Take-home message

- Dimension dominates behaviour, membranes are not like random walks
- Membrane models on different scales can only exhibit properties present in the model
- Vesicle fusion exhibits competing relaxation processes under non-equilibrium membrane deformations

Macroscopic membrane models

- Membranes as fluctuating surfaces
- Random walks and phantom membranes (1d and 2d)
- Elasticity theory of membranes
- Discretized membranes, triangulated surfaces, Monte Carlo simulations
- Polymerized membranes, auxetic materials, cytoskeleton
- Membrane as a barrier, multicomponent membranes
- Domains, budding and fusion in membranes

Membranes on macroscopic scale

Cell size / membrane thickness ~ 10 micron/4 nm = 2500

the PM is a very thin, flexible fluid sheet when viewed on the scale of the whole cell. We can ignore the molecules and treat the membrane as an infinitely thin elastic sheet.

How do we mathematically describe a membrane at this scale?

A 2-dimensional surface requires 2 in-plane coordinates (x_1, x_2).

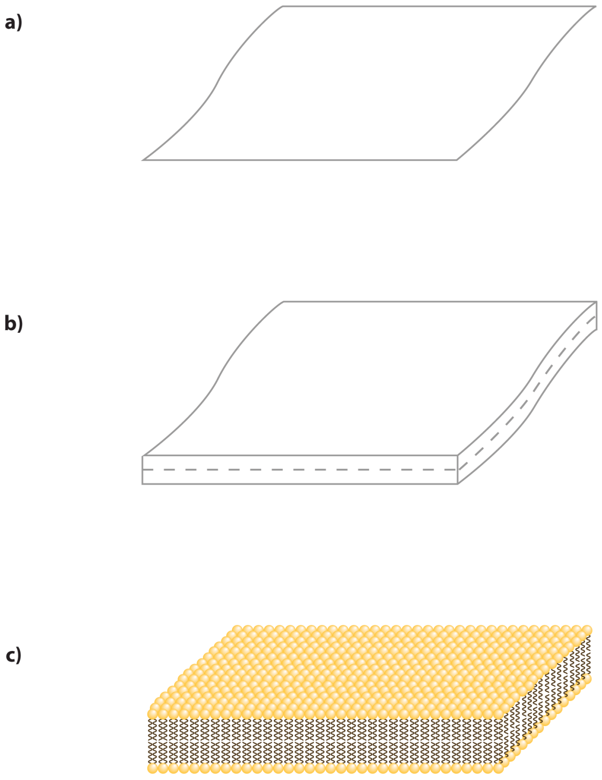
But in 3D, each point has 3 coordinates.

Nearly flat membranes can be described by Monge representation, where the height is specified at the projected (x,y) coordinates

$h(x_1, x_2)$ = function of (x, y) in the projected plane.

e..g, $h(x, y) = h0*\sin(k_x x)*\cos(k_y y)$

Now we have a coordinate system for the membrane surface, but what controls its dynamics?



Membrane models

We can construct a range of membrane models similar to the range of random walks (see Lecture 2 - random walk, Gaussian chain, self-avoiding walk, wormlike chain, rigid polymer)

RW	$< R_{ee}^2 > = N.a^2$
Gaussian chain	$p(R) = \mathrm{const.}\ \exp(-d.R^2/2.N.a^2)$
Wormlike chain	$p(\{\mathbf{r}(s)\}). \sim e^{-\beta H(\mathbf{r}(s))}$ $H(\mathbf{r}(s)) = \kappa/2 \int \mid \partial\mathbf{r}/\partial s\mid^2 ds$
Rigid polymer	$< R_{ee} > = N.a$

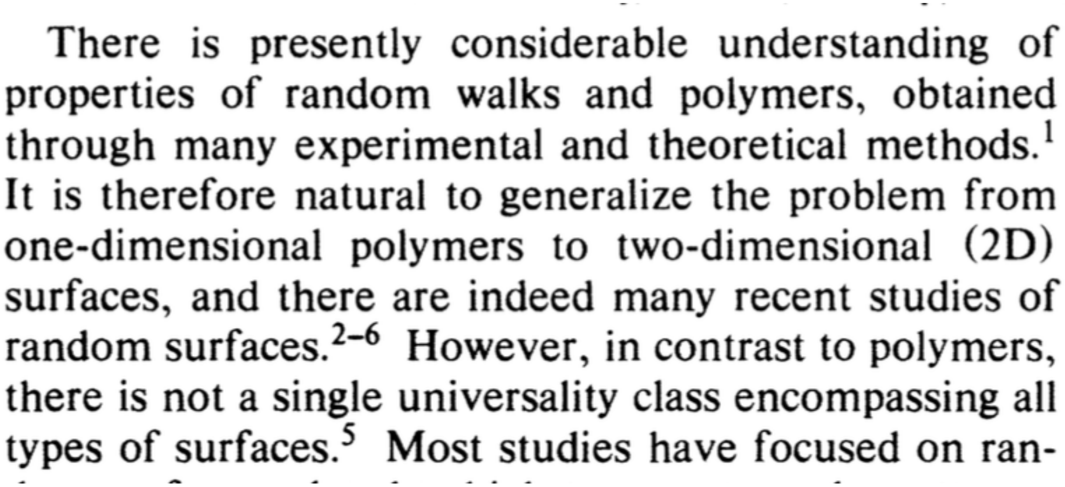
The properties of each type of walk are determined by the energy (if non-zero) associated with conformations.

The shape of a 2d membrane (or surface) is similarly determined by the energy associated with its conformations (or entropy if there is no energy function). But there is extra connectivity in 2d.

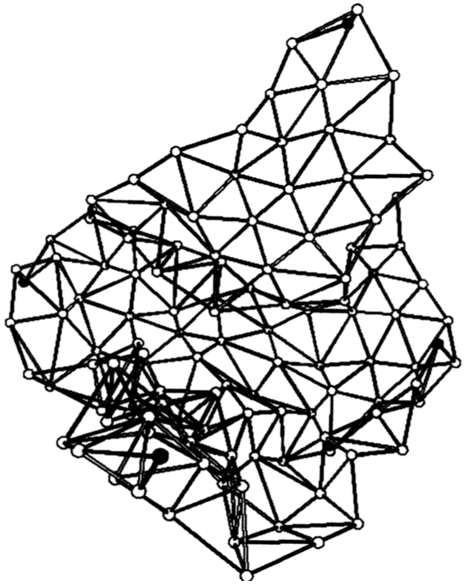
Membranes as random surfaces

In 1980s, a lot of interest in random surfaces, usually triangulated networks.

Quoting Kantor, Kardar and Nelson, PRL 57:791 (1986)



There is presently considerable understanding of properties of random walks and polymers, obtained through many experimental and theoretical methods.[1] It is therefore natural to generalize the problem from one-dimensional polymers to two-dimensional (2D) surfaces, and there are indeed many recent studies of random surfaces.[2–6] However, in contrast to polymers, there is not a single universality class encompassing all types of surfaces.[5] Most studies have focused on ran-



If the surface is self-avoiding, its radius of gyration is:

$$R_g \sim L^{\nu}$$

where Flory theory gives: $\nu = 4 / (d + 2)$ cp. $3 / (d + 2)$ for RWs

Flory theory gives: $\nu = D + 2 / (d + 2)$ for D-dim. objects embedded in d-dim. space.

Statistical Mechanics of Membranes and Surfaces, eds. D. Nelson, T. Piran, S. Weinberg, World Scientific Publishing, Singapore, 2004

Phantom membrane

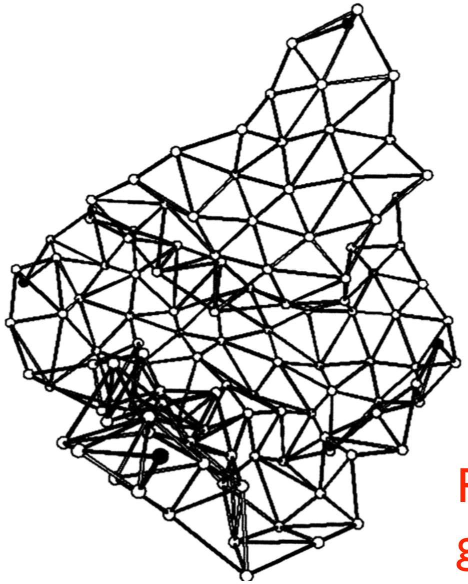
A phantom membrane or random surface is the generalisation of a simple RW to 2d

It has some unusual properties …

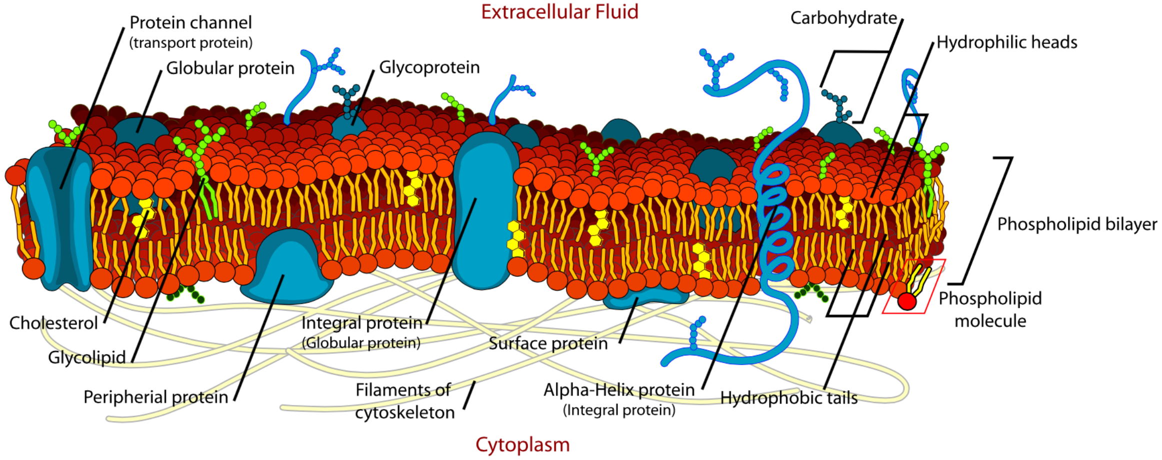
… it can collapse …

… it is non-volume preserving
(has a negative Poisson ratio)

more on this later today



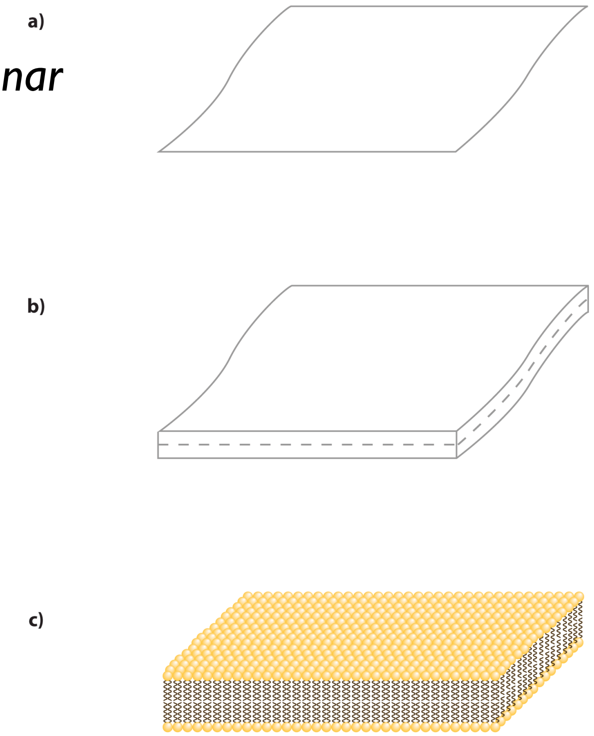
Random surfaces are **not** a good model for biomembranes



Elasticity theory of membranes

To go beyond a random surface model, we need to know the *energy* of membrane conformations: biological membranes do not self intersect!

Canham-Helfrich Hamiltonian gives the energy cost of smooth *near-planar* shapes of the cellular plasma membrane in terms of two parameters: a **bending stiffness κ** and **spontaneous curvature c_0**



$$H = \kappa/2 \oiint dA \, (c_1 + c_2 - c_0)^2$$

where $c_1(x,y)$, $c_2(x,y)$ are the local curvatures at any point (x,y) of the surface. If $c_0 = 0$, the preferred membrane conformation is planar.

For symmetric lipid membranes $\kappa \sim 10 - 25\ k_BT$, $c_0 \sim 0$

This is the first term in an expansion of the energy in powers of the curvature, recall definition of curvature: let a curve be defined parametrically by $\mathbf{r}(s)$ and the local tangent $\mathbf{t}(s)$

$$\mathbf{c}(s) = d\mathbf{t} / ds = d^2\mathbf{r}(s) / ds^2$$

P. B. Canham, The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cells, J. Theor. Biology 26:61-81 (1970)

W. Helfrich, Elastic properties of lipid bilayer membranes: theory and possible experiments, Z. Naturforschung C 28:693-703 (1974)

Red blood cells are dominated by symmetry

RBCs take up a wide variety of shapes controlled by physical constraints arising from environment and the energy of deforming the PM:

Inner volume is fixed
Plasma membrane area is fixed (# lipids constant)

(at constant temperature, osmolarity)

Canham-Helfrich Hamiltonian gives the energy cost of near-spherical shapes of the cellular plasma membrane:

$$H = \kappa/2 \oiint dA\ (c_1 + c_2 - c_0)^2$$

where $c_1(x,y)$, $c_2(x,y)$ are the local curvatures at any point (x,y)of the surface, $c_0(x,y)$ is the spontaneous curvature, and κ is the bending rigidity (units of energy); for pure lipid membranes $\kappa \sim 10$ - 25 k_BT

P. B. Canham, The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cells, J. Theor. Biology 26:61-81 (1970)

W. Helfrich, Elastic properties of lipid bilayer membranes: theory and possible experiments, Z. Naturforschung C 28:693-703 (1974)

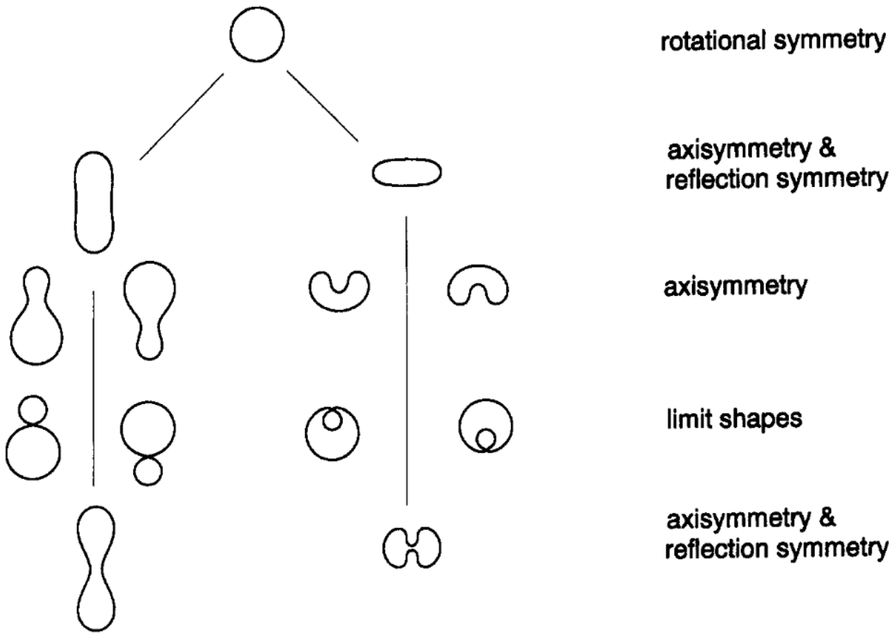


Figure 11. Bifurcation scheme with stationary shapes and their symmetry.

U. Seifert, Advances in Physics, 46:13-137 (1997)

RBC Lifetime ~ 120 days,
circulation ~ 20 sec,
bend through capillaries 1/2 its diameter;

polystyrene balls of the same size fall apart after ~10 deformations

Theory or Monte Carlo simulations?

Given a Hamiltonian, we can do two things:

1) Try and find an analytic solution to membrane properties

2) Try doing Monte Carlo simulations of the membrane

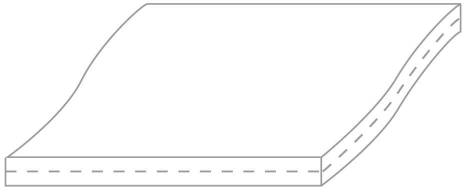
The first one has the advantage that we can understand the whole range of membrane behaviour for all parameter values;

The MC simulations have the advantage that we can apply them even when we cannot find solutions to 1).

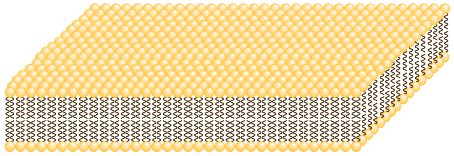
But we need to discretize the membrane if we want to go beyond very simple (i.e., symmetric) shape calculations and particularly if we want to do simulations.

a)

b)



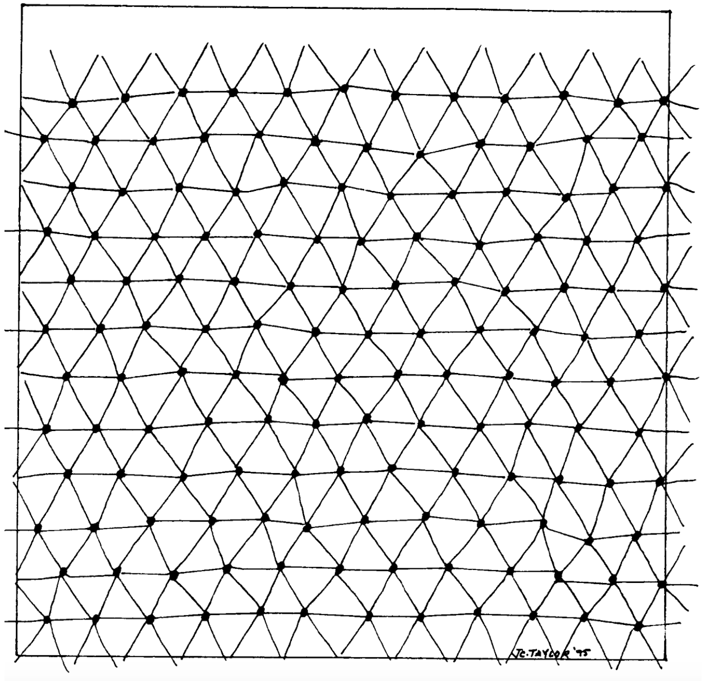
c)



Discretized membranes

Analytic theories of membrane shape are only useful for highly symmetric cases.
MC simulations have been used for many purposes because they are general and powerful.

- Consider a 2d tethered (i.e. non-fluid) hexagonal membrane (e.g. fish net)
- N_V vertices (x, y) connected by $N_E = 3 N_V$ Hookean springs to 6 neighbours
- Applied pressure p ($p < 0$ stretches the membrane)
- Use NpT Monte Carlo simulations to study the membrane properties



Planar membrane

$$V(s_{ij}) = \frac{1}{2} k \left| s_{ij} - s_h \right|^2, \qquad 0 < \left| s_{ij} \right| < \infty,$$

$$A_V = \frac{\sqrt{3}}{2} s^2$$

$$E_V = \frac{3}{2} k (s - s_h)^2,$$

MC Moves

Each vertex (x, y) moves in plane ~ $e^{-\beta H(\{\mathbf{x,y}\})}$

Area changes ~ $e^{-\beta \Delta A}$

What does it do?

Springs in 1d and 2d

Before we look at a network, let's compare simple Hookean springs in 1 and 2 dimensions.

We *just* need to calculate the partition function to get mean values.

$Z = \int d\mathbf{x}\, e^{-\beta H(\{\mathbf{x}\})}$ $\langle A \rangle = 1/Z \int d\mathbf{x}\, A(\{\mathbf{x}\})\, e^{-\beta H(\{\mathbf{x}\})}$

1) 1d Hookean spring with an unstretched length $s_h = 0$ under tension subject to thermal fluctuations?

What are $\langle s \rangle$, $\langle s^2 \rangle$? (Blackboard calculation)

$$V(s_{ij}) = \frac{1}{2} k \left| s_{ij} - s_h \right|^2, \qquad 0 < \left| s_{ij} \right| < \infty,$$

$Z = \sqrt{(k_B T / 2k)}$

$\langle s \rangle = \sqrt{(2 k_B T / \pi k)}$

$\langle s^2 \rangle = k_B T / k$

which predicts: 1) average length increases with increasing temperature, 2) decreases with increasing spring constant. This is just an elastic spring subject to thermal fluctuations.

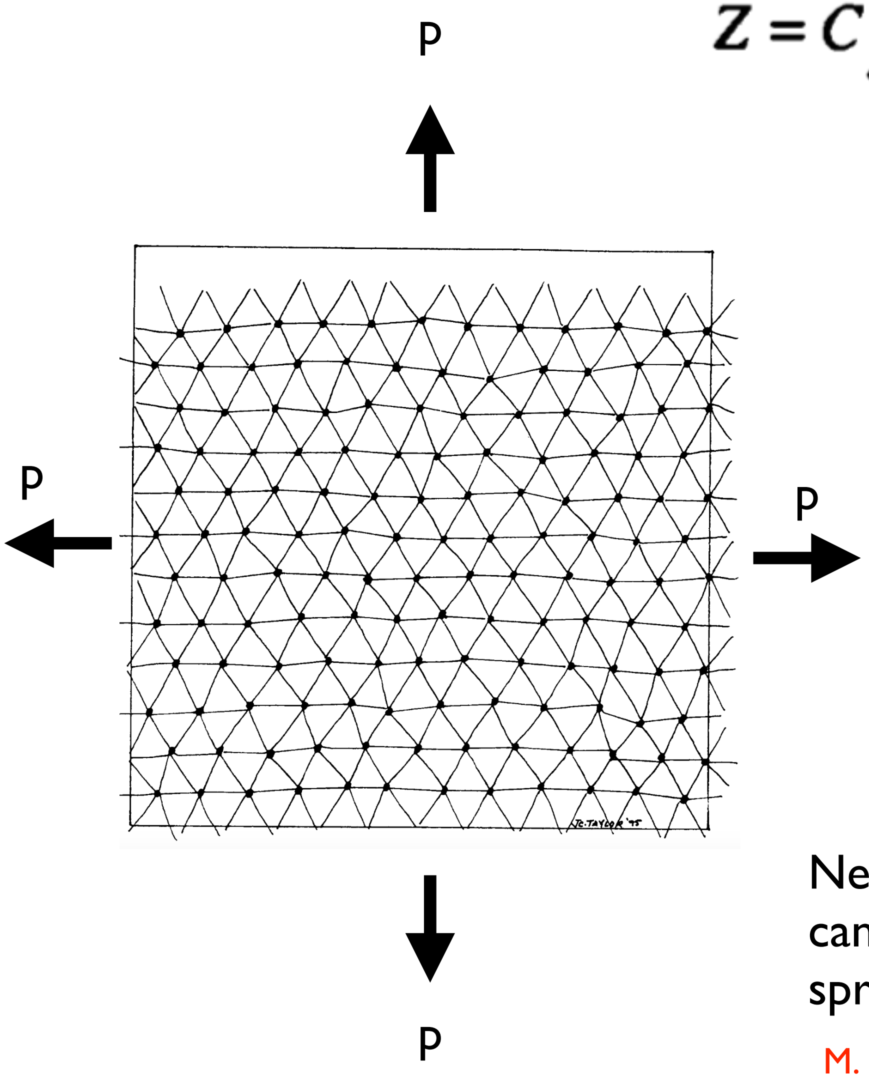
2) With a non-zero unstretched length or applied force we cannot calculate it in closed form in 1d.

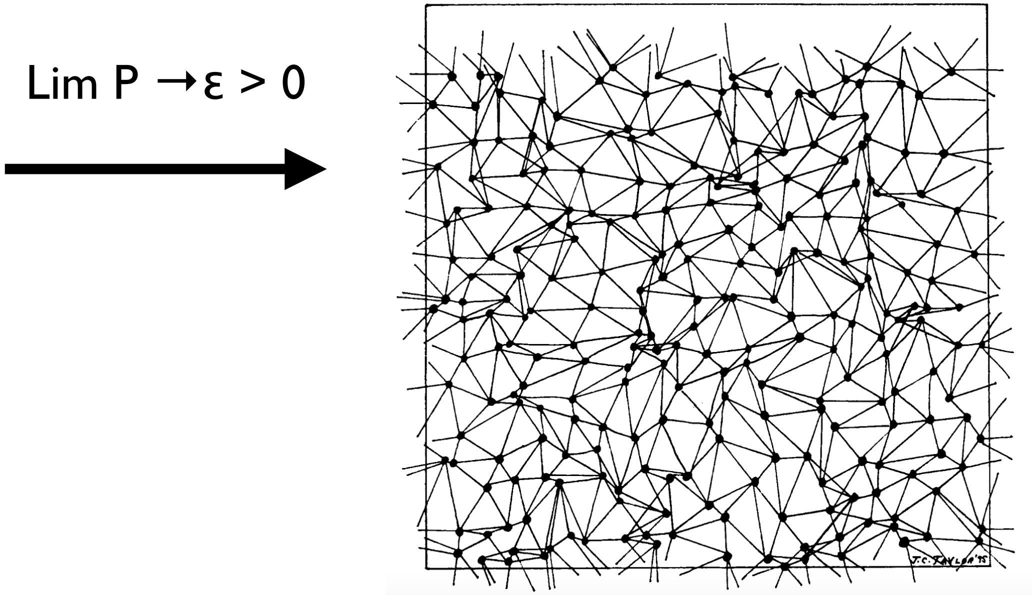
Collapse transition of phantom network in 2d

$$Z = C\int_0^\infty e^{-\beta p A_v} . e^{-\beta E_v} \, dA_v$$

$$A_v = \frac{\sqrt{3}}{2} s^2$$

$$E_v = \frac{3}{2} k (s - s_h)^2,$$





Network collapses at a finite (stretching) pressure because it can maximise its entropy while not costing energy by allowing springs to overlap and produce "lines" of equal length springs

M. F. Thorpe and E. J. Garboczi, Elastic properties of central-force networks with bond length mismatch, Phys. Rev. B. 14:4771 4775 (1990)

Expansion of 1d spring

A 1d Hookean spring under a (finite) tension F simply oscillates about a mean length. But even this is hard to calculate.

Given $H(x, F) = 1/2\, k\, x^2 - F\, x$

when $F = 0$, $\langle x \rangle = k_B T / k$

$F > 0$, $\langle x \rangle = \exp(\beta F^2 / 2k) \,.\, \int dy \exp(-1/2\, \beta k y^2)$ from $y = -F/k$ to ∞

where $y = x - F/k$. But this integral cannot be done in closed form because of the lower limit. But $\langle x \rangle$ remains finite for any finite force.

Unlike the 1d RW case, the 2d phantom network has unusual behaviour because the energy density due to the stretched springs can be less than the (negative) energy density of the applied (stretching) tension. This is a result of the phase space factor in 2d ($dA = s\, ds$) compared to 1d (dx).

But a 2d network is even harder to calculate … this is the partition function:

$$Z = \sqrt{3}C\, e^{-\frac{3}{2}\beta k \bar{p}(1+\bar{p})\bar{s}^2} \left[\bar{s}\sqrt{\frac{2\pi}{3\beta k(1+\bar{p})}} + \frac{2}{3\beta k(1+\bar{p})} e^{-\frac{3}{2}\beta k(1+\bar{p})\bar{s}^2} + 2\bar{s}\int_0^{\bar{s}} e^{-\frac{3}{2}\beta k(1+\bar{p})y^2}\, dy \right],$$

Expansion instability of 2d network

$$s_p = \langle s \rangle = \frac{s_h}{1+\bar{p}} + \frac{1}{3\beta k s_h} + \cdots,$$

$\bar{p} = p / \sqrt{3}\,k$, $p < 0$ for tension

$$\Delta s_p^2 = \langle s^2 \rangle - \langle s \rangle^2 = \frac{1}{3\beta k(1+\bar{p})} + \cdots,$$

$\langle s \rangle \to \infty$ when $p = \sqrt{3}\,k$

Bulk modulus K = -1/A ∂A / ∂p

$$K = \frac{\sqrt{3}}{2} k \left(1 + \frac{p}{\sqrt{3}k} \right)$$

Shear modulus

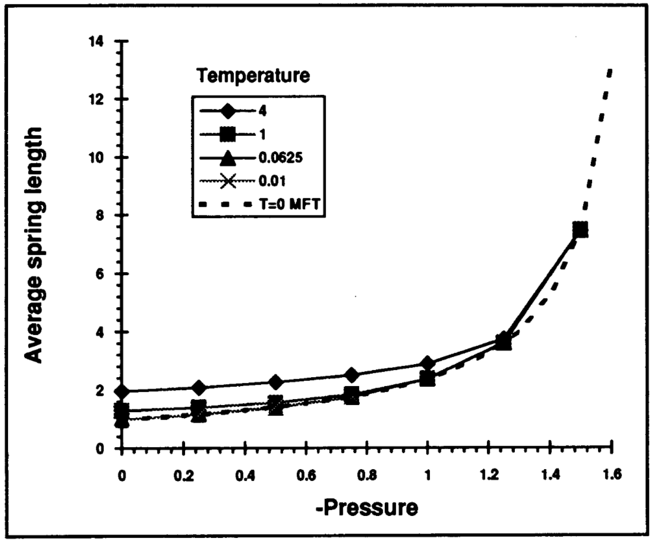
$$\mu = \frac{\sqrt{3}k}{4} \left(1 - \frac{\sqrt{3}p}{k} \right)$$

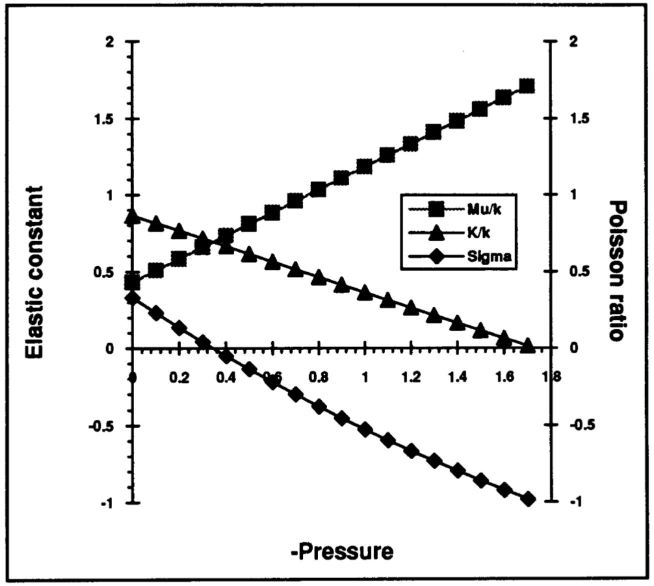
(K and μ must be positive for stability)

Poisson ratio = K - μ / (K + μ)

$$\sigma = \frac{1 + 5\frac{p}{\sqrt{3}k}}{3 - \frac{p}{\sqrt{3}k}}$$

This is the ratio of the transverse strain to the longitudinal strain and is usually positive for, e.g., rubber or steel.





Auxetic materials

The Poisson ratio does NOT have to be positive for stability; a negative ratio means that a substance *expands* transversely when *stretched* longitudinally. Such materials are called "auxetic".

R. Cuerno et al. Universal behaviour of crystalline membranes: Crumpling transition and Poisson ratio of the flat hase, Phys. Rev. E 93:022111 (2016)

A crumpled newspaper
has a negative poisson ratio!

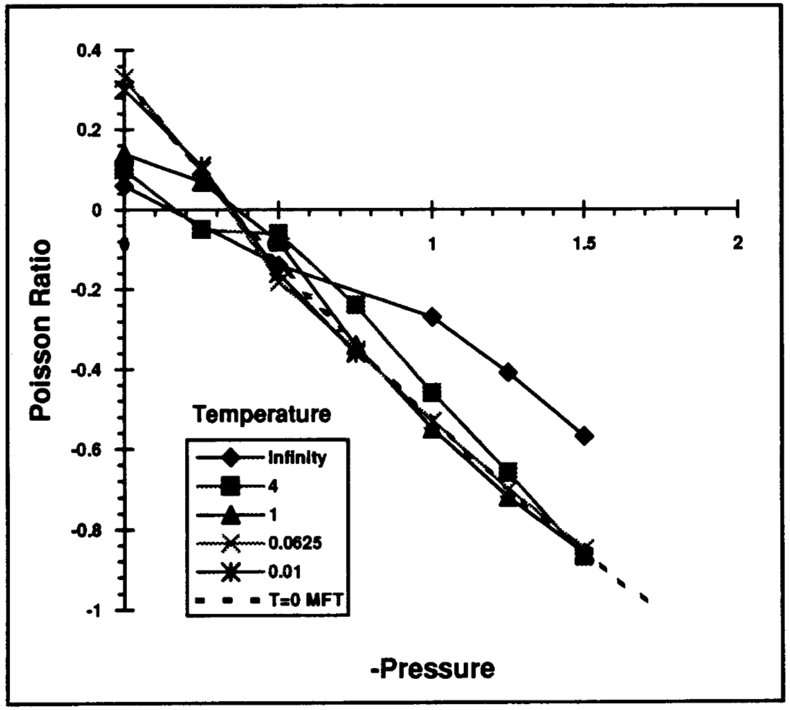


Figure 4.10 Poisson ratio σ against pressure p/k for self-avoiding Hooke's law network at all temperatures k_BT/ks_h^2 considered. N=144 vertices. The T=0 MFT prediction from Eq. (3.33) is shown for comparison.

Cellular cytoskeleton is a polymerized network

Beneath the plasma membrane is a 2d, polymerized network of "springs" made of spectrin/actin/ankyrin, etc. Although the contour length of spectrin is ~ 200 nm, the vertex-to-vertex distance is only about ~50 nm in the RBC (Nans et al. 2011)

It provides shear rigidity and compression resistance to the fluid lipid bilayer and has a negative Poisson ratio!

Simulations of RBC membranes (lipid bilayer + spectrin network) have been done, and micropipette experiments have been simulated.

A. Nans, N. Mohandas, and D. L. Stokes, Natice ultrastructure of the red cell cytoskeleton by cryo-electron tomography, Biophys. J. 101:2341-235- (2011).

FIG. 2. Sample membrane configuration at $\kappa = 2$ in the flat phase for $L = 24$.

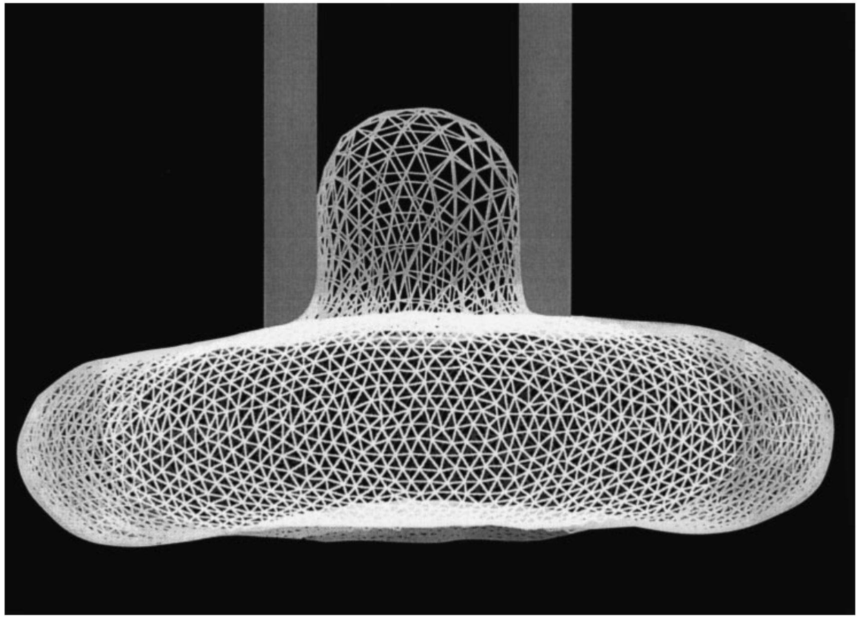


FIGURE 1 Simulation of a small erythrocyte under aspiration. The micropipette, indicated by the solid gray shading, has an inside diameter of $12s_R = 0.9$ μm. The surface of the cell is triangulated with 6110 vertex nodes that represent the spectrin-actin junction complexes of the erythrocyte cytoskeleton. The volume of the cell is 0.6 times the fully inflated volume, and the simulation is drawn from the stress-free model in the free shape ensemble, as described in the text.

D. Discher, D. H. Boal and S. K. Boey, Simulations of the erythrocyte membrane at large deformation, Biophys. J. 75:1584 - 1597 (1998).

Relevance of negative poisson ratio???

Breaching the barrier

Cellular membranes form an impermeable barrier for good reason:

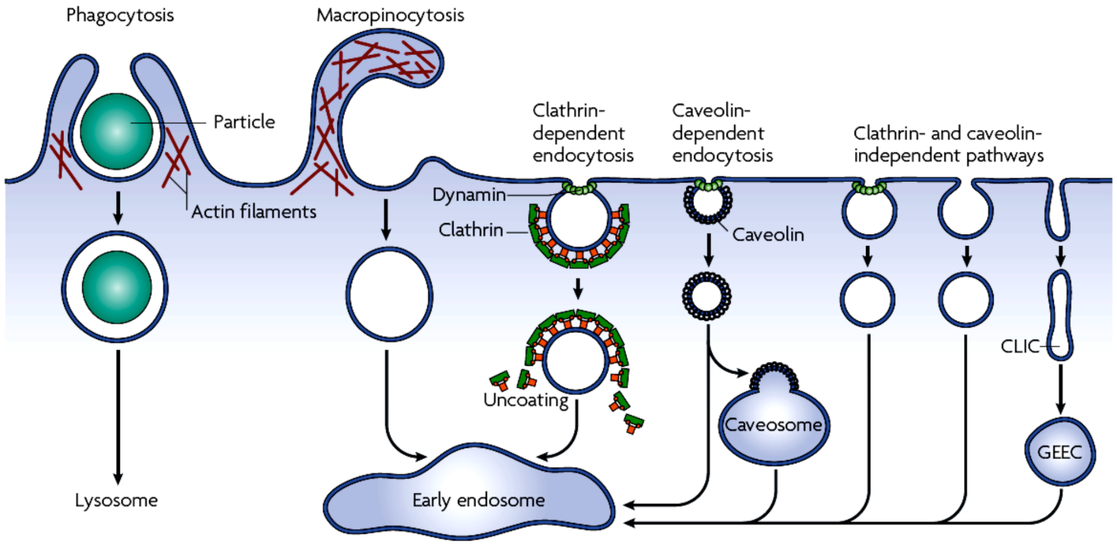
- ionic concentrations inside and out are different (esp. for neurons)
- Lysosomes contain low pH and would kill itself, endosomes carry materials, mitochondria need a large electron gradient
- bacteria and viruses would invade and kill cell
- DNA, RNA and proteins would diffuse away
- osmotic stress or other gradients would swell or shrink the cell

But the cell still needs to breach membranes for its own purposes:

- uptake of nutrients (endocytosis)
- expulsion of waste products (exocytosis)
- fusion (sperm and agg)
- transport vesicle fusion (ER to Golgie transport)

So, how does a cell stop unwanted pores appearing but create them when necessary?

Endocytosis



Retrograde transport, C. Wunder, Ludger Johannes, Curie Inst. France

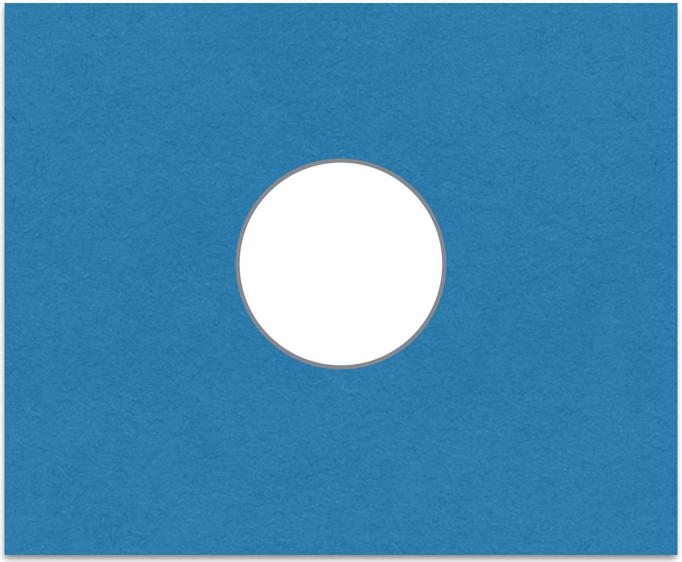
How can we quantify the energy cost of pore formation? What work is done?

Transmembrane proteins span the bilayer and some peptides (e.g., magainin, mellitin are anti-bacterial agents) enable pore formation, how?

- exert force on surrounding membrane - “tear it apart”?
- modify lipid environment and so reduce the hydrophobic effect, membrane “dissolves”?
- interact with each other and form a pore, e.g., barrel stave?

Classical theory of pore formation

Litster in 1975 proposed a simple theory for how pores should behave in the plasma membrane of cells. A pore in a flat membrane has an area and circumference. Assume:



- Membrane is flat and incompressible
- Pore is circular with radius R
- Pore is energy dominated (temperature is irrelevant)
- Membrane is under tension Γ (energy/area)
- Pore rim has energy cost λ (energy/length)

Energy cost of a pore is then

$$E(R) = 2\pi R\lambda - \pi R^2\Gamma$$

Minimising this gives a critical pore radius:

$$R^* = \lambda / \Gamma$$

Pores smaller than this shrink while larger ones grow until they rupture the membrane. But there is an energy barrier to the pore growing:

$$E(R^*) = \pi\lambda^2 / \Gamma$$

Litster, J. D. 1975. Stability of lipid bilayers and red blood cell membranes. ***Phys. Lett.*** A. 53: 193-194.

Classical theory of pore formation

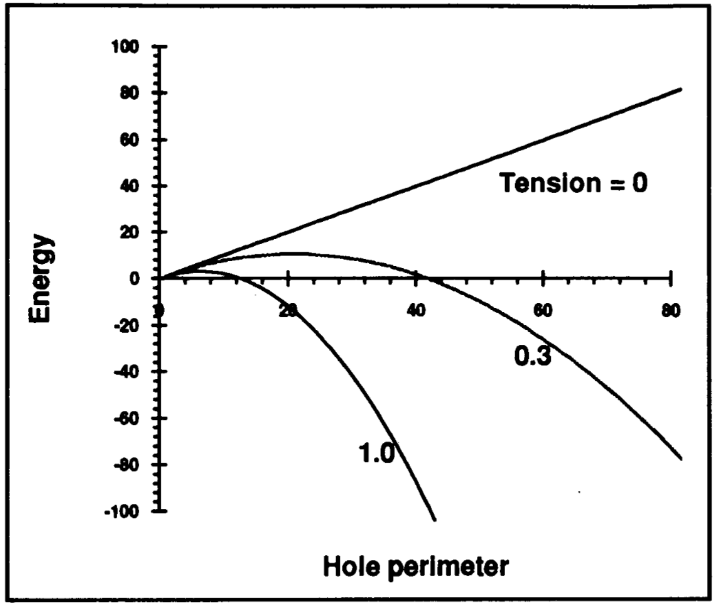
Relevance to biological membranes?

- Cells can be under osmotic stress ⇒ tension

- Lipid bilayer can only stretch a tiny amount before it ruptures
- A pore requires that lipids on the boundary rearrange

  ⇒ edge energy or line tension (stretched and tilted)

- What is the effect of temperature?
- Are holes stationary? Can they merge?



From experiments:

$\Gamma \sim 3$ dyn/cm for SOPC[1], 10 dyn/cm for RBCs[2]

$\lambda \sim 10^{-6}$ dyn for SOPC[3]

$R^* = \lambda / \Gamma \sim 1/3\ 10^{-6}$ cm ~ 3 nm

$\Delta E(R^*) = \pi\lambda^2 / \Gamma \sim 10^{-19}$ J ~ 24 $k_B T$

So, spontaneous pores are very unlikely in an unperturbed SOPC membrane.

[1] Evans and Needham, J. Phys. Chem. 91:4219-4228 (1987)

[2] Needham and Hochmuth, Biophys. J. 55:1001-1009 (1989)

[3] Zhelev and Needham, BBA. 1147:89-104 (1993)

Extended theory of pore formation

Revisit the assumptions

- Litster’s theory assumed circular pores so only **energy** is relevant: at finite temperature, the membrane fluctuates both in plane and out of plane, so the pore boundary can change shape and may not be circular; the **free energy** is then important: $G = E + PV - TS$
- Lipids on the boundary have greater freedom to move than those in the bulk membrane, so the entropy of their extra configurations reduces the pore edge energy
- Proteins and peptides may bind to/insert into the membrane and nucleate pores or aggregate at their boundary modifying the edge energy, this can stabilise membrane or make pores grow
- Nothing prevents spontaneous pore formation so multiple pores can appear
- Lipid membrane is a fluid, so pores can “diffuse” and merge to form larger ones

Can we extend the theory to multiple pores and include the effects of temperature, pore shape fluctuations and proteins?

Thermodynamics of pores in lipid bilayers

Consider an MC simulation of a 2d network of N vertices connected by *fluid* edges

- pores are formed by removal of a single edge with a probability $e^{-\beta q}$ controlled by a barrier height **q**.

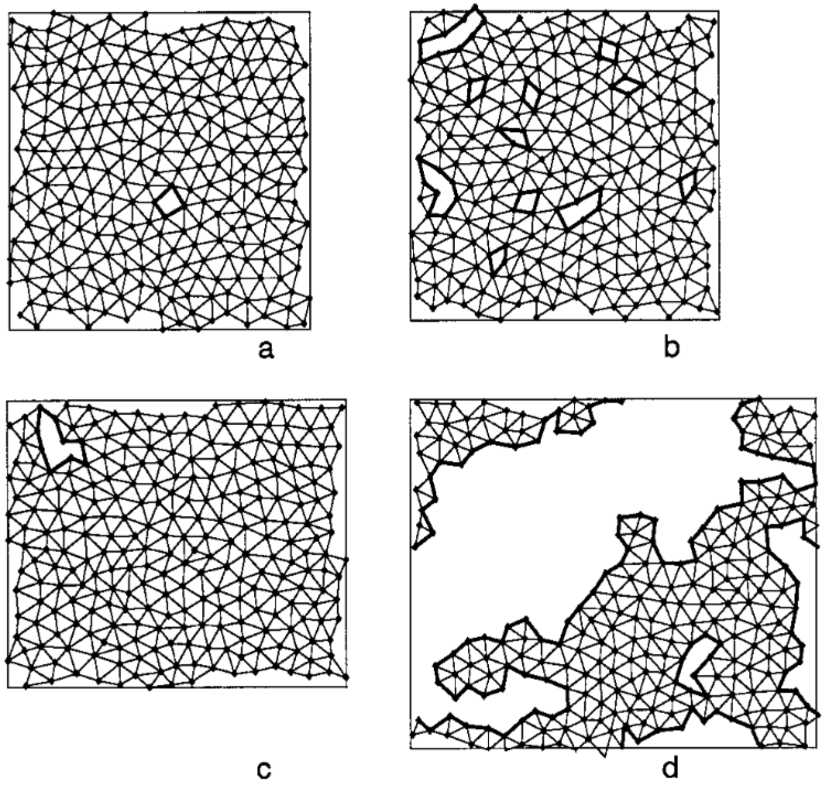
- pores grow/shrink by removal/addition of edges around their rim with an edge energy cost λL controlled by a line tension parameter **λ**

Gibbs free energy of a single pore:

$$G(\lambda, p, T) = \lambda\langle L\rangle + p\langle A\rangle - TS(\langle L\rangle)$$

λ = line tension around pore edge (J / m)
p = stretching pressure (tension) (J / m^2)
T = temperature (J)

L = length of pore edge
A = area of membrane including pore
S(<L>) = entropy of fluctuations of pore edge



Pores in membrane:
a) q large, λ large
b) q small, λ large
c) q large, λ small
d) q small, λ small

Network has a 2d phase space controlled by the probability of pore creation, and the edge energy of the pore boundary.

Pore edge is a self-avoiding random walk

Once a pore appears what does it do? Will it grow or shrink and disappear?

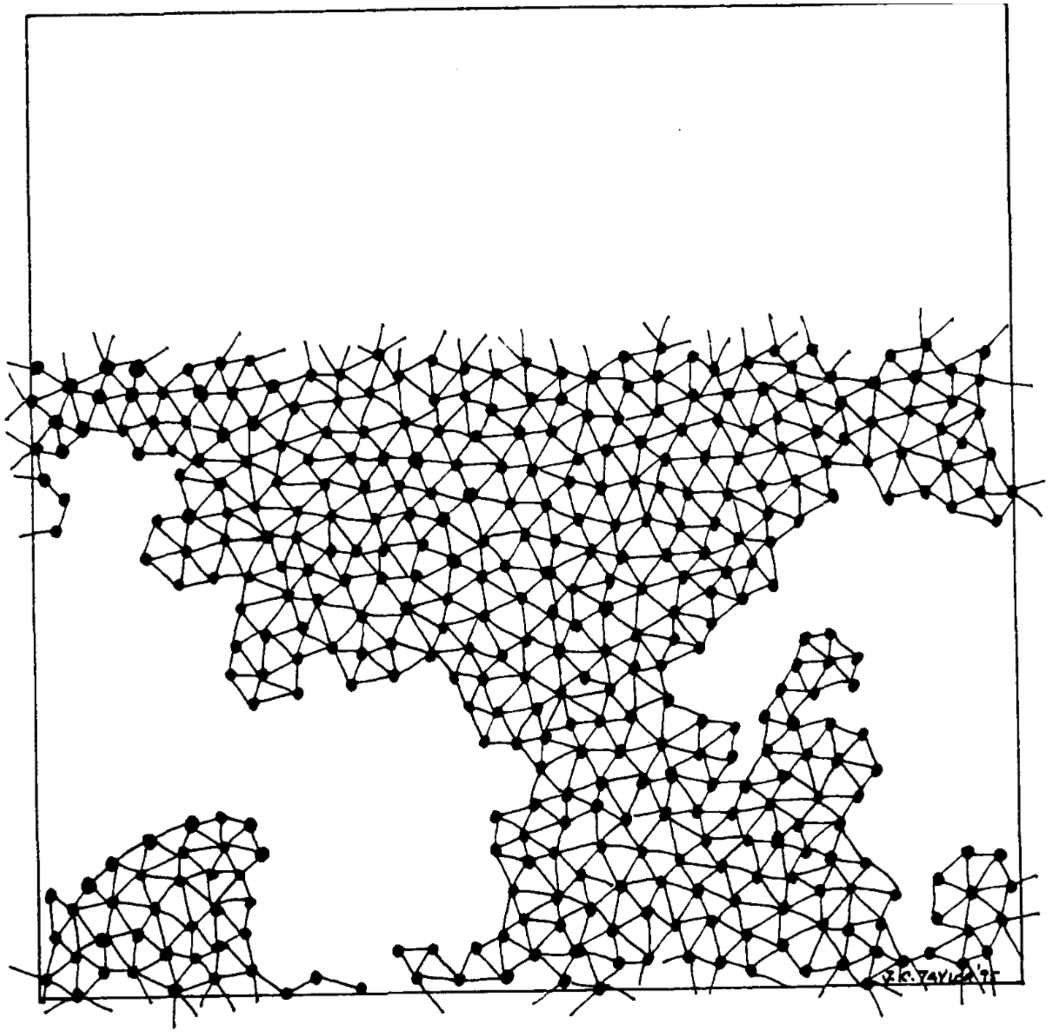
To answer this, we need to calculate the free energy of a pore and minimise it. Assume a single pore for now and let it fluctuate in shape and size in two dimensions. Its energy is just proportional to the edge length.

From lattice calculations, the number of configurations of a pore with n steps is[1]:

$$\Omega(n) = \Omega_0 z^n n^{\alpha-2}$$

where n is the length (L/a_0) of the edge, z is the lattice coordination number, α is an exponent and Ω_0 is a prefactor independent of n.

What is the Gibbs free energy of the pore? (Blackboard calculation)



[1] D. S. McKenzie Physics Reports 27:35-88 (1976)

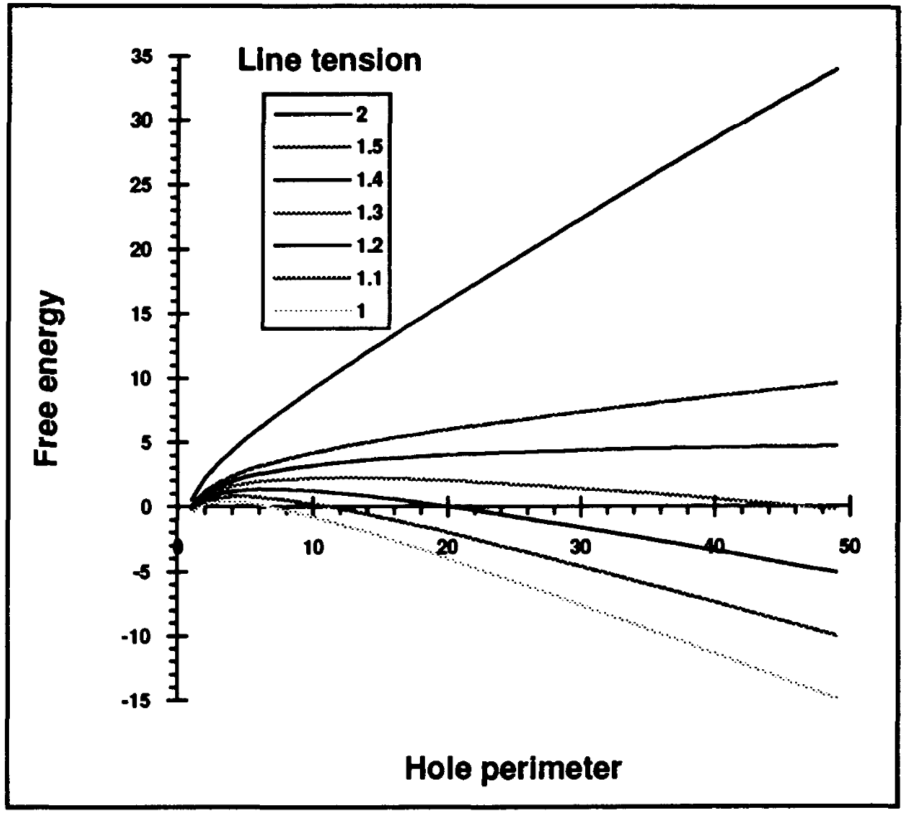
Barrier height against pore growth

$\beta G = n (\beta\lambda a_0 - \ln z) - (\alpha - 2) \ln n - \ln \Omega_0$

We have a similar behaviour to the Litster theory but now a pore can appear *without* any stretching tension if the line tension is small enough.

The *entropy* of the shape fluctuations of the pore's edge destabilises the membrane.

The Litster theory is a zero temperature theory, and here we include entropy. What are the finite temperature equivalents to the barrier height and critical hole size?



Litster model: T = 0

$R^* = \lambda / \Gamma$

$\Delta E(R^*) = \pi\lambda^2 / \Gamma$

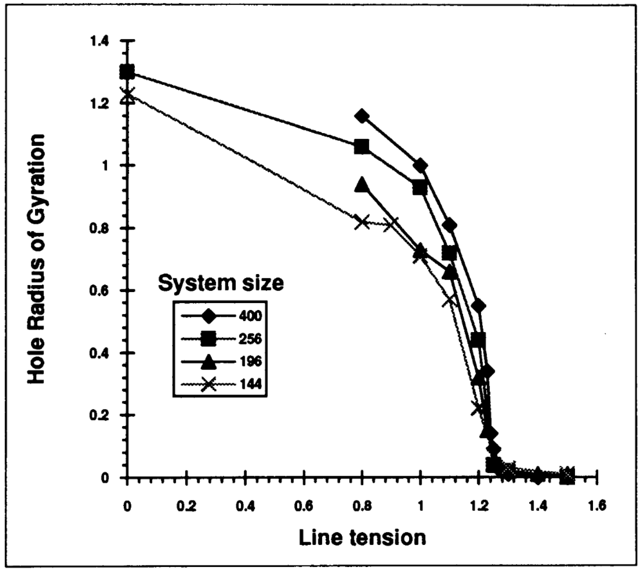
Free energy model with T > 0

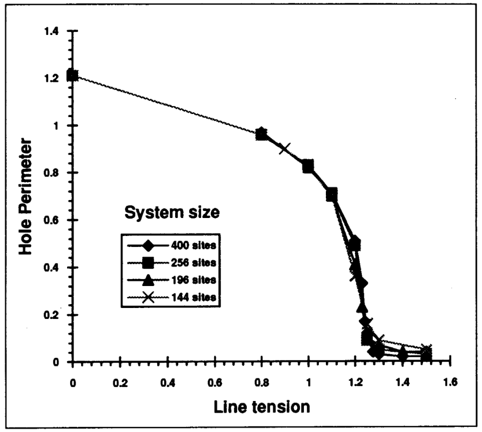
$n^* = (\alpha - 2) / (\beta\lambda a_0 - \ln z) = 1.5 / (\ln z - \beta\lambda a_0)$

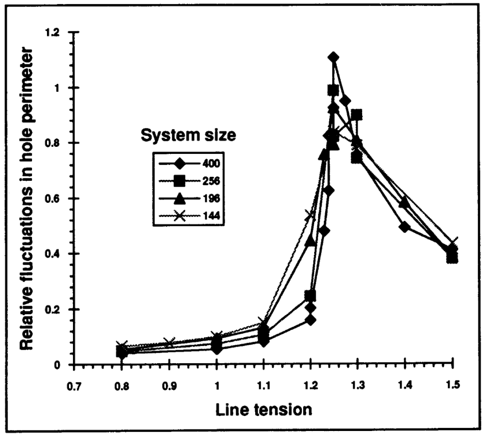
$\Delta\beta G(n^*) = (\alpha - 2)(1 - \ln((\alpha - 2) / (\beta\lambda a_0 - \ln z))$

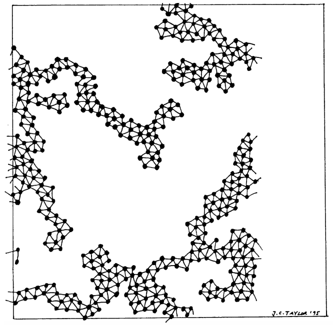
Triangular lattice
$z = 4.15$
$\alpha = 0.5$
McKenzie, 1976

Single pore shape dynamics

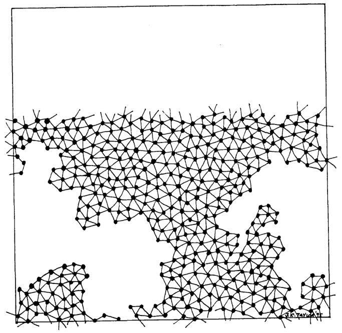




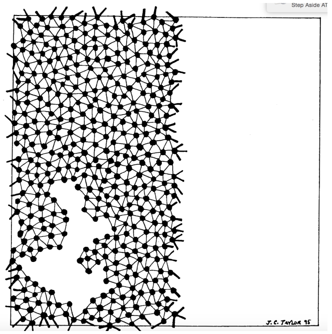




$\beta\lambda a_0$ ~ 0.8



$\beta\lambda a_0$ ~ 1.24



$\beta\lambda a_0$ ~ 1.24

Fluctuations grow at the critical value of $\beta\lambda a_0$

⇒ phase transition from stable membrane to ruptured membrane as $\beta\lambda a_0$ decreases

Multiple pores in a membrane

If we allow multiple pores, whose number is controlled by a barrier height βq, and whose subsequent growth by $\beta\lambda a_0$, we can calculate the phase diagram as shown.

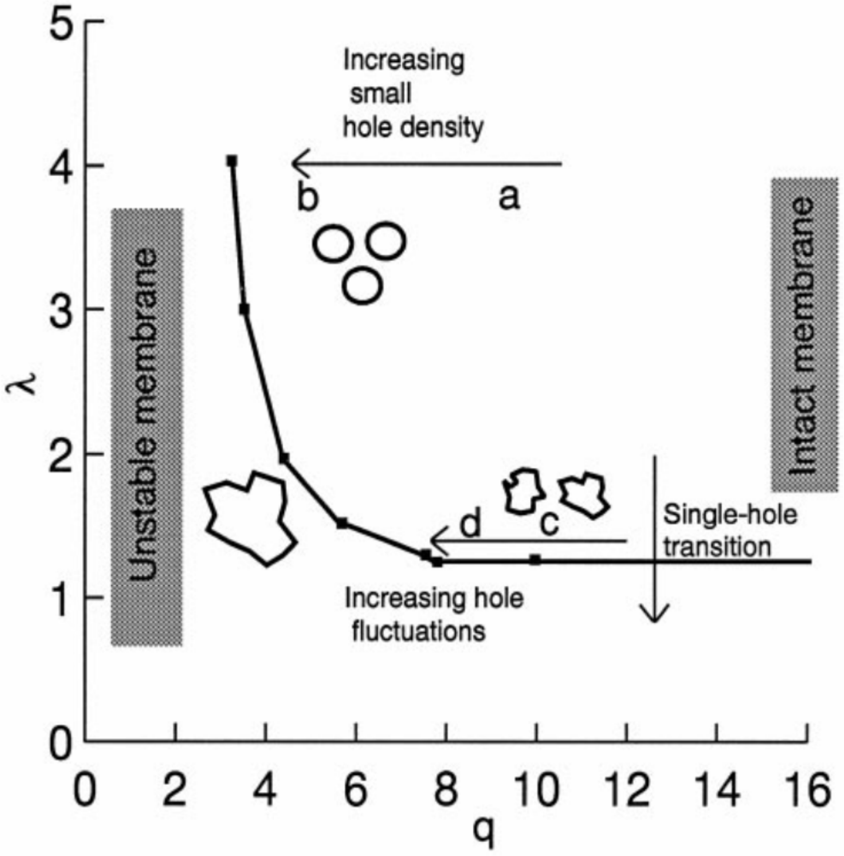
There are two limiting cases:

1) Ideal pore "gas"

Assume: $q > q_{rupt}$ but also not too large, so lots of holes, but $\beta\lambda a_0$ is large so each hole is small, circular and unlikely to merge with others but can have a range of sizes.

2) Chemical reaction of multiple interacting pores

Assume: pores are of two types: small hydrophobic pores form spontaneously and can shrink and disappear, or transform into hydrophilic pores and grow and/or merge.



J. C. Shillcock and U. Seifert, Biophys. J. 74:1754-1766 (1998)

Biological significance

What does this imply for a cell?

Clearly a cell is always at T > 0, and cannot easily change its temperature.

But the model says that the only important parameters are the dimensionless barrier height to pore formation βq, and the line tension of the pore boundary, $\beta \lambda a_0$.

So a cell can modify the number of pores, and their subsequent behaviour by modulating βq and $\beta \lambda a_0$. It can do this by changing the composition of the membrane: lipids with shorter tails or larger headgroups weaken the lamella state, and molecules that can easily change their orientation (single tailed lysolipids or peptides) reduce the energy cost of the pore boundary.

Animals use this mechanism to kill bacteria; they secrete peptides that pack in the bacterial membrane and lower the barrier height against pore formation and expansion.

Synaptic vesicle fusion relies on being able to create a pore "on demand" so that the neurotransmitter packed inside a vesicle can be released swiftly and reliably.

Ideal pore "gas"

Assume: q > q* ~ 3.3 so lots of holes and $\beta\lambda a_0 > 2$ so each hole is small, circular and unlikely to merge with others but can have a range of areas.

Hamiltonian of one pore = $H = \sum_{j=1}^{N} 2\pi r_j \Lambda.$

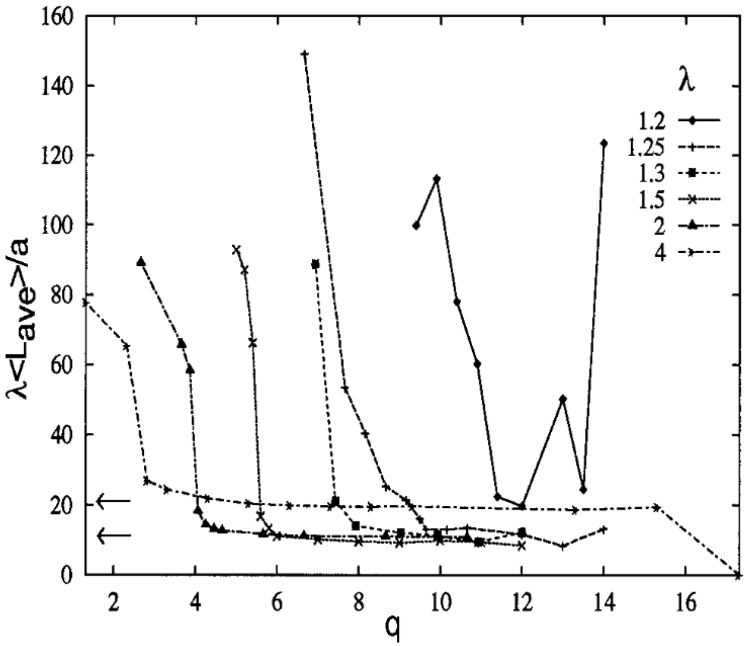
Partition function of "gas" of pores = $Z(T, A, \mu, \Lambda) = \sum_{N=0}^{\infty} e^{\beta\mu N} \sum_{\text{states}} e^{-\beta H},$

Grand potential = $\beta\Omega(T, A, \mu, \Lambda) = -\frac{1 + 2\pi\beta\Lambda r_0}{(2\pi\beta\Lambda a)^2} e^{-(2\pi\beta\Lambda r_0 - \beta\mu)}.$

We calculate the thermodynamic properties from derivatives of Ω(T, A, μ, λ):

$<L> = \partial\Omega / \partial\lambda$

$<N> = -\partial\Omega / \partial\mu$

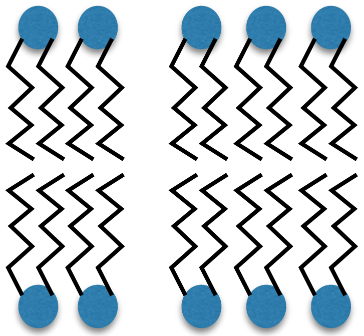


Horizontal arrows show predictions of the average pore perimeter for $\beta\lambda a_0$ = 4 and 2.

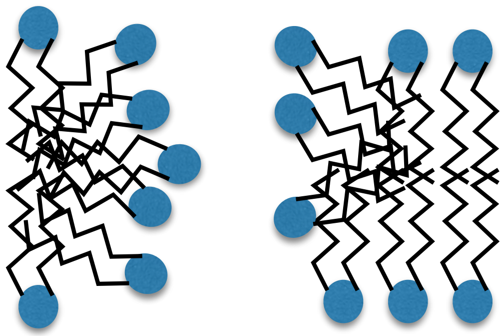
$$\frac{\beta\Lambda\langle L\rangle}{\langle N\rangle} = 1 + 2\pi\beta\Lambda r_0 + \frac{1}{1 + 2\pi\beta\Lambda r_0}.$$

Chemical reaction model of pores

Assume: $q \sim q^* \sim 3.3$ so lots of holes and $\beta\lambda a_0 < 2$ so holes fluctuate in shape, grow/shrink and merge/break up. Small (< 1nm) hydrophobic pores *spontaneously* appear and can transform into hydrophilic ones where the lipids rearrange at the pore edge to minimise the exposure of hydrophobic tails to water; only hydrophilic pores grow larger and merge.



Hydrophobic pore HO



Hydrophilic pore HI

x = HO-HI conversion

d = HO resealing

y = HI-HO conversion

r = HI-HO merging

c = HO creation

$N_1(t)$ = # lipids around HO pores at time t

$N_2(t)$ = # lipids around HI pores at time t

$$\dot{N}_1(t) = c - (d + x)N_1 + yN_2 - rN_1N_2$$

$$\dot{N}_2(t) = xN_1 - yN_2 + rN_1N_2 .$$

Steady state solutions

Consider the linearised steady state, and set $rN_1N_2 = 0$, then

$$dN_1/dt = 0 \quad \text{and } dN_2/dt = 0$$

gives:

$$N_1^* = c / d \quad \text{and } N_2^* = x/y\ N_1^*$$

We assume that HO pores must cross an energy barrier to transform into HI pores, so $x \leq y$, and $N_2^* < N_1^*$ and the membrane is stable against HI pores growing (NB N_2 is number of lipids on pore edge not number of pores)

But when the non-linear term is included, HI pores can grow without limit:

$$N_1^* = c / d$$

$$N_2^* = x\ N_1^* / (\ y - r\ N_1^*\ )$$

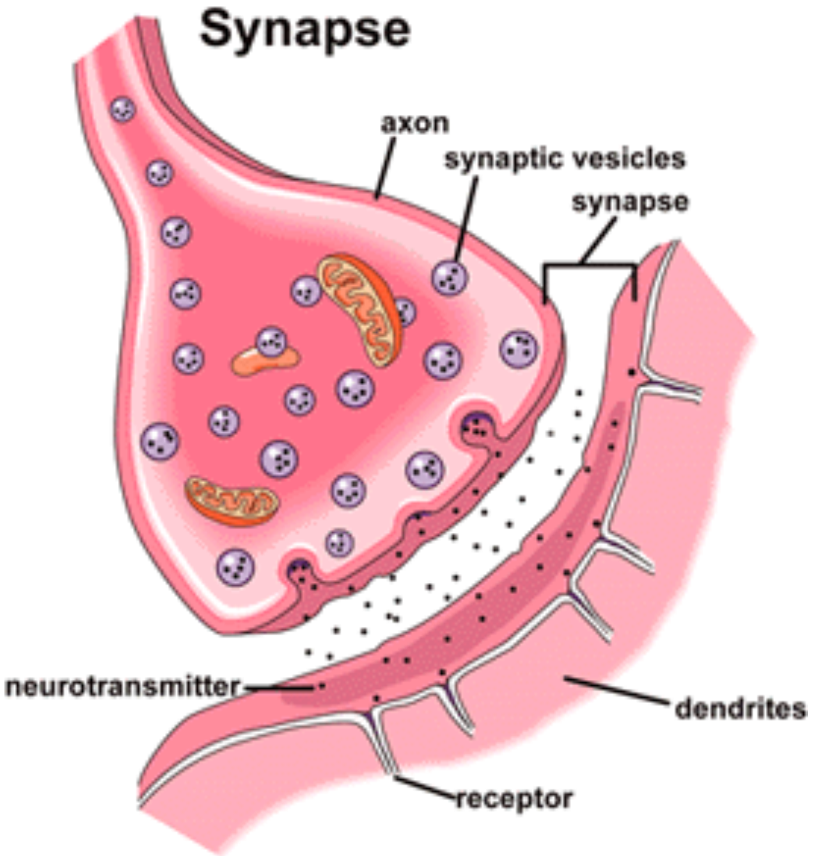
The key parameter is r, which we can relate to the line tension as it controls the rate of absorption of HO pores by HI pores due to the shape fluctuations of HI pores.

Synapses

Synapses are chemical connections between neurons (often between an axon and a dendrite).

A tightly-regulated sequence of steps:

- Arrival of AP at axon terminal
- Opening of Ca channels
- SNARE-mediated fusion of vesicles
- Release of neurotransmitter (NT) into the synaptic cleft (20-50 nm wide)
- Binding of NT to receptors
- Modification of post-synaptic neuron's membrane potential
- Transport of membrane voltage to soma of post-synaptic neuron



Synapses are involved in: learning, memory, mental disorders, drug actions,… They appear to do computations depending on their state, and modify this state and produce new proteins from RNA located near dendritic spines.

Fusion in reality...

Vesicle fusion requires several steps:

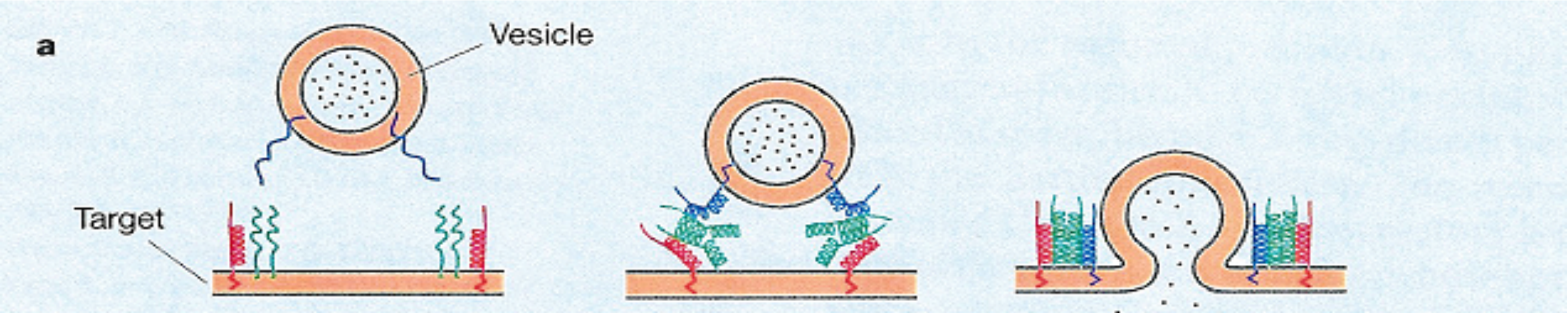
- membrane bending
- membrane merging
- membrane rupture or fission

Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins

Mathieu Pinot,[1,2] Stefano Vanni,[1] Sophie Pagnotta,[3] Sandra Lacas-Gervais,[3]
Laurie-Anne Payet,[4] Thierry Ferreira,[4] Romain Gautier,[1] Bruno Goud,[2]
Bruno Antonny,[1*] Hélène Barelli[1]

Pinot M et al. Science 345:693-697 (2014)

PUFAs in synaptic vesicle membranes lower the energy cost of bending, so facilitating fusion.



Close Is Not Enough: SNARE-dependent Membrane Fusion Requires an Active Mechanism that Transduces Force to Membrane Anchors

James A. McNew, Thomas Weber, Francesco Parlati, Robert J. Johnston, Thomas J. Melia,
Thomas H. Söllner, and James E. Rothman

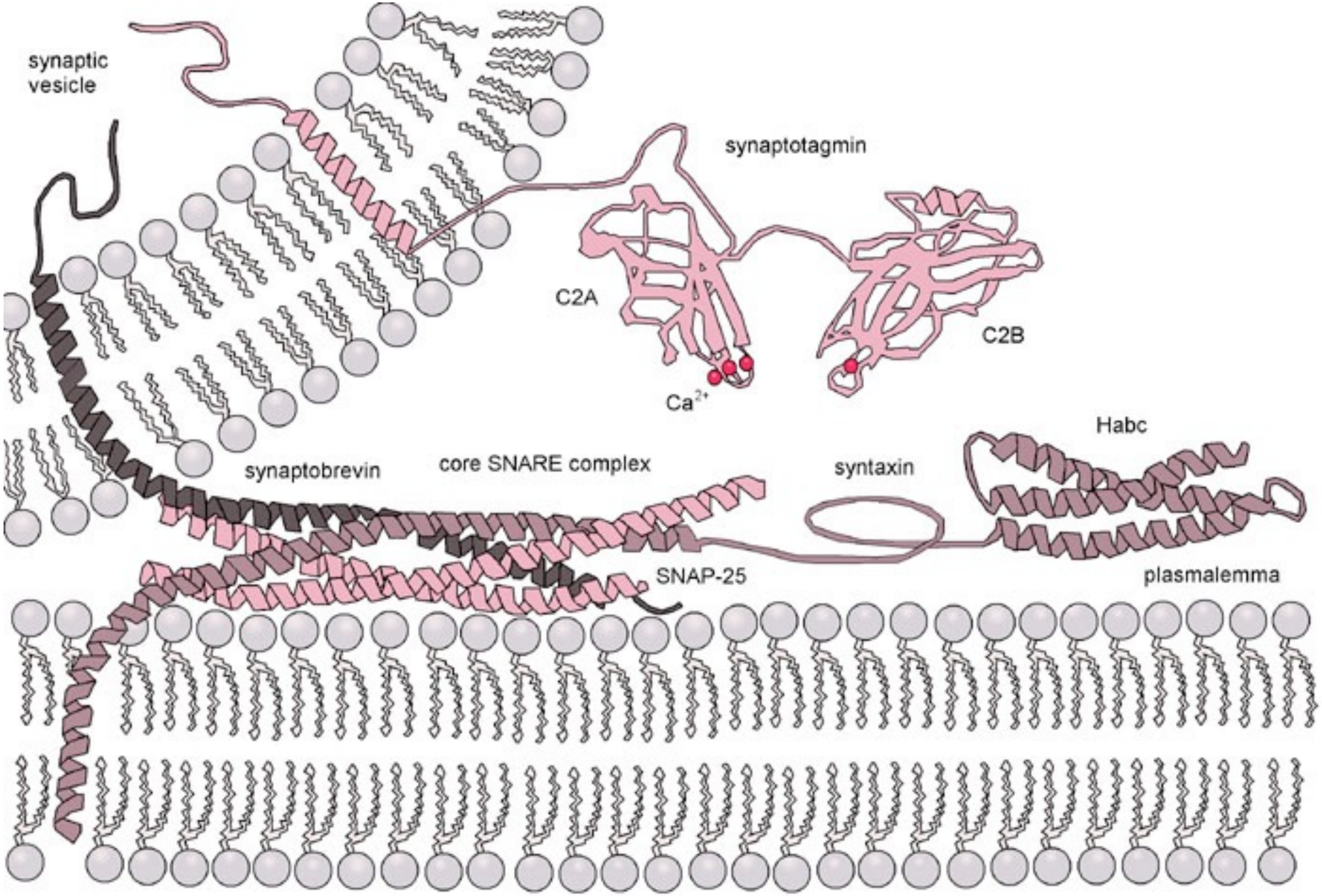
Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

McNew JA et al. J. Cell Biology 150:105-117 (2000)

SNARE proteins present in both membranes pull them together and drive the formation of the fusion pore.

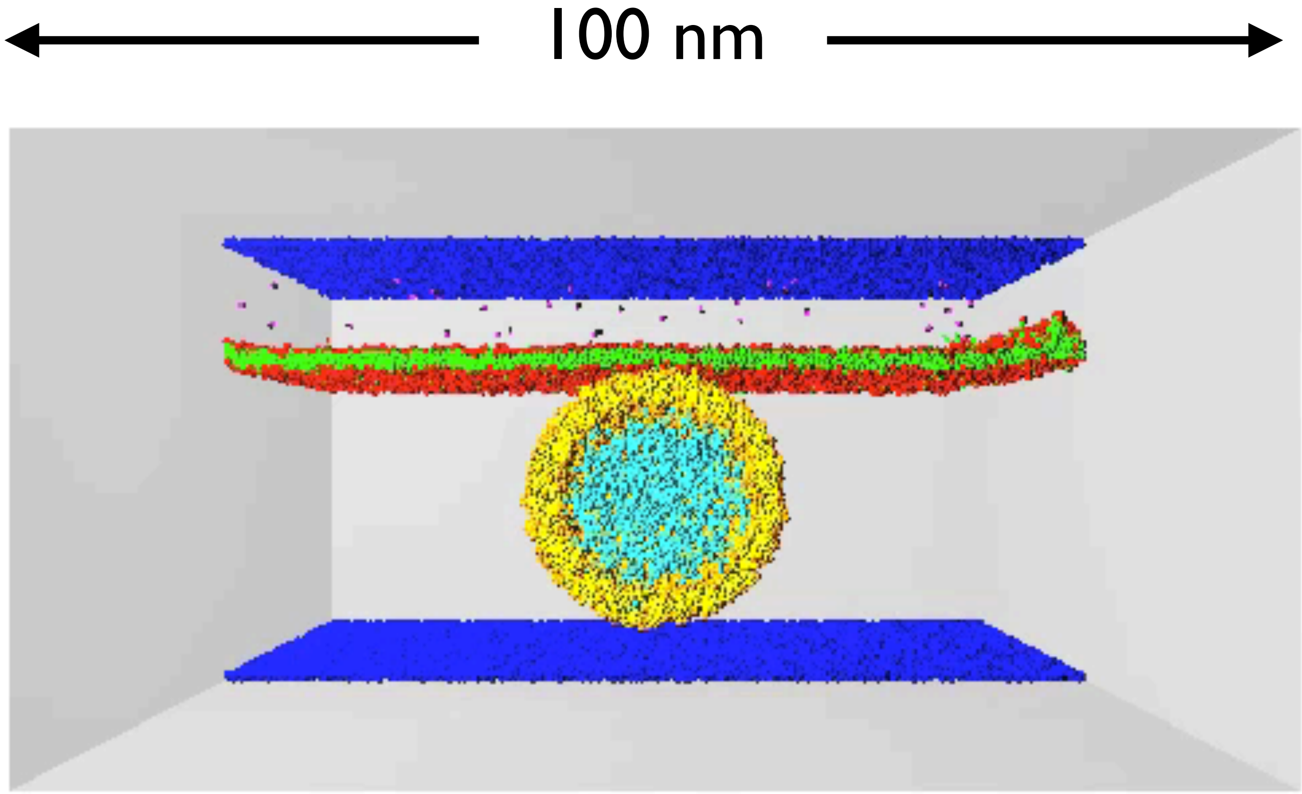
But… what do they actually do? Force, torque, displacement…?

Exocytosis Machinery



Molecular machinery driving exocytosis in neurotransmitter release: the core SNARE complex (formed by four α-helices contributed by synaptobrevin, syntaxin and SNAP-25) and the Ca2+ sensor synaptotagmin.

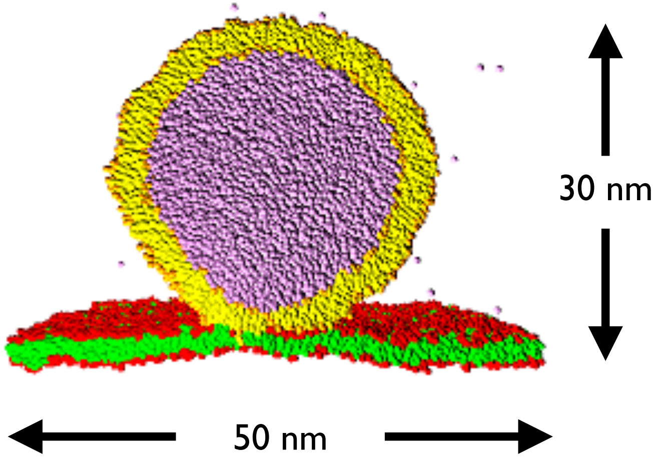
and in simulations …

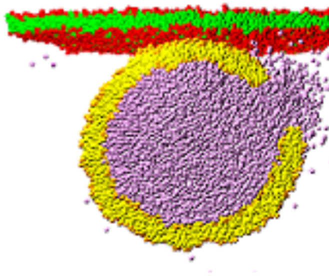


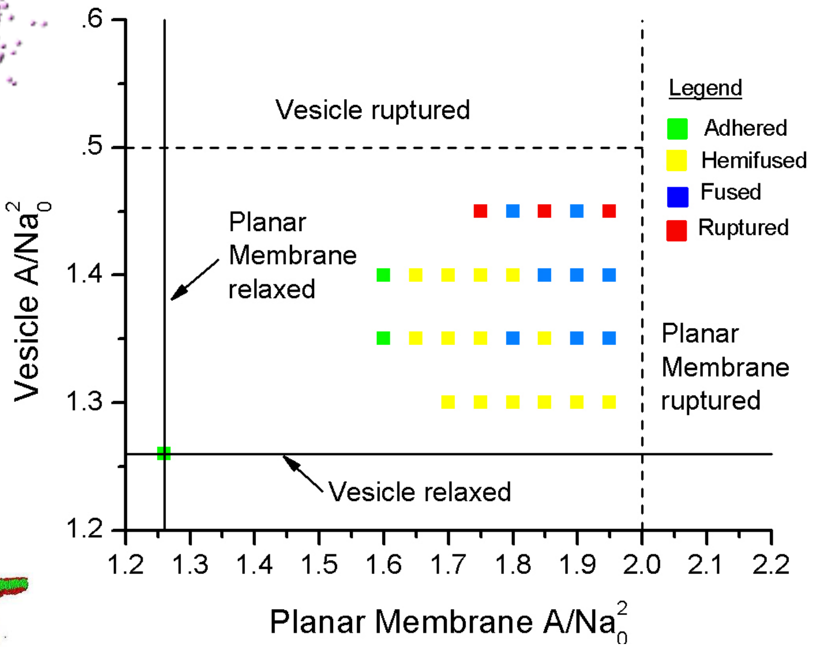
DPD simulation - water invisible, cut through vesicle and simulation box.

Cyan beads are “glutamate”, stationary pink dots are “receptors”

Vesicle fusion protocol: tension





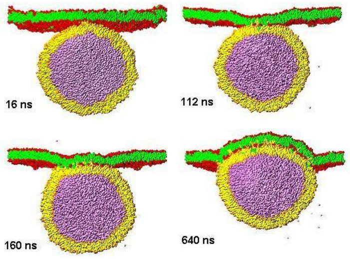


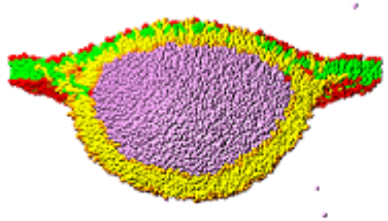
Create a bilayer and a vesicle under tension initially close together and let them evolve

Bilayer and vesicle lipids: $H_3(T_4)_2$

Relaxed N_{ves} = 6542

Relaxed N_{bil} = 8228

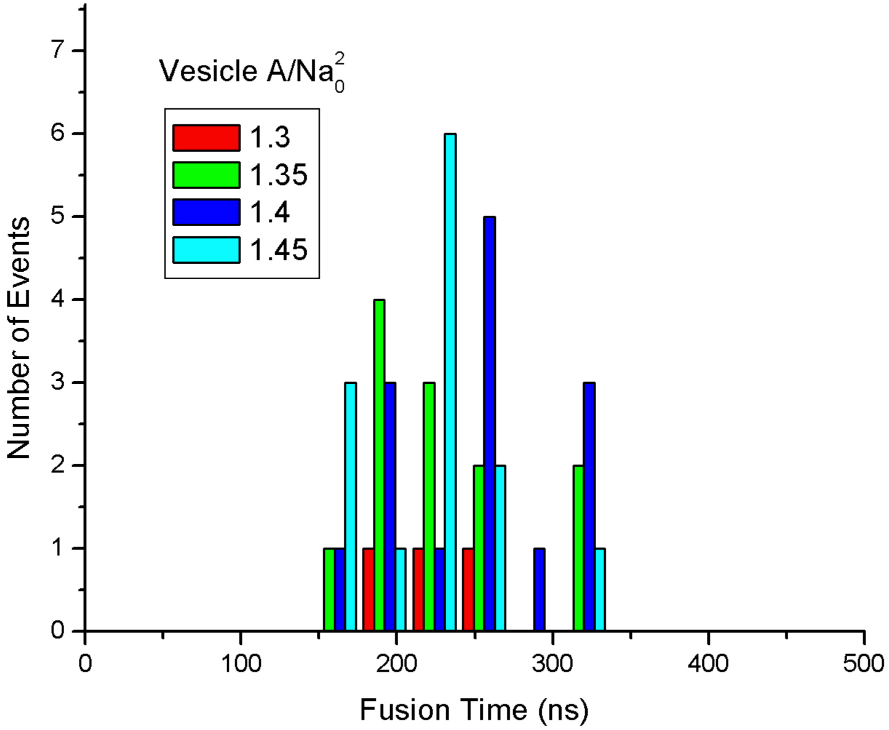




Only 42 successful fusion events out of 93 attempts

Shillcock JC and Lipowsky R, *Nature Mat.* **4**:225 (2005)

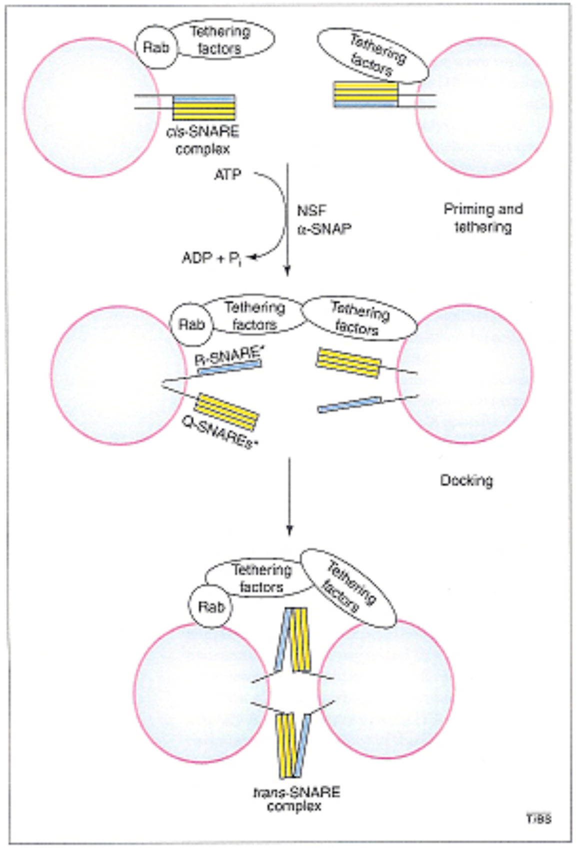
Fusion time distribution



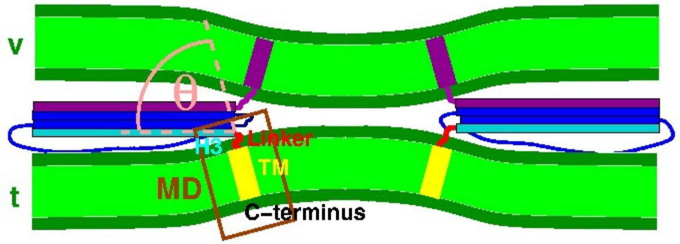
No fusion events between 350 ns and 1.6 µs

Why not?

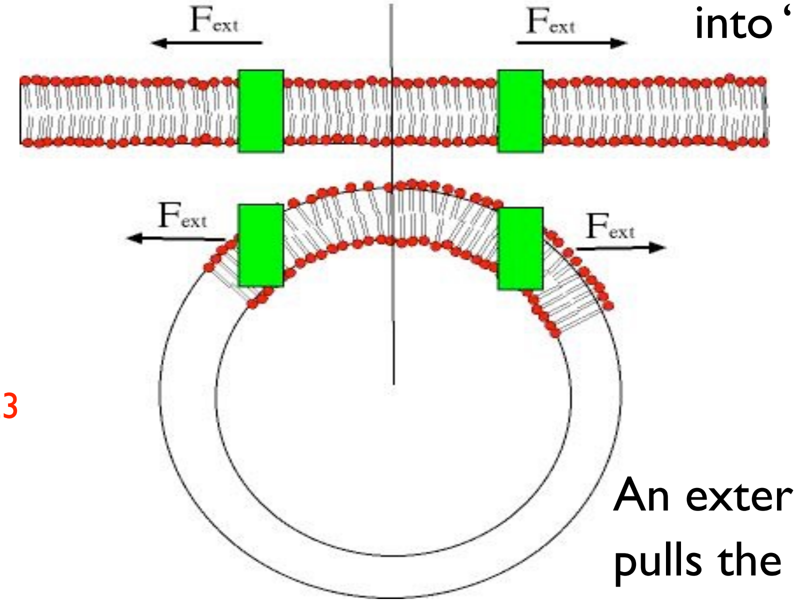
Vesicle fusion protocol: proteins



Vesicle tethers then docks prior to fusion
(Mayer, TRENDS in Biochemical Sci. 26:717-723



SNAREs hold the vesicle close to the membrane and promote fusion,
Knecht & Grubmueller, Biophys. J. **84**:1527-1547(2003)



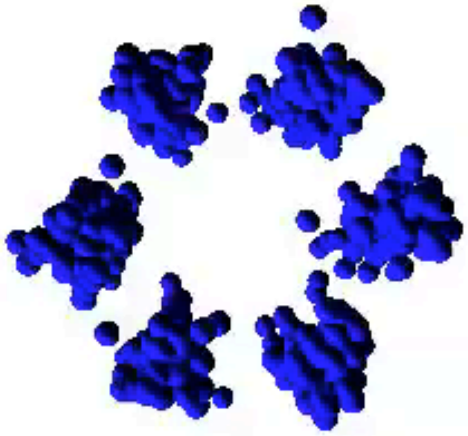
Lipid tail beads are polymerised into "rigid" cylinders, of radius r

An external force, of magnitude F_{ext}, pulls the barrels radially apart

We create a force protocol that applies forces to membrane-bound anchors (or barrels) to perturb it in order to drive fusion.

“Protein-driven” vesicle fusion in DPD

Forces applied to membrane-spanning barrels **bend** and **stretch** the membrane leading to fusion. Is this what SNARE complexes do?



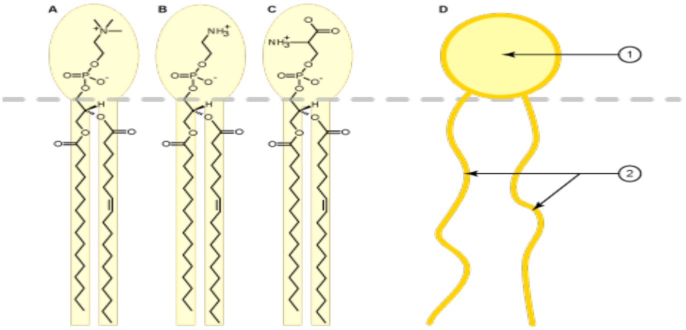
Box = 100 x 100 x 42 nm^3
3.2 x 10^6 beads in total

Planar membrane ~ (100 nm)^2
28,000 lipids

Vesicle ~ 6000 lipids

Duration ~ 500 ns

Simulation Notes



Water is present in all movies, but invisible to reveal dynamics

Periodic Boundary Conditions are used, which means that a molecule leaving one face of the simulation box re-enters at the opposite face.

Lipids have a **headgroup** (red/orange) and oily **tails** (green/yellow); **proteins** are blue; bending forces are applied to white lipids

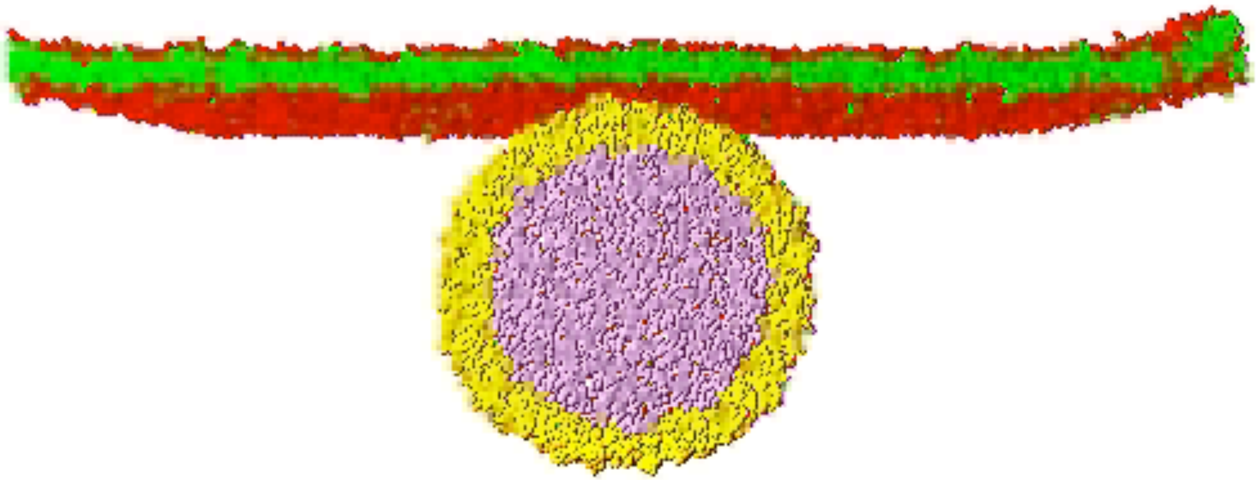
6 proteins in a circle per membrane
- area per protein matches expts.

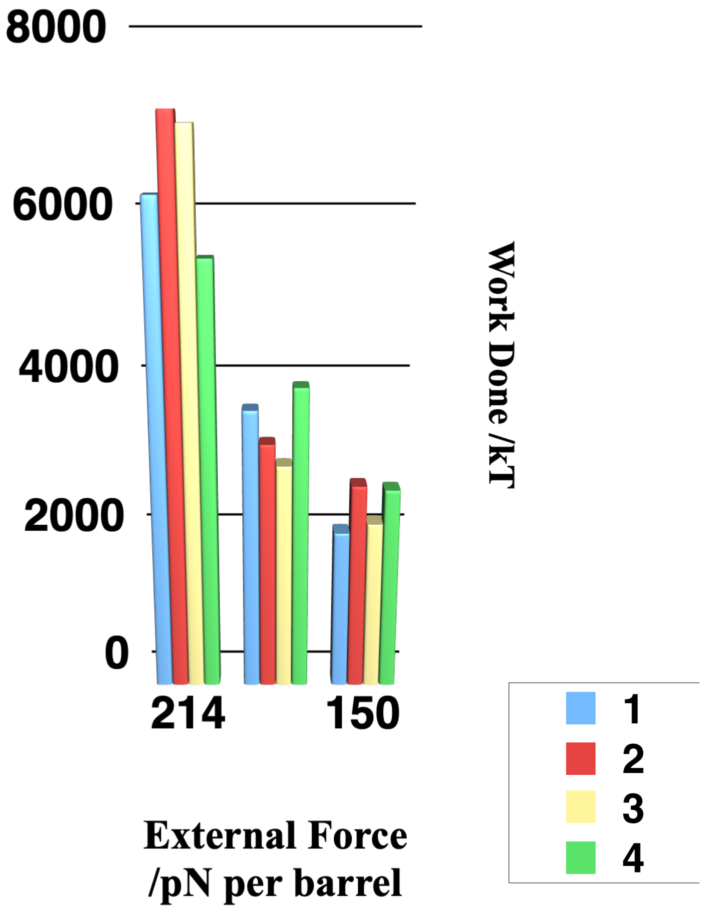
Schuette et al. PNAS 101:2858 (2004)

Shillcock & Lipowsky, J Phys Cond Matt.18:S1191 (2006)

Computational resources: 50 cpu-hours per fusion event

How much work is required for fusion?





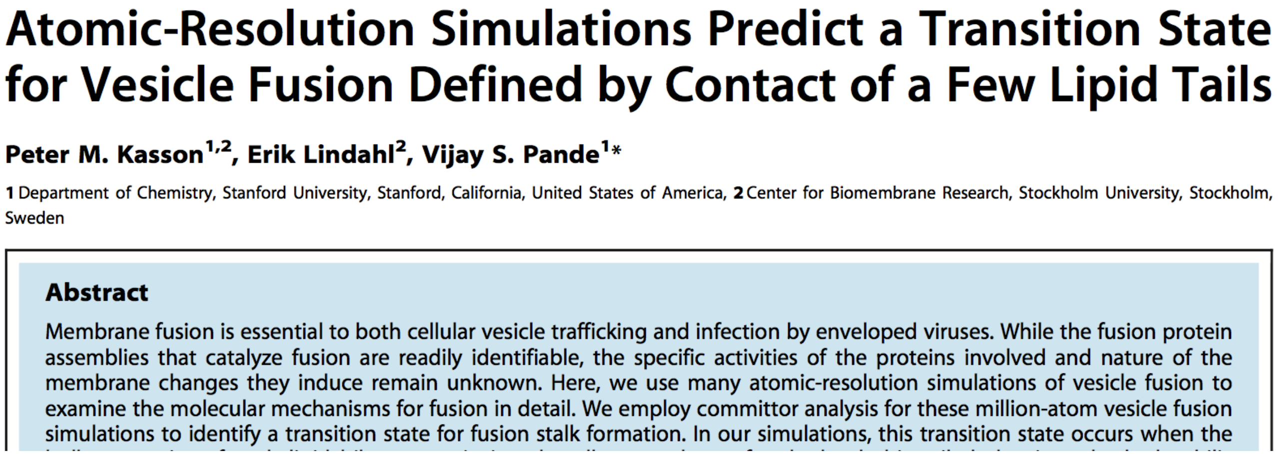
Yersin A et al, PNAS 100:8736-8741 (2003)

McNew JA et al. J. Cell Biology 150:105-117 (2000)

NB. Work done is for all 12 barrels

Vesicle fusion simulation: a short history

Müller M, Katsov K, Schick M, *Biophys. J.* **85**:1611-1623 (2003)	- MC
Stevens MJ et al, *Phys. Rev. Lett.* **91**:188102 (2003)	- MD
Shillcock JC and Lipowsky R, *Nature Mat.* **4**:225 (2005)	- DPD
Grafmueller, Shillcock and Lipowsky, *PRL* **98**:218101 (2007)	- DPD
Kasson PM et al, *PLoS Comp. Biol.* **6**:c1000829 (2010)	- aaMD



Atomic-Resolution Simulations Predict a Transition State for Vesicle Fusion Defined by Contact of a Few Lipid Tails

Peter M. Kasson[1,2], **Erik Lindahl**[2], **Vijay S. Pande**[1]*

1 Department of Chemistry, Stanford University, Stanford, California, United States of America, **2** Center for Biomembrane Research, Stockholm University, Stockholm, Sweden

Abstract

Membrane fusion is essential to both cellular vesicle trafficking and infection by enveloped viruses. While the fusion protein assemblies that catalyze fusion are readily identifiable, the specific activities of the proteins involved and nature of the membrane changes they induce remain unknown. Here, we use many atomic-resolution simulations of vesicle fusion to examine the molecular mechanisms for fusion in detail. We employ committor analysis for these million-atom vesicle fusion simulations to identify a transition state for fusion stalk formation. In our simulations, this transition state occurs when the

"Our results thus suggest that the specific molecular properties of individual lipids are highly important to vesicle fusion…"