LETTER TO THE EDITOR

Energetics of Fluctuation in Lipid Bilayer Thickness

Dear Sir:

A breathing model of bilayer membranes has been recently proposed (1, 2). According to the model the fluctuation in thickness should not be accompanied by any volume compression and the volume deficiency in an indentation is balanced by the volume excess in an adjacent swelling. Hladky and Green criticized the model (3) claiming that, in the case of short range fluctuations, expansion in surface area with concomitant increase in surface energy makes the model energetically untenable. For long range fluctuations they applied the continuous treatment introduced by Vrij for soap lamella (4) using the measured compressibility values for the interaction term between the two interfaces. Macroscopic compression of a solventless bilayer involves either volume compression, or area expansion, or both, while compression of solvated bilayer can be achieved by squeezing out of the solvent. The consequent difference in the compressibilities led the authors to conclude that solvated bilayer are more susceptible to thickness fluctuations than the nonsolvated ones.

In the following discussion we shall show that fluctuations in thickness can occur within a restricted range without any appreciable variation in exposed area or volume occupied per molecule. In this case there has to be a shrinkage in the area of the domain fluctuating in thickness and an increase in its average thickness. The size of the thinned region will be restricted by the requirement that the excess hydrocarbon volume attached to its bounding head groups can be accommodated in the adjacent thicker than average regions.

Let's consider fluctuations from planarity in the form of swellings and of identations (Fig. 1 *a*) shaped as spherical cups of radius r and half angle θ_m (Fig. 1 *b*). Let v_{hc} be the volume of hydrocarbon chains (including solvent) per lipid molecule, then the average half thickness of a domain of swellings and indentations will be

$$\delta_{av} = v_{hc}\, n/A_{ap} \tag{1}$$

where A_{ap} is the apparent geometric area of this region containing n molecules, while A equals na is the new area. A_{ap} of an identation and a swelling of equal size is

$$A_{ap} = \frac{na}{2}\,\frac{\sin^2\theta_m}{(1-\cos\theta_m)} \tag{2}$$

and hence

$$\delta_{av} = 2v_{hc}\,(1-\cos\theta_m)/a\sin^2\theta_m = \delta_{min} + r(1-\cos\theta_m), \tag{3}$$

where $a = \ell^2$ is the cross-sectional area of a lipid molecule. There is another restriction that $\delta_{max} = \delta_{av} + r(1-\cos\theta_m)$ cannot be larger than a stretched hydrocarbon chain. Assume that the molecules are arranged in concentric rings of width ℓ within the indentations or swellings (Fig. 2). The attachment of the hydrocarbon chains to the polar groups imposes the condition that the excess hydrocarbon volume carried over from the center of the indentation to the subsequent rings up to the maximal one, never exceeds the volume of the hydrocarbons in the next ring. The volume of hydrocarbons carried along by the head groups in a molecular ring at an angle θ is

$$V_c = (2\Pi r\, v_{hc}/\ell)\sin\theta. \tag{4}$$

The area of the head groups in a ring at an angle θ (see Fig. 2) is $2\Pi r\ell\sin\theta$ and its projection on the plane of the bilayer is $2\Pi r\ell\delta\sin\theta\cos\theta$.

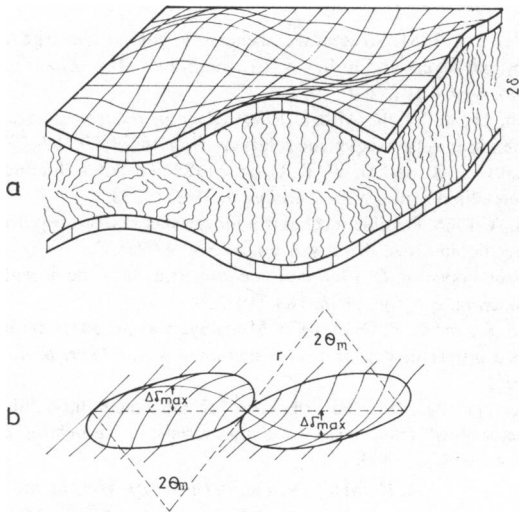

FIGURE 1 (*a*) Model of a lipid bilayer membrane with concerted spherical-cup shaped fluctuations; (*b*) dimensions of an indentation and a swelling.

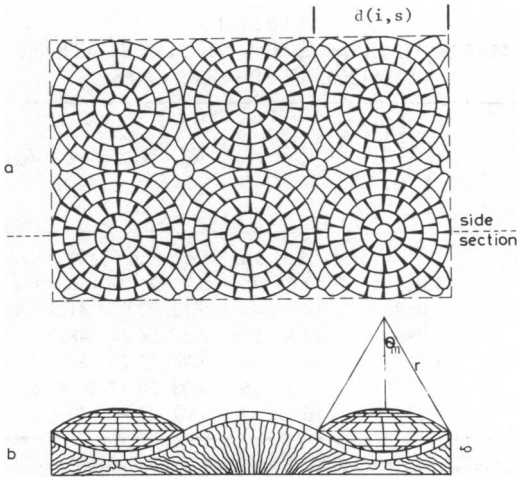

FIGURE 2 (*a*) Top view of a fluctuating membrane with the lipid molecules arranged in concentric rings; (*b*) side section of a membrane at the dashed line indicated in *a*.

The volume of hydrocarbons accommodated underneath each ring is

$$V_a = 2\Pi r \ell \delta \sin\theta \cos\theta, \quad (5)$$

where

$$\delta = \delta_{av} - r(\cos\theta - \cos\theta_m) \text{ for an identation}$$

and

$$\delta = \delta_{av} + r(\cos\theta - \cos\theta_m) \text{ for a swelling.} \quad (6)$$

The part of this volume that cannot be accommodated underneath the head groups of a ring in the identation is then

$$V_c - V_a = 2\Pi r(\sin\theta)[(v_{hc}/\ell) - \ell\delta\cos\theta],$$

and similarly this is also the excess volume underneath the head groups of a molecular ring in a swelling that cannot be filled by the hydrocarbons of these molecules alone and accommodates the excesses from the indentation. Starting at the middle of an indentation, the sum of the unaccommodated volumes increases up to $\theta = \theta_m$ where it starts to decrease in an adjacent swelling. The size of the indentations and thus also of the swellings is limited by the condition that the sum of the unaccommodated volumes underneath the indentation, which equals its volume, does not exceed the value of V_c of its outer ring. This condition can be written as

$$\frac{\Pi r^3}{3}[2 - \cos\theta_m(\sin^2\theta_m + 2)] \leqq \frac{2\Pi r}{\ell} v_{hc} \sin\theta_m. \quad (7)$$

In Table I the maximal possible values of the calculated diameters of the indentations or swellings $d_{(s,i)}$, the maximal radii of curvature r_o, as well as the maximal and minimal thickness, δ_{max} and δ_{min}, of a half bilayer are given for different δ_{av} values. Eqs. 1–7 were used for the calculations taking $a = 39.7$ Å^2, $v_{hc} = 252$ Å^3, and 23 Å the maximal extension of the hydrocarbon chain. These values fit the glycerylmono-oleate and for a planar molecule monolayer yield $\delta = 13.2$ Å. In Table I for some values of δ_{av}, δ_{max} is larger than the maximal extension of the chains and the values of r_o, δ_{min}, and $d_{(i,s)}$ were recalculated for $\delta_{max} = 23$ Å

TABLE I
MAXIMAL DIMENSIONS OF FLUCTUATIONS SHAPED AS SPHERIC CUPS

Roughness factor θ	$= \left(\frac{A}{A_{ap}}\right)$	δ_{av}	r'_{max}	δ'_{min}	δ'_{max}	r_0	δ_{min}	$d_{(i,s)}$
degree		Å	Å	Å	Å	Å	Å	Å
19	1.028	13.6	136	6.2	21	136	6.2	88.5
27	1.058	14	81.5	5.12	22.88	81.5	5.12	74
34.5	1.096	14.5	57	4.56	24.44	48.3	6	54.7
40	1.132	15	46	4.24	25.76	34.2	7	44
45	1.172	15.5	39	4.08	26.92	25.6	8	36.2
49.3	1.211	16	34.3	4.07	27.93	20.1	9	30.5

r'_{max}, δ'_{min}, and δ'_{max} were calculated disregarding the limitation of maximal extension of the hydrocarbon chains. r_0, δ_{min}, and $d_{(i,s)}$ calculated for $\delta_{max} = 23$ Å.

(three last columns). As in this model the actual area per molecule does not vary, there is no variation in surface energy per unit area and no energy changes due to these fluctuations in nonsupported bilayer membranes, e.g., liposomal vesicles. In a planar bilayer membrane supported on a septum, there may be a total area change with a concomitant energy change of the order of kT per fluctuation. This energy will be lower for indentations of smaller diameters. There is, however, also bound to be a lower limit to the radius of curvature of a fluctuating deviation from planarity. As in this case, the areas of the head groups would not shield the hydrocarbon/water interfaces.

On the other hand, surface isotherms of different lipids indicate that close to the equilibrium spreading pressure there is a region of sizable compression without large variations in surface pressure (5, 6). This suggests that some variations in area per molecule may be tolerated during fluctuations. Large differences in areas per molecule in the outer and the inner layers of small bilayer vesicles are in keeping with this possibility.

The question remains open whether these fluctuations extend over the whole surface and whether they can be detected. Comparison of capacitance measurements of lipid bilayer membranes, which yield an average over the reciprocal of the thickness with reflectance measurements that yield an average over the square of the thickness, have been attempted with conflicting results (2, 7). The model does not give preference to any particular size of fluctuation. Moreover the fluctuating units may be of different shapes, giving wavy structures thus producing a jumbled overall structure. Could fluctuation be induced by some external influence? Possibly it could be done by applying a transmembrane potential. If one considers the polarization surface charge as discrete charges, then the field across the membrane is nonuniform with higher field strengths near the charges. It would therefore be interesting to measure capacitance and reflectance of bilayer membranes as a function of imposed polarization potentials.

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