Electric Field Effects in Multicomponent Fluid Lipid Membranes

Jay T. Groves,† Steven G. Boxer,* and Harden M. McConnell

Department of Chemistry, Stanford University, Stanford, California 94305-5080

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A thermodynamic model of multicomponent fluid membranes under the influence of lateral forces is described. Membrane components are characterized by their effective molecular areas, molecular charges, and the set of critical demixing coefficients (differential interaction energies) between each of the different species present. This model is useful in the analysis of electric field induced concentration gradients in supported lipid bilayer membranes. It has been employed to determine the critical temperature for spontaneous lateral phase separation of a mixture of cardiolipin and phosphatidylcholine from field-induced concentration profiles of a fluorescent probe. More generally, it provides a convenient way of interpreting critical demixing effects in multicomponent membranes and exploring the way these influence the response of a membrane to lateral forces.

Introduction

The fluid nature of lipid membranes enables lateral composition gradients to be generated in response to external forces. The dynamical rearrangement depends on the mobilities of the membrane components,[1−3] while the steady-state configuration is determined by a balance between entropy and the relevant forces.[4−6] Such membrane reorganizations are of particular interest since they play an important role in a variety of cellular processes.[7−13] In this paper, we describe a thermodynamic model of lateral reorganization in multicomponent membranes which has been developed in conjunction with experiments on supported lipid bilayers.

Lateral reorganization of fluid membranes in response to applied forces can be observed and studied with precision in the supported bilayer membrane configuration.[1,2,4,14] This provides a highly controlled system in which physical properties of membranes can be quantitatively characterized. Bilayer lipid vesicles spontaneously adsorb and fuse together on a clean silica surface to form a supported membrane.[15−17] These membranes are typically separated from the substrate by a thin aqueous layer and retain many properties of free membranes.[18−20] Most notably, the lateral diffusion is preserved with mobile components diffusing freely over the entire supporting surface. At the same time, the substrate affords a significant degree of control over the membrane. Barriers to lateral diffusion can be created by manually scratching the membrane-coated surface or by the use of micropatterned substrates.[1,21−23] These barriers can be used to corral off sections of the fluid membrane. Application of a tangential electric field to a confined section of membrane induces lateral reorganization of charged (and uncharged) membrane components (Figure 1).

Electric field induced concentration profiles in confined sections of supported membranes have been studied quantitatively with epifluorescence microscopy.[4,14] Measured profiles were found to be in good agreement with calculations based on a two-component thermodynamic model. Analytical solutions for the equilibrium distribution in these systems are readily obtained and have been discussed previously. However, many informative experiments and calculations require three or more components. In the present work, a general thermodynamic model for multicomponent membrane systems is described.

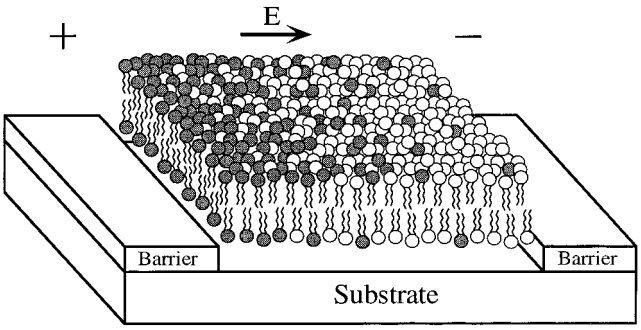

Figure 1. Schematic diagram of an electric field induced concentration gradient in a confined section of a supported membrane. A two-component system is depicted in which the negatively charged lipid (shaded) has congregated on the anode side of the corral. This represents an equilibrium distribution in the fluid membrane where the field-induced drift is balanced by back diffusion.

This thermodynamic model has been used previously to analyze equilibrium concentration profiles in three-component membranes consisting of egg phosphatidylcholine (egg-PC), cardiolipin, and a small percentage of a fluorescently labeled probe lipid.[14] These experiments were performed to investigate critical demixing phenomena in membranes. Critical demixing is a collective molecular interaction which ultimately results in spontaneous phase separation at appropriate temperatures and compositions. It also has the effect of amplifying the lateral reorganization of fluid membranes in response to external forces. This can be observed in the field-induced concentration profile. By fitting calculated concentration profiles to measured profiles of the fluorescent probe, it is possible to determine the critical temperature (T_c) for lateral phase separation of the lipid mixture. The use of a dilute probe to query the mixing behavior of two other membrane components is quite sensitive. For example, a T_c of 75 °K was easily detected in the cardiolipin/egg-PC mixture by an experiment carried out at room temperature. This amounts to a measurement of the sensitivity of a fluid membrane to lateral forces; a higher critical temperature implies greater sensitivity. Amplification of membrane sensitivity by critical

† Current address: Physical Bioscience Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720.

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demixing effects can have significant consequences on the field-induced reorganization of membranes in living cells.[24]

The thermodynamic model described here provides a relatively simple framework for the interpretation and prediction of lateral reorganization phenomenon in fluid membranes. The fundamental parameters included in this treatment (molecular size, charge, and binary critical demixing coefficients) render it suitable for modeling the basic behavior of biological membranes. It is also adaptable to alternative field geometries such as the inhomogeneous field gradient used in some lipid monolayer experiments[25,26] or physiological electric fields occurring during normal cell function.[24]

Thermodynamic Model for Multicomponent Systems

A thermodynamic model of the electric field induced reorganization of lipids in two-component fluid bilayer membranes has been described previously.[4] This model employs Flory's approximation for the free energy of mixing in binary liquids composed of differently sized molecules and accounts for electrostatic interactions within the membrane. It has further been shown that critical demixing effects can be included by addition of an interaction term in the expression for the free energy of mixing.[14] This model specifically describes fluid mixtures, or regions thereof, consisting of a single thermodynamic phase. It may be applied to phase-separated systems by treating the individual phase domains separately. Equations for the chemical potential of each component have been constructed and used to solve for the equilibrium concentration profiles. Here we generalize this model to describe field-induced redistribution of lipids in multicomponent membranes.

Flory's approximation for the free energy of mixing[27] can be extended to describe multicomponent liquids by allowing each component itself to be a binary mixture and carrying out the logical expansion,

$$\Delta G_{\mathrm{mix}} = k_{\mathrm{B}}T\sum_i N_i \ln \varphi_i + \frac{A_{\mathrm{T}}}{A_{\mathrm{u}}}\sum_{i,j}\frac{\gamma_{ij}}{2}\varphi_i\varphi_j \qquad (1)$$

Here k_{B} is the Boltzmann constant, T is the temperature, and N_i is the number of molecules of component i. The area occupied by each molecule in the plane of the membrane is $A_{\mathrm{m}i}$, A_{T} is the total are of the system ($A_{\mathrm{T}} = \sum_i A_{\mathrm{m}i}N_i$), and the unit area of the lattice, A_{u}, is equal to the smallest of the molecular areas. The area fraction, φ_i, is given by

$$\varphi_i = \frac{A_{\mathrm{m}i}N_i}{A_{\mathrm{T}}} \qquad (2)$$

Critical demixing effects are included via the set of interaction elements, $\{\gamma_{ij}\}$; these are symmetric, with γ_{ij} and γ_{ji} each representing the interaction energy of an ij contact relative to an ii contact. Thus, γ_{ij} is the critical demixing coefficient for a pure binary mixture of components i and j. It uniquely determines the critical temperature for lateral phase separation, T_{c}^{ij}, of the two-component mixture. The relationship between $\{\gamma_{ij}\}$ and critical points in multicomponent mixtures will be discussed in more detail below. A similar model has been used to describe the phase behavior of multicomponent lipid monolayer membranes at the air−water interface and found to be consistent with experimental observations.[28] The first term in eq 1 is the entropic contribution ($T\Delta S$) to the free energy of mixing. The second term, which is here referred to as the demixing term, accounts for critical demixing and gives rise to critical points for lateral phase separation.

The entropic and demixing contributions to the chemical potential of component k can be obtained by differentiating eq 1 with respect to N_k. First considering the entropic term in the free energy of mixing, the entropic contribution to the chemical potential, μ_{Sk}, is given by

$$\mu_{\mathrm{S}k} = k_{\mathrm{B}}T\left(\ln \varphi_k + \sum_i\left(\frac{A_{\mathrm{m}i} - A_{\mathrm{m}k}}{A_{\mathrm{m}i}}\right)\varphi_i\right) \qquad (3)$$

Differentiation of the demixing term in eq 1 with respect to N_k provides its contribution to the chemical potential, $\mu_{\mathrm{D}k}$:

$$\mu_{\mathrm{D}k} = \frac{A_{\mathrm{m}k}}{A_{\mathrm{u}}}\sum_{i,j}\gamma_{ij}\left(\delta_k^i\,\varphi_j - \frac{\varphi_i\varphi_j}{2}\right) \qquad (4)$$

Here δ_k^i represents the Kronecker delta function defined as $\delta_k^i = \{1 \text{ for } i = k;\ 0 \text{ for } i \neq k\}$; we also make use of the fact that $\{\gamma_{ij}\}$ is symmetric.

Additional terms in the chemical potential arise from lateral pressure in the membrane (Π), the electrophoretic force (f_k = net force per area applied to component k), and electrostatic interactions within the membrane. Lateral pressure contributes $A_{\mathrm{m}k}\Pi$ to the chemical potential, while the electrophoretic contribution is $A_{\mathrm{m}k}\int_{r_0}^{r} f_k\,\mathrm{d}r'$, where r represents position along the direction of the electrophoretic field. The integral is evaluated from the boundary, r_0, to the position r. The electrostatic term in the chemical potential can be written as $z_{\mathrm{m}k}e\psi$, where e is the elementary charge, $z_{\mathrm{m}k}e$ is the molecular charge of component k, and ψ represents the position-dependent surface potential of the membrane. Summing up the five contributing factors outlined above, the following set of equations is obtained for the chemical potentials in a multicomponent membrane:

$$\mu_k = \mu_k^0 + k_{\mathrm{B}}T\left(\ln \varphi_k + \sum_i\left(\frac{A_{\mathrm{m}i} - A_{\mathrm{m}k}}{A_{\mathrm{m}i}}\right)\varphi_i\right) + \frac{A_{\mathrm{m}k}}{A_{\mathrm{u}}}\sum_{i,j}\gamma_{ij}\left(\delta_k^i\,\varphi_j - \frac{\varphi_i\varphi_j}{2}\right) + A_{\mathrm{m}k}\Pi - A_{\mathrm{m}k}\int_{r_0}^{r} f_k\,\mathrm{d}r' + z_{\mathrm{m}k}e\psi \qquad (5)$$

The constants, μ_k^0, represent the nonelectrostatic portion of the chemical potential in a pure membrane of component k. The gradients of the chemical potentials, used to solve for the dynamic evolution of the system, are given by

$$\nabla\mu_k = k_{\mathrm{B}}T\left(\frac{1}{\varphi_k}\frac{\partial\varphi_k}{\partial r} + \sum_i\left(\frac{A_{\mathrm{m}i} - A_{\mathrm{m}k}}{A_{\mathrm{m}i}}\right)\frac{\partial\varphi_i}{\partial r}\right) + \frac{A_{\mathrm{m}k}}{A_{\mathrm{u}}}\sum_{i,j}\gamma_{ij}(\delta_k^i - \varphi_i)\frac{\partial\varphi_j}{\partial r} + A_{\mathrm{m}k}\frac{\partial\Pi}{\partial r} - A_{\mathrm{m}k}f_k + z_{\mathrm{m}k}e\frac{\partial\psi}{\partial r} \qquad (6)$$

The requirement of mechanical equilibrium can be used to relate the pressure gradient, $\partial\Pi/\partial r$, to other terms in eq 6. Even when the system is not at chemical equilibrium, Newton's second law requires that the sum of all forces acting on any section of the stationary membrane be zero,

$$\sum_k \frac{\varphi_k}{A_{\mathrm{m}k}}\nabla\mu_k = 0 \qquad (7)$$

Imposing this mechanical restriction on eq 6 and solving for

the pressure gradient reveals

$$\frac{\partial \Pi}{\partial r} = \sum_k \left(f_k - \frac{z_{mk} e}{A_{mk}} \frac{\partial \psi}{\partial r} \right) \varphi_k \tag{8}$$

Only the applied electrophoretic force, f_k, and electrostatic interactions contribute to the pressure gradient; the entropic and demixing effects drop out. Note that f_k is the net force per unit area experienced by component k as a result of the applied field. It includes contributions from hydrodynamic and frictional coupling between the membrane and the bulk aqueous phase.[3,29] This force can be determined directly by measurement of the corresponding drift velocity.[1,3] Here, we implicitly assume that the drag on the external medium experienced by each species in the membrane is uniformly proportional to the molecular area. In this case, the drag forces exactly balance and can thus be omitted. If there is substantial variation among the drag interactions within a given membrane, these forces should be explicitly included since they will not necessarily balance and thus can contribute to the pressure gradient.

Gouy−Chapman theory provides a reasonable model of the electrostatic environment near the membrane.[30,31] It can be used to evaluate the effective lateral field, $\partial\psi/\partial r$, produced by an inhomogeneous distribution of charged membrane components. The surface potential is related to the surface charge density, σ, by the Gouy equation,

$$\psi(\sigma) = \left(\frac{2k_B T}{ze} \right) \sinh^{-1} \left(\frac{\sigma z e L_D}{2k_B T \epsilon_w} \right) \tag{9}$$

where z is the valence of the symmetrical electrolyte solution, e is the elementary charge, ϵ_w is the dielectric constant of water, and L_D is the Debye length defined as $L_D = (k_B T \epsilon_w / 2Iz^2e^2)^{1/2}$ with I denoting the ionic strength of the bulk solution. At low ionic strengths ($I \leq 1$ mM), the total surface charge density of the multicomponent supported membrane is given by

$$\sigma = 2 \sum_i \frac{z_{mi} e}{A_{mi}} \varphi_i + \sigma_0 \tag{10}$$

where the factor of 2 comes from the two leaflets of the bilayer membrane and σ_0 is the surface charge density on the substrate.[32] By this definition, any net charge trapped in the water layer between the membrane and the supporting substrate should be included in σ_0. In the high ionic strength of physiological environments, the Debye length is so short ($L_D < 1$ nm) that the two leaflets of the membrane may be treated independently thus eliminating the factor of 2 in eq 10; in this case, σ_0 can be neglected as well. Differentiating eqs 9 and 10 with respect to r and combining we obtain

$$\frac{\partial \psi}{\partial r} = \frac{L_D}{\epsilon_w} \frac{2 \sum_i \frac{z_{mi} e}{A_{mi}} \frac{\partial \varphi_i}{\partial r}}{\sqrt{1 + \left(\frac{\sigma z e L_D}{2k_B T \epsilon_w} \right)^2}} \tag{11}$$

A system of equations describing the chemical potential gradients in terms of $\{\varphi_i(r)\}$ and $\{\partial\varphi_i/\partial r\}$ is generated by incorporating eqs 8 and 11 into eq 6.

Critical Points

Critical demixing effects are included in this model via the set of pairwise critical demixing coefficients, $\{\gamma_{ij}\}$, mentioned above. In a binary system, there is a unique miscibility critical point (composition and temperature) which is related to the critical demixing coefficient. In the case of two neutral components, it can been shown that

$$\gamma_{ij} = \frac{k_B T_c^{ij}}{2} \left(1 + \sqrt{\frac{A_{mj}}{A_{mi}}} \right)^2 \tag{12}$$

where A_{mi} is the larger of the two molecular areas.[14] Incorporating electrostatic effects from charged components, this relation becomes

$$\gamma_{ij} = \frac{k_B T_c^{ij}}{2} \left[\frac{A_u}{A_{mi}} \left(\frac{1}{\varphi_i} \right) + \frac{A_u}{A_{mj}} \left(\frac{1}{1 - \varphi_i} \right) + \frac{A_u}{2} \left(\frac{z_{mi} e}{A_{mi}} - \frac{z_{mj} e}{A_{mj}} \right) \frac{\partial \psi}{\partial \varphi_i} \right]_{\varphi_{icrit}} \tag{13}$$

evaluated at the critical composition (φ_{icrit}), which corresponds to the minimum value of the expression in square brackets over the interval $\{0 < \varphi_i < 1\}$. In multicomponent systems, there are generally an infinite number of binary critical points forming a continuous locus in the composition−temperature phase space. Since critical demixing phenomena strongly influence the lateral reorganization of membranes, it is important to consider the relation between $\{\gamma_{ij}\}$ and the corresponding set of critical points. A convenient way of surveying this is to calculate the spinodal surface.

The spinodal is the boundary between stable or metastable and unstable regions of the phase space. For the membrane systems described here, this amounts to the set of temperatures and compositions at which the lipid mixture becomes absolutely unstable with respect to lateral phase separation. Collectively, these points define an $(n - 1)$-dimensional surface in the composition−temperature phase space of an n-component system. Critical points are a subset of the spinodal points. A membrane mixture that is near a spinodal point will show an enhanced propensity toward lateral reorganization; it is not necessary to be near a critical point to experience this effect. For example, consider a binary mixture at a temperature below T_c but at a composition far from the critical composition (the position marked P in Figure 2). The sensitivity of this mixture to lateral forces is related to proximity of the nearest spinodal

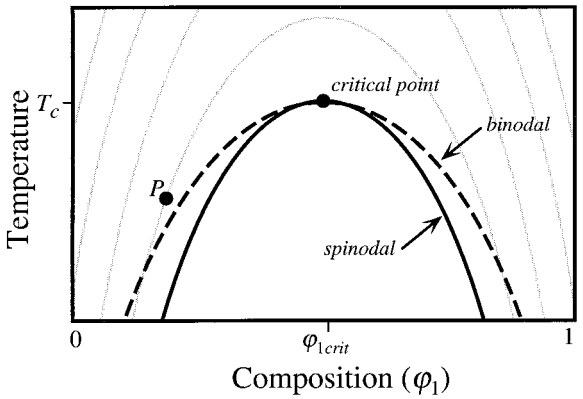

Figure 2. Phase diagram illustrating the relative sensitivity of membranes with respect to lateral reorganization at different positions in phase space. Points further outside the spinodal have lower sensitivities. Contours of equal sensitivity are drawn with light lines illustrating the appropriate metric. Here we define the sensitivity as the multiplicative factor which relates the net lateral force on a membrane component to the concentration gradient that can be maintained in equilibrium by this force (see eq 4 in ref 14). The example system discussed in the text is at point P in this phase diagram.

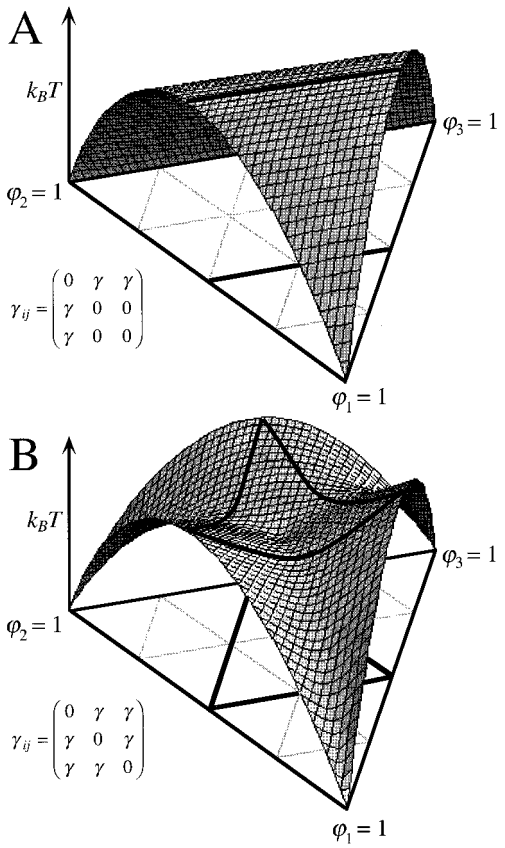

Figure 3. (A) Calculated spinodal surface for a ternary mixture in which two components (φ_2 and φ_3) mix ideally with each other and have equivalent demixing coefficients (γ) with respect to the third component (φ_1). This system can be treated as a two-component mixture in which the ideally mixing components behave as a single component. The locus of critical points lie along the line $\varphi_2 + \varphi_3 = 0.5$ in the composition phase space. These are marked with a heavy line on the composition phase space and on the spinodal surface. (B) Spinodal surface of a ternary mixture in which all three components have equivalent demixing coefficients with each other. The locus of critical points is marked with heavy lines. Note that critical temperatures in this ternary system are not linear combinations of the binary critical temperatures.

point. Contours of equal sensitivity have been drawn with light lines in Figure 2 to illustrate the appropriate metric. Raising the temperature at this composition (*P*) toward the critical temperature actually lowers the sensitivity of the system. Note also that it is essentially impossible to reach a spinodal point at temperatures well below T_c, since a binodal point will be encountered first. Between the binodal and the spinodal, the system is metastable and will tend to separate into two phases with appropriate nucleation.

The spinodal surface of a system can be calculated from the chemical potentials. The onset of instability occurs when the demixing terms exactly balance entropy and other terms that favor mixing. In this situation, the gradients of the chemical potentials all go to zero independent of composition changes in the membrane along a particular path. Each point on the spinodal surface is associated with a direction in the composition space. This is the path along which the composition of the system may change without change in the energy, hence there is no energetic barrier to phase separation.

Two spinodal surfaces for ternary systems with different $\{\gamma_{ij}\}$ are illustrated in Figure 3. The locus of critical points is marked with a solid line.[33,34] The surface pictured in Figure 3A corresponds to a system in which two components mix ideally with each other and have equivalent demixing coefficients with respect to the third component. In this case, the ideally mixing components behave as a single component and the system becomes a quasi-two-component system. This is similar to the situation observed experimentally in lipid extracts from red blood cell membranes.[35] If there is significant nonideality among all three components, the spinodal surface takes on a different shape (Figure 3B). There are several alternative ways the system may split into two or three phases and the critical temperatures of the mixture are not simply linear combinations of the binary critical temperatures.

Dynamic Solutions

An informative and relatively convenient method of calculating solutions to the system of equations constructed above is to set up a dynamic equation describing the time evolution of the system. Since there is no net flow of the membrane, the area flux of component *k*, $\mathbf{J}_k$, is related to the gradient of the chemical potential by $\mathbf{J}_k = \eta_k \varphi_k \nabla \mu_k$, where η_k is the corresponding electrophoretic mobility. The dynamic equation is given by the divergence of the flux,

$$\frac{\partial \varphi_k}{\partial t} = \eta_k \nabla \cdot (\varphi_k \nabla \mu_k) \tag{14}$$

Typical mobility coefficients for double chain phospholipid molecules in a supported bilayer are around 1.1×10^{21} $\mu m^2/J$ s.[1,3] This corresponds to a diffusion coefficient of 4.5 $\mu m^2/s$ and is comparable to the diffusion of lipids in free bilayer membranes.[36] In a mixture of two or more components, mobility becomes somewhat more complicated; it is not a molecular property, but rather a property of the system. There is a necessary dependence of mobility on the effective area occupied by a molecule in the membrane. This is a consequence of symmetry requirements on the net flux and viscous drag within the membrane.

For a stationary membrane, the net flux is zero and the net force due to dissipative drag within the membrane must also be zero:

$$\sum_k \mathbf{v}_{dk} \varphi_k = 0 \quad \text{and} \quad \sum_k \frac{\mathbf{v}_{dk} \varphi_k}{\eta_k A_{mk}} = 0 \tag{15}$$

where $\mathbf{v}_{dk}$ is the drift velocity and $\mathbf{v}_{dk}/\eta_k A_{mk}$ corresponds to the dissipative drag per unit area experienced by component *k* due to its relative motion within the membrane. This dissipative drag results from interactions between molecules in the membrane as well as coupling to the external medium.[37] It is a well-defined quantity since the mobility coefficient, η_k, can be determined by measuring the diffusion coefficient, D_k, and making use of the Einstein relation ($D_k = \eta_k k_B T$). The dissipative drag is distinct from the bulk electroosmotic drag which can be treated as a conservative force and has already been included in the chemical potential via the electrophoretic force term. The equality involving dissipative drag in eq 15 holds as long as coupling to the external medium results in an equivalent drag per unit area on each species in the membrane. This assumption is expected to be valid for the lipid mixtures described here. A second assumption, which is also expected to be valid for these lipid mixtures, is that the mobility coefficients are independent of composition. Under the restrictions described above, the

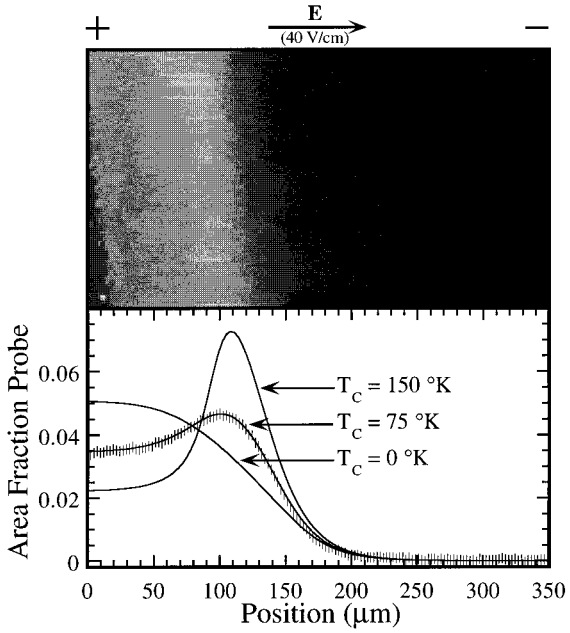

Figure 4. Experimental characterization of the mixing behavior of two lipid components by the dilute probe method. The upper panel contains an image of a steady-state concentration profile of the NBD-PE fluorescent lipid probe (1% by area) in a mixture of cardiolipin (14%) and egg-PC (85%). The negatively charged cardiolipin and NBD-PE have been driven toward the left side of this corral by the 40 V/cm electric field. A concentration gradient has built up along the scratch boundary which is roughly at the zero position. The boundary on the right-hand side of this corral lies outside of the image at the ~900 μm position. A trace of the fluorescence intensity, which linearly correlates with the NBD-PE concentration, is plotted with vertical bars in the lower panel. Calculated profiles for different degrees of critical mixing are plotted with solid curves. It can be seen that calculations closely match the data for a critical temperature of 75° K. These data were compiled from ref 14.

mobility coefficients are all determined as

$$\eta_k = \frac{A_u}{A_{mk}}\eta_0 \tag{16}$$

where η_0 is the mobility of the smallest component in the mixture (molecular area A_u). For the calculations presented below, η_0 was taken to be 1.1×10^{21} μm^2/J s and the other η_k were scaled according to eq 16.

Although the mobility coefficients appear in the dynamic equation, it is important to note that the equilibrium concentration profiles are independent of the electrophoretic mobilities. Consequently, equilibrium profiles are insensitive to factors that may produce composition dependence or other spatial variation of the mobility coefficients within the membrane. The equilibrium concentration profile is a relatively robust observable that directly probes the balance of forces affecting the organization of molecules in a bilayer membrane.

Supported Bilayers

We have previously introduced the technique of using a dilute, fluorescently labeled lipid to probe the mixing behavior of two other components in a bilayer membrane.[14] Results from experiments on three-component supported membranes consisting of cardiolipin, egg-PC, and a fluorescent probe (NBD-PE) are briefly reviewed below. In this work, a tangentially applied electric field was used to induce lateral reorganization of the lipids in confined regions of the supported membrane. Both the cardiolipin and the NBD-PE probe lipid are negatively charged and were driven toward the anode side of corralled sections of the membrane. Steady-state distributions, such as the one shown in Figure 4, can be reached in roughly 1 h under the conditions of these experiments.

A fluorescence image of the field-induced concentration profile of the probe is shown in the upper panel of Figure 4. At dilute levels, fluorescence from the probe is linearly proportional to its concentration; thus, the fluorescence intensity provides a direct measure of the probe distribution. The measured concentration of the probe (vertical bars) along with calculated profiles for varying degrees of critical demixing are plotted in the lower panel of Figure 4. These results suggest a critical demixing coefficient for cardiolipin/egg-PC mixtures corresponding to a T_c of 75 °K. It is interesting to compare this result to similar experiments carried out on mixtures of phosphatidylserine (PS) and egg-PC, where nearly ideal mixing ($T_c = 0$ °K) was observed.[4] An advantage of the dilute probe method is that subtle changes in the concentration profiles of the major components can produce exaggerated differences in the probe concentration profile. This effect is particularly prominent in the case at hand. Cardiolipin and egg-PC concentration profiles (inferred from the model) do not show as large or as distinctive of a dependence on the critical demixing coefficient as was seen in the probe profile.[14] The field-induced concentration profile provides an observable which allows direct measurement of the propensity of a membrane to reorganize under the influence of lateral forces. As seen here, critical demixing effects persist in membranes even at temperatures and compositions far from a critical point.

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