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The Egg Carton: Theory of a Periodic Superstructure of Some Lipid Membranes

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Abstract. — A model calculation of the square membrane superstructure of some mixed lipid bilayers is presented. It involves bending elasticity of higher than quadratic order in the principal curvatures and employs Monte Carlo simulation.

Some years ago, Meyer et al. [1] found a periodically curved, square texture in the membranes of L-form (i.e. wall-less) cells of *Streptomyces hygroscopicus*. The same pattern appeared in bilayers made of the lipids extracted from the cell membrane and even in those containing only the phospholipid fraction. As mentioned in [1], Verkleij and Wilschut also saw it in a mixture of egg yolk phosphatidylcholine and bacterial cardiolipin in the presence of Ca⁺⁺ ions. The general method of detection was freeze fracture electron microscopy. The square texture resembles an egg carton and looks equal from both sides of the membrane. The period of this ordered membrane superstructure as observed by Meyer et al. varied between 15 and 75 nm. While the square texture occurred in fluid membranes, Verkleij et al. had found a less regular texture in the apparently crystalline bilayers of a mixture of lipids obtained from cell membranes of *Staphylococcus aureus* [2].

The square pattern may be related to an anomalous roughness of the fluid bilayers of common lipids such as phosphatidylcholines, phosphatidylethanolamines and digalactosyldiacylglycerol. The roughness was inferred from studies of mutual bilayer adhesion induced by weak lateral tensions [3]. It was explained in terms of a postulated membrane superstructure that consists of a disordered arrangement of cooperative local saddle deformations due to higher order bending elasticity [4]. An important reason to propose such a model was the finding of a grainy texture, with cryo-transmission electron microscopy, in vesicular bilayers of egg yolk phosphatidylcholine [5]. Induced adhesion and other properties of lipid bilayers pointing to a disordered superstructure have been the subject of a recent review [6]. Of particular interest in the present context is the possibility that the ordered superstructure represented by the egg carton and the disordered superstructure producing anomalous roughness have the same origin, one being the

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crystalline version of the other [4]. In the following we present a model calculation yielding egg carton textures. It is based on bilayer bending elasticity, in particular energy terms of higher than quadratic order in the principal curvatures. Only a minimal number of such energy terms will be taken into account, with values of the new elastic moduli as suggested by order of magnitude estimates. Stable or metastable equilibrium membrane shapes are obtained by Monte Carlo simulation including simulated annealing.

Let us express the bending energy per unit area of membrane, $g$, by the simple formula

$$g = \frac{1}{2} \kappa J^2 + \bar{\kappa} K + \bar{\kappa}_2 K^2 + \bar{\kappa}_4 K^4$$

(1)

Here $J = c_1 + c_2$ and $K = c_1 c_2$ are the total and Gaussian curvatures, respectively, $c_1$ and $c_2$ being the principal curvatures. Odd powers of the principal curvatures are omitted in (1) as we are dealing with symmetric bilayers. The two standard terms, quadratic in the principal curvatures, contain the bending rigidity $\kappa$ and the modulus of Gaussian curvature, $\bar{\kappa}$. The bending rigidities of lipid bilayers are known to be on the order of $\kappa = 10^{-19}$ J [7]. Considering periodic deformations of the flat membrane, we omit the $\bar{\kappa}$ term because of the Gauss-Bonnet theorem. The next term, quartic in the principal curvatures, is assumed to be negative, thus giving rise to the formation of saddles. An estimate based on a molecular model (see Appendix) shows $\bar{\kappa}_2 \approx \gamma h^4 \approx 0.8 \times 10^{-26}$ J m$^2$. Here $\gamma \approx 50$ mN/m is the surface tension at the lipid-water interface and $h \approx 2$ nm is the monolayer thickness. We disregard other quartic terms such as $J^4$ and $K J^2$, which vanish for pure saddle curvature ($c_2 = -c_1$), and several gradient terms of the same order [8]. However, two gradient terms, one of them of fourth order, will be adopted in the simulations to prevent unphysical deformations of very small periods. The last term of (1) is introduced to limit the amplitude of the egg carton. We take $K^4$ instead of $K^3$ because only an even power of $K$ with a positive modulus suppresses the saddles as well as the highs and lows between them. An order of magnitude estimate is given by the dimensional relationship $\bar{\kappa}_4 \approx \gamma h^8 \approx 1 \times 10^{-71}$ J m$^6$.

For the Monte Carlo simulations we described the membrane shape by the Monge representation $z = z(x, y)$. The first and second derivatives of $z$ with respect to $x$ and $y$ were discretized as

$$z_x(x, y) = \frac{z(x + h, y) - z(x - h, y)}{2a}$$

$$z_{xx}(x, y) = \frac{z(x + h, y) - 2z(x, y) + z(x - h, y)}{a^2}$$

$$z_{xy}(x, y) = \frac{z(x + h, y + h) - z(x, y + h) - z(x + h, y - h) + z(x - h, y - h)}{4a^2}$$

e.tc., where $a$ is the lattice parameter of the square grid. These expressions were inserted into the formulas for the total and Gaussian curvatures obtained from differential geometry.

$$J = \frac{(1 + z_x^2) z_{xx} - 2 z_x z_y z_{xy} + (1 + z_y^2) z_{yy}^2}{m^3}$$

$$K = \frac{z_{xx} z_{yy} - z_{xy}^2}{m^4}$$

where the metric $m$ is, in principle,

$$m = \sqrt{1 + z_x^2 + z_y^2}.$$
In part of the calculations we expressed the metric through

\[ m = \sqrt{1 + \frac{z_{x+}^2 + z_{y-}^2}{2} + \frac{z_{y+}^2 + z_{y-}^2}{2}}, \]

using the one-sided derivatives

\[ z_{x+}(x, y) = \frac{z(x + h, y) - z(x, y)}{2}, \]
\[ z_{y-}(x, y) = \frac{z(x, y) - z(x - h, y)}{2}, \]

and so on. This replacement improved the counting of area in ragged shapes, but was not matched by similar corrections in the nominator of the formula for \( J \). It turned out to have very little effect on the energies of smooth shapes.

The calculations were done with periodic boundary conditions and, in most cases, on a \( 16 \times 16 \) lattice. We used the standard Metropolis algorithm and simulated annealing. As the temperature was lowered we decreased the interval of permitted changes at a gridpoint so that the acceptance rate was always near 50\%. Normally, 100 000 to 200 000 sweeps were performed for each calculation of an equilibrium shape. The elastic moduli \( \kappa, \bar{\kappa}_2 \) and \( \bar{\kappa}_4 \) were set equal or similar to the values given above. The temperature was varied stepwise, typically from \( kT = 4 \times 10^{-21} \) J (room temperature) to \( 10^{-23} \) J, with 10 000 to 20 000 sweeps for each temperature.

Our first attempts to obtain the egg carton started from the flat state and led to a closely packed square structure in which the unit cell consisted of four gridpoints. Two of them were saddles and the other two a high and a low. Adding a positive gradient term of the type \((\nabla K)^2\) removed the saddles so that the unit cells were reduced to two gridpoints, one a high and the other a low. A further gradient term, \((\nabla J)^2\), had to be introduced to suppress all of these artefacts.

In the supplemented formula for the bending energy,

\[ g = \frac{1}{2} \kappa J^2 + \bar{\kappa} K + \bar{\kappa}_2 K^2 + \bar{\kappa}_4 K^4 + \kappa_{gr}(\nabla J)^2 + \bar{\kappa}_{gr}(\nabla K)^2, \]

the moduli \( \kappa_{gr} \) and \( \bar{\kappa}_{gr} \) were chosen such that the gradient terms contributed relatively little to the total bending energies of the smooth square textures that we now obtained. Usually they were about a hundred times smaller than \( \kappa_{gr} = \gamma h^4 = \bar{\kappa}_2 = 1 \times 10^{-36} \) Jm\(^2\) and \( \bar{\kappa}_{gr} = \gamma h^6 = 4 \times 10^{-55} \) Jm\(^4\), the values of the fourth and sixth order moduli suggested by dimensional analysis. The gradient terms could often be turned off without much effect after the smooth square texture had developed. However, the long-term stability of these situations is still unclear.

A square equilibrium structure of the smooth type is shown in Figure 1, together with the values taken for the elastic moduli and their contributions to the total bending energy. All these energies refer to one quarter of the figure since only one quarter of the shape (with \( 16 \times 16 \) gridpoints) was calculated and then doubled in both directions. In this particular case the total bending energy is negative in sign and a few times \( 4 \times 10^{-21} \) J in size.

Varying the number of gridpoints between \( 8 \times 8 \) and \( 32 \times 32 \) at a constant unit cell size of \((6.6 \text{ nm})^2\), we checked the practical unchangeability of the shape and the convergence of its energy and area. (At \( 16 \times 16 \) the average energy per unit area was about 0.5mJ/m\(^2\) below the result at \( 32 \times 32 \) where the dependence on size seemed to have come to an end.)
Fig. 1.—Square equilibrium structure obtained with the moduli $\kappa = 1 \times 10^{-19}$ J, $\bar{k}_2 = -1 \times 10^{-36}$ J, $\bar{k}_4 = 8.4 \times 10^{-72}$ J, $\kappa_{gr} = 1 \times 10^{-38}$ J and $\bar{k}_{gr} = 1 \times 10^{-56}$ J. A single unit cell, i.e. a quarter of the surface shown, was actually calculated. The total bending energy of the unit cell, $E = -9.2 \times 10^{-21}$ J, is composed of the following contributions from the five moduli: $E[\kappa] = 885.0 \times 10^{-21}$ J, $E[\bar{k}_2] = -1467.3 \times 10^{-21}$ J, $E[\bar{k}_4] = 428.7 \times 10^{-21}$ J, $E[\kappa_{gr}] = 93.5 \times 10^{-21}$ J and $E[\bar{k}_{gr}] = 50.9 \times 10^{-21}$ J. The membrane area is 1.27 times the projected area; the final annealing temperature was $10^{-23}$ J.

The total deformational energy becomes slightly lower when we subtract a fluctuation energy of $1/2 kT$ per gridpoint. Simulated annealing of smooth square equilibrium shapes showed the fluctuation energy to agree with this value predicted by the equipartition theorem. The last annealing temperature of the square structure of Figure 1 being $kT = 10^{-23}$ J, the fluctuation energy amounts to $128kT = 1.3 \times 10^{-21}$ J.

Continuing the simulation after relatively small changes of the elastic moduli $\bar{k}_2$ and $\bar{k}_4$ leads to negative or positive total bending energies which can be much larger than $4 \times 10^{-21}$ J. The persistence of the square texture at positive energies reveals an energy barrier between the egg carton and the flat state. Such a barrier was suspected earlier for the isolated saddle structure in an otherwise flat membrane [4].

It is interesting to compare the simulated egg carton of Figure 1 with a sinusoidal deformation of equal amplitude $b$ and period,

$$z(x, y) = b[\cos(qx) + \cos(qy)],$$

which is depicted in Figure 2. Despite its similarity the latter has a strongly positive total bending energy of $546 \times 10^{-21}$ J for the same elastic moduli. Inspection shows that the saddles are larger and, in a sense, flatter in Figure 1 than in Figure 2. The local energy densities of the $J^2$ and $K^2$ terms in the simulated egg carton of Figure 1 are plotted in Figures 3 and 4, respectively.

A structure resembling the sinusoidal deformation of Figure 2 was used to start the simulation leading to the egg carton. The fact that the flat state used to remain unchanged apart from its fluctuations again demonstrates the existence of an energy barrier between the two states. Transitions from the flat state to the egg carton occurred only if the energy of the latter was made extremely negative, e.g. $-2.4 \times 10^{-18}$ J at 70 K.
Fig. 2. — A sinusoidal surface representing the sum of two orthogonal sine waves. It has the same period and amplitude as the similar surface of Figure 1. The total bending energy of the unit cell, $E = 546 \times 10^{-21}$ J, calculated with the same set of elastic moduli, is positive and more than 50 times higher than in the case of Figure 1.

Fig. 3. — The contributions $\epsilon[\kappa] = (1/2)\kappa J^3 a^2$ of the gridpoints to the energy $E[\kappa]$ as obtained for the surface of Figure 1. Note that they vanish in the saddles.

The egg carton of minimum energy per unit area of bilayer at fixed elastic moduli was found by letting the square texture adjust to small changes of the lattice parameter. The configuration of Figure 1 is of minimal energy with respect to this length variation. The optimal period of 6.6 nm, obtained for the given set of moduli, is not much larger than the lipid bilayer thickness of nearly 4 nm. It agrees well with the typical spacing of the bright spots in the grainy membranes [5], which supports the assumption that the graininess represents a disordered (or “melted”) egg carton [4]. A lattice spacing near the bilayer thickness may have
been expected since the elastic moduli employed are not far from their order of magnitude estimates, that for the bending rigidity being \( \kappa = \gamma h^2 \).

It should be noted that a minimization of the bending energy (without gradient terms) in the usual “quasi planar” approximation (which puts \( m = 1 \)) gives only the optimal curvatures of the sinusoidal pattern (2), but leaves the period undetermined. Allowing for the variation of \( m \) and again expanding the bending energy up to the eighth power of the amplitude, we did find periods similar to those obtained by Monte Carlo simulation. However, if the sinusoidal pattern was stable, its energy was much less negative than that of the egg carton resulting from Monte Carlo simulation with the same values of the elastic moduli.

The larger repeat distances of 15 to 75 nm, as found by Meyer et al., pose a problem. Changing the three moduli \( \kappa, \tilde{\kappa}_2 \) and \( \tilde{\kappa}_4 \) by the same factor leaves the equilibrium shape unchanged. Changing the moduli by the factors 1/4, 1 and 16, respectively, (or multiples thereof) magnifies the egg carton by the factor 2. This kind of scaling may explain periods near 15 nm, but its extrapolation to account for a further increase to 75 nm seems hardly acceptable. The third degree of freedom in changing the moduli should affect mainly the maximum slopes of the egg carton which we chose to be near 45°, in apparent agreement with experiment [1]. Periods above 15 (or 7) nm and, in particular, their wide variation seem difficult to explain. One may speculate that the larger periods are due to a delay in the formation of the equilibrium number of saddles and that in the course of time they would evolve into a stable, finer egg carton. (In this case the saddles should be sharp regardless of the repeat distance.)

Summing up, we have found that a model involving higher order bending elasticity and essentially containing only three elastic moduli yields the egg carton as an equilibrium membrane structure when reasonable values are inserted for the moduli. The calculated period of ca. 7 nm agrees well with a disordered membrane superstructure of presumably equal origin. The wide range of larger repeat distances found in the well-ordered square textures can not be understood without additional assumptions.
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Appendix A

We want to estimate the elastic modulus \( \tilde{K}_2 \) of the \( K^2 \) term in (1). Let us follow a previous attempt based on a particular molecular model of the monolayer \[4\]. On this occasion, we will correct earlier mistakes and introduce an alternative method of computing \( \tilde{K}_2 \).

Our two basic assumptions are constant bulk density of the lipid and a particular form of monolayer elasticity that derives from a two-sheet model proposed by Israelachvili et al. \[9\]. They write for the chemical potential of a lipid molecule in the flat monolayer

\[
\mu = \gamma a + \frac{a_0^2}{a} + \mu_0
\]

where \( a \) is the molecular cross section, \( a_0 \) being its equilibrium value, and \( \gamma \) the lateral tension at the hydrocarbon/water interface. The formula predicts the Gibbs free energy of stretching per unit area to be

\[
g_s = \frac{1}{2}(2\gamma) \left( \frac{a - a_0}{a_0} \right)^2
\]

for the monolayer. Accordingly, the stretching modulus of the monolayer is \( 2\gamma \). With \( \gamma = 50 \text{ mN/m} \) one obtains \( 4\gamma = 200 \text{ mN/m} \) for the stretching modulus of lipid bilayers, in good agreement with experimental data \[10\]. In the following we assume the interfacial tension to be concentrated at the height \( h \) above (or below) the bilayer mid-plane at \( z = 0 \), \( z \) being a normal coordinate. The hydrocarbon chain region in the range \( 0 < z < h \) (or \( 0 > z > h \)) is taken to be uniform in flat layers so that the tension \( s(z) \), a force per unit area, is everywhere \( s(z) = \gamma/h \) at equilibrium.

With constant bulk density, bending will affect the thickness of the monolayers. On the simplifying assumption that the monolayers preserve their area at the bilayer mid-plane, the height \( \tilde{h} \) of the interface will obey, to first order in \( K \),

\[
\tilde{h} = (1 - \frac{1}{3}K\tilde{h}^2)h
\]

in the presence of pure saddle curvature \((K < 0, J = 0)\). An analogous formula describes the new position \( \tilde{z} \) of lipid material originally at \( z \). Recalling an equation for \( \tilde{K} \) in terms of the stress profile of the flat state of the monolayer \[11\],

\[
\tilde{K} = \int (z - z_0)^2 s(z) \, dz
\]

where \( z_0 \) is the position of the surface of inextension, we find for \( z_0 = 0 \) the following contributions to the bending energy per unit area of pure saddle curvature. The terms linear in \( K \) are

\[
\gamma h^2 K - \frac{\gamma}{h} \int_0^h z^2 \, dz \, K
\]  \[A.1\]
where the first comes from the interface and the second from the hydrocarbon chains. The terms quadratic in $K$ are

$$-\frac{2}{3} \gamma h^3 K^2 + \frac{2 \gamma}{h} k_1 \int_0^h z^4 \, dz \, K' \, dK' + \frac{2 \gamma}{3h} \int_0^h z^4 \, dz \, K^2 + \frac{\gamma}{h} \int_0^h z^4 \, dz \, K' \, dK'$$  \hspace{1cm} (A.2)

Here the first term, originating from the interface, represents the correction of $h^2 K$, i.e. the change in area, due to the shift of the interface from $h$ to $\tilde{h}$. The second term accounts for the change of $s(z)$, i.e. increase of pressure, in the chain region due to lateral chain compression with the bulk modulus $2\gamma/h$. The third term is for the chains what the first is for the interface and the fourth term stems from $d\tilde{z} = (1 - z^2 K) \, dz$ in the chain region.

The final formulas for the bilayer moduli $\bar{\kappa}$ and $\bar{\kappa}_2$ obtained from (A.1) and (A.2), respectively, after integrating, summing, and doubling are

$$\bar{\kappa} = \frac{4}{3} \gamma h^2$$  \hspace{1cm} (A.3)

and

$$\bar{\kappa}_2 = -\frac{7}{15} \gamma h^4$$  \hspace{1cm} (A.4)

The magnitude of $\bar{\kappa}_2$ thus derived should be a lower limit in the present model as lifting the constraint of area conservation at the bilayer mid-plane can only lower the bending energy.

Another way of estimating $\bar{\kappa}_2$ is to employ the monolayer bending tension which is identical to the bending energy per unit area and compresses or dilates each monolayer to lower the bending energy of a saddle of fixed shape. This approach, similar in spirit to a previous correction of $\kappa$ [12], leads at first sight to

$$\bar{\kappa}_2 = -\left(\frac{\bar{\kappa}}{2}\right)^2 \lambda$$  \hspace{1cm} (A.5)

where the monolayer stretching modulus $\lambda$ is $2\gamma$ in our model. Inserting $\lambda = 2\gamma$ and (A.3) into (A.5) then results in

$$\bar{\kappa}_2 = -\frac{8}{9} \gamma h^4,$$  \hspace{1cm} (A.6)

which is about twice as large as (A.4). It should be noted, however, that the saddle curvature of the monolayer with the surface of inextension at $z = 0$ may itself produce a nonvanishing lateral tension. In fact, inspection of our model shows that the additional tension should be negative like the expected bending tension. Therefore, part of the bending tension is anticipated by the additional tension so that (A.6) should be an upper limit to the magnitude of $\bar{\kappa}_2$.

In any event, both formulas for $\bar{\kappa}_2$, (A.4) and (A.6) predict a negative sign and agree rather well with the magnitude $\gamma h^4$ suggested by dimensional analysis.

References


