

THICKNESS FLUCTUATIONS IN BLACK LIPID MEMBRANES

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ABSTRACT Because a black lipid membrane is compressible, there will be spontaneous fluctuations in its thickness. Qualitative arguments are given that the preferred configuration of the membranes is flat and that thickness fluctuations are smaller in amplitude than the differences in mean thickness observed using different hydrocarbon solvents. Fluctuations with short characteristic lengths will not be large as a result of the large amounts of oil-water contact these would entail. Quantitative analysis based on an extension of the treatment for soap films, predicts that the root mean square (rms) amplitude for fluctuations of wavelength longer than ~ 10 nm is negligible for glyceryl monoleate membranes with squalene ($< 3\%$) but may be $\sim 20\%$ with *n*-decane. rms fluctuations of 20% would lead to a discrepancy between the rms thickness of the core and the mean reciprocal thickness of only 6% .

I. INTRODUCTION

The hydrophobic core of a black lipid membrane has generally been assumed to have uniform thickness over most of its area (Hanai et al., 1964; Andrews et al., 1970; Fettiplace et al., 1971; Benz et al., 1975; Requena et al., 1975a; White, 1977; Gruen and Haydon, 1981), but the arguments for and against this assumption appear never to have been discussed. There is no known technique that can reveal the distribution of thicknesses present at any moment, but different averages of the thickness are available from three sources. First, the capacitance per unit area, C , is related to the mean reciprocal thickness,

$$1/d_c = \langle 1/d \rangle, \quad (1)$$

by

$$C = \epsilon_0/d_c, \quad (2)$$

where $\epsilon \approx 2.14$ is the dielectric constant of the core medium and ϵ_0 is the permittivity of free space. Second, for light incident nearly perpendicular to the plane of the film, the reflectance, R , is related to the mean-square thickness

$$d_r^2 = \langle d^2 \rangle \quad (3)$$

by (Cherry and Chapman, 1969)

$$R = [2\pi(n_m - n_o + \Delta)d_r/\lambda]^2, \quad (4)$$

where λ is the wavelength of the light, n_m is the refractive index in the plane of the membrane (i.e., perpendicular to the optical axis), n_o is the refractive index of the aqueous

phase, and

$$\Delta = (n_m - n_o)^2/(n_m + n_o). \quad (5)$$

Third, for a solventless membrane, the average thickness can be calculated from the number of lipid molecules per unit area, N , the molecular weight of their hydrocarbon chains, M , and the density of liquid hydrocarbon, ρ , as

$$d_{av} = N \cdot M/\rho. \quad (6)$$

For a membrane core of uniform thickness, the inverse of the mean reciprocal thickness, d_c , should be the same as the average thickness d_{av} and these should both be less than the thickness determined from reflectance d_r by the thickness of the polar head-group layers. For a membrane with a range of thicknesses, d_c will be less than d_{av} , whereas d_r will exceed d_{av} by more than the height of the head groups. However, very large variations in thickness are required (see Appendix I) to produce observable changes in the averages.

In black lipid membranes containing solvent there may be a number of thick inclusions containing some mixture of the bulk solution from which the membrane is formed and solvent squeezed out of the black membrane proper. These lenses, which range in diameter from submicroscopic to half the diameter of the membrane, are not part of the equilibrium structure of the membrane and Plateau border. Once the large lenses have fused with the border, the remaining lenses have a negligible effect on the capacitance, presumably because their capacitance per unit area is small and they replace only a small proportion of the

membrane area (Requena et al., 1975b). Any remaining lenses, will, however, reflect light much more strongly than the same area of black membrane and thus may increase the thickness calculated from the reflectance. Such an increase was proposed by Pagano et al. (1973), to explain the ~20% discrepancies they observed between d_c + polar groups and d_r for membranes made from glyceryl monooleate with either *n*-tetradecane or *n*-hexadecane. The recent careful experiments by Dilger (1981) show no such discrepancies.

While lenses are not part of the equilibrium structure of the black lipid membrane, the proper structure may still have a variable thickness. It is this possibility that must now be considered. With glyceryl monooleate and the short-chain solvent *n*-decane, the capacitance is 3.9 nF/mm² at 0 mV and 4.1 nF/mm² at 100 mV (Andrews et al., 1970; Requena and Haydon, 1975). However for a long-branched chain solvent, squalene, the capacitance is much higher, 7.8 nF/mm², but varies much less with potential (White, 1978). It is possible to contend that the capacitance of a membrane is less with decane than with squalene for any of three reasons or some compromise. The difference could result (a) if the uniform thickness of the core of the membrane were greater with shorter chain solvents, (b) if short-chain solvents cause portions of the membrane to be substantially thicker than a solventless bilayer, and (c) if there is a decrease in the amount of membrane with a thickness that is twice the extended chain length as the solvent size increases. In the second hypothesis, solventless monoglyceride membranes are flat; in the third hypothesis, decane-containing membranes are flat at approximately twice the extended chain length, whereas solventless membranes are wavy.

In the first hypothesis (Andrews et al., 1970) the decrease in capacitance is interpreted as a uniform increase in the thickness of the membrane core from 2.5 nm with squalene to a maximum of 4.8 nm seen with decane. This increase occurs with constant area per lipid molecule. The extra volume is provided by individual molecules of the solvent mixed in with the lipid chains. The thicker membranes, which contain solvent, are relatively compressible since the solvent can be squeezed out into the border and into lenses (Requena et al., 1975b). This hypothesis provides a ready explanation for the observation that the maximum thickness calculated from the capacitance is nearly twice the extended chain length of the lipid, since neither a layer nor pockets of bulk solvent form within the membrane. It also allows a simple and plausible mechanism for exclusion of larger molecules. When placed in the environment of the partially ordered lipid chains, large molecules can only exist in a small proportion of the conformations available to them in bulk solution. This unfavorable entropy term grows rapidly with molecular size. The effect on the amount of solvent within the membrane is cooperative, inasmuch as adding more solvent to the membrane decreases the proportion of chains

anchored at the surface, which decreases the ordering, which makes it easier to add more solvent. Thus a large change in incorporation is expected over a small range in molecular sizes (Gruen and Haydon, 1981).

In the second hypothesis, to explain a reduction in capacitance from 7.8 to 3.9 nF/mm² while still retaining portions of the membrane which are as thin as for squalene, about half the membrane must become substantially thicker in the presence of *n*-decane than twice the extended length of the lipid chains. Thus a large range of thicknesses must occur. In turn this implies that the extent of the thick and thin patches must also be comparatively large. Thus in a thick region there would almost be a pocket of bulk solvent sandwiched between two monolayers. If such pockets were to exist with *n*-decane, it is difficult to understand their absence with *n*-hexadecane and squalene, yet these membranes are thin and much less compressible, which implies that such pockets are absent. The possibility that with decane the whole membrane is thicker and thickness fluctuations become greater will be considered in section IV.

The third hypothesis, based on a proposal by Bach and Miller (1980), produces the unusual result that those membranes that are least compressible, i.e., that most resist changes in their mean reciprocal thickness, are the membranes whose structure displays the largest range of core thicknesses. The possibility that such a range might occur either as the preferred structure of the membrane or as fluctuations from a weakly preferred flat structure will be considered in the second and third sections, respectively.

II. PREFERRED CONFORMATION OF BLACK LIPID MEMBRANES

The minimum area that can be occupied by a hydrocarbon chain can be estimated as ~0.21 nm² from the common limiting areas for insoluble monolayers of alcohols, carboxylic acids (at low pH), and amides at the air-water interface (Adam, 1968). The limiting area for saturated monoglycerides is larger, ~0.24 nm² (0.26 nm² at zero pressure), which suggests that here the size of the head group is important. However, when the saturated lipid chains are replaced by oleate chains the behavior is quite different. Thus, at the air-water interface oleic acid (on 0.005 M H₂SO₄) gives expanded films with areas always substantially larger than those above, while glyceryl monooleate at oil-water interfaces in equilibrium with aggregated lipid has an area of 0.39 nm² (Fettiplace et al., 1971). These large areas per molecule (projected onto the planar area), observed at high surface pressures, must reflect repulsion between the chains since only the chains and their environment have changed. This repulsion in turn is entropic in origin, representing the tendency above the transition temperature for the chains to take up conformations many of which have large areas when projected onto the surface plane and small extension perpendicular to that

plane (Marčelja, 1974; Gruen, 1980). The area per chain that affects the structure of the membrane is not the minimum, which is smaller than a head group, but the area that excludes, on average, other chains by the probable conformations of the chain. By the arguments just given and by theoretical calculations (Marčelja, 1974; Gruen, 1980) this area for one chain exceeds that of a glycerol headgroup and the combined area for two chains exceeds the area of a phospholipid head group. Similarly the optimal length of the chains is substantially less than the extended chain length.

The effect of area changes on the free energy of the membrane can be thought of in terms of pressures due to the head groups, Π_{HG} , and the chains, Π_{C} , and a tension due to avoidance of oil-water contact, $\gamma_o \approx 50 \text{ mN/m}$. Since the net membrane tension is much smaller than γ_o , $\Pi_{\text{HG}} + \Pi_{\text{C}} - \gamma_o \approx 0$. From theoretical calculations neither Π_{HG} nor Π_{C} should be very small above the melting temperature for the chains (Gruen, 1980). By the arguments given above, the pressure due to the chains, Π_{C} , must be considerable for glyceryl monooleate membranes, and thus γ_o is significantly greater than Π_{HG} . Thus, increases in the area of the polar group layer will have a free energy cost of the order of $(\gamma_o - \Pi_{\text{HG}})$ per unit area (not 1–2 mN/m as claimed by Bach and Miller, 1980). Decreases in the area per chain will cost $\sim \Pi_{\text{C}}$ per unit area. The preferred conformation will minimize the area of the polar group layer while avoiding excessive compression of the chains. This conformation is flat for a membrane.

Black lipid membranes are metastable. Therefore it might be expected that the membrane structure would reflect some aspects of a more stable state. For the monoglycerides in the presence of decane or, presumably, squalene, the stable state is a micellar solution of the lipid in the hydrocarbon solvent (Andrews et al., 1970). In this solution there are monomers and micelles in which the head groups will be clustered on the inside with the chains in contact with the oil, i.e., in these the area per head group will be much less than the area per chain. There is, however, no plausible way to incorporate a structure with this feature into the bilayer structure, since inward dimpling of the surface reduces the volume of the membrane while increasing the area. Appendix II shows that micelles incorporated within the membrane should exist at a negligible concentration.

III. SHORT-WAVELENGTH FLUCTUATIONS

To estimate the probability of occurrence of fluctuation events, an estimate is required for the free-energy cost. When the wavelengths or typical dimensions along the surface of such events are comparable to the distance between head groups, the cost cannot be estimated using macroscopic properties such as the surface tension. However, the $-\text{OH}$ groups of glycerol will normally be strongly attracted to water and effectively repelled from oil. Thus conformations in which the polar group of one lipid

molecule is adjacent to the chain of another are very improbable. Molecular roughness of the surface, of height comparable to the dimensions of the ester linkage, may still occur. Such roughness, $\sim 5\%$ of the thickness of the solventless membranes, would produce a $< 3\%$ root mean square variation in thickness, which would presently defy detection.

Fluctuations of somewhat larger lateral dimensions, e.g. 2.5 nm or five head groups across, are intermediate between the molecular and the macroscopic and are thus difficult to treat. Fortunately it is possible to conclude that they will not be large for solventless membranes. If such fluctuations are common, then for the capacitative thickness of the membrane to be 2.5 nm, the average thickness cannot increase much above this value; thus the fluctuations cannot increase the number of molecules per unit area of midplane much above the number for a flat membrane that is 2.5-nm thick. By contrast the area following the surface, A_s , can be increased substantially by the fluctuations. This area increase is unfavorable. Sinusoidal profiles are considered in Appendix I, where d_{av}/d_c and A_s/L^2 are tabulated as functions of $\Delta h/h_o$ and $\Delta h/L$, respectively, where $h_o = d_{av}$, Δh is the amplitude of the sine wave, and L is the apparent wavelength or extent of the disturbance. For $L = d_c = 2.5 \text{ nm}$, a sinusoidal variation with $\Delta h/h_o = 0.4$ if present everywhere would produce a root-mean-square thickness deviation of 20% and a difference between the root-mean-square thickness (of the core) and the capacitative thickness of 6%, which at present is too small to detect. For this waveform the membrane will have only 4.4% more molecules and volume than a flat membrane with the same capacitance and midplane area, but 33% more surface area. This area represents increased oil-water contact but not increased area per chain (i.e., there is little or no increase in number of conformations available to the chains). The free-energy cost per unit area will therefore (presumably) be in the range $\gamma_o - \Pi_{\text{HG}}$ to γ_o . Since the extra area is $(2.5)^2 \times (1.33 - 1.04) = 1.8 \text{ nm}^2$ and the cost per unit area will be of the order of 20 mJ/m^2 , the cost of a pair of one thick and one thin patch will be $> 8 kT$ where k is Boltzmann's constant and T is temperature. The cost increases rapidly with increasing Δh and decreasing L . Thus significant short-wavelength wavy structures are unlikely in solventless membranes, even as fluctuations. Long-wavelength fluctuations are considered in section IV.

For solvent-containing membranes, the average thickness still cannot increase much above the capacitative thickness, but now the number of lipid molecules might increase in parallel with increases in area if the volume were held constant by elimination of some of the solvent. The principal costs of such fluctuations would be the increased exclusion of solvent, an adverse entropic term, and a decrease in the area available per chain. For the profile parameters considered above ($\Delta h/h_o = 0.4$, $\Delta h/L = 0.4$) and constant area per head group along the surface,

the midplane area per chain would be decreased by a factor $1/1.33 = 0.75$ relative to a membrane with the same average thickness. The area decrease for the molecule in a patch will thus be $\sim 0.25 \times (2.5)^2 = 1.6 \text{ nm}^2$. The cost per unit area of compressing the chains from their equilibrium area is at least Π_c (see Appendix A in Gruen, 1981). As a low estimate, if we assume the cost to be 20 mJ/m^2 , the cost for one patch will exceed $7.5 kT$. Again the cost increases rapidly with amplitude, i.e., with $\Delta h/L$. Thus again, significant short-wavelength fluctuations are unlikely.

IV. CONTINUUM THEORY

As argued in section III, the fluctuations that matter for both solventless and solvent-containing membranes will have apparent wavelengths that are larger than the thickness of the membrane and substantially larger than the distance across a lipid chain. It is thus reasonable to consider, as an approximation, a continuum normal-mode analysis for these fluctuations. This method has one distinct advantage; it avoids the arbitrary use of "cooperative units" of any particular size.

Vrij (1964, 1966, 1968) has used a continuum normal-mode analysis in his treatment of the light scattering from soap films. He calculates the relative free energies for the possible conformation of the surfaces of the membrane from the assumptions that each surface has a constant tension, γ , and that for each small area of the membrane there is a free energy of interaction between the surfaces that depends only on the separation of the surfaces in that area. He then shows that if a conformation is not too far from planar it can be expressed as a Fourier series of normal modes, each of which refers either to bending of the two surfaces in phase or to a change in thickness. Each mode contributes a term to the series expression for the free energy which is quadratic in the amplitude. If these modes are independent, as they are with his assumptions, then the theorem of equipartition of energy (see, e.g., Landau and Lifshitz, 1958) allows the determination of the time average of the square of the amplitudes and the corresponding mean increase in area of the membrane surfaces. In Vrij's work with soap films (1964, 1966, 1968) the wavelengths of the Fourier terms that contributed to the light scattering ($>200 \text{ nm}$) were much larger than the film thickness ($>15 \text{ nm}$) and both were larger than the molecular size of the material in the film core (water). Thus his assumptions were reasonable.

For black lipid membranes it is necessary to consider wavelengths that are not much longer than the membrane thickness. Fluctuation modes at these wavelengths may be strongly coupled which will lead to overcounting and hence an overestimate for the root mean square (rms) fluctuation amplitude. However, even an upper estimate for the fluctuation amplitude is of considerable interest.

In Vrij's analysis (1964, 1966, 1968) the difference in free energy between a given conformation of the mem-

brane and the planar state has two components. For both bending and thickness changes a free energy, $\gamma\Delta A$, is required to increase the area of one surface of the film by ΔA where γ is approximately one half the bilayer tension or somewhat larger (see Appendix III). For thickness changes there is a second component to the free energy change $\frac{1}{2}[d^2U/dh^2]_{h_0}(h - h_0)^2$, where h_0 is the mean thickness and $U(h)$ is the free energy of interaction between the surfaces per unit area for a film of thickness h_0 . Thus the free energy increase due to changes in thickness becomes

$$\Delta F^T = \int \int_{\text{area of membrane}} \left\{ \frac{1}{4} \gamma \left[\left(\frac{\partial h}{\partial x} \right)^2 + \left(\frac{\partial h}{\partial y} \right)^2 \right] + \frac{1}{2} \left(\frac{d^2U}{dh^2} \right)_{h_0} (h - h_0)^2 \right\} dx dy, \quad (7)$$

where the quantity in square brackets is four times the sum of the associated area increases for the two surfaces (see, e.g., Thomas, 1953).

As long as the membrane diameter is large compared with typical dimensions of the fluctuations, the fluctuations will not depend on the overall shape or size of the membrane. Thus, even though the membranes are nearly circular, it is permissible to simplify the mathematics of the normal mode analysis by assuming that the membrane is square. For such a membrane the fluctuations are expressed as sums of sinusoidal deformations with differing wavelengths and varying amplitudes. The wavelengths are in turn expressed as fractions of the length of a side. Thus a mode ρ, σ has a wavelength a/ρ in the x -direction and a/σ in the y -direction. The expression for the thickness thus becomes

$$h(x, y) = h_0 + \sum_{\rho=-r_{\max}}^{r_{\max}} \sum_{\sigma=-r_{\max}}^{r_{\max}} T_{\rho,\sigma} \exp \left(i \frac{2\pi}{a} (\rho x + \sigma y) \right), \quad (8)$$

where $T_{\rho,\sigma}$ is the complex amplitude. Since $h(x, y)$ must be real it is necessary that

$$T_{\rho,\sigma} = T_{-\rho,-\sigma}^* \quad \text{for} \quad -r_{\max} \leq \rho, \sigma \leq r_{\max}. \quad (9)$$

The limits on the summations, $\pm r_{\max}$, specify the shortest permitted wavelengths. Since the lipid chains are $\sim 0.5 \text{ nm}$ across, it is clear that r_{\max} will be $< a/1 \text{ nm}$. The value of r_{\max} to be used is discussed further below. From Eqs. 7-9 the free-energy increase associated with the ρ, σ th thickness mode is

$$\Delta F_{\rho,\sigma}^T = T_{\rho,\sigma} T_{\rho,\sigma}^* \left\{ \pi^2 (\rho^2 + \sigma^2) \gamma + \frac{1}{2} (d^2U/dh^2)_{h_0} a^2 \right\} \quad (10)$$

and similarly for the corresponding bending mode,

$$\Delta F_{\rho,\sigma}^B = B_{\rho,\sigma} B_{\rho,\sigma}^* \pi^2 (\rho^2 + \sigma^2) \gamma. \quad (11)$$

Vrij then invokes the theorem of equipartition of energy

TABLE I
PARAMETERS FOR GLYCERYL MONOOLEATE MEMBRANES

Solvent	Capacitance at 0 mV: C	Increase in capacitance at 100 mV: $\Delta C/C$	Area per lipid molecule: A_o	Surface tension: γ	Compressibility parameter: d^2U/dh^2
	$nF\text{ mm}^{-2}$		nm^2	mN m^{-1}	$J\text{ m}^{-4}$
<i>n</i> -decane	3.9*	0.073*	0.395‡	3.8*	$1.2 \times 10^{13}\S$
<i>n</i> -hexadecane	5.9‡	<0.02	0.38‡	2.5*	—
squalene	7.8	<0.0003	0.38	1.5	$2.0 \times 10^{16}***$

*Requena and Haydon (1975).

‡Andrews et al (1970).

§Calculated as shown in Appendix III from data of Requena and Haydon (1975).

|White (1978).

||See Appendix III.

***Calculated as shown in Appendix III from the compressibility reported by White (1978).

whereby ΔF for each mode equals $kT/2$ and obtains

$$T_{\rho,\sigma}^2 = kT/[2\pi^2\gamma(\rho^2 + \sigma^2) + a^2(d^2U/dh^2)_{h_0}] \quad (12)$$

and

$$B_{\rho,\sigma}^2 = kT/[2\pi^2\gamma(\rho^2 + \sigma^2)]. \quad (13)$$

There is no bending mode for $\rho = \sigma = 0$, hence $B_{0,0} = 0$. The mean-square deviation of thickness and the mean increase in area may now be calculated as

$$\langle (h - h_0)^2 \rangle = \sum_{\rho} \sum_{\sigma} T_{\rho,\sigma}^2 \quad (14)$$

and

$$\Delta A = \sum_{\rho} \sum_{\sigma} \pi^2(\rho^2 + \sigma^2)(T_{\rho,\sigma}^2 + B_{\rho,\sigma}^2). \quad (15)$$

To proceed further it is useful to note that there are very many terms in each of these sums and that no individual terms are unusually large. Thus the summations can be replaced by integration over the area of a square in ρ, σ space with side $2r_{\max}$. Each of these integrals can be broken into an integral over a circle of radius r_{\max} and another over the remaining corners. Thus, defining $r^2 = \rho^2 + \sigma^2$,

$$\langle (h - h_0)^2 \rangle = \int_0^{r_{\max}} \frac{2\pi r k T dr}{2\pi^2\gamma r^2 + a^2(d^2U/dh^2)_{h_0}} + H(r_{\max}) \quad (16)$$

and

$$\Delta A = \int_0^{r_{\max}} 2\pi^3 r^3 k T \cdot \left(\frac{1}{2\pi^2\gamma r^2} + \frac{1}{2\pi^2\gamma r^2 + a^2(d^2U/dh^2)_{h_0}} \right) dr + \Gamma(r_{\max}). \quad (17)$$

For the arguments that follow, all that is required for ΔA is an underestimate, thus Γ will be ignored and ΔA approximated by the integral over the circle, ΔA_c . For the values of the parameters in Table I, $H(r_{\max})$ is negligible for squalene and may be estimated for *n*-decane as

$$H(r_{\max}) \leq (4 - \pi)r_{\max}^2 T_{r_{\max},0}^2 \quad (18)$$

$$\leq (4 - \pi) \frac{kT}{2\pi^2\gamma}. \quad (19)$$

Thus after carrying out the integrals and defining

$$\phi = [1 + 2r_{\max}^2\pi^2\gamma/(a^2d^2U/dh^2)], \quad (20)$$

$$\langle (h - h_0)^2 \rangle = \frac{kT}{2\pi\gamma} \ln \phi + H(r_{\max}) \quad (21)$$

and

$$\Delta A_c = \frac{\pi kT}{\gamma} \left(r_{\max}^2 - \frac{a^2}{4\pi^2\gamma} \frac{d^2U}{dh^2} \ln \phi \right). \quad (22)$$

Since r_{\max} is proportional to a , ϕ and hence $\langle (h - h_0)^2 \rangle$ are independent of the size of the membrane and ΔA is proportional to the size of the membrane.

Adequate values of the tension for half a bilayer are available experimentally while an estimate for the change in free energy of interaction may be calculated from the compressibility (Appendix III). They have been taken to have the values in Table I. The predictions for $\langle (h - h_0)^2 \rangle^{1/2}$ and ΔA_c for cutoff wavelengths of 10 nm, where the theory should be at least a crude approximation, and 1 nm, where it should provide an overestimate are given in Table II. It should be noted that, with a cutoff wavelength

TABLE II
PREDICTED FLUCTUATIONS OF GLYCEROL
MONOOLEATE MEMBRANES FORMED IN TWO
SOLVENTS

Solvent	Squalene		<i>n</i> -Decane	
Cutoff wavelength, nm	10	1	10	1
rms fluctuations in thickness $\langle (h - h_0)^2 \rangle^{1/2}$, nm	0.08	0.6	0.9	1.2
Relative area expansion, A_c/A_t	0.02	3.0	0.02	1.7

A_t is the sum of the areas of the two surfaces when flat.

of 10 nm, the calculation for squalene includes the particular mode which Bach and Miller (1980) discussed. Including all modes down to 10 nm leads to a predicted $\langle (h - h_0)^2 \rangle^{1/2} = 0.08$ nm (see Table II) which is very small. Predicted shorter wavelength fluctuations from 10 to 1 nm, although individually even smaller are much more numerous leading to a predicted rms deviation of 0.6 nm (~25%). In this region, however, as discussed above and in Appendix III, the continuum theory has clearly failed.

For *n*-decane, the mean of the reciprocal thickness is known to vary with the applied potential. This observation is inserted into the calculation via a comparatively small value of d^2U/dh^2 . With this value the theory predicts, at realistic wavelengths, 0.9-nm rms fluctuations (~20%) about a mean of ~5.0 nm, which leads to only a small difference in the predictions for the various averages of the core thickness: $d_c = 4.80$, $d_{av} = 4.97$, and $d_r = 5.05$ + polar groups.

For glycerol monooleate membranes formed in both squalene and in *n*-decane, the theoretical arguments presented here suggest strongly that estimates of the membrane core thickness from capacitance and from reflectance measurements should be very similar. The experiments of Dilger (1981) support this conclusion.

V. CONCLUSION

Long-wavelength fluctuations in membrane thickness are only possible if the membrane is macroscopically compressible. Quantitatively the rms amplitude of fluctuations with characteristic lengths longer than ~1,000 nm are negligible with decane, whereas for squalene (membranes which are much less compressible), this length is near 10 nm. Short-wavelength fluctuations (<3 nm) in thickness are severely limited by the oil-water contact they create and by the crowding of the lipid chains. Intermediate wavelength fluctuations with lateral extent of ~10 nm may well occur in lipid membranes. They are expected to be greater in amplitude for the more compressible membranes but for all the membranes they should be too small to produce large differences between the average thickness determined from capacitance, reflectance, and composition.

APPENDIX I

Mean Thickness, Mean Reciprocal Thickness, Mean-Square Thickness, and Surface Area for a Membrane with Sinusoidal Variations in Thickness

Perhaps the simplest smoothly varying profile for membrane thickness is given by

$$h(x, y) = h_0 + \Delta h \sin \frac{2\pi x}{L} \sin \frac{2\pi y}{L}, \quad (1.1)$$

where h_0 is the mean thickness, L is the wavelength, Δh is the maximum excursion from the mean, and $h(x, y)$ is the thickness at point x, y on the

midplane. The mean thickness is thus $d_{av} = h_0$. The mean-square thickness of the core is also simply obtained:

$$\langle h^2 \rangle = \frac{1}{L^2} \int_0^L \int_0^L \left(h_0^2 + 2h_0\Delta h \sin \frac{2\pi x}{L} \sin \frac{2\pi y}{L} + \Delta h^2 \sin^2 \frac{2\pi x}{L} \sin^2 \frac{2\pi y}{L} \right) dx dy \quad (1.2)$$

$$= h_0^2 + \frac{\Delta h^2}{4\pi^2} \left(\int_0^{2\pi} \sin^2 \theta d\theta \right)^2 \quad (1.3)$$

$$= h_0^2 + \frac{\Delta h^2}{4}. \quad (1.4)$$

Thus the rms amplitude variation in core thickness is just

$$\sqrt{\langle h^2 \rangle - h_0^2} = \Delta h/2 \quad (1.5)$$

and the reflective thickness is

$$d_r = \sqrt{(h_0 + \text{polar groups})^2 + (\Delta h^2/4)}. \quad (1.6)$$

Values of d_r are tabulated as a function of $\Delta h/h_0$ in Table III. It should be noted that when averaging over patches of membrane characterized by different Δh , d_r^2 not d_r must be summed. The mean reciprocal thickness $d_c = 1/\langle 1/h \rangle$ may be calculated from

$$\langle 1/h \rangle = \frac{8}{L^2 h_0} \int_{-L/4}^{L/4} \int_{-L/4}^{L/4} dx \left[1 + \frac{\Delta h}{h_0} \sin \frac{2\pi x}{L} \sin \frac{2\pi y}{L} \right] dy \quad (1.7)$$

$$= \frac{2}{\pi^2 h_0} \int_{-\pi/2}^{\pi/2} \int_{-\pi/2}^{\pi/2} d\theta \left[1 + \frac{\Delta h}{h_0} \sin \theta \sin \phi \right] d\phi. \quad (1.8)$$

The ratio $h_0 \langle 1/h \rangle = d_{av}/d_c$ has been evaluated numerically by dividing the area of integration into 128 equal patches, evaluating the integrand at the center of each and summing. The results are given in Table III.

TABLE III
RATIOS OF THE AVERAGE THICKNESS
 $d_{av} = h_0$, CAPACITIVE THICKNESS, $d_c = 1/\langle 1/h \rangle$,
AND THE RMS CORE THICKNESS, $\langle h^2 \rangle^{1/2}$

$\Delta h/h_0$	d_{av}/d_c	$\langle h^2 \rangle^{1/2}/d_{av}$	d_r/d_{av}	d_r/d_c
0.05	1.0006	1.000	1.400	1.401
0.1	1.0025	1.001	1.401	1.404
0.2	1.0102	1.005	1.404	1.418
0.3	1.024	1.011	1.408	1.442
0.4	1.044	1.020	1.414	1.476
0.5	1.073	1.031	1.422	1.526
0.6	1.11	1.044	1.432	1.589
0.65	1.14	1.051	1.437	1.638
0.7	1.17	1.059	1.443	1.688
0.75	1.22	1.068	1.449	1.768
0.8	1.27	1.077	1.456	1.849
0.85	1.34	1.087	1.463	1.961
0.9	1.45	1.097	1.471	2.132
0.95	1.65	1.107	1.478	2.439

To calculate the reflective thickness, d_r , it has been assumed that the combined thickness of the polar group layers in 40% of the mean core thickness.

The area of each surface above a square of area L^2 on the midplane can be calculated as

$$A_s/L^2 = 16/L^2 \int_0^{L/4} \int_0^{L/4} \sqrt{1 + (\partial z/\partial x)^2 + (\partial z/\partial y)^2} dx dy, \quad (1.9)$$

where $z = h/2$ is the distance from the midplane to the surface. Thus

$$\frac{A_s}{L^2} = \frac{16}{L^2} \int_0^{L/4} \int_0^{L/4} \left[1 + \frac{\pi^2 \Delta h^2}{L^2} \left(\cos^2 \frac{2\pi x}{L} \sin^2 \frac{2\pi y}{L} + \sin^2 \frac{2\pi x}{L} \cos^2 \frac{2\pi y}{L} \right) \right]^{1/2} dx dy. \quad (1.10)$$

Using

$$\cos^2 \theta \sin^2 \phi + \sin^2 \theta \cos^2 \phi = \frac{1}{2}(1 - \cos 2\theta \cos 2\phi), \quad (1.11)$$

Eq. 1.10 may be rewritten

$$\frac{A_s}{L^2} = \frac{4}{\pi^2} \int_0^{\pi/2} \int_0^{\pi/2} \left(1 + \frac{\pi^2 \Delta h^2}{2L^2} (1 - \cos 2\theta \cos 2\phi) \right)^{1/2} d\theta d\phi. \quad (1.12)$$

This integral has been evaluated numerically by dividing the area of integration into 64 equal patches, evaluating the integrand at the center of each and summing. The results are given in Table IV. For $(\Delta h/L) < 0.3$,

$$\frac{A_s - L^2}{L^2} \approx \frac{\pi^2}{4} \left(\frac{\Delta h}{L} \right)^2, \quad (1.13)$$

while for large $\Delta h/L$, A increases proportionally with $\Delta h/L$.

To achieve a rms core thickness that is just 20% larger than the capacitive thickness $(h^2)^{1/2}/d_c = 1.2$, large fluctuations are required, $(\Delta h/h_0 = 0.65)$. Thus if the capacitive thickness were 2.5 nm, the

average core thickness would be 2.85 nm, the minimum 0.99 nm, the maximum 4.7 nm, and the reflective thickness (d_r , which includes polar groups) 4.1 nm. Twice the extended chain length is ~ 4.7 nm.

APPENDIX II

Micelles

For the monoglycerides there may be an equilibrium number of micelles, which have their chains pointing outwards, inserted between the leaflets of the membrane. A micelle, ~ 3 nm across, would create a bulge in the membrane with a radius of curvature ~ 3 nm. Thus within this bulge there will be excess pressure, $P = 2\gamma/r$. For a squalene membrane, $\gamma = 1.5$ mN/m (Table I) and hence $P \sim (3 \times 10^{-3}/3 \times 10^{-9}) = 10^6$ N/m². Thus the pressure-volume work of inserting the micelle will be of the order of $10^6 \times 10 \times 10^{-27}/4 \times 10^{-21} = 2.5$ kT. Thus, the concentration of micelles in the "free" volume of the membrane core is expected to be ~ 0.1 that in the solution of the Plateau border. The free volume will be small, perhaps 0.2 nm times the membrane area. Thus for a micelle concentration 50 mmol/m³ in the Plateau border, there might be $\sim 5 \times 10^{-3} \times 6 \times 10^{23} \times 2 \times 10^{-10} = 6 \times 10^{11}$ micelles/m² which would occupy roughly one part in 10^6 of the membrane area. This proportion is too small to matter.

APPENDIX III

Values for the Tension and Compressibility Parameters in the Continuum Theory

The tension used to calculate free-energy cost of area changes is a compromise. However, for long-wavelength fluctuations, changes in the area per molecule are small and whole molecules may move along the surface to minimize variations in the local value of the tension. The appropriate tension will thus be close to that of a half bilayer, which in turn is similar to that of the monolayers on the surfaces of the Plateau border. For short-wavelength fluctuations the change in area per molecule is large and the appropriate tensions are also much larger. By retaining the half-bilayer tension for all wavelengths the theory is again biased towards overestimating the importance of fluctuations at wavelengths less than ~ 10 nm. The tensions listed in Table I for *n*-decane- and *n*-hexadecane-containing membranes are half the membrane tensions. For squalene, the stated value, 1.5 mN/m, should underestimate the half-membrane tension, which in turn is somewhat less than the monolayer tension, 2.3 mN/m (Elliott and Haydon, 1979).¹

The compressibility parameter may be estimated from the measured changes in capacitance with voltage. For squalene, White (1978) reports $C = C_0 (1 + 3 \times 10^{-2} V^2)$ while the data for Requena and Haydon (1975) for decane can be fitted by a procedure similar to that used by Requena et al. (1975a) as $C = C_0 (1 + 7.3 V^2)$. Benz et al. (1975) report for decane $C = C_0 (1 + 5.4 V^2)$. For present purposes the difference between 5.4 and 7.3 is irrelevant. At each applied potential the sum of the electric compressive force and all others must be zero at the (mean) equilibrium thickness, i.e.,

$$F_{\text{other}}(h) = -\frac{dU}{dh} = -F_e(h) = +\frac{CV(h)^2}{2h}, \quad (3.1)$$

where $V(h)$ is the potential at which the thickness h is reached and positive force tends to make h increase. Using $C(V) = C_0 (1 + \alpha V^2)$ to eliminate V^2 from Eq. 3.1, we have

$$\frac{dU}{dh} = -\frac{\epsilon\epsilon_0}{2\alpha h^2} \left(\frac{h_0}{h} - 1 \right) \quad (3.2)$$

¹D.N. Needham, personal communication.

TABLE IV
AREA OF EACH
SURFACE PER UNIT
AREA OF MIDPLANE
AS A FUNCTION OF
THICKNESS
VARIATIONS

$\Delta h/L$	A_s/L^2
0	1
0.01	1.0002
0.1	1.02
0.2	1.09
0.3	1.20
0.4	1.33
0.5	1.48
0.6	1.64
0.7	1.82
0.8	2.00
0.9	2.18
1.0	2.37
1.2	2.77
1.5	3.37
2.0	4.39
3.0	6.48
10	21.32
100	212.87

and thus,

$$\left(\frac{d^2U}{dh^2}\right)_{h_0} = \frac{\epsilon\epsilon_0}{2\alpha h_0^3}. \quad (3.3)$$

Substitution of the appropriate values into Eq. 3.3 gives the compressibility parameters in Table I.

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