

Spontaneous curvature and thermodynamic stability of mixed amphiphilic layers

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The elastic behavior of mixed bilayers composed of two amphiphilic components with different chain length (and identical head groups) is studied using two molecular level models. In both, the bilayer free energy is expressed as a sum of chain, head group and interfacial contributions as well as a mixing entropy term. The head group and interfacial terms are modeled using simple phenomenological but general expressions. The models differ in their treatment of the chain conformational free energy. In one it is calculated using a detailed mean-field molecular theory. The other is based on a simple "compression" model. Both models lead to similar conclusions. Expressing the bilayer free energy as a sum of its two monolayer contributions, a thermodynamic stability analysis is performed to examine the possibility of spontaneous vesicle formation. To this end, we expand the bilayer free energy as a power series (up to second order) in terms of the monolayer curvatures, their amphiphilic compositions and the average cross sectional areas per molecule; all variables are coupled, with the molecular composition and areas treated as degrees of freedom which are allowed to relax during bending. Using reasonable molecular interaction parameters we find that a second order transition from a planar to a curved (vesicle) geometry in a randomly mixed bilayer is unlikely. Most of our analysis is devoted to calculating the spontaneous curvature and the bending rigidity of the bilayer as a function of its amphiphile chain composition. We find that adding short chain amphiphiles to a layer of long chain molecules reduces considerably its bending rigidity, as already known from calculations involving only the chain contributions. However, we find that inclusion of head group and interfacial interactions moderates the effect of the added short chains. We also find that the bending rigidity of pure monolayers is approximately linear in chain length, as compared to the nearly cubic dependence implied by the chain free energy alone (at constant head group area). Our main result involves the calculation of the spontaneous curvature as a function of composition. We find, for different chain mixtures, that upon adding short chains to long chain monolayers, the spontaneous curvature first increases nearly linearly with composition and then (beyond mole fraction of about 0.5) begins to saturate towards the spontaneous curvature of a pure short chain layer. Qualitative arguments are provided to explain this behavior. © 1995 American Institute of Physics.

I. INTRODUCTION

A planar bilayer of amphiphilic molecules is composed of two monolayers, stuck to each other through their hydrophobic interfaces, thus avoiding direct contact between their amphiphile hydrocarbon tails and water.¹ Yet, each monolayer is characterized by an equilibrium, *spontaneous*, curvature which, in general, is nonzero. The sign of the spontaneous curvature (say positive when the hydrocarbon-water interface is convex towards the water) and its magnitude are governed by the balance of the moments associated with the lateral forces acting in the monolayer. These are the repulsive forces (steric and/or electrostatic) between the amphiphile polar heads, the entropic (conformational) repulsion between the hydrocarbon tails, and the attractive hydrocarbon-water interfacial tension resulting from the van der Waals attraction between the tails. The monolayer spontaneous curvature is generally not very different from the planar one, otherwise the amphiphiles would rather form micelles (or inverted mi-

cellar phases). The curvatures of the two monolayers composing a bilayer are opposite in signs, and equal in magnitude (provided the two monolayers are identical). At least one of the monolayer curvatures is different from the spontaneous one. Thus the formation of a bilayer involves a "frustration" free energy associated with this difference. In the case of symmetric bilayers, i.e., bilayers composed of identical monolayers, the planar geometry corresponds, by symmetry, to the minimal frustration energy. These qualitative notions explain why vesicle formation is generally not a spontaneous process, requiring the input of mechanical energy (e.g., by sonication) or by other means.

From the considerations above it follows that the spontaneous curvature of the *bilayer* is generally zero (although finite thickness effects can lead to spontaneous saddlelike instabilities²). This is obviously the case in a pure (single component) bilayer as well as in a mixed bilayer, provided the amphiphile compositions of its two constituent monolayers are forced to be identical. However, if the compositions of the two monolayers are allowed to differ then, possibly, the spontaneous bilayer geometry will be nonplanar, thus favoring the formation of finite size vesicles. Indeed, sponta-

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neous vesicle formation has been experimentally observed in several mixed systems. For instance, a few years ago, Kaler *et al.*³ have reported spontaneous vesicle formation in mixtures of surfactants with oppositely charged head groups but similar hydrocarbon tails. Earlier, spontaneous vesicle formation has also been reported by Gabriel and Roberts^{4,5} for mixtures of long and short chain lecithins. In this latter system, the two amphiphilic components have the same head group but differ in their hydrophobic tail length.

To explain the experiments on the mixed ionic systems, Safran *et al.*^{6,7} have formulated a mean-field theory based on a free energy involving an elastic energy term and mixing entropy contribution. In the elastic energy, nonlinearity of the spontaneous curvature as a function of composition was taken into account by an interaction parameter. (This is equivalent to assuming a spontaneous curvature which varies linearly with composition, and a nonideal interaction energy contribution to the free energy). For strong enough interactions an instability of the symmetric bilayer state was predicted, implying spontaneous vesicle formation.

In a more recent paper, Porte and Ligoure⁸ have presented a detailed theoretical stability analysis of a mixed bilayer, emphasizing the effects of finite membrane thickness and the coupling between the bilayer curvature and the composition difference between the two monolayers. They express the free energy as a sum of a mixing term, an interaction term and a packing (elastic) free energy. The packing free energy is equivalent to a curvature elastic energy with a composition independent bending constant and a spontaneous curvature which varies linearly with composition.

Both experiment⁹ and theory¹⁰ show that the bending modulus of a mixed amphiphilic layer can vary significantly with composition. Similar behavior is observed in diblock copolymer layers. Based on a self-consistent field analysis and scaling arguments Dan and Safran¹¹ have recently shown that lamellar bilayers of diblock copolymers can be destabilized and form vesicles by adding a small fraction of short chains into the bilayer. In this theory both the bending rigidity and the spontaneous curvature depend on the bilayer composition. The mixing entropy plays a minor role in this, polymeric, system. Similar behavior has earlier been predicted by Wang.¹²

In this paper we analyze the free energy of a mixed "binary" bilayer in which the two amphiphilic components have identical head groups but their hydrophobic tails are of different length. As usual we write the bilayer free energy as a sum of two terms corresponding to the free energies of the constituent monolayers and allow, as in Ref. 8, for molecular exchange between the monolayers. Each monolayer free energy is expressed as a sum of an elastic energy and an ideal mixing entropy term. In the elastic energy we explicitly consider the composition dependence of both the bending modulus and the spontaneous curvature, and calculate them by two approaches. One is based on a detailed *molecular* (mean-field) theory for chain packing statistics in membranes. The other one is a simple phenomenological (compression) model which accounts for the most relevant qualitative trends of the molecular theory. One of the central results of our calculations is that the spontaneous curvature of the

mixed monolayer is, at least over a certain range of compositions, a linear function of the short chain mole fraction. Over this range the effective bending rigidity is a decreasing function of the short chain content.

As in the case of diblock copolymers we find that the curvature elastic free energy indeed favors the formation of vesicles. However, for the calculated values of spontaneous curvature and bending rigidity we find that this is not sufficient to overcome the loss in mixing entropy. Based on these calculations we conclude that spontaneous vesicle formation in a bilayer composed of amphiphiles which only differ in their chain length cannot take place unless the mixing is nonideal.

Our analysis of the bilayer stability with respect to curvature deformations is based on expanding the bilayer free energy as a power series involving the curvature and a composition order parameter. The latter measures the difference in the distribution of the short chain component between the two monolayers. Using this expansion we derive a stability criterion, appropriate only to the occurrence of second order transitions. The absence of a second order instability according to this approach does not exclude the possibility of a first order transition. It is well known, for example, that addition of surfactants to lipid membranes can destabilize the bilayer¹³ and result, above a certain concentration, in a first order transition to cylindrical or spherical micelles containing a larger fraction of surfactants. It should be noted however, that this kind of phase transition cannot be accurately analyzed using a single elastic free energy expression for the very different packing geometries involved in such transitions.^{14,15}

II. THEORY

Consider an amphiphilic bilayer consisting of a long chain (L) and a short chain (S) component, having in mind a phosphatidylcholine (PC) mixture of two different chain lengths.^{4,5} By $N^{\text{bl}} = N_L^E + N_S^E + N_L^I + N_S^I$ we denote the total number of molecules in the bilayer, N_L^E being the number of molecules of type L in the external (E) monolayer, N_L^I being the number of molecules of type L in the internal (I) monolayer, etc. To examine possible transitions of the bilayer from its symmetric state to an asymmetric equilibrium state, it is convenient to introduce the quantities

$$\begin{aligned} m &= (N_S^E + N_S^I) / N^{\text{bl}}, \\ \lambda &= (N_S^E - N_S^I) / N^{\text{bl}}, \\ \chi &= (N_L^E + N_S^E - N_L^I - N_S^I) / N^{\text{bl}}. \end{aligned} \quad (1)$$

Here, m is the mole fraction of short chain molecules in the bilayer, λ is an "asymmetry" order parameter measuring their distribution between the two monolayers, and χ measures the difference between the total number of molecules in the two monolayers. When a bilayer of a given composition m undergoes a curvature deformation both χ and λ can vary to reduce the bending free energy involved. It is also possible that the bilayer is destabilized, i.e., undergoes a spontaneous curvature deformation associated with a change in these two degrees of freedom. To this end we consider a

spherical deformation of the bilayer in which $c^{\text{bl}}=c_1^{\text{bl}}=c_2^{\text{bl}}$, where c_1^{bl} and c_2^{bl} are the principal curvatures, measured at the midplane of the bilayer. At this plane the average area per molecule is $a^{\text{bl}}=2A^{\text{bl}}/N^{\text{bl}}$, where A^{bl} is the area of the midplane, around which all N^{bl} molecules are assembled.

Now, we express the free energy of the bilayer F_{bl} as a sum of the external and internal monolayer free energies $F_{\text{bl}}=F_{\text{ml}}^E+F_{\text{ml}}^I$. Defining free energies per molecule by $f_{\text{bl}}=2F_{\text{bl}}/N^{\text{bl}}$, $f_{\text{ml}}^E=F_{\text{ml}}^E/(N_L^E+N_S^E)$, and $f_{\text{ml}}^I=F_{\text{ml}}^I/(N_L^I+N_S^I)$, this reads

$$f_{\text{bl}}=(1+\chi)f_{\text{ml}}^E+(1-\chi)f_{\text{ml}}^I. \quad (2)$$

Consider now a mixed monolayer composed of $N=N_L+N_S$ molecules, and let A denote the monolayer area measured at some dividing surface with, (spherical) curvature c . The free energy per molecule in the mixed monolayer $f_{\text{ml}}(a,c,\phi)$ is a function of the area per molecule $a=A/N$, curvature c , and composition $\phi=N_S/N$. We write f_{ml} as a sum of an elastic energy term and a mixing entropy contribution

$$f_{\text{ml}}(a,c,\phi)=f_{\text{el}}(a,c,\phi)+f_{\text{mix}}(\phi). \quad (3)$$

with

$$\frac{f_{\text{el}}(a,c,\phi)}{a_0(\phi)}=\frac{1}{2}\psi(\phi)(a-a_0(\phi))^2+2k(\phi)(c-c_0(\phi))^2, \quad (4)$$

$$f_{\text{mix}}(\phi)=\phi \ln(\phi)+(1-\phi)\ln(1-\phi), \quad (5)$$

where in Eq. (5) and hereon all energies will be measured in units of $k_B T$ (k_B is Boltzmann's constant, T is the Temperature). In Eq. (4), the elastic energy is expressed as a power series up to second order in a and c , where $\psi(\phi)$ is the stretching constant of the monolayer, $k(\phi)$ is the bending rigidity with respect to a spherical deformation, $c_0(\phi)$ denotes the spontaneous curvature of a spherically bent monolayer, and $a_0(\phi)$ is the equilibrium area. All these constants may be functions of composition. In Eq. (4), the dividing surface is chosen to be the neutral surface, corresponding to the absence of a mixed term $\sim(a-a_0)(c-c_0)$ in Eq. (4). We note that k and c_0 are simply related to the canonical mean curvature modulus k' and the spontaneous curvature c'_0 in the Helfrich¹⁶ form of the bending free energy $f_{\text{bend}}=(1/2)k'(c_1+c_2-c'_0)^2+\bar{k}'c_1c_2$. Namely, $k=k'+\bar{k}'/2$ and $c_0=2c'_0k'/k$.

The mixing entropy term (5) always favors a disordered state of the system. Later, it will be argued that in a mixture of long and short lipids, both having the same head group structure, the average area per molecule (measured at the neutral surface) is nearly the same for both components. Therefore, in Eq. (5) we have expressed f_{mix} in terms of the mole fractions rather than area fractions.

We denote the distance of the bilayer midplane from the neutral surface, of both the external and internal monolayer by ξ . For nonsymmetric bilayers this definition fixes the position of the midplane to be in the middle between the monolayers neutral surfaces.

Taking into account first order changes in ξc^{bl} of the area and curvature at distance ξ from the midplane, we write

$$f_{\text{bl}}(a^{\text{bl}},c^{\text{bl}},m,\chi,\lambda) \\ = (1+\chi)f_{\text{ml}}\left[a^{\text{bl}}\frac{1+2c^{\text{bl}}\xi}{1+\chi},c^{\text{bl}}(1-c^{\text{bl}}\xi),\frac{m+\lambda}{1+\chi}\right] \\ + (1-\chi)f_{\text{ml}}\left[a^{\text{bl}}\frac{1-2c^{\text{bl}}\xi}{1-\chi},-c^{\text{bl}}(1+c^{\text{bl}}\xi),\frac{m-\lambda}{1-\chi}\right]. \quad (6)$$

In writing this equation we have noted that the compositions of the external and internal monolayers are given by $\phi^E=(m+\lambda)/(1+\chi)$ and $\phi^I=(m-\lambda)/(1-\chi)$, respectively, and that the curvatures of the two monolayers have opposite signs.

To a good approximation the area per molecule in the monolayer is kept constant during a bending deformation. From Eq. (6) we see that to first order in ξc^{bl} this condition is fulfilled by coupling the number of molecules to curvature through $\chi=2c^{\text{bl}}\xi$. It can be shown that this condition is satisfied if $c_0k/(a_0^2\psi\xi)\ll 1$. Later on, in Sec. V we confirm this assumption by molecular calculations.

Adopting the constant area condition, we find that the area and curvature changes of the *bilayer* are decoupled. Setting $a^{\text{bl}}=a_0^{\text{bl}}(m,\lambda)$ and $a=a_0(\phi)$ (for each monolayer), the bilayer and monolayer free energies are area independent. Denoting

$$\tilde{f}_{\text{bl}}(c^{\text{bl}},m,\lambda)=f_{\text{bl}}(a_0^{\text{bl}}(m,\lambda),c^{\text{bl}},m,2c^{\text{bl}}\xi,\lambda), \\ \tilde{f}_{\text{ml}}(c,\phi)=f_{\text{ml}}(a_0(\phi),c,\phi). \quad (7)$$

we find that, to first order in $c^{\text{bl}}\xi$ and λ , Eq. (6) reads

$$\tilde{f}_{\text{bl}}(c^{\text{bl}},m,\lambda)=(1+2c^{\text{bl}}\xi)\tilde{f}_{\text{ml}}(c^{\text{bl}}(1-c^{\text{bl}}\xi),m+\lambda-2c^{\text{bl}}\xi) \\ + (1-2c^{\text{bl}}\xi)\tilde{f}_{\text{ml}}(-c^{\text{bl}}(1+c^{\text{bl}}\xi),m-\lambda \\ + 2c^{\text{bl}}\xi). \quad (8)$$

From the symmetry of \tilde{f}_{bl} it follows immediately that the symmetric planar bilayer ($c^{\text{bl}}=0, \lambda=0$) corresponds to a free energy extremum. Whether this state is stable or not, can be determined by the thermodynamic stability conditions involving the second free energy derivatives

$$\tau(m)=\left(\frac{\partial^2\tilde{f}_{\text{bl}}}{\partial c^{\text{bl}}\partial\lambda}\right)_{\lambda=0,c^{\text{bl}}=0}, \\ \sigma(m)=\left(\frac{\partial^2\tilde{f}_{\text{bl}}}{\partial^2\lambda}\right)_{\lambda=0,c^{\text{bl}}=0}, \\ \kappa(m)=\left(\frac{\partial^2\tilde{f}_{\text{bl}}}{\partial^2c^{\text{bl}}}\right)_{\lambda=0,c^{\text{bl}}=0}. \quad (9)$$

The stability conditions are $\sigma>0$, $\kappa>0$, and $k_{\text{eff}}>0$ where the effective bending rigidity k_{eff} is defined by

$$\frac{1}{4a_0}k_{\text{eff}}(m)=\kappa(m)-\frac{\tau^2(m)}{\sigma(m)}. \quad (10)$$

This effective bending rigidity expresses the resistance of the monolayer against curvature deformations for optimal

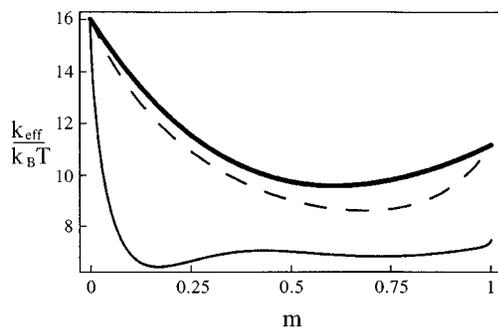


FIG. 1. Effective bending rigidity k_{eff} of a C-16/C-8 bilayer as a function of the short chain mole fraction according to Eq. (10); for $k_L = 8k_B T$, $\rho = 0.7$, $a_0 = 30 \text{ \AA}^2$, and $s = 0.02 \text{ \AA}^{-1}$. Thick line: no coupling ($k_{\text{eff}} = 2k$). Dashed line: limit of vanishing monolayer thickness. Full line: finite monolayer thickness.

coupling of changes in λ to changes in curvature given by $\lambda = -\tau c / \sigma$. Note that k_{eff} reduces to $2k$ in Eq. (4) when there is no coupling between λ and c .

The stability condition can be expressed equivalently in terms of an effective resistance σ_{eff} against changes in λ , that is

$$\sigma_{\text{eff}}(m) = \sigma(m) - \frac{\tau^2(m)}{\kappa(m)} > 0. \quad (11)$$

There are still the two unknown functions, $k(\phi)$ and $c_0(\phi)$, contained in k_{eff} or σ_{eff} . The main part of this paper presents a simple phenomenological model and a molecular calculation for determining $c_0(\phi)$ and $k(\phi)$. It will be shown that for a rather wide range of ϕ values ($\phi < 0.5$), c_0 varies roughly linearly with ϕ

$$c_0(\phi) = s\phi, \quad (12)$$

where s is a constant (smaller than the spontaneous curvature of the short chain component, i.e., $s < c_0(\phi = 1)$).

The expression for $k(\phi)$ that will be derived in Sec. III using the phenomenological compression model is

$$k(\phi) = k_L [\phi \rho^3 + (1 - \phi)(\phi \rho + (1 - \phi))^4], \quad (13)$$

with $\rho = (k_C / k_L)^{1/3}$, where k_L and k_C denote the bending rigidities of the long and short chain components.

We use Eqs. (12) and (13) to illustrate the softening effect of the composition degree of freedom λ . Reasonable values for mixtures of say 16 and 8 carbon chains (C-16/C-8) are $k_L = 8k_B T$, $\rho = 0.7$, $a_0 = 30 \text{ \AA}^2$, and $s = 0.02 \text{ \AA}^{-1}$. The last value implies a radius of curvature of 50 \AA for the pure short chain monolayer if extrapolation of (12) to $\phi = 1$ holds true. Figure 1 shows the effective bending rigidity as a function of the short chain mole fraction in the bilayer. One calculation takes explicitly into account the finite thickness of the monolayer, ξ . The other corresponds to the limit $\xi \rightarrow 0$, often taken as a first order approximation in phenomenological models.^{6,7,12} The thickness of the mixed monolayer, corresponding to the distance of the neutral surface from the bilayer midplane, is taken as $\xi \approx d$, (see Sec. V). In the $\xi \rightarrow 0$ limit the bilayer free energy, Eq. (8) becomes

$$\tilde{f}_{\text{bl}}(c^{\text{bl}}, m, \lambda) = \tilde{f}_{\text{m}}(c^{\text{bl}}, m + \lambda) + \tilde{f}_{\text{m}}(-c^{\text{bl}}, m - \lambda). \quad (14)$$

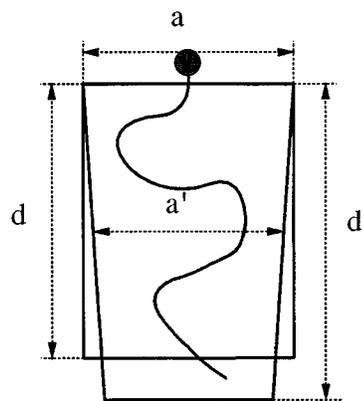


FIG. 2. Schematic representation of the average shape of a hydrocarbon chain in a monolayer and its changes upon bending. The fixed hydrocarbon-water interface is denoted by a . Curvature deformation leads to changes in the cross sectional area ($a \rightarrow a'$) and to a stretching deformation ($d \rightarrow d'$).

This expression was used, together with Eqs. (4) and (7) to obtain the dashed curve in Fig. 1. One of the curves in Fig. 1 describes $k_{\text{eff}}(m)$ with finite membrane effects explicitly taken into account. It has recently been shown⁸ that first order finite thickness corrections to the bending energy enter only through the saddle-splay (Gaussian) modulus and not through the splay (mean curvature) modulus in the Helfrich expression. Recall that the bending constant corresponding to a spherical deformation introduced in Eq. (4) indeed contains \bar{k}' , the saddle splay modulus. We note from Fig. 1 that the finite thickness corrections strongly reduce k_{eff} , especially for small m values. These should be attributed to \bar{k}' . Most importantly we note that even with the finite thickness correction included, k_{eff} remains positive for all m values. Thus at least according to our model calculations, spontaneous vesiculation in a randomly mixed bilayer of different chain lengths is not a likely possibility.

Finally we note that Eq. (14) can be used to derive general expressions for k_{eff} and σ_{eff} . For example,

$$2\sigma_{\text{eff}} = \frac{1}{m(1-m)} - 2a_0 c_0(m)^2 k(m)^2 \frac{d^2}{dm^2} \left(\frac{1}{k(m)} \right) + 4a_0 c_0(m) k(m) \frac{d^2 c_0(m)}{dm^2}. \quad (15)$$

III. COMPRESSION MODEL FOR A MIXED MONOLAYER

The average shape of a hydrocarbon chain in a planar monolayer is that of a cylinder of length d , equal to the monolayer thickness, and cross sectional area a . Upon bending, the shape of the volume occupied (on the average) by the chain becomes wedgelike (for cylindrical deformation) or truncated conelike (for spherical deformation). Thus the splaylike deformation of the chain due to bending can be related to the changes in its average length, from d to d' , and its area, from a to a' ; see Fig. 2. These considerations, together with the assumption that the hydrophobic region is incompressible have been used¹⁷ to derive a simple scaling relation for the bending rigidity in terms of the chain length

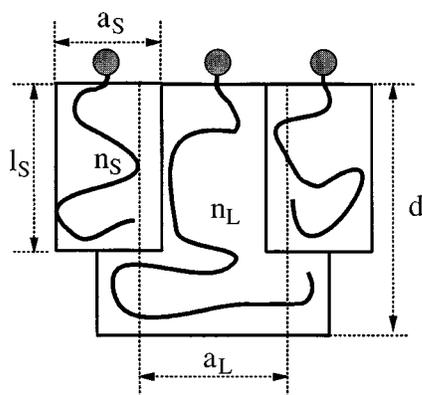


FIG. 3. Schematic representation of a mixed planar monolayer. The monolayer is divided into two regions; a mixed chain region of thickness l_S and a long chain region of thickness $d - l_S$. The average cross sectional area per molecule in the mixed chain region is denoted by a_S . The long chain has an average cross sectional area a_L in the monolayer. n_S and n_L are the short and long chain volumes, respectively.

n and the area a , for pure surfactant layers. In this section we generalize these ideas to derive a simple scaling form for $k(\phi)$ and $c_0(\phi)$, for mixed layers.

A. Chain contribution to the bending rigidity

We begin with the long chain limit for a flat, one component, monolayer. The free energy per molecule f_{ml} of chain stretching can be derived based on random walk arguments.¹⁸ It scales as

$$f_{ml} \sim \frac{d^2}{n} \sim \frac{n}{a'^2}, \quad (16)$$

where d is the monolayer thickness, n is the number of chain segments, and a' the area per chain measured, say, in the middle of the chain region. Since the hydrophobic core is incompressible $ad = a'd' = n\nu$ with ν denoting the volume per chain segment (hereafter $\nu = 1$) and the other quantities as defined above, see Fig. 2. We assume that the neutral surface coincides with the hydrocarbon-water interface, so that $a = a_0$ is kept constant in curvature deformations. For a monolayer of curvature c we have $a' \approx a(1 - dc/2)$. Then, comparing the scaling relation (16) to $f_{ml}/a = (k/2)(c - c_0)^2$ one finds¹⁷

$$k \sim \frac{n^3}{a^5}. \quad (17)$$

Consider now the mixed monolayer. It can be roughly divided into two regions, of thickness l_S and $d - l_S$. The first is occupied by both long and short chains, the other only by long chains, as shown in Fig. 3.

Let a_L and a_S denote the average cross sectional areas of the long and short chains, respectively. The average is taken over the whole monolayer for the long chain component, and only over the mixed chain region for the short chains. Chain incompressibility implies $a_S l_S = n_S$ and $a_L d = n_L$. Let a be the average interfacial area per head group. Then, compactness of the monolayer requires $ad = \bar{n}$, where $\bar{n} = \phi n_S$

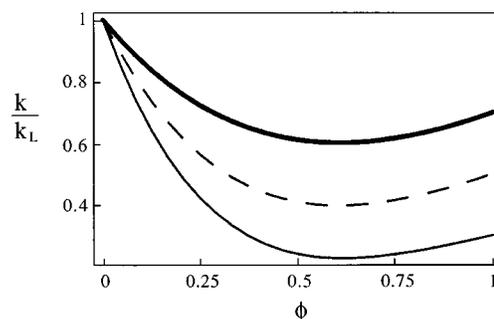


FIG. 4. Normalized bending rigidity k/k_L as a function of composition ϕ according to Eq. (13) for: $\rho = 0.3$ (full line), $\rho = 0.5$ (dashed line), and $\rho = 0.7$ (thick line).

$+ (1 - \phi)n_L$ is the average number of segments per chain. The average free energy per chain f_{ch} may be written as

$$f_{ch} \sim \phi \frac{n_S}{a_S'^2} + (1 - \phi) \frac{n_L}{a_L'^2}. \quad (18)$$

Here, a_S' is the short chain cross sectional area measured in the middle of the mixed chain region. Similarly, a_L' is the average area of the long chain, measured in the middle of the monolayer. We consider now a bent monolayer. As above, we assume the interfacial area per molecule to be constant. Changes in the cross sectional areas are determined geometrically, implying $a_S' \approx a_S(1 - l_S c/2)$ and $a_L' \approx a_L(1 - dc/2)$. This leads to the scaling

$$ak(\phi) \sim \phi l_S^2 \frac{n_S}{a_S'^2} + (1 - \phi) d^2 \frac{n_L}{a_L'^2}. \quad (19)$$

Molecular calculations of chain packing in mixed bilayers¹⁹ reveal that the average areas of long and short chain near the interface are roughly the same. Therefore, we use the approximation $a_S = a$, which leads to $a_L = a n_L / \bar{n}$. Using these expressions for a_S and a_L in Eq. (19) yields the scaling form (13) for the bending rigidity of the mixed monolayer. Note that Eqs. (13) and (19) reduce to $k(\phi = 0) = k_L \sim n_L^3 / a^5$ and $k(\phi = 1) = k_S \sim n_S^3 / a^5$, as expected. The variation of the bending rigidity with ϕ is depicted in Fig. 4 for three different chain mixtures. It can be seen that inclusion of a small amount of short chain molecules is very effective in lowering k as already found experimentally and predicted by detailed molecular calculations.¹⁰

B. Spontaneous curvature

The elastic monolayer energy (4) can be separated into chain, head group, and interfacial contributions, $f_{el} = f_{ch} + f_{int} + f_{hg}$. The minimum of f_{el} with respect to c dictates the spontaneous curvature. For f_{int} we use the simple form $f_{int} = \gamma a_{int}$ with $\gamma = 0.12 kT / \text{\AA}^2$ denoting the effective hydrocarbon water surface tension,²⁰ and a_{int} is the area per chain measured at the interface. The chain contribution was already discussed in the previous section. For simplicity we assume that chain repulsions are concentrated in only one interaction plane for each chain type, as in Eq. (18). In the system considered here, all molecules have the same head

group structure, thus the head group interaction term is independent of composition. But even in this case, head group interactions are difficult to model. Therefore, quite often head group interactions are written as a series expansion²⁰ in $1/a_{\text{hg}}$. In order to keep the system analytically solvable we chose the form $f_{\text{hg}} \sim 1/a_{\text{hg}}^2$, where a_{hg} is the head group area per molecule. A similar approximation has been used by Ennis.²¹ Using B to measure the head group interaction strength and β the proportionality constant in Eq. (18) we write for the elastic energy

$$f_{\text{el}} = \gamma a_{\text{int}} + \frac{B}{a_{\text{hg}}^2} + \beta \left(\phi \frac{n_S}{a_S'^2} + (1 - \phi) \frac{n_L}{a_L'^2} \right). \quad (20)$$

Let b , h_L , and h_S denote the distances of the head group, long chain, and short chain interaction planes from the interface, respectively. Also, let ζ denote the distance between the interface and the neutral surface, taken to be positive if the neutral surface lies inside the chain region. The areas per molecule at the planes of interaction change as a function of the (spherical) curvature c and the area of the neutral surface a according to

$$\begin{aligned} a_{\text{hg}} &= a(1 + 2c(\zeta + b) + c^2(\zeta + b)^2), \\ a_{\text{int}} &= a(1 + 2c\zeta + c^2\zeta^2), \\ a_S' &= a(1 + 2c(\zeta - h_S) + c^2(\zeta - h_S)^2), \\ a_L' &= a \frac{n_L}{\bar{n}} (1 + 2c(\zeta - h_L) + c^2(\zeta - h_L)^2), \end{aligned} \quad (21)$$

where we have used again the approximation $a_S = a$ and $a_L = a n_L / \bar{n}$. The free energy Eq. (20) is now used to find the elastic constants in Eq. (4), as well as a_0 and c_0 as a function of composition.

Treating the monolayer as a part of a slightly bent bilayer, we expand Eq. (20) with respect to c and a around $c=0$ and $a=a_0$. This yields

$$\begin{aligned} a_0^3 &= \frac{2}{\gamma} G_0, \quad c_0 = \frac{G_1 G_0}{5G_2 G_0 - 4G_1^2}, \\ k &= \frac{\gamma}{G_0} \left(5G_2 - \frac{G_1^2}{G_0} \right), \quad \psi = \frac{\gamma}{3a_0'^3}, \end{aligned} \quad (22)$$

where we have defined

$$\begin{aligned} G_i &= B b^i + \beta \left[\phi n_S (-h_S)^i + (1 - \phi) \frac{\bar{n}^2}{n_L} (-h_L)^i \right]; \\ i &= 1, 2, 3. \end{aligned} \quad (23)$$

We also find that the position of the neutral surface is given by $\zeta = -(2G_1)/(3G_0)$.

To implement Eq. (20) we need to choose a_S' and a_L' . A reasonable choice for the chain interaction planes is the position of maximal lateral pressure for each component inside the hydrophobic core.¹⁰ Molecular level calculations reveal that this plane remains, roughly, at the same position for all chain lengths. We use this fact to keep the chain interaction planes simply at constant distance h from the hydrocarbon-water interface, namely,

$$h_S = h_L = h. \quad (24)$$

We find the remaining constants in such a way that for a pure long chain system $\bar{a}_L = a_0(\phi=0)$, $\bar{c}_L = c_0(\phi=0)$, and $k_L = k(\phi=0)$ adopt certain given values (see Sec. V). From Eqs. (22) and (24) we find

$$k_L = 2\gamma b h / 5. \quad (25)$$

Now, using Eqs. (22), (24), and (25) all for $\phi=0$, we obtain for the remaining unknown constants

$$\begin{aligned} h &= \frac{2k_L}{5b\gamma}, \\ B &= \frac{\bar{a}_L^3 \gamma h}{2(b+h)}, \\ \beta &= \frac{\bar{a}_L^3 \gamma b}{2n_L(b+h)}. \end{aligned} \quad (26)$$

The volumes of the acyl chains, n_S and n_L in Eqs. (20) and (23) are calculated using the common formula for $-(\text{CH})_{2N-1}-\text{CH}_3$ chains: $n = (N+1)\nu$ with $\nu = 27 \text{ \AA}^3$.¹⁰ Once, k_L , b , and \bar{a}_L are determined the other relevant parameters, h , B , and β can be calculated from Eq. (26), for all combinations of chain lengths. Calculations based on this procedure are reported in Sec. V.

IV. MOLECULAR MEAN-FIELD CALCULATIONS

In this section we outline a detailed molecular theory of chain packing in amphiphilic aggregates, which in Sec. V is used to calculate the elastic properties of the monolayers composing a bilayer.²² The sole assumption of this mean-field theory is that the hydrophobic bilayer core is liquidlike and hence uniformly packed by chain segments. Our goal is to apply the molecular chain packing theory to calculate the spontaneous curvature $c_0(\phi)$ and bending rigidity $k(\phi)$ of the monolayer. To this end we must also include in our free energy expression the contributions due to head group repulsion, surface tension and the mixing entropy. Thus we write $f_{\text{ml}} = f_{\text{ch}} + f_{\text{hg}} + f_{\text{int}} + f_{\text{mix}}$. All these terms except f_{ch} are calculated as in the previous section. The calculation of the chain contribution is outlined next.

A. Theoretical background

The average conformational free energy per molecule in the mixed bilayer is given by

$$\begin{aligned} f_{\text{ch}}^{\text{bl}} &= (1 + \chi) [\phi^E f_{\text{ch},S}^E + (1 - \phi^E) f_{\text{ch},L}^E] + (1 - \chi) \\ &\quad \times [\phi^I f_{\text{ch},S}^I + (1 - \phi^I) f_{\text{ch},L}^I] \\ &= (1 + \chi) f_{\text{ch}}^E + (1 - \chi) f_{\text{ch}}^I, \end{aligned} \quad (27)$$

where $f_{\text{ch},L}^E$ is the (composition dependent) free energy per long chain in the external monolayer, etc. In the mean-field theory $f_{\text{ch},L}^E$ is given by¹⁹

$$f_{\text{ch},L}^E = \sum_{\alpha_L} P_L^E(\alpha_L) [\tilde{\epsilon}(\alpha_L) + P_L^E(\alpha_L) \ln P_L^E(\alpha_L)]. \quad (28)$$

Here, $P_L^E(\alpha_L)$ denotes the probability of finding a long chain in the external layer in conformation α_L . $\tilde{\epsilon}(\alpha_L)$ is the inter-

nal, *trans/gauche*, energy for conformation α_L . The expressions for $f_{\text{ch},S}^E$ as well as for $f_{\text{ch},L}^I$ and $f_{\text{ch},S}^I$ are defined analogously. The chains must fill up the volume of the hydrophobic region, which changes its shape in the course of a bending deformation. This shape can be expressed by a function $a(x)$ where $a(x)dx$ is the volume of a thin shell $x, x+dx$; x denoting the normal distance of this shell from the bilayer midplane. For a spherically bent bilayer with curvature $c^{\text{bl}}=c_1^{\text{bl}}=c_2^{\text{bl}}$, we have

$$a(x) = a^{\text{bl}}(1 + 2c^{\text{bl}}x + (c^{\text{bl}}x)^2). \quad (29)$$

We denote by $\Phi_L^E(x, \alpha_L)$ the number of segments belonging to a long chain in conformation α_L originating at the external monolayer. Analogously we define $\Phi_S^E(x, \alpha_S)$, $\Phi_L^I(x, \alpha_L)$, and $\Phi_S^I(x, \alpha_S)$. Summing up over all possible conformations gives the average number of segments at position x , that is

$$\langle \Phi_L^E(x) \rangle = \sum_{\alpha_L} P_L^E(\alpha_L) \Phi_L^E(x, \alpha_L). \quad (30)$$

With these definitions, the packing constraints on the singlet probability distributions, $P_L^E(\alpha_L)$, etc., are

$$a(x) = (1 + \chi) [\phi^E \langle \Phi_S^E(x) \rangle + (1 - \phi^E) \langle \Phi_L^E(x) \rangle] \\ + (1 - \chi) [\phi^I \langle \Phi_S^I(x) \rangle + (1 - \phi^I) \langle \Phi_L^I(x) \rangle]. \quad (31)$$

Minimization of $f_{\text{ch}}^{\text{bl}}$ for all $-d \leq x \leq d$ subject to Eq. (31) yields

$$P_L^E(\alpha_L) = \frac{1}{q_L^E} \exp \left[-\tilde{\epsilon} - \int_{-d}^d \pi(x) \Phi_L^E(x, \alpha_L) dx \right], \text{ etc.} \quad (32)$$

Here, the Lagrange multipliers $\pi(x)$ represent the lateral pressure profile that forces the chains to adopt, on average, a geometry dictated by Eq. (29). In Eq. (32), q_L^E is the partition function of the long chain component in the external monolayer. The lateral pressure can be evaluated by substituting Eq. (32) into Eq. (30) and this into the packing constraints (31). The result is an integral (self-consistency) equation for $\pi(x)$ that can be solved numerically. For this, a convenient procedure is to divide the bilayer core into a number of layers (usually of width $\approx 1.5 \text{ \AA}$). All possible bond sequences for a given chain length are generated according to the RIS (rotational isomeric state) scheme.²³ For each bond sequence, some (usually 20–30) combinations of the overall chain orientation and head group altitude (randomly chosen within a region of $\pm 1 \text{ \AA}$ from the interface) are sampled. Then, each conformation for both the long and short chain is classified according to its segment distribution and internal energy. Given this chain model, the only input parameters in numerically solving the integral equation are a^{bl} and c^{bl} .

After inserting $P_L^E(\alpha_L)$, etc., back into Eq. (28), the chain free energy per molecule in the external monolayer f_{ch}^E , as defined in Eq. (27), becomes

$$f_{\text{ch}}^E = \phi^E \left(-\ln q_S^E - \int_{-d}^d \pi(x) \langle \Phi_S^E(x) \rangle dx \right) + (1 - \phi^E) \\ \times \left(-\ln q_L^E - \int_{-d}^d \pi(x) \langle \Phi_L^E(x) \rangle dx \right). \quad (33)$$

Note that $f_{\text{ch}}^E(a^{\text{bl}}, c^{\text{bl}}, \phi^E)$ is expressed as a function of the area per molecule and the curvature, both measured at the bilayer midplane. If measured with respect to area and curvature at the position of the neutral surface of the monolayer, we may use $f_{\text{ch}}^E(a^{\text{bl}}, c^{\text{bl}}, \phi^E) = f_{\text{ch}}(a, c, \phi)$ as the chain contribution to the monolayer free energy. This can be compared to Eq. (4) in order to find c_0 and k as a function of composition.

As already pointed out, we use a $1/a^2$ dependence for the head group interactions. Then the free energy per molecule in the monolayer is

$$f_{\text{ml}}(a, c, \phi) = \gamma a_{\text{int}} + \frac{B}{a_{\text{hg}}^2} + f_{\text{ch}}(a, c, \phi) + f_{\text{mix}}(\phi). \quad (34)$$

The elastic part in this free energy (i.e., its first three terms) is analogous to the compression model free energy (20), except that here the chain contribution is calculated in detail. The area per molecule at the interface and the head group interaction plane are given by Eq. (21)

V. RESULTS AND ANALYSIS

In this section we present numerical calculations for the bending rigidity $k(\phi)$ and the spontaneous curvature $c_0(\phi)$ as a function of composition in different mixtures of long and short chain amphiphiles, ranging from C-6 to C-16. We first discuss the results obtained using the molecular (mean-field) calculations and then compare them to the compressional model.

In the mean-field treatment the calculations include the entire bilayer, but we focus on the free energy of one of its constituent monolayers.

Once the elastic characteristics of such a monolayer are known, the bilayer energy can be evaluated via Eq. (6). In most of the continuum models the elastic energy of the monolayer is represented by a quadratic expansion in curvature $f_{\text{el}}/a_0 = 2k(c - c_0)^2$. Here, c_0 is the curvature for which the monolayer energy is minimal. This expression provides a reasonable approximation to the monolayer free energy in a planar bilayer provided c_0 is small, that is $|c_0 d| \ll 1$ where d is the monolayer thickness. Note that kc_0 and $2k$ are the coefficients of the linear and quadratic terms of f_{el} . If $|c_0 d|$ is not small, the elastic free energy of a moderately bent (i.e., $c \approx 0$) monolayer can still be expanded in power series of c , but now around $c=0$. Now the coefficients of c^2 and c can be used to define an *apparent* bending rigidity and spontaneous curvature, but these will differ from the k and c_0 corresponding to expansion around the spontaneous geometry. Most of the results presented below for $k(\phi)$ and $c_0(\phi)$ refer to those *apparent* curvature elastic constants. Eventually we will also compare the apparent and “real” quantities.

In all our molecular calculations we have fixed the head group interaction parameters B and b at $B = 1300 k_B T \text{ \AA}^4$

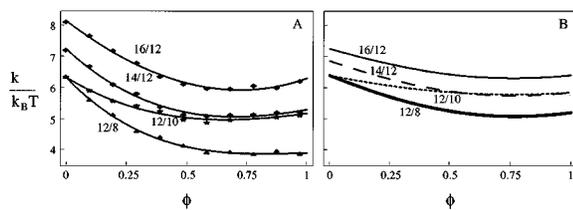


FIG. 5. Bending rigidity as a function of ϕ of a mixed monolayer, for several combinations of long and short chains. (A) Molecular calculations. (B) Compression model calculations.

and $b=2.25 \text{ \AA}$. These values were chosen to calibrate a pure C-12 monolayer to have vanishing spontaneous curvature. This implies that pure monolayers composed of longer chains will have negative spontaneous curvature (stronger chain repulsion). Similarly, monolayers of shorter chains will have positive spontaneous curvatures. Of course, choosing another chain length to have zero spontaneous curvature would simply shift the set of results but would not modify the qualitative trends.

Using the above numerical values of b and B for C-12 chains also imply an equilibrium area per chain of 32.6 \AA^2 . We may use these values as input parameters in the compressional model. In this model, as in the molecular calculations we assume that a C-12 monolayer has a vanishing spontaneous curvature. From Eq. (26) we obtain a head group interaction parameter $B = 1600 k_B T \text{ \AA}^4$ which is somewhat higher than the one used in the molecular calculations.

The apparent bending rigidity of several different combinations of mixed monolayers are shown in Fig. 5. The corresponding spontaneous curvatures of these mixtures, and several additional ones are shown in Fig. 6. The figures contrast the results obtained from the detailed molecular calcu-

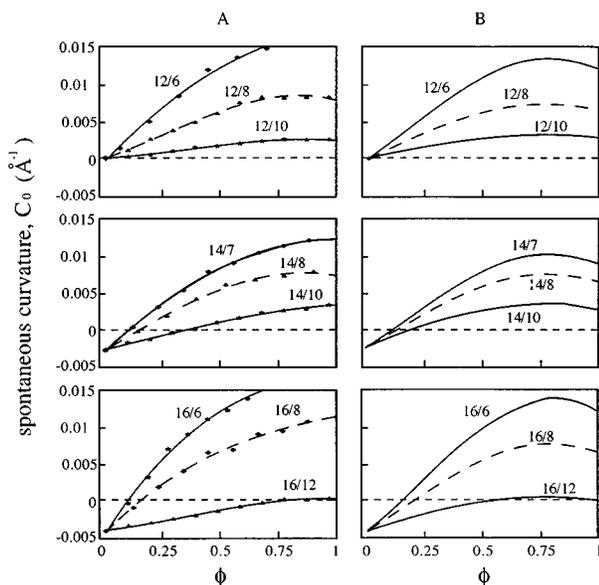


FIG. 6. Spontaneous curvature as a function of ϕ for a mixed monolayer, for several combinations of long and short chains. (A) Molecular calculations. (B) Compression model calculations.

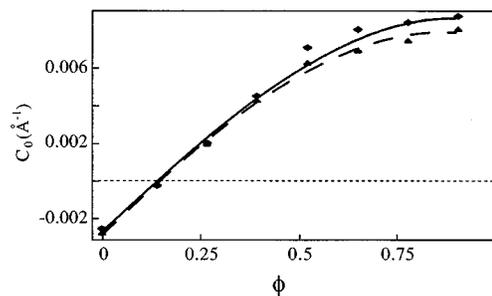


FIG. 7. Comparison of real (diamonds) and apparent (triangles) spontaneous curvature in a C-16/C-8 monolayer as a function of ϕ .

lation and the simplified compression model. We note that despite the rather crude approximations involved in formulating the compression model, its predictions reveal reasonably good agreement with the more detailed molecular calculations.

The main result of our calculations involves the composition (ϕ) dependence of the monolayer spontaneous curvature $c_0(\phi)$, as shown in Fig. 6. As noted in previous sections, it is often assumed in phenomenological models^{6,8,14} of membrane elasticity that $c_0(\phi)$ varies linearly with ϕ ; in our case from $c_0^L = c_0(\phi=0)$ to $c_0^S = c_0(\phi=1)$. From Fig. 6 it is apparent that a linear variation of $c_0(\phi)$ is indeed a reasonable approximation for low, up to moderate, mole fractions of the short chain component, especially for mixtures with small chain length disparity. At higher values of short chain mole fractions $c_0(\phi)$ exhibits a saturation behavior, often passing through a weak maximum on the way to $c_0(\phi=1) = c_0^S$. From Fig. 6, which shows $k(\phi)$ vs ϕ we note that a similar behavior characterizes the bending rigidity. After a rapid falloff of $k(\phi)$ at low ϕ values the bending rigidity approaches, slowly, the short chain limit $k(\phi=1) = k_S$.

From these results it follows that adding a small amount of short chains into a layer of long ones modifies the elastic behavior of the film to a considerably larger extent than in the opposite case (of adding long chains to a short chain monolayer). The lowering of the bending rigidity of long chain amphiphilic films by adding short chain (cosurfactant) amphiphiles has already been noted theoretically¹⁰ (also for diblock copolymer bilayers^{11,12}) and observed experimentally.⁹ On the other hand, our findings concerning the ϕ dependence of c_0 is, to our knowledge, new.

An approximate, qualitative explanation of the ϕ dependencies of $c_0(\phi)$ and $k(\phi)$ may be given as follows. The short chain amphiphiles incorporated into a layer of long chains can be visualized as wedges, or spacers, pushing apart the head groups of the long amphiphiles and the first few chain segments (those connected to the head groups). They hardly affect the terminal parts of the long chains. Accordingly, the monolayer tends to bend 'inwards', towards the hydrophobic region, implying a (positive) increase in the monolayer spontaneous curvature. Also, beyond the region where the short and long chains overlap (i.e., away from the interface), the average cross sectional area of the long chains increases (see Fig. 3) implying a rapid decrease in k with ϕ .

TABLE I. Bending rigidities and spontaneous curvatures for pure monolayers of different chain length. k^c and c_0^c are derived from the compression model, k^m and c_0^m refer to the molecular calculations.

Chain	C-7	C-8	C-10	C-12	C-14	C-16
$k^c/k_B T$	4.8	5.2	5.9	6.4	6.9	7.3
$k^m/k_B T$	3.8	4.2	5.2	6.4	7.2	8.1
c_0^c (\AA^{-1})	0.0090	0.0065	0.0030	0	-0.0022	-0.0040
c_0^m (\AA^{-1})	0.0120	0.0080	0.0030	0	-0.0020	-0.0040

On the other hand, when a small amount of long chain amphiphiles are incorporated into a short chain monolayer, their terminal parts will protrude beyond the short chain regime. (In a bilayer the protruding segments will interdigitate into the opposite monolayer and/or occupy the central region of the bilayer, around the midplane, see Fig. 3). Thus, the area per chain and the lateral pressure profile in the short chain regime will hardly be affected, and one does not expect a significant change in $c_0(\phi)$ or $k(\phi)$ as ϕ begins to fall below 1.

Another result of our calculations concerns the changes in molecular areas during bending deformations. Recall that in our analysis of the bilayer stability with respect to bending deformations (Sec. II) it was assumed that the average cross sectional area per molecule is kept at its equilibrium value, independent of composition. The results shown in Fig. 1 are based on this assumption. The constant area assumption has not been employed in our compression model nor in the molecular mean-field calculations. Namely, the area has been allowed to adjust so as to minimize the curvature free energy. Yet, in both types of calculations it was found that the constant area assumption is fulfilled to a high accuracy, and for all compositions. Typically, when $a = a_0 \approx 30 \text{ \AA}^2$ for the planar layer the variations in a during bending were no more than $\Delta a \sim 0.5 \text{ \AA}^2$.

Earlier in this section we have commented on the difference between the real and apparent spontaneous curvatures and bending rigidities. In Fig. 7 we compare the apparent spontaneous curvature and the real one. Since the curvatures involved are small compared to the thickness of the monolayer we expect the quadratic expansion of the free energy with respect to curvature to be a good approximation. In Fig. 7 we show, for the case of a C-14/C-8 mixture that this is indeed the case.

Another interesting aspect of the calculations concerns the chain length dependence of the elastic properties of pure monolayers. In Table I we present the numerical values obtained using the compression model and the mean-field theory for the bending moduli and spontaneous curvatures of pure C-7 to C-16 monolayers.

Apart from the satisfactory agreement between the two types of calculations we note that k increases roughly linearly with chain length. This should be contrasted with the n^3 dependence of the chain contribution to the bending rigidity suggested by the compressional model, see Eq. (17). The difference should be attributed to the inclusion of the head group and interfacial contributions to the bending free energy. Qualitatively, since these two contributions are independent of chain length, they obviously moderate the strong

n dependence implied by the chain calculations alone. The trends observed for the spontaneous curvature are quite obvious. Calibrating c_0 to be zero for C-12 chains, it is expected that as the chain length (and hence chain repulsion) increases beyond $n=12$ the monolayer would tend to bend negatively, with an opposite trend for $n < 12$.

VI. CONCLUDING REMARKS

In Sec. I we have presented a thermodynamic stability analysis to examine the possibility of spontaneous vesicle formation in mixed bilayers composed of amphiphiles of different chain length. Using reasonable molecular values for head group and chain interactions we concluded that a second order transition from a planar to a curved vesicle geometry in such systems is unlikely in a randomly mixed bilayer. However, this does not exclude the possibility of spontaneous vesiculation in nonideally mixed layers. It would therefore be of interest to formulate a molecular level model which may predict nonideal chain mixing and subsequent lateral phase separation in mixed amphiphilic monolayers. It is also possible that the bilayer to vesicle transition will be of first order, especially in view of the experiments reported in Ref. 4, where the two aggregation geometries were found to coexist in solution. Theoretical calculations of first order transformations have recently been presented for the vesicle to micelle^{14,16} transition and could in principle be also applied to the transition from a planar bilayer to a vesicle.

As noted at several junctures in previous sections the spontaneous curvature of mixed amphiphilic layers is often modeled as a linear combination of the spontaneous curvatures of the pure components. Our molecular level calculations suggest that this is a reasonable approximation up to moderate short chain mole fractions, with a saturation behavior towards the pure short chain limit. These results, apart of being of interest in their own right, could be implemented in subsequent phenomenological models of mixed amphiphilic layers.

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- ¹ See, for example, C. Tanford, *The Hydrophobic Effect*, 2nd ed. (Wiley-Interscience, New York, 1980); J. N. Israelachvili, *Intermolecular and Surface Forces* (Academic, New York, 1985).
- ² G. Porte, J. Appell, P. Bassereau, and J. Marignan, *J. Phys. (Paris)* **50**, 1335 (1989).
- ³ E. W. Kaler, A. K. Murthy, B. E. Rodriguez, and J. A. N. Zasadzinski, *Science* **245**, 1371 (1989).
- ⁴ N. E. Gabriel and M. F. Roberts, *Biochemistry* **23**, 4011 (1984).
- ⁵ N. E. Gabriel and M. F. Roberts, *Biochemistry* **25**, 2812 (1986).
- ⁶ S. A. Safran, P. A. Pincus, and D. Andelman, *Science* **248**, 354 (1990).
- ⁷ S. A. Safran, P. A. Pincus, D. Andelman, and F. C. MacIntosh *Phys. Rev. A* **43**, 1071 (1991).
- ⁸ G. Porte and C. Ligoure *J. Chem. Phys.* (to be published).
- ⁹ C. R. Safinya, E. B. Sirota, D. Roux, and G. S. Smith *Phys. Rev. Lett.* **62**, 1134 (1989). See also P.-O. Quist, *Langmuir* (to be published).
- ¹⁰ I. Szleifer, D. Kramer, A. Ben-Shaul, W. M. Gelbart, and S. A. Safran, *J. Chem. Phys.* **92**, 6800 (1990).
- ¹¹ N. Dan and S. A. Safran, *Europhys. Lett.* **21**, 975 (1993).
- ¹² Z.-G. Wang *Macromolecules* **25**, 3702 (1992).
- ¹³ S. Almog, B. J. Litman, W. Wimley, J. Cohen, E. J. Wachtel, Y. Barenholz, A. Ben-Shaul, and D. Lichtenberg *Biochemistry* **29**, 4582 (1990).
- ¹⁴ D. Andelman, M. M. Kozlov, and W. Helfrich *Europhys. Lett.* **25**, 231 (1994).
- ¹⁵ D. R. Fattal, D. Andelman and A. Ben-Shaul, *Langmuir* **11**, 1154 (1995).
- ¹⁶ W. Helfrich *Z. Naturforsch. Teil C* **28**, 693 (1973).
- ¹⁷ W. M. Gelbart and A. Ben-Shaul, in *Physics of Amphiphilic Layers*, edited by J. Meunier, D. Langevin, and N. Boccaro (Springer, New York, 1987), p. 9.
- ¹⁸ P. G. deGennes, *Scaling Concepts in Polymer Physics* (Cornell University, Ithaca, 1979).
- ¹⁹ I. Szleifer, A. Ben-Shaul, and W. M. Gelbart, *J. Chem. Phys.* **86**, 7094 (1987).
- ²⁰ J. N. Israelachvili, S. Marcelja, and R. G. Horn, *Q. Rev. Biophys.* **13**, 121 (1980).
- ²¹ J. Ennis, *J. Chem. Phys.* **97**, 663 (1992).
- ²² For reviews see: A. Ben-Shaul in *Structure and Dynamics of Membranes*, edited by R. Lipowsky and E. Sackmann (Elsevier, Amsterdam, 1995), Chap. 7; A. Ben-Shaul and W. M. Gelbart, in *Micelles, Membranes, Microemulsions and Monolayers*, edited by W. M. Gelbart, A. Ben-Shaul, and D. Roux (Springer, New York, 1994), Chap. 1
- ²³ P. J. Flory, *Statistical Mechanics of Chain Molecules* (Wiley-Interscience, New York, 1969).