Eur. Phys. J. B **1**, 481–489 (1998)

THE EUROPEAN PHYSICAL JOURNAL B

Stiffening of fluid membranes and entropy loss of membrane closure: Two effects of thermal undulations

W. Helfrich[a]

Fachbereich Physik, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany

Received: 10 June 1997 / Revised: 7 October 1997 / Accepted: 2 December 1997

Abstract. The problem of membrane softening by thermal undulations is revisited. In contrast to general belief, fluid membranes are predicted to be stiffened, not softened, by their undulations. Equal values of the effective bending rigidity are calculated from the interplay of local mean curvature modes (hats) on the basically flat membrane and from the coupling of spherical harmonic modes with spherical curvature. In addition, a conjecture is made on the entropy of membrane closure. It relies on a similarity of membrane closure to periodic boundary conditions.

PACS. 68.10.Et Interface elasticity, viscosity and viscoelasticity – 82.65.Dp Thermodynamics of surfaces and interfaces

1 Introduction

Fluid membranes undergo microscopically visible thermal undulations. If the undulations are strong enough, they are expected to destroy long-range orientational correlation within a membrane [1] and the equilibrium shapes of large membrane vesicles [2]. A simple parameter describing the strength of undulations is the relative excess area $\Delta A/A$, where A is the area of an averaged surface, and ΔA is the area stored by the undulations in excess of A. To lowest order, which is quadratic in the undulation mode amplitudes, the relative excess area was calculated to be [2–6]

$$\frac{\Delta A}{A} = \frac{kT}{4\pi\kappa} \ln \frac{L}{a} = \frac{kT}{8\pi\kappa} \ln M. \tag{1}$$

In the two equivalent formulas k is Boltzmann's constant, T temperature and κ the "bare" bending rigidity of the membrane. A noteworthy feature of (1) is the logarithmic dependence of $\Delta A/A$ on the size of the membrane. The membrane area enters either through the number M of fluctuation modes, typically the number of molecules (in a bilayer pairs of opposite molecules), or through the ratio of the lengths L and a characterizing, respectively, the size of a (quadratic) piece of membrane and the molecular diameter. Fluctuations are likely to become "destructive" when $\Delta A/A$ as given by (1) approaches unity and has ceased to be a good approximation. Solving (1) for L and putting $\Delta A/A = 1/2$, one finds L to be equal to the persistence length of membrane orientation introduced by de Gennes [1].

[a] e-mail: Wolfgang.Helfrich@physik.fu-berlin.de

If a polymer is longer than its persistence length it can be strongly bent by thermal energies, thus losing its stiffness on this scale. Accordingly, it seems natural to anticipate that a membrane is floppy when its size exceeds the orientational persistence length. Some theoretical work about bicontinuous microemulsions is based on this idea [1,7,8]. In addition, attempts have been made to deal with the transition from the stiff state to the floppy state by introducing an effective bending rigidity $\kappa_{\rm eff}$ that depends on the size of a bent piece of undulating membrane [5,9–13]. All authors agree on the form

$$\kappa_{\rm eff} = \kappa(1 - \alpha \frac{\Delta A}{A}), \tag{2}$$

with $\Delta A/A$ given by (1), to describe the softening as a function of membrane size. However, there is disagreement on the value of the numerical factor α. Most authors obtained $\alpha = 3$, while we predicted $\alpha = 1$. The higher value is a consequence of using the normal displacement of the fluctuations as statistical measure. We argued that (mean) curvature is the correct measure of integration, but made serious mistakes in our calculations.

All previously proposed theories of the effective bending rigidity employed global modes to describe the membrane fluctuations, such as sine waves for the flat base or spherical harmonics for the spherical base. In the meantime, local mean curvature modes, called hats, have been found useful in dealing with the bending rigidity of mixed membranes [14,15]. A precise formulation of the hat model exists as yet only for the flat base surface. In this case the hat modes have been shown to reproduce all the features of the usual linear theory of thermal undulations in terms of sinusoidal modes [14,15]. In the simplest version of the hat model, each molecule in a monolayer (or pair

of opposite molecules in a bilayer) forms the center of a hat with uniform mean curvature. The center is surrounded by a brim of pure saddle curvature which is part of a catenoidal minimal surface.

Armed with an additional model and having noted our earlier mistakes, we decided to examine once more the effects of bending fluctuations on the large-scale bending properties of fluid membranes. Therefore, we consider how hat modes affect each other in the basically flat membrane and how spherical harmonic modes couple with spherical curvature. The question of the right statistical measure does not come up explicitly in the first calculation and will only be treated before we start the second. Most surprisingly, we find in both calculations a stiffening instead of a softening of the membrane. Formula (2) remains valid, but with a negative numerical factor, $\alpha = -1$. The new result is not in conflict with the concept of a persistence length of membrane orientation. However, it suggests that the loss of orientational correlation at large enough distances is brought about not by a transition to a floppy state, but by a progressive crinkling of the membrane.

In the present study, we also propose an entropy loss ΔS of membrane closure (or vesicle formation). To derive it, we start from the hat model with doubly periodic boundary conditions and exploit a similarity of spherical membrane closure to periodicity. It may come as another surprise that the value of the entropy of closure,

$$\Delta S = -2k \ln M, \tag{3}$$

as conjectured for spheres is very similar to the entropy loss of a polymer chain being closed to form a loop. For a diffusion path the entropy of closure is known to be

$$\Delta S = -\frac{d}{2} k \ln N$$

where N is the number of steps on a simple rectangular lattice and d is dimensionality. The same formula holds for self-avoiding random walks in two and three dimensions, with slightly different numerical prefactors [16]. (All these formulas are good only for large enough M or N and omit additive constants.) We mention that Fisher raised the question of an entropy loss of membrane closure already ten years ago [17]. Very recently, Kegel *et al.* [18] have extracted from a 30-year-old article of his [19] a possible answer. It is of type (3) but with an unknown numerical factor. We will briefly return to these matters at the end of the article.

2 Membrane stiffening by thermal undulations

In order to calculate the stiffening of fluid membranes by bending fluctuations, we first study the flat membrane in terms of the hat model. Subsequently, we consider the freely fluctuating sphere, using the global undulation modes expressed by spherical harmonics. The first calculation is novel, while the second is based on previous work and focuses on correcting a mistake. As is common in dealing with thermal undulations, the validity of most of the results is restricted to rather weakly fluctuating membranes which will be called almost flat or almost spherical.

Both calculations are based on the bending energy g per unit area of fluid membrane in the standard form

$$g = \frac{1}{2}\kappa(c_1 + c_2)^2 - \kappa c_\mathrm{s}(c_1 + c_2) + \bar{\kappa} c_1 c_2. \tag{4}$$

Here c_1 and c_2 are the principal curvatures, κ is the bending rigidity, and $\bar{\kappa}$ is the bending modulus of Gaussian curvature $K = c_1 c_2$. The spontaneous curvature c_s is taken to be zero and, specifically, bilayers are assumed to be symmetric, unless otherwise stated. We will frequently use the sum of principal curvatures (or "splay" in liquid crystal language) $J = c_1 + c_2$ and, correspondingly, $J_\mathrm{s} = c_\mathrm{s}$. J is related to the mean curvature H through $J = 2H$. In the following, the membrane is assumed to be unstretchable and, in the case of vesicles, permeable to water.

2.1 The almost flat membrane and the hat model

The base surface of the almost flat membrane is taken to coincide with the xy plane of a Cartesian xyz coordinate system. An instantaneous shape of the fluctuating membrane can then be described by $z = u(x, y)$, *i.e.* the height of the membrane relative to the base plane as a function of position. Each local bending fluctuation or hat is made of a central part of uniform J, to be called cap, and a brim of zero J. The membrane is thought to be completely divided into caps which, for simplicity, are approximated by (slightly bent) circular disks. If only a single such hat is "excited" in an infinite membrane, it will consist of a cap with pure spherical curvature and a brim with pure saddle curvature which is part of a catenoid. The continuous slope $\psi(\rho)$ of the axisymmetric hat depends on the distance ρ in the xy plane from the hat's center, having its extremum $\psi_0 = \psi(\rho_0)$ at the boundary $\rho = \rho_0$ of the cap. For generally small slopes, *i.e.* for $\psi_0^2 \ll 1$, the brim drops or rises logarithmically with ρ, depending on whether the hat points upwards or downwards [14,15].

In the case of the almost flat membrane ($|\mathrm{grad}\, u| \ll 1$ or $\Delta A/A \ll 1$), the shape of the freely fluctuating membrane is, in the usual linear approximation, the superposition of the displacements u of all the hats. The superposition principle applies equally to the gradients and the mean curvatures. Summing up the deformations leaves the uniform mean curvature of each cap unchanged, but may alter its positive Gaussian curvature because of the saddle curvature residing in the brims of other hats. Standard Fourier transformations lead from the hat modes to sinusoidal curvature modes and, with good accuracy, back to the hat modes. Each sinusoidal curvature mode is at the same time a displacement mode where $J = q^2 u$, q being the wave number of the mode. This relationship enables us to go from hats to displacements and *vice versa* in the linear approximation. The diameter of the cap, $2\rho_0$, is equivalent to the length a in the upper wave vector cutoff π/a and may be identified with a molecular diameter.

For a membrane of zero spontaneous curvature the equipartition theorem of the hat model has the general form

$$\frac{1}{2}\kappa\langle J^2\rangle A_0 = \frac{1}{2}kT \tag{5}$$

where $A_0 = \pi\rho_0^2$ is the area of the slightly bent disk representing the cap of a hat. $J = c_1 + c_2$ is related to the cap radius $\rho_0 (= a/2)$ and the cap boundary angle ψ_0 by

$$J = \frac{2\psi_0}{\rho_0}\,. \tag{6}$$

If the membrane is a monolayer or bilayer consisting of a single species of amphiphilic molecules, equation (5) is simply the equipartition theorem valid for a membrane disk of fluctuating mean curvature. The Gaussian curvature term in (3) can be omitted since the fluctuations do not change the topology of the membrane. Inserting (6) in (5) leads to

$$\langle\psi_0^2\rangle = \frac{kT}{4\pi\kappa}\,. \tag{7}$$

Taking $kT = 4\times10^{-21}$ J (room temperature) and $\kappa = 1\times10^{-19}$ J (typical of lipid bilayers), one finds $\langle\psi_0^2\rangle^{1/2} \approx 3^\circ$, in agreement with the assumption of an almost flat membrane. The fact that $\langle\psi_0^2\rangle$ is independent of ρ_0 reflects the well-known scale invariance of membrane bending energies and permits some freedom in defining the size of the spherical caps.

In many situations it makes no difference whether the statistical mechanics of out-of-plane membrane fluctuations is based on a Fourier mode model with displacement, gradient angle, or mean curvature as statistical measure, or on the hat model. The latter was introduced because it can be generalized to allow a straightforward calculation of the bending rigidity of mixed membranes made of very different molecules [14,15]. For this purpose it is attractive to rewrite (5) in the form

$$\frac{1}{\kappa} = \langle J^2\rangle\frac{A_0}{kT} \tag{8}$$

which emphasizes that the flexibility $1/\kappa$ is proportional to the mean-square strength $\langle J^2\rangle$ of the local bending fluctuations. $\langle J^2\rangle$ contains the weighted sum of the hat bending fluctuations of the various molecules. In addition, it includes any local bending fluctuations resulting from the diffusion of molecules (or pairs of molecules in a bilayer) of different spontaneous curvature. (To keep things simple, we assume uniformity of both A_0 and $\bar\kappa$ in the present work.)

We would like to stress that at least in equilibrium statistical mechanics the hat modes are in no way inferior to the usual sinusoidal modes. Like the latter, they may be regarded as a complete set of energetically decoupled fluctuation modes in the linear approximation of the almost flat membrane. The cutoff problems of the Fourier representation translate into the problems of disk size and shape when the hat modes are used. Periodic boundary conditions for the hat model will be discussed below in connection with the entropy of membrane closure. It is an advantage of the hat modes, particularly useful in the case of mixed membranes, that they relate the (macroscopic) bending rigidity to the (microscopic) bending properties of the molecules composing the membrane.

Before calculating by means of (8) the effective increase of the bending rigidity due to thermal undulations we write down the very direct derivation of the excess area δA bound by a hat of cap boundary angle ψ_0 [14]. With $\psi(\rho) = \psi_0\rho_0/\rho$ for $\rho > \rho_0$, one has

$$\delta A = \int_{\rho_0}^{\rho_{\max}} \frac{1}{2}\psi_0^2\frac{\rho_0^2}{\rho^2}2\pi\rho\mathrm{d}\rho$$

$$= \pi\rho_0^2\psi_0^2\ln(\rho_{\max}/\rho_0) \tag{9}$$

if the contribution from the cap is neglected. Dividing δA by $A_0 = \pi\rho_0^2$, taking the statistical average (7) for ψ_0^2, and replacing $\rho_{\max}$ and ρ_0 by L and a, respectively, lead back to (1) as expected. It is sufficient to consider a fixed position accommodating a single molecule since in a membrane of freely diffusing and fluctuating hats, the local bending fluctuations can be regarded as statistically independent.

Let us now employ the hat model to calculate the effective bending rigidity of the weakly fluctuating membrane. Because of the presence of other hats, the cap of each hat will in general be (slightly) tilted by an angle τ from the direction normal to the xy plane. Taking this effect into account, we correct the linear hat model to lowest order. The tilt of a cap reduces the strength of the associated brim. To show this, we start from the relationship

$$J = \mathrm{div}\ \mathbf{n} = \frac{\partial n_x}{\partial x} + \frac{\partial n_y}{\partial y} \tag{10}$$

where

$$\mathbf{n}(x,y) = \frac{(-\partial u/\partial x, -\partial u/\partial y, 1)}{[1+(\partial u/\partial x)^2 + (\partial u/\partial y)^2]^{1/2}}$$

is the director, a unit vector normal to the membrane. Note that (10) is valid at any tilt τ of the membrane. Next we make use of an electrostatic analogy, comparing $\partial n_x/\partial x + \partial n_y/\partial y$ with a charge density and (n_x, n_y) with an electric field. In this strictly two-dimensional analogy, the tilt of the cap does not affect the charge density, but it does reduce the area which the cap projects on the xy plane. Accordingly, the tilt of the cap lowers the total charge of a cap on average by the factor

$$1 - \frac{1}{2}\langle\tau^2\rangle = 1 - \frac{\Delta A}{A}.$$

The mean strength of the associated brim decreases by the same factor. It is proportional to the total charge of the cap in the electrostatic analogy because it varies as

the number of field lines (or "tilt lines") emanating from the cap. The (slight) deviation of the projection of the cap from axisymmetry should be irrelevant at large distances.

The electrostatic analogy suggests that the effect of tilt can be taken into account to lowest order by reducing in (8) the area A_0 of the molecules while keeping $\langle J^2 \rangle$ fixed. Replacing A_0 by $A_0(1 - \Delta A/A)$ in this equation, one obtains the effective bending rigidity κ_{eff} from

$$\frac{1}{\kappa_{\text{eff}}} = \langle J^2 \rangle \frac{A_0}{kT}(1 - \frac{\Delta A}{A}).$$

Evidently, κ_{eff} is related to the "bare" bending rigidity κ through

$$\kappa_{\text{eff}} = \kappa/(1 - \frac{\Delta A}{A}).$$

Because of $\Delta A/A \ll 1$ and (1) this may be rewritten as

$$\kappa_{\text{eff}} = \kappa + \frac{kT}{4\pi} \ln \frac{L}{a} \tag{11}$$

for the almost flat membrane. According to this result, the bending rigidity *increases* logarithmically with the scale on which the membrane is bent.

2.2 The almost spherical membrane and spherical harmonics

In the case of fluctuating spherical vesicles, to be treated next, we will use the global fluctuation modes expressed by spherical harmonics. (Hat modes on the sphere pose the problem that they are not completely decoupled; see below.) Since we will refer to our earlier calculation [5] and only change the measure of integration, let us first look into the problem of the measure.

The natural measure of the hat model and the sinusoidal curvature modes related to it by Fourier transformations, is the mean curvature H or, as we prefer, $J = 2H = c_1 + c_2$. The logarithmic divergence of the height u in the brim of a hat rules out, in the case of an infinite membrane, the use of normal displacement even as a substitute for the true measure. Another argument for the curvature measure is the fact that the strain of the membrane is curvature and not displacement or tilt. Also, curvature in terms of the angle made by subsequent links is the accepted measure of integration in the similar case of fluctuating polymer chains [20]. The appropriateness of mean curvature in the case of membranes becomes particularly evident if one builds up the surface from single molecules, adding them row by row along a membrane edge. The positional freedom of a new molecule is very much restricted by the position and orientation of the membrane edge to which it is added. Essentially, we can only choose the height of the molecule relative to a membrane patch. If the patch consists of at least four preexisting adjacent molecules, this height determines the mean curvature of the membrane at the center of the complemented patch.

We emphasize that these local measures are not in conflict with the basic measure of classical statistical mechanics which is displacement (after integrating out momentum). It is the limitation to small *relative* displacements, because of the cohesion of the material, that leads to the redefinition of position as the angle difference between subsequent polymer links or the mean curvature of a group of molecules.

To elucidate this point, let us briefly have recourse to the basics of statistical mechanics. A well-known method to visualize and count microstates in classical statistical mechanics is to represent the membrane molecules by mass points, each attached to a lattice point of a three-dimensional cubic lattice. The lattice parameter equals the thermal de Broglie wavelength of the mass points. In this simple picture, which disregards internal microstates of the molecules, we can describe the fluctuating membrane in a satisfactory manner if the distance between neighboring molecules is at least several lattice parameters and fluctuates over a length larger than one lattice parameter. In such a model it is evident that the factor by which each molecule increases the number of participating membrane configurations equals the number of lattice points that are thermally accessible to the molecule complementing the patch. Accordingly, the total entropy of membrane bending fluctuations is the sum of the local entropies of mean curvature. It is independent of the absolute displacement u of the mass point from a base plane. (Of course, there is also a molecular entropy of lateral distance fluctuations. It can be separated from that of bending and is of no interest in the present context.)

The bending fluctuations of a (practically) unstretchable membrane are associated with lateral flows. Lateral motion does not need to be analyzed in equilibrium statistical mechanics and can often be neglected entirely as it is quadratic in the mode amplitudes. However, a one-to-one mapping of the deformed membrane states on a reference surface and *vice versa* has to be possible, for small and large deformations, to ensure the proper counting of microstates in terms of molecular mean curvatures. Fortunately, invertible mappings satisfying local area conservation and fully characterized by local mean curvatures appear, in special cases, easy to devise. A basically flat infinite membrane with nonvanishing mean curvature only in a finite region may serve as an example. Choosing the strictly flat state as reference state, one can return the deformed membrane to it in a unique way by controlling the lateral flow of membrane material. A satisfactory prescription (in the absence of overhangs) would be purely radial flow from a center, *e.g.* the origin of the xy coordinate system. After the flattening of the membrane, we have a pattern of original mean curvatures in the xy plane. To make it visible, we could imagine each membrane molecule to carry a little tag indicating its original mean curvature. Inspection shows that any change of the deformed surface, apart from a general change of height, leads to a different pattern of tags. This strongly suggests that the mapping of the deformed surface on the xy plane and *vice versa* is unique in both directions.

From a broader perspective, it seems attractive to invoke the fundamental theorem of surfaces which allows three degrees of freedom in surface mapping. They are needed in the present case to specify mean curvatures and to guarantee local area conservation and invertibility, but it is not generally clear how to incorporate the obviously important boundary conditions.

We do not repeat here in detail our previous calculation of the bending energies of weakly fluctuating spherical vesicles without volume constraint [5]. The same results were obtained in similar forms by others [3,6] and also once more by us [21], in connection with different problems.

Describing the surface of the slightly deformed sphere by

$$r(\theta,\phi) = r_0' + u(\theta,\phi)$$

where r, θ, ϕ are the usual polar coordinates and r_0' is a renormalized radius of the sphere (see below), one has the director

$$\mathbf{n}(\theta,\phi) = \frac{(1, -\partial u/r\partial\theta, -\partial u/r\sin\theta\ \partial\phi)}{[1+(\partial u/r\partial\theta)^2+(\partial u/r\sin\theta\ \partial\phi)^2]^{1/2}}$$

and the curvature

$$J = \operatorname{div}\mathbf{n} = \frac{1}{r^2}\frac{\partial}{\partial r}(r^2 n_r) + \frac{1}{r\sin\theta}\frac{\partial}{\partial\theta}(\sin\theta\ n_\theta) + \frac{1}{r\sin\theta}\frac{\partial n_\phi}{\partial\phi}.$$

It is advantageous to adopt the operators

$$\nabla_2 = \left(\frac{\partial}{\partial\theta}, \frac{1}{\sin\theta}\frac{\partial}{\partial\phi}\right)$$
$$\Delta_2 = \frac{1}{\sin\theta}\frac{\partial}{\partial\theta}\left(\sin\theta\frac{\partial}{\partial\theta}\right) + \frac{1}{\sin^2\theta}\frac{\partial^2}{\partial\phi^2},$$

and to introduce the relative radial displacement of the surface

$$f(\theta,\phi) = u(\theta,\phi)/r_0'.$$

Keeping here and in the following only terms up to quadratic order in f, one finds

$$\operatorname{div}\mathbf{n} = \frac{2}{r_0'}\left[1 - f - \frac{1}{2}\Delta_2 f + f^2 + f\Delta_2 f\right] \tag{12}$$

after some algebra. The differentials of surface area and solid angle $\mathrm{d}\Omega = \sin\theta\ \mathrm{d}\phi\mathrm{d}\theta$ are related through

$$\mathrm{d}A = r_0'^2\left[1 + 2f + f^2 + \frac{1}{2}(\nabla_2 f)^2\right]\mathrm{d}\Omega.$$

The last two equations lead to the total bending energy

$$G = \frac{1}{2}\kappa\oint(\operatorname{div}\mathbf{n})^2\mathrm{d}A$$
$$= 2\kappa\oint\left[1 - \Delta_2 f + \frac{1}{4}(\Delta_2 f)^2 + f\Delta_2 f + \frac{1}{2}(\nabla_2 f)^2\right]\mathrm{d}\Omega \tag{13}$$

where the Gaussian curvature term $4\pi\bar{\kappa}$ is omitted. The linear term $\Delta_2 f$ will be dropped in the following as its integral over the sphere vanishes. Global fluctuation modes are defined by expanding f in spherical harmonics

$$f(\theta,\phi) = \sum_{l=2}^{\infty}\sum_{m=-l}^{+l} a_{lm}Y_{lm}(\theta,\phi).$$

The $l = 0$ and the three $l = 1$ modes are left out as they represent uniform radial displacement and vesicle translation, respectively. The spherical harmonics are an orthonormal set of functions and fulfill the eigenvalue equation

$$\Delta_2 Y_{lm} = -l(l+1)Y_{lm}.$$

Any function $f(\theta,\phi)$ satisfies

$$\oint(\nabla_2 f)^2\mathrm{d}\Omega = -\oint f\Delta_2 f\mathrm{d}\Omega, \tag{14}$$

a formula to be used below.

In the earlier paper [5] we claimed erroneously that on a sphere it does not matter whether curvature or displacement is taken as the measure of integration and for simplicity chose displacement. The resulting partition function for the fluctuating sphere is the functional integral

$$\oint\mathcal{D}f\exp\left[-G(f,\nabla_2 f,\Delta_2 f)/kT\right]$$

where it makes no difference, apart from an irrelevant factor, whether we write $\mathcal{D}u$ or $\mathcal{D}f$. With f as statistical measure, it is attractive to cast (13) in the form

$$G = 2\kappa\oint\left[1 + \frac{1}{4}(\Delta_2 f)^2 - \frac{1}{2}(\nabla_2 f)^2\right]\mathrm{d}\Omega \tag{15}$$

and regard the second term as the "regular" bending energy of the fluctuations. The third term of (15) lumps together, by means of (14), the last two terms of (13). It is regarded as a "correction" due to a coupling of the fluctuation modes with spherical curvature. The particular definition of the "regular" bending energy may seem to be justified by the close correspondence of $\Delta_2 u/(r_0')^2$ on a sphere to $\partial^2 u/\partial x^2 + \partial^2 u/\partial y^2$ on a plane. The expansion

$$\oint\frac{1}{2}\kappa(\Delta_2 f)^2\mathrm{d}\Omega = \frac{1}{2}\kappa l^2(l+1)^2\sum_{l,m}|a_{lm}|^2$$

displays the "regular" bending energies of the fluctuation modes.

Relying on the displacement measure, other authors dealing with the effective bending rigidity of vesicle membranes appear to have been guided by similar ideas. However, the fact that $\Delta_2 f$ is not the only linear term in J as given by (12) may be taken as a warning that f is not the right measure of integration.

Selecting curvature as the measure leads to a different partition function,

$$\oint\mathcal{D}J\exp\left[-G(f,\nabla_2 f,\Delta_2 f)/kT\right]\ .$$

In our quadratic approximation, $\mathcal{D}J$ may be replaced by $\mathcal{D}(-\Delta_2 f - 2f)/r_0'$, the total contribution in (12) to $J = \text{div } \mathbf{n}$ that is linear in f. It is now appropriate to cast (13) in the form

$$G = 2\kappa \oint \left[1 + (\frac{1}{2}\Delta_2 f + f)^2 - f^2 + \frac{1}{2}(\nabla_2 f)^2\right] \mathrm{d}\Omega \quad (16)$$

and to consider the second term

$$\oint \frac{1}{2}\kappa(\Delta_2 f + 2f)^2 \mathrm{d}\Omega = \frac{1}{2}\kappa[l(l+1) - 2]^2 \ |a_{lm}|^2$$

to be for the regular bending energy of the thermal undulations, with $[l(l+1)-2]a_{lm}$ being the curvature mode amplitude. The new mode energies are seen to differ from their counterparts in (15). The last two terms of (16) are the corrections due to the coupling of the fluctuation curvatures with the spherical base.

To corroborate the assignments let us for a moment subtract from J a spontaneous curvature $J_\mathrm{s} = c_\mathrm{s} = 2/r_0'$ just canceling the first term in (12). In this particular case, only the second term of (16) survives as is to be expected in the absence of coupling. Also, if the global fluctuation modes are approximated by vectors in a molecular curvature space (J space) of dimensionality M, it becomes apparent that contributions to the curvature J of the mode lm that are quadratic in a_{lm} disappear in our quadratic approximation when the curvature vector is squared. (In order to see this, it helps to shift the origin of the molecular curvature space to the point of uniform spherical curvature.)

Inspection shows that the third term in (16) is negligible in comparison with the fourth unless the number of vesicular undulation modes (or molecules) is unrealistically small. When it is omitted the correction terms in (15) and (16) differ only in sign.

Previously, we derived the effective bending rigidity of the sphere with a standard method of statistical mechanics. For each mode, we computed the slight change of its mean-square amplitude arising from the bending energy correction term in (15). The resulting change in mode entropy translates into a change of mode free energy. Integration over all modes leads to a free energy, negative in the old calculation, which has to be added to $8\pi\kappa$, the bending energy of the nonfluctuating sphere, to obtain the total free energy of bending and, from it, the effective bending rigidity. An outline of this type of calculation, now with J as measure and employing (16), is given in the Appendix.

As an attractive alternative we take here a shortcut which should be equally valid as it means an exchange of roles between spherical curvature and bending fluctuation mode. Imagining the thermal undulations to "freeze" in their instantaneous amplitudes, we treat the $(1/2)(\nabla_2 f)^2$ term in (16) as a correction of the bending energy of the sphere due to the frozen undulations. Because of

$$\langle \oint \frac{1}{2}(\nabla_2 f)^2 \mathrm{d}\Omega \rangle = \frac{\Delta A}{A}$$

and the second form of (1) this leads, after taking thermal averages and subtracting the regular mode energies, to the total (free) energy of membrane bending

$$\Delta G = 8\pi\kappa \left(1 + \frac{kT}{8\pi\kappa} \ln M\right),$$

and thus to

$$\kappa_\mathrm{eff} = \kappa + \frac{kT}{8\pi} \ln M. \quad (17)$$

The result suggests a convenient interpretation: The undulations of the spherical vesicle produce the relative excess area $\Delta A/A$ which has to assume the curvature $J = 2/r_0'$ of the sphere. The excess area could be supplied by a reservoir, but in our case of constant membrane area it results from a shrinkage of the sphere radius. The prime on r_0 in our formulas is to indicate the renormalized, shrunken radius as opposed to the radius of the nonfluctuating sphere.

We have obtained in two entirely different ways equivalent results, equations (11, 17), for the effective bending rigidity as a function of the scale of bending. Both of them may be expressed by equation (2), the numerical factor being $\alpha = -1$. Clearly, a negative value of α means that the bending fluctuations make fluid membranes stiffer, not softer.

It is not self-evident that the stiffening should be the same for the large-scale bending fluctuations of a planar membrane and for spherical curvature in the presence of fluctuations. The stiffening is independent of geometry because the correction of the bending modulus $\bar{\kappa}$ of Gaussian curvature, predicted to exist and to be of type (2) by other authors [9,12,13], vanishes if J is the statistical measure. This may be seen by superimposing a (weak) undulation on a base with pure saddle curvature ($J = c_1 + c_2 = 0, K = c_1 c_2 < 0$). An undulation of fixed mean curvatures cannot interact with a base having no mean curvature of its own. The displacement measure fakes a coupling because at fixed normal displacement the associated J varies with saddle curvature. As an illustration, we write down J for a base which is of pure and uniform saddle curvature $c_1 = -c_2 = 1/r_0$ and parallel to the xy plane in the origin of the xy coordinate system. (Since there are no surfaces of uniform pure saddle curvature, uniformity can only only be approximated.) With u being the normal displacement from the base, we find up to second order in u and its derivatives

$$\begin{aligned} J &= \frac{1}{r_0 + u} - \frac{1}{r_0 - u} - \Delta u \\ &= -2\frac{u}{r_0^2} - \frac{\partial^2 u}{\partial x^2} - \frac{\partial^2 u}{\partial y^2}, \end{aligned} \quad (18)$$

if only a small enough vicinity of the origin is considered. The first term in (18), $-2u/r_0^2$, couples u and $K = -1/r_0^2$ in the displacement measure, but it is part of the curvature of the undulation itself if J is the measure.

3 Entropy loss of membrane closure

Before studying the entropy loss of membrane closure it seems useful to digress on a related system, the finite almost flat membrane with periodic boundary conditions in two directions. For a special version of the hat model, *i.e.* hats with a fixed cap boundary angle ψ_0 that point either upwards or downwards, periodic boundary conditions require equal numbers of positive and negative hats. Conversely, any periodic arrangement of positive and negative hats in equal numbers satisfies periodic boundary conditions. If the restriction is lifted and the hats can turn around in a bilayer (free flip-flop) or move in and out of equal reservoirs for positive and negative hats, the number of positive hats, M_+, satisfies the Gaussian distribution function

$$w(M_+) = (\pi M/2)^{-1/2} \exp\left[-\frac{(M_+ - M/2)^2}{M/2}\right]$$

where $M \gg 1$ is the total number of hats in the membrane as above. The same distribution applies, of course, to the number of negative hats, M_-. Since the distribution function is normalized such that its integral is unity, the imposition of periodic boundary conditions changes the fluctuation entropy of the membrane by

$$\Delta S = k \ln w(0) = -\frac{1}{2} k \ln M \qquad (19)$$

apart from an additive constant, $-(k/2)\ln(\pi/2)$ in this particular case. If there are n thermally accessible molecular curvature states, we have to add to (19) a term $-k \ln n$ (and change the additive constant). In some cases curvature states could, perhaps, be defined in terms of a quantum mechanical description. In the classical case of continuous cap curvature there is still such a term, with $n \gg 1$ characterizing the number of participating "smeared" microstates. We do not try here to estimate n. (Also, we do not discuss the relationship between molecules as mass points on a lattice, the picture used above, and molecules as caps of hats.) We are interested mainly in the part of the entropy of periodic boundary conditions that depends on M and is given by equation (19). All other parts of this entropy, including one of mixing, should depend on molecular properties and be independent of M.

If a Fourier expansion of mean curvature is used instead of hat modes, it will in principle include a zero wave vector mode of uniform mean curvature. Periodic boundary conditions suppress this mode, while they seem not to interfere with any others. The suppression of the uniform curvature mode is equivalent to the previous condition of equal numbers of positive and negative hats. Therefore, we can return to the hat model for an evaluation of the associated entropy loss. In this way we arrive again at equation (19).

A little reflection reveals that we have obtained a curious result. The entropy loss of (19) comes in addition to that of suppressing the mean-curvature fluctuations of a single molecule, $-k \ln n$. Evidently, the suppression of the zero wave vector mode can have a much stronger effect than the suppression of the bending fluctuations of a single molecule.

The entropy of membrane closure appears to be similar, at least in the case of spheres, to the entropy of periodic boundary conditions. In order to show this, we first note that the constraint of fixed membrane area rules out fluctuations of the uniform spherical curvature in a linear approximation. Using the two-state hat model, we then create a sphere by producing an excess of positive hats in a piece of membrane. The required excess number of positive hats should increase linearly with the radius of the sphere. It will be much smaller than the total number M of molecules if the vesicle is large enough. Accordingly, we may expect (19) to be a good approximation for the entropy of suppressing the fluctuations of uniform curvature of such vesicles. The model can again be generalized by introducing multistate or continuous cap curvatures. If the molecular bending energies are continuous and quadratic in J, equation (19) should be exact for weakly fluctuating spheres of all sizes, apart from an unknown additive constant.

Since spherical closure suppresses fluctuations not only of the $l = 0$ but also of the three $l = 1$ curvature modes, we may expect the total entropy of vesicle closure to consist of altogether four contributions of type (19). In this way we arrive at equation (3), announced in the Introduction, which differs from (19) only by its numerical factor (2 instead of 1/2). We cannot exactly calculate the additional contributions as the suppression of a harmonic curvature mode is difficult to define for flat and spherical membranes.

Hat modes on a sphere pose a further problem. As an illustration, we consider a membrane with only two hats. If their caps are of equal but opposite curvature (in addition to a base curvature), the mean curvature will vanish on the rest of a periodically planar membrane, but it will not be generally uniform on the rest of a sphere. This is another consequence of the absence of the $l = 1$ spherical harmonic modes of curvature. It implies that there are no fully decoupled hat modes on a sphere even in the linear approximation. The problem seems irrelevant for large spheres and can be circumvented by using spherical harmonic modes. This makes us confident that the entropy loss of spherical membrane closure is indeed given by equation (19), apart from an additive constant depending on molecular properties. Very small vesicles may be an exception for the reasons given above.

4 Concluding remarks

In the present work we derived the effective bending rigidity in two entirely different ways, using local bending modes (hats) in one case and global bending modes (spherical harmonics) in the other. The calculation based on hat modes in almost flat membranes indicates that these local bending modes weaken each other's contribution to the bending fluctuations of the membrane in the large. The weakening is due to the tilt which one hat imposes

on another residing in its brim. It appears to be the beginning of a self-screening of hats which becomes extreme when an aggregation of hats of one sign produces a nearly spherical bud in the membrane. In such a case, the action of those hats on the rest of the membrane can, in principle, vanish completely. The relatively weak self-screening within a single spherical cap has been neglected in our calculations.

The other calculation of $\kappa_{\rm eff}$ employs spherical harmonic modes to obtain the effective energy of spherical curvature in the presence of bending fluctuations. The result of it depends on whether mean curvature or displacement is taken as measure of integration. We selected mean curvature and gave several reasons for this choice. The fact that the two different calculations of the effective bending rigidity lead to the same fluctuation induced membrane stiffening lends additional support to the choice of mean curvature H or $J = 2H$.

To obtain a formula for the entropy loss of membrane closure we first considered the planar membrane with periodic boundary conditions in two directions. The entropy loss due to these boundary conditions, which amount to the suppression of the global fluctuation mode of uniform curvature, was calculated in terms of the hat model. Starting from molecular mean curvature fluctuations seems to be indispensable in deriving this entropy. The result was adopted as a conjecture for the sphere and, in particular, applied to all of its four suppressed fluctuation modes. The entropy loss obtained should be independent of spontaneous curvature unless the latter is strong enough to deform the sphere. Nonspherical equilibrium shapes and fixed vesicle volumes raise additional problems which remain to be studied.

Both fluctuation effects, the stiffening of membranes and the entropy of membrane closure, will have consequences for the stability of microemulsions and the size distribution of vesicles. Presently available experimental data seem to be inconclusive in both respects. As a relatively recent result we would like to mention an analysis of data collected on droplet microemulsions in thermodynamic equilibrium with an amphiphilic interface. Assuming a correction of the type $z'kT\ln M$ to the bending energy of a closed spherical membrane (with $J_{\rm s} = 2/r_0'$), Kegel *et al.* [18] estimated the numerical prefactor to be in the range $0 < z' < 3/4$ which does not quite agree with our prediction $z' = 2$. The idea of a logarithmic correction with a yet to be determined numerical factor was taken from Fisher's early theory of condensation and the critical point [19].

I am grateful to R. Lipowsky, R. Netz, H. Pinnow and T. Weikl for discussions.

Appendix

We give here in outline the derivation of the effective bending rigidity of a fluctuating spherical membrane by way of a standard method of statistical mechanics. The starting point is equation (16) and its discussion.

The equipartition theorem for a bending fluctuation mode expressed by the relative displacement $f = u/r_0'$ reads without coupling to spherical curvature ($J_{\rm s} = 2/r_0'$)

$$\frac{1}{2}\kappa\left[l(l+1)-2\right]^2\left\langle|a_{lm}|^2\right\rangle_{J_{\rm s}=2/r_0'} = \frac{1}{2}kT \qquad (20)$$

and with full coupling to spherical curvature ($J_{\rm s} = 0$)

$$\frac{1}{2}\kappa\left\{\left[l(l+1)-2\right]^2 + 2l(l+1)\right\}\left\langle|a_{lm}|^2\right\rangle_{J_{\rm s}=0} = \frac{1}{2}kT. \qquad (21)$$

We are interested in the ratios R_l of the mean-square curvature mode amplitudes without and with coupling. Since the curvature amplitudes equal $[l(l+1)-2]$ times the displacement amplitudes, we can replace them in each ratio by the displacement amplitudes. From

$$\begin{aligned} R_l &= \frac{\left\langle|a_{lm}|^2\right\rangle_{J_{\rm s}=2/r_0'}}{\left\langle|a_{lm}|^2\right\rangle_{J_{\rm s}=0}} \\ &= \frac{\left[l(l+1)-2\right]^2}{\left[l(l+1)-2\right]^2 + 2l(l+1)} \end{aligned}$$

we obtain the change in entropy of a single mode due to the coupling

$$\begin{aligned} \Delta S_l = \frac{1}{2}k\ln R_l &\approx -\frac{1}{2}k\frac{2l(l+1)}{\left[l(l+1)-2\right]^2} \\ &\approx -k\frac{1}{l^2}, \end{aligned}$$

where the approximations are valid for $l \gg 1$.

Summation over all modes lm leads to the total entropy change

$$\begin{aligned} \Delta S &\approx -k\sum_{l=2}^{l_{\max}}\frac{2l+1}{l^2} \\ &\approx -2k\int_1^{l_{\max}}\frac{1}{l}{\rm d}l \approx -k\ln M \end{aligned}$$

where use is made of $M \approx l_{\max}^2$. Accordingly, the interaction with spherical curvature increases the free energy of the thermal undulations by $kT\ln M$. Inserting this in

$$8\pi\kappa - T\Delta S = 8\pi\kappa_{\rm eff},$$

an equation defining the effective bending rigidity of the spherical membrane, we arrive at equation (17).

References

1. P.G. de Gennes, C. Taupin, J. Phys. Chem. **86**, 2294 (1982).
2. W. Helfrich, Z. Naturforsch. **30c**, 841 (1975).
3. S.A. Safran, J. Chem. Phys. **78**, 2073 (1983).

4. W. Helfrich, R.M. Servuss, Il Nuovo Cimento D **3**, 137 (1984).
5. W. Helfrich, J. Physique **47**, 321 (1986).
6. S.T. Milner, S.A. Safran, Phys. Rev. A **36**, 4371 (1987).
7. D. Andelman, M.E. Cates, D. Roux, S.A. Safran, J. Chem. Phys. **87**, 7229 (1987); D. Andelman, S.A. Safran, D. Roux, M.E. Cates, Langmuir **4**, 802 (1988).
8. L. Golubovic, T.C. Lubensky, Phys. Rev. A **41**, 43 (1990).
9. L. Peliti, S. Leibler, Phys. Rev. Lett. **54**, 1960 (1985).
10. W. Helfrich, J. Physique **46**, 1263 (1985); *ibidem* **48**, 285 (1987).
11. D. Förster, Phys. Lett., **114A**, 115 (1986); Europhys. Lett. **4**, 65 (1987).
12. H. Kleinert, Phys. Lett. **114A**, 263 (1986); *ibidem* **116A**, 57 (1986).
13. F. David, in *Statistical Mechanics of Membranes and Interfaces*, edited by D. Nelson, T. Piran, S. Weinberg (World Scientific, 1989).
14. W. Helfrich, Liq. Crystals **5**, 1647 (1989).
15. W. Helfrich, M.M. Kozlov, J. Phys. II France **4**, 1427 (1994).
16. P.G. de Gennes, *Scaling Concepts in Polymer Physics* (Cornell University Press, 1979).
17. M.E. Fisher, remark in the discussion following a talk of the author at the Spring Meeting of the American Physical Society in 1987, New York, NY.
18. W.K. Kegel, H. Reiss, Ber. Bunsenges. Phys. Chem. **100**, 300 (1996); W.K. Kegel, Th.G. Overbeck, H.N.W. Lekkerkerker, to appear in *Microemulsions: Fundamental and Applied Aspects*, edited by P. Kumar, K.L. Mittal.
19. M.E. Fisher, Physics **3** 253 (1967).
20. K.F. Freed, in *Advances in Chemical Physics XXII*, edited by. I. Prigogine, S.A. Rice (Wiley-Interscience, 1972)
21. Ou-Yang Zhong-can, W. Helfrich, Phys. Rev. A **39**, 5280 (1989).