Published in final edited form as:

Phys Rev E Stat Nonlin Soft Matter Phys. 2013 December ; 88(6): 062701.

Teardrop Shapes Minimize Bending Energy of Fusion Pores Connecting Planar Bilayers

Rolf J. Ryham*,

Fordham University, Department of Mathematics, 441 E. Fordham Road, Bronx, NY 10458

Mark A. Ward†, and

Fordham University, Department of Mathematics, 441 E. Fordham Road, Bronx, NY 10458

Fredric S. Cohen‡

Rush University Medical Center, Department of Molecular Biophysics and Physiology, 1750 W. Harrison St., Chicago, IL 60612

Abstract

A numerical gradient flow procedure was devised to characterize minimal energy shapes of fusion pores connecting two parallel planar bilayer membranes. Pore energy, composed of splay, tilt, and stretching, was obtained by modeling each bilayer as two monolayers and treating each monolayer of a bilayer membrane as a freely deformable surface described with a mean lipid orientation field. Voids between the two monolayers were prevented by a steric penalty formulation. Pore shapes were assumed to possess both axial and reflectional symmetry. For fixed pore radius and bilayer separation, the gradient flow procedure was applied to initially toroidal pore shapes. Using initially elliptical pore shapes yielded the same final shape. The resulting minimal pore shapes and energies were analyzed as a function of pore dimension and lipid composition. Previous studies either assumed or confined pore shapes, thereby tacitly supplying an unspecified amount of energy to maintain shape. The shapes derived in the present study were outputs of calculations and an externally provided energy was not supplied. Our procedure therefore yielded energy minima significantly lower than those reported in prior studies. The membrane of minimal energy pores bowed outward near the pore lumen, yielding a pore length that exceeded the distance between the two fusing membranes.

I. INTRODUCTION

Membrane fusion involves the formation and then expansion of a fusion pore that connects two formerly separate membranes. The fusion pore is a circular, hourglass shaped membrane bilayer whose inner rim forms a channel between the respective interior membrane compartments. From a theoretical point of view, the energies required for pore formation and pore enlargement depend on the precise shape of the fusion pore, and consequently the pore shape that minimizes energy must be sought. An assumed pore shape will generally not be one of minimal energy, and consequently external energy must be

*rryham@fordham.edu.

†mward1@fordham.edu

‡Fredric_Cohen@rush.edu

provided to maintain the artificially imposed shape. Methods to calculate shapes of fusion pores of minimal energy would therefore greatly aid in understanding the mechanisms of fusion. A suitable method must be based on physical principles and should have few adjustable parameters. But a suitable method has yet to be developed. In this study we develop a method that does meet these criteria.

In past studies, the pore has often been assumed, for analytical convenience, to be toroidal [5, 6]. However, this assumption leads to energies required for pore enlargement to be inordinately large, and perhaps, experimentally unrealizable. Improvements have been made by making the pore elliptical [21] or piecewise catenoidal [25, 30]. Relatively recently, a course-grained molecular dynamics calculation was used to obtain a minimum energy pore shape and an explicit functional form was assumed that allowed continuum membrane mechanics to obtain the same energy-minimized shape [40]. This study showed that a minimum energy pore bows out somewhat near the lumen to increase pore length, and the membranes relax to smaller separations as the distance from the lumen increases. To date, all continuum approaches in the study of fusion pores have used a small, finite number of degrees of freedom to construct pore shapes. Pore shapes, however, have infinitely many degrees of freedom and the energy of a pore is extremely sensitive to the precise shape. This implies that pore shapes derived from any calculation—from all atom, to course-grained, and through continuum—must carefully evaluate whether the derived shape is sensitive to any underlying parameter, such as a force field which is inferred from macroscopic (rather than molecular) experimental data. In the present study, we devise a procedure, without assuming any functional forms, to minimize the energy of a fusion pore within the context of continuum membrane mechanics. The procedure is general, allowing minimal energy pore shapes to be obtained for any continuum Hamiltonian. For example, the role of proteins in controlling pore shape can be incorporated once experimentalists measure (for example, by Atomic Force Microscopy) the forces exerted by the proteins.

There is a strong connection between the continuum energies used to describe liquid crystals and the energies used to describe bilayers [27]. A lipid bilayer is a lyotropic phase that possesses both orientational and positional structure [8, 34], and bilayer elastic energy is determined both by the shape of its monolayer neutral surfaces and the mean orientation of the lipid molecules [19, 22, 24]. Monolayer bending energy is analogous to the splay energy from the theory of liquid crystals [19, 35]. Drawing on this connection, we describe a monolayer by its neutral surface and a director field for the mean lipid orientation. We let the bending energy of the bilayer be the sum of the two monolayer energies. In addition to the elastic deformation of the neutral surfaces, surface stretching and tilt deformations of individual lipid molecules are allowed. The splay energy competes with the tilt energy which aligns the lipid tails to the neutral surface normal. The monolayers are coupled by a steric interaction penalizing any interstices (voids) between opposing hydrocarbon tails. The description of a bilayer through its neutral surfaces and directors has the advantage that arbitrary configurations of two opposing monolayers can be calculated; additional interactions between monolayers can also be readily introduced. The resulting minimized energy yields a pore with membranes that bow outward near the lumen, as was reported in preliminary findings [29].

In the past two decades, significant efforts have been devoted by mathematicians and biophysicists in modeling the dynamics and minimal shapes of bilayers. Mathematically, this is challenging because great care must be taken in discretizing long range deformations, the higher order membrane surface energies, and possible changes in topology. Modern mathematical methods, such as the implicit interfaces methods [7, 10–12] and direct boundary integral and immersed boundary approaches [3, 9, 28, 32], calculate the elastic energy of a bilayer in terms of the principal curvatures of the neutral surfaces, assuming that lipid orientation is normal to the neutral surface. Mathematical studies minimizing the Helfrich mean curvature squared energy of surfaces have been presented [31, 38, 39]. These approaches describe the bilayer by one mathematical surface; they give an accurate determination of membrane energy when curvatures are small compared to inverse membrane thickness, as is the case for relatively large vesicles and liposomes. Fusion pores, however, are highly curved and deform on scales comparable to the bilayer thickness. Here, the bilayer must be modeled as two monolayers: the calculation of fusion pore bilayer energy must account for differences in shape assumed by the two opposing monolayers.

A stalk is a fusion intermediate that leads to hemifusion, [22]. In stalks and in the hemifused state, interstices would form in the bilayer core if only membrane curvature were considered. But the formation of interstices is energetically unfavorable because of the large surface tension of the hydrocarbon-water interface. Consequently, monolayers undergo deformations that create sharp corners, thereby filling in the interstices that would have been created by monolayer curvature alone [23, 24]. Unrelated to fusion, bilayers can exist in rippled laminar phases that contain sharp kinks [26]. Thus, in stalk formation, hemifusion, and rippled phases, the mean curvature square energy gives an unrealistic evaluation of membrane elastic energy. Instead, defining the elastic energy in terms of a lipid director that is different than the surface normal alleviates this problem [19, 23], giving an accurate determination of membrane energy. Only a few mathematical treatments have allowed the director to deviate from the perpendicular direction of the surface when calculating elastic energy [20, 33]. Investigators have devised finite element models for almost flat membranes driven by competition between in-plane orientational order and bending energies [1, 16, 37]. Motivated by recent studies in surfactant monolayers, [2] have performed a finite element analysis of a nonlinear surface-director model for membranes where the lipid director induces a spatially varying spontaneous curvature. In the biophysical literature, the deviation between the lipid director and surface normal has been allowed by introducing the deformation of tilt [18, 24]. But in the biophysical literature, elastic energies have been obtained predominantly for specific geometries [5, 6, 21]. Specifying a geometry implicitly assumes that an undefined, external force—which varies with conditions—is applied to maintain the shape of the bilayer. Models with undefined variable energies can be misleading, as pointed out in other contexts [13, 14, 36].

In the next Section we define the bilayer elastic energy and energy of pore formation. A discretization and minimization scheme is presented in Section 3. In Section 4, we analyze the shapes and energies of fusion pore bilayers as a function of pore dimension and lipid composition.

II. ELASTIC ENERGY FUNCTIONAL

The elastic energy of the fusion pore is calculated by the integral

$$E_B = \int_{\Sigma} \frac{k_B}{2} (\nabla \cdot \mathbf{d} - k_0)^2 + \frac{k_T}{2} |\mathbf{d} \times \mathbf{n}|^2 dS + \int_{\Sigma'} \frac{k_B}{2} (\nabla \cdot \mathbf{d}' - k'_0)^2 + \frac{k_T}{2} |\mathbf{d}' \times \mathbf{n}'|^2 dS'. \quad (1)$$

Integrations are performed along the neutral surfaces of each monolayer: the non-primed mathematical surface Σ is created from the proximal monolayers and the primed surface Σ' is derived from the distal monolayers (Figure 1). In the case of cell-cell fusion, the proximal and distal monolayers are the outer and inner monolayers, respectively. A lipid bilayer membrane is represented by two surfaces. It is known that modeling a bilayer by a single surface incorrectly yields fusion pore energies that are lower, sometimes significantly, than the true energy of the bilayer [21]. Within each surface, the vector field $\mathbf{d}$ (resp. $\mathbf{d}'$) is the mean orientation of lipid molecules: $|\mathbf{d}| = |\mathbf{d}'| = 1$. The lipids in opposing bilayers point in opposite directions but are not necessarily anti-parallel. The first term in the two integrands is the splay energy for the bending modulus k_B and spontaneous curvatures k_0 and k'_0 . The value of the spontaneous curvature depends on the lipid composition and may be different in the two monolayers. The second term in the two integrands is the tilt energy for the tilt modulus k_T . The tilt energy density vanishes when the lipid molecules are parallel to the neutral surface normal $\mathbf{n}$ (resp. $\mathbf{n}'$).

It is useful to compare the elastic energy (1) in our formulation and the model used in [2]. Both models involve a penalty term—referred to here as tilt—that provides the energy resulting from the deviation of the director field from the surface normal. Here, the leading order terms $(\nabla \cdot \mathbf{d} - k_0)^2$ and $(\nabla \cdot \mathbf{d}' - k'_0)^2$ permit the formation of corners, such as the Y-shaped configurations formed at the rim of a hemifusion diaphragm [22]. In [2], the leading order terms involve the surface curvatures $(\nabla \cdot \mathbf{n} - \delta \nabla \cdot \mathbf{d})^2$ and the full surface gradient $|\nabla_{\Sigma} \mathbf{d}|^2$. Including the surface gradient energy has the advantage that one is able to derive discrete *a priori* estimates and prove the existence of minimal configurations. Here, the term $\nabla \cdot \mathbf{d}$ gives the splay density while in [2], the term $\nabla \cdot \mathbf{d}$ plays the role of a spatially varying spontaneous curvature.

We assume that Σ , Σ' , $\mathbf{d}$, and $\mathbf{d}'$ are axisymmetric. The surface divergence is

$$\nabla \cdot \mathbf{d} = \frac{\partial \mathbf{d}}{\partial s} \cdot \mathbf{e}_s + \frac{1}{r} \mathbf{d} \cdot \mathbf{e}_r,$$

where s is the arclength parameter of the curve, of length l , that parametrizes the cross section of the surface. Here, $\mathbf{e}_s$ is the unit tangent to the curve, and $\mathbf{e}_r$ and $\mathbf{e}_z$ are the radial and height basis vectors, respectively. The integral is computed with area differential $dS = 2\pi r ds$ for $0 < s < l$. In the absence of lipid tilt ($\mathbf{d} = \mathbf{n}$, $\mathbf{d}' = \mathbf{n}'$), fusion pore energy reduces to the usual Helfrich bending energy

$$E_B = \int \sum \frac{k_B}{2} (k_1 + k_2 - k_0)^2 dS + \int \sum' \frac{k_B}{2} (k'_1 + k'_2 - k'_0)^2 dS',$$

where k_1, k_2 and k'_1, k'_2 are the principal curvatures of Σ and Σ' respectively.

Monolayers are almost volumetrically incompressible, and hence as the area per lipid increases (stretching), monolayer thickness decreases; area compression leads to an increase in monolayer thickness. Consequently, the thickness of each monolayer is not generally half of the bilayer thickness. The resulting local increase in the number of head groups per surface area is at the cost of an increasing local stretching energy. To arrive a suitable definition of the local stretching energy, we model the proximal and distal lipid molecules by vector fields $\mathbf{D}$ and $\mathbf{D}'$ where $\mathbf{d} = \mathbf{D}/|\mathbf{D}|$ and $\mathbf{d}' = \mathbf{D}'/|\mathbf{D}'|$. An infinitesimal volume v of monolayer contains roughly $n = v/v_l$ lipid molecules where v_l is the volume per lipid ($\sim 1.1 \times 10^3 \text{ \AA}^3/\text{molecule}$, [26]). A monolayer at rest consisting of n lipid molecules has surface area $a_0 = nv_l/h_0 = v/h_0$ (Figure 2.) The actual surface area a of the volume v may, however, differ from a_0 , resulting in a local stretching energy density

$$k_s \frac{(a - a_0)^2}{2a_0}. \quad (2)$$

The modulus of stretching k_s for a monolayer is large, 30 kT nm^{-2} , (60 kT nm^{-2} or 240 dyn cm^{-1} for the bilayer, [15]) compared to the other moduli. Consequently, the area per lipid is almost constant even when tension is applied to a monolayer. Dividing (2) by a and summing over the proximal and distal monolayers yields the total stretching energy

$$E_s = \int \sum \frac{k_s}{2} e(\mathbf{p}, \mathbf{D}) dS + \int \sum' \frac{k_s}{2} e(\mathbf{p}', \mathbf{D}') dS. \quad (3)$$

For axisymmetric surfaces, the local stretching energy density is made explicit by the limit

$$e(\mathbf{p}, \mathbf{D}) := \lim_{a \rightarrow 0} \frac{(a - a_0)^2}{aa_0} = \frac{\left(1 - \frac{\mathbf{D} \cdot \mathbf{n}}{h_0} \left(1 + \frac{\mathbf{D} \cdot \mathbf{e}_r}{2\mathbf{p} \cdot \mathbf{r}}\right)\right)^2}{\frac{\mathbf{D} \cdot \mathbf{n}}{h_0} \left(1 + \frac{\mathbf{D} \cdot \mathbf{e}_r}{2\mathbf{p} \cdot \mathbf{r}}\right)}.$$

For moderate pore radii, the local stretching energy density $e(\mathbf{p}, \mathbf{D})$ is small if the lipid director $\mathbf{D}$ is simultaneously normal to the neutral surface and has length h_0 . We have arrived at (2) and (3) by applying the (global) stretching energy formula—which implicitly assumes homogeneity of lipid density—locally to monolayers where lipid density may vary as the bilayer deforms.

To couple the monolayers and prevent the formation of voids in the bilayer core, we incorporate a void penalty term

$$E_V = \int_M k_V |\mathbf{s}|^2 dS, \quad (4)$$

where M is the midplane surface between monolayers, $\mathbf{p}$ and $\mathbf{p}'$ are opposing points in the distal and proximal neutral surfaces, $\mathbf{s} = \mathbf{p} - \mathbf{p}' + \mathbf{D} - \mathbf{D}'$ is the separation between the ends of the distal and proximal acyl chains, and k_V is a large void penalty parameter. The penalty energy E_V vanishes only when the two monolayers touch everywhere. Alternative interactions between monolayers, such as interdigitation, can be accounted for by modifying the steric penalty (4) and replacing the bending modulus k_B with a function that depends on the depth of penetration of lipids of one monolayer into the other. For example, replacing the constant k_B in (1) with

$$k_B(\mathbf{p}, \mathbf{p}', \mathbf{D}, \mathbf{D}') = \tilde{k}_B \left(1 + \frac{\lambda}{2|\mathbf{s}|} (\mathbf{s} \cdot \mathbf{D} + |\mathbf{s} \cdot \mathbf{D}|) \right),$$

models a linear increase in bending modulus as a function of penetration depth of interdigitation. The parameter λ is a proportionality constants between the bending modulus and depth of penetration. Molecular dynamics simulations might provide these parameters as part of a larger multiscale analysis.

We use boundary conditions that model a fusion pore connecting two infinite, flat bilayers. In order to fuse, separated bilayers must be brought into close proximity, ranging from 2 to 3 nm. At this length scale, the balance between attractive Van der Waals and repulsive fluctuation forces ([26]) causes a considerable portion of the opposing bilayers to be parallel and lie at a fixed separation. Experimentally, prior to fusion between cells, cell-cell binding yields an extended region of two parallel membranes; fusion occurs within this region (Figure 3). Fusion in model systems, such as stacks of laminar bilayers, also occurs between effectively infinite, parallel membranes. The deformations associated with pore formation are therefore essentially localized to the site of pore nucleation. By confining the bilayer geometry exterior to the computational domain, minimal energies can be parametrized in terms of pore dimensions (r_p and r_b). Also, our results can be directly compared to prior theoretical studies ([5, 6, 21, 30, 40]) which also assumed that the fusion pore connects two infinite, flat bilayers. Therefore, we assume that the fusion pore lies within the computational region $0 < r < L$ and possesses reflectional symmetry about the plane $\{z = 0\}$. We further assume that for radii L large compared to the fixed pore radius r_p (the distance from the z -axis to the pore lumen) the bilayer is planar and maintained at a fixed distance of separation r_b between the respective proximal neutral surfaces. Eight constraints (four for each monolayer) provide the boundary conditions needed to formulate the minimization problem:

$$\mathbf{p}(0) = (r_p + 2h_0, 0), \quad \mathbf{p}'(0) = (r_p, 0), \quad \mathbf{p}(l) = (L, r_b/2), \quad \mathbf{p}'(l) = (0, r_b/2 + 2h_0), \quad (5)$$

$$\mathbf{D}(0) \cdot \mathbf{e}_z = \mathbf{D}'(0) \cdot \mathbf{e}_z = \mathbf{D}(l) \cdot \mathbf{e}_r = \mathbf{D}'(l) \cdot \mathbf{e}_r = 0. \quad (6)$$

The first two boundary conditions in (5) position the proximal and distal neutral surfaces along the pore rim at distances $r = r_p + 2h_0$ and $r = r_p$ respectively. The third and fourth boundary conditions in (5) connect the pore with a planar bilayer separated by a distance r_b . The boundary conditions (6) fulfill the requirement that the bilayer has reflectional symmetry and is horizontal at the extremities of the computational domain.

A. The Energy of Pore Formation

The energy of pore formation is defined as:

$$E_{PF} = \int \sum \frac{k_B}{2} (\nabla \cdot \mathbf{d} - k_0)^2 - \frac{k_B}{2} k_0^2 + \frac{k_T}{2} |\mathbf{d} \times \mathbf{n}|^2 dS + \int \sum' \frac{k_B}{2} (\nabla \cdot \mathbf{d}' - k'_0)^2 - \frac{k_B}{2} (k'_0)^2 + \frac{k_T}{2} |\mathbf{d}' \times \mathbf{n}'|^2 dS'. \quad (7)$$

We use two planar bilayers with the same area as the fusion pore as the reference state to calculate pore energies. Energy of pore formation accounts for the change in energy due to conservative deformations but does not evaluate the energy lost through viscous dissipation. Minimization of E_{PF} subject to boundary conditions (5-6) determines the conditions that permit energetically favored pore expansion as a function of pore radius r_p .

In order to show that E_{PF} is the appropriate quantity to minimize, we relate it to the total energy of the fusion pore. Consider the reference energy

$$E_{Ref} = \frac{k_B}{2} (A_0 k_0^2 + A'_0 (k'_0)^2), \quad (8)$$

of two planar bilayers with proximal and distal areas A_0 and A'_0 respectively which deform into the shape of a fusion pore. The fusion pore lying within the computational region $0 < r < L$ has proximal and distal areas A and A' respectively and the planar portions lying outside the computational region $L - r$ have area $A_0 - A$ and $A_0 - A'$ respectively. Then, the elastic energy of the fusion pore plus the energy of the remaining planar part is

$$E_{Total} = E_B + \frac{k_B}{2} ((A_0 - A)k_0^2 + (A_0 - A')(k'_0)^2) = E_{PF} + E_{Ref}. \quad (9)$$

We have thus established that E_{PF} is the total energy of the pore, E_{Total} , relative to the reference energy E_{Ref} . Since the reference energy is constant, minimizing the energy of pore formation is equivalent to minimizing the total energy. We therefore minimize the energy of pore formation (7) plus the penalty terms for stretching membranes (3) and preventing voids (4):

$$E = E_{PF} + E_S + E_V. \quad (10)$$

Hence, pore energy consists of splay, tilt, local stretching, and void penalty energies.

III. DISCRETIZATION AND MINIMIZATION

To numerically evaluate fusion pore energy, the bilayer was divided into N discrete elements, forming a piecewise linear approximation of the neutral surfaces. The basic element consisted of two quadrilaterals bound on four sides—by a neutral surface, the bilayer mid-plane, and two edges parallel to the lipids. The energies E_{PF} and E_V were calculated by standard numerical quadrature and finite difference formulas using piecewise linear functions to track the grid points $\mathbf{p}$ (resp. $\mathbf{p}'$) of the head group on the neutral surface and the end-points $\mathbf{p}+\mathbf{D}$ (resp. $\mathbf{p}'+\mathbf{D}'$) of the acyl chains. The stretching energy E_S was calculated by

$$E_S = \sum_{n=1}^N k_S \frac{(a_n - a_{n,0})^2}{2a_{n,0}} + k_S \frac{(a'_n - a'_{n,0})^2}{2a'_{n,0}}, \quad (11)$$

where $a_{n,0} = v_n/h_0$ resp. $a'_{n,0} = v'_n/h_0$ and v_n, a_n resp. v'_n, a'_n are the volume and head group surface area of the n th proximal resp. distal monolayer elements (see Equation 2). The neutral surfaces are mathematically smooth and we found that a few hundred elements give a highly accurate approximation of the energy.

It was helpful to partition the more curved portions of the bilayer with more grid points. Points were spaced evenly along the midplane in the parallel portion of the membrane. A finer discretization was chosen along the midplane near the rim of the pore, where the membrane is necessarily more curved (Figure 1). We spaced the grid points quadratically (as a function of arc length) in the curved portion. This ensured the quality of the representation near the highly curved portion of the pore and guaranteed an accurate calculation of the curvature dependent elastic terms. We found that in practice, too few representative elements at the rim caused the discretization to fail, resulting in an unphysical fissure near the pore rim during the minimization procedure. The mesh quality was maintained throughout the minimization procedure by requiring that the grid points approximating the midplane maintain their initial spacing. We note that the mathematical grid points specify the representative elements and not necessarily the location of lipid or head groups. This redistribution of representative elements (analogous to moving the rings of a shower curtain on a curved rod), does not alter the value of the calculated energy of the pore shape and allows the minimization to be performed without the need for re-discretization.

The method of steepest gradient descent was applied to the membrane shape subject to boundary conditions (5-6) and fixed parameters $r_p, r_b, h_0, k_0, k'_0, L$, and N . To iteratively minimize energy, a toroidal fusion pore was used as the initial shape. The proximal rim of the toroidal pore was modeled as a quarter of a circle with radius $r_b/2$. The membrane extended parallel to the r -axis for the planar portion of the initial pore (Figure 1A). Let $\mathbf{p}(s, t)$, $\mathbf{p}'(s, t)$, $\mathbf{D}(s, t)$, and $\mathbf{D}'(s, t)$ define the proximal and distal monolayers at time t ; s is a parametrization which is no longer necessarily arc-length. Then the (continuous) gradient flow parabolic system is

$$\frac{\partial \mathbf{p}}{\partial t} = -\frac{\delta E}{\delta \mathbf{p}}, \quad \frac{\partial \mathbf{p}'}{\partial t} = -\frac{\delta E}{\delta \mathbf{p}'}, \quad \frac{\partial \mathbf{D}}{\partial t} = -\frac{\delta E}{\delta \mathbf{D}}, \quad \frac{\partial \mathbf{D}'}{\partial t} = -\frac{\delta E}{\delta \mathbf{D}'}, \quad (12)$$

where δ denotes the Euler-Lagrange derivative. Equations (12) form a system of second order parabolic equations and, along with boundary conditions (5-6), solutions satisfy the relevant energy monotonicity property $\frac{d}{dt}E \leq 0$. In particular,

$$\begin{aligned} \frac{\delta E_{PF}}{\delta \mathbf{p}} = & 2\pi k_B \left\{ -\frac{\partial}{\partial s} \left[(\nabla \cdot \mathbf{d} - k_0)(\mathbf{p} \cdot \mathbf{e}_r) \left| \frac{\partial \mathbf{p}}{\partial s} \right|^{-1} \left(\frac{\partial \mathbf{d}}{\partial s} - 2 \frac{\partial \mathbf{d}}{\partial s} \cdot \tau \tau \right) \right] \right. \\ & - (\nabla \cdot \mathbf{d} - k_0) \frac{\mathbf{d} \cdot \mathbf{e}_r}{\mathbf{p} \cdot \mathbf{e}_r} \left| \frac{\partial \mathbf{p}}{\partial s} \right| \mathbf{e}_r - \frac{\partial}{\partial s} \left[\left(\frac{1}{2} (\nabla \cdot \mathbf{d} - k_0)^2 - \frac{1}{2} k_0^2 \right) (\mathbf{p} \cdot \mathbf{e}_r) \tau \right] \\ & + \left(\frac{1}{2} (\nabla \cdot \mathbf{d} - k_0)^2 - \frac{1}{2} k_0^2 \right) \left| \frac{\partial \mathbf{p}}{\partial s} \right| \mathbf{e}_r \left. \right\} \\ & + 2\pi k_T \left\{ -\frac{\partial}{\partial s} [(\mathbf{d} \cdot \tau)(\mathbf{p} \cdot \mathbf{e}_r)(\mathbf{d} - \mathbf{d} \cdot \tau \tau)] - \frac{\partial}{\partial s} \left(\frac{1}{2} (\mathbf{d} \cdot \tau)^2 (\mathbf{p} \cdot \mathbf{e}_r) \tau \right) \right. \\ & + \frac{1}{2} (\mathbf{d} \cdot \tau)^2 \left| \frac{\partial \mathbf{p}}{\partial s} \right| \mathbf{e}_r \left. \right\}, \quad \left(\text{where } \mathbf{d} = \frac{\mathbf{D}}{|\mathbf{D}|} \right) \end{aligned}$$

and

$$\begin{aligned} \frac{\delta E_{PF}}{\delta \mathbf{D}} = & 2\pi k_B \left\{ -\frac{\partial}{\partial s} \left[(\nabla \cdot \mathbf{d} - k_0)(\mathbf{p} \cdot \mathbf{e}_r) \left(\frac{\tau - (\tau \cdot \mathbf{d})\mathbf{d}}{|\mathbf{D}|} \right) \right] + (\nabla \cdot \mathbf{d} - k_0) \left| \frac{\partial \mathbf{p}}{\partial s} \right| \left(\frac{\mathbf{e}_r - (\mathbf{e}_r \cdot \mathbf{d})\mathbf{d}}{|\mathbf{D}|} \right) \right\} + 2\pi k_T (\mathbf{d} \\ & \cdot \tau) \frac{\tau - (\tau \cdot \mathbf{d})\mathbf{d}}{|\mathbf{D}|}, \end{aligned}$$

are second order operators. By substituting the non-primed variables with the primed variables, one arrives at the analogous Euler-Lagrange derivatives of E_{PF} with respect to $\mathbf{p}'$ and $\mathbf{D}'$. Here we have used that $|\mathbf{d} \times \mathbf{n}| = |\mathbf{d} \cdot \tau|$ where $\tau = \frac{\partial \mathbf{p}}{\partial s} / \left| \frac{\partial \mathbf{p}}{\partial s} \right|$. If the monolayers are decoupled and it is assumed that $\mathbf{D} = \mathbf{d} = \mathbf{n}$, then the 2nd order parabolic system in the position variable $\mathbf{p}$ and director variable $\mathbf{D}$ is related to the Willmore flow, a 4th order parabolic elasticity equation in the position variable $\mathbf{p}$, [39]. Newton's method was used to solve the fully implicit semi-discretization of the 2nd order system of parabolic equations (12) with time step Δt and the Euler-Lagrange derivatives were calculated by numerical differentiation. The subiterations of Newton's method produced large, symmetric systems of linear equations which were solved by the conjugate gradient method. The system of linear equations were well conditioned since Δt was chosen sufficiently small. The scheme produced a decreasing sequence of values $E_0 > E_1 > E_2 > \dots$ where E_0 was the energy of pore formation (10) for the initial toroidal fusion pore. The minimum energy was taken to be $\lim_{i \rightarrow \infty} E_i$.

To validate the accuracy of the numerical quadrature, convergence tests were performed; the numerically obtained energy converged linearly in the number of grid points. The value of minimum energy was stable with respect to the number of grid points. For example, taking $r_p = 2.5$ nm, $r_b = 3$ nm, $h_0 = 1.5$ nm, $k_0 = -0.15$ nm⁻¹, $k'_0 = -0.05$ nm⁻¹, gave the minimum values $E = 13.12$ kT, 13.14 kT, and 13.26 kT for $N = 180, 200$, and 220 respectively. To validate the precision of the numerical quadrature, the numerically obtained energy of the

initial shape was calculated for the case $k_0=k'_0=0$ and compared to the analytically determined exact value of energy of a toroidal fusion pore [5]. The two energies agreed for all parameters r_p , r_b , and h_0 that were used.

Energy minimization of any single fusion pore shape can be computationally costly, taking up to a few hours on a single computing processor. For the first ~ 10 – 20 iterations, a small value of $\Delta t = 0.1$ was required for Newton's method to converge. However, energy minima were typically reached after a total descent time on the order of $t = 10^5$. To speed up convergence to minimal energy, we employed an adaptive time stepping scheme: the time step Δt was doubled if Newton's method converged within tolerance in less than four steps and Δt was halved if the Newton's method did not converge within ten steps. By analyzing the rate at which energy converged, we estimated the number of iterations needed to achieve a sufficiently close approximation of minimal energy. As a stopping criterion, the minimization was terminated if further iterations reduced energy by at most 1.0 kT (0.5% relative error). This was satisfied when $i > -3 \log(10)/\log \rho$ where i is the number of iterations and $\rho = \lim_{i \rightarrow \infty} (E_{i+1} - E_i)/(E_i - E_{i-1})$. We found that the rate of energy decay ρ ranged between 0.96 and 0.995.

Shapes minimizing energy were uniquely determined by the physical constants and boundary conditions, and were independent of the assumed initial shape. An elliptical fusion pore was formed by using an ellipse connected to a line parallel with the r -axis in place of the circular arc used to form the toroidal fusion pore. Although the energies of elliptical and toroidal fusion pores are quite different [21], the gradient descent procedure we employed deformed both initial shapes into the same teardrop shape; one which minimizes E .

IV. MINIMAL ENERGY SHAPES

Minimal energy shapes were calculated for pore radii in the range $1.5 \text{ nm} < r_p < 8.5 \text{ nm}$, bilayer separation $r_b = 2.5 \text{ nm}$, 3 nm , 3.4 nm , 4 nm and 5 nm , and bilayer thicknesses $2h_0 = 2.0 \text{ nm}$, 3.0 nm , and 4.0 nm . The values of spontaneous curvature k_0 and k'_0 were chosen to model a symmetric bilayer ($k_0=k'_0$), an asymmetric bilayer ($|k_0| \neq |k'_0|$), and an anti-symmetric bilayer ($k_0=-k'_0$). We used a monolayer bending modulus of $k_B = 10 \text{ kT}$ and tilt modulus $k_T = 10 \text{ kT nm}^{-2}$, [18]. For the void penalty parameter, we used $k_V = 100 \text{ kT nm}^{-2}$, a value sufficient to prevent the formation of voids between two monolayers.

We used $N = 200$ elements to discretize the bilayer for all simulations. In applying the time adaptive, iterative gradient descent scheme to the initial shape, the energy dropped rapidly, by tens of kT, over the first few iterations ($\Delta t = 0.1$), and then continued decreasing but at a decelerated rate. The rapid initial decrease in energy was due to a combination of lipid tilt and a bowing of bilayer out of the planar configuration. Bowing continued until the final bilayer configuration consisted of untilted lipids lying along a teardrop shaped profile. In other words, lipid tilt contributed zero energy to minimal shapes; all the energy was contributed by bending of the proximal and distal surfaces.

A. Symmetric Bilayers

The spontaneous curvature in both monolayers is equal in a symmetric bilayer. We considered three possibilities for the values of spontaneous curvature:

$k_0=k'_0=0 \text{ nm}^{-1}$, $k_0=k'_0=-0.1 \text{ nm}^{-1}$, and $k_0=k'_0=0.1 \text{ nm}^{-1}$, [17, 21]. Increasing the spontaneous curvature in both monolayers had the effect of increasing the energy of pore formation. E was an increasing function for zero or positive spontaneous curvature for $r_p > 3 \text{ nm}$, prohibiting pore expansion. For these symmetric bilayers, pore energy is upwardly convex and possesses a minimum value in the range $1 \text{ nm} < r_p < 4.5 \text{ nm}$, which is comparable to bilayer thickness. For negative spontaneous curvature, e.g.

$k_0=k'_0=-0.1 \text{ nm}^{-1}$, E is a decreasing function of r_p . This means that once a pore is formed, it will reach a state of lower total energy by widening independently of external forces, such as those exerted by proteins (Figure 4).

A change in spontaneous curvature also resulted in a change of the minimized shape. Physically, the shape achieved its minimum energy by adjusting the extent of the bowing out of the pore so as to match the mean curvature (splay) with the spontaneous curvature at the pore rim. As a consequence of this physical principle, bowing was most severe for negative spontaneous curvature (Figure 5A), intermediate for zero spontaneous curvature, and least-pronounced for positive spontaneous curvature. This order follows the order of meridian curvature: least for negative mean curvature, greater for zero mean curvature, and greatest for positive mean curvature. We thus arrive at the rule that bowing of the bilayer increases with pore radius but decreases with spontaneous curvature (Figure 5).

To test the generality of the rule that negative spontaneous curvature leads to pore expansion, and zero and positive spontaneous curvature prohibits pore expansion, we analyzed the dependence of E on membrane thickness (Figure 4) and bilayer separation (Figure 6). Increasing bilayer thickness, by letting $h_0 = 1.0, 1.5$, and 2.0 nm respectively, led to a slight upward shift and steepening of slope in the dependence of E on r_p . Thus the effect of spontaneous curvature on pore expansion and suspension is amplified by increasing membrane thickness (Figure 4). Increasing bilayer separation, by letting $r_b = 2.5 \text{ nm}, 3.0 \text{ nm}, 3.4 \text{ nm}, 4 \text{ nm}$, and 5 nm respectively, led to a relatively uniform decrease in E as a function of r_p , with energy decreasing the most for pores with symmetric, negative spontaneous curvature (Figure 6).

B. Anti-symmetric Bilayers

The spontaneous curvature in the respective monolayers has the same magnitude but opposite sign for an anti-symmetric fusion pore. We used the values $k_0=-k'_0=0.1 \text{ nm}^{-1}$ and $-k_0=k'_0=0.1 \text{ nm}^{-1}$, [17, 21]. The meridian curvature near the pore rim is larger in the proximal layer than in the distal layer. Consequently, energy decreases when the curvature of the distal layer has negative spontaneous curvature and the proximal layer has positive spontaneous curvature (Figure 7A–C). By the same reasoning, energy increases if the sign of these curvatures is switched. But even the energetically most favorable combination of spontaneous curvature does not lead to pore expansion. In all cases for anti-symmetric fusion pores, E was an increasing function of pore radius when $r_p > 3 \text{ nm}$. As was the case

for symmetric bilayers with zero or positive spontaneous curvature, the energy of a pore was upwardly convex and possesses a minimum value and the location of the minimum, $1.5 < r_p < 3$ nm, a value again comparable to the bilayer thickness.

C. Asymmetric Bilayers

In the symmetric and anti-symmetric cases, positive spontaneous curvature in at least one of the monolayers resulted in E increasing with r_p . We thus consider the opposite extreme, where the spontaneous curvature in both the distal and proximal layer is negative, but not equal. We studied the combinations $k_0 = -0.05$, $k'_0 = -0.15 \text{ nm}^{-1}$ and $k_0 = -0.15$, $k'_0 = -0.05 \text{ nm}^{-1}$, [17, 21]. Here, the energy of pore formation does not have a minimum; instead energy is a decreasing function of pore radius for all values of r_p . The same held for the case of a symmetric bilayer with negative spontaneous curvature. The overall energy decreased if the distal layer has the more negative value of spontaneous curvature (Figure 8). We thus arrive at the rule: pore expansion in symmetric and asymmetric bilayers is promoted by negative spontaneous curvature. Pores in bilayers with negative spontaneous curvature in both monolayers lower their total energy by widening, independently of external forces.

D. Discussion

We found minimal energy shapes amongst all possible shapes having a pore radius r_p and bilayer separation r_b as a function of spontaneous curvature and membrane thickness. Energies are presented in terms of pore radius to be able to understand whether a fusion pore in bilayers of specific compositions, with fixed separation, will spontaneously expand or not. Because all shapes considered by prior theories are captured by our method, we have found energy minima which are less than those reported previously. The difference between our minima and those reported previously [6, 21, 40], is significant—on the same order as the total energy of the pore itself. The results of this study also show that the energy of the reference state must be treated in a self-consistent manner in order to obtain minimal pore energies.

Minimizing E_{PF} (Equation 7) will give a smaller energy of pore formation than if one minimized E_B (Equation 1) first and subtracts the energy $A \frac{k_B}{2} (k_0^2 + (k'_0)^2)$ of a planar bilayer with the same area A . That is, perhaps surprisingly, the energy of the reference bilayer must be subtracted before applying the minimization procedure if a true minimum is to be obtained. The calculated minimum energy is higher if the reference energy is subtracted subsequent to minimization [21, 40], because the energy of pore formation can decrease by incorporating more of the planar bilayer into the fusion pore, an effect that is only captured if the energy of the planar bilayer is consistently subtracted before minimizing.

We have found E is a decreasing function of pore radius when the spontaneous curvature of both layers is negative (Figures 1 and 4). Explicitly, when $k_0, k'_0 < 0$, E decreases sharply for small values of r_p and continues to decrease, albeit less steeply, for all larger values of r_p . This occurs because the energy of the bilayer is reduced by deforming the planar portion, with nonzero energy density, into a curvature that matches spontaneous curvature. Because a

highly curved axisymmetric membrane has a large meridian curvature within the pore, the bilayer energetically prefers a negatively curved configuration. Conversely, lipids with positive and zero spontaneous curvature inhibited pore expansion as E was an increasing function of r_p for moderate values of pore radius. Previous investigations have studied shapes constrained to certain geometries. Because these constrained minimization focused only on a low dimensional subset of all possible membrane shapes, this potentially important dependence of energy on pore radius and spontaneous curvature was missed. Increasing the thickness of the membrane (i.e., increasing h_0) and keeping other parameters fixed leads to a moderate, but uniform, increase in energy of pore formation.

Fusion pore energies and energy of pore formation are highly sensitive to membrane shape.

The energy of a toroidal fusion pore with $k_B = 10$ kT, $k_0 = k'_0 = 0.0 \text{ nm}^{-1}$, membrane thickness 2 nm, midplane separation $r_b = 3.0$ nm, and pore radius $r_p = 2.0$ is 222 kT. Minimizing among three or four degrees of freedom, [21] found a minimum E of roughly 130 kT. Using a detailed piecewise elemental representation of the bilayer, we found that the minimum energy is 58 kT (solid line, Figure 4A). These values show that calculated fusion pore energy can double or even triple by artificially constraining membrane shape. The shape of a toroidal fusion pore is completely determined by the boundary conditions yielding zero degrees of freedom. In the continuum approaches [21, 40], the fusion pore geometry was defined in terms of polynomials; polynomial coefficients yielded three or four degrees of freedom. In the present study, we used arbitrarily many degrees of freedom capable of representing any smooth shape, yielding an absolute minimum energy.

Experimentally, an energy of pore formation $E = 58$ kT for zero spontaneous curvature is large when compared to thermal energy (~ 1 kT). The spontaneous curvature of DOPC (dioleoylPC) is close to zero, at the slightly negative value -0.05 nm^{-1} [4], and bilayers composed of DOPC are stable for hours and even days; they do not spontaneously fuse. The spontaneous curvature of DOPE is approximately -0.35 nm^{-1} , significantly more negative than DOPC, and bilayers composed of DOPE readily fuse. For membranes with negative spontaneous curvature, we found that the energy for pore formation is close to zero or negative (hashed line, Figure 4 and solid line, Figure 8). Therefore, the dependence of our numerically evaluated minimal pore energies on spontaneous curvature is in accord with the experimental observations.

We performed two simulations to determine whether the minimum pore energy was sensitive to surface tension. In one simulation we applied a stretching tension $k_S = 30 \text{ kT nm}^{-2}$; in the other we set $k_S = 0 \text{ kT nm}^{-2}$, substituting the stretching energy E_S with

$$\tilde{E}_S = \int \tilde{k}_S \frac{(|D| - h_0)^2}{2h_0} dS + \int \tilde{k}'_S \frac{(|D'| - h_0)^2}{2h_0} dS'.$$

The functional $\tilde{E}_S$ models a spring constant for the tail groups for which we used the value $\tilde{k}_S = 20 \text{ kT nm}^{-3}$. In the first simulation, the minimum energy was $E = 58$ kT. In the second simulation, the minimum energy was $E = 55.5$ kT. For bilayers with constant lipid length $|D|$

$= |\mathbf{D}'| = h_0$, energy of pore formation is slightly smaller than bilayers with varying lipid length when stretching tension is applied. We conclude that splay is dominant and that tilt and stretching contribute relatively little to the total energy of minimal pore shapes.

Spontaneous curvature is determined by lipid composition. Additional internal and external forces also control pore expansion. Surface tension is one such force, and it can be varied and controlled in model and biological systems. We considered an energy of pore formation $\tilde{E} = E + \sigma(A + A' - 4\pi L^2)$ for surface tension σ in the case of a symmetric bilayer

$k_0 = k'_0 = 0 \text{ nm}^{-1}$ and an anti-symmetric bilayer $-k_0 = k'_0 = -0.1 \text{ nm}^{-1}$, for both $r_b = 3 \text{ nm}$ and $h_0 = 1.5 \text{ nm}$. For these cases, in the absence of surface tension, energy of pore formation was an increasing function of pore radius r_p (Figure 4B and Figure 7B). For $\sigma = 0.8 \text{ dyn/cm}$, a value typical of model planar bilayers [6], the energy of pore formation decreases for increasing r_p , implying that once a pore forms, it would spontaneously widen (Figure 9).

We have dealt with the boundary conditions (5–6) for an infinite, planar bilayer. This was done in order to be able to compare our minimal shapes and energies with those found in the literature, which also assume infinite flat membranes [5, 6, 21, 30, 40]. By varying the boundary conditions, the shapes of fusion pores connecting curved membranes can be studied. For example, we considered the same boundary conditions, except that the fusion pore connects two spheres of the identical radii R with their closest points separated by 3 nm. This required specifying an angle α and distance r_b where the fusion pore meets the boundary of the computational domain (Figure 10). For fixed $L = 20 \text{ nm}$, $\alpha = 0, 0.1$, and 0.2 and $r_b = 3, 4.02$, and 5.02 nm for the cases $R = \infty, 200 \text{ nm}$, and 100 nm . For pore radius $r_p = 3.5 \text{ nm}$, the energies for the three cases are $E = 192 \text{ kT}$, 160 kT , and 152 kT respectively. Pore energy is smaller for more highly curved spheres because the angle and separation match that of a minimally curved fusion pore as R becomes smaller. The shapes of the pore do not, however, appreciably change; the pores have teardrop shapes in all cases.

Because the elastic energy function is highly sensitive to membrane shape, assuming a specific shape or functional form for the fusion pore may lead to theoretical conclusions which do not reflect the real system. The major advantage of our method is that it predicts minimal energy shapes by continuum mechanics without prior knowledge of what the fusion pore should look like. As an important consequence, our variational method does not artificially inject energy into the system.

[40] found by coarse-grained molecular dynamics that, energetically, fusion pores should bow perpendicularly from the bilayers near the pore rim. This coarse-grained determined geometry was captured by continuum mechanics by assuming rational functions (instead of polynomials) tailored to produce the desired membrane shape. To reliably arrive at theoretical predictions and/or principles, it is important that shapes not depend on artificially imposed functional forms, but instead be outputs of a calculation. Precise membrane shapes of plasma and organelle membranes are continually changing. In some cases, such as exo- and endocytosis, the changes are not just in shape, but in topology. A procedure that does not make a priori assumptions regarding membrane shape should therefore find wide applicability in membrane biophysics.

It is possible that there are other axisymmetric pore shapes which are local minima and have elastic energies even smaller than the minimal energies found by our gradient flow procedure. We have calculated minimal energy shapes which evolve from initially toroidal shapes by a path of steepest descent. The surface geometry and director fields are coupled through nonlinear interactions. This makes a determination of global minimality a challenging analytical problem beyond the scope of this study.

Our study used a time variable in the steepest gradient descent procedure. When formulated as the dissipation equation, steepest gradient descent allows calculations of time dependent phenomena that are not energetically conservative. We used the simplified dissipation function $T|\mathbf{v}|^2$ where $\mathbf{v}$ is the discrete velocity vector of the grid point positions. However, it is possible to use the dissipation equation to extend our energetic analysis to derivations for realistic time courses of pore evolution. This is achieved by defining a dissipation function to account for the membrane viscosity. This would allow us to evaluate the dynamics of pore enlargement in an energetically self consistent manner.

V. CONCLUSION

A variational procedure was developed to find minimal energy shapes of fusion pores. The bilayer was described using a surface-director model where bending energy—comprised of splay and tilt—was minimized, and two opposing, volumetrically incompressible monolayers were coupled through steric and stretching interactions. Minimal energy shapes were found and the energy was analyzed as a function of pore dimension and lipid composition. The fact that the minimal energies found by our method are significantly less than those reportedly previously underscores the need for numerical procedures to calculate precise energies of membrane shapes with arbitrary geometry, a factor which is especially important for accurately predicting membrane dynamics in experimental systems. In the present study, we limited our focus to the static, minimization problem. Most biological membrane processes are dynamic, and therefore are strongly regulated by the viscous environment in which they occur. A major goal of biophysics and biomathematics is to describe the physical forces that control these processes and their dynamics. The variational approach we have now developed can be expanded to account for the consequences of energy dissipation that result from viscous forces.

Acknowledgments

We thank Robert Eisenberg for critical readings of the manuscript and hosting R.J.R. and M.A.W. in the Rush Department of Molecular Biophysics and Physiology. Computing resources at Argonne National Lab were made available by Gary Leaf. We thank Ruben Markosyan for providing the images in Figure 3. M.A.W. was supported by the Fordham Summer Science Internship. F.S.C. was supported by NIHR01 GM066837 and GM101539.

References

1. Bartels S, Dolzmann G, Nochetto RH. A finite element scheme for the evolution of orientational order in fluid membranes. *ESAIM: Mathematical Modelling and Numerical Analysis*. 2010; 44:1–31.
2. Bartels S, Dolzmann G, Nochetto RH, Raisch A. Finite element methods for director fields on flexible surfaces. *Interfaces and Free Boundaries*. 2012; 14(2):231–272.

3. Canham PB. The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. *Journal of Theoretical Biology*. 1970; 26(1):61–81. [PubMed: 5411112]
4. Chen Z, Rand RP. The influence of cholesterol on phospholipid membrane curvature and bending elasticity. *Biophys J*. Jul; 1997 73(1):267–276. [PubMed: 9199791]
5. Chizmadzhev YA, Cohen FS, Shcherbakov A, Zimmerberg J. Membrane mechanics can account for fusion pore dilation in stages. *Biophys J*. Dec; 1995 69(6):2489–2500. [PubMed: 8599655]
6. Chizmadzhev YA, Kuzmin PI, Kumenko DA, Zimmerberg J, Cohen FS. Dynamics of fusion pores connecting membranes of different tensions. *Biophys J*. May; 2000 78(5):2241–2256. [PubMed: 10777723]
7. Dai S, Promislow K. Geometric evolution of bilayers under the functionalized Cahn-Hilliard equation. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Science*. 2013; 469(2153):1471–2946.
8. de Gennes, PG.; Prost, J. *The Physics of Liquid Crystals*. Clarendon Press; Oxford, U.K: 1993.
9. Döbereiner H-G, Evans E, Kraus M, Seifert U, Wortis M. Mapping vesicle shapes into the phase diagram: A comparison of experiment and theory. *Phys Rev E*. Apr.1997 55:4458–4474.
10. Droske M, Rumpf M. A level set formulation for Willmore flow. *Interfaces Free Bound*. 2004; 6(3):361–378.
11. Du Q, Liu C, Ryham R, Wang X. Modeling the spontaneous curvature effects in static cell membrane deformations by a phase field formulation. *Commun Pure Appl Anal*. 2005; 4(3):537–548.
12. Du Q, Liu C, Wang X. A phase field approach in the numerical study of the elastic bending energy for vesicle membranes. *J Comput Phys*. 2004; 198(2):450–468.
13. Eisenberg RS. Computing the field in proteins and channels. *J Membr Biol*. Mar; 1996 150(1):1–25. [PubMed: 8699474]
14. Eisenberg RS. From structure to function in open ionic channels. *J Membr Biol*. Sep; 1999 171(1):1–24. [PubMed: 10485990]
15. Evans E, Rawicz W. Entropy-driven tension and bending elasticity in condensed-fluid membranes. *Phys Rev Lett*. Apr; 1990 64(17):2094–2097. [PubMed: 10041575]
16. Fournier JB, Galatola P. Sponges, tubules and modulated phases of para-antennemal membranes. *J Phys II France*. 1997; 7(10):1509–1520.
17. Fuller N, Rand RP. The influence of lysolipids on the spontaneous curvature and bending elasticity of phospholipid membranes. *Biophys J*. Jul; 2001 81(1):243–254. [PubMed: 11423410]
18. Hamm M, Kozlov MM. Elastic energy of tilt and bending of fluid membranes. *The European Physical Journal E*. 2000; 3(4):323–335.
19. Helfrich W. Elastic properties of lipid bilayers-theory and possible experiments. *Zeitschrift für Naturforschung C*. 1973; 28
20. Hu D, Zhang PWE. Continuum theory of a moving membrane. *Phys Rev E Stat Nonlin Soft Matter Phys*. Apr.2007 75(4 Pt 1):041605. [PubMed: 17500905]
21. Jackson MB. Minimum membrane bending energies of fusion pores. *J Membr Biol*. Oct; 2009 231(2–3):101–115. [PubMed: 19865786]
22. Kozlovsky Y, Chernomordik LV, Kozlov MM. Lipid intermediates in membrane fusion: formation, structure, and decay of hemifusion diaphragm. *Biophys J*. Nov; 2002 83(5):2634–2651. [PubMed: 12414697]
23. Kozlovsky Y, Kozlov MM. Stalk model of membrane fusion: solution of energy crisis. *Biophys J*. 2002 Feb; 82(2):882–895. [PubMed: 11806930]
24. Kuzmin PI, Akimov SA, Chizmadzhev YA, Zimmerberg J, Cohen FS. Line tension and interaction energies of membrane rafts calculated from lipid splay and tilt. *Biophys J*. Feb; 2005 88(2):1120–1133. [PubMed: 15542550]
25. Markin VS, Albanesi JP. Membrane fusion: stalk model revisited. *Biophys J*. Feb; 2002 82(2):693–712. [PubMed: 11806912]
26. Tristram-Nagle S, Nagle JF. Structure of Lipid Bilayers. *Biochim Biophys Acta*. Nov; 2000 1469(3):159–195. [PubMed: 11063882]

27. Ou-Yang, Z-C.; Liu, J-X.; Xie, Y-Z. Geometric Methods in the Elastic Theory of Membranes in Liquid Crystal Phases, Volume 2 of Advanced Series on Theoretical Physical Science. World Scientific; Singapore: 1999.
28. Pozrikidis C. Resting shape and spontaneous membrane curvature of red blood cells. *Math Med Biol.* 2005; 22:34–52. [PubMed: 15716299]
29. Ryham, R.; Ward, M.; Cohen, F.; Eisenberg, R. Calculating minimal energy shapes of fusion pores. Presented at the 97th Meeting of the Biophysical Society; Philadelphia, PA. Feb 2013;
30. Siegel DP. The Gaussian curvature elastic energy of intermediates in membrane fusion. *Biophys J.* Dec; 2008 95(11):5200–5215. [PubMed: 18805927]
31. Simon L. Existence of surfaces minimizing the Willmore functional. *Comm Anal Geom.* 1993; 1(2):281–326.
32. Sohn J, Tseng Y-H, Li S, Voigt A, Lowengrub J. Dynamics of multicomponent vesicles in a viscous fluid. *Journal of Computational Physics.* 2010; 229(1):119–144. [PubMed: 20808718]
33. Song P, Hu D, Zhang P. Numerical simulation of fluid membranes in two-dimensional space. *Commun Comput Phys.* 2008; 3(4):794–821.
34. Steigmann DJ. Fluid films with curvature elasticity. *Archive for Rational Mechanics and Analysis.* 1999; 150:127–152.
35. Stephen MJ, Straley JP. Physics of liquid crystals. *Rev Mod Phys.* Oct.1974 46:617–704.
36. Syganow A, von Kitzing E. (In)validity of the constant field and constant currents assumptions in theories of ion transport. *Biophys J.* Feb; 1999 76(2):768–781. [PubMed: 9929480]
37. Uchida N. Dynamics of orientational ordering in fluid membranes. *Phys Rev E.* Oct.2002 66:040902.
38. Wang W, Zhang P, Zhang Z. Well-posedness of hydrodynamics on the moving elastic surface. *Archive for Rational Mechanics and Analysis.* 2012; 206(3):953–995.
39. Willmore, TJ. Riemannian geometry. Oxford Science Publications, The Clarendon Press Oxford University Press; New York: 1993.
40. Yoo J, Jackson MB, Cui Q. A comparison of coarse-grained and continuum models for membrane bending in lipid bilayer fusion pores. *Biophys J.* Feb; 2013 104(4):841–852. [PubMed: 23442963]

FIG. 1.

Dimensions and discretization of axisymmetric fusion pores in the computational domain $0 < r < L = 40$ nm. In Panel A, the initial toroidal fusion pore used for calculations has pore radius $r_p = 7.0$ nm, bilayer separation $r_b = 3.0$ nm, and membrane thickness $2h_0 = 3.0$ nm. In Panel B, the fusion pore, with the same parameters, has deformed into a teardrop shape that minimizes energy.

FIG. 2.

Three potential configurations of a portion of monolayer with the same volume v . At rest (panel A), the monolayer has height h_0 and the unstretched surface has area a_0 . In panel B, flattening the monolayer can lead to a stretched surface; $a > a_0$. In panel C, extending the monolayer can lead to a compressed surface; $a < a_0$.

FIG. 3.

Cells expressing a viral fusion protein (known as effector cells) were loaded with a green fluorescent aqueous dye (calcein) and bound to unlabeled target cells. In the bound state, the effector and target cells flatten against each other over an extended region to effectively create two parallel membranes that abut. The left-hand panels show bound cells prior to fusion; the right-hand panel shows the same cell pairs post-fusion. The calcein has spread from effector to target cell post-fusion, so both effector and target cells appear green. The three cell pairs are from different experiments.

FIG. 4.

Energy of pore formation for symmetric bilayers as a function of pore radius r_p . The solid line is the case $k_0=k'_0=0 \text{ nm}^{-1}$, the hashed line represents $k_0=k'_0=-0.1 \text{ nm}^{-1}$, and the dashed line is for $k_0=k'_0=0.1 \text{ nm}^{-1}$. In panel A, $h_0 = 1.0 \text{ nm}$ and $r_b = 3.0 \text{ nm}$; in panel B, $h_0 = 1.5 \text{ nm}$ and $r_b = 3.0 \text{ nm}$; and in panel C, $h_0 = 2.0 \text{ nm}$ and $r_b = 3.0 \text{ nm}$. Energy of pore formation is a decreasing function of r_p when the spontaneous curvature of both monolayers is negative.

FIG. 5. Bowing shape of fusion pore changes with pore radius and spontaneous curvature. The pairs of lines are the distal and proximal neutral surfaces with $r_b = 3.0$ nm and $h_0 = 1.5$ nm for $r_p = 1.7$ nm (thick lines) and $r_p = 5.7$ nm (thin lines). Panel A: $k_0 = k'_0 = -0.1$ nm⁻¹; panel B, $k_0 = k'_0 = 0$ nm⁻¹; panel C, $k_0 = k'_0 = 0.1$ nm⁻¹. Bowing of the bilayer increases with pore radius, but decreases with spontaneous curvature.

FIG. 6.

Energy of pore formation for symmetric bilayers as a function of pore radius r_p for $h_0 = 1.5$ nm with varying bilayer separation. The light solid line is the case $r_b = 2.5$ nm, the heavy solid line is the case $r_b = 3.0$ nm, the dashed line is the case $r_b = 3.4$ nm, the hashed line is the case $r_b = 4$ nm, and the dotted line is the case $r_b = 5$ nm. Panel A: $k_0 = 0.0$, $k'_0 = 0.0 \text{ nm}^{-1}$; panel B, $k_0 = -0.1$, $k'_0 = -0.1 \text{ nm}^{-1}$; panel C, $k_0 = 0.1$, $k'_0 = 0.1 \text{ nm}^{-1}$. Note that energy of pore formation decreases with increasing bilayer separation.

FIG. 7.

Energy of pore formation for anti-symmetric bilayers as a function of pore radius r_p . The solid line is the case $k_0 = -0.1 \text{ nm}^{-1}$ and $k'_0 = 0.1 \text{ nm}^{-1}$ and the dashed line represents $k_0 = 0.1 \text{ nm}^{-1}$ and $k'_0 = -0.1 \text{ nm}^{-1}$. In panel A: $h_0 = 1.0 \text{ nm}$ and $r_b = 3.0 \text{ nm}$; panel B, $h_0 = 1.5 \text{ nm}$ and $r_b = 3.0 \text{ nm}$; panel C, $h_0 = 2.0 \text{ nm}$ and $r_b = 3.0 \text{ nm}$. Energy of pore formation decreases when the spontaneous curvature is positive (resp. negative) in the proximal (resp. distal) monolayer. In either case, pore expansion increases E for $r_p > 3 \text{ nm}$.

FIG. 8.

Energy of pore formation for asymmetric bilayers as a function of pore radius r_p . The solid line is the case $k_0 = -0.05$, $k'_0 = -0.15 \text{ nm}^{-1}$ and the dashed line represents $k_0 = -0.15$, $k'_0 = -0.05 \text{ nm}^{-1}$. Panel A: $h_0 = 1.0 \text{ nm}$ and $r_b = 3.0 \text{ nm}$; panel B, $h_0 = 1.5 \text{ nm}$ and $r_b = 3.0 \text{ nm}$; panel C, $h_0 = 2.0 \text{ nm}$ and $r_b = 3.0 \text{ nm}$. Energy of pore formation is a decreasing function of pore radius. The pore is more likely to expand if the spontaneous curvature is more negative in the proximal layer than in the distal layer.

FIG. 9.

Surface tension, σ , promotes pore expansion. In panel A, $k_0 = k'_0 = 0 \text{ nm}^{-1}$, and in panel B $-k_0 = k'_0 = 0.1 \text{ nm}^{-1}$, the hashed lines ($\sigma = 0.8 \text{ dyn cm}^{-1}$) slope downward, whereas the solid lines ($\sigma = 0 \text{ dyn cm}^{-1}$) (see also Figure 4B and Figure 7B), slope upward. The dashed lines represent $\sigma = 0.4 \text{ dyn cm}^{-1}$. For all curves, $h_0 = 1.5 \text{ nm}$, $r_b = 3 \text{ nm}$.

FIG. 10.

Teardrop shapes minimize energy when bilayers meet the boundary of the computational domain at an angle α ; $L = 20$, $N = 100$, $h_0 = 1.5$, $r_p = 3.5$ nm, and $k_0 = k'_0 = 0 \text{ nm}^{-1}$. In panel A, $\alpha = 0$ rad, $r_m = 3$ nm, and $E = 192$ kT; in panel B, $\alpha = 0.1$ rad, $r_m = 4.02$ nm, and $E = 160$ kT; in panel C, $\alpha = 0.2$ rad, $r_m = 5.02$ nm, and $E = 152$ kT.