



Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and
subscription information:

<http://www.tandfonline.com/loi/gmcl19>

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P. B. Sunil Kumar^a & Madan Rao^a

^a Institute of Mathematical Sciences, Taramani, Madras, 600 113,
India

Version of record first published: 24 Sep 2006.

To cite this article: P. B. Sunil Kumar & Madan Rao (1996): Kinetics of Phase Ordering in a Two-Component Fluid Membrane, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 288:1, 105-118

To link to this article: <http://dx.doi.org/10.1080/10587259608034588>

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KINETICS OF PHASE ORDERING IN A TWO-COMPONENT FLUID MEMBRANE

P.B.SUNIL KUMAR AND MADAN RAO
Institute of Mathematical Sciences, Taramani, Madras 600 113, INDIA

Abstract We present a novel Monte Carlo simulation of the phase separation dynamics of a model fluid membrane. Such a phase segregation induces shape changes of the membrane and results in local ‘budding’ under favourable conditions. We present a preliminary investigation of the nucleation regime and compute a variety of growth laws. Our study indicates that membranes with high viscosity, buckle on account of phase segregation. We discuss the relation between this dynamical buckling and the phenomenon of ‘capping’ seen in biological membranes.

INTRODUCTION

Owing to strong hydrophobic interactions, phospholipid molecules like DMPC or SOPC, spontaneously aggregate in an aqueous medium to form a bilayered fluid membrane. Over length scales larger than its bilayer thickness ($\sim 50\text{\AA}$), the membrane can be represented by a two-dimensional closed surface (vesicle) of dimension $10 - 20\mu\text{m}$, embedded in a 3-dimensional aqueous medium. In equilibrium, the vesicle exhibits a well-defined shape and topology when subject to constraints of fixed surface area and volume. Unlike soap films, these equilibrium conformations are primarily determined by bending elasticity.¹ Shape and topology changes can be induced by changing parameters like the temperature or osmotic conditions. Experiments like phase contrast video micrography have established an entire taxonomy of equilibrium shapes and shape transitions as these parameters are varied, the most dramatic being the ‘budding’ transition at which a large ‘parent’ vesicle forms a spherical appendage attached to it by a narrow umbilical.² An extensive study of certain theoretical models³ have resulted in complicated shape-phase diagrams, with shape changes occurring at discontinuous or continuous phase boundaries. The sequence of shape changes predicted by these theories are largely in conformity with the experiments mentioned above.

Although the above shape changes have been observed in artificial membranes, similar and more complex shape changes have been seen in ‘real’ biological membranes (e.g., plasma membrane of animal cells⁴). These changes, often triggered by a

change in the chemical environment, are associated with distinct biological functions (e.g., endocytosis, exocytosis). Unlike the simple single-component artificial membranes discussed above, biological membranes possess around a 1000 different species of lipids. These lipids may differ in their head groups, length of chains, or number and position of the unsaturated bonds within the chain. Such multi-component lipid membranes can also exhibit shape and topology changes, as the area fractions of the constituent lipids are altered. Indeed, recent theoretical⁵ and experimental⁶ studies on the shapes of two-component artificial membranes, have shown that ‘budding’ and even ‘fission’⁶ may be induced, following the phase segregation of the lipid species along the membrane.

In this paper we argue that the time scale over which the shape of the membrane changes, is of the same order as the time scale over which phase segregation occurs. This implies that the study of shape changes induced by phase separation is a nonequilibrium problem. In the last few years there have been some studies on the dynamical behaviour of fluid membranes (see Ref. 7 and references therein). Most of the effort has focussed on evaluating dynamical correlation functions (and renormalized transport coefficients) of membranes slightly away from equilibrium. On the other hand, we are concerned with the shape dynamics of membranes far from equilibrium. In this paper, we present preliminary results of a Monte Carlo study of the dynamics of phase separation of a two-component lipid fluid membrane, that incorporates the momentum density of the lipids. As we shall see below, the relative lipid concentration is coupled to the local curvature — thus the shape of the membrane changes as phase separation proceeds. Our study is restricted to the nucleation regime, where the generic lipid concentration profile shows patches of the minority species in the background of the other. Following a temperature quench, the membrane sprouts buds locally, with the minority species forming the bud. At these early times, coalescence does not occur. From the domain interfacial energy density and the total mean curvature density, we extract two length scales R_ϕ and R_H , which we show are related to the average ‘neck’ radius and the average curvature of the bud respectively. The length R_H , increases with time as $t^{1/4}$. Subsequent coarsening happens with the buds coalescing or moving towards the edge (the latter is an artifact of our ‘free’ boundary conditions).

We next investigate the time evolution of a single isolated patch of the minority species starting from a flat configuration. We find that the late time morphology depends crucially on the in-plane viscosity of the lipids. We find that an increase in the viscosity causes the fluid membrane to buckle on phase segregation. This buckled shape is thus an effect of dynamics; it is not an equilibrium configuration of

the fluid membrane. This buckling comes about because of the presence of positive disclinations localised at the domain interface.⁸ This clustering induced buckling of a fluid membrane, whose in-plane viscosity is high, might be of relevance in the phenomenon of ‘capping’.⁴

DYNAMICS OF PHASE SEPARATION OF TWO COMPONENT FLUIDS

Let us recount that when two immiscible fluids, like water+toluene, are cooled below their coexistence curve, they phase segregate into water-rich and toluene-rich regions, separated by sharp interfaces. For equal volume fractions of water and toluene (critical quench), the homogenous phase is linearly unstable to long wavelength fluctuations of the concentration variable (spinodal decomposition). The concentration profile at intermediate times reveals bicontinuous regions of one fluid in the other. When the relative concentrations of the two fluids are widely different (off-critical quench), phase separation proceeds by the nucleation of domains of the ordered phase, which grow and coalesce in time. In both cases, the late time dynamics is universal - the system enters a dynamical scaling regime,⁹ where the equal-time concentration correlation function behaves as $g(r, t) = g(r/t^z)$, with a growth exponent z which is independent of microscopic details. The scaling form defines a characteristic length scale $R(t) = t^z$, interpreted as the distance between interfaces. Scaling of the correlation function implies the existence of only one diverging length scale. The phase separation dynamics of a binary fluid is described by a (conserved) concentration density $\phi(\mathbf{r}, t)$ coupled to a (conserved) momentum density $\pi(\mathbf{r}, t)$. A variety of theoretical and numerical techniques⁹ have been employed to obtain the form of the scaling function $g(r/t^z)$ and the exponent z . Although there has been some controversy in two dimensions, our recent Monte Carlo simulation and theoretical analyses¹⁰ show that there is an extended intermediate regime (for high viscosity fluids) when $z = 1/2$, with a crossover to $z = 2/3$ at late times.

TWO COMPONENT FLUID MEMBRANES

Biological membranes generically possess several (~ 1000) different lipid species, and may exhibit complex shapes and shape transformations as a function of chemical environment or concentration of the lipid species. In spite of their obvious interest, a systematic experimental investigation of the shapes of artificial multi-component membranes has not been carried out. Let us for the moment, consider an isolated

bilayered fluid membrane made up of two distinct lipid species, e.g., DMPC and SOPC. These phospholipids, typically differ in their degree of saturation of the length of their hydrocarbon chains (this affects the shape of the molecule), which in turn crucially affects the transition temperature T_m below which the lipid fluid freezes into a gel state. Typical values of this *main transition temperature* are $T_m(\text{DMPC}) \sim 23^\circ\text{C}$ and $T_m(\text{SOPC}) \sim 5^\circ\text{C}$. Since we are interested in the effects of phase separation of the lipids on the shape of the membrane in the fluid state, we should ensure that the phase segregation temperature lies above T_m . This is done by choosing lipids with short chains or with a lot of double bonds.

At equilibrium,^{5,11} the energy of a 2-component vesicle, is a unique function of the concentration of lipids $\phi(u_1, u_2)$ and the shape of the membrane (parametrized by a 3-dim vector of the internal coordinates on the surface, $\mathbf{R}(u_1, u_2)$), subject to constraints of fixed surface area and volume. At length scales larger than the thickness of the bilayer ($d \sim 50\text{\AA}$), the shape-energy is determined by curvature elasticity,¹

$$\mathcal{H}_c = \frac{\kappa_c}{2} \int (H - H_0(\phi))^2 \sqrt{g} d^2u + \kappa_g \int K \sqrt{g} d^2u, \quad (1)$$

where the first term contains the extrinsic curvature H , and the second, the intrinsic curvature K . The extrinsic bending modulus $\kappa_c \approx 10^{-12}$ ergs, is around $100k_B T_{\text{room}}$ and so thermal fluctuations are small, on the scale of $100\mu\text{m}$, the typical size of vesicles. At length scales larger than the persistence length $\xi \approx d \exp(4\pi\kappa_c/3k_B T)$, however, thermal fluctuations are violent enough to drive the vesicle into a self-avoiding branched polymer phase. This sets the upper length scale cutoff in the theory. Though the intrinsic bending modulus κ_g has not been measured for lipid membranes, fluctuations which change the topology of the membranes are discouraged because of the large hydrophobicity of the lipids. In principle the moduli κ_c and κ_g can depend on the local concentration ϕ . We shall ignore this, not being so crucial, and so the second term integrates to a constant for a closed surface. The curvature hamiltonian, Eq. 1, replaces the bilayer by a monolayer with a spontaneous curvature (indeed the area difference between the two leaves is, to leading order in the thickness d , given by $\Delta A \approx d H_0$). We shall comment of the legitimacy of this model later. The shape asymmetry between the two lipid species gives rise to an inhomogenous spontaneous curvature which biases the sign of the local extrinsic curvature,

$$H_0 = c_{00} + c_{01}\phi \quad (2)$$

We choose the constants c_{00} and c_{01} (this choice reflects the nature of the lipid molecules) in such a way that $\phi = +1$ favours a positive local curvature and $\phi = -1$

favours zero local curvature (Fig. 1).



FIGURE 1: The shape of the lipid determines the local spontaneous curvature.

The hamiltonian describing the concentration profile of the lipids on the membrane has the standard Landau-Ginzburg form,

$$\mathcal{H}_\phi = \int \left[\frac{\sigma}{2} (\nabla \phi)^2 - \frac{\phi^2}{2} + \frac{\phi^4}{4} + \mu \phi \right] \sqrt{g} d^2 u \quad (3)$$

Though the area fractions of the individual species may be different in the two leaves of the bilayer, we shall, in the spirit of replacing the bilayer by a monolayer with a spontaneous curvature, ignore this fact. Given the hamiltonian, $\mathcal{H} = \mathcal{H}_c + \mathcal{H}_\phi$, one can determine the equilibrium shape of the membrane subject to constraints of constant area and volume. It was shown in Ref. 5 that when the area fraction of one of the lipid components is small, phase segregation results in a budding transition, with the minority phase forming the bud. This arises because the interfacial (domain wall) energy between the two species, can be made arbitrarily small (upto the lower length scale cutoff, d) by forming a bud at the cost of curvature energy. It was demonstrated, that for a wide range of parameters, this budded configuration is favoured. Recent experiments⁶ have confirmed such a shape transition in artificial membranes made of a mixture of natural brain (sphingomyelin) lipids .

We note that the above scenario, is valid only when phase segregation precedes shape change, i.e., the time scale over which the local lipid concentration relaxes is much smaller than the time scales over which shape changes occur. In fact in membranes, both artificial and “real”, these relaxation time scales are comparable. The lateral diffusion coefficients of lipids in a fluid membrane are around $10^{-8} - 10^{-7} \text{cm}^2/\text{sec}$ and so it takes a second for a tagged lipid to traverse the entire length of the vesicle! Global shape changes of the membrane also occur on these time scales. This implies that we have to address a nonequilibrium problem, which involves the dynamics of the shape coupled to the local lipid concentration. Indeed, it is quite plausible that the shape assumed by membranes may be governed more by dynamics than by equilibrium physics, much like the shapes of crystals produced from a melt. The full dynamical equations clearly involve the following “slow modes”, the conserved total density of the lipids $\rho = \rho_a + \rho_b$, the conserved relative concentration

of the lipids $\phi = (\rho_a - \rho_b)/\rho$, the ‘broken symmetry’ shape variable $\mathbf{R}(u_1, u_2)$, the density of the solvent, the momentum density of the lipid π (lipid hydrodynamics) and the momentum density of the solvent (solvent hydrodynamics). In what follows, we will not consider the effect of the solvent hydrodynamics — we shall however make precise where its inclusion would change our results. In this paper, we will study the dynamics of the 2-component fluid membrane by a novel Monte Carlo simulation, which incorporates the *overdamped hydrodynamics* of the two-component lipid mixture. We will not go into a detailed justification of how our Monte Carlo method includes the momentum density of the lipids, and refer the reader to Ref. 10 for a more complete account.

CONSTRUCTION OF A TWO-COMPONENT FLUID MEMBRANE

As a first step to the study of the Monte Carlo dynamics of a 2-component fluid membrane, let us learn how to “prepare” the system. Our model 2-component fluid membrane consists of two types of hard spherical beads (vertices), A and B (the total number of A (N_A) and B (N_B) beads are fixed, $N = N_A + N_B$), lying on a smooth, compact, 2-dim surface embedded in \mathcal{R}^3 . The beads are linked together by straight flexible tethers (bonds), in such a way as to triangulate this compact 2-dim surface. Thus every configuration of the surface, is represented by a *graph*. The tethers do not intersect each other — *this ensures the planarity* of the graph. The potential $V(r)$ between any two beads is infinitely repulsive at distances less than a bead diameter $l_{min} = a$ (for all vertex pairs) and greater than $l_{max} = \sqrt{3}a$ (for vertices connected by tethers), and so the length of each tether can vary between these two limits. This value of l_{max} imposes self-avoidance locally.¹² We restrict the local coordination number of every bead to lie between 3 and 9; this ensures that the entire planar graph is *connected*; which forces all the beads to lie on the 2-dim surface. A variety of ensembles suggest themselves — closed (spherical topology) and open (with a rigid (frame) or free boundary). In this paper we shall study the dynamics of open membranes with a free boundary. With this choice of ensemble, the distribution of local coordination numbers is peaked at and symmetric about 6. We distinguish between external (edge) vertices (V^E) and internal (bulk) vertices (V^I). Likewise an external (edge) tether (T^E) connects two external vertices, while an internal (bulk) tether (T^I) connects at least one internal vertex. The topology of the surface is maintained by demanding that every internal bond T^I is shared by two triangles and every external bond is associated with only one triangle.

Deformations of this fluid membrane consist of shape changes of the membrane (particle moves), motion of particles along the membrane (fluidity moves) and phase separation of the A and B beads (exchange moves).

Shape changes of the membrane involve movement of the beads, which is effected by randomly choosing a bead i (of either type A or B) and then translating i to a random point (with a uniform distribution) within a cube (of size $2l$) centered on the old position of i . The movement is accepted if (a) the new bond lengths lie between l_{min} and l_{max} , (b) *the graph remains planar* (i.e., no bond intersections) and (c) the topology of the surface is maintained. A single Monte Carlo sweep is N attempted particle moves. These shape changes of the membrane, cost energy \mathcal{H}_c (Eq. 1), and so the new configurations generated by bead movements are sampled using a Metropolis algorithm. To implement this, we write \mathcal{H}_c in the following discrete form,

$$\mathcal{H}_c = \kappa_c \sum_i \sum_{(ij)} \left[H_{(ij)} + \frac{K_i}{\sqrt{3}} \right] - \kappa_0 \kappa_c \sum_i (\pm) \sqrt{\sum_{(ij)} \left[H_{(ij)} + \frac{K_i}{\sqrt{3}} \right]} (1 + \phi_i) + \kappa_0^2 \sum_i \frac{(1 + \phi_i)^2}{4} a_i. \quad (4)$$

In the above equation i is the vertex index, (ij) is the bond connecting the vertices i and j and $\sum_{(ij)}$ is a sum over all bonds emanating from i . The term $H_{(ij)} = 1 - \hat{\mathbf{n}}_\alpha \cdot \hat{\mathbf{n}}_\beta$, where $\hat{\mathbf{n}}_\alpha$ is the local *outward* normal to the triangle labelled α and (ij) is the common bond between the two adjacent triangles α and β . In the continuum limit, however, $H_{(ij)}$ goes over to $\frac{1}{\sqrt{3}}(2H^2 - K)$ and so the term $K_i = 2\pi - \sum \delta_\alpha$, which is the deficit angle at i , must be added to $H_{(ij)}$ to obtain the hamiltonian, Eq. 1 (without the intrinsic curvature term), in the continuum limit. The \pm sign in the second term, denotes the sign of the local mean curvature — it is positive if the *outward* normals on adjacent triangles (determined consistently) point away from each other and negative otherwise. The concentration variable ϕ_i defined at every vertex i , takes values ± 1 depending on whether the vertex i is occupied by an A or a B bead. The a_i in the last term, is sum of the areas of the triangles with i as the vertex.

Motion of particles along the membrane is impeded by the tethering constraints. To simulate fluidity, it is necessary to break-and-reconnect bonds, in addition to moving beads. A successful method to achieve this is the bond reconnection algorithm,¹² which works as follows. An internal bond T_{ij}^I (connecting vertices i and j) is picked at random. With every internal bond T_{ij}^I , we can identify two triangles ijv_1 and ijv_2 . This defines a quadrilateral with i and j as one pair of opposite vertices. Clearly though i and j are connected by a bond T_{ij}^I , the vertices v_1 and v_2 are not. The bond T_{ij}^I is now flipped, so that it connects v_1 and v_2 (the vertices i and j now, do not have a tether connecting them). The bond flip clearly changes the triangulation

dynamically, and is associated with a curvature energy cost. This flip is accepted with a Boltzmann probability, provided the length of $T_{v_1 v_2}^I$ satisfies the tethering constraint, and *does not intersect any other tether (planarity)*. Let us denote the number of flip moves per Monte Carlo sweep by N_{flip} . Clearly the total number of internal bonds is conserved during this flip operation and none of the external bonds are flipped.

The above algorithm has been employed in Ref. 12 to study the equilibrium shapes of thermally fluctuating self-avoiding fluid membranes. We have extended this algorithm¹⁰ to study the *phase ordering kinetics* of a two component fluid in two dimensions. As we have argued in detail,¹⁰ this Monte Carlo simulation includes the momentum density of the fluid and so probes the late-time hydrodynamical regime quite accurately. Here we just note that a determination of the single-particle diffusion coefficient D from a computation of the ratio of the mean-square displacement of a tagged particle to the excursion time (in MCS), shows that D increases on increasing N_{flip} (Fig. 2). This implies that the viscosity of the lipid can be tuned by N_{flip} .

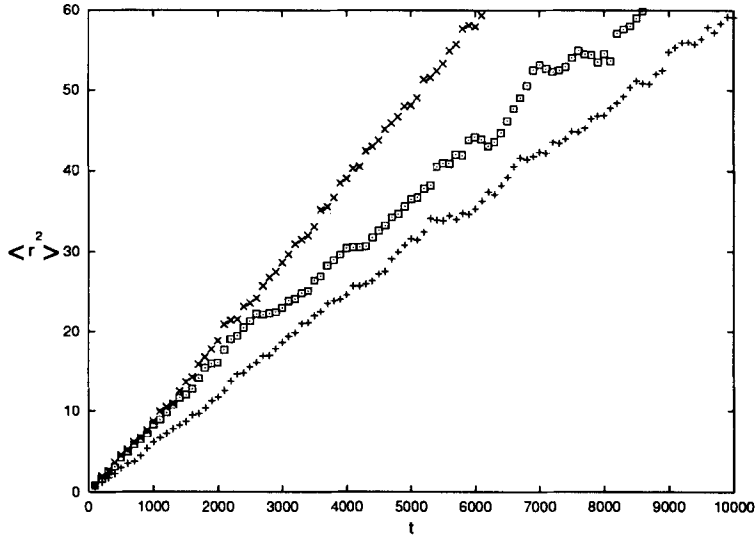


FIGURE 2: Single-particle diffusion of a tagged particle for different values of N_{flip} . Symbols correspond to $N_{flip} = 20N$ (\times), $N_{flip} = 10N$ (\square) and $N_{flip} = N$ ($+$), where $N = 6000$.

Phase separation of the two species A and B, labelled by a local concentration ϕ_i (+1 for A and -1 for B), is modelled by the the usual Kawasaki exchange dynamics⁹

which conserves $\sum_i \phi_i$. Thus locally ϕ_i evolves by exchanging particles at vertices i and j , where j is connected to i by a tether, with a transition probability $W(i \leftrightarrow j) = [1 - \tanh(\Delta E/2k_B T)]/2$, where ΔE is the energy difference between the final and the initial configuration, calculated from the discrete form of the hamiltonian Eq. 3,

$$\mathcal{H}_\phi = -J \sum_{\langle ij \rangle} \phi_i \phi_j, \quad (5)$$

where the sum over $\langle ij \rangle$ is over vertex pairs connected by a tether. The number of attempted exchanges in one Monte Carlo sweep is N_{ex} . This completes the discussion on the rules governing the dynamics of a 2-component fluid membrane.

NUCLEATION DYNAMICS — BUDDING AND BUCKLING

In this paper we shall confine our attention to the case when the concentration of A is much smaller than B. An initial mixed phase is unstable to large amplitude, small wavelength fluctuations of the concentration variable. This phase separation, known as nucleation, results in isolated patches of the A-rich phase in a background of the B-rich phase.

We start with a flat, mixed configuration with the following parameters $\kappa_c = 3$, $k_B T = 1/20$, $\kappa_0 = 3$ and $J = 30$. This temperature is smaller than the critical temperature of the 2-dim Ising model on a triangular lattice, and so is unstable to the nucleation of A domains. We display results for $N = 640$, where the number of A lipids is 20% of the total. The viscosity of the lipids is low; the number of flip moves is $N_{flip} = 15N$ (N_{ex} is maintained at N). The size R_ϕ of each A patch increases as more A lipids cluster together. When the size of the patch is such that $\kappa_c \approx 4\pi z J R_\phi$ (where z is the average coordination number at the A|B interface), the region locally sprouts a bud of radius R_H . We evaluate the interfacial energy density, $\langle E \rangle = N^{-1} \sum_{\langle ij \rangle} (1 - \phi_i \phi_j)$, and the total mean curvature density $\langle H \rangle = N^{-1} \sum_i (\pm) \sqrt{\sum_{(ij)} [H_{(ij)} + \frac{K_i}{\sqrt{3}}]}$ (averaged over several runs). It is clear that at early times, before domain coalescence has occurred, the interfacial energy density $\langle E \rangle \approx N_{bud} R_\phi / N$, while the total mean curvature density $\langle H \rangle \approx N_{bud} A_{bud} / N R_H$, where N_{bud} and A_{bud} , the average number and area of the buds, are constants in time. From a computation of $\langle H(t) \rangle$, averaged over 10 runs, we extract $R_H(t)$ (Fig. 3), which we fit to $R_H(t) \sim A(t - t_0)^z$. We obtain a $z \approx 0.25$ as a best fit. This $R_H(t) \sim t^{1/4}$ growth can be understood from a dimensional analysis of $\partial h / \partial t = -\nabla^4 h + \dots$, which is a highly simplified dynamical equation for the surface in the Monge representation $h(x_1, x_2, t)$. This simplification ignores all the other

slow modes in the theory, and is valid at these early times (before hydrodynamics and coalescence become prominent).

We can parametrize the intermediate shapes of an evolving bud, from a circular patch (disc) of radius R_ϕ to a sphere of radius R_H (attached to the rest of the membrane by an infinitesimally narrow neck), by spherical-cap shapes, with an area,

$$A_{bud} = 2\pi R_H^2 \left[1 \pm \sqrt{1 - \frac{R_\phi^2}{R_H^2}} \right]. \quad (6)$$

Thus for fixed A_{bud} , the two length scales are related by $R_\phi^2 = C_1 - C_2/R_H^2$, where C_1 and C_2 are positive constants. Even if the intermediate shapes are not exactly spherical-caps (for instance, it can be shown that these spherical cap configurations are not Euler shapes¹³), the above relation between R_H and R_ϕ should still hold for arbitrary positive constants C_1 and C_2 (Fig. 3, inset).

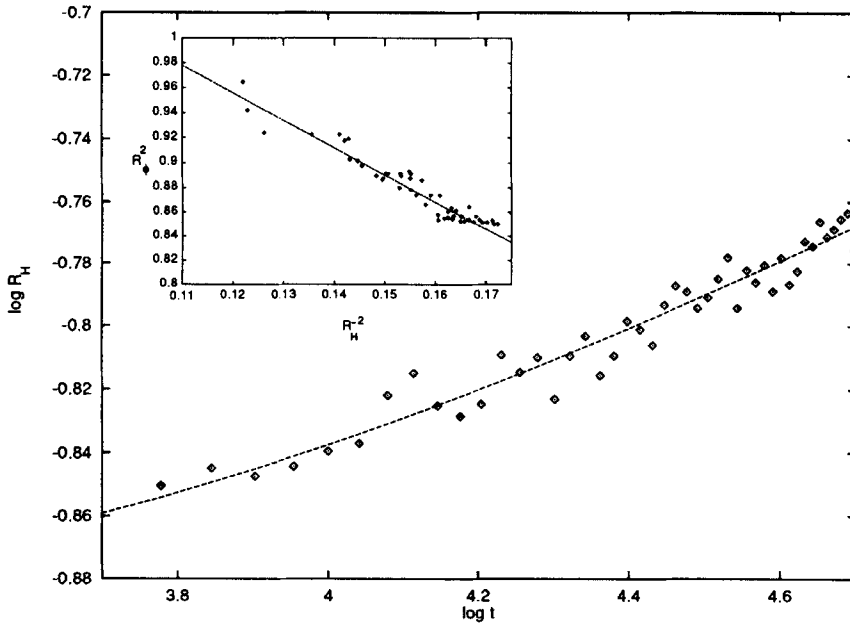


FIGURE 3: Time dependence of the characteristic length scale R_H , showing the $t^{1/4}$ growth (solid line is a best fit to $A(t-t_0)^z$). *Inset*: The length scales, R_ϕ and R_H are related by Eq. 6.

Subsequent coarsening occurs by these buds moving towards each other or towards the boundary (artifact of our 'free' boundary conditions !), and so the late time growth laws would be drastically altered. It is at these advanced stages in the

time evolution, as buds of typical size R_H move towards each other, that solvent hydrodynamics would be relevant. The motion of these buds would experience a viscous drag which would slow down the coarsening rate. We may define two kinds of equal time concentration correlation functions — an Extrinsic correlation function, $g(r_3, t) \equiv \frac{1}{N} \sum_{\mathbf{x}} \langle \phi(\mathbf{x} + \mathbf{r}_3, t) \phi(\mathbf{x}, t) \rangle$ (where $|\mathbf{r}_3|$ is the euclidean distance between points \mathbf{x} and $\mathbf{x} + \mathbf{r}_3$ defined in \mathbf{R}^3) and the Intrinsic correlation function $g(r_2, t) \equiv \frac{1}{N} \sum_{\mathbf{u}} \langle \phi(\mathbf{u} + \mathbf{r}_2, t) \phi(\mathbf{u}, t) \rangle$ (where $|\mathbf{r}_2|$ is the geodesic distance between points \mathbf{u} and $\mathbf{u} + \mathbf{r}_2$ defined on the 2-dim manifold). Light scattering experiments would probe the fourier tranform of the extrinsic correlation function. Which of these quantities, if any, exhibit dynamical scaling as defined in Section II ? It is clear that the configuration of the membrane would deviate appreciably from flatness at late times, and so it is likely that more than one length scale would be operative. This would indicate a breakdown of the usual dynamical scaling at late times. We defer an investigation of this important aspect to a later paper.

Our next initial configuration is a flat membrane with a single patch of A in a sea of B. This initial condition mimics the conditions of Ref. 5, i.e., phase segregation is faster than shape changes. We know that the equilibrium shape consists of a single (or multiple) bud(s),⁵ attached to the rest of the membrane with an arbitrarily small neck. However as we shall see now, the final shape, is crucially dependent on the viscosity of the lipid fluid, which can be tuned by N_{flip} .

When the viscosity is low, the single patch of A sprouts out as a bud (Fig. 4), and evolves steadily towards the equilibrium configuration. The radius of the bud is related to the amount of A lipids in the patch. When the initial configuration consists of two isolated patches of A, then two buds sprout locally, which move towards each other (eventually they might coalesce and form a single (or multiple) bud(s)), or towards the edge (because of our ‘free’ boundary condition).



FIGURE 4: Low viscosity, $N_{flip} = 15N$, causes Budding.

The late time shapes are dramatically different, when the viscosities are large. An initial configuration consisting of a single patch of A on a flat membrane, evolves into a *buckled* conical shape (Fig. 5), with the A species at the tip (which has positive

mean curvature). This buckled surface is asymptotically flat. This shape is clearly not an equilibrium configuration, but a very long-lived intermediate structure. The angle of buckling is related to the amount of A species. When the initial configuration consists of two isolated patches of A, the late time configuration has two buckled conical structures (with the A species at the tips), with a region of negative mean curvature in between.



FIGURE 5: High viscosity, $N_{flip} = N$, causes Buckling.

There is a simple reason for this dynamical buckling. The interfacial energy can be reduced by both shrinking the interface perimeter and lowering the local coordination number of the beads at the interface to smaller than 6. This introduces a preponderance of positive orientational disclinations at the interface, in an otherwise 6-fold coordinated network.¹⁴ Having forced a high viscosity on the lipids, these positive disclinations will be relatively immobile. Further, the high viscosity allows the shear modulus to be nonzero over small time scales (being a fluid, it will ofcourse be zero over infinitely long time scales). Thus over small time scales, the fluid membrane will respond elastically. As shown in Refs. 8 an elastic membrane with a positive disclination density s (proportional to the perimeter of the A domain), will buckle to relieve the elastic strain due to the disclinations, though at the cost of curvature energy. The negative curvature observed in the late-time configuration of the fluid membrane starting from an initial configuration having two patches of A, is due to the occurrence of regions where the local coordination number is greater than 6.

We must stress that even at these high viscosities, the membrane is fluid. We have computed the disclination density correlation function $\langle n_5(\mathbf{u})n_5(\mathbf{0}) \rangle$ (where $n_5(\mathbf{u})$ is the local density of 5-membered rings) and find no evidence of bond orientational order. The observed buckling is therefore truly a long-lived metastable phenomenon generated by the slow dynamics. This dynamical buckling is reminiscent of the shapes of crystals which are governed not by equilibrium physics but by the dynamics of heat extraction from the melt.

This dynamical buckling phenomenon should be observed in artificial fluid mem-

branes comprising of two lipids which undergo phase separation. After the minority species have clustered to form a domain, one may induce a photochemical crosslinking of the lipids. This would lead to an enhancement of the in-plane viscosity η of the membrane, by a factor, $\eta \sim M^{3.5}$ (where M is the molecular weight of the crosslinked unit¹⁵). We expect the membrane to buckle under these conditions.

RELEVANCE TO BIOLOGICAL MEMBRANES

In this section we offer the tentative suggestion that the two scenarios — *budding* and *buckling* — may be observed in real biological membranes. We must stress that the ideas expressed in this section are speculative.

It is not clear whether, the mixture of lipids present in the plasma membrane of cells, phase separate under normal physiological conditions. However clustering of lipids and/or proteins can and do occur, and are associated with a variety of biological functions.

The relation between phase-segregation induced budding and the formation of coated vesicles involving clathrin proteins⁴ was first pointed out by Lipowsky.⁵ We note that the structure of the clathrin coat resembles a polyhedral network of pentagons and hexagons, bound to the vesicle. We feel that the detailed physics of clathrin coated buds, may be more complex than the simple suggestion of phase segregation induced budding.

We would like to suggest that the phenomenon of ‘capping’ observed in the plasma membrane of cells,⁴ may be related to the dynamical buckling discussed in the previous section. When bivalent antibodies bind to specific plasma membrane proteins, they induce these proteins to aggregate in patches (the membrane is still fluid). These patches collect over one pole of the cell, which contains actin filaments, to form a ‘cap’. The role of actin filaments is largely unknown,⁴ and we suggest that it may induce partial polymerization of the lipids. This would increase the viscosity, leading to buckling akin to the phenomenon described above.

We must caution, that any comparison with real (artificial or biological) membranes, must take cognisance of the fact that real membranes are bilayered having differing area fractions of the two species. Would the qualitative features of the phase separation dynamics of such bilayered membranes, be different from those obtained from our model? How independent are the lipids on the two leaves of the bilayer? We note that friction between the two leaves of the bilayer is extremely large $\sim 10^7$ dynes-sec/cm⁴. Thus lateral diffusion of lipids on the two leaves of the bilayer must

be strongly correlated. Moreover, the energy cost to change the difference in the areas ΔA of the two leaves of the bilayer is given by $(\kappa_c \pi / 8 A d^2)(\Delta A - \Delta A_0)^2$, where A is the area of the vesicle. The area difference modulus for a typical $10 \mu\text{m}$ sized artificial membrane is of the order of $10^{17} \kappa_c \text{ ergs/cm}^4$.³ This may imply that shape changes induced by phase segregation on the outer leaf would pull the inner leaf along with it, to maintain the area difference between the two leaves at a constant value ΔA_0 . Clearly there are a lot of aspects to be understood in the late-time phase separation dynamics of lipid bilayers consisting of two lipid species.

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