_c^{AB} = -c_0 (1 - c_0) G^2,$$

(19)

where $J^{AB}$ is the critical coupling parameter between A and B components when a third component C is present. 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. These behaviors are seen in the upper panel of Fig. 6, where we explicitly show the way in which increasing $G$ lowers the necessary $J$ for phase separation to occur. Accordingly, increasing $J$ for a given set of concentrations lowers $G_c$ since it is now easier to separate the A and B components (note that all $J$’s in the figure are below the critical value $J_c^{AB}$ in the absence of C). This panel also shows that the minimal interaction $J$ required for phase separation occurs when $\phi_0=0$ and $c_0=1/2$. In fact, $\partial^2 \Delta J/\partial c_0^2|_{c_0=1/2} = 0$ and $\phi_0$. Other points concerning the linearization analysis are also noteworthy. While 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 variation of $G_c$, with $c_0$, the average amount of C present, is interestingly (but intuitively) nonmonotonic (see lower panel of Fig. 6) as suggested by the MC simulations. If $c_0$ is very small, then a very strong $G$ is required to induce phase separation, with $G_c$ decreasing as $c_0$ increases. However, this trend does not continue indefinitely. Beyond a certain point, when $c_0$ moves beyond 1/2, C’s stop being as effective as separators, and a stronger and stronger $G$ is required as $c_0$ increases. 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.

C. Numerical simulations

We numerically solve Eqs. (17) in a $128 \times 128$ 2D square lattice by means of a forward time centered space discretization scheme and a first order Euler algorithm. We impose periodic boundary conditions, a mesh size $\Delta x=1$, and time step $\Delta t=10^{-4}$. These time and space steps insure numerical accuracy and fulfill the Von Neumann stability criteria. Simulations are started from a slightly randomly (Gaussian) perturbed distribution around the homogeneous solution $\phi(\mathbf{r})=\phi_0$ and $\epsilon(\mathbf{r})=c_0$ such that a conserved dynamics is also satisfied for the initial condition, that is, $\{\phi(\mathbf{r}, t)\} = \{\phi(\mathbf{r}, 0)\} = \{\phi(\mathbf{r}, 0)\} = \{\epsilon(\mathbf{r}, 0)\} = \{c_0\}$. Here $\{ \cdots \}$ indicates a spatial average over the whole system. Finally, the line tension parameter $\gamma$ and the diffusivities $D$ are set to unity in all numerical simulations.

We explore the two main results suggested by the MC simulations and by the linear stability analysis concerning the effect of a third component C. On the one hand, when C is present, by increasing the interaction parameter $G$ between the two lattices, phase separation is achieved even if $J < J_c^{AB}$. Figure 7 shows numerical simulation results when $\phi_0=0$, $c_0=0.5$, $J=0.4 < J_c^{AB}=0.5$, and $G=1.5$. In agreement with the linear stability analysis (see Fig. 6) and the Monte Carlo simulations, phase separation is observed even though $J < J_c^{AB}$.

On the other hand, we check the nonmonotonic (reentrant) behavior upon an increase of the concentration of the third component C. Setting $\phi_0=0$, $J=0.2 < J_c^{AB}=0.5$, and $G=1.8$, Fig. 6 reveals the reentrant behavior as $c_0$ is increased. This result is confirmed by numerical simulations, as shown in Fig. 8. Thus, note that when $c_0=0.2$ no phase separation occurs. As $c_0$ is increased up to $c_0=0.5$, components A and B (and C) become segregated. However, if $c_0$ is further increased up to $c_0=0.8$, phase separation is no longer observed.
In the context of model membranes with cholesterol, such reentrant behavior is obtained experimentally (see discussion below).

V. CORRESPONDENCE WITH BIOLOGICAL FACTS

We start by commenting on the intrinsic length and time scales in our modeling scheme. Obviously, the mean-field nature of the model precludes any reference to the single molecule scale. The spatial discretization step $\Delta x$ in the numerical simulations is chosen to correspond to the typical interfacial width of $\approx 5$ nm. Since we have chosen $\Delta x=1$, the simulations length unit slu $=5$ nm, so that each discretized lattice site contains about 50 lipid molecules, validating the coarse-grained nature of the model. The simulation time units $stu$ can be extracted from the value of the diffusion coefficient. The choice $D=1$ in our simulations is given in slu $^2/stu$. Since for a generic lipid or cholesterol molecule in a bilayer the diffusivity is of the order of $\mu m^2/s$, this implies that a $stu=10^{-5}$ s. Simulations in Figs. 7 and 8 have been then performed in $0.64 \times 0.64 \mu m^2$ membranes up to $0.5$ s. Typical length and time scales in raft phenomena or macroscopic phase separation processes are, therefore, accessible.

A second level of correspondence concerns the free energy parameters. A simple estimation of the interaction strengths allows a preliminary understanding of the system behavior that, apart from particular details that depend on the specific molecular components, may be considered of general applicability. In the context of lipid membranes and based on the Regular Solution Theory (see, for example, Lupis), the parameters $J$ and $G$ in Eq. (11) are related to the lipid-lipid and cholesterol-lipid differential interactions, $\omega_{il} = 4J$ and $\omega_{ij} = G$ respectively. Actually, $\omega_{il}$ is defined as the difference between the interaction energy of a pair AB and the interaction energy of a pair AA (considered equal to the energy of a pair BB). $\omega_{ij}$ accounts for the energy cost of the formation of a BC pair (for simplicity considered equal to the energy gain of a pair AC). These parameters can be obtained from experiments with different lipid systems, and a reasonable estimation for our model (with generic saturated and unsaturated lipids) leads to $J \approx 0.15$ and $G \approx 1.8$, in $k_B T$ energy units. These values reflect the fact that, generally, the
lipid binary mixture is highly miscible ($J = J_{AB} = 0.5$), but the presence of cholesterol strongly alters the stability of the mixed state.

By considering the critical condition in Eq. (18) and identifying the system molar fractions, $\chi_{c} = 2c_{0}/(1 + 2c_{0})$ and $\chi_{h} = \frac{1}{2}(1/(1 + 2c_{0}) \pm \phi_{0})$, we construct the ternary phase diagram in Fig. 9 for the estimated values of $J$ and $G$ (solid boundary). As a general result also found in the experiments, a two-phase coexistence region appears. Obviously, the shape of an experimental phase diagram depends on the particular lipid mixture considered. In our model, similarly, the values of the interaction parameters determine the shape of the coexistence region. For example, in Fig. 9 we have also plotted the miscibility boundary for the case $J = 0.48$ and $G = 0.5$ (dashed boundary), where the lipid mixture is, in the absence of cholesterol, closer to phase separation. Notice, however, that in both cases, the coexistence region occupies a rather central position in the diagram and displays a well-defined symmetry. Both features reflect the simplifications assumed for the particle-particle interactions when constructing the free energy; namely, the energies of AA and BB pairs are chosen the same (equal chemical potential for the pure components), the energy gain to form an AC pair is considered equal to the energy lost when forming a BC pair, and interactions between C molecules are disregarded. Adding more interaction parameters to avoid these simplifications would allow us to obtain coexistence regions with different shapes and shifted to different zones of the phase diagram, so that an adjustment of the model interaction parameters to the particular lipid system would be possible.

The generic case, $J = 0.15$ and $G = 1.8$, proposed here implies an additional fact that is relevant in a biological context. Although the lipids are rather miscible, the presence of cholesterol in the lipid mixture places the system very close to the phase separation boundary. In the upper panel of Fig. 6, we show the initial thermodynamic state of the pure lipid mixture (cross) and the state of the mixture after adding cholesterol (black spot). Far from being a coincidence, this situation is biologically plausible since lipid cell mixtures are generally accepted to be close to a phase separation boundary. According to this view, any slight perturbation may drive the system across the phase boundary, inducing large scale effects on the lipid membrane organization.

In the previous paragraphs, we referred to basic equilibrium concepts of a ternary mixture. Next we introduce some nonequilibrium considerations. An undisputed fact is that the cell membrane is an open and highly regulated system. A large variety of external perturbations may be considered: the inclusion of proteins in the membrane that may either nucleate ordered lipids shells around them (the *umbrella* model) or may place themselves at the interfaces of ordered and disordered lipid domains acting as effective surfactants, the effect of cytoskeleton-induced curvature modifications, the existence of fluxes of lipids and/or cholesterol across the membrane, etc. The kinetic formalism proposed here can easily be complemented with extra terms to account for these perturbations acting on the membrane system. The *umbrella* hypothesis may be implemented by introducing a spatial dependence of the interaction parameters $J$ and $G$. The case of surfactantlike proteins may be modeled by using a concentration dependent line tension parameter $\gamma$. Curvature effects are usually implemented by adding the standard Helfrich term to the free energy functional. The existence of fluxes through the membrane, particularly of cholesterol, seem to be strongly involved in the regulation of raft organization and can be introduced in the kinetic equations by simple influx and efflux contributions. All these possibilities make our model a suitable approach to test different proposals to explain raft formation in cell membranes.

VI. CONCLUSIONS

The main goal of this paper has been to derive a simple kinetic model to describe the phase separation process of a two-component miscible system that demixes due to the inclusion of a third component that interacts preferentially with one of the two components. This scenario is interesting in the context of raft phenomena in cell membranes, and for ternary model membranes where it has been observed that the presence of cholesterol can cause the lipids to separate even under conditions that do not lead to separation in the absence of cholesterol.

Starting from an Ising-like Hamiltonian for the three component system, we have attacked the problem from two fronts. On the one hand, kinetic and nonkinetic MC simulations pictorially exhibit the way in which the system goes to its appropriate equilibrium as well as the nature of the equilibrium. More quantitative results were obtained with a mean-field Landau-like free energy functional and associated Ginzburg-Landau continuum equations for the evolution of our phase-separating system. We have presented numerical solutions of these equations showing conditions under which the predicted phase separation takes place and the results of the MC simulations are recovered. In particular, we have carried out a linear stability analysis that provides a prediction for critical conditions that lead to phase separation and reveals the existence of a two-phase coexistence region.
Besides the possibility of obtaining analytical information, the advantage of our continuum kinetic equations is that their numerical simulations can access the time and length scales involved in collective membrane phenomena with modest computational resources. Additionally, simple implementation of extra kinetic terms into the equations would make it possible to address other relevant biological questions. For example, our modeling approach considers a closed system in equilibrium where external perturbations have been disregarded. However, nonequilibrium contributions (inclusion of proteins, curvature modifications, transversal component fluxes, etc.) indeed lead to more complex dynamical effects in terms of membrane organization.\textsuperscript{25,40,41}

In fact, the connection of this work with more realistic processes of raft formation in biological membranes relies on these nonequilibrium considerations. Work along these directions is in progress.

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