CALCULATING MINIMAL ENERGY SHAPES OF FUSION PORES

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OVERVIEW

Fusion between membranes involves large scale deformations and in its final stage leads to an hourglass shaped membrane called a fusion pore. Creation of a fusion pore from two initially separated, planar bilayers requires energy (kT) but the process facilitating fusion is poorly understood and the minimum amount of energy required is not presently known. Continuum elastic theory quantifies the energy of the membrane through the generalized Helfrich bending energy [3]

$$E = \int_{\Sigma_P \cup \Sigma_D} \frac{k_B}{2} |\text{div } \mathbf{d} - k_{0,D,P}|^2 + \frac{k_T}{2} |\mathbf{d} - \mathbf{n}|^2 \, dA$$

$k_B = 10$ kT is splay modulus
$k_T = 10$ kT nm^{-2} is tilt modulus [7]
$k_{0,D,P}$ nm^{-1} is spont. curv.; -0.1 (DOPC), 0.07 (DOPS), -0.33 (DOPE) [2]
Σ_P, Σ_D are proximal and distal neutral surfaces
$\mathbf{d} = \mathbf{D}/|\mathbf{D}|$, $\mathbf{n}$ are lipid and normal directors
$R_p =$ radius, $R_b =$ length are pore dimensions

NUMERICAL METHOD AND CALIBRATION

The bending energy is approximated by discretizing the fusion pore into piecewise linear finite elements:

$$E_{\text{Total}} = \sum_{j=1}^{N} \boxed{\frac{k_B}{2} \left(|\text{div } \mathbf{d}_{j,D} - k_{0,D}|^2 A_{j,D} + |\text{div } \mathbf{d}_{j,P} - k_{0,P}|^2 A_{j,P} \right)} \text{ (splay)}$$

$$+ \boxed{\frac{k_T}{2} \left(|\bar{\mathbf{d}}_{j,D} - \mathbf{n}_{j,D}|^2 A_{j,D} + |\bar{\mathbf{d}}_{j,P} - \mathbf{n}_{j,P}|^2 A_{j,P} \right)} \text{ (tilt)}$$

$$+ \boxed{k_S |\bar{\mathbf{D}}_{j,P} + \bar{\mathbf{x}}_{j,P} - \bar{\mathbf{D}}_{j,D} - \bar{\mathbf{x}}_{j,D}|^2 \frac{A_{j,P} + A_{j,D}}{2}} \text{ (steric)}$$

$$+ \boxed{k_E \left(g(\bar{\mathbf{D}}_{j,D}, h_0) A_{j,D} + g(\bar{\mathbf{D}}_{j,P}, h_0) A_{j,P} \right)} \text{ (extensional)}$$

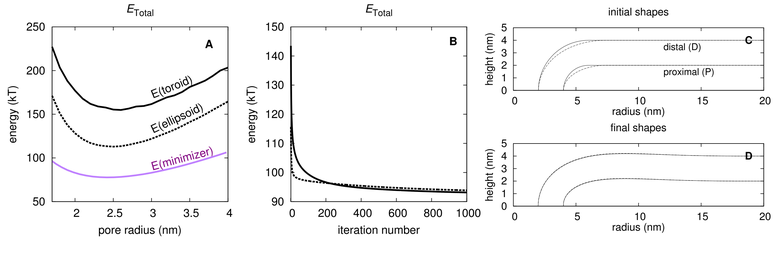


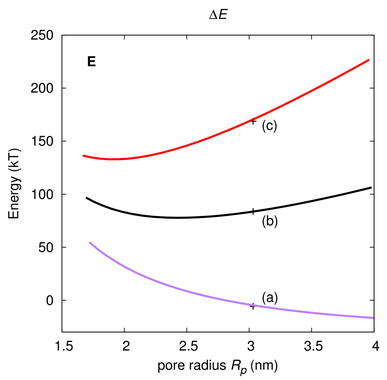
Figure A: Energy as a function of pore radius R_p (with fixed length $R_b = 3.0$ nm and $k_{0,D} = k_{0,P} = 0.0$) for toroidal (solid), ellipsoidal (dashed), and minimal (purple) fusion pore. Figure B: Energy as a function of the number of gradient descent iterations for initially toroidal (solid) and ellipsoidal (dashed) pores. The two energies converge monotonically to the same, stable value as the the iteration number becomes large. Although the initial shape varied, (Figure C, initial shape of toroidal (solid) and ellipsoidal (dashed) pores) the final, energy minimizing shape of the pore is the same (Figure D, overlapping curves).

MINIMAL ENERGIES OF FUSION PORES

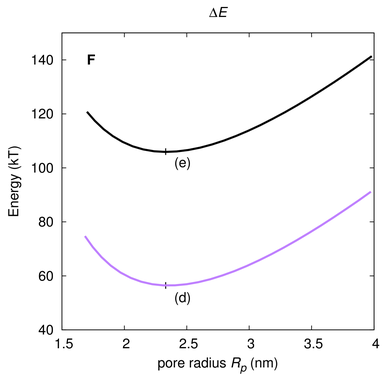
Lipid composition determines the value of spontaneous curvature $k_{0,D}$ and $k_{0,P}$ and affects the energy of the minimizing shape. The energy of a planar monolayer need not be 0, but depends on the area A. We define energy of pore formation ΔE by

$$\Delta E = E - \frac{k_B}{2} k_{0,D}^2 A(\Sigma_I) - \frac{k_B}{2} k_{0,P}^2 A(\Sigma_O)$$

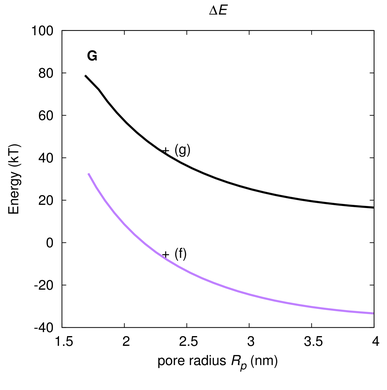
Symmetric Bilayers: Energy of pore formation, $R_b = 3.0$. When $k_{0,D} = k_{0,P} = 0.1$ (red curve) and 0.0 (black curve), energy possess a minimum. When $k_{0,D} = k_{0,P} = -0.1$, energy is decreasing, promoting pore expansion. In the present study, the energies (a) -4.4, (b) 82, and (c) 165 kT were recorded at $R_p = 3.0$ nm, and each is smaller than the previously reported energies of 78, 125, and 182 kT [4].



Anti-Symmetric Bilayers: Energy of pore formation, $R_b = 3.0$. When $-k_{0,D} = k_{0,P} = 0.05$ (black curve), energy is greater than when $k_{0,D} = -k_{0,P} = 0.05$ (purple curve), promoting fusion but not pore expansion. In the present study, the energies (d) 57 and (e) 106 kT were recorded at $R_p = 2.3$ nm, and each is smaller than the previously reported energies of 82 and 150 kT [4].



Asymmetric Bilayers: Energy of pore formation, $R_b = 3.0$. When $k_{0,D} = -0.15$, $k_{0,P} = -0.05$ (black curve), energy is greater than when $k_{0,D} = -0.05$ and $k_{0,P} = -0.15$ (purple curve), but both are less than the anti-symmetric case. Pore formation and expansion are promoted by negative spontaneous curvature. In the present study, the energies (f) -4 and (g) 44 kT were recorded at $R_p = 2.3$ nm, and each is smaller than the previously reported energies of 37 and 100 kT [4].

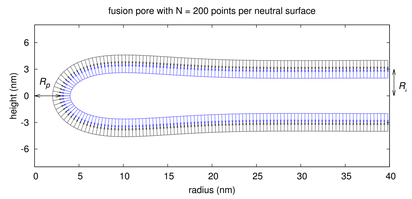


DISCUSSION

It is well known that the fusion pore energy E depends heavily on membrane shape [1, 4, 8] and can lead to variances of up to tens to hundreds of kT for the same pore dimensions R_b and R_p. In prior studies, membrane shapes have either been assumed [1, 6, 9] or treated by self consistent approximations constrained to low-order polynomials [4].

Here, fusion pore energy minimization allows for a full range of deformations. Energy minima have been observed (Figures E, F, and G) that are significantly smaller than previously reported values.

In all cases, the bilayer corrects for the large deformation at the pore lumen by overshooting the pore length, sometimes by several nm's. Tilt energy is negligible in fusion pores for radii larger than 1.5 nm and accounts for at most 6% of the total energy density (data not shown).



Energy of pore formation can be negative (Figures E and G). Generally, energy decreases when the proximal monolayer has negative spontaneous curvature (Figure F). More consequently, if both proximal and distal monolayers have negative spontaneous curvature, whether symmetric or asymmetric, minimal energy decreases as a function of pore radius R_p (Figures E and G), predicting that some fusion pores will widen independently of external forces.

For other lipid compositions, pore energy is minimal for a critical radius $R_p \approx 2.3$ nm and follows the general trend observed in other studies, although with significantly smaller values of energy.

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