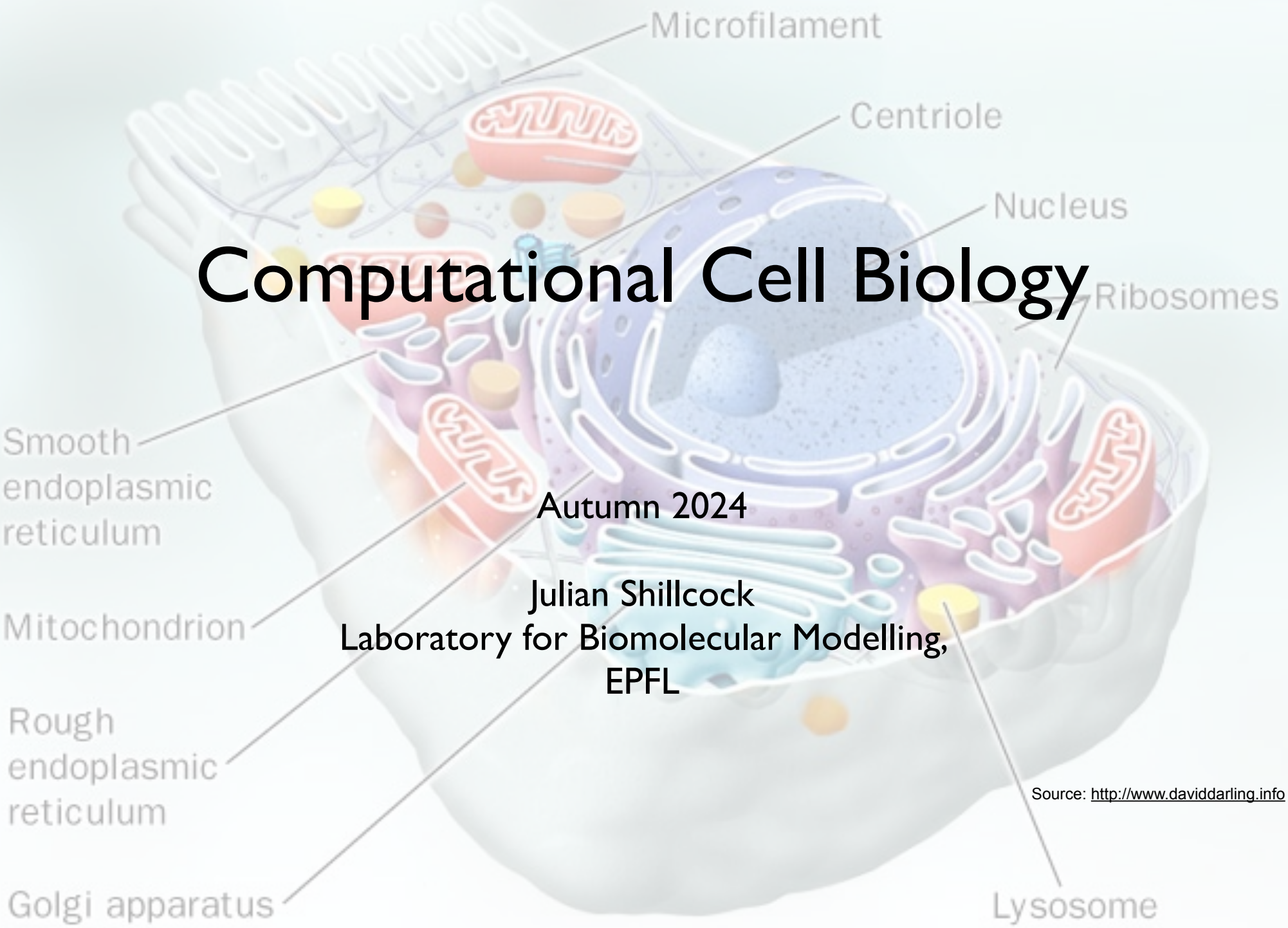


Computational Cell Biology

Autumn 2024

Julian Shillcock
Laboratory for Biomolecular Modelling,
EPFL

Source: http://www.daviddarling.info

Core Concepts

Membranes play contradictory roles:

- Reliably separate the inner and out worlds, and be a solvent for proteins that mediate signals

- Open up pores for material transport on demand

They do this by *pre-stressing* their membrane/proteins to favour fast pore formation and leave the recovery phase for later

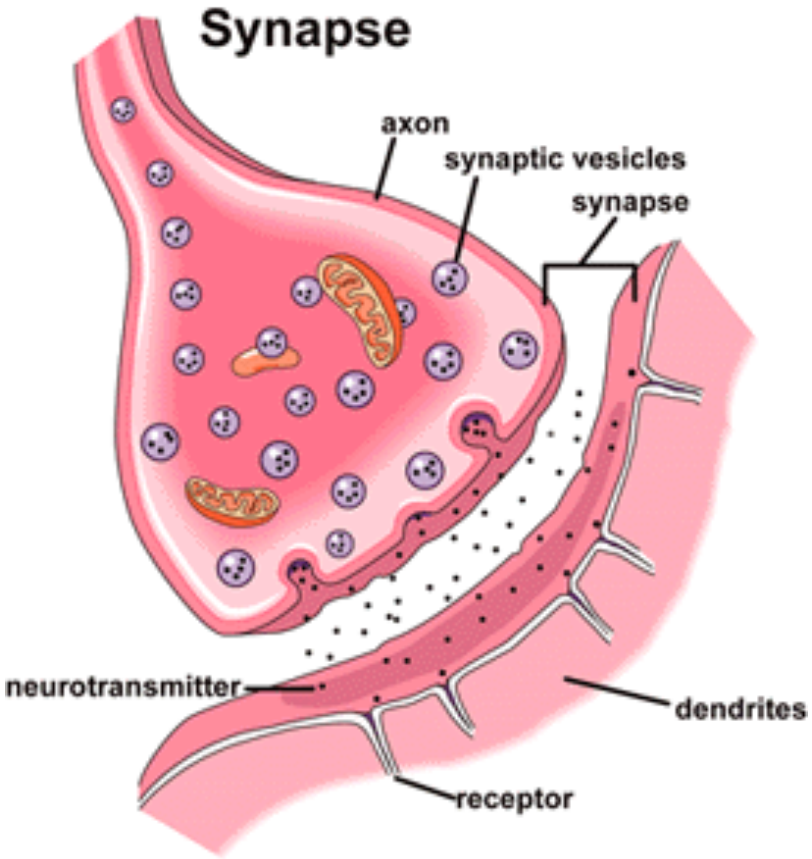
Neurons do this continuously at synapses to allow neurotransmitter diffusion to bind to receptors on post-synaptic membrane

Synapses in the brain…

Synapses are chemical connections between neurons (usually 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.

Steps in endo- and exocytosis

What does a synapse do? - transduce an *electrical* signal from axon to dendrite by *chemical* means (electrochemical synapse)

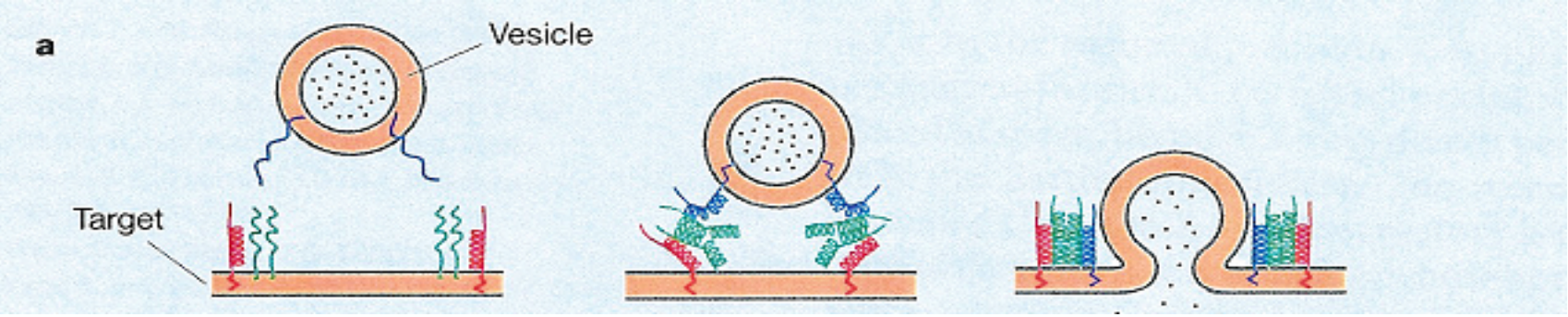
How does it do it? - membrane rupture, diffusion, receptor activation, post-synaptic density (PSD) biochemistry, ion channel openening/Ca ion influx

How can we build a model of endo-/exocytosis that makes predictions? - break the problem into a sequence of smaller ones:

From pores to vesicle fusion - Litster, entropy, and protein-induced fusion

How to rupture a membrane?	Litster, lipids, proteins
How to model diffusion?	PDE or simulation
How to model receptor activation?	ODE or simulation

Why don't neurons blow up?



Typical neuron: ~ 10,000 synapses

Each synapse fires at ~ 1 Hz

Vesicle radius ~ 50 nm

Cell radius ~ 10 micron

Group calculation
5 mins
discuss

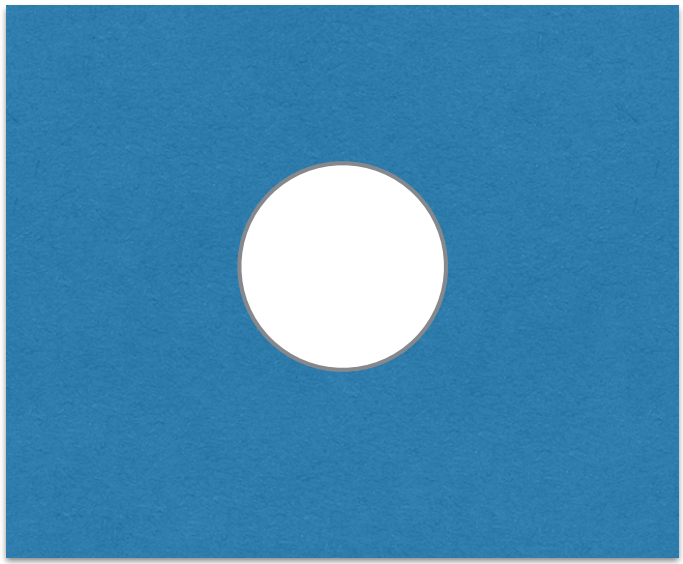
1) How much membrane is added to the neuron's plasma membrane per second?
2) Why doesn't it get bigger?

Classical theory of pore formation

Litster in 1975 proposed a simple theory for how pores could form 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

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

Litster, J. D. 1975. Stability of lipid bilayers and red blood cell membranes

Phys. Lett. A. 53: 193-194.

Can a membrane spontaneously rupture?

Energy cost of a pore is: $E(R) = 2\pi R\lambda - \pi R^2\Gamma$

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: membrane phase changes from intact to ruptured.

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

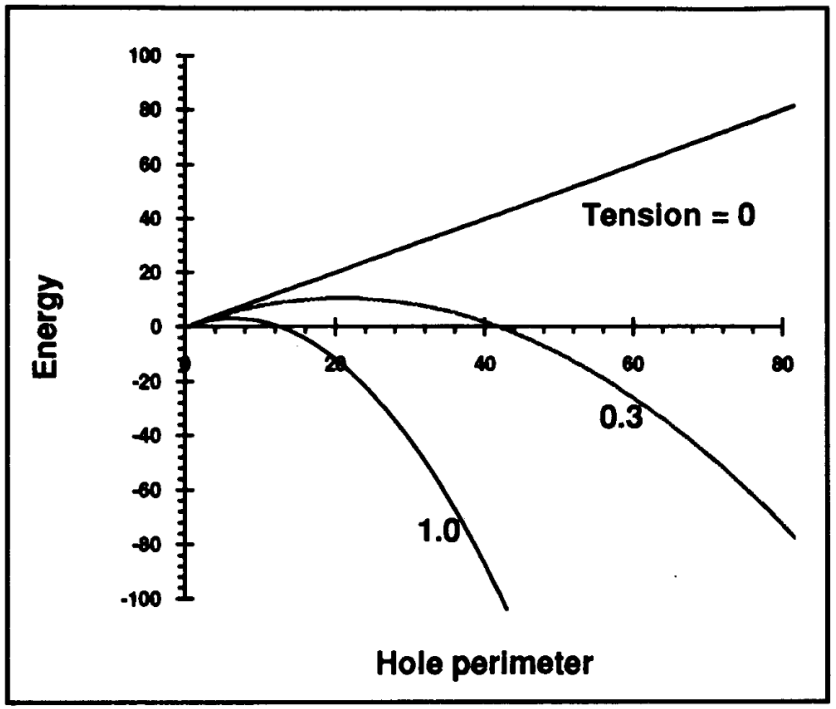
$\Gamma \sim 3$ dyn/cm for SOPC[1], 10 dyn/cm for RBCs[2]

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

NB 1 dyne = 10**-5 N

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

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



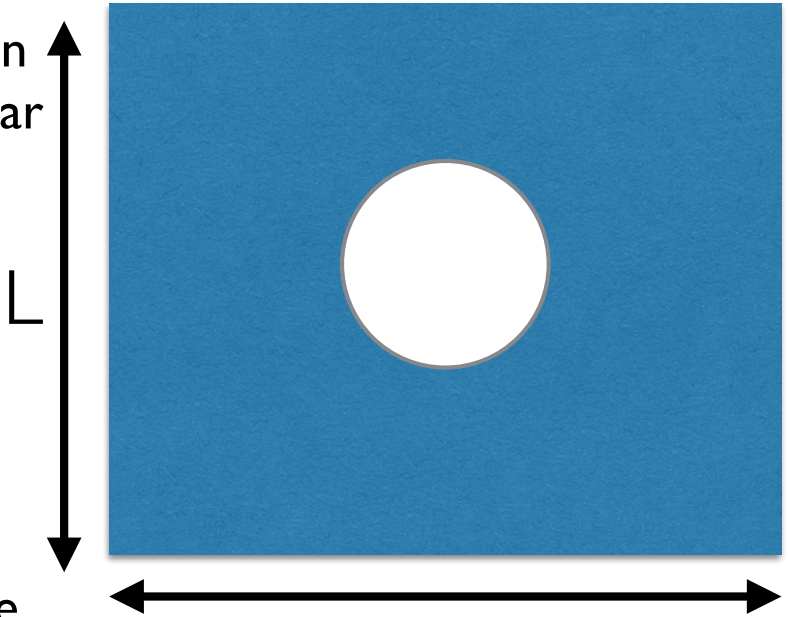
[1] Evans and Needham, J. Phys. Chem. 91:4219-4228 (1987)

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

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

How big is a tension-induced pore?

In the Litster theory, a single circular pore appears in a planar lipid membrane when it is stretched. This question asks how the pore size depends on the tension as a planar membrane is stretched.



Parameters

L = Box size
R = Pore radius
a_0 = area per lipid in the tensionless membrane state
a_1 = area per lipid in the initial stretched membrane state

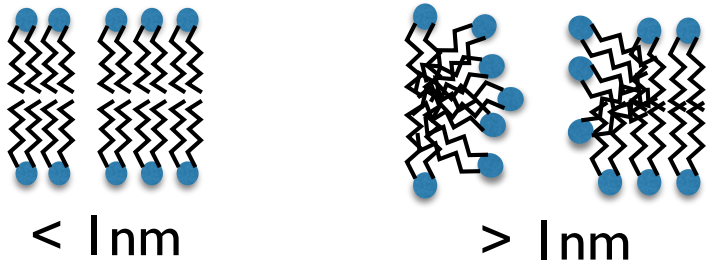
Assume the initial state is N lipids in the membrane in box of size L (ignore the double monolayer complication for this question), and assume the membrane is under tension because L is larger than is consistent with each lipid having its relaxed area per molecule.

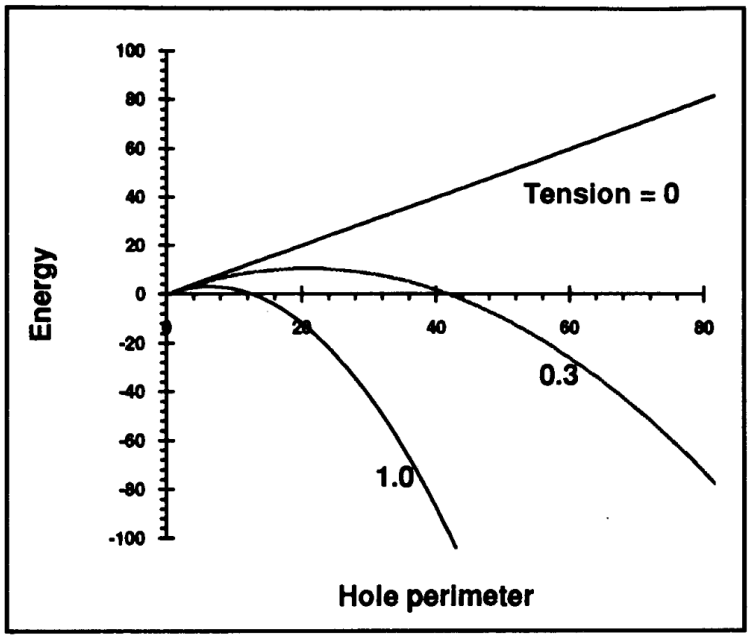
Group calculation
10 mins
discuss

1) How does R depend on stretched area per lipid (a_1) and equilibrium area per lipid (a_0)?
2) Do you expect intuitively that R increases continuously from zero as L increases or does it jump at some value of L? What does your equation predict?

Relevance to biological membranes?

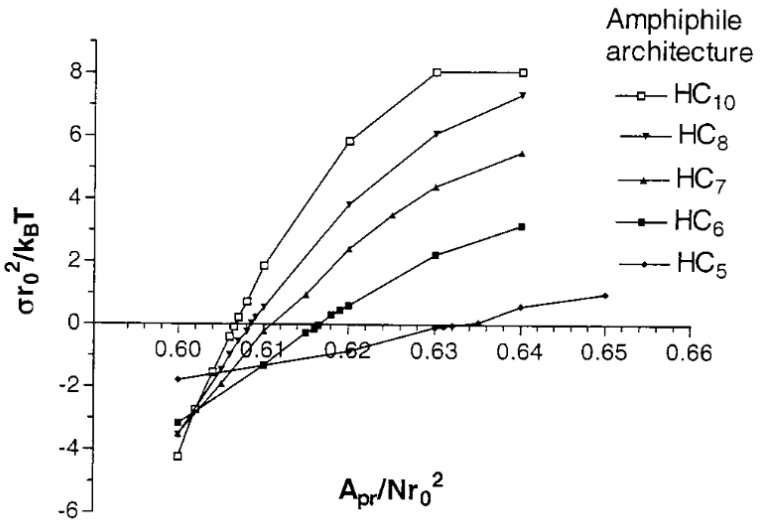
- Cells can be under osmotic stress ⇒ tension ✓
- Lipids in L_α phase rearrange around pore circumference ⇒ edge energy or line tension (stretched and tilted) ✓





- Are pores circular?
- What is the effect of temperature?
- What if there are multiple pores? Can they merge?

Cell membranes are fragile: under ~ 5% area strain they rupture.



Shillcock, JC, Lipowsky, R. J. Chem. Phys 117:5048 (2002)

Membranes: more than a barrier

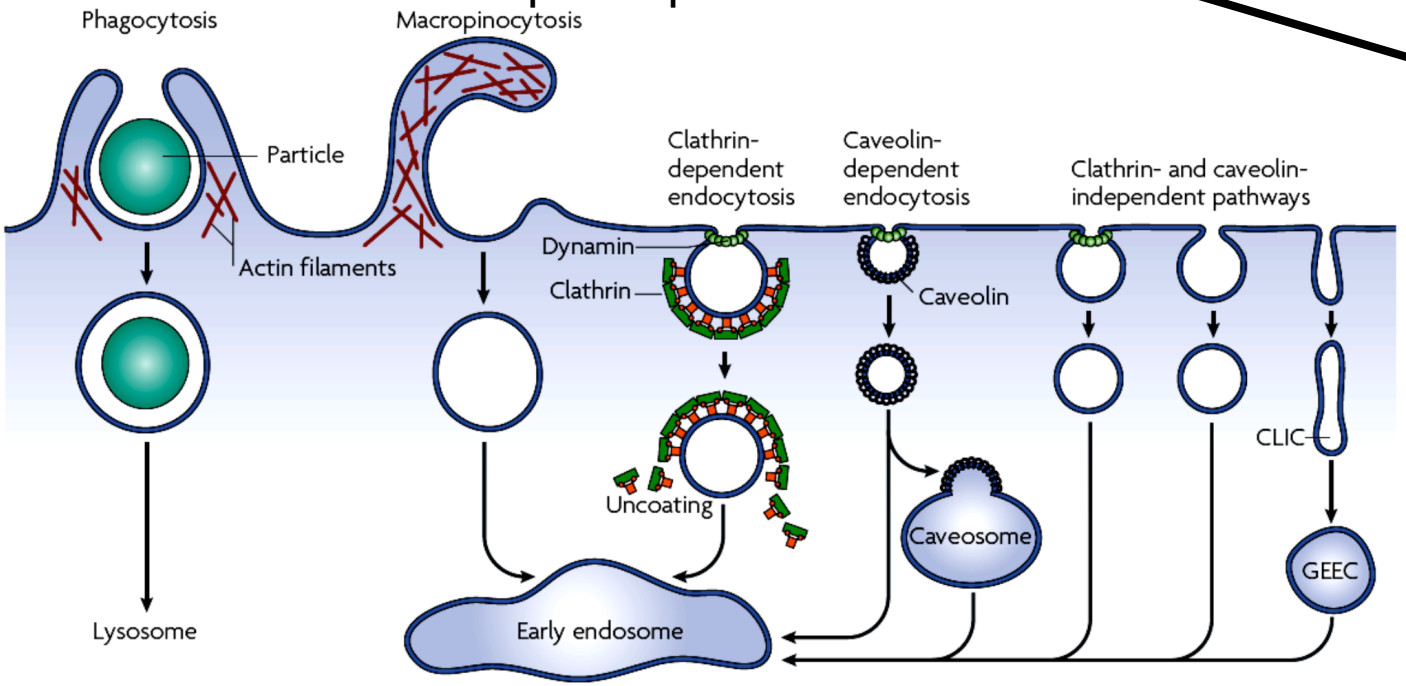
Why would we want to extend Litster's theory?

The hydrophobic effect means that lipid bilayers tend to resist pore formation. But cells sometimes *need* to allow pores to form.

It's hard to control membrane tension (as in Litster's theory), so cells use specialised proteins to create pores in the membrane in a tightly-regulated process that is highly conserved across species.

We'll see later that bacteria co-opt this process to infect cells.

Why?



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

Revisit the assumptions T > 0

Which free energy do we use? E (Litster theory only for T = 0 — does this mean -273 K?)

$$F = E - TS$$

$$G = E + PV - TS$$

$$\Omega = E - TS + \mu N$$

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

What is the system and what are the boundary conditions? Assume a single pore for now.

- Membrane is stabilised by the hydrophobic effect that inhibits small pores
- If a pore grows > 1 nm, lipids on the boundary have greater freedom to move than those in the bulk membrane
- proteins and peptides may bind to/insert into the membrane and nucleate pores, or aggregate at their boundary. so and reduce the pore edge energy: λL where λ is the edge energy per unit length.
- If the membrane is tense, a pore experiences a force trying to enlarge it due to surface tension

Pore free energy

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)
k_BT = temperature (J)

L = length of pore edge
A = area of membrane including pore
$S(\langle L\rangle)$ = entropy of fluctuations of pore edge

So now we have 3 parameters (λ, p, T), and an as yet unknown function $S(\langle L\rangle)$ - the entropy of a pore with mean edge length $\langle L\rangle$.

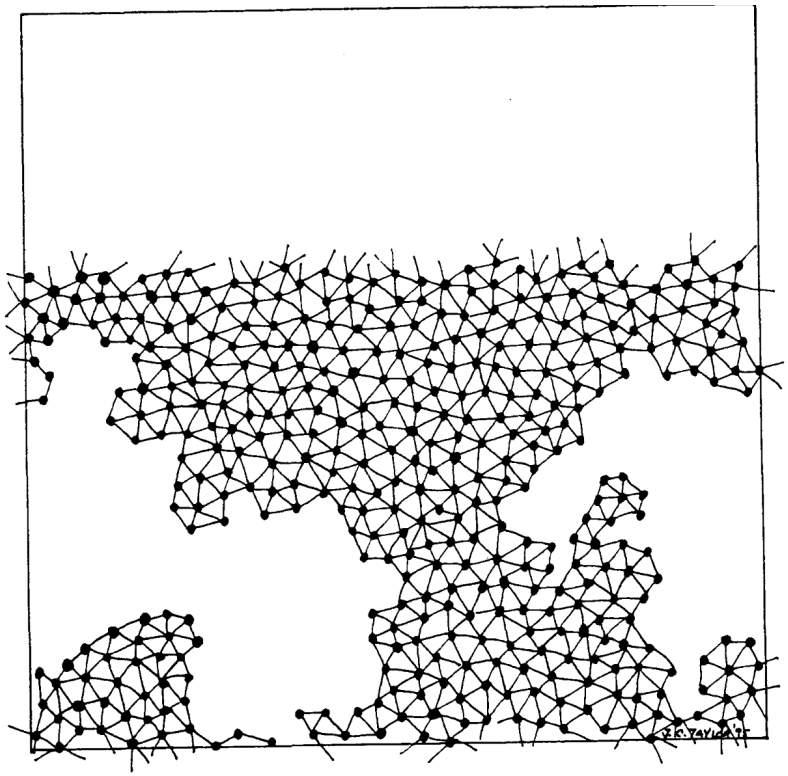
We need a model of the pore shape and its shape fluctuations to enable us to find the entropy of the boundary.

Pore edge is a self-avoiding random walk

Assume a single pore for now that fluctuates in shape and size in two dimensions. Its energy is proportional to the edge length, and its entropy is related to the number of configurations it has.

From lattice calculations, the number of configurations of a closed loop 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 this pore?

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

Barrier height against pore growth

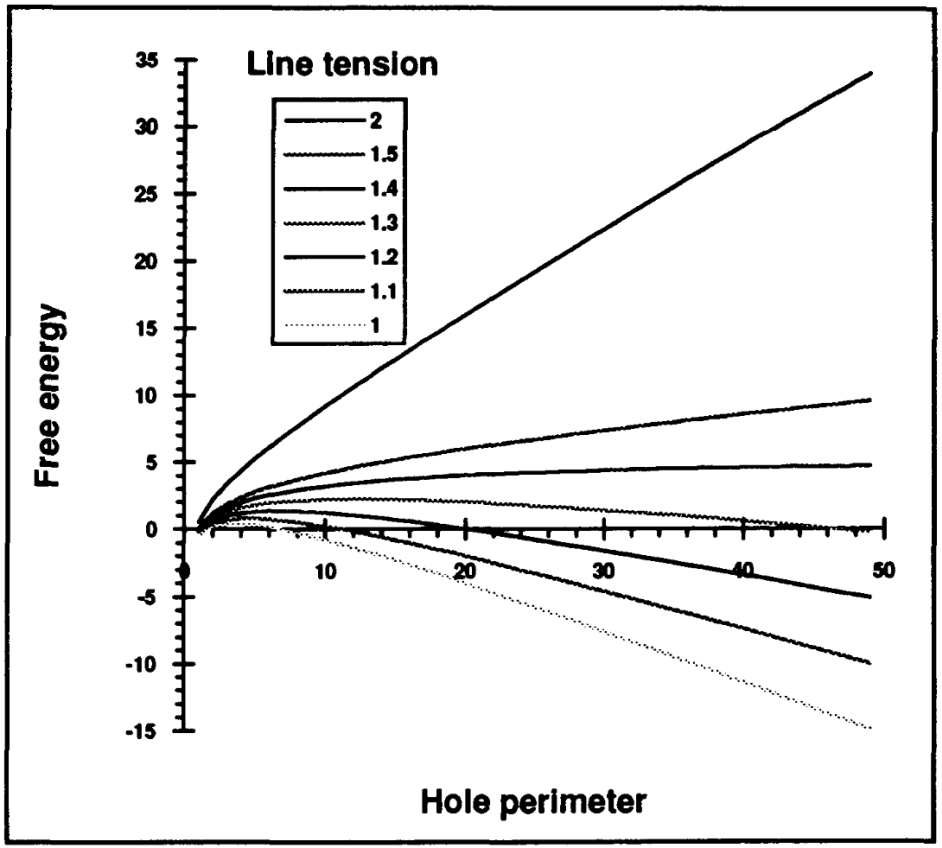
Assume $S = k_B T \ln \Omega(\langle L \rangle)$, and $\langle L \rangle \sim n\, a_0$ then:

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

A pore can now 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

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

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

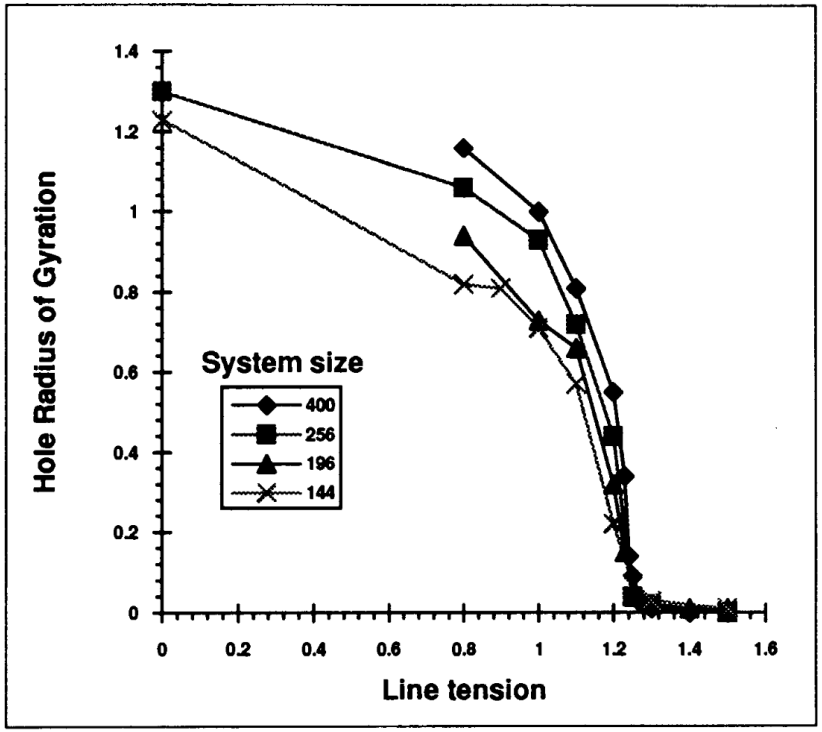
Free energy model with T > 0

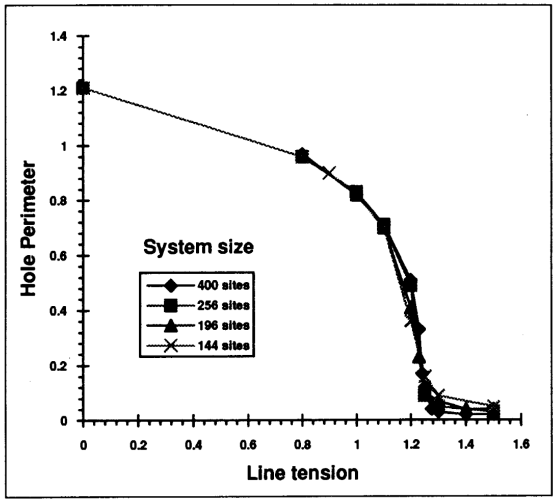
$$n^* = (\alpha - 2) / (\beta \lambda a_0 - \ln z) = 1.5 / (\ln z - \beta \lambda a_0)$$

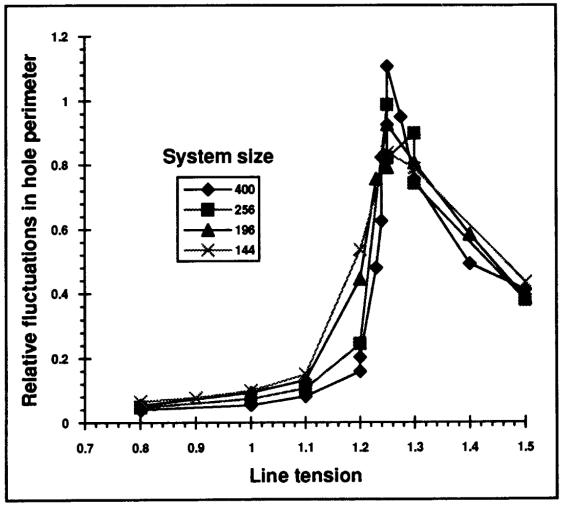
$$\Delta \beta G(n^*) = (\alpha - 2)(1 - \ln((\alpha - 2) / (\beta \lambda a_0 - \ln z))$$

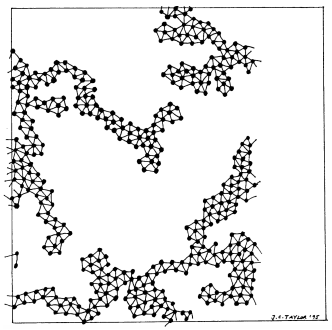
Triangular lattice
$z = 4.15$
$\alpha = 0.5$
McKenzie, 1976

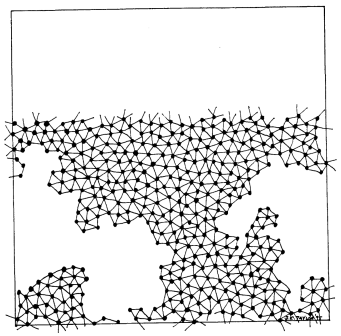
Single pore shape dynamics

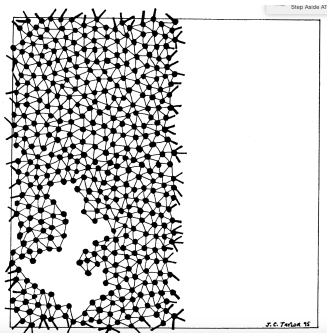












$\beta\lambda a_0 \sim 0.8$ $\beta\lambda a_0 \sim 1.24$ $\beta\lambda a_0 > 1.24$

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

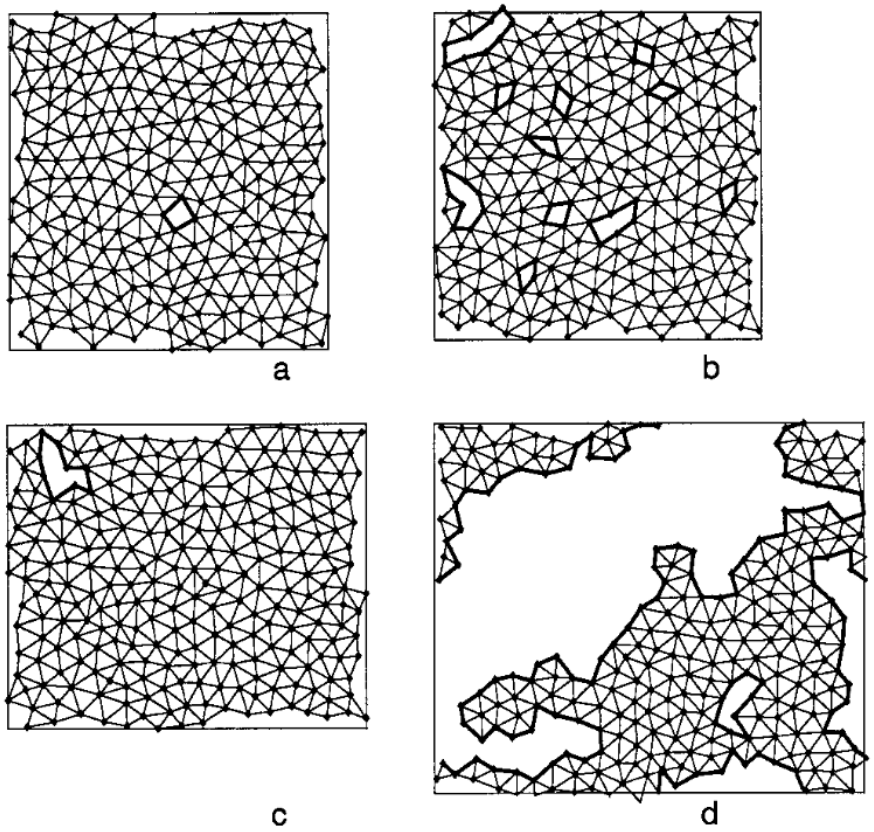
$\Rightarrow$ phase transition from stable membrane to ruptured membrane as $\beta\lambda a_0$ decreases

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**.

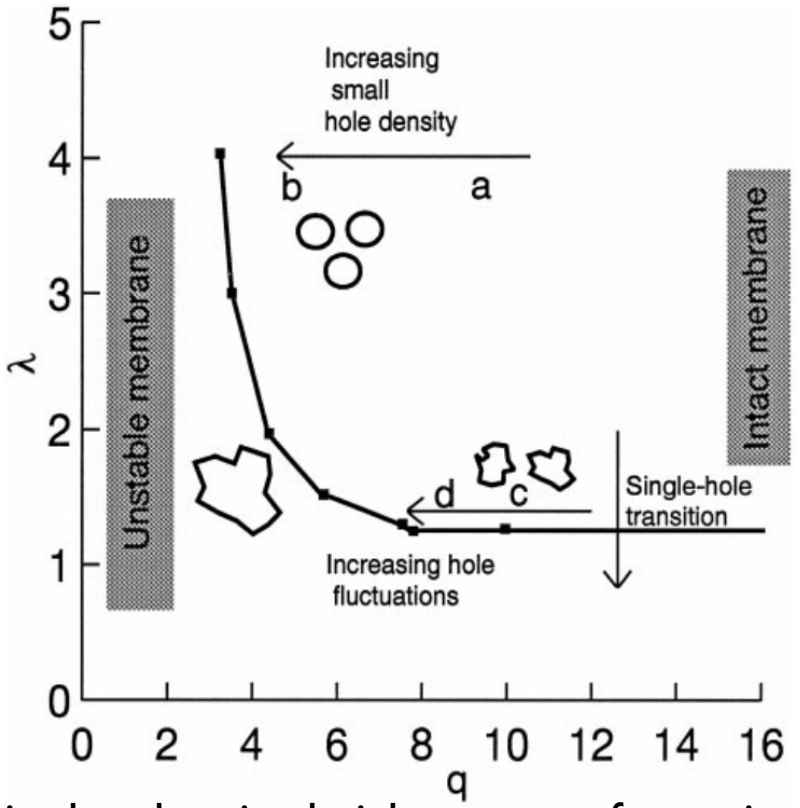
- pores grow/shrink by removal/addition of edges around their rim with an edge energy cost λL controlled by a line tension parameter **λ**



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

Rupture transition in a membrane

Multiple pores are controlled by a barrier height βq, and subsequent growth by $\beta\lambda a_0$, we can calculate the phase diagram shown.



A) 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.

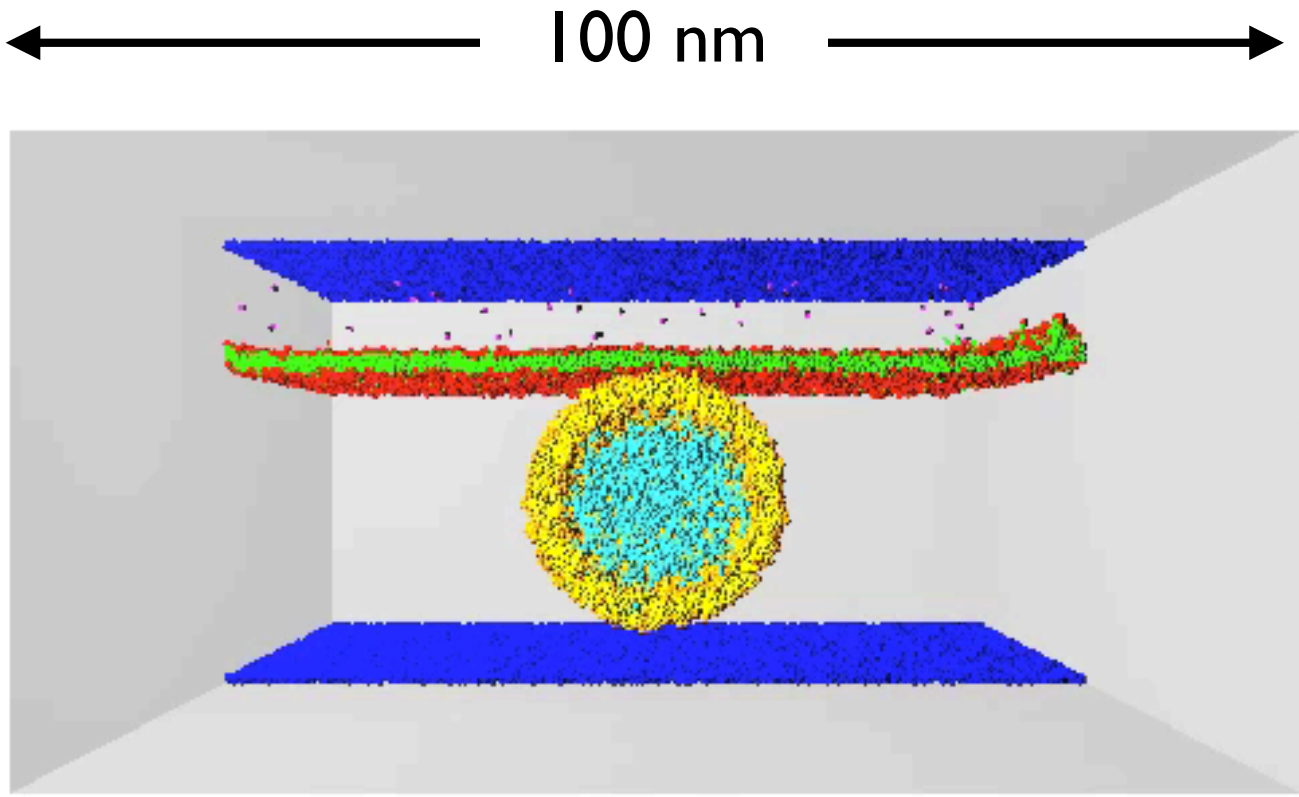
B) Single large fluctuating pore

Model says the only important parameters are the dimensionless barrier height to pore formation **βq**, and the line tension of the pore boundary, **$\beta\lambda a_0$**. A cell can make lipids with shorter tails or larger headgroups to weaken the lamella state or non-lamellar forming molecules (single tailed lysolipids or peptides) to reduce the energy cost of the pore boundary.

Antibacterial peptides use this mechanism to rupture cells; they enter the membrane, aggregate, and lower the barrier height against pore formation and expansion thereby killing the bacterium.

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

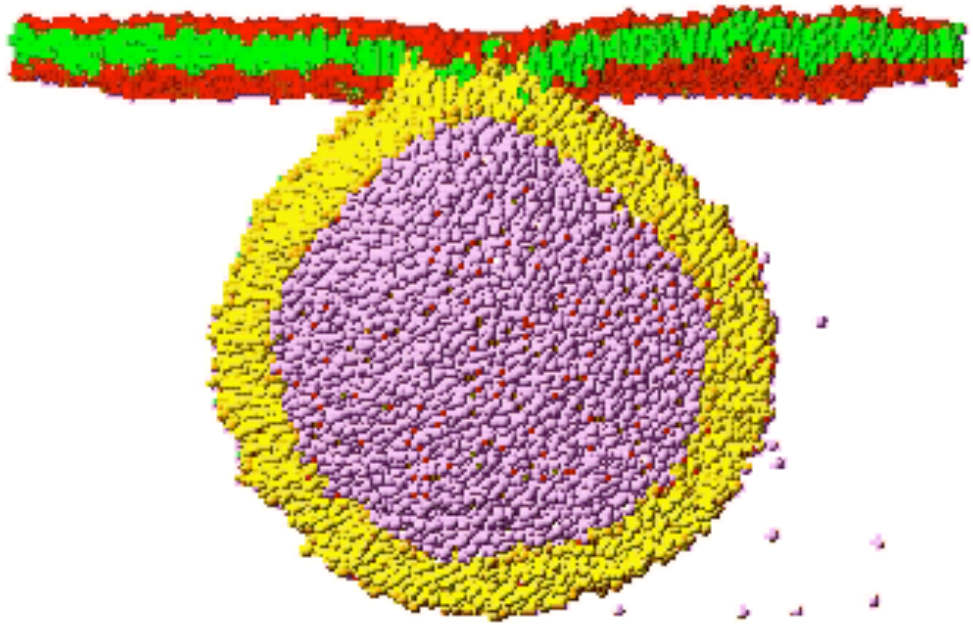
and in DPD simulations …



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

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

Tension-induced vesicle fusion



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

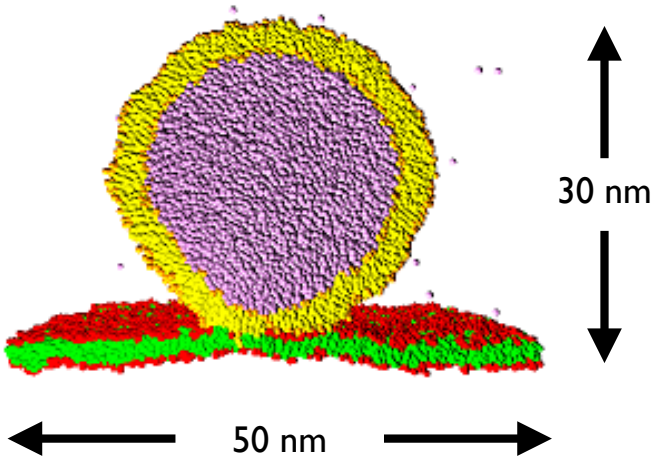
Grafmueller, Shillcock and Lipowsky, *PRL* **98**:218101 (2007) - DPD

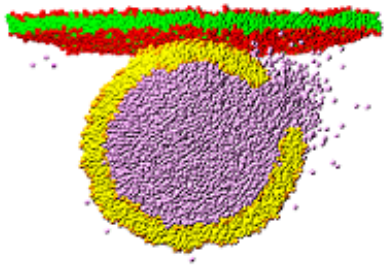
Müller M, Katsov K, Schick M, Biophys. J. 85:1611-1623 (2003) - MC

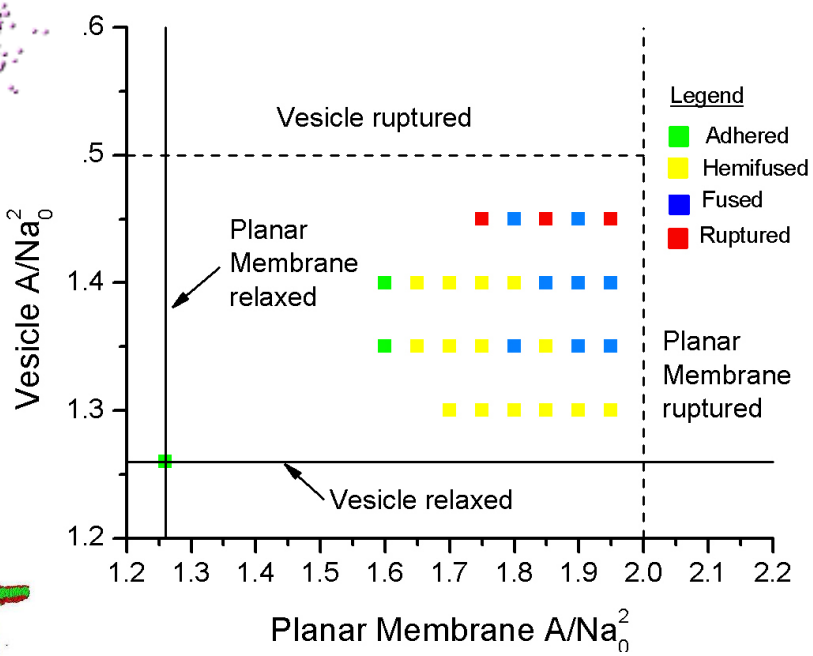
Stevens MJ et al, Phys. Rev. Lett. 91:188102 (2003) - MD

Kasson PM et al, PLoS Comp. Biol 6:c1000829 (2010) - MD

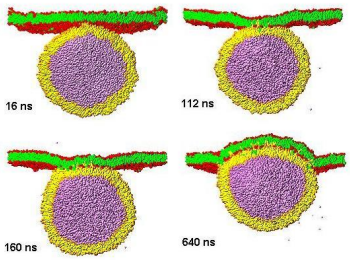
Vesicle fusion protocol: several outcomes







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

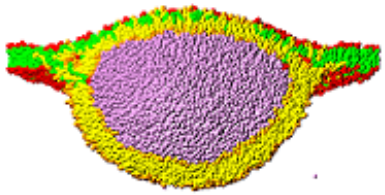


Bilayer and vesicle lipids: $H_3(T_4)_2$

Relaxed N_{ves} = 6542

Relaxed N_{bil} = 8228

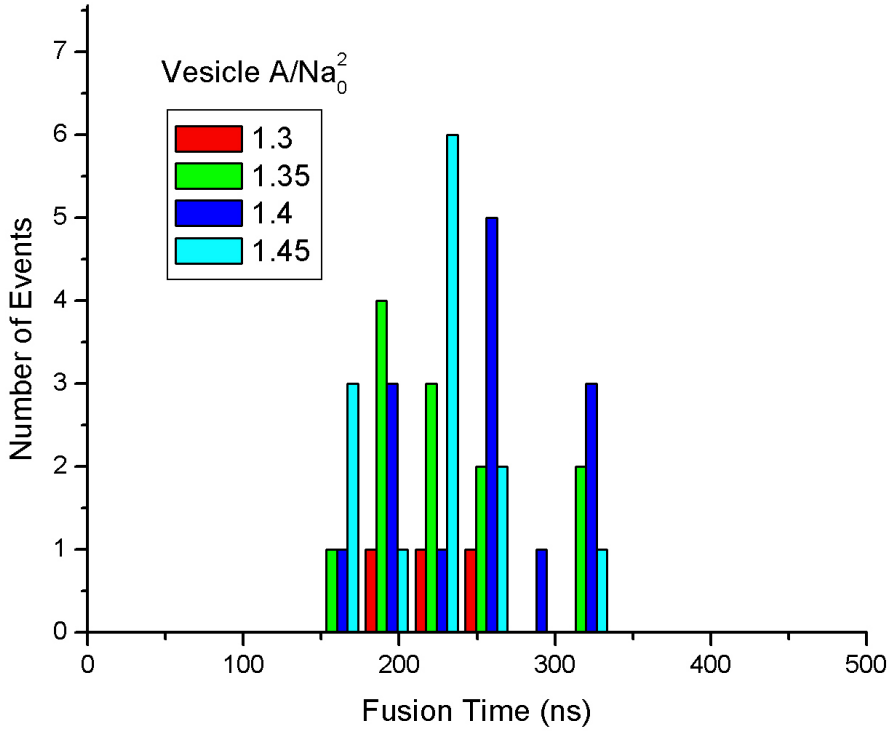
Why?



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 observed between 350 ns and 1.6 µs

Why not?

Fusion in reality...

Vesicle fusion requires:

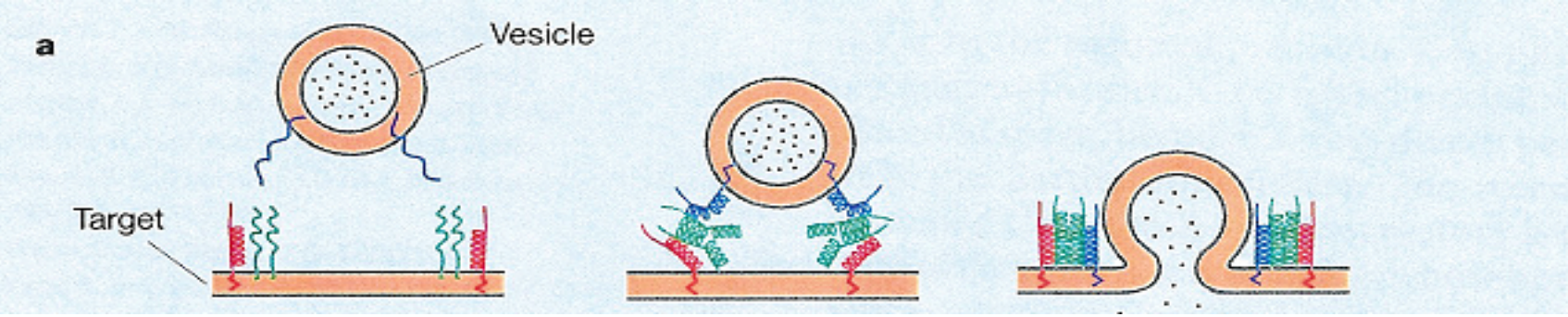
- membrane bending
- membrane merging
- membrane rupture or fission

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

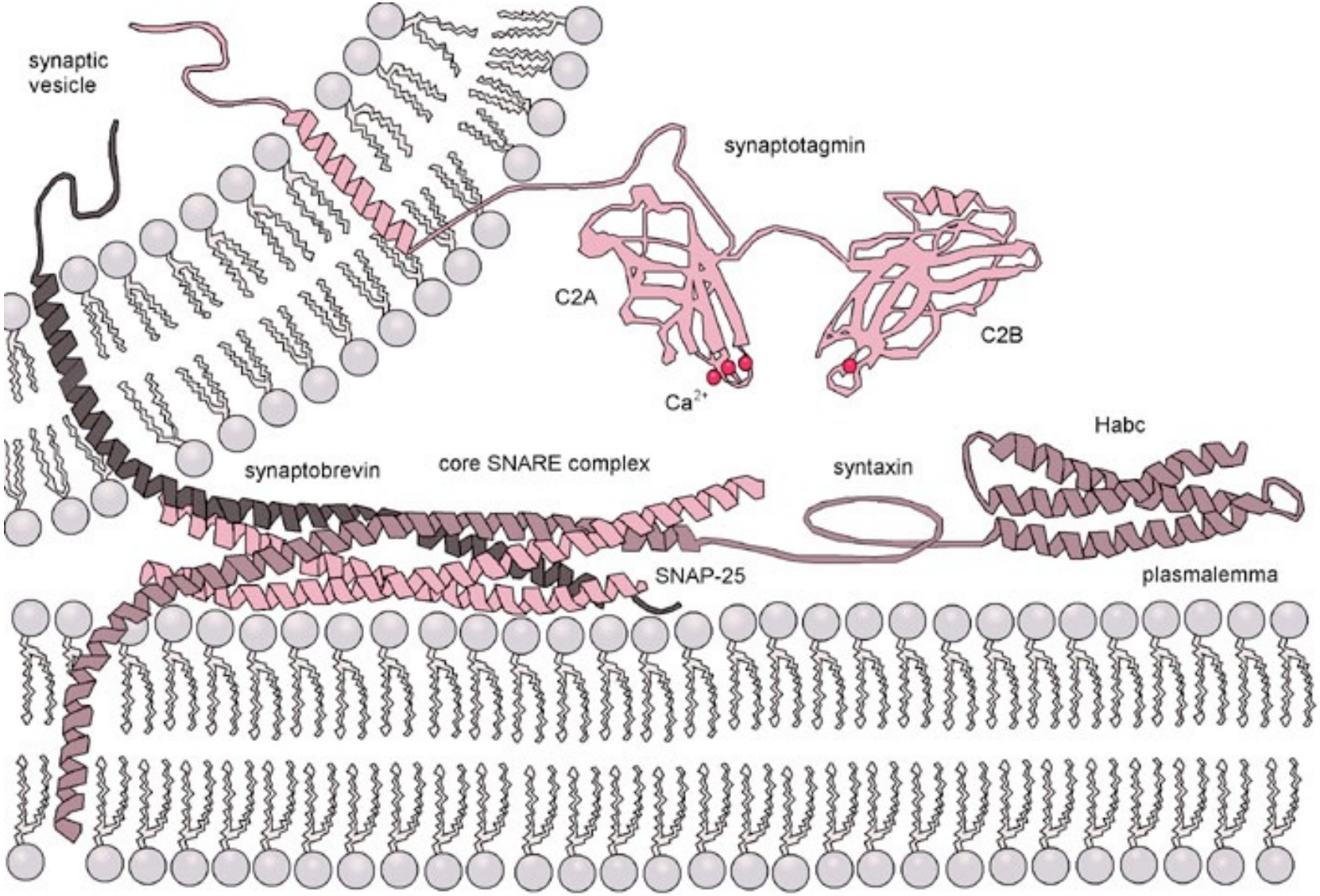
Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

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.

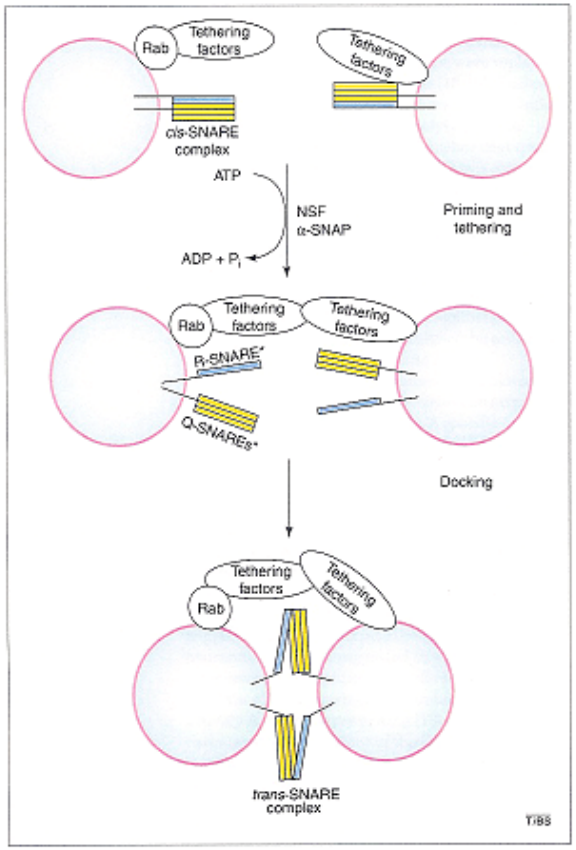
But… what do they actually do? Force, torque, displacement…?

Exocytosis Machinery

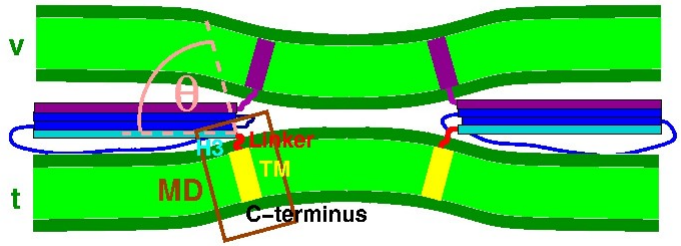


Molecular machinery driving exocytosis in neurotransmitter release: the core SNARE complex (formed by four α-helices contributed by synaptobrevin, syntaxin and SNAP-25) and the Ca2+ sensor synaptotagmin.

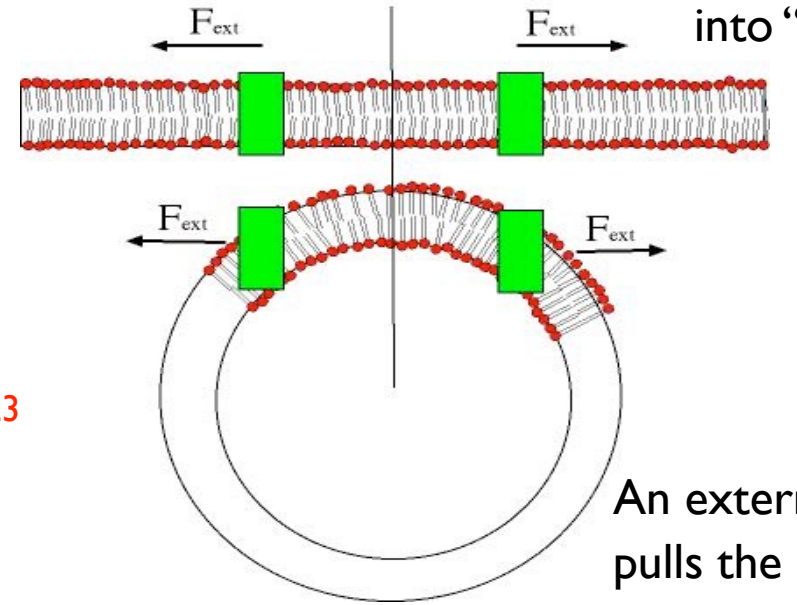
Protein-driven vesicle fusion



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

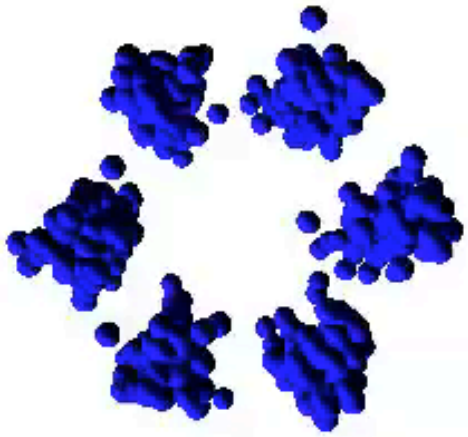
An external force, of magnitude F_{ext}, pulls the barrels radially apart

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

Transmembrane proteins span the axon and vesicle membranes, 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



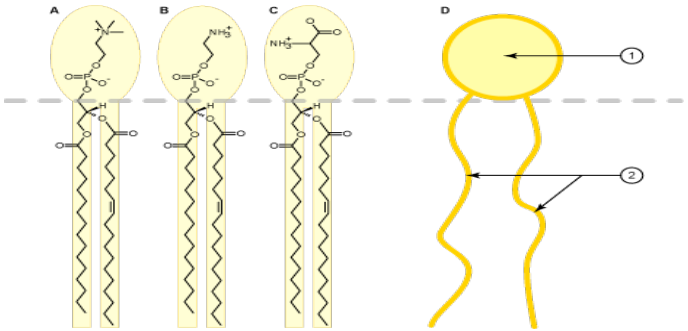
Box = 100 x 100 x 42 nm^3
3.2 x 10^6 beads in total

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

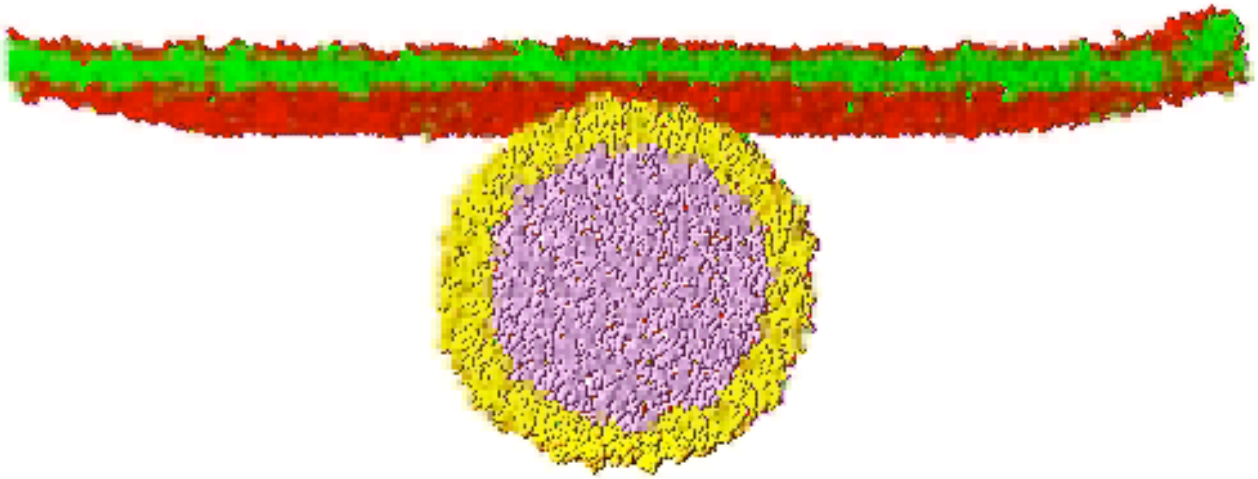
6 proteins in a circle per membrane
- area per protein matches expts.

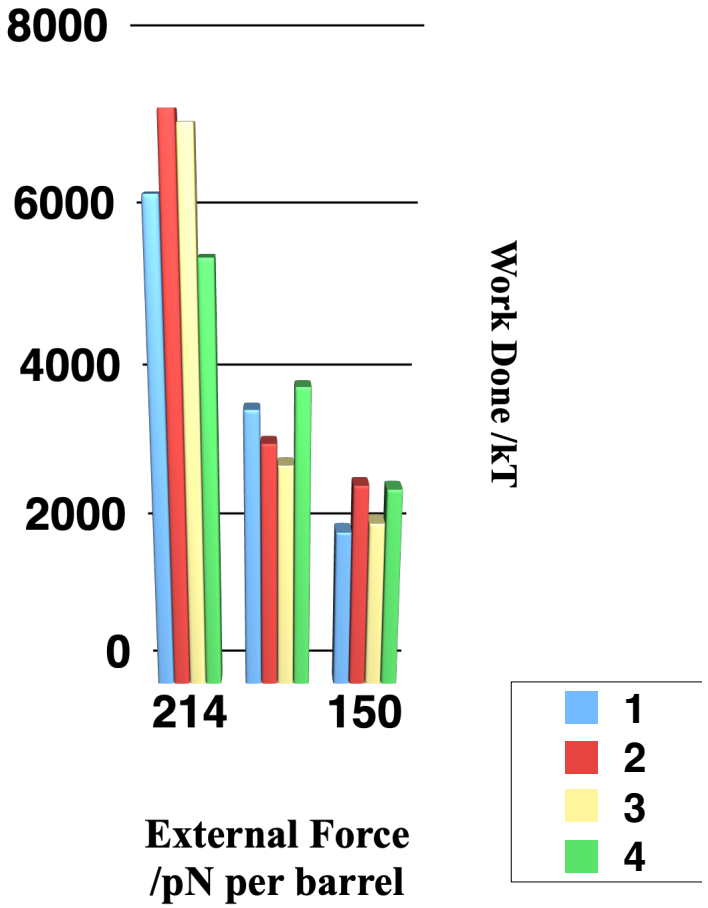
Schuette et al. PNAS 101:2858 (2004)

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

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

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

Synapses operate by a combination of generic processes (membrane rupture, diffusion) coupled to precise molecular structures (lipids, proteins, positioning)

Summary

Membranes play contradictory roles: stability versus transport:

Stability is the equilibrium state (hydrophobic effect, low tension)

Global destabilisation by tension is too unreliable to use for endo- and exocytosis

Cell **locally** manipulates the natural response of a membrane by controlling composition/tension to create localised pores that allow transmembrane transport

Break

10 mins.