

Elastic Constants of Polymer-Grafted Lipid Membranes

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ABSTRACT The surface expansion that is induced by the lateral pressure in the brush region of lipid membranes containing grafted polymers is deduced from the scaling and mean-field theories for the polymer brush, together with the equation of state for a lipid monolayer at the equivalence pressure with fluid lipid bilayers. Depending on the length and mole fraction of the polymer lipid, the membrane expansion can be appreciable. Direct experimental evidence for this lateral expansion comes from recent spin-label measurements with lipid membranes containing poly(ethylene glycol)-grafted lipids. The expansion in lipid area modifies the elastic constants of the polymer-grafted membranes in a way that opposes the direct elastic response of the polymer itself. Calculations as a function of polymer lipid content indicate that the net change in isothermal area expansion modulus of the membrane is negative but small, in contrast to previous predictions. A similar situation applies to the curvature elastic moduli of membranes containing short polymer lipids. For longer polymer lipids, however, the direct contribution of the polymer brush to the bending elastic constants dominates, and the increase in bending moduli with increasing polymer lipid content rapidly exceeds the basal values of the bare lipid membrane. The spontaneous (or intrinsic) curvature of the component monolayer of polymer lipid-containing membranes is calculated for the first time. The polymer brush contribution to spontaneous curvature scales quadratically with the polymer length, and at least quadratically with the mole fraction of polymer lipid.

INTRODUCTION

Lipids with hydrophilic polymers grafted at the polar head-group, e.g., the *N*-poly(ethylene glycol) derivatives of phosphatidylethanolamine (PEG lipids), now find extensive application as components of sterically stabilized liposomes that are designed as drug delivery systems (see, e.g., Lasic and Needham, 1995). The polymer brush region formed at the liposome surface serves to stabilize the liposome against interactions with serum proteins and diverse elements of the immune system, hence prolonging the lifetime in the circulation. In practice, ethylene glycol polymers that are used in grafting commonly have high molecular weights relative to those of the parent grafted lipid, e.g., PEG-2000 or PEG-5000 of molecular weights 2000 and 5000, respectively. In addition to their desired transverse repulsive interactions, they will therefore exert lateral interactions of considerable strength that are likely to modify the properties of the host liposomal membrane. This is typified by the known tendency of PEG lipids to induce micelle formation (see, e.g., Hristova and Needham, 1995; Belsito et al., 2000). Indeed, lateral expansion of lipid membranes resulting from the lateral pressure exerted within the surface polymer brush has recently been inferred directly by the increase in motional freedom of spin-labeled lipid chains in lipid membranes, on incorporating polymer-grafted lipids (Montesano et al., 2001). Such lateral expansion of the membrane surface area will also affect the elastic properties of the lipid

membrane. These are characterized specifically by the area expansion modulus, K_A , which plays a significant role in liposome permeability and by the curvature elastic moduli, k_c , which can affect both the morphology and stability of the lamellar liposomal membrane.

Both the area extension elasticity (Hristova and Needham, 1994) and the curvature elasticity (Milner and Witten, 1988; Hristova and Needham, 1994) have been considered previously. In the latter work, scaling laws were established for the direct contribution of the polymer brush to the elastic moduli. In both cases it was tacitly assumed that the lipid contribution to the elastic moduli was unchanged by the presence of the polymer brush. Because, as argued above, the grafted polymer exerts a lateral pressure on the lipids, this must not necessarily be the case. Recent work already mentioned has shown that the motional freedom of spin-labeled lipid chains is increased by the membrane admixture of polymer-grafted lipids with chainlength identical to that of the host lipids (see also Belsito et al., 2000). The purpose of this paper is to treat the membrane stretching and bending by including these effects on the lipids, and to estimate their relative importance. It is found that the lateral expansion induced by the polymer brush can be appreciable and that this has important consequences for the elastic expansion moduli, but less so for the elastic curvature moduli if the grafted polymers are relatively long. Spontaneous curvature of the component monolayers containing polymer-grafted lipids is also treated quantitatively for the first time.

RESULTS AND DISCUSSION

Lateral pressure in the polymer brush

The first question is whether the lateral pressure exerted by the grafted polymer is sufficient to have a significant effect

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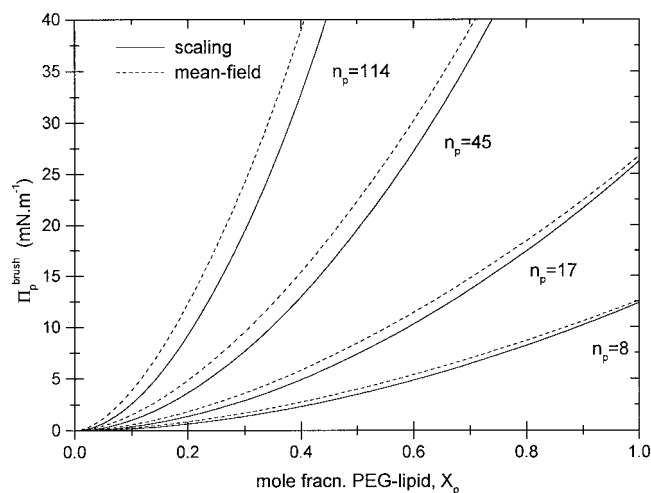


FIGURE 1 Lateral pressure, Π_p , exerted by the polymer brush, predicted from Eq. 2 as a function of mole fraction, X_p , of polymer lipid in the membrane. Calculations are for $T = 293$ K, $a_m = 0.39$ nm, $A_1 = 0.65$ nm², and the following degrees of polymerization: $n_p = 8, 17, 45$, and 114 , which correspond to PEGs with molar masses of 350, 750, 2000, and 5000 Da, respectively. Solid lines are predictions of scaling theory and dashed lines are predictions of mean-field theory.

on the membrane lipids. Theories derived from polymer physics (De Gennes, 1979; Milner et al., 1988; Hristova and Needham, 1994, 1995) give the free energy of the polymer brush, expressed per lipid molecule, as:

$$F_p^{\text{brush}} = X_p \cdot k_B T n_p a_m^{2m_F} (X_p/A_1)^{m_F} \quad (1)$$

where n_p is the number and a_m the size of monomer units in the polymer, A_1 is the membrane area per lipid molecule, and X_p is the mole fraction of polymer lipids. A value of $a_m = 0.39$ nm, corresponding to the volume of an oxyethylene monomer for PEG in solution (Evans et al., 1996), is assumed throughout. This corresponds quite closely with the value required by the latter authors to fit experimental adhesion data. As usual, k_B is Boltzmann's constant and T is the absolute temperature. The exponent m_F is $5/6$ in the de Gennes scaling theory, and is $2/3$ in the mean-field theory. The lateral pressure ($\Pi_p = -\partial F_p / \partial A_1$) that is created by the polymer brush is therefore:

$$\Pi_p^{\text{brush}} = m_F k_B T n_p a_m^{2m_F} (X_p/A_1)^{m_F+1} \quad (2)$$

The dependence on polymer lipid content for membranes containing PEG lipids of representative PEG molecular masses is given in Fig. 1. For comparison, the equivalence surface pressure between lipid bilayers and monolayers is in the region of $\Pi_{\text{eq}} \approx 35$ mN m⁻¹, which is comparable to the hydrophobic free energy density (Marsh, 1996a). Depending on polymer lipid content and chain length, it is clear from Fig. 1 that the polymer brush

may exert a significant lateral pressure on the membrane lipids. Values given for the shorter polymers (especially for $n_p = 8$), which are in any case small, must be treated with some caution, however. For these, neither the mean-field treatment nor scaling theory is likely to be a good approximation.

Equilibrium area per lipid molecule

The net tension, τ_{lat} , in the membrane is the resultant of the cohesive hydrophobic lateral tension (or free energy density), γ_{phob} , and the opposing lateral pressures, Π_{lipid} and Π_p^{brush} , which are contributed by the lipids and by the polymer brush, respectively (Evans and Waugh, 1977; Cevc and Marsh, 1987):

$$\tau_{\text{lat}} = \gamma_{\text{phob}} - \Pi_{\text{lipid}} - \Pi_p^{\text{brush}} \quad (3)$$

This equation is the condition for mechanical equilibrium of the membrane under tension. The lateral pressure contributed by the polymer is given by Eq. 2. That contributed by the lipid can be obtained from the equation of state for a lipid monolayer under conditions of equivalence with the bilayer (see Marsh, 1996a). It is expressed most generally by a virial expansion:

$$\Pi_{\text{lipid}} = k_B T \left(\frac{1}{A_1} + \frac{B_2}{A_1^2} + \frac{B_3}{A_1^3} + \dots \right) \quad (4)$$

where A_1 is the area per lipid molecule and B_2, B_3 , etc. are the virial coefficients. Terms up to third-order, with $B_2 = 2.51$ nm² and $B_3 = 0.779$ nm⁴, are found sufficient to parameterize the Π - A isotherms of a dioleoyl phosphatidylcholine monolayer at the oil-water interface (Marsh, 1996a). The latter reproduces well the area per lipid molecule and area expansion modulus of fluid-phase lipid bilayers at the monolayer-bilayer equivalence surface pressure: $\Pi_{\text{eq}} = \gamma_{\text{phob}}$ (Cevc and Marsh, 1987; Marsh, 1996a). It should be noted that, although representing the Π - A isotherm very well at pressures around the bilayer-equivalence value, B_2 and B_3 so determined are not true virial coefficients because of the truncation of the expansion.

In the presence of the polymer brush, the equilibrium area per lipid molecule, A_1 , in the tension-free state is obtained by putting $\tau_{\text{lat}} = 0$ in Eq. 3. This corresponds to the free energy minimum, because the membrane tension is given by the first derivative with respect to the membrane area: $\tau_{\text{lat}} = \partial F_{\text{tot}} / \partial A_1$ (Evans and Skalak, 1980; Cevc and Marsh, 1987). Correspondingly, the equilibrium area per lipid molecule, $A_{1,0}$, in the absence of the polymer is given by the same condition, but with $\Pi_p^{\text{brush}} = 0$ in Eq. 3. This can be used to eliminate γ_{phob} , and to work instead with the direct experimental observable, $A_{1,0}$. The equilibrium area per lipid, A_1 , in the presence of

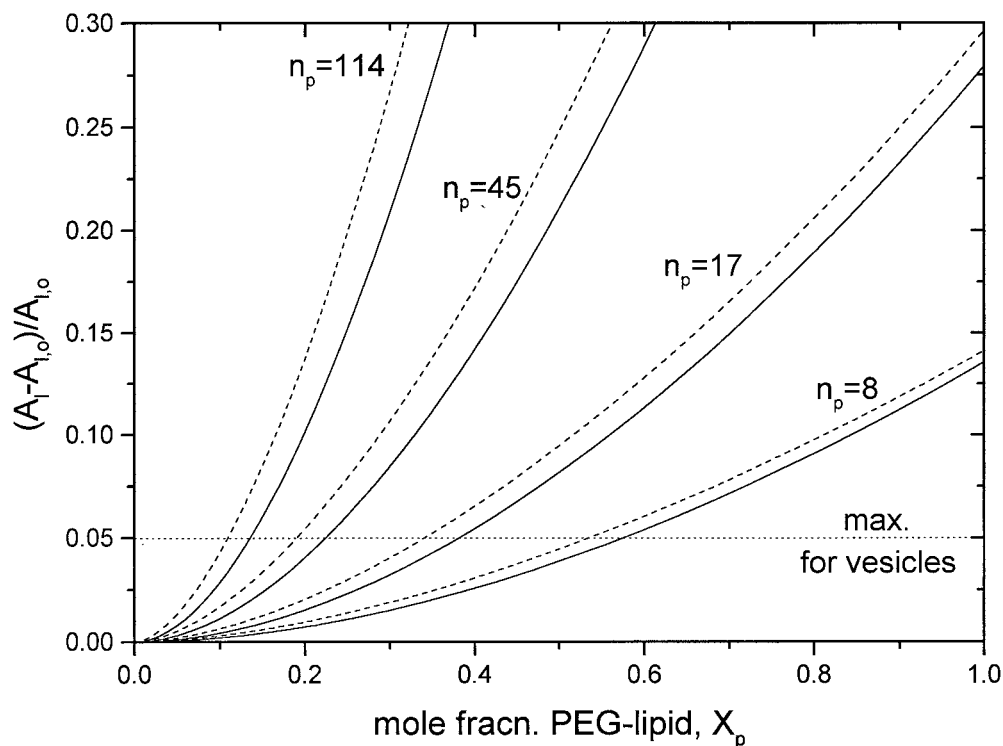


FIGURE 2 Dependence of the fractional increase, $(A_1 - A_{1,0})/A_{1,0}$, in area per lipid molecule induced by the lateral pressure in the polymer brush, as a function of the mole fraction, X_p , of polymer lipid. Values are obtained from the numerical solution of Eq. 5, with $B_2 = 2.51 \text{ nm}^2$, $B_3 = 0.779 \text{ nm}^4$, $a_m = 0.39 \text{ nm}$, and $A_{1,0} = 0.65 \text{ nm}^2$. The degree of polymerization, n_p , of the grafted polymer is indicated on the figure. Solid lines are predictions from scaling theory and dashed lines are those from mean-field theory.

the polymer brush is then given by the solution of the following equation:

$$\left(\frac{1}{A_{1,0}} - \frac{1}{A_1}\right) + B_2\left(\frac{1}{A_{1,0}^2} - \frac{1}{A_1^2}\right) \times B_3\left(\frac{1}{A_{1,0}^3} - \frac{1}{A_1^3}\right) - m_F a_m^{2m_F} n_p \left(\frac{X_p}{A_1}\right)^{m_F+1} = 0 \quad (5)$$

Because the lateral pressure in the polymer brush can reach relatively high values (see Fig. 1), an exact numerical solution is needed for A_1 to cover all polymer lipid sizes and contents. Results are given in Fig. 2, as a function of polymer lipid mole fraction in the membrane, for various representative PEG lipids. The range of polymer lipid concentrations shown in Fig. 2 encompasses those for which micelles are formed (see, e.g., Belsito et al., 2000). An increase in area of 5% is achieved at mole fractions of polymer lipid of $X_p \approx 0.575$ (0.535), 0.38 (0.34), 0.22 (0.19), and 0.135 (0.11) for polymer lengths of $n_p = 8, 17, 45$, and 114, respectively, as predicted by scaling (mean-field) theory. This is the maximum area extension supported by giant lipid vesicles under externally applied tension. It was used by Hristova and Needham (1995) as a criterion to define the saturation concentration of polymer lipid in a lamellar phospholipid membrane. These values are all

greater than those for the onset of micelle formation in PEG lipid/phosphatidylcholine mixtures, but less than those for complete micellization (see, e.g., Belsito et al., 2000).

Analytical solutions for low lateral pressures exerted by the polymer brush are also of interest. These apply to short polymer lipids and/or low polymer lipid contents. Expanding Eq. 5 up to first-order in the area increase, $\Delta A = A_1 - A_{1,0}$, induced by the polymer brush, yields the following approximate solution:

$$\frac{\Delta A}{A_{1,0}} = \frac{m_F a_m^{2m_F} n_p (X_p/A_{1,0})^{m_F+1}}{(A_{1,0}^{-1} + 2B_2 A_{1,0}^{-2} + 3B_3 A_{1,0}^{-3}) + m_F(m_F + 1) a_m^{2m_F} n_p (X_p/A_{1,0})^{m_F+1}} \quad (6)$$

This equation is simply the first-order elastic response of the membrane to the presence of the polymer brush. For mean-field theory (i.e., $m_F = 2/3$), it coincides with the result obtained by Hristova and Needham (1995). It will be seen below that the first term in parentheses of the denominator in Eq. 6 is simply the area elastic modulus, K_A° , of a lipid layer divided by $k_B T$. To the lowest order, the area expansion induced by the polymer brush scales directly with the polymer length, n_p , and with the $m_F + 1$ power of the mole fraction of polymer lipid, viz. $X_p^{m_F+1}$.

Equation 6, with explicit inclusion of K_A° , is a suitable approximation for membranes with very high elastic mod-

uli. Examples are membranes in the gel phase or containing near-saturating amounts of cholesterol (see, e.g., Evans and Needham, 1987). In these cases, the area expansion induced by the polymer brush will be considerably smaller than the values given in Fig. 2. From Eq. 6, it is predicted that the reduction factor is approximately the inverse ratio of the area elastic moduli.

Stretching elasticity

The isothermal elastic constant for area expansion, K_A , is given by the derivative of the tension with respect to the fractional change in membrane area (Evans and Skalak, 1980):

$$K_A = A_l \left(\frac{\partial \tau_{lat}}{\partial A_l} \right)_T \quad (7)$$

where, as already defined, A_l is the area per lipid molecule in the tension-free state. The calculation performed below is for a single lipid and polymer layer, i.e., for half a membrane thickness. For a bilayer membrane, the values of K_A are simply twice this. From Eqs. 2–4, the area expansion modulus of a single layer in the presence of the polymer brush is given by:

$$K_A = k_B T \left(\frac{1}{A_l} + \frac{2B_2}{A_l^2} + \frac{3B_3}{A_l^3} \right) + m_F(m_F + 1)k_B T a_m^{2m_F} n_p \left(\frac{X_p}{A_l} \right)^{m_F+1} \quad (8)$$

In the absence of grafted polymer, the expansion modulus (K_A^o) of the lipid layer is given simply by the first term on the right with $A_l = A_{l,o}$. The change in expansion modulus induced by the polymer lipid is therefore:

$$\frac{K_A - K_A^o}{k_B T} = - \left[\frac{1}{A_{l,o}} - \frac{1}{A_l} + 2B_2 \left(\frac{1}{A_{l,o}^2} - \frac{1}{A_l^2} \right) + 3B_3 \left(\frac{1}{A_{l,o}^3} - \frac{1}{A_l^3} \right) \right] + m_F(m_F + 1)a_m^{2m_F} n_p \left(\frac{X_p}{A_l} \right)^{m_F+1} \quad (9)$$

The term in square brackets on the righthand side of Eq. 9 represents the reduction in the lipid expansion modulus. This arises because the expansion induced by the polymer brush shifts the lipid assembly to a lower point on its II-A isotherm, at which K_A is smaller. The second term on the right of Eq. 9 represents the direct elastic response of the polymer brush. This tends to increase the expansion modulus.

The results of direct numerical calculations, according to Eqs. 5 and 9, are given for PEG lipids in Fig. 3. The virial

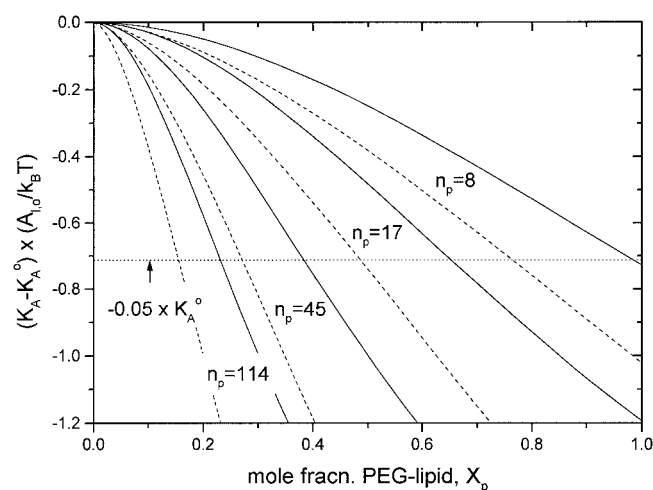


FIGURE 3 Dependence of the change, $K_A - K_A^o$, in isothermal area expansion modulus on mole fraction, X_p , of polymer lipid. Values are calculated from Eq. 9 for a lipid monolayer by using the numerical solutions of Eq. 5 that are given in Fig. 2. Solid lines are predictions from scaling theory and dashed lines are those from mean-field theory. At 293 K, the factor scaling the ordinate is $k_B T/A_{l,o} = 6.2 \text{ mN m}^{-1}$ with $A_{l,o} = 0.65 \text{ nm}^2$.

coefficients used are those obtained for dioleoyl phosphatidylcholine monolayers in the fluid regime at the oil-water interface (Marsh, 1996a). From this it is seen that the decrease in the lipid expansion modulus dominates over the elasticity contributed directly by the polymer brush. The result is a net decrease in K_A under all conditions for which the lipid equation of state is adequately represented by the dioleoyl phosphatidylcholine II-A isotherms. The magnitude of the decrease is, however, relatively small. The maximum extent of the ordinate in Fig. 3 corresponds to $K_A - K_A^o \approx -15 \text{ mN m}^{-1}$ (at 293 K with $A_{l,o} = 0.65 \text{ nm}^2$) for a bilayer, or $\sim 8\%$ of the expansion modulus for the bare lipid membrane. There is a high degree of compensation between the change in expansion modulus of the lipid and the direct contribution of the polymer brush. It is seen immediately from Eqs. 9 and 2 that the latter is positive and $(m_F + 1)$ times the lateral pressure in the brush. Reference to Fig. 1 shows that this direct contribution from the polymer brush can reach values that approach those of bare lipid membranes. The latter has a value of $K_A^o \approx 180 \text{ mN m}^{-1}$ for a bilayer in the present calculations, which would require $\Pi_p^{\text{brush}} \approx 49\text{--}54 \text{ mN m}^{-1}$ for a contribution equal to this from the polymer brush.

The situation depicted in Fig. 3 is likely to be reasonably representative of fluid lipid bilayer membranes, and possibly of membranes containing up to 30 mol % cholesterol. For the latter, the values of the lipid K_A^o are not changed appreciably relative to fluid membranes without cholesterol (Needham and Nunn, 1990). At high cholesterol contents, K_A^o is increased considerably, however, by a factor of six at saturation (Needham and Nunn, 1990). In these cases, as-

suming a constant K_A ($\approx K_A^o$) for the lipid is a reasonable approximation. Equation 3 for the membrane tension is then replaced by $\tau_{\text{lat}} = K_A^o(A_1 - A_{1,o})/A_{1,o} - \Pi_p^{\text{brush}}$, where the lateral expansion induced by the polymer is given by the condition $\tau_{\text{lat}} = 0$. Using this expression for the tension, the change in expansion modulus by the polymer is found to be positive and given by:

$$\frac{K_A - K_A^o}{k_B T} = m_F(m_F + 2)a_m^{2m_F}n_p \left(\frac{X_p}{A_1}\right)^{m_F+1} \quad (10)$$

This is greater, by a factor of $(m_F + 2)/(m_F + 1)$, than the direct contribution to K_A from the polymer brush because of the expansion $(A_1 - A_{1,o})$ of the lipid area by the brush at constant K_A (cf. Eq. 8). Nevertheless, the overall increase in K_A by the polymer is not large compared with the high intrinsic values of K_A^o ($\sim 1200 \text{ mN m}^{-1}$ or more) that are characteristic of lipid membranes with saturating contents of cholesterol (see, e.g., Lasic and Needham, 1995). For polymer lipid contents corresponding to the extreme ordinate in Fig. 3, Eq. 10 predicts that $K_A - K_A^o \approx 220\text{--}240 \text{ mN m}^{-1}$ for a bilayer, i.e., 20% of the intrinsic K_A^o (with $A_{1,o} \approx 0.45 \text{ nm}^2$ and $T = 293 \text{ K}$). Not until a mole fraction $X_p = 0.75\text{--}0.8$ of the polymer lipid with $n_p = 114$ is the increase in K_A predicted to reach the intrinsic value of membranes with high cholesterol.

Again, an approximate expression can be derived that corresponds to short polymer lipids and/or low polymer-lipid contents. Expanding Eq. 9 to first order in ΔA , and making use of Eq. 5, gives the following approximate solution:

$$K_A - K_A^o = -\frac{k_B T}{A_{1,o}} \left[2(1 - m_F) \frac{B_2}{A_{1,o}} + 3(2 - m_F) \frac{B_3}{A_{1,o}^2} - m_F \right] \frac{\Delta A}{A_{1,o}} \quad (11)$$

This predicts that the change in expansion modulus scales with the polymer lipid parameters in the same way as does the area expansion, $\Delta A/A_{1,o}$, but with the opposite sign. From Eq. 6 to lowest order, this represents a direct dependence on the degree of polymerization, n_p , and an $X_p^{m_F+1}$ dependence on polymer lipid content. This agrees with the scaling results obtained by Hristova and Needham (1994). The latter authors assumed that $K_A - K_A^o$ was negative, which as seen above corresponds to the reduction in lipid K_A dominating. Strictly speaking, however, their calculation refers to the direct elastic response of the polymer brush, and should be given by Eq. 10. The latter has a very simple relation to the area expansion induced by the polymer brush that is given by $K_A - K_A^o = (m_F + 2)K_A^o(\Delta A/A_{1,o})$. This result is exact to within the degree of approximation inherent in Eq. 10.

Curvature elasticity

The elastic parameters that govern the free energy of bending the membrane, F_{curv} , are the mean curvature modulus, k_c , the Gaussian curvature modulus, $\bar{k}_c$, and the spontaneous curvature, c_o (Helfrich, 1973):

$$F_{\text{curv}}(c_1, c_2) = \frac{1}{2} k_c (c_1 + c_2 - 2c_o)^2 + \bar{k}_c c_1 c_2 \quad (12)$$

where c_1 and c_2 ($\equiv 1/R_1, 1/R_2$) are the principal curvatures at a given point. All three curvature elasticity parameters are related to integrals over the lateral pressure distribution, $\Pi'_{\text{tot}}(z) \cdot dz$, transverse to the membrane. The total lateral pressure is given by $\Pi_{\text{tot}} = \Pi_{\text{lipid}} + \Pi_p^{\text{brush}}$, where the lipid and polymer contributions are given by Eqs. 4 and 2, respectively. The required lateral pressure density is then defined by $\Pi'_{\text{tot}} = \partial \Pi_{\text{tot}} / \partial z$. In terms of these definitions, the curvature elastic parameters are given by (Szeifer et al., 1990; Milner and Witten, 1988):

$$k_c = - \int z \frac{\partial \Pi'(z)}{\partial c} \Big|_{c=0} \cdot dz \quad (13)$$

$$\bar{k}_c = - \int z^2 \Pi'(z) \cdot dz \quad (14)$$

$$2k_c c_o = \int z \Pi'(z) \cdot dz \quad (15)$$

where z is in the direction of the membrane normal, c ($\equiv 1/R$) is the total mean curvature of the membrane, and the integration extends over the entire width of the membrane. It is evident from Eqs. 13–15 that the contributions of the lipids and the polymer brush to the bending elastic parameters of the membrane are additive. They correspond to integrations over the bilayer and polymer brush regions, respectively. The mean curvature modulus, k_c , depends on the stress distribution in the bent membrane, whereas the Gaussian curvature modulus, $\bar{k}_c$, and the spontaneous bending moment, $k_c c_o$, depend only on that of the planar unbent membrane. Simple models for the stress distribution are considered in the following subsections. As regards the lipid curvature elastic moduli, these are justified in so far as they are referred back ultimately to an experimental equation of state, or equivalently to an experimental area expansion modulus. For the lipid spontaneous curvature, however, this is not the case, and detailed consideration of the stress profile (see, e.g., Szeifer et al., 1990; Cantor, 1999) is necessary. However, in this latter case emphasis is placed primarily on the polymer contribution to spontaneous curvature.

Mean curvature elastic modulus

We consider first the mean curvature elastic modulus, k_c , for the outer monolayer of the membrane. This is the bending

modulus that is normally measured experimentally. For a bilayer membrane, by symmetry it has twice the value for a monolayer. The evaluation of k_c requires explicit allowance for the membrane curvature, as given in Eq. 13. To do this, A_1 in Eqs. 2 and 4 must be replaced by its z -dependent value, $A_1(z)$, and the total lateral pressure becomes $\Pi_{\text{tot}}(z) = \Pi_{\text{lipid}}(z) + \Pi_{\text{p}}^{\text{brush}}(z)$. The area per lipid molecule at a distance z from the lipid surface is given from simple cylindrical geometry appropriate to mean curvature by

$$A_1(z) = A_1(1 + zc) \quad (16)$$

where A_1 is the area at the lipid surface (strictly speaking the neutral surface). The sign of z is taken as positive in the direction of the polymer brush. In the case of non-zero Gaussian curvature, an extra term $A_1 z^2 c_1 c_2$ is added to Eq. 16, but that does not concern us here. From Eqs. 2, 4, and 16, the required derivative of the lateral stress (see Eq. 13) is:

$$\left. \frac{\partial \Pi'_{\text{tot}}(z)}{\partial c} \right|_{c=0} = -k_B T \left(\frac{1}{A_1} + \frac{2B_2}{A_1^2} + \frac{3B_3}{A_1^3} \right) - m_F(m_F + 1)k_B T a_m^{2m_F} n_p \left(\frac{X_p}{A_1} \right)^{m_F+1} \quad (17)$$

where $\Pi'_{\text{tot}}(z) = [\partial \Pi_{\text{tot}} / \partial A_1(z)] [\partial A_1(z) / \partial z]$. It is assumed that the only variation in lateral stress arises from the differential expansion of the area per lipid that is caused by the curvature according to Eq. 16. This approximation is in the spirit of the approach used by De Gennes (1979, 1980) and the conventional elastic analysis for lipid membranes. The integration in Eq. 13 is then straightforward. It has limits from $-d_1$ to $+L_p$, where d_1 is the thickness of the lipid monolayer and L_p is the height of the polymer brush. The latter is given in both scaling and mean-field theories by (Daoud and De Gennes, 1977; Hristova and Needham, 1994):

$$L_p = n_p a_m^{5/3} (X_p / A_1)^{1/3} \quad (18)$$

From Eqs. 13, 17, and 18 the mean curvature elastic modulus for the monolayer is given by

$$k_c = \frac{k_B T}{2} \left(\frac{1}{A_1} + \frac{2B_2}{A_1^2} + \frac{3B_3}{A_1^3} \right) d_1^2 + \frac{m_F(m_F + 1)}{2} k_B T a_m^{2m_F+10/3} n_p^3 \left(\frac{X_p}{A_1} \right)^{m_F+5/3} \quad (19)$$

The first term on the right of Eq. 19 is the lipid contribution to the bending modulus and the second term is that from the polymer brush. If it is alternatively assumed that the stress distribution is given by $\Pi'_{\text{tot}}(z) = \Pi_{\text{lipid}}/d_1 + \Pi_{\text{p}}^{\text{brush}}/L_p$ for the lipid and polymer regions, respectively, an expression very similar to Eq. 19 is obtained; k_c is simply multiplied by an overall factor of $2/3$.

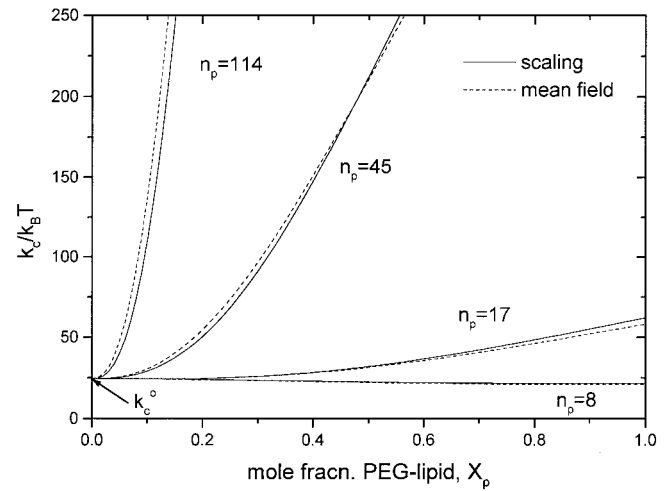


FIGURE 4 Dependence of the mean curvature elastic modulus, k_c , on mole fraction, X_p , of polymer lipid. Values are calculated from Eq. 19 for a lipid monolayer by using the numerical solutions of Eq. 5 that are given in Fig. 2, together with $d_{1,0} = 1.5$ nm. The degree of polymerization, n_p , of the grafted polymer is indicated on the figure. Solid lines are predicted from scaling theory and dashed lines are those from mean-field theory.

The contribution from the lipid in Eq. 19 is classical. It has the general form $k_c \sim K_A^L d_1^2$, where K_A^L (see Eq. 8) is the area elastic constant of the lipid (see, e.g., Evans and Skalak, 1980; Cevc and Marsh, 1987; Bloom et al., 1991). The value of K_A^L is modified from that of a bare lipid bilayer, viz., K_A^0 . This is because of the area expansion arising from the lateral pressure exerted by the polymer brush, as discussed in the previous section (see Eq. 9). Correspondingly, the lipid thickness, d_1 , is reduced from that of a bare lipid bilayer, $d_{1,0}$, without grafted polymer. Assuming constant lipid volume, $d_1 = d_{1,0}(A_{1,0}/A_1)$. These two features represent the indirect effect of the polymer brush on the bending modulus. The lipid curvature elasticity, k_c^0 , is reduced by a factor $(K_A^L/K_A^0)(A_{1,0}/A_1)^2$. This contribution was neglected in previous treatments.

From the second term on the right of Eq. 19 it is seen that the polymer contribution to the bending modulus, k_c , scales as the third power of the degree of polymerization, i.e., as n_p^3 , and as the $m_F + 5/3$ power of the polymer lipid content. This is in agreement with the scaling theory result obtained by Hristova and Needham (1994) and the mean-field result of Milner and Witten (1988). It is also expected from quite general scaling arguments. The bending modulus is related to the area modulus, K_A , and membrane thickness, d_t , according to $k_c \sim K_A \cdot d_t^2$ (see above and Evans and Skalak, 1980; Bloom et al., 1991). For the polymer contribution: $K_A - K_A^0 \sim n_p \cdot X_p^{m_F+1}$ and $d_t \sim L_p \sim n_p \cdot X_p^{1/3}$ (see Eqs. 11 and 18), and hence the bending modulus scales as $k_c \sim n_p^3 \cdot X_p^{m_F+5/3}$.

The mean-curvature modulus predicted by Eq. 19 as a function of polymer lipid content is given for representative PEG lipids in Fig. 4. The calculation uses the numerical

solutions of Eq. 5 for the area per lipid molecule and a value of $d_{l,o} = 1.5$ nm for the effective elastic thickness of a lipid molecule. At $X_p = 0$, this value of $d_{l,o}$ (together with the virial expansion for a dioleoyl phosphatidylcholine monolayer) predicts a value of $k_c^o \approx 1 \times 10^{-19}$ J for the bare lipid monolayer at 293 K. Twice this value is within the range of mean curvature elastic constants measured experimentally for fluid lipid bilayer membranes (see, e.g., Marsh, 1990). The rapid increase in k_c with degree of polymerization that is evident in Fig. 4 reflects the strong n_p^3 dependence. For the short polymer lipid with $n_p = 8$, the decrease in lipid k_c slightly outweighs the direct contribution from the polymer brush, producing a small net decrease in k_c with increasing polymer lipid content. The value of k_c remains close to the bare lipid value, k_c^o , and the situation is analogous to that for the area expansion modulus treated in the previous section. For higher degrees of polymerization this is not the case, however. The direct contribution of the polymer brush then dominates; k_c increases with increasing polymer lipid content and, for large n_p , the polymer brush contribution to k_c quickly becomes much larger than the bare lipid value, k_c^o .

Gaussian curvature elastic modulus

Calculation of the elastic modulus for saddle-splay bending, $\bar{k}_c$, is more straightforward than that for the mean curvature because it depends only on the lateral stress distribution, $\Pi'_{tot}(z) \cdot dz$, of the unbent membrane. For a constant stress profile throughout the lipid membrane and the separate constant profile in the polymer brush (i.e., both linearly dependent on z), $\Pi'_{tot}(z)$ is given by Π'_{lipid}/d_l and Π'_p^{brush}/L_p , respectively. In this case (see Eqs. 2 and 4), Π_p^{brush} and Π_{lipid} take their z -independent values corresponding to constant A_l . From Eqs. 2, 4, 14, and 18, the Gaussian curvature modulus is then given by:

$$\bar{k}_c = -\frac{k_B T}{3} \left(\frac{1}{A_l} + \frac{B_2}{A_l^2} + \frac{B_3}{A_l^3} \right) d_l^2 - \frac{m_F}{3} k_B T a_m^{2m_F+10/3} n_p^3 \left(\frac{X_p}{A_l} \right)^{m_F+5/3} \quad (20)$$

where again the calculation is for one monolayer of the membrane. The value of $\bar{k}_c$ for a bilayer membrane is twice this. The sign of $\bar{k}_c$ is negative, and the contribution of saddle-splay bending (i.e., $c_1 = -c_2$) to the elastic free energy is positive (see Eq. 12), just as for cylindrical bending with positive k_c (see also Milner and Witten, 1988).

It is seen from comparison of the second terms on the right of Eqs. 19 and 20 that the direct polymer contribution to $\bar{k}_c$ scales in exactly the same way as does that to k_c . This scaling result was derived previously both for

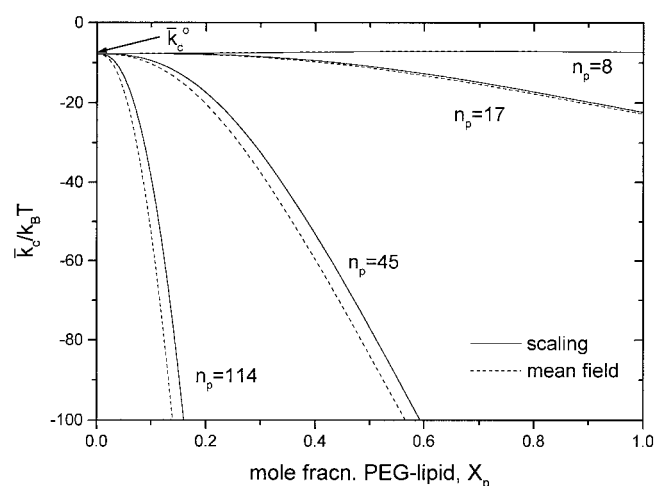


FIGURE 5 Dependence of the Gaussian curvature modulus, $\bar{k}_c$, on mole fraction, X_p , of polymer lipid. Values are calculated from Eq. 20 for a lipid monolayer by using the numerical solutions of Eq. 5 that are given in Fig. 2, together with $d_{l,o} = 1.5$ nm. The length, n_p , of the grafted polymer is indicated on the figure. Solid lines are predictions from scaling theory and dashed lines are those from mean-field theory.

mean-field theory (Milner and Witten, 1988), and for scaling theory (Hristova and Needham, 1994). The lipid contribution to $\bar{k}_c$ scales with d_l^2 , just as does that to k_c . However, for a realistic virial expansion, $\bar{k}_c$ scales directly with the lipid lateral pressure, whereas k_c scales with the lipid area expansion modulus, K_A^L , as seen above. The indirect effect of the polymer on the lipid Gaussian modulus therefore differs from that on the mean curvature modulus. The bare lipid value, $\bar{k}_c^o$, is reduced by a factor $\Pi_{lipid}/\Pi_{lipid}^o (A_{l,o}/A_l)^2$ as a result of the lateral expansion induced by the polymer brush.

The dependence of the Gaussian curvature modulus, $\bar{k}_c$, on polymer lipid content that is predicted by Eq. 20 is given in Fig. 5 for representative PEG lipids. Qualitatively, the dependence on polymer length is similar to that for the mean curvature modulus, k_c (cf. Fig. 4). There are, however, quantitative differences that arise from the different ways in which the lipid contribution to the curvature modulus is modified by the polymer brush. The value of the Gaussian curvature modulus predicted for a bare lipid monolayer from the lipid equation of state is $\bar{k}_c^o = -3 \times 10^{-20}$ J at 293 K. For $X_p \rightarrow 1$, this is decreased in magnitude by $\sim 5\%$, whereas the corresponding decrease in mean curvature modulus is $\sim 12\text{--}15\%$ (cf. Fig. 4).

Spontaneous curvature

As for the Gaussian modulus, calculation of the spontaneous curvature, c_o , depends only on the lateral stress distribution of the unbent membrane (see Eq. 15). This quantity, although important to the stability of bilayer membranes, has not been addressed previously for polymer lipids. From

integration of Eq. 15 in exactly the same manner as for $\bar{k}_c$, the spontaneous curvature becomes:

$$c_o = \frac{k_B T}{4k_c} \left[-\left(\frac{1}{A_1} + \frac{B_2}{A_1^2} + \frac{B_3}{A_1^3} \right) d_1 + m_F a_m^{2m_F+5/3} n_p^2 \left(\frac{X_p}{A_1} \right)^{m_F+4/3} \right] \quad (21)$$

where k_c is the total mean curvature modulus that contains both lipid and polymer contributions. The polymer tends to induce positive curvature, outward from the membrane. In this simple calculation, the sign of the lipid spontaneous curvature opposes that of the polymer. This must not necessarily be so, however, when the contributions of the lipid headgroup and chains to the lateral stress profile are considered separately (see, e.g., Marsh, 1996a). In general, the spontaneous curvature induced by the lipid is reduced by a factor $\Pi_{\text{lipid}}/\Pi_{\text{lipid}}^0(A_{1,o}/A_1)$, as compared to a bare lipid membrane, by the action of the polymer brush.

In the case that the lipid contribution to k_c still dominates, the polymer contribution scales quadratically with the polymer length, i.e., as n_p^2 , and with the $m_F + 4/3$ power of the polymer lipid content. This represents a strong tendency of long polymers to induce outward curvature of the monolayer. When k_c is dominated by the polymer contribution, i.e., the second term on the right of Eq. 19 is greater than the first, the spontaneous curvature contributed by the polymer is:

$$c_o^p = \left[2(m_F + 2)a_m^{2m_F} n_p \left(\frac{X_p}{A_1} \right)^{1/3} \right]^{-1} \quad (22)$$

The spontaneous curvature then scales inversely, rather than directly, with the height of the polymer brush, L_p . Outward spontaneous bending is then less favored than in the dilute case because of the stronger dependence of k_c on the polymer length and density. Note, however, that it is the product $k_c c_o^2$ that determines the free energy associated with the tendency of the lamellar membrane to bend (see Eq. 12); also, the product $k_c c_o$ given by Eq. 15 represents the spontaneous bending moment. Correspondingly, under circumstances where the polymer contribution (k_c^p) to k_c dominates, the spontaneous curvature contribution to the lipid component is reduced by a factor $(k_c^o/k_c^p)(A_{1,o}/A_1)$.

The dependence on polymer lipid content of the direct contribution, c_o^p , of the polymer to the spontaneous curvature of a lipid monolayer is given in Fig. 6. These values are calculated from the final term on the righthand side of Eq. 21, together with the expression for k_c that is given in Eq. 19. The latter contains both lipid and polymer contributions to the mean curvature modulus, including modifications of the former by the lateral pressure in the polymer brush. Results are given for representative PEG lipids of different polymer lengths, n_p . At low contents, X_p , of polymer lipid, the longer the polymer is the more effective it is at inducing spontaneous curvature in the lipid layer. As the content of

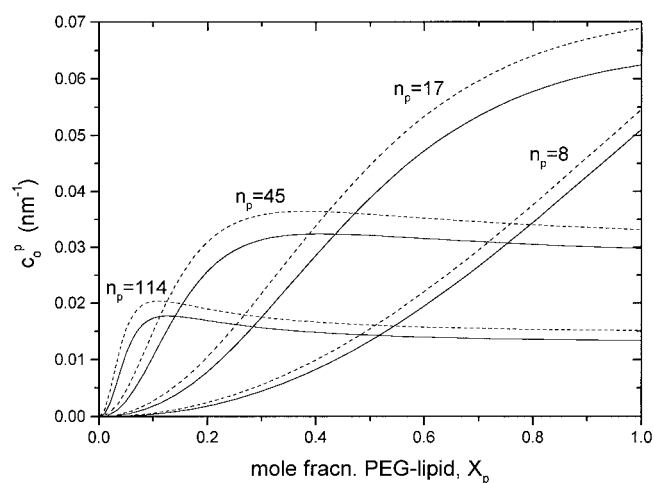


FIGURE 6 Dependence of the polymer brush contribution, c_o^p , to the monolayer intrinsic curvature on mole fraction, X_p , of polymer lipid. Values are calculated from the rightmost term in Eq. 21, with $d_{1,o} = 1.5$ nm and Eq. 19 for k_c , by using the numerical solutions of Eq. 5 that are given in Fig. 2. The length, n_p , of the grafted polymer is indicated on the figure. Solid lines are predictions from scaling theory and dashed lines are those from mean-field theory.

polymer lipid increases, however, the contribution of the polymer brush to the mean curvature modulus, k_c , comes to dominate. The spontaneous curvature reaches a maximum and then begins to decrease, as depicted by Eq. 22. This effect is greater with increasing length of the polymer. Progressively, first the polymer lipid with $n_p = 114$, then that with $n_p = 45$, and finally that with $n_p = 17$ induces the largest spontaneous curvature as the polymer lipid content increases. These polymer lipid contents are larger, however, than those at which micelle formation is induced. The mole fraction of PEG lipid at the onset of micelle formation is $X_{\text{PEG}}^{\text{on}} = 0.03 \pm 0.01$, 0.07 ± 0.03 , and 0.22 ± 0.07 for $n_p = 114$, 45, and 17, respectively (Belsito et al., 2000; Montezano et al., 2001), which corresponds to an approximately common threshold value in spontaneous curvature of $c_o^p \sim 0.005\text{--}0.01$ nm⁻¹.

The above calculations are for a monolayer of the membrane. For a bilayer membrane, the spontaneous curvature is zero by symmetry. Nevertheless, the monolayer value of c_o is important for lamellar membranes because it expresses the spontaneous tendency of polymer lipid-containing systems to form micelles (Marsh, 1996a,b).

CONCLUSIONS

The present calculations indicate that the lateral pressure from the brush region of polymer-grafted lipids induces an appreciable expansion in area of fluid lipid membranes. For very stiff membranes, such as those in the gel phase or containing near-saturating amounts of cholesterol, the lateral expansion is considerably smaller. The area expansion

induced in fluid membranes results in a net decrease of the area extension elastic modulus, but this change is small. For stiff membranes, the area extension modulus is increased and the magnitude of the change is much greater. However, the fractional change remains relatively modest for membranes with intrinsically high values of K_A^0 . The area expansion by the polymer brush is a determining feature in the curvature elastic moduli, k_c and $\bar{k}_c$, only for fluid membranes and short polymers. For longer polymers, the direct contribution from the brush region quickly comes to dominate the curvature moduli, and achieves values several times greater than the intrinsic lipid curvature moduli. The predicted contribution of the polymer brush to the spontaneous lipid monolayer curvature remains relatively modest because of the opposing increase in the mean curvature modulus. This prediction assumes that the neutral surface remains close to the lipid polar-apolar interface, which may not be the case at the onset of micellization, which has been treated already in some detail in Hristova and Needham (1995).

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