Renormalization of the Tension and Area Expansion Modulus in Fluid Membranes

Derek Marsh
Abteilung Spektroskopie, Max-Planck-Institut für biophysikalische Chemie, D-37077 Göttingen, Germany

ABSTRACT Renormalization of the membrane tension and elastic area expansion modulus by thermally induced bending fluctuations is treated in terms of the formalism of Brochard, De Gennes, and Pfeuty (*J. de Phys. (France).* 37:1099–1104, 1976). The dependence of the renormalized tension on the bare membrane tension parallels the dependence on the fractional area extension of giant vesicles found experimentally by Evans and Rawicz (*Physiol. Rev. Lett.* 64:2094–2097, 1990), and suggests conditions for molecular dynamics simulations with membrane patches of limited size that might best represent the properties of macroscopic vesicles.

INTRODUCTION

The question of the membrane tension that is appropriate to molecular dynamics simulations of lipid bilayer systems has been raised recently (Jähnig, 1996; Jakobsson et al., 1996; Marsh, 1996). Considerations of the interfacial membrane equilibrium (e.g., Cevc and Marsh, 1987) and elastomechanical experiments (Evans and Skalak, 1980) suggest that the net tension in stress-free bilayer membranes should be zero. It has been pointed out, however, that the relatively small cell sizes inevitably employed in molecular dynamics simulations preclude the long-wavelength bending fluctuations that are thermally excited in membrane vesicles of macroscopic size (Feller and Pastor, 1996). In consequence, the tension required in a molecular dynamics simulation to emulate this effect of long-range elastic excitations may be nonvanishing.

By including elastic stretching in the membrane free energy, Brochard et al. (1976) have shown that the thermal excitations (undulations) give rise to a renormalization of the bare membrane tension. Results were presented for the tension-free state. The purpose here is to explore the consequences of this renormalization in a membrane under net tension. The importance of such effects has been demonstrated in both the elastic dilation of the membrane (Evans and Rawicz, 1990; Evans, 1991) and the adhesive properties of vesicles (Rädler et al., 1995; Servuss and Helfrich, 1989; Evans and Metcalfe, 1984). This treatment leads naturally to a consideration of the membrane tensions under which molecular dynamics simulations for limited membrane patches may best represent the properties of macroscopic membranes undergoing elastic fluctuations. The results obtained are also in agreement with the classic experiments and analysis of Evans and Rawicz (1990) on the area dilation of giant lipid vesicles under a wide range of applied tensions, from extremely low values to those approaching the elastic limit.

RESULTS AND DISCUSSION

Membrane tension

In terms of the transverse displacement amplitude, $u(\mathbf{r})$, of the fluctuating membrane, the local curvature is given by $c(\mathbf{r}) = \nabla^2 u$ and the local extension of the membrane area by $\delta A(\mathbf{r}) = \frac{1}{2}(\nabla u)^2 \delta^2\mathbf{r}$, for small angular displacements (see Fig. 1). The total elastic free energy for a membrane of area A is therefore given by (Brochard et al., 1976)

$$F_{el}(u(\mathbf{r})) = \int d^2\mathbf{r}\left\{\frac{1}{2}k_c(\nabla^2 u)^2 + \frac{1}{2}\bar{\tau}(\nabla u)^2\right\} + \frac{1}{2}\frac{K_A}{A}\left(\int d^2\mathbf{r}\,\frac{1}{2}(\nabla u)^2\right)^2 \quad (1)$$

where k_c is the bending modulus, $\bar{\tau}$ is the "bare" tension component within the membrane, and K_A is the area expansion modulus. The last term on the right of Eq. 1 is an elastic stretching free energy component that is frequently omitted. It represents the local extension, $\delta A(\mathbf{r})$, of the membrane area beyond that of a perfectly flat membrane with an area (specified by ΔA) that is equal to the projected area of the fluctuating membrane. This serves to define the reference state in which the area dilation of the fluctuating membrane, relative to this state, is suppressed by the "bare" tension component, $\bar{\tau}$, which turns out to be negative (see below). It is automatically assumed by this definition that the membrane is constrained in lateral extent within the plane that contains the vectors $\mathbf{r}$ (see Fig. 1). In the treatment given by Evans and Rawicz (1990), a different reference state is taken that corresponds to zero net tension of the stress-free fluctuating membrane. The expression for the free energy, following the approach of Helfrich and Servuss (1984)

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Address reprint requests to Dr. Derek Marsh, MPI für Biophysikalische Chemie, Abteilung 010 Spektroskopie, Am Fassberg, D-37077 Göttingen-Nikolausberg, Germany. Tel.: 49-551-201-1285; Fax: 49-551-201-1501; E-mail: dmarsh@gwdg.de.

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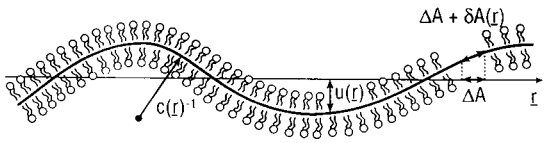

FIGURE 1 Thermal elastic fluctuations of a flat lipid membrane. The amplitude of the local deviation from the flat membrane is $u(\mathbf{r})$ and the local radius of surface curvature is $c(\mathbf{r})^{-1}$. The local extension in the surface area is $\delta A(\mathbf{r})$, relative to the flat membrane of area specified by ΔA. The reference state is the flat membrane that, relative to the tension-free fluctuating state, experiences the "bare" membrane tension, $\bar{\tau}$.

(except that the contribution from the reference state must be subtracted), is then correspondingly different.

Expanding Eq. 1 in terms of the Fourier components of the transverse excitations: $u(\mathbf{r}) = \Sigma_q u_q e^{i\mathbf{q}\cdot\mathbf{r}}$, yields the following expression for the free energy of the membrane:

$$F_{el}(u_q) = A\int d^2\mathbf{q}\,\frac{1}{2}(k_c q^4 + \bar{\tau}q^2)|u_q|^2 + \frac{1}{2}K_A A\left(\int d^2\mathbf{q}\,\frac{1}{2}q^2\left|u_q\right|^2\right)^2 \tag{2}$$

The central result obtained from the partition function corresponding to Eq. 2 is that the "bare" tension component, $\bar{\tau}$, is renormalized by the thermal fluctuations to yield the net "observable" tension, $\tilde{\tau}$, which is given by (Brochard et al., 1976)

$$\tilde{\tau} = \bar{\tau} + \frac{K_A k_B T}{2A}\sum_q \frac{1}{\tilde{\tau} + k_c q^2} \tag{3}$$

For a net tension-free membrane (i.e., $\tilde{\tau} = 0$), the "bare" membrane tension component is given by

$$\bar{\tau}_o = -\frac{K_A k_B T}{2A}\sum_q \frac{1}{k_c q^2} \tag{4}$$

which corresponds to a positive effective lateral pressure that is required to balance the tension induced by the thermal excitations. Typically, this effective lateral pressure component has values around 2 mN · m^{-1} (Marsh, 1996). It is tempting to suggest that this "bare" lateral pressure is that which should be employed for molecular dynamics simulations of flat membrane patches to reproduce the conditions for a macroscopic fluctuating membrane. This, however, would be the incorrect reference state, because for an already flat membrane it corresponds to a compression of the true membrane area to a value that is not characteristic of the equilibrium area per lipid molecule at the chosen temperature (cf. Marsh, 1996). To obtain an appropriate reference state, it is necessary to consider the membrane under tension.

For a membrane with net tension (i.e., $\tilde{\tau} \neq 0$), it is useful to define $\bar{\tau} = \bar{\tau}_o + \Delta\bar{\tau}$, where $\Delta\bar{\tau}$ is the amount by which the "bare" tension component must be augmented to support a given net "observable" tension in the fluctuating membrane. The latter tension is then given by combining Eqs. 3 and 4:

$$\tilde{\tau} = \Delta\bar{\tau} - \frac{K_A k_B T}{2Ak_c}\sum_q \frac{\tilde{\tau}}{\tilde{\tau}q^2 + k_c q^4} \tag{5}$$

If this equation is decoupled by assuming that $\tilde{\tau}$ is independent of q in making the summation on the right-hand side, then relatively straightforward results are obtained. Summation is performed by the usual integration over q-space:

$$\sum_q \ldots \rightarrow \frac{A}{(2\pi)^2}\int_{q_{min}}^{q_{max}} \ldots \pi d(q^2) \tag{6}$$

where the cutoff wave vectors are given by $q_{max} \approx \pi/\sqrt{a_l}$ and $q_{min} \approx \pi/\sqrt{A}$, where a_l is the area per lipid molecule. The result for the renormalized tension is

$$\tilde{\tau} = \Delta\bar{\tau} - \frac{K_A k_B T}{8\pi k_c}\ln\left(\frac{\tilde{\tau}A/\pi^2 k_c + 1}{\tilde{\tau}a_l/\pi^2 k_c + 1}\right) \tag{7}$$

For tensions $\tilde{\tau} \ll \pi^2 k_c/a_l$ ($\approx 2 \times 10^3$ mN · m^{-1}), the denominator in the logarithm is close to unity.

The dependence of the renormalized tension on the incremental change in the "bare" tension, obtained from numerical solution of Eq. 7, is given in Fig. 2. Results are presented for a 20-μm-diameter giant vesicle with a typical value of the area expansion modulus, $K_A = 140$ mN · m^{-1}, and values of the curvature modulus in the usual experimental range: $k_c = 0.5$–2.0×10^{-19} J (see, e.g., Marsh, 1990, 1996). Initially, the renormalized tension changes by relatively very little as $\Delta\bar{\tau}$ increases, and remains close to zero (see Fig. 2 *A*). This regimen corresponds predominantly to damping of the long-wavelength membrane fluctuations. At higher values of $\Delta\bar{\tau}$, the renormalized tension increases more rapidly. Finally, the dependence of $\tilde{\tau}$ on $\Delta\bar{\tau}$ becomes approximately linear, with a slope that is close to, but slightly less than, unity. As might be expected from the structure of Eq. 7, the extent of renormalization depends rather strongly on the curvature modulus, especially with small values of k_c for which thermal fluctuations are readily excited. The renormalization is also affected almost equally strongly by the value of the area expansion modulus. Results for $K_A = 500$ mN · m^{-1}, typical of cholesterol-containing lipid bilayers, and $k_c = 2.0 \times 10^{-19}$ J are given by the dotted line in Fig. 2. In fact, the renormalization is determined predominantly, but not entirely, by the ratio K_A/k_c. For example, for $K_A = 350$ mN · m^{-1} and $k_c = 2.0 \times 10^{-19}$ J, the results lie close to but slightly above those given for $K_A = 140$ mN · m^{-1} and $k_c = 0.8 \times 10^{-19}$ J in Fig. 2 *A*. It will be noted that the curvature modulus scales as $k_c \sim K_A d^2$, where d is the bilayer thickness. Therefore the ratio K_A/k_c might be expected to remain approximately constant. Experimentally, values are found to lie in the range $K_A/k_c \approx 2$–3 nm^{-2} (Evans and Rawicz, 1990). This would correspond approximately to values of $k_c = 0.5$–0.8×10^{-19} J for $K_A = 140$ mN · m^{-1} in Fig. 2.

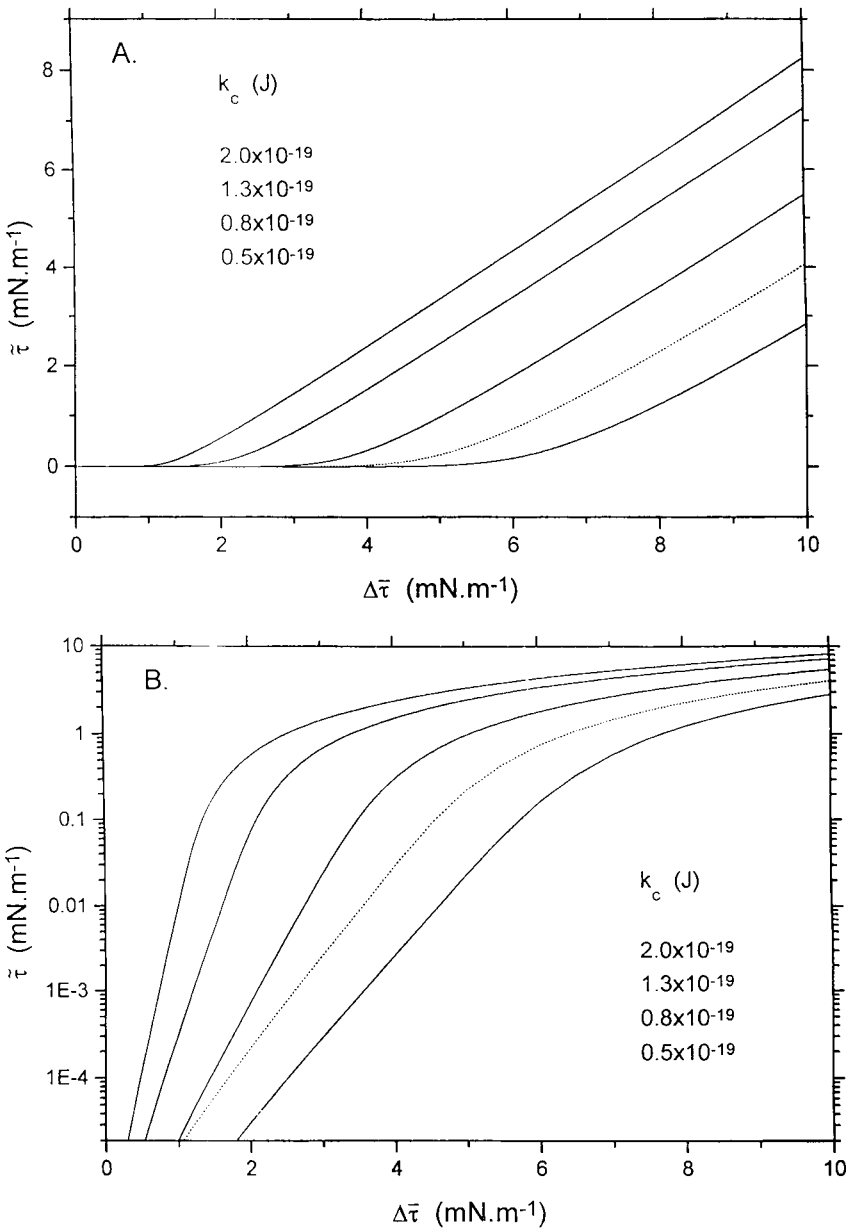

FIGURE 2 Dependence of the renormalized membrane tension, $\tilde{\tau}$, on the increment, $\Delta\tilde{\tau}$, in the "bare" tension component, relative to the value for $\tilde{\tau} = 0$. Values of $\tilde{\tau}$ are obtained from the numerical solution of Eq. 7 for $\tilde{A}_o = 1.26 \times 10^3$ μm^2, $a_l = 0.6$ nm^2, $T = 20°C$, and various values of k_c (from upper to lower: 2.0, 1.3, 0.8, and 0.5×10^{-19} J) with $K_A = 140$ mN · m^{-1} (——), and for $K_A = 500$ mN · m^{-1} with $k_c = 2.0 \times 10^{-19}$ J (· · · · ·). (*A*) Linear plot; (*B*) logarithmic plot. The higher values of k_c support a higher tension, $\tilde{\tau}$, for a given $\Delta\tilde{\tau}$, and vice versa for K_A.

Implications for molecular dynamics simulations

From Fig. 2 *A* it is possible to suggest conditions under which molecular dynamics simulations might reasonably well approximate the mesoscopic properties of a macroscopic lipid membrane. The finite size of the simulation cell means that long-wavelength thermal excitations are suppressed (cf. Feller and Pastor, 1996). A similar situation is achieved for membranes of macroscopic size at values of $\Delta\tilde{\tau}$ corresponding to the intersection point of the quasilinear region in Fig. 2 *A* with the $\tilde{\tau} = 0$ axis. At this point, the thermal excitations are largely suppressed, but without appreciable extension of the true membrane area beyond that induced by the fluctuations themselves (relative to the "bare" tension reference state defined above by Eq. 1). From Fig. 2 *A* it can be seen that this condition is achieved for values of $\Delta\tilde{\tau}$ in the range of 1–6 mN · m^{-1}, depending on the values of the elastic constants (and to a lesser extent on the membrane area). If restriction is made to values $K_A/k_c \approx 2$–3 nm^{-2}, the critical values of $\Delta\tilde{\tau}$ are confined to the range of 4–6 mN · m^{-1}. It is therefore suggested that values of the applied tension in this range may be required for molecular dynamics simulations to approximate best the properties of macroscopic bilayer membranes. These values are of the same magnitude as the surface tension obtained from a molecular dynamics simulation for 72 lipids at a constant mean area per molecule of 0.655 nm^2 (Feller et al., 1995), but more recent simulations using different potentials and a different method for calculating the electrostatic interactions with the same system have yielded considerably larger values (Feller and Pastor, 1996).

It is pertinent to estimate the degree to which the long-wavelength modes are suppressed by the applied tension. The mean square amplitude of the transverse fluctuations is given by (Brochard et al., 1976)

$$\langle u^2 \rangle = \frac{k_B T}{A} \sum_q \frac{1}{\bar{\tau} q^2 + k_c q^4} \tag{8}$$

where summation is over the wave vectors, q, of all modes. From Eq. 5, this may be expressed simply in terms of the tension by

$$\langle u^2 \rangle \approx \frac{2k_c}{K_A}\left(\frac{\Delta\bar{\tau}}{\tilde{\tau}} - 1\right) \tag{9}$$

As the increment in the bare tension, $\Delta\bar{\tau}$, increases, the mean square fluctuation amplitude therefore decreases linearly with the ratio $\Delta\bar{\tau}/\tilde{\tau}$, which is dependent on the total area, A, of the membrane patch. For the conditions given in Fig. 2 (~20-μm-diameter vesicle), it can be estimated that $\Delta\bar{\tau}/\tilde{\tau} \approx 10$ at the linear extrapolation point, which for $K_A/k_c = 3\ \mathrm{nm}^{-2}$ results in a root mean square displacement of $\langle u^2 \rangle^{1/2} \approx 2.5$ nm. This is considerably larger than the vertical fluctuations obtained in molecular dynamics simulations with currently realizable cell sizes (Feller and Pastor, 1996). It does represent, however, a very effective suppression of the long-wavelength fluctuations that are present in the tension-free state. For the latter, under conditions corresponding to those of Fig. 2 with $k_c = 0.5 \times 10^{-19}$ J and $A = 1.26 \times 10^3\ \mu\mathrm{m}^2$, the r.m.s. amplitude is $\langle u^2 \rangle^{1/2} = (k_B T A/4\pi^3 k_c)^{1/2} \approx 1\ \mu$m (see, e.g., Helfrich and Servuss, 1984; Marsh, 1996). Considerably larger simulation cells would be required to achieve the residual level of undulation associated with the tension criteria for near-equivalence that are proposed here. There also remains the related issue of the time scale required for simulation of large-scale cooperative motions, which will increase with cell size. A possible means of emulating the effects of long-wavelength bending excitations by using simulation cells of small size has been suggested by an anonymous reviewer. This is to apply separate tension boundary conditions to the inner and outer monolayers, so that the areas in the two are free to fluctuate independently, accompanied by unrestricted motion in the transverse direction.

Membrane expansion modulus

According to the formulation in Eq. 1, $\bar{\tau}$ obeys the normal elastic relations, and the true area expansion modulus, defined relative to the "bare" tension reference state, is given by $K_A = A(\partial\bar{\tau}/\partial A)$. The incremental change in $\bar{\tau}$ can therefore be approximated by the elastic equation of state:

$$\Delta\bar{\tau} = K_A \Delta A/\tilde{A}_0 \tag{10}$$

where $\Delta A = A - \tilde{A}_0$, A is the projected area of the membrane and $\tilde{A}_0$ is the value of A at which $\tilde{\tau} = 0$. Not surprisingly, therefore, the dependence of the renormalized tension on $\Delta\bar{\tau}$ parallels that found experimentally for the observed (i.e., applied) tension in individual lipid macrovesicles as a function of the fractional area extension (Evans and Rawicz, 1990). According to the above reasoning (viz., Eq. 10), the fractional area extension is related directly to $\Delta\bar{\tau}$ by the proportionality constant $1/K_A$. In particular, $\tilde{\tau}$ depends approximately logarithmically on $\Delta\bar{\tau}$ at low tensions (see Fig. 2 *B*). Rearranging Eq. 7 and from Eq. 10, one obtains

$$\frac{\Delta A}{\tilde{A}_0} = \frac{\tilde{\tau}}{K_A} + \frac{k_B T}{8\pi k_c} \ln(\tilde{\tau} A/\pi^2 k_c + 1) \tag{11}$$

where it is assumed that $\tilde{\tau} \ll \pi^2 k_c/a_l$, which is valid for all realistic tensions. This expression has exactly the same structure as that of the general expression obtained previously by Evans and Rawicz (1990), which was shown to represent accurately the experimentally measured area extension as a function of applied tension. Here the plane-wave approximation is used. For $K_A \gg \tilde{\tau} \gg \pi^2 k_c/A$ ($\approx 10^{-6}$ mN · m^{-1}, for a 20-μm vesicle), a logarithmic dependence is obtained, as is observed experimentally at low membrane tensions (Evans and Rawicz, 1990). For extremely low tensions ($\tilde{\tau} \ll \pi^2 k_c/A$), a linear dependence is eventually reached. Equation 11 differs from the expression derived originally by Helfrich and Servuss (1984), in the plane-wave approximation, because a different reference state was used. Evans and Rawicz (1990) redefined the reference state of the latter authors by subtracting the contribution from the fluctuating state at zero tension, thus obtaining an expression for the plane-wave approximation with which Eq. 11 is in agreement.

The renormalization of the measured area expansion modulus, $\tilde{K}_A = A(\partial\tilde{\tau}/\partial A)$, can be derived by differentiating Eq. 7. Remembering that $A = n_l a_l/2$, where n_l is the total number of lipids in the membrane, the result for the renormalization is

$$\tilde{K}_A = K_A\left(\frac{1 - (k_B T A\tilde{\tau}/8\pi^3 k_c^2)/(\tilde{\tau} A/\pi^2 k_c + 1)}{1 + (K_A k_B T A/8\pi^3 k_c^2)/(\tilde{\tau} A/\pi^2 k_c + 1)}\right) \tag{12}$$

where the condition $\tilde{\tau} \ll \pi^2 k_c/a_l$ has again been used. The value obtained at zero initial tension is given by

$$\tilde{K}_A(\tilde{\tau} = 0) = \frac{K_A}{1 + (K_A k_B T A/8\pi^3 k_c^2)} \approx \frac{8\pi^3 k_c^2}{k_B T A} \tag{13}$$

Because $K_A k_B T A/8\pi^3 k_c{}^2 \gg 1$ when $A \gg 10^{-2}\ \mu\mathrm{m}^2$, this initial value is independent of K_A for micron-sized vesicles. For a 20-μm-diameter vesicle with a bending modulus of $k_c = 1.3 \times 10^{-19}$ J, the initial value of the modulus is extremely small: $\tilde{K}_A(\tilde{\tau} = 0) \approx 8 \times 10^{-4}$ mN · m^{-1}. For finite tensions where $\tilde{\tau} \gg \pi^2 k_c/A$ ($\approx 4 \times 10^{-4}$ mN · m^{-1}, for a 1-μm-diameter vesicle), the renormalized surface-

expansion modulus depends on the tension according to

$$\tilde{K}_A = K_A\left(\frac{1-(k_BT/8\pi k_c)}{1+(K_Ak_BT/8\pi k_c\tilde{\tau})}\right) \approx \frac{K_A}{1+(K_Ak_BT/8\pi k_c\tilde{\tau})} \quad (14)$$

Because $k_BT/8\pi k_c \approx 10^{-3}$, this latter expression agrees with the renormalization derived originally by Evans and Rawicz (1990). For typical values of $K_A = 140$ mN · m^{-1} and $k_c = 1.3 \times 10^{-19}$ J, the renormalized modulus is less than the true value by roughly a factor of 2.6 for a tension of $\tilde{\tau} = 0.1$ mN · m^{-1}, but this correction decreases to ~16% and 3% at tensions of $\tilde{\tau} = 1$ mN · m^{-1} and $\tilde{\tau} = 5$ mN · m^{-1}, respectively, in the quasilinear regimen, as has been pointed out previously (Evans and Rawicz, 1990).

CONCLUSIONS

With the membrane reference state implied by Eq. 1, the thermal elastic fluctuations in macroscopically sized membranes are visualized as inducing a tension that is balanced by a negative "bare" surface tension in the relaxed state. These fluctuations are largely suppressed in molecular dynamics simulations by the restricted cell size that is employed. In the formulation for the renormalization given by Brochard et al. (1976), the net observed tension is seen as being reduced, relative to increments in the notional bare surface tension, by the thermal fluctuations. The renormalized tension remains very low up to increments in the bare tension of 4–6 mN · m^{-1}, for ratios of the elastic constants $K_A/k_c = 2$–3 nm^{-2}. Increments in the bare tension beyond these values result in an elastic extension of the area per lipid molecule. This suggests that tensions of these values may be required in molecular dynamics simulations to produce the additional membrane extension associated with thermal fluctuations. Values of the renormalized membrane tension and renormalized area extension modulus defined here are in accordance with pipette-aspiration experiments on giant vesicles, and are in agreement with the analysis of such experiments that has been given previously (Evans and Rawicz, 1990).

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