The central role of line tension in the fusion of biological membranes

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Abstract

Recent progress in the fusion of biological membranes is reviewed to highlight the central role played by the line tension, which permits exquisite control of the process.

La plus expresse marque de la sagesse, c'est une esjouïssance constante; son etat est comme des choses au dessus de la Lune; toujours serein.

Michel de Montaigne

The importance of the fusion of biological membranes is sufficiently clear that only a few keywords, such as endocytosis, intracellular trafficking, synaptic release, and viral entry, should suffice to remind the reader of it. For all its importance, the physics of this topological rearrangement is not well understood at all. In fact, what we see as the central conundrum which fusion presents seems, with notable exceptions (1), not to have been addressed explicitly. That conundrum is the following. In order for any vesicle to be useful, it must be relatively stable. In particular, its enclosing membrane must be stable to the occurrence of long-lived holes which are thermally activated. Yet in order to undergo fusion, just such long-lived holes must occur at some point along the fusion pathway. It would seem that vesicles could *either* be stable, *or* they could undergo fusion, but not both. How they actually manage to exhibit these two conflicting properties is the conundrum. Because of recent work on this problem, some to be published elsewhere (2), we believe we understand the resolution of this puzzle. Because line tension is at the heart of this resolution, we thought it a very appropriate subject to be included in a volume honoring Ben Widom whose interest in, and explication of, this concept is long-standing (3).

Let us briefly review the situation. We begin with two membranes, each consisting of two layers of amphiphiles, or lipids. In general the head groups of the lipids like to be surrounded by water. To bring the membranes sufficiently close together so that fusion can occur, the interposed water must be removed, at least in some region between the membranes. To remove this water takes energy which, presumably, is provided *in vivo*, by fusion proteins. Due to the loss of water between membranes, the free energy per unit area, or surface tension, of the membranes increases. One possible response of the system to this increase is to undergo fusion because this process, by making holes in the membranes, decreases the membrane area and thus the free energy. The canonical way this has been thought to occur (see (1) and references therein) was first suggested by Kozlov and Markin (4), and is illustrated in Fig. 1.

In panel a) we see two bilayers under zero tension, They are composed of amphiphiles, block copolymers in this case, which contain a fraction $f = 0.35$ of the hydrophilic com-

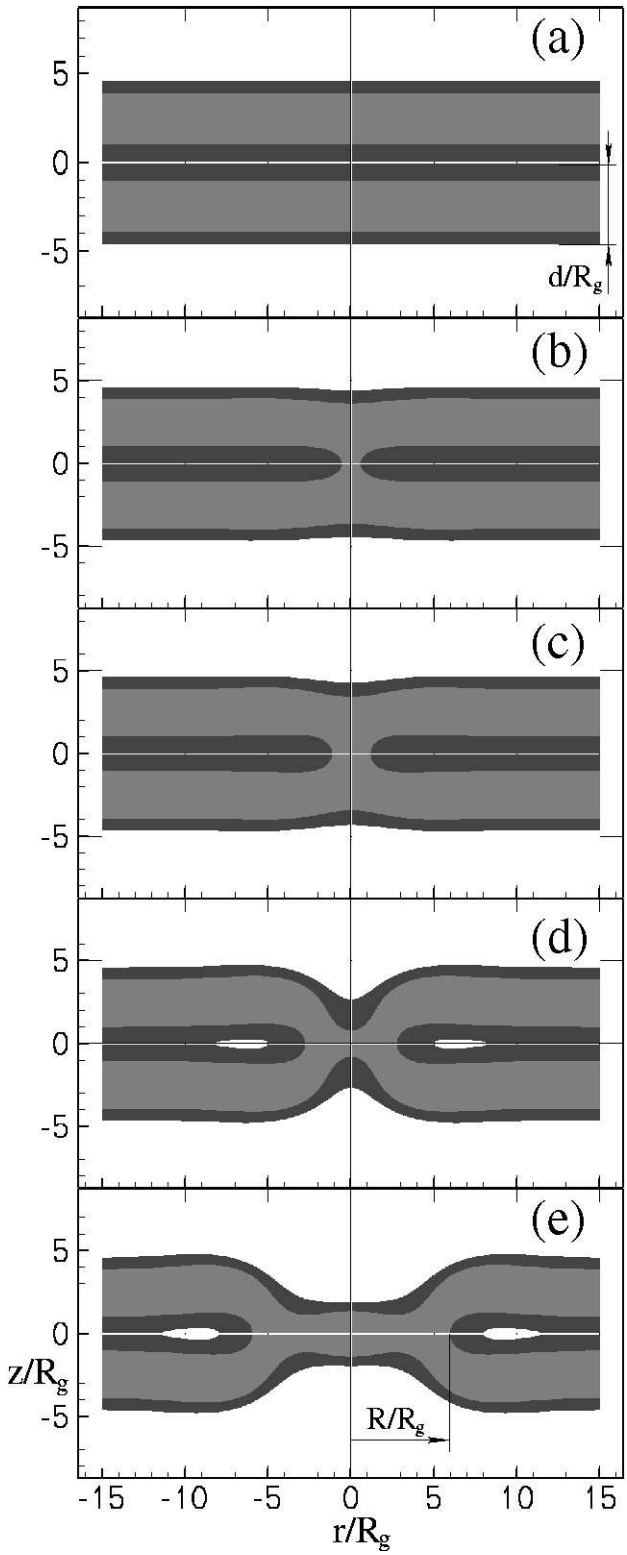

FIG. 1 Density profiles of structures from bilayers in apposition, (a), passing through a metastable stalk (c), to a hemifusion diaphragm (e). Figures are shown in the r, z plane of cylindrical coordinates.

ponent. Only the majority component is shown at each point: solvent segments are white, hydrophilic and hydrophobic segments of the amphiphile are dark and light correspondingly. Distances are measured in units of the polymer radius of gyration, R_g, which is the same for both the amphiphiles and for the homopolymer solvent. In (b), tails of some amphiphiles in a small region have turned over, attempting to form an axially-symmetric "stalk". This panel shows the transition state to the formation of the stalk, and panel (c) shows the metastable stalk itself. After the stalk forms, the layers pinch down and expand, pass through a second intermediate, shown in (d), and arrive at a hemifusion diaphragm, (e). A hole then forms in this diaphragm, which completes formation of the fusion pore. Note that the conundrum is not addressed explicitly by this scenario. However one can observe that this mechanism requires a hole to form only in the *one* hemifusion diaphragm rather than in the *two* bilayers separately.

Sometime ago, we decided to watch, via Monte Carlo simulation, the fusion process unfold in a system of bilayers formed by block copolymers in homopolymer solvent (5). Our choice of this system of non-biological amphiphiles was motivated by the fact that we had experience in simulating such amphiphilic copolymers, and our belief, as physicists, that the fusion process was probably universal. The time and energy scales would vary from system to system, but not the pathway of the process itself. Furthermore vesicles of block copolymer form a novel family which is currently being investigated for its technological possibilities (6). Details of the simulation can be found in (5) and (7), but the results can be summarized as follows. Upon putting the bilayers under tension and in close apposition, we did see the formation of an axially symmetric stalk. We expected to see the stalk expand radially, but it did not. Instead, it expanded asymmetrically, forming a worm-like structure which moved about. We also observed that once the stalk formed, the rate of hole formation in either bilayer rose dramatically. This is shown in Fig. 2 where, in the lower panel, the rate of hole formation in each bilayer, one in black, the other in gray, is seen to rise dramatically after about 200 time steps when we know, independently, that a stalk had formed. The rate of hole formation in a *single* bilayer is shown in the upper panel for comparison.

Furthermore, we could determine that the stalk and the newly-created holes were correlated; that is, for the most part, the holes formed very near to the stalk. Once a hole formed in one bilayer next to the stalk, the latter, which we had observed to be quite mobile, proceeded to walk around the hole, thereby forming something like a hemifusion diaphragm.

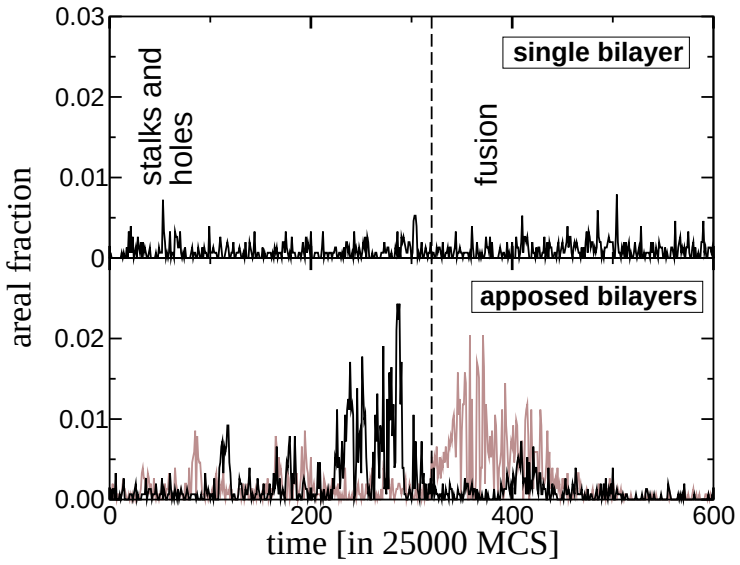

FIG. 2 Area of holes vs. time in the system of two apposed bilayers, bottom, and in an isolated bilayer, top.

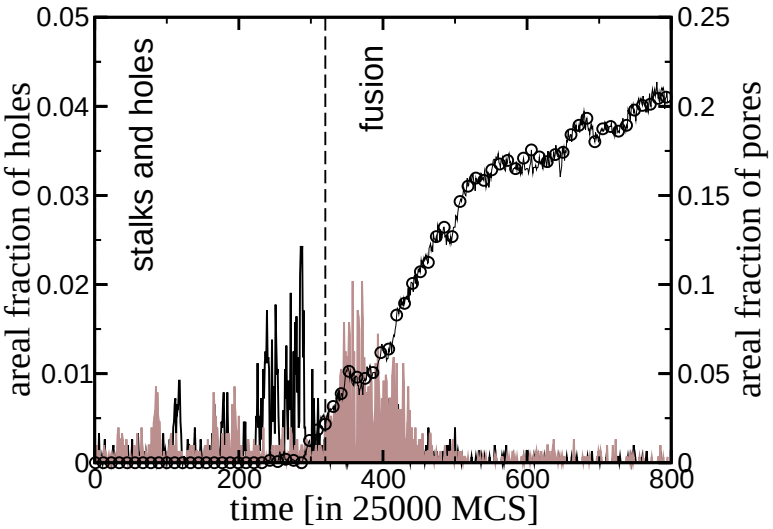

FIG. 3 Area of pore (symbols) and of holes (lines) for one simulation run, the same as shown in Fig. 2. Note the different scale for pore and hole areas.

Once a second hole pierced this diaphragm, the fusion pore was complete. In a slightly different scenario, we saw a hole form in one bilayer, and the stalk begin to walk around it. Before it completely surrounded the first hole, a second one appeared in the other bilayer near the stalk. The stalk then had to corral the two holes, walking around them both, to complete the fusion pore.

It is clear that in the mechanism we saw, the formation of a fusion pore is closely correlated, in space and time, with hole creation. This correlation is seen in Fig. 3; the formation

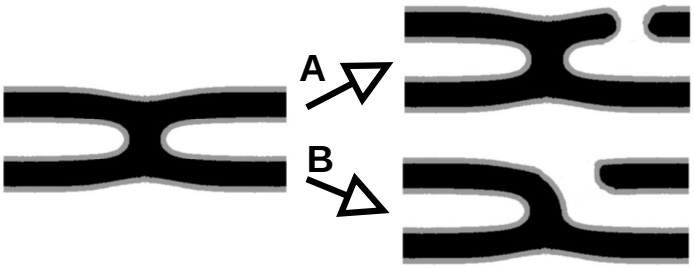

FIG. 4 A schematic diagram which makes plausible that the line tension of a hole which forms near a stalk, as in path B, is less than if it forms far from a stalk, as in A.

of pores closely follows in time the onset of hole formation triggered somehow by stalk formation . There is a clear experimental differentiation between this new mechanism, and the standard hemifusion mechanism discussed earlier. This consequence, transient leakage, can be understood from Fig. 4 which shows that for a certain period of time, there is a hole from at least one of the vesicles to the outside during the fusion process. How much leakage there is depends on what molecule one is observing, as each will have its own characteristic time to diffuse through the hole. If this time is significantly greater than the time for the stalk to surround the hole and seal it up, there will be little, if any, observable leakage. However if the time to diffuse to the hole is much less than the sealing time, there will be. Just such leakage, correlated with fusion in the manner of Fig. 3, was recently observed in an elegant experiment (8).

How do we understand the behavior we have seen in our model, and by others (9) in a more simplified model? We had an idea as to what was going on, and to verify it, we embarked on a series of self-consistent field calculations (2; 10) on the same system as had been simulated, from which various free energies could be calculated explicitly.

First of all, we can understand the wandering of the elongated stalk by calculating its free energy per unit length, that is, its line tension. Not surprisingly, we find that it varies with the architecture of the block copolymer, which is described by the fraction, f, of hydrophilic monomers in the diblock. For values of f in the vicinity of 0.5, the system makes bilayers. As f is reduced, the majority hydrophobic component wants more space to explore more configurations. Eventually this will cause the system to undergo a phase transition to an inverted hexagonal phase consisting of cylinders with the minority hydrophilic parts confined to the smaller region inside the cylinders and the majority hydrophobic part filling the larger region outside. These cylinders are separated by structures that look very much like stalks

that are stretched out parallel to them. The phase transition occurs at a value of $f \approx 0.31$. The line tension, λ_{linear}, of a linear stalk which we have calculated is shown in Fig. 5. It is given there in units of $\gamma_0 d$, where γ_0 is the surface free energy per unit area between coexisting regions of hydrophobic and hydrophilic molecules, and d is the thickness of a bilayer. We note that we have found (10) successful fusion can occur over an interval of architectures from about $0.31 < f < 0.35$. In this interval the line tension of the linear stalk does not exceed 0.06, and becomes extremely small as f approaches the transition to the inverted hexagonal phase. This is, of course, no coincidence, and tells us that this fusion mechanism becomes more favorable as the architecture of the lipids in the system become more like those of "hexagonal formers". The upshot is that, as this transition is approached, it costs very little energy for the stalk to walk around as we had observed.

Secondly it seems intuitively clear from Fig. 4 that the line tension of a hole when it forms near a stalk is less than that of a hole formed far from a stalk. To verify this intuition, we have calculated the line tension, λ_{bare}, of a "bare" hole, that is, the line tension associated with the formation of a linear edge of a bilayer membrane. We have also calculated the line tension, $\lambda_{dressed}$, of a hole in one bilayer which is next to a linear stalk; that is, the line tension associated with the linear defect at which two bilayers join to form one bilayer, as in Fig. 4. These quantities are also shown in Fig 5. We note that the line tension of a hole formed next to a stalk is indeed lower than that of a bare hole. Depending upon the architecture, the reduction in line tension is at least a factor of two. Further, the dependence on architecture is such that the reduction is greater the smaller the value of f, that is, the more hexagonal-forming the amphiphiles are.

We can now understand the conundrum posed by fusion. Let us consider the simple approximate phenomenolgical expression for the free energy of a hole of radius R and line tension λ_{bare} in a membrane of surface tension γ, which generally is much smaller than γ_0,

$$F_{bare}(R) = 2\pi R\lambda_{bare} - \pi R^2\gamma. \tag{1}$$

In order for the hole to expand, and become long-lived, it must pass the barrier of free energy

$$F^*_{bare}(R^*_{bare}) = \pi\lambda^2_{bare}/\gamma \tag{2}$$

which occurs at $R^*_{bare} = \lambda_{bare}/\gamma$. Stability of a membrane is guaranteed by the fact that the

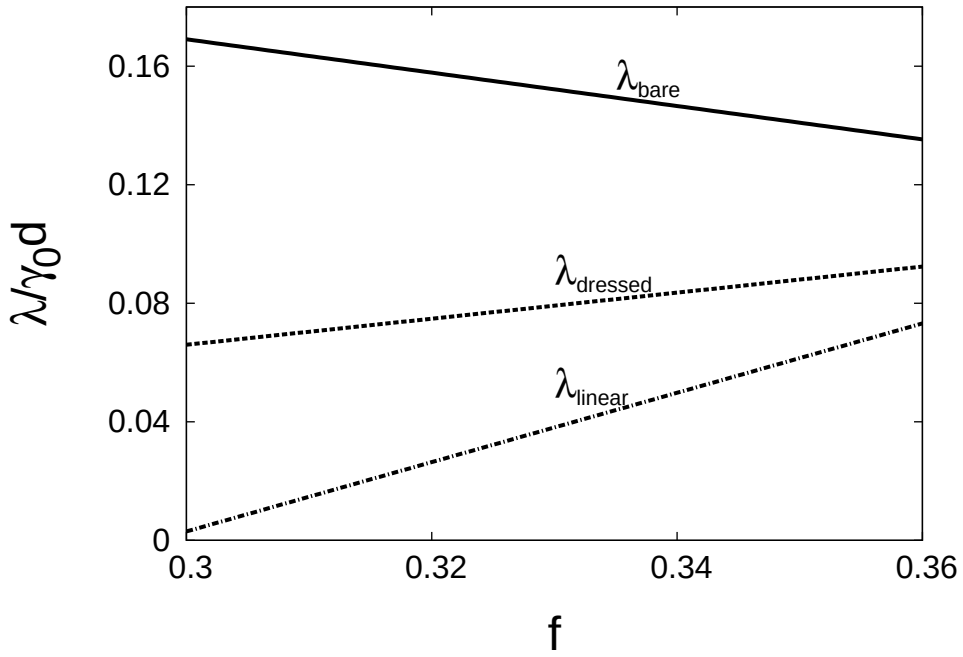

FIG. 5 Line tensions of a linear stalk, λ_{linear}, of a bare hole in a membrane, λ_{bare}, and of a hole which forms next to a stalk, $\lambda_{dressed}$. All line tensions are in units of $\gamma_0 d$, the bare hydrophilic-hydrophobic surface tension multiplied by the bilayer thickness.

rate of hole formation, which is proportional to the Boltzmann factor

$$P_{bare} \sim \exp\left\{-\frac{\pi\lambda_{bare}^2}{\gamma k_B T}\right\} \tag{3}$$

is small. For example, for the system simulated, $\lambda_{bare}/\gamma_0 d \approx 0.15$, $\gamma_0 d^2/kT \approx 60$, and $\gamma/\gamma_0 \approx 0.75$, from which we obtain $P_{bare} \approx 4 \times 10^{-3}$.

Now however, let the hole form next to a stalk which almost completely surrounds it, reducing its line tension from λ_{bare} to $\lambda_{dressed}$. The barrier to stable hole formation is now only

$$F^*_{dressed}(R^*_{dressed}) = \pi\lambda^2_{dressed}/\gamma, \tag{4}$$

and the relative rate of formation of a long-lived hole compared to what it was without the stalk is

$$\frac{P_{dressed}}{P_{bare}} = \exp\left\{\frac{\pi\lambda_{bare}^2}{\gamma k_B T}\left(1 - \frac{\lambda^2_{dressed}}{\lambda^2_{bare}}\right)\right\} \tag{5}$$

With $(\lambda_{dressed}/\lambda_{bare})^2$ much less than unity, the rate of hole formation increases almost by the large factor $P^{-1}_{bare} >> 1$. In particular, taking the reduction in line tension to be a modest

$\lambda_{dressed}/\lambda_{bare} = 1/2$, and $P_{bare} = 4 \times 10^{-3}$, we obtain

$$\frac{P_{dressed}}{P_{bare}} \approx 60, \tag{6}$$

so that the rate of hole formation rises by over an order of magnitude, in qualitative agreement with the results from simulation.

This increase in the rate of hole formation is predicted to be far more dramatic for a biological membrane. In such a system, $\lambda_{bare} \approx 2.6 \times 10^{-6}$erg/cm (11; 12). To obtain an order of magnitude for the local tension in a biological membrane undergoing fusion, we consider a scenario (13) in which six hemagglutinin molecules release their energy of conformational change, about 60 kT per molecule, within a circular area of radius 4 nm. This yields an estimate of 30 erg/cm^2. For illustration we shall take a γ of 10 erg/cm^2 which is not unreasonable. We then find that $P_{bare} \approx 3 \times 10^{-22}$. A single membrane is stable indeed! The relative rate of formation of a long-lived hole in the presence of a stalk compared to the rate without it becomes, for the same modest reduction in line tension by a factor of two,

$$\frac{P_{dressed}}{P_{bare}} \sim 1 \times 10^{16} \; ! \tag{7}$$

Of course the exact expression for the free energy of the intermediate in which the hole is only partially surrounded by the stalk will differ in detail from the simple expression given in Eq 4. Nevertheless, the key determinant in this free energy, the quadratic dependence upon the line tension, will remain. It is the fact that *fusion is a thermally excited event for which the rate is proportional to the exponential of the square of the line tension* which explains the conundrum of fusion. As long as the line tension is "normal", a membrane is *extremely* stable to thermally excited holes. But because the membrane *is* so stable, and because the line tension appears *squared* in the exponent, any mechanism, such as the one we have proposed, which even slightly affects the line tension will greatly affect the rate of hole formation, and therefore the rate of fusion. Thus does fusion become possible!

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