SIZE DISTRIBUTION OF SPHERICAL VESICLES

H. KLEINERT [1,2]

Department of Physics, University of California, San Diego, La Jolla, CA 92093, USA

Received 6 January 1986; revised manuscript received 19 March 1986; accepted for publication 21 March 1986.

We show that mean, gaussian, and spontaneous curvature constants κ, $\bar{\kappa}$, and $c_s\kappa$ of spherical vesicles change with temperature by $-[T/8\pi]\alpha \log N$, where N is the number of molecules and α takes the values 3, $-\frac{10}{3}$, -1, in the three cases. For bilipid vesicles, κ, $\bar{\kappa}$, and c_s have two sources, the area elasticity of monolayers and the liquid crystal orientational bending energy. If $\rho \in [0,1]$ denotes the elastic fraction in κ, we find the size distribution $P(N) \propto N^{7\rho^2-6\rho+4/3}\,\mathrm{e}^{-\mathrm{const}\times N}$ such that for pure liquid crystal bending $P(N) \propto N^{4/3}\,\mathrm{e}^{-\mathrm{const}\times N}$.

In a recent paper, Helfrich [1] points out that the thermal softening of the curvature elastic constants has simple observable consequences for the size distribution of spherical vesicles in equilibrium. Since such distributions are in the process of being measured rather accurately [2], it is worth obtaining a reliable theoretical prediction. If the bare curvature elastic constants are defined as in Helfrich's original paper [3]

$$E_{\mathrm{curv},0} = \tfrac{1}{2}\kappa_0 \int \mathrm{d}^2\xi\, \sqrt{g}\,(c_1 + c_2 - c_0)^2 + \kappa_0 \int \mathrm{d}^2\xi\, \sqrt{g}\, c_1 c_2, \tag{1}$$

where $(c_1 + c_2)/2$ is the mean, and $c_1 c_2$ the gaussian and $c_0 \equiv 2R_0$ the spontaneous curvature, the energy of a spherical vesicle receives a contribution

$$E_{\mathrm{curv},0} = 8\pi\kappa_0(1 - R/R_0)^2 + 4\pi\bar{\kappa}_0. \tag{2}$$

Thermal fluctuations lead to a renormalization of the constants κ_0, $\bar{\kappa}_0$, c_0, linear the temperature and with a logarithmic dependence on the number N of the lipid molecules in the vesicle.

$$\begin{aligned}
&\kappa = \kappa_0 - (T/8\pi)\alpha \log N, \qquad \bar{\kappa} = \bar{\kappa}_0 - (T/8\pi)\bar{\alpha} \log N,\\
&c_s\kappa = c_0\left[\kappa_0 - (T/8\pi)\alpha_s \log N\right], \qquad c_s = c_0\left[1 - (T/8\pi\kappa_0)(\alpha_s - \alpha) \log N\right].
\end{aligned} \tag{3}$$

The parameters α, $\bar{\alpha}$, α_s will be given below.

Using the renormalized constants (3), the curvature energy becomes

$$E_{\mathrm{curv}} = 8\pi\kappa(1 - R/R_s)^2 - T(\alpha - \alpha_s)\left(R^2/R_s^2\right) \log N - 4\pi\bar{\kappa}, \tag{4}$$

rather than (2). When inserted into the Boltzmann factor $\mathrm{e}^{-E/T}$, the new terms give rise to a characteristic prefactor in the equilibrium size distribution at a given temperature.

$$P(N) \propto N^{\alpha(1-R/R_s)^2+\bar{\alpha}/2} N^{(\alpha-\alpha_s)R^2/R_s^2} \exp\left[-(8\pi\kappa_0/T)(1 - R/R_s)^2\right], \tag{5}$$

where $R \propto \sqrt{N}$. Helfrich in his paper calculates $\alpha = 1$, assumes $\bar{\alpha} = 0$, and obtains for $R_s = \infty$ a distribution $N\,\mathrm{e}^{-\mathrm{const}\times N}$ which he says to agree fairly well with experimental data [2].

---

[1] Supported in part by Deutsche Forschungsgemeinschaft under grant no. Kl 256 and UCSD/DOE contract DEAT-03-81ER40029.

[2] Permanent address: Institut für Theorie der Elementarteilchen, Freie Universität Berlin, Arnimallee 14, 1000 Berlin 33, Germany.

0375-9601/86/$03.50

The purpose of this note is threefold:

First, motivated by the fact that Helfrich's result for α is at variance with two independent calculations by other authors (who obtain $\alpha = 3$ [4], and $\alpha = 2$ [5] [#1], respectively), we show once more in a simpler way than in ref. [6] that the correct value of α is $\alpha = 3$.

Second, we calculate the parameters $\bar{\alpha}$, α_s which govern the change of the gaussian and spontaneous curvatures. They turn out to be $\bar{\alpha} = -4 + \frac{2}{3} = -\frac{10}{3}$, $\alpha_s = -1$, respectively. The term $\frac{2}{3}$ was missed in refs. [1–6]. As a consequence, the prefactor $N^{\alpha+\bar{\alpha}/2}$ in the size distribution (5) of $R_s = \infty$ vesicles is $N^{4/3} \times \mathrm{e}^{-\mathrm{const} \times N}$, somewhat different from the result of Helfrich [1] [#2]. Notice also that our $\alpha_s - \alpha$ which governs the renormalization of c_s is twice as large as Helfrich's.

Third, and most important, we point out that the prefactor $N^{4/3}$ is not valid for all types of bilipid vesicles for the following reason. The bending energy of a bilayer has, in general, physically completely different components: One component is caused by the liquid crystal property of the monolayers. The molecules are rod-like and resist *directional* changes. The bending constants of this type will be denoted by $\kappa_0^{\mathrm{l.c.}}$, $\bar{\kappa}_0^{\mathrm{l.c.}}$, $c_0^{\mathrm{l.c.}}$. The constant of spontaneous curvature $c_0^{\mathrm{l.c.}}$ is an *intrinsic* quantity determined by the asymmetry of the rod-like molecules. The other component has its origin on the area elasticity of the two monolayers. If the bilayer is bent, the outer layer is stretched, the inner compressed. If K and μ are the area elastic moduli of the monolayers it is easy to see hat the joint bending is governed by the elastic bending constants [#3]

$$\kappa_0^{\mathrm{el}} = \tfrac{1}{2}(K-\mu)d^2/2, \qquad \bar{\kappa}_0^{\mathrm{el}} = -2\mu d^2/2. \tag{6}$$

where d is the distance between the monolayers.

The important point is now that the constant of spontaneous curvature c_0^{el} caused by the elastic forces is *not intrinsic* as its liquid crystal counterpart. For a membrane in equilibrium it is always equal to the actual equilibrium curvature $c = 1/R$. For a flat membrane, $c_0^{\mathrm{el}} = 0$ since the densities in the two monolayers are equal. If the same membrane is brought into a spherical shape, by sonication, the pressures in the inner and outer layers will, during the formation process, equilibrate in such a way that the inner layer has fewer molecules than the outer layer (see fig. 1 for an illustration of the different number of molecules in the two shells). Thus, when deforming a spherical vesicle away from its equilibrium shape, the elastic component of the bending forces always try to restore the equilibrium curvature (for times smaller than τ). Thus the total bending energy can therefore be written as follows:

$$\begin{aligned} E &= \tfrac{1}{2}\kappa^{\mathrm{el}}\left(c_1 + c_2 - c_0^{\mathrm{el}}\right)^2 + \tfrac{1}{2}\kappa^{\mathrm{l.c.}}\left(c_1 + c_2\right)^2 + \left(\bar{\kappa}^{\mathrm{el}} + \bar{\kappa}^{\mathrm{l.c.}}\right)c_1 c_2 \\ &= \tfrac{1}{2}\left(\kappa^{\mathrm{el}} + \kappa^{\mathrm{l.c.}}\right)\left(c_1 + c_2 - \frac{\kappa^{\mathrm{el}}}{\kappa^{\mathrm{el}} + \kappa^{\mathrm{l.c.}}} c_0^{\mathrm{el}}\right)^2 + \frac{\kappa^{\mathrm{el}}\kappa^{\mathrm{l.c.}}}{\kappa^{\mathrm{el}} + \kappa^{\mathrm{l.c.}}} \frac{\left(c_0^{\mathrm{el}}\right)^2}{2} + \left(\bar{\kappa}^{\mathrm{el}}\bar{\kappa}^{\mathrm{l.c.}}\right)c_1 c_2, \end{aligned} \tag{7}$$

where $c_0^{\mathrm{el}} = 1/R$. The combination of the two terms is governed by a constant spontaneous curvature $1/R_s = \rho/R$ where the ratio $\rho = \kappa^{\mathrm{el}}/(\kappa^{\mathrm{el}} + \kappa^{\mathrm{l.c.}})$ may be called *elastic fraction*.

Inserting this result into our expression (6) we obtain the distribution

$$P(N) \propto N^{a}\, \mathrm{e}^{-\mathrm{const} \times N}, \tag{8}$$

where

$$(2\alpha - \alpha_s)\rho^2 - 2\alpha\rho + (\alpha + \bar{\alpha}/2) = 7\rho^2 - 6\rho + \tfrac{4}{3}. \tag{9}$$

[#1] The value for α agrees with that of ref. [4] (who gave no result for $\bar{\alpha}$) but this agreement is fortuitous since the authors use an unphysical measure in the functional integral. Ref. [5] has the correct measure. For details see ref. [6].

[#2] For a more detailed discussion of the measure and the T dependence of the gaussian curvature term, see ref. [7].

[#3] For details on this source of curvature energy, see ref. [8].

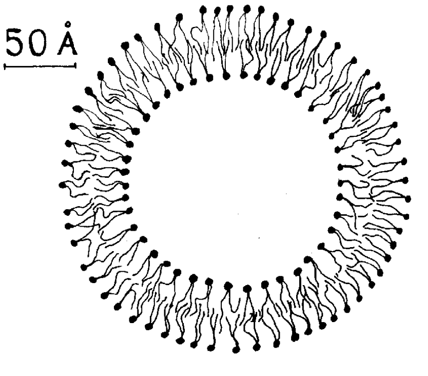

Fig. 1. A bilayer vesicle. The inside layer contains less molecules than the outside layer. The elastic part c_0^{el} of the constant of spontaneous curvature is equal to the actual curvature. Only the directional part of c_0^{el} tries to restore the planar state.

The experimental data of Holzwarth and Groll [2] show, among 1478 DPPC vesicles, a maximum in the radial distribution $\mathrm{d}P/\mathrm{d}R \propto R^{2a+1}\,\mathrm{e}^{-\mathrm{const}\times R^2}$ at $R_{\mathrm{m}} \approx$ 35 nm while the radius R_{h} at which the number has dropped to half the maximum is $R_{\mathrm{h}} \approx 50$ nm. From the ratio $r = R_{\mathrm{h}}/R_{\mathrm{m}} \approx 1.43$ we calculate $f = r\exp(-r^2) \approx 0.515$ and $a = -\frac{1}{2}\{1 + \log(2)/[\log(f) + 1/2]\} \approx 1.6$. The determination is not very precise. We could easily have extracted $R_{\mathrm{h}}/R_{\mathrm{m}} \approx 1.513$ or 1.393 which would have given $a = 1$ or $a = 2$. Thus, on the basis of the present data, the ratio ρ cannot yet be determined and is comparable with purely liquid crystalline bending ($\rho = 0$, $a = 4/3$).

Let us now turn to the calculation of the renormalization equations (3), which is quite straightforward. We consider fluctuations of the surface $x^a(\xi^i)$ along its normal unit vector N^a

$$x^a(\xi^i) \to x^a(\xi^i) + \nu(\xi^i)N^a(\xi^i) \quad (a = 1, 2, 3;\ i = 1, 2), \tag{10}$$

and find that the metric $g_{ij}(\xi^i) = D_i x^a D_j x^a$ changes as follows

$$g_{ij} \to g_{ij} - 2\nu C_{ij} + D_i\nu D_j\nu + \nu^2 C_{il}C^l_j, \tag{11}$$

where

$$C_{ij} = N^a D_i D_j x^a = C_{ji} \tag{12}$$

is the curvature matrix whose trace $C \equiv C^i_i \equiv g^{ij}C_{ij}$ and determinant $K \equiv \det(C^j_i) = \frac{1}{2}(C^2 - C^j_i C^i_j)$ are equal to $c_1 + c_2$ and $c_1 c_2$, respectively. From (11), we find the well-known result [8] that the surface element changes as follows

$$\sqrt{g} \to \sqrt{g}\left[1 - C\nu + \tfrac{1}{2}(D\nu)^2 + K\nu^2 + \mathrm{O}(\nu^3)\right] \tag{13}$$

and calculate the variations [#4]

$$\begin{aligned} N^a &\to \left[1 - \tfrac{1}{2}(D\nu)^2\right]N^a - D\nu Dx^a + \ldots, \\ D^2x^a &\to \big[C + D^2\nu + C^{ij}C_{ij}\nu + C^{ij}D_i\nu D_j\nu - CD\nu D\nu \\ &\qquad + 2C^{ij}\nu D_i D_j\nu + \nu^2 C^j_i C^k_j C^i_k + (D^k C)\nu D_k\nu\big]N^a - CD\nu Dx^a + \ldots . \end{aligned} \tag{14}$$

When multiplied together, this gives [#5]

$$C \to C + D^2\nu + C^{ij}C_{ij}\nu - \tfrac{1}{2}C(D\nu)^2 + C^{ij}D_i\nu D_j\nu + 2C^{ij}\nu D_i D_j\nu + \nu^2 C^j_i C^k_j C^i_k + D^k C\nu D_k\nu + \mathrm{O}(\nu^2). \tag{15}$$

[#4] Notice that in each of the equations (12), the coefficient of the tangential vectors Dx^a need to be known only to first order in ν.

[#5] The linear piece of eq. (13) being well known, see eq. (43) of §101 in ref. [7].

Combining (13)–(15) leads to

$$-2\sqrt{g}\,Cc_0 \to -2\sqrt{g}\,c_0\big[C + D^2\nu + C_{ij}C^{ij}\nu - C^2\nu - C\nu D^2\nu + C^{ij}\big(D_i\nu D_j\nu + 2\nu D_iD_j\nu\big)$$
$$+\big(C_i^jC_j^kC_k^i + CK - CC_{ij}C^{ij}\big)\nu^2 + D^kC\nu D_k\nu\big] + \ldots, \qquad (16)$$

$$\sqrt{g}\,C^2 \to \sqrt{g}\,\big[C^2 + 2C\big(D^2\nu + C_{ij}C^{ij}\nu - \tfrac{1}{2}C^2\nu\big) + \nu\big(D^2\big)^2\nu + 2CC^{ij}\big(D_i\nu D_j\nu + 2\nu D_iD_j\nu\big)$$
$$-\tfrac{1}{2}C^2(D\nu)^2 - 2\big(C^2 - C_i^jC_j^i\big)\nu D^2\nu + \big(2CC_i^jC_j^kC_k^i + 4K^2 + C^2K - C^4\big)\nu^2 + 2CD^kC\nu D_k\nu\big] + \ldots. \qquad (17)$$

In equilibrium, the linear terms do not contribute. The quadratic fluctuations can be rewritten, for large momenta, as

$$\int D^2\xi\,\sqrt{g}\,\nu\big[D^4 + 2CC^{ij}D_iD_j - \tfrac{3}{2}C^2D^2 + 2C_i^jC_j^iD^2 + 2c_0\big(CD^2 - C^{ij}D_iD_j\big) - \tfrac{1}{2}c_0^2D^2 + \ldots\big]\nu$$
$$\approx \int d^2\xi\,\sqrt{g}\,\nu D^4\big[1 + \big(\tfrac{3}{2}C^2 - 4K\big)/D^2 + \big(c_0C - \tfrac{1}{2}c_0^2\big)/D^2 + \ldots\big]\nu. \qquad (18)$$

This produces an additional free energy

$$\approx \tfrac{1}{2}T\ \mathrm{Tr}\log\ D^4 + \tfrac{1}{2}TD^{-2}(0)\int d^2\xi\,\sqrt{g}\big(\tfrac{3}{2}C^2 - 4K + Cc_0 - \tfrac{1}{2}c_0^2\big). \qquad (19)$$

For a flat surface, we may calculate

$$D^{-2}(0) = -\int_{q_{\min}}^{q_{\max}} \frac{d^2q}{(2\pi)^2}\,\frac{1}{q^2} \equiv -L = -\frac{1}{2\pi}\log(q_{\max}/q_{\min}). \qquad (20)$$

For a sphere of radius r_0, $\boldsymbol{k}^2$ becomes $l(l+1)/r_0^2$ and

$$\int \frac{d^2q}{(2\pi)^2} = \sum_l \frac{(2l+1)}{4\pi r_0^2}, \qquad (21)$$

such that $D^{-2}(0) \approx -(1/2\pi)\log(l_{\max}/l_{\min})$.

The argument of the logarithm can be related to the total number of modes on the vesicle

$$M = \sum_{l_{\min}}^{l_{\max}} (2l+1) \sim l_{\max}^2, \qquad (22)$$

which, due to its microscopic bilipid nature, is proportional to N, the total number of lipid molecules [1]. Hence, we can write , for large N,

$$D^{-2}(0) = -L \sim -(1/4\pi)\log N. \qquad (23)$$

Consider now the term

$$\tfrac{1}{2}\mathrm{Tr}\log(D^4) = \mathrm{Tr}\log(-D^2). \qquad (24)$$

For a sphere of radius r_0 it is given by

$$\sum_l (2l+1)\log\big[l(l+1)/r_0^2\big].$$

This sum diverges and requires regularization. For this we consider the partition function

$$Z(\beta) = \sum_l (2l+1)\exp\left[-\tfrac{1}{2}\beta l(l+1)/r_0^2\right] \tag{25}$$

and take

$$\operatorname{Tr}\log(-D^2) = -\int_0^\infty \frac{d\beta}{\beta} Z(\beta). \tag{26}$$

Being only interested in the short distance fluctuations, the sum may start with some $l = l_{\min} \gg 1$. The divergences can be isolated by evaluating $Z(\beta)$ via the Euler–McLaurin formula

$$Z(\beta) = \int_{l_{\min}}^\infty d[l(l+1)]\exp\left[-\tfrac{1}{2}\beta l(l+1)/r_0^2\right] + \tfrac{1}{2}g(l_{\min}) - (B_2/2!)g'(l_{\min}) - (B_4/4!)g'''(l_{\min}),$$

where

$$g(l) \equiv (2l+1)\exp\left[-\tfrac{1}{2}\beta l(l+1)/r_0^2\right],$$

and B_l are the Bernoulli numbers. This gives

$$Z(\beta) = \exp\left[-\tfrac{1}{2}\beta l_{\min}(l_{\min}+1)/r_0^2\right]\left\{2r_0^2/\beta + \tfrac{1}{2} - \tfrac{1}{12}\left[2 - (\beta/2r_0^2)(2l_{\min}+1)\right]\right\} + \dots. \tag{27}$$

This is an expansion of the partition function in powers of $1/r_0^2$, starting from a flat reference membrane. This property is exhibited by rewriting $Z(\beta)$ in a form in which the first term is the partition function of random motion in a plane with a total area $4\pi r_0^2$

$$-4\pi r_0^2 \int_{k_{\min}}^\infty \frac{d^2k}{(2\pi)^2}\exp(-\tfrac{1}{2}\beta k^2)\left[1 + \beta/6r_0^2 + O(\beta^2)\right].$$

Inserting this into eq. (26) and integrating over β gives

$$\operatorname{Tr}\log(-D^2) = 4\pi r_0^2\left(\int_{k_{\min}}^\infty \frac{d^2k}{(2\pi)^2}\log(k^2) - \frac{1}{3r_0^2}\int_{k_{\min}}^\infty \frac{d^2k}{(2\pi)^2}\frac{1}{k^2} + \dots\right). \tag{28}$$

The first term is the quadratically divergent trace log of the flat reference membrane. This is absorbed into the definition of the measure of the path integral.

The second term gives a further logarithmically divergent contribution $-L(1/3r_0^2) = -L(K/3)$ to the energy density [7].

Adding all terms in eq. (19) together [#6] we find our result (3) with $\alpha = 3$, $\bar{\alpha} = -4 + \frac{2}{3}$, $\alpha_s = -1$. Inserted into (5) gives the distribution (6) with $a = \frac{4}{3}$ for a pure directional bending energy and the further consequences discussed in the beginning of this note.

The author is grateful to Dr. S. Ami for a careful check of our calculations and for many useful discussions. He also thanks Professors N. Kroll and J. Kuti for their kind hospitality at UCSD.

[#6] If $L \equiv (1/8\pi)\log N$, the renormalization of the mean curvature energies goes as follows: $\frac{1}{2}\kappa_0(C - c_0)^2 - \frac{1}{2}TL[\alpha C^2 - 2\alpha_s(Cc_0 - \frac{1}{2}c_0^2)] = \frac{1}{2}(\kappa_0 - TL\alpha)C^2 - (\kappa_0 - TL\alpha_s)Cc_0 + \frac{1}{2}(\kappa_0 - TL\alpha_s)c_0^2 = \frac{1}{2}\kappa(C - c_s)^2 - \frac{1}{2}\kappa c_s^2 + \frac{1}{2}\kappa c_s c_0 = \frac{1}{2}\kappa(C - c_2)^2 - \frac{1}{2}\kappa c_s(c_s - c_0)$ leading directly to the first two terms in [4].

References

[1] W. Helfrich, Berlin preprint (July 1985).
[2] J.F. Holzwarth and R. Groll, Fritz Haber Institut Berlin, preprint, in preparation;
S. Kim and G.M. Martin, Biochim. Biophys. Acta 646 (1981) 1;
K. Hammond, M.D. Reboiras, I.G. Lyle and M.N. Jones, Biochem. Biophys. Acta 774 (1984) 19;
T.S. Aurora, W. Li, H.Z. Cummins and T.H. Haines, Biochem. Biophys. Acta 820 (1985) 250;
R.A. Parente and B.R. Leutz, Biochemistry 23 (1984) 2353.
[3] W. Helfrich, Z. Naturforsch. B 103 (1975) 67.
[4] W. Helfrich, J. Phys. (Paris) 46 (1985) 1263;
L. Peliti and S. Leibler, Phys. Rev. Lett. 54 (1985) 1690.
[5] D. Förster, Berlin preprint (August 1985).
[6] H. Kleinert, Physics Lett. A 114 (1985) 263.
[7] S. Ami and H. Kleinert, UCSD preprint (March 1986).
[8] R. Skalak, Mechanics and thermodynamics of biomembranes (CRC Press, Baton Rouge, 1980) Sec. 4.9;
M. Kléman, Proc. R. Soc. A 347 (1976) 387.
[9] W. Blaschke, Differential geometry (Springer, Berlin, 1926) §100, eq. (33).