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Fusion of biological membranes

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Abstract. The process of membrane fusion has been examined by Monte Carlo simulation, and is found to be very different than the conventional picture. The differences in mechanism lead to several predictions, in particular that fusion is accompanied by transient leakage. This prediction has recently been verified. Self-consistent field theory is applied to examine the free energy barriers in the different scenarios.

Keywords. Fusion; membranes.

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1. Introduction

Fusion of membranes is involved in basic biological processes but its mechanism remains poorly understood. That different proteins trigger different fusion events obscures the possibility of a stage common to them, one which depends only on the properties of membrane bilayers themselves. The existence of such a universality is made plausible by the realization that, in all fusion processes, membranes must merge, and the properties of the membranes will most likely determine the nature of this stage, the fusion intermediate [1]. Time and length scales of fusion events are of the order of microseconds [2] and nanometers, [3,4] so that direct measurements of intermediate structure have not been possible. Limited theoretical treatment of this problem, based on membrane elasticity theory, has focused on the so-called *stalk* mechanism [5–7]. In this scenario, the leaves of the two *cis* membranes, closest to one another, fuse, as shown in figure 1A and expand slightly to form a *metastable*, axially-symmetric stalk, figure 1B. Further radial expansion of the stalk, figure 1C, causes it to thin creating a single bilayer diaphragm from the *trans* leaves, figure 1D. Hole formation in this bilayer completes the fusion pore. Due to the lack of direct experimental confirmation, this mechanism, although plausible, remains hypothetical.

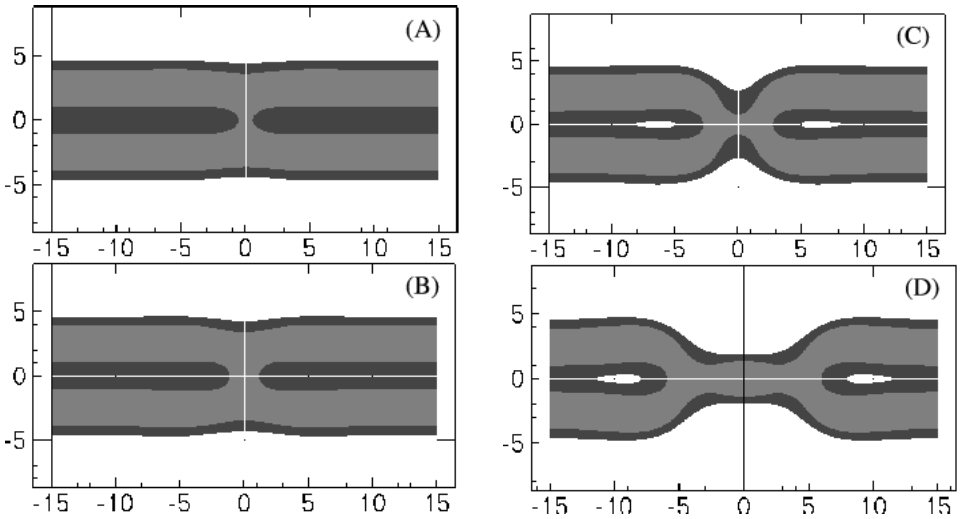

Figure 1. Density profiles of the stalk-like structures shown in the r, z plane of cylindrical coordinates as obtained in self-consistent field theory. The amphiphiles contain a fraction $f = 0.35$ of the hydrophilic component. The bilayers are under zero tension. (**A**) Unstable transition state to the formation of the initial stalk. (**B**) The metastable stalk itself. (**C**) Unstable transition state between the metastable stalk and the hemifusion diaphragm. (**D**) A small hemifusion diaphragm.

To obtain a direct view of the fusion process, we have carried out extensive simulations of two bilayers, composed of block copolymers, which are immersed in a solvent which favors one of the blocks. As in the biological case, the membranes are placed under tension. This is essential as fusion is one possible response of the system to the increase in free energy per unit area caused by bringing the membranes close to one another, eliminating the solvent. What was seen is a fusion process very different from the standard mechanism above. In the next section, we briefly describe the model, the fusion process observed, and our understanding of it. In the final section we describe ongoing analytic calculations which permit the estimate of the fusion barriers both in the conventional picture, and in the scenario we have observed.

2. The fusion process

Our model describes membranes formed by single-chain amphiphiles, like block copolymers which exhibit the same phases as do biological lipids, and also form vesicles [8]. It has the advantage that it has been well studied, permits detailed analysis of molecular configurations, and is well suited to processes occurring on the small time and length scales characteristic of fusion. The amphiphiles are treated using the bond fluctuation model [9] in which each molecular segment occupies a

cube of a three-dimensional lattice. The eight lattice sites defining the cube cannot be occupied by another segment centered on neighboring sites. Segments along an amphiphile are connected by one of 108 bond vectors of lengths $2, \sqrt{3}, \sqrt{5}, 3$ or $\sqrt{10}$, measured in units of the lattice spacing a_0. Mapping this model onto lipids in solution [10], we find the lattice spacing to correspond to approximately 1Å. The amphiphilic molecules consist of $N = 32$ segments, of which 11 are hydrophilic and 21 are hydrophobic. This particular choice of the fraction of hydrophilic segments, $f = 0.34$, results in the diblock system being relatively close to coexistence of the lamellar and inverted hexagonal phases. The solvent is represented by a homopolymer, chains consisting of 32 hydrophilic segments. Like segments attract each other and unlike segments repel each other via a square well potential which comprises the nearest 54 lattice sites. Each contact changes the energy by an amount $\epsilon = 0.177 k_{\mathrm{B}}T$. The particular choice of the interaction parameter ϵ guarantees that the interfacial width between hydrophilic and hydrophobic segments is not too small to be comparable to the lattice spacing, and at the same time results in well-defined bilayers. It is for this same reason that the solvent is chosen to be homopolymers rather than monomers.

The simulation cell is $L \times L$ in the x, y directions and of length D in the z direction, with $L = 156a_0$ and $D = 96a_0$. Periodic boundary conditions are utilized in all three directions. The monomer density of the system is $\rho = 1/(16a_0^3)$, corresponding to 146,016 segments within the volume, or 2376 amphiphiles and 2187 homopolymers. Further details of the simulation can be found in [11].

What we see is the following. The initial event in the fusion process is indeed the formation of a stalk, as in the conventional scenario. However we do not see the stalk expand radially, instead it expands axially, forming a worm-like object. Once the stalk appears, the rate of occurrence of simple holes, formed in either of the two bilayers, increases markedly, as shown in figure 2. It is compared there with the rate of hole formation in a single bilayer under the same tension. In the absence of a stalk, the rate of hole formation is small because the energy of hole formation in a bilayer is large due to the line tension of the hole. However it is not difficult to see

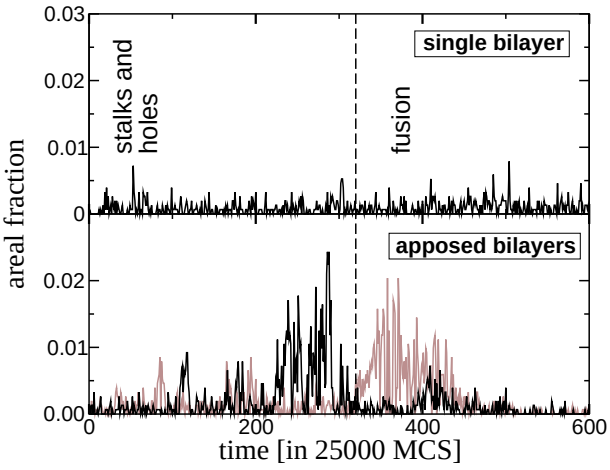

Figure 2. Area of holes vs. time in the system of two apposed bilayers (gray for one bilayer and black for the other on the bottom panel) and in an isolated bilayer (top panel).

that if a hole forms next to a stalk, then the line tension of that part of the hole next to the stalk is reduced. Given this, what occurs next is understandable; the system reduces its free energy by having the worm-like stalk proceed to surround the hole completely. Once it has done so, there is only a single bilayer which remains within the enclosure formed by the worm-like stalk. A second hole appears in this bilayer which completes formation of the fusion pore. It is possible, of course, that the second hole appears in the other bilayer before the stalk completely surrounds the first hole. When this occurs, the worm-like stalk simply aligns and surrounds both holes completing the fusion pore. At the same time our work was carried out [12], a similar mechanism was observed by Noguchi and Takasu [13] in a very different and simplified model system. It has since been observed in molecular dynamics simulations [14,15].

3. Experimental consequences

There are several consequences of this new fusion mechanism, of which we list three.

First, due to the presence of the stalk, there is mixing of lipids in the *cis* leaf of one bilayer with the lipids of the *cis* leaf of the other bilayer. This mixing has been observed [16–18]. Second, due to the formation of holes in each bilayer near a stalk, our scenario allows for the mixing of lipids in the *cis* and *trans* leaves of the same bilayer, and also of lipids in the *cis* leaf of one bilayer with those in the *trans* leaf of the other. The standard hemifusion mechanism does not permit either process. Third, our mechanism allows for *transient leakage* during fusion, something the standard mechanism does not. The amount of leakage depends on the size of the transient holes formed in the bilayer, the time between the formation of the initial stalk and the completion of the fusion pore, and the diffusion constant of the molecules which leak. This constant introduces another time scale whose magnitude, relative to that of fusion pore formation, determines whether the fusion process is *observed* to be leaky or tight. It is clear that within our mechanism, leakage via transient holes, and fusion via pore formation, are correlated in space and time. This is shown in figure 3 which presents, as a function of time, the area

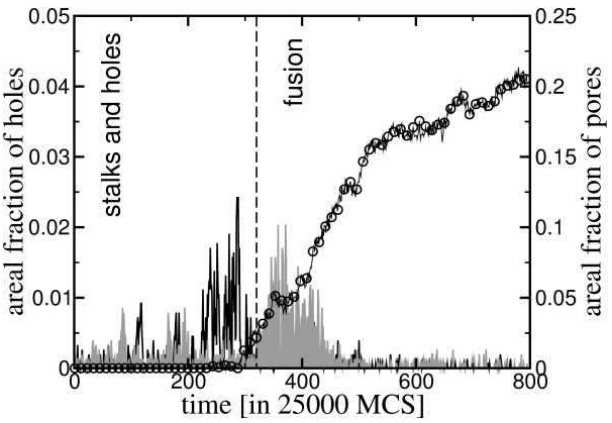

Figure 3. Area of pore (symbols) and of holes (lines) vs. time for one simulation run (identical to figure 2). Note the different scale for pore and hole areas.

of holes and that of fusion pores from one of the simulation runs. One sees in this figure that the rate at which holes appear, and therefore the rate at which leakage should occur, *increases significantly before, and is correlated with, the formation of fusion pores.* An experiment to determine whether fusion is correlated in space and time with transient leakage has been carried out recently [19]. It is observed that leakage is, in fact, correlated spatially and temporally with the process of fusion. Indeed, results of the experiment comparing the time sequence of the electrical conductance arising from leakage with that arising from fusion, shown in figure 5 of ref. [19] displays a remarkable similarity to our results comparing the time sequence of the areal fraction taken up by holes with that taken up by fusion pores (figure 3).

4. Analytic calculations

The Monte Carlo simulations show very clearly the nature of the fusion process. However they are not well-suited for calculating free energy barriers of the intermediates, information needed for fusion rates, and for the dependence of these rates on architecture. Further it would be interesting to compare the barriers in the new mechanism to those in the standard mechanism, again as a function of architecture.

In order to do so, we have begun a series of calculations utilizing almost the same model as employed in the simulations, but in which we solve it analytically within the self-consistent field approximation. A nice review of this method is provided by Schmid [20]. We have first applied it to the conventional fusion scenario in which the stalk expands radially to form a bilayer diaphragm which is then pierced by a hole to form the fusion pore. We have obtained [21] solutions of the self-consistent field equations in real space which describe two bilayers, under a specified tension, connected by an axially-symmetric stalk-like structure of a specified radius, R. The free energy of this constrained system is then obtained as a function of the radius [22,23]. Results for the free energy of the stalk-like structure are shown in figure 4a for bilayers of zero tension. The amphiphiles comprising the bilayer are characterized by the volume fraction, f, of the amphiphile which is hydrophilic. This parameter is directly correlated with the spontaneous curvature, with $f < 0.5$ corresponding to negative spontaneous curvatures. One sees that for sufficiently small values of f there is a metastable stalk. If f is too large, however, the stalk is no longer metastable. As fusion is a thermally activated process, the disappearance of this intermediate would make the time scale for fusion extremely long. As f decreases, the free energy of the metastable stalk decreases which favors fusion. However one also sees that if f is too small the stalk free energy becomes negative relative to that of the unconnected bilayers, which signals an instability either to a phase of stalks [24] or to an inverted hexagonal phase. Once again there would be no fusion. Thus the span of different architectures over which fusion can occur is quite limited. This may be one of the reasons that lipid composition of membranes is tightly regulated.

The free energy of stalk-like structures for a given architecture, $f = 0.35$, for different tensions is shown in figure 4b. Tensions there, are in units of that of the bare hydrophilic, hydrophobic interface, γ_{int}. One sees that the low free energy barrier to making the initial stalk of small radius is almost unaffected by tension.

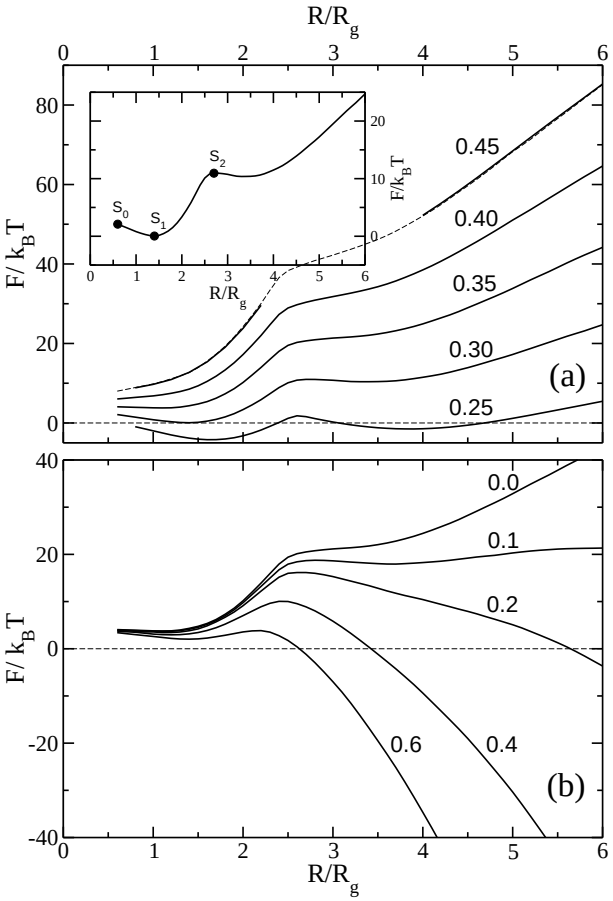

Figure 4. (**a**) The free energy, F, of the stalk-like structure connecting bilayers of fixed tension, zero, is shown for several *different* values of the amphiphile's hydrophilic fraction f. The zero of free energy is that of similar bilayers without the stalk connection. In the inset we identify the metastable stalk, S_1, the transition state, S_0, between the system with no stalk at all and with this metastable stalk, and the transition state, S_2, between the metastable stalk and a hemifusion diaphragm. The architectural parameter is $f = 0.30$ for the inset. No stable stalk solutions were found for $f = 0.45$ in the region shown with dashed lines. (**b**) The free energy of the expanding stalk-like structure connecting bilayers of amphiphiles with fixed architectural parameter $f = 0.35$ is shown for several different bilayer tensions. These tensions, $\gamma/\gamma_{\rm int}$, are shown next to each curve.

However the barrier to expansion of the structure into a bilayer diaphragm depends very much upon it, and decreases with increasing tension. As a corollary, one sees that as the tension decreases to zero, the energy to expand this structure increases due to the line tension, and the rate of successful fusion would diminish.

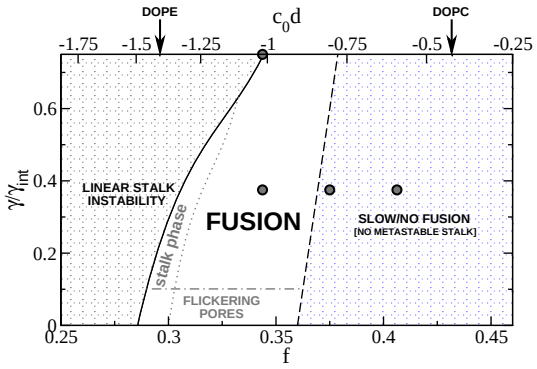

Figure 5. A 'phase diagram' of the hemifusion process in the hydrophilic fraction-tension, (f, γ), plane. Circles show points at which previous, independent, simulations were performed by us. Successful fusion can occur within the unshaded region. As the tension, γ, decreases to zero, the barrier to expansion of the pore increases without limit as does the time for fusion. As the right-hand boundary is approached, the stalk loses its metastability causing fusion to be extremely slow. As the left-hand boundary is approached, the boundaries to fusion are reduced, as is the time for fusion, but the process is eventually pre-empted due to the stability of either the radial stalks forming the stalk phase, or the linear stalks forming the inverted hexagonal phase.

To determine at what radius a hole would pierce the bilayer diaphragm, we calculated the free energy of fusion pores with a specified radius. We assume that a hole is formed in the diaphragm at the radius at which the free energy of an intact and a pierced diaphragm are the same. The result is that, except for very small tensions, the diaphragm converts to a fusion pore at a radius larger than that of the barrier to expansion. As a consequence *the barrier to stalk expansion to a diaphragm remains the maximum barrier for the entire process* in this conventional scenario. Only at very low tensions is this barrier exceeded by that corresponding to the expansion of the fusion pore itself.

A phase diagram showing the region where successful fusion can be expected to occur as a function of architecture, f, and tension, γ, is shown in figure 5.

We are currently carrying out a similar calculation for the newer fusion mechanism seen in the simulations in which the process occurs via a very different route. In spite of the differences, we note that both mechanisms begin with the formation of a stalk. As the phase diagram shown in figure 5 is determined only by the properties of stalk-like structures, we do not expect it to be affected greatly. However the intermediates to fusion and their associated free energy barriers will certainly be different. We shall report on the results of this calculation elsewhere.

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