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**LATERAL TENSIONS AND PRESSURES IN MEMBRANES AND LIPID MONOLAYERS**DAVID W.R. GRUEN<sup>a</sup> and JOE WOLFE<sup>b</sup><sup>a</sup> Department of Applied Mathematics, Research School of Physical Sciences, Australian National University, Canberra 2601 and<sup>b</sup> Division of Plant Industry, CSIRO, Canberra 2601 (Australia)

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The effects of lateral tension on the properties of membranes are often explained in comparison with analogous experiments on monolayers, which yield more detailed data. To calculate the effects of changes in tension on the composition of, or incorporation of amphiphiles into membranes we examine (i) the fidelity of the monolayer analogy, (ii) the range of possible tensions in a membrane, and the way in which tensions arise and (iii) the equilibrium partitioning of amphiphiles between aqueous solution and a bilayer under tension. We argue that, at the same areas per molecule, a monolayer at an *n*-alkane/water interface is a closer analogy of the lipid bilayer than a monolayer at an air/water interface. Next, we show from a thermodynamic argument that changes in membrane tension can affect the absorption of very large amphiphiles such as proteins, but that physiological tensions are unlikely to affect the absorption of lipids or drugs. Finally we consider the possibility that the measured bulk tension in a complicated membrane such as that of the erythrocyte may be larger than the local tension in the fluid mosaic portions, and suggest a model which explains the ability of the erythrocyte membrane to withstand much higher tensions than other biological membranes and lipid bilayers.

**1. Introduction**

Biological membranes are generally believed to be extensible, two-dimensional fluids and the inter-related mechanical parameters of membrane tension, area density and lateral extensibility are functionally important. Lateral extensibility (or compressibility) and area density are thought to affect membrane permeability [1–3], and the partitioning of molecules between membranes and the aqueous phase is assumed to be dependent on membrane tension and area density [4]. It is difficult, however, to manipulate the area and tension of membranes with precision and so the mechanical and phase behaviour of membranes (especially lipid bilayers) and their effects on permeability and partitioning are often deduced from analogous experiments on amphiphilic monolayers. For example, Van Deenen et al. [4] show that penetration of a phosphatidylcholine monolayer by phos-

pholipase is critically dependent on the lateral pressure on the monolayer and use their results to estimate the area density of erythrocyte membranes.

In this paper we consider three different but related questions: (i) Which amphiphile monolayer is a closer analogy to half a bilayer: one at an air/water interface or one at an oil/water interface? In either case, what is the appropriate monolayer pressure?

(ii) What ranges of tensions may be expected in artificial or biological membranes, and how do these tensions arise?

(iii) How does the tension in a membrane (or the lateral pressure in a monolayer) affect the partitioning of added amphiphiles?

This paper is arranged as follows. Section 2 part I describes the physical conditions under which we will compare monolayers with bilayers. We present a naïve argument which suggests (for both

air/water and *n*-alkane/water monolayers) that a surface pressure of  $50 \text{ mN} \cdot \text{m}^{-1}$  is required to achieve the same area per lipid as found in bilayers. This suggestion is compared with experiment. In section 2 part II we present reasons for preferring the *n*-alkane/water monolayer to the air/water monolayer as an analogy to the bilayer. Section 3 presents a thermodynamic analysis which describes the origin of the surface tension in 'black' lipid films. This analysis is then used to determine the tension dependence of partitioning in a membrane of amphiphiles with finite aqueous solubility. We find that, over the possible range of tensions in biological membranes, this dependence is considerable only for very large molecules.

## 2. The closest monolayer analogy to the lipid bilayer

### I. Appropriate monolayer pressure

Lipid monolayers may be formed at either an oil/water or air/water interface and may be subject to an external lateral (or surface) pressure  $\Pi$ . Once the interface is chosen, and  $T$  (temperature) and  $\Pi$  are fixed, the area per amphiphile in the plane of the interface ( $A$ ) is determined uniquely (as long as the monolayer is not at a first-order phase transition). By contrast, external lateral pressure cannot be applied to a macroscopic bilayer and it can only be formed between two water layers. (The problem of interaction between bilayers in a multilayered system doesn't concern us here, we shall consider only single membranes or fully hydrated multilayers in which interaction between bilayers is weak [5]).

Since physiological temperatures are well above the transition temperatures of biological membranes (see, for example, Ref. 7), we shall demand of our monolayer analogue that, at the same temperature, its state resemble that of a bilayer in the liquid crystalline state, i.e. above the main transition temperature,  $T_c$ . Further, the only oil/water interfaces we shall consider will be *n*-alkane/water interfaces. This choice is suggested by the chemical similarity between the lipid and *n*-alkane chains. Not surprisingly, changing the oil phase to something very different from *n*-alkane (e.g., 2,2,4-trimethylpentane) changes the  $\Pi$ - $A$  behaviour of the lipid monolayer [5].

In large lipid vesicles, the bifacial surface tension is zero so long as there is no hydrostatic pressure difference between the inside and outside. The lipid molecules are free to vary their area and minimize their free energy ( $F$ ) [8], i.e.

$$2\gamma = \left( \frac{\partial F}{\partial A} \right)_{n,V,T} = 0$$

where  $2\gamma$  is the membrane bifacial tension,  $n$  the number of lipids and  $V$  and  $T$  the volume and temperature of the system. In 'black' lipid films containing some alkane solvent, the membrane is constrained in area and the tension need not be zero, but it is small ( $\lesssim 5 \text{ mN} \cdot \text{m}^{-1}$ ) in comparison with the competing surface energies of the system's components (*n*-alkane/water surface tension is approx.  $50 \text{ mN} \cdot \text{m}^{-1}$ ). We shall compare monolayers with bilayers in which the tension is zero.

In order for lipid molecules in two systems to be in the same physical state it is necessary that both the headgroup environment and the relative probabilities of the chain conformations be the same. To satisfy the first requirement, it is necessary that the area per headgroup be the same in the two systems so that headgroup freedom and the amount of water in the headgroup layer are identical in the two systems. We shall therefore compare monolayers and bilayers at the same area per lipid.

It is possible to consider a lipid monolayer at an air/water (*n*-alkane/water) interface as consisting of two interfaces: a lipid/water interface and a lipid chain/air (lipid chain/*n*-alkane chain) interface. In a similar way, lipid molecules in one monolayer of a bilayer have a lipid/water interface and an interface with the opposing monolayer chains. It is now useful to consider each interface having an associated free energy cost of formation, i.e., interfacial tension. (The macroscopic concept of interfacial tension cannot strictly be applied to two interfaces separated by a single layer of molecules. Nevertheless, as we shall see, this is a simple and useful way of looking at these systems.)

We now present a simple argument which suggests that a surface pressure of  $50 \text{ mN} \cdot \text{m}^{-1}$  must be applied to a monolayer (either air/water or *n*-alkane/water) to achieve the area per lipid found in bilayers. We must assume that (i) the lipid

chain/lipid chain interface in the bilayer and the lipid chain/*n*-alkane chain interface at the *n*-alkane/water monolayer have no tension, (ii) the lipid chain/air interface (at the area per lipid found in bilayers) has a tension comparable to the *n*-alkane/air interface i.e. approx.  $20 \text{ mN} \cdot \text{m}^{-1}$ , and (iii) a monolayer and a bilayer exhibit the same area per lipid when their lipid/water interfaces have the same tension (zero in this case as our bilayer has no tension).

The *n*-alkane/water interface has a tension of approx.  $50 \text{ mN} \cdot \text{m}^{-1}$  in the absence of an amphiphile monolayer. Using (i) above, we require a surface pressure of approx.  $50 \text{ mN} \cdot \text{m}^{-1}$  for the lipid/water interface to be without tension. Similarly, the air/water interface has a tension of approx.  $70 \text{ mN} \cdot \text{m}^{-1}$  in the absence of lipid. Using (ii) above, again,  $50 \text{ mN} \cdot \text{m}^{-1}$  surface pressure is required on the monolayer to have a lipid/water interface without tension [9]. Using assumption (iii) above completes the argument.

This very simple idea can be tested. The area per molecule found in phosphatidylcholine bilayers is  $0.60$  to  $0.70 \text{ nm}^2$ . For the monolayer, we are dealing with the steep part of the  $\Pi$ - $A$  curve, and hence small changes of  $A$  require large changes in  $\Pi$ . Nevertheless, it appears that a surface pressure of somewhat less than  $50 \text{ mN} \cdot \text{m}^{-1}$  is necessary to give an area per lipid equal to that in a bilayer. Thus, for a monolayer of dioleoylphosphatidylcholine at  $3.1^\circ\text{C}$  (above the fully hydrated bilayer  $T_c$  of  $-22^\circ\text{C}$  [34]) at the *n*-heptane/water interface,  $\Pi \sim 33 \text{ mN} \cdot \text{m}^{-1}$  for  $A = 0.70 \text{ nm}^2$  and  $\Pi \sim 46 \text{ mN} \cdot \text{m}^{-1}$  for  $A = 0.60 \text{ nm}^2$  [29]. For a monolayer of dipalmitoylphosphatidylcholine at  $42.8^\circ\text{C}$  (above the fully hydrated bilayer  $T_c$  of  $41.4^\circ\text{C}$  [30]) at the air/water interface,  $\Pi \sim 18 \text{ mN} \cdot \text{m}^{-1}$  for  $A = 0.70 \text{ nm}^2$  and  $\Pi \sim 35 \text{ mN} \cdot \text{m}^{-1}$  for  $A = 0.60 \text{ nm}^2$  [16]. In this paper, we wish to compare bilayers with monolayers at the same area per lipid, even we cannot predict exactly what surface pressure is required to achieve this.

## II. The advantages of the *n*-alkane/water monolayer over the air/water monolayer

The results of X-ray diffraction experiments on stacked lipid bilayers (see, for example, Ref. 10) suggest that the  $\text{CH}_3$  groups at the end of the chains are significantly less well localised to the

bilayer midplane above  $T_c$  than below. Thus, above  $T_c$ , the interface between opposing monolayers is fairly rough. This conclusion is supported by the results of neutron diffraction experiments [11]\*. To make a more quantitative statement one can appeal to a model which seems to fit the available spectroscopic and diffraction data quite well [6]. This suggests (for dipalmitoylphosphatidylcholine) that the standard deviation of the length of a chain is  $0.27 \text{ nm}$ : i.e. the root mean square difference between the length of chains is  $0.27 \text{ nm}$ .

Consider a monolayer of lipid molecules at the air/water interface at the same area per lipid as in the bilayer. If the chains were in the same state in the two systems, the monolayer chain ends would expose a large surface area to air (because of the roughness of this surface). A simple calculation (see Appendix) suggests that this roughness should have an associated free energy cost of about  $1.7 kT$  per lipid tail where  $k$  is Boltzmann's constant.

Therefore at an air/water interface, neighbouring chains are more likely to adopt similar than dissimilar conformations. Lipid chain conformations which are near the average chain length will be strongly favoured over those which are not (see Appendix). These constraints do not apply in a bilayer, and hence, even at equal areas per lipid, the air/water monolayer must be viewed as an imperfect analogue of the bilayer.

Consider now a monolayer of lipid at an *n*-alkane/water interface, which has the same area per molecule as does the bilayer. The ends of the monolayer chains are immersed in other hydrocarbon chains, presumably at liquid hydrocarbon density. In the bilayer, the ends of the lipid hydrocarbon chains are also surrounded by chains which are chemically identical to alkane hydrocarbon chains (i.e., lipid chains from the same monolayer or the opposing one) and the packing density and hence the van der Waals interactions are very close to liquid hydrocarbon values [12]. Further, in the bilayer, the ends of the lipid chains are the least

\* The diffraction data could be explained if the interface between opposing monolayers was smooth and undulating. However, the disorder in the bilayer core is very similar to a bulk *n*-alkane liquid [38], strongly suggesting that the lipid chain ends should be similarly disordered.

ordered segments in the chain [13], and hence (presumably) are only slightly perturbed by immersion in disordered *n*-alkane. This conclusion is supported by model calculations on 'black' lipid films [15].

Nagle [5] has argued that van der Waals attractions between lipid chains are effectively reduced at the oil/water interface. This follows because oil molecules can enter between two adjacent [lipid] chains that are pulled apart laterally and the energy of such a state is not increased as much as if no oil molecules could enter as at the air/water interface or in bilayers [5]. This is true, but, at the areas per lipid found in bilayers, it is a very small effect. In order to reduce hydrocarbon-water contact, the chains in a bilayer are straightened somewhat (relative to liquid hydrocarbon). There is a free energy cost associated with this straightening, and the lipid chains exert a surface pressure on each other [14,15]. At an oil/water monolayer at the same area per lipid as the bilayer, no oil can enter between two adjacent lipid chains without further straightening the lipid chains. Above  $T_c$ , the chains will not freeze and hence this process is very costly in free energy terms [15]. Also, if the oil is an *n*-alkane, there is a further free energy cost involved in oil molecules entering between lipid chains. In bulk hydrocarbon, the molecules are relatively free to take up any spatial orientation and, apart from an internal energy barrier, to change between trans and gauche conformers. Lying between the ordered lipid chains, both these freedoms are substantially curtailed [15].

Fig. 1 compares the results of mean-field calculations on a solventless bilayer and a substantially thickened bilayer containing *n*-nonane. (The latter should closely resemble two non-interacting lipid monolayers at nonane/water interfaces.) It is clear that very little nonane interdigitates between the lipid chains at the interface, and that the distribution of the ends of the lipid chains is very similar in the two systems. The authors therefore believe that an *n*-alkane/water monolayer at a comparable area per molecule is the closest analogy to the lipid bilayer.

Thus we argue that quantitative predictions about the behaviour of bilayers and other membranes should be made from experiments on monolayers at the *n*-alkane/water interface rather

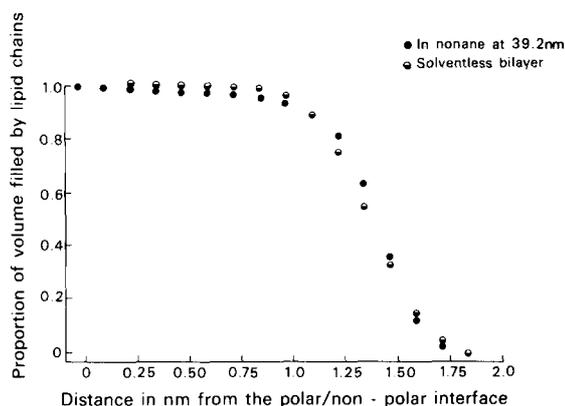


Fig. 1. Proportion of volume filled by lipid chains versus distance from polar/non-polar interface.  $\odot$ , one lipid monolayer in a solventless bilayer [6].  $\bullet$ , one lipid monolayer in a bilayer of thickness 3.92 nm containing *n*-nonane [15]. At a thickness of 3.92 nm, fully extended lipid chains just reach the middle of the bilayer. Less than 1% of the lipid chains are in this state [15] and hence interaction between opposing lipid monolayers is minimal. This system should be a close analogy to a lipid monolayer at the nonane/water interface. The area per lipid for both systems was  $0.633 \text{ nm}^2$ .

than on monolayers at the air/water interface. Nevertheless, qualitative features of air/water monolayers, especially features which are insensitive to packing at the tail-air interface, suggest the appearance of similar features in a bilayer. Van Deenen et al. [4] found that the ability of phospholipase to penetrate an air/water monolayer varied dramatically with a change in lateral pressure of only  $5 \text{ mN} \cdot \text{m}^{-1}$  \*. Can a change in mem-

\* This article appears to have been misunderstood by Conrad and Singer [18]. They confuse surface pressure (measured in  $\text{N} \cdot \text{m}^{-1}$ ) with bulk pressure (measured in  $\text{N} \cdot \text{m}^{-2}$ ), and misquote Ref. 4 to support the proposition that there is a hydrostatic pressure excess in the interior of membranes. As the membrane is not a solid, the pressure within a flat membrane must be the same as that in the aqueous phase, and that in a curved membrane not very different from that in the aqueous phase. The average curvature of the erythrocyte membrane is small and the hydrostatic pressure in the membrane must be close to atmospheric pressure.

If a bulk pressure difference  $\Delta P$  were to change the partitioning of an amphiphile by a factor  $f$ , then  $\ln f = \Delta P \cdot v/kT$  where  $v$  is the volume of the molecule. Conrad and Singer report  $f$  values in the order of 200 for molecules with  $v \sim 0.4 \text{ nm}^3$ . The required  $\Delta P$  is  $5 \cdot 10^7 \text{ Pa}$ , or 500 atmospheres.

brane tension of this order affect the penetration of membranes in a similar fashion? Are such changes in membrane tension possible? We shall consider these questions in the following section.

### 3. Surface tension of bilayers and membranes

Though it is difficult to imagine how a substantial area of a bilayer membrane could be subjected to a surface pressure without buckling, it is relatively easy to apply small tensions (negative surface pressures), often by producing a hydrostatic pressure across a spherical membrane. Two different responses are possible. If the number of molecules in the membrane is conserved, the membrane stretches elastically and the tension increases with area until it lyses; this is exemplified by the osmotic swelling of erythrocytes [19]. If, however, there is a reservoir of membrane material which can be incorporated into the membrane upon deformation, then the equilibrium tension\* will have a value  $2\gamma = 2 \Delta G/a$  where  $\Delta G$  is the free energy difference per molecule between the reservoir and the unstretched membrane, and  $a$  is the area per molecule in the plane of the membrane [17]. If  $\Delta G$  is constant for all areas, then a constant surface tension will be measured. Thus a 'black' lipid film, in equilibrium with a reservoir of inverted micelles in a Plateau-Gibbs border has a tension of the order of  $1-5 \text{ mN} \cdot \text{m}^{-1}$ , independent of the degree of stretching [17,20-22,32]. The plasma membrane of plant protoplasts has an equilibrium tension of about  $0.1 \text{ mN} \cdot \text{m}^{-1}$  which is approached after deformations in area [23] and the reservoir in this case is probably vesicles associated with the membrane.

It is possible to make more quantitative statements about the tension in an artificial membrane. Consider a 'black' lipid film formed from glycerol monooleate dissolved in an  $n$ -alkane. The film is stable for some time if the concentration of glycerol monooleate in the film-forming solution of  $n$ -alkane is greater than the critical micelle concentration (CMC) [32]. The glycerol monooleate forms inverted micelles in the film-forming solution and

\* This is not a very stable equilibrium, since membranes are unstable with respect to large vesicles, however they may have lifetimes of many hours.

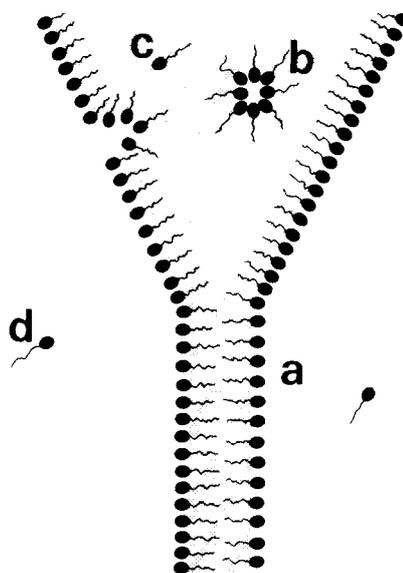


Fig. 2. This picture, after Ref. 17, depicts the equilibrium between a bilayer of glycerol monooleate (a), inverted micelles in the Plateau-Gibbs border (b) and monomers in the hydrocarbon solvent (c) and aqueous solution (d).

also in the Plateau-Gibbs border of the black film. Fig. 2 shows a schematic diagram of the system. We define the chemical potential of a glycerol monooleate molecule in a hypothetical bilayer at zero tension to be  $\mu_B^0$ , and that of a glycerol monooleate molecule in a bilayer with a bifacial tension of  $2\gamma$  to be  $\mu_B(\gamma)$ . Now

$$\left. \frac{\partial \mu_B(\gamma)}{\partial \gamma} \right|_{T, V, n} = -a$$

where  $a$  is the area per glycerol monooleate molecule in the bilayer. If we assume that  $a$  is independent of  $\gamma$  (which, for small tension, should be a good approximation), then

$$\mu_B(\gamma) = \mu_B^0 - \gamma a$$

We define  $\mu_M^0$  to be the standard chemical potential of glycerol monooleate molecules in the inverted micelles and assume that the micelles are monodisperse with  $N$  glycerol monooleate molecules per micelle. If the mole fraction of glycerol monooleate in the inverted micelles is  $X_M$ , then

$$\mu_B^0 - \gamma a = \mu_M^0 + \frac{kT}{N} \ln \frac{X_M}{N} \quad (1)$$

and  $a \sim 0.39 \text{ nm}^2$  [32]. Eqn. 1 is given in Ref. 17. It follows, straightforwardly, from the analysis of Israelachvili et al. [33]. The second term on the right hand side gives the contribution to the free energy of the entropy of mixing of the inverted micelles in the Plateau-Gibbs border. Rearranging Eqn. 1 gives:

$$\gamma = \frac{1}{a} \left( (\mu_B^0 - \mu_M^0) - \frac{kT}{N} \ln \frac{X_M}{N} \right) \quad (2)$$

For *n*-alkanes hexadecane to heptane, the one-sided bilayer tension varies from 2.50 to 4.70  $\text{mN} \cdot \text{m}^{-1}$  [32]. Table I demonstrates that if the inverted micelles in the Plateau-Gibbs border are not too big ( $N \lesssim 100$ ), the loss of mixing entropy of micelles when glycerol monooleate molecules move into the bilayer contributes substantially to the free energy cost of forming the bilayer. Part of the difference in tension between bilayers formed in different alkanes may be due to differences in the size of the inverted micelles in the Plateau-Gibbs borders (Coster, H.G.L., Israelachvili, J.N., Pailthorpe, B. and Wolfe, J., in preparation). Biological membranes which exhibit constant surface tensions are likely to be in equilibrium with much larger aggregates in aqueous solution, but since their tensions are very small [23], the mixing entropy of these aggregates may still be an important contribution.

A similar analysis may determine the partitioning between a membrane and an aqueous solution of a small concentration of a different amphiphile

(including membrane-soluble drugs or proteins) added to the solution containing the membrane. Using dashes to signify properties of the added amphiphile, and introducing  $X'_B$  its (monomer) concentration in the bilayer, the equilibrium partitioning is described by

$$\mu_B^{0'} + kT \ln X'_B - \gamma a' = \mu_N^{0'} + \frac{kT}{N'} \ln \frac{X'_M}{N'} \quad (3)$$

$$\frac{X'_B}{(X'_M)^{1/N'}} = K' e^{\gamma a' / kT} \quad (4)$$

where  $K'$  is the equilibrium constant for the reaction of incorporating the amphiphile in the membrane at zero tension.

In the simplest case, let  $N' = 1$ : the added amphiphile either does not form aggregates or else is added in a concentration below its CMC. From Eqn. 4 we see that an  $e$ -fold change in the ratio  $X'_B/X'_M$  requires that  $\gamma$  change by  $kT/a = 4.1 \cdot 10^{-21} \text{ J}/a$  at room temperature. For a lipid,  $a$  is  $\sim 0.6 \text{ nm}^2$  and so an  $e$ -fold change in partitioning requires a change in the bifacial tension ( $2\gamma$ ) of about  $14 \text{ mN} \cdot \text{m}^{-1}$ . The partitioning of larger amphiphiles would be affected by smaller tensions: a protein of molecular weight 100000 might occupy an area of tens of  $\text{nm}^2$  in the plane of the membrane and thus could be absorbed into or excluded from the membrane by changes in tension of less than  $1 \text{ mN} \cdot \text{m}^{-1}$ . The experiments of Van Deenen et al. [4] exemplify this: A change in monolayer surface pressure of  $5 \text{ mN} \cdot \text{m}^{-1}$  causes a large change in the concentration of phospholipase in a radioactively labelled phosphatidylcholine monolayer, as measured by the change in surface radioactivity as labelled groups are released by the action of the phospholipase. The value of the surface pressure at which this large change occurs cannot be simply related to the tension in a membrane because of the imperfection of the analogy between air/water monolayers and membranes. The change in the equilibrium partitioning, however, could be calculated as above if the area of phospholipase molecules in the plane of the monolayer were known. Thus the composition of membranes, especially with respect to large molecules, may be dependent on the tension applied to them.

TABLE I  
CONTRIBUTION OF THE ENTROPY OF MIXING OF INVERTED MICELLES TO THE ONE-SIDED BILAYER TENSION,  $\gamma$

$N$	$-\frac{kT}{aN} \ln \frac{X_M}{N} \text{ (mN} \cdot \text{m}^{-1}\text{)}$			
	$X_M$	0.01	0.001	0.0001
1		49	74	98
10		7.4	9.8	12.3
50		1.8	2.3	2.8
100		1.0	1.2	1.5
500		0.23	0.28	0.33
1000		0.12	0.15	0.17

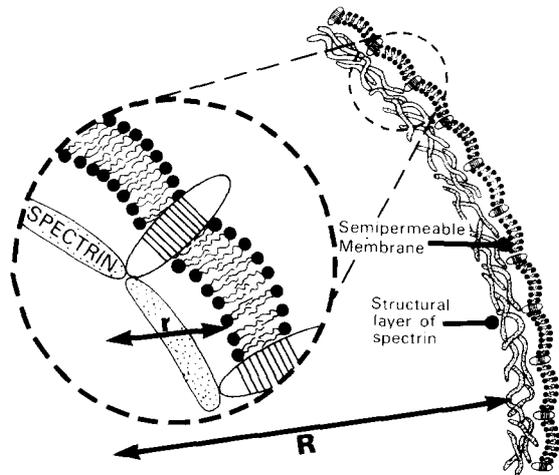


Fig. 3. This speculative depiction of the membrane of an osmotically swollen erythrocyte demonstrates that, even though the membrane as a whole may sustain a large tension (i.e. resist a hydrostatic pressure difference with a region of low curvature), the putative semipermeable layer of the membrane may be subject to a lower tension. In our idealized representation, the semi-permeable layer is a bilayer interspersed with intrinsic proteins which are the anchorage points for the extrinsic web of spectrin. (We have omitted from this diagram the external glycoprotein layer described by Tanner [31]). Though the membrane sustains a global tension of  $\Delta P \cdot R/2$ , this tension is supported by the crosslinking of the relatively rigid spectrin layer. The semipermeable layer, which has high curvature locally, sustains a tension of only  $\Delta P \cdot r/2$ , where  $r \ll R$  (e.g.,  $R \sim 5 \mu\text{m}$ ,  $r \sim 50 \text{ nm}$  say). (This model may be compared to a steel frame, plastic lined geodesic dome in which the frail plastic, which is impermeable to air, sustains a pressure difference with high local curvatures and the frame, which may withstand higher tensions, has a much lower curvature.)

What variation of tension is possible under physiological conditions? The tensions required to lyse phospholipid vesicles [24] and plant plasma membranes [23] are similar, about  $4 \text{ mN} \cdot \text{m}^{-1}$ . The erythrocyte has an atypically rigid membrane [25] and can withstand tensions of 10 to greater than  $20 \text{ mN} \cdot \text{m}^{-1}$ , depending on the time of exposure [26]\*. We remark that it is possible that this macroscopic tension may be sustained prin-

\* Rand and Burton [27] suggest area expansions of approx. 10% in the erythrocyte membrane. If this were elastic, and the modulus were  $450 \text{ mN} \cdot \text{m}^{-1}$  [28] then the lysing tension would be at least  $45 \text{ mN} \cdot \text{m}^{-1}$ .

cipally by the spectrin cytoskeleton and so the tension in the membrane at the microscopic level, in the lipid and intrinsic protein regions, could be substantially lower. This is illustrated in Fig. 3.

With area elastic moduli in the range  $230 \text{ mN} \cdot \text{m}^{-1}$  [23] to  $450 \text{ mN} \cdot \text{m}^{-1}$  [28], elastic expansions in area of the order of a few percent induce tensions of several  $\text{mN} \cdot \text{m}^{-1}$ . In the plant plasma membrane, the tension relaxes with time as new material enters [23], however, the erythrocyte can retain a tension for a longer period (after the initial excess tension is dissipated during viscous flow [19]). Thus relatively mild osmotic expansions are expected to cause changes in the partitioning of large amphiphilic molecules between membranes and the aqueous phase. The very large differences in the partitioning of small amphiphiles into different membranes reported by Conrad and Singer [18] however are not likely to be a result of different membrane tensions or lateral pressures.

## Appendix

The calculation presented here is fairly naive and can only be trusted to give a rough estimate for the free energy cost associated with air/water monolayer chains exposing an uneven surface to air. We assume that these chains exist in the same conformational state as in the bilayer (i.e., any given chain conformation has the same probability in the two systems). The calculation will be performed for model dipalmitoylphosphatidylcholine chains [6].

As the standard deviation of the length of a chain is  $0.27 \text{ nm}$ , we shall estimate the free energy cost of a chain extending  $0.27 \text{ nm}$  from the average chain-air interface. Fig. 4 shows a schematic diagram. Such a chain will extend  $(1.365 + 0.27) \text{ nm}$  from the water-chain interface; a fully extended chain extends  $1.97 \text{ nm}$  from the water-chain interface [6]. The distance between carbon atoms, resolved along the normal to the interface is  $0.125 \text{ nm}$ . Therefore in the given chain, three carbon atoms sit above the average chain-air interface; at distances of  $X_3 = 0.27 \text{ nm}$ ,  $X_2 = (0.27 - 0.104) \text{ nm}$  and  $X_1 = (0.27 - 2 \times 0.104) \text{ nm}$  above this interface.

We assume that the major contribution to the free energy cost of exposing the chain to air is the

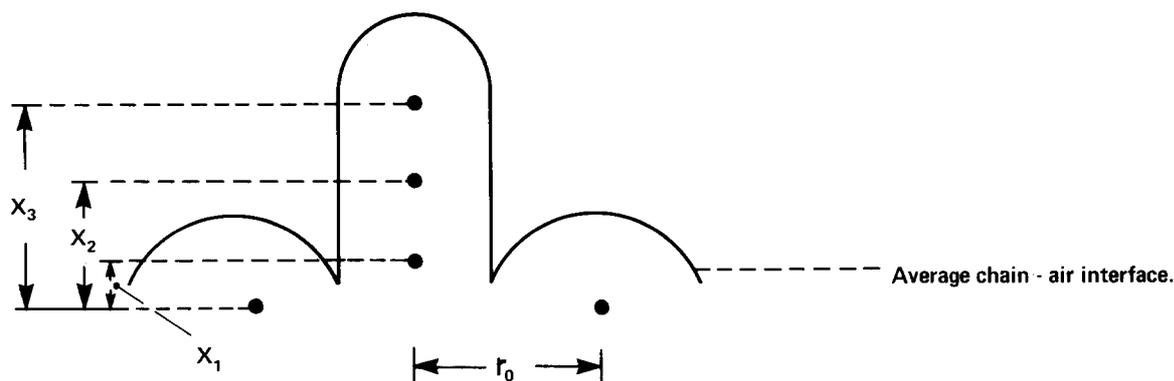


Fig. 4. Schematic drawing of a chain extending 0.27 nm beyond the average chain-air interface ( $X_3 = 0.27$  nm) at an air/water monolayer. The dots represent the centres of carbon atoms.

loss of van der Waals attractions with other chains. Chains in the bilayer are packed at liquid hydrocarbon density and hence should have van der Waals attractions which are very similar to those in liquid hydrocarbon [12]. Liquid hydrocarbon chains are almost completely disordered [35,36], and hence liquid and gaseous hydrocarbon chains (at the same temperature) should have almost the same proportion of *gauche* bonds and configurational entropy. Given these facts, measurement of  $\Delta H$ (vapourization) of liquid hydrocarbons as a function of chain length should give an accurate measure of the van der Waals energy between chains in the bilayer. Such measurement yields  $\Delta H$ (vapourization) = 1.169 kcal/mol of  $\text{CH}_2$  groups [37].

We assume a  $d^{-6}$  van der Waals attraction between  $\text{CH}_2$  groups, and assume that only nearest neighbours contribute to the sum. If  $r_0$  is the mean nearest neighbour distance between non-bonded  $\text{CH}_2$  groups in liquid hydrocarbon and the geometry shown in Fig. 4 is assumed, the loss of van der Waals energy for the given chain is

$$1.169 \sum_{i=1}^3 \left( 1 - \left[ 1 / \left( 1 + \frac{X_i^2}{r_0^2} \right)^3 \right] \right) \text{ kcal/mol}$$

Substituting the values  $X_3 = 0.27$  nm,  $X_2 = 0.166$  nm,  $X_1 = 0.062$  nm and  $r_0 = 0.502$  nm (from Table II in Ref. 12) leads to an estimate of 0.99 kcal/mol or 1.7  $kT$  per chain.

A chain which extends 0.27 nm less than the average chain-air interface will not experience reduced van der Waals attractions, but its neighbours will. (Using the same assumptions as above, the van der Waals energy cost for such a chain and its neighbours would again be 1.7  $kT$ .) Hence, chain conformations which deviate in length by 0.27 nm from the average chain-air interface are about  $e^{1.7}$  (approx. 5) times less likely, in an air/water monolayer, than they would be in a bilayer (relative to average length of chains). This is a large effect.

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