Variations on membrane fusion mechanism

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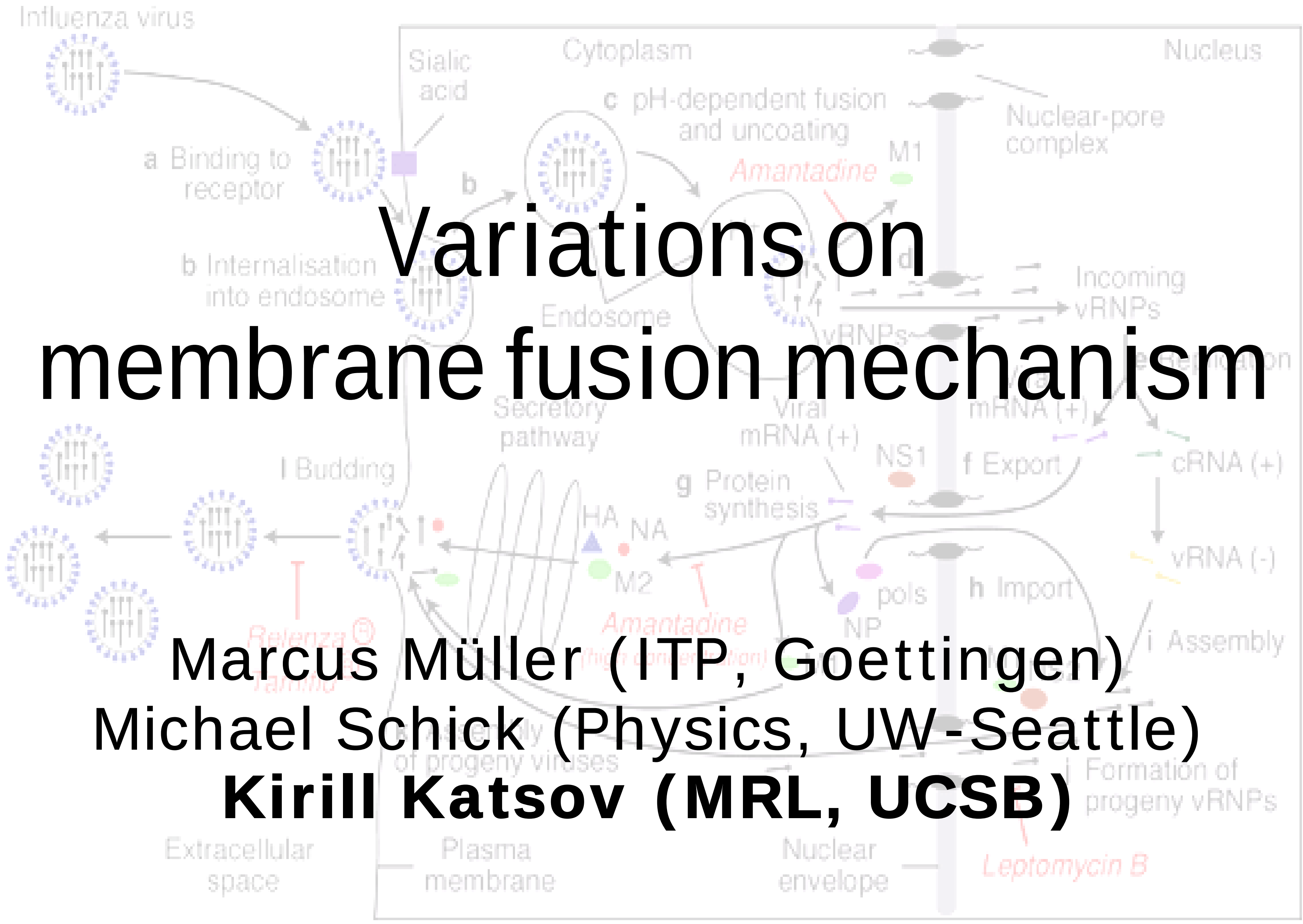
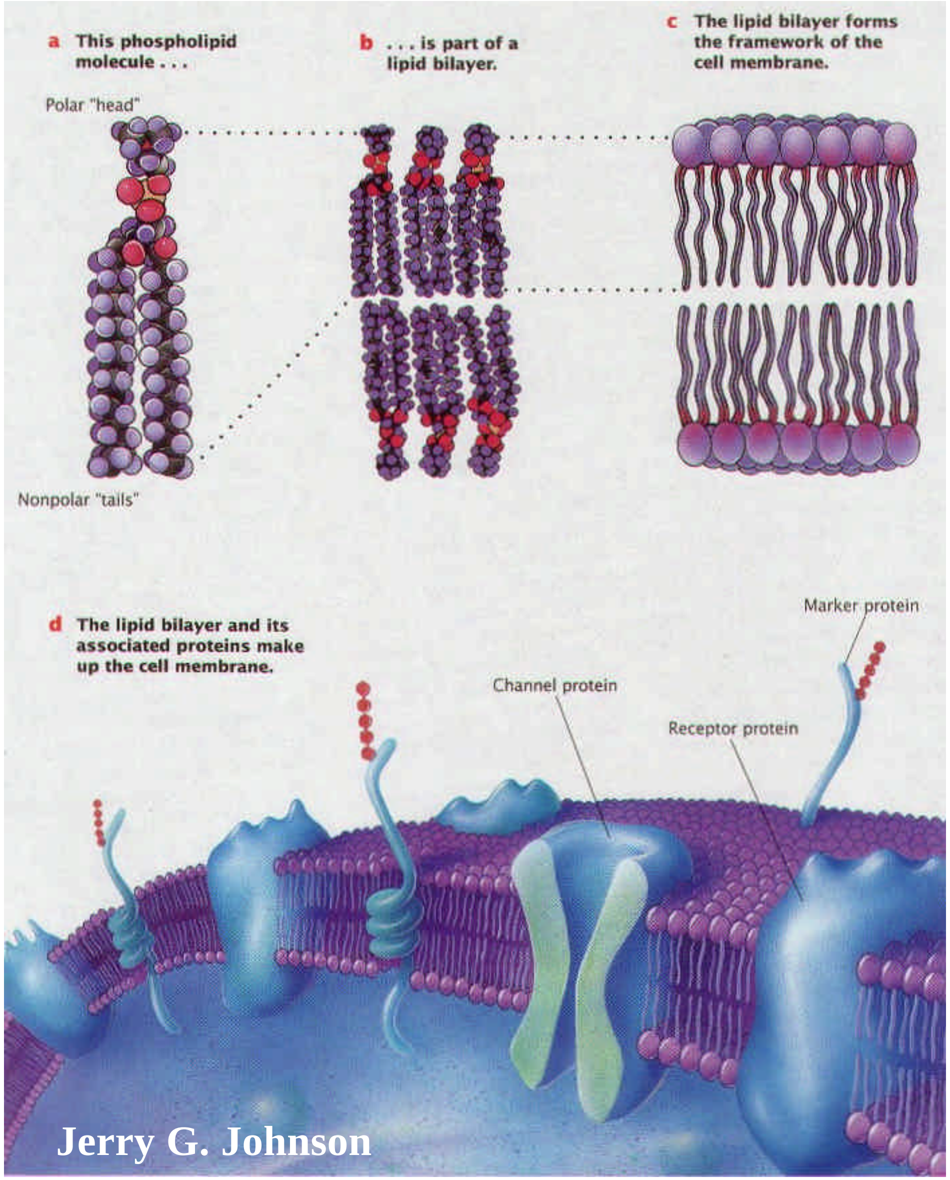
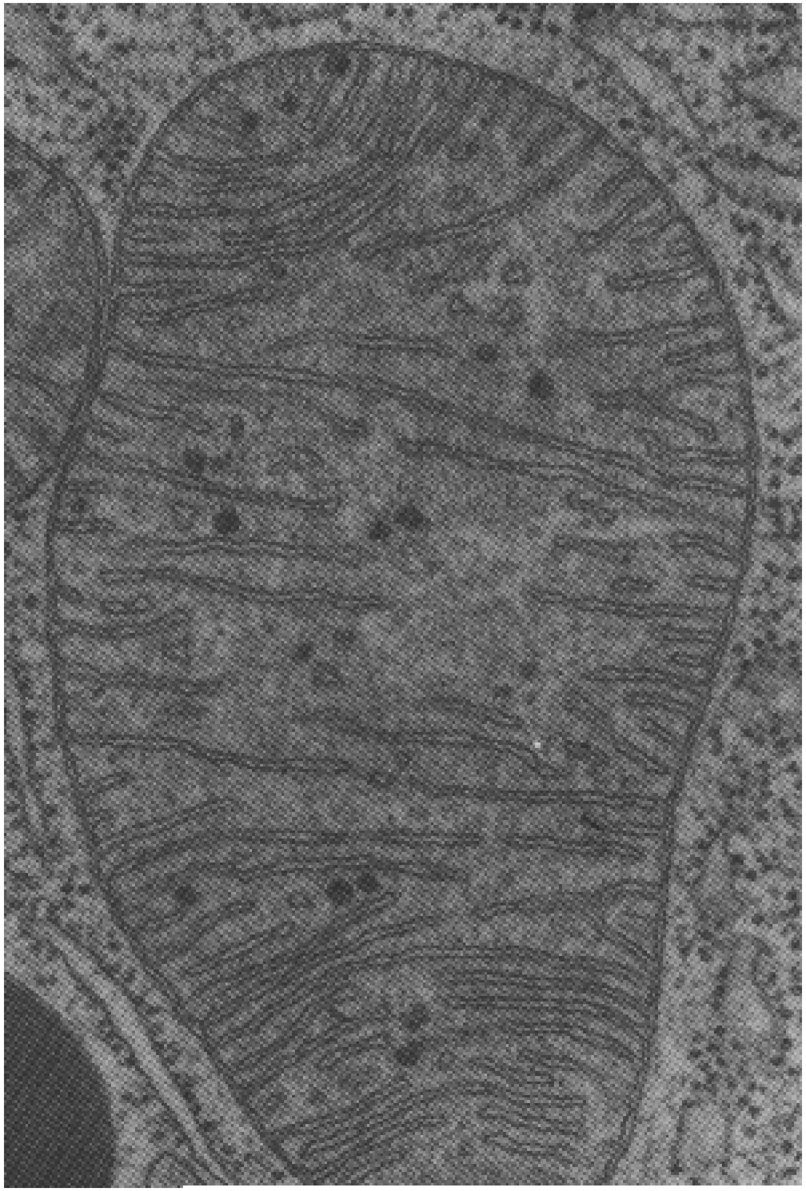


Replication cycle of an influenza virus

Lipid membrane

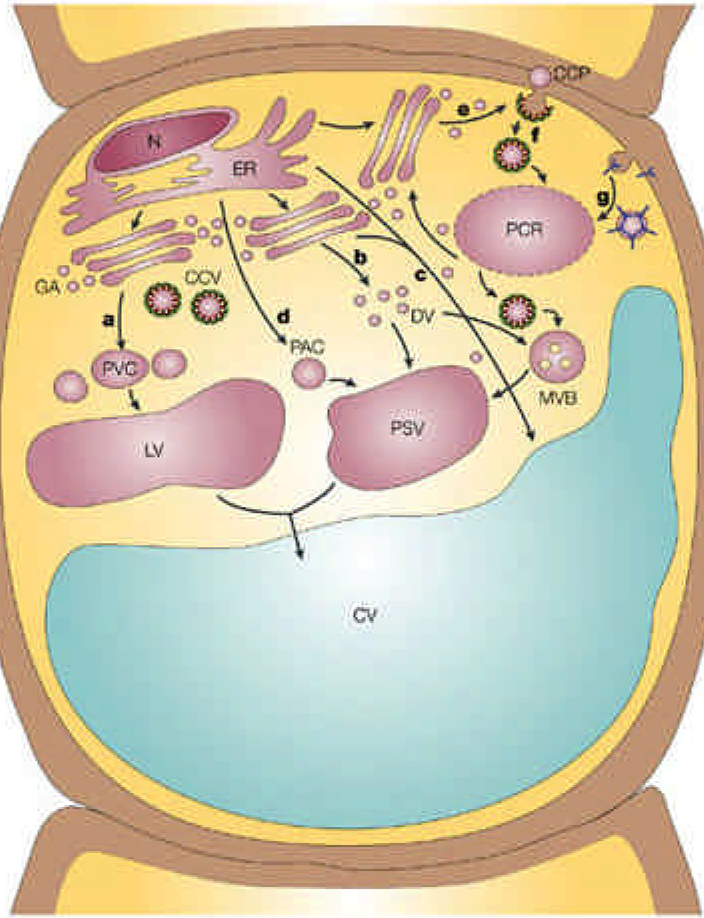




Lipid membrane fusion

Basic biological process:

cell trafficking



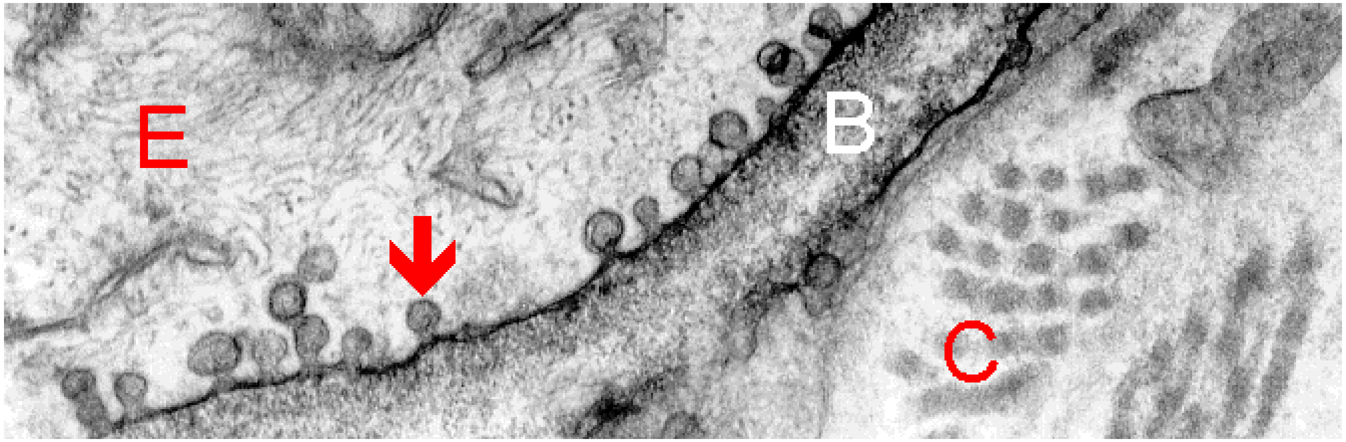
www.cepceb.ucr.edu

Lipid membrane fusion

Basic biological process:

cell trafficking

exocytosis (synaptic release)



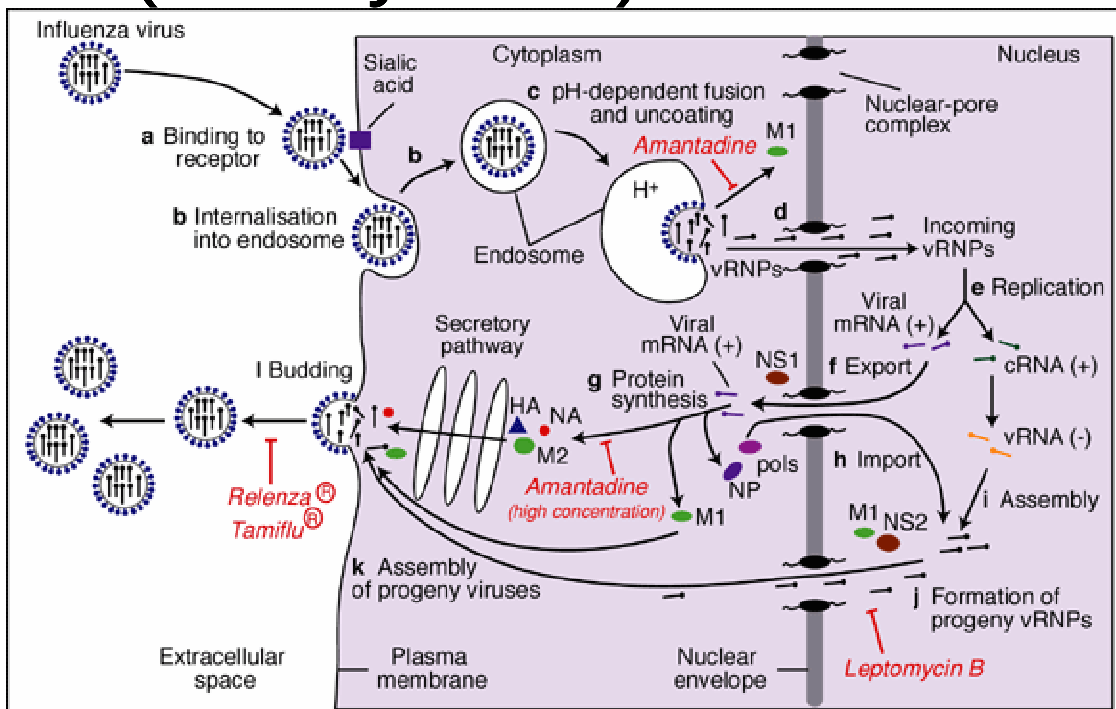
Lipid membrane fusion

Basic biological process:

cell trafficking

synaptic release (exocytosis)

viral entry



Replication cycle of an influenza virus

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Lipid membrane fusion

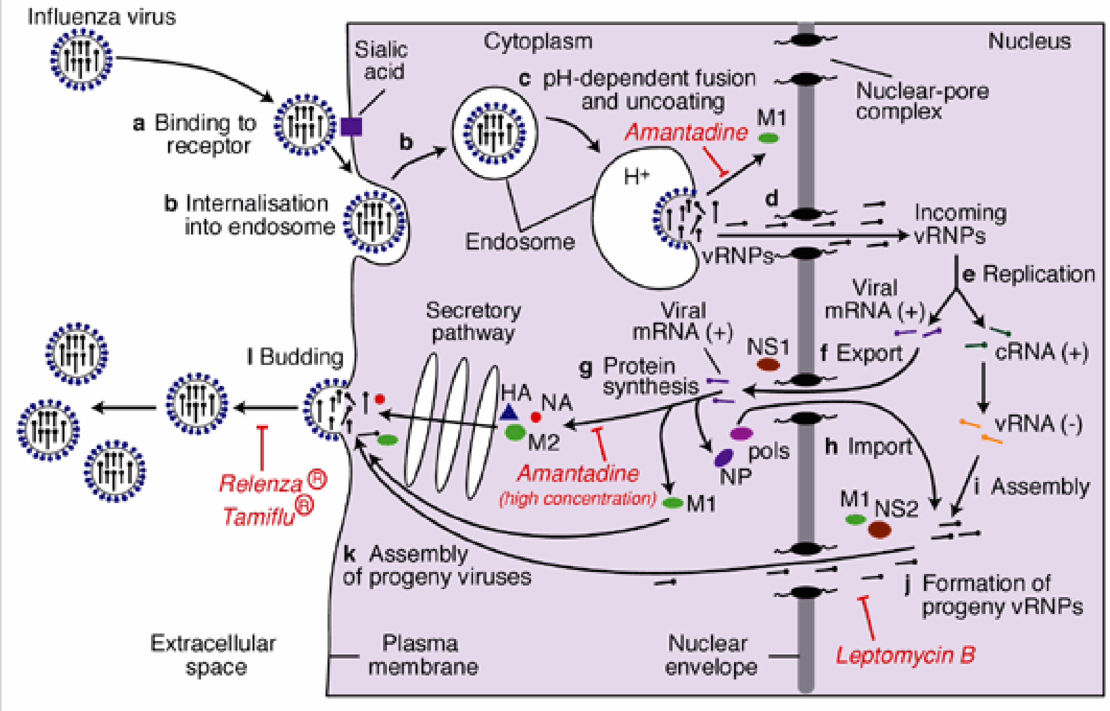
Basic biological process:

cell trafficking

synaptic release (exocytosis)

viral entry

drug delivery



Replication cycle of an influenza virus

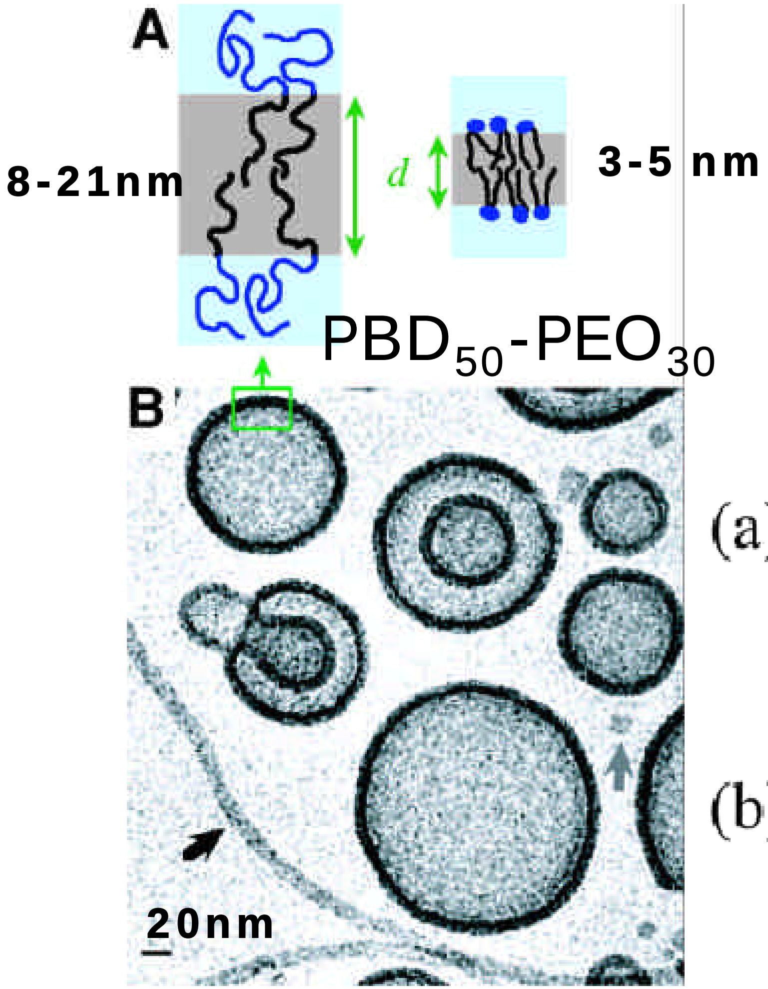
Expert Reviews in Molecular Medicine ©2001 Cambridge University Press

Fusion: experimental facts

- Short timescales – *micro-seconds*
- Small length scales – *nano-meters*
- No direct observation of intermediates
- Indirect evidence via dependence on:
  - lipid composition
  - mechanical/electrical stress
  - temperature
- Microscopic mechanism is not well understood
- Universality(?): fusion peptides or other "machinery" are not necessary for fusion of lipid membranes

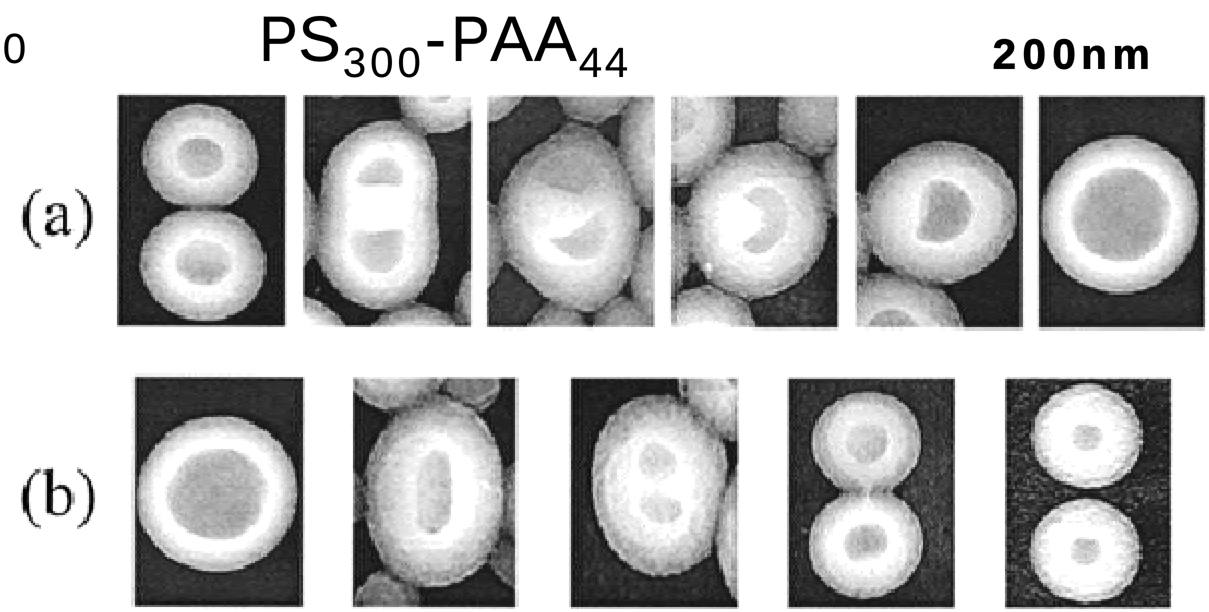
Not only in lipids!

Simple amphiphiles:



Discher et al., Science (1999)

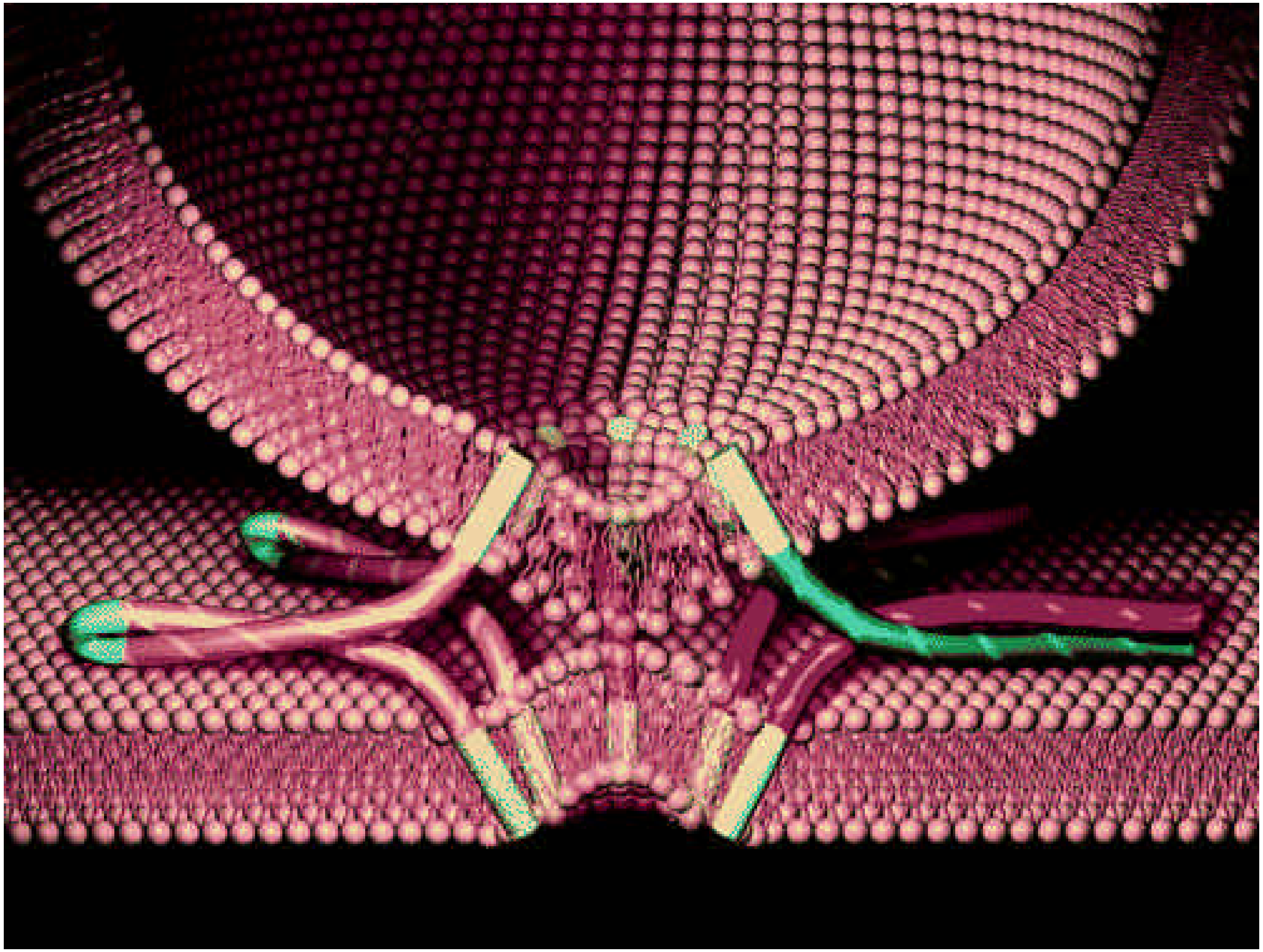
Polymersomes



Luo and Eisenberg, Langmuir (2001)

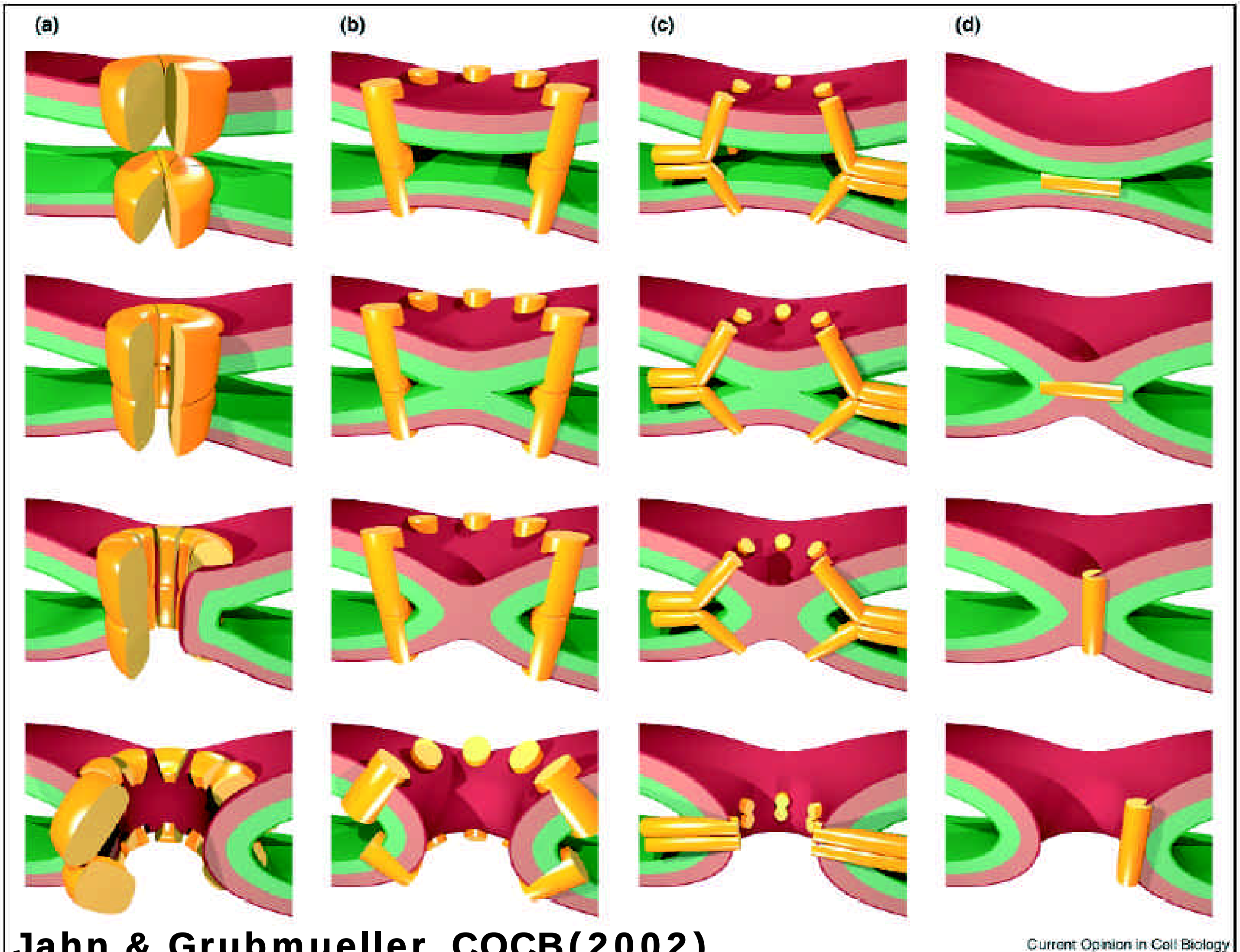
How do things fuse?

A biologist's view



How do things fuse?

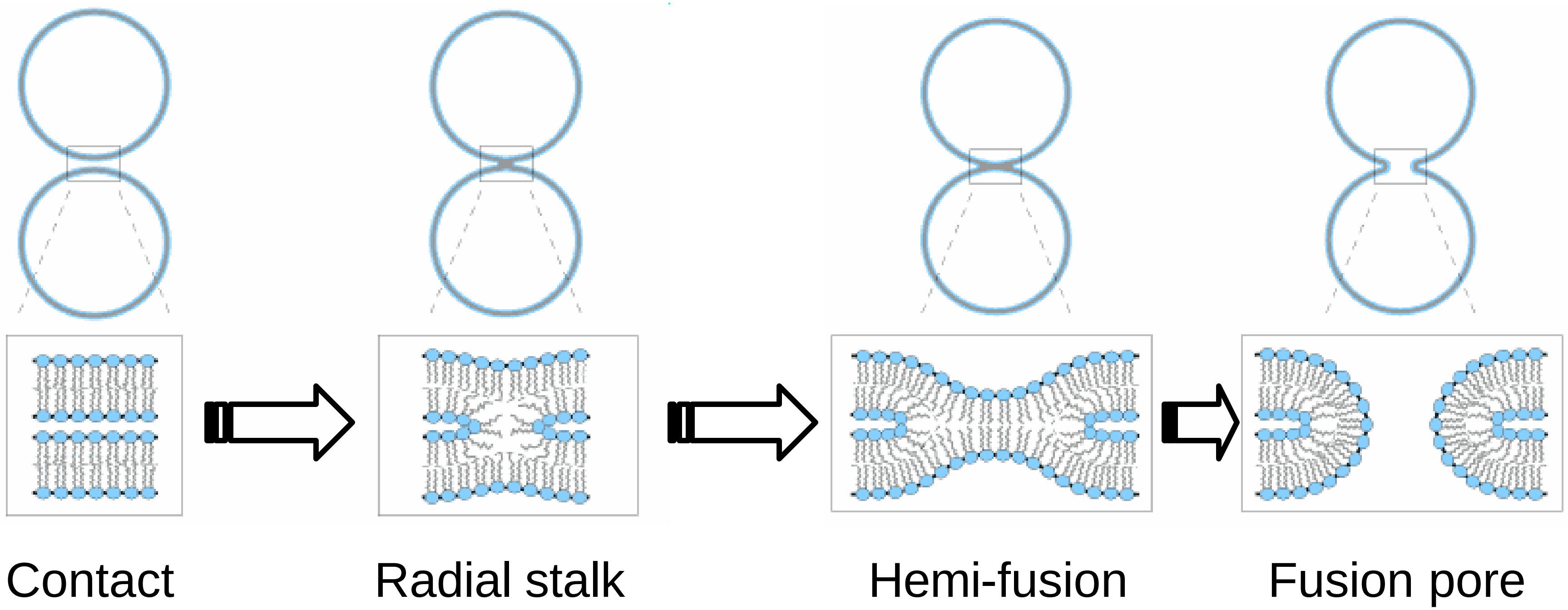
A biologist's view



Jahn & Grubmueller, COCB(2002)

How do things fuse?

A physicist's view



http://www.bnl.gov/bnlweb/pubaf/pr/2002/bnlpr091202.htm

Conceptual difficulty

Membrane stability:

long lived holes must be difficult to form.

Membrane fusion:

long-lived holes must be easy to form.

Modelling membranes

-ab initio:

MD [1nm, 10ns]

-coarse-grained:

MD, MC, (D)DFT, (D)SCFT, DPD

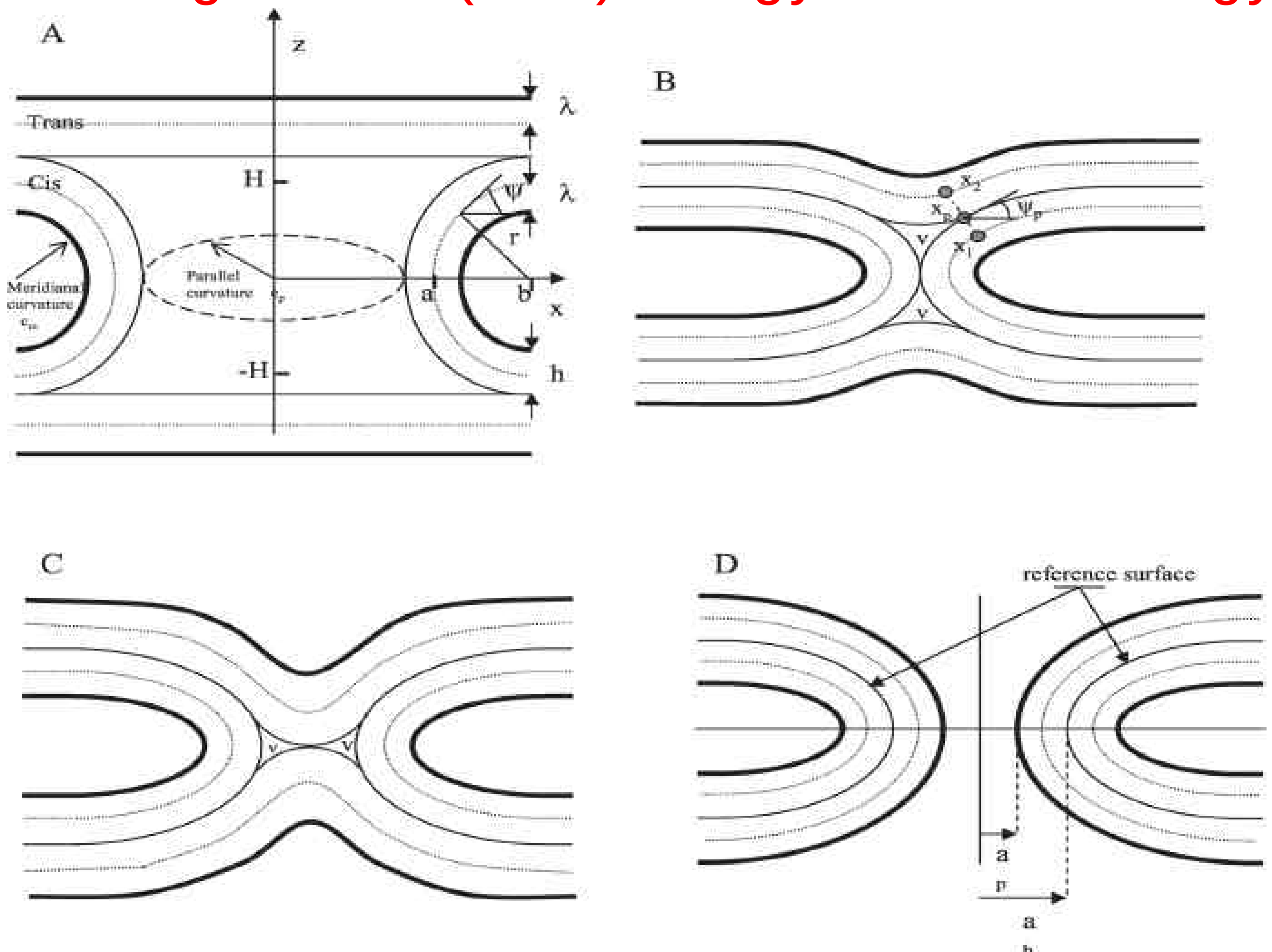
-phenomenological:

2D elasticity theory

First attempt: Kozlov & Markin (1983)

Phenomenological approach

Bending elastic (free)energy + Void energy

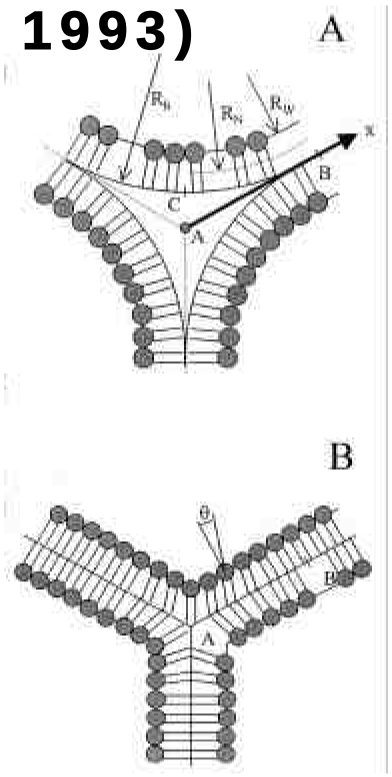


“Energy crisis”

200 kT

40 kT

- Torroidal shape (Kozlov et al., 1983)
- “Void” energy estimates (Siegel, 1993)
- Stress free stalk
  (Markin et al., 2001)
  Torroid-to-Catenoid
- Tilt and Splay deformations
  (Kozlovsky et al.,
  2001; Kuzmin et al., 2001)

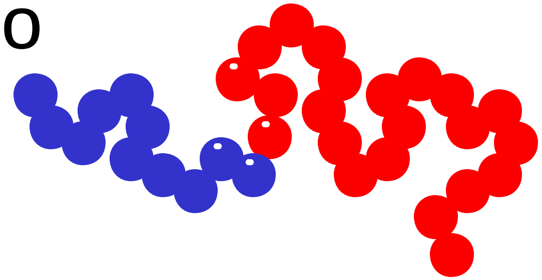


Simulating fusion

System: diblock co-polymer + solvent

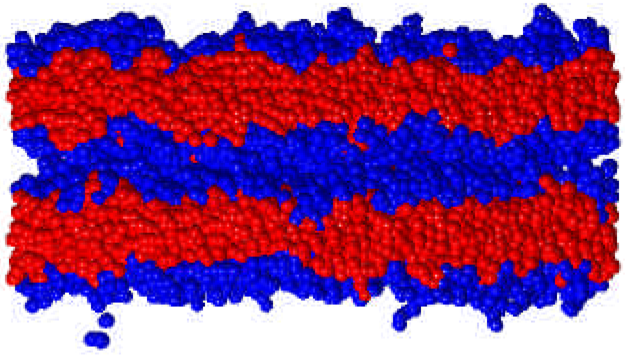
Algorithm: Bond Fluctuation Model

Lattice Monte Carlo

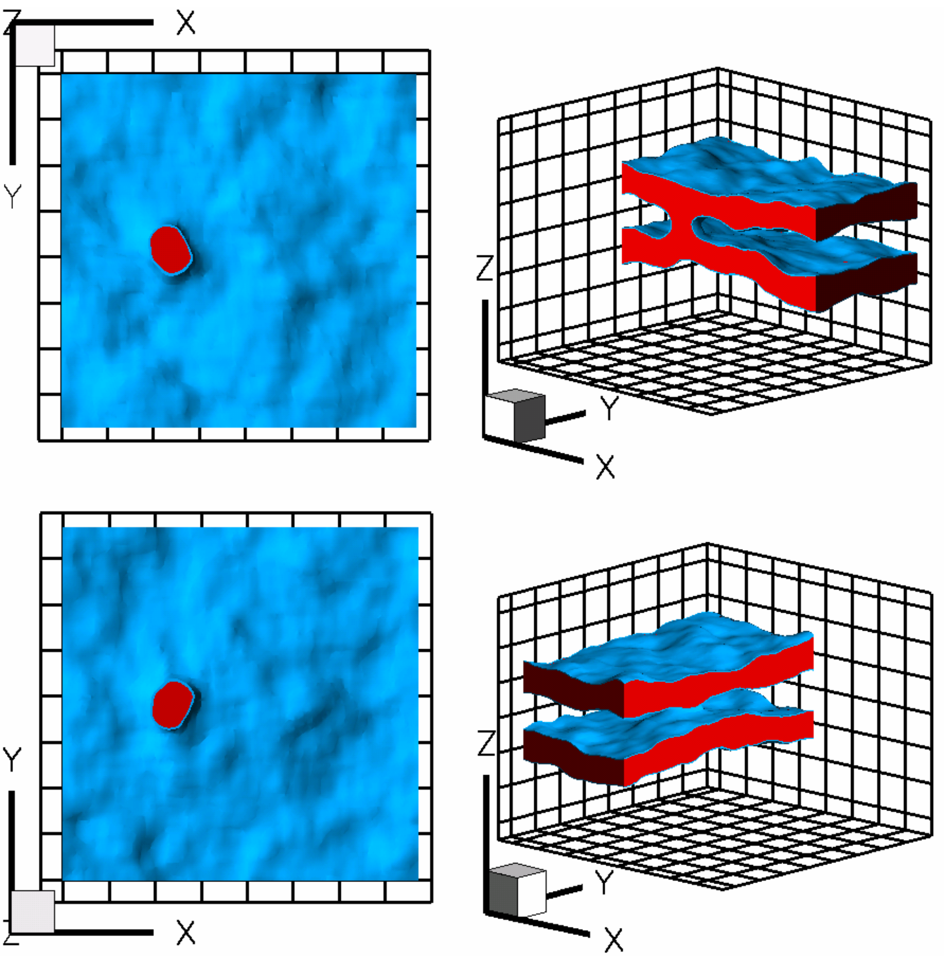


Advantages:

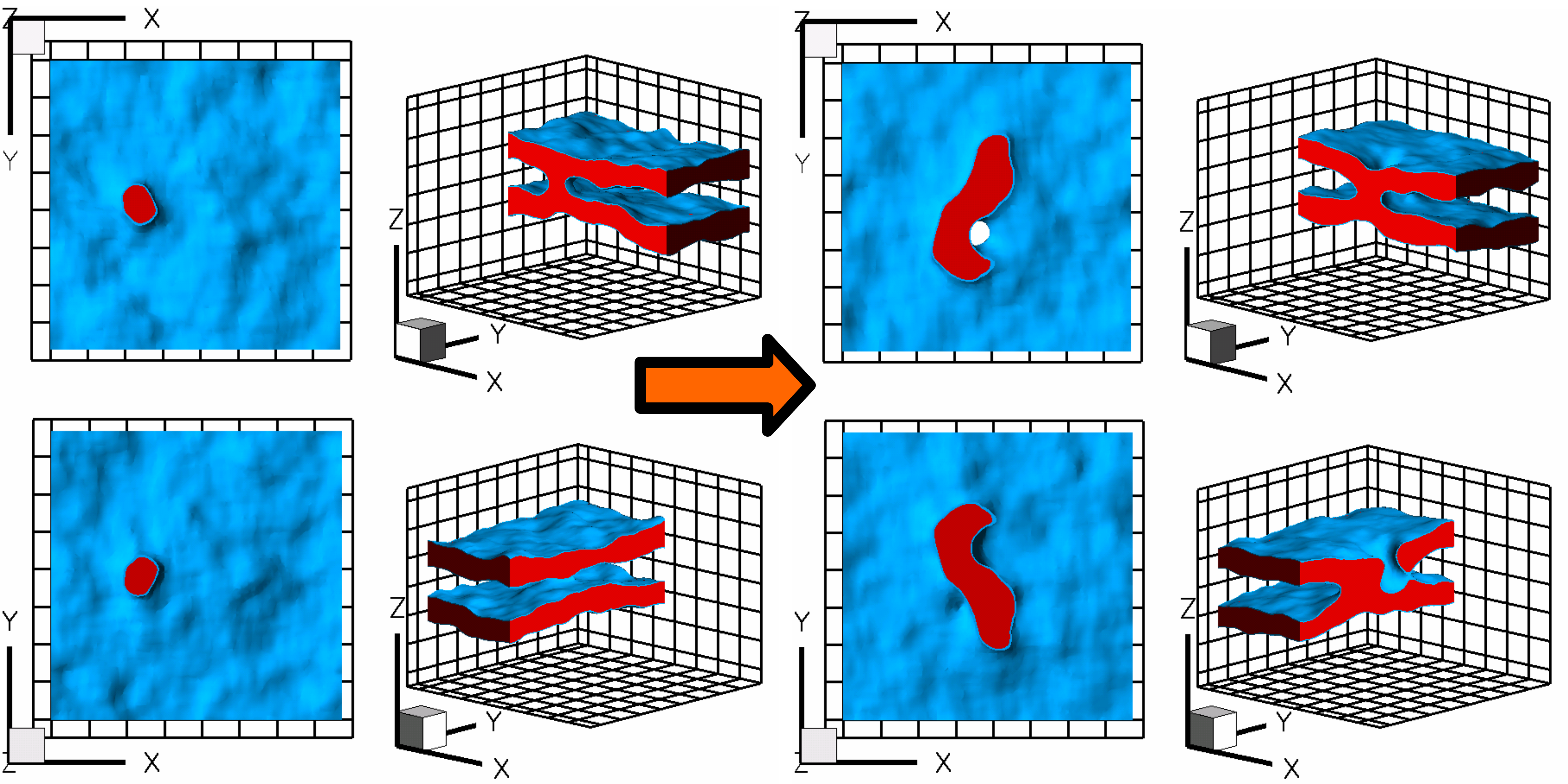
- **fast** (fusion does happen!)
- **large** system size (~250 nm^2,2500 "lipids")
- **diffusive** dynamics (local moves)



Stalk formation

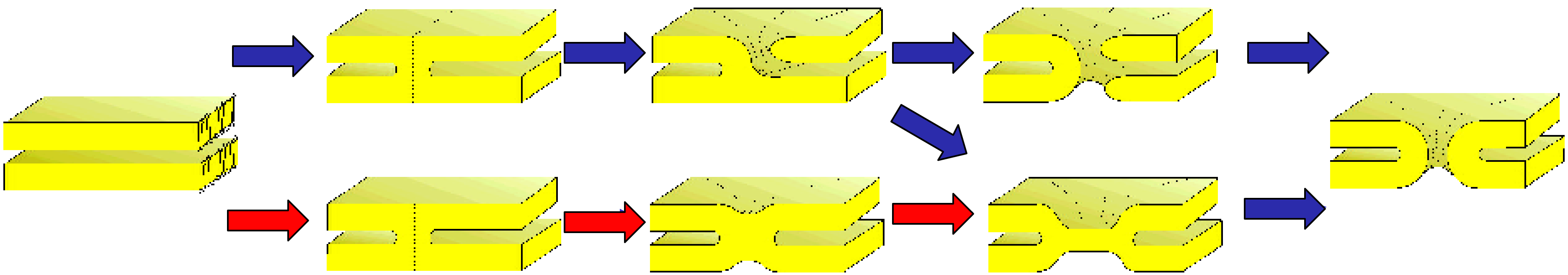


Stalk expansion
+
hole formation



Fusion Mechanisms

Mechanisms observed in simulation



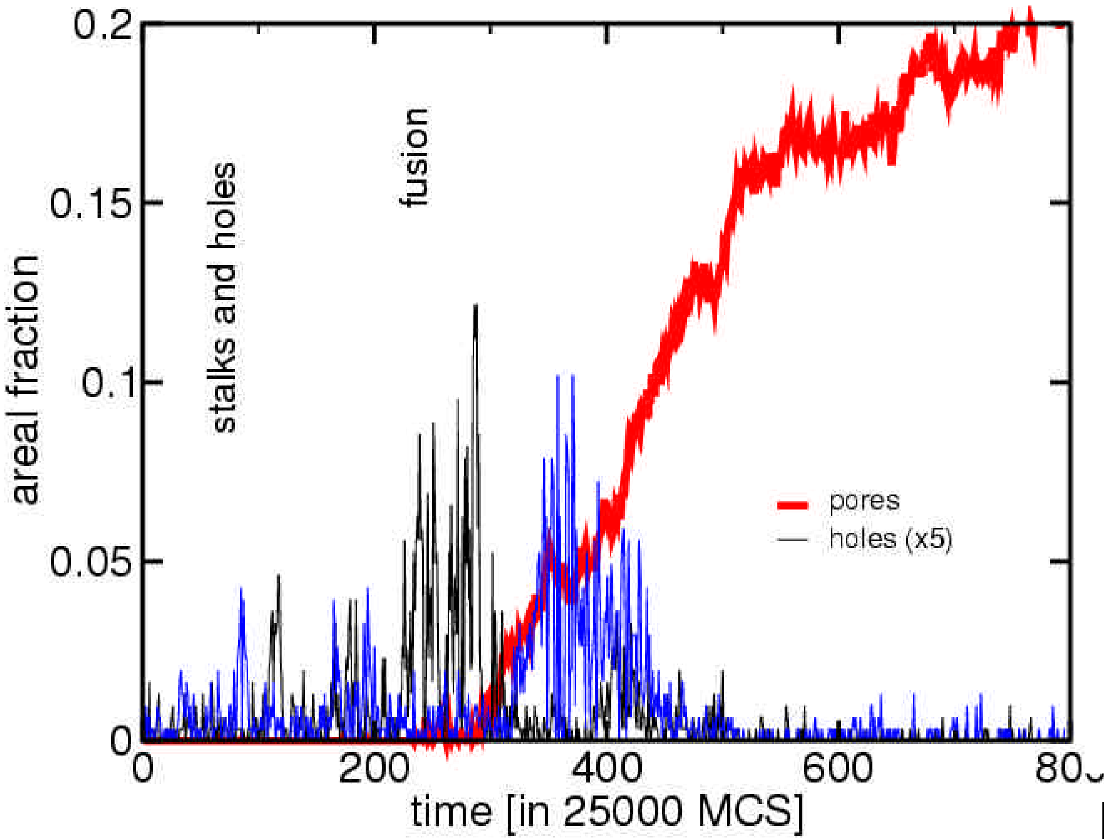
Hypothesized “stalk” mechanism

Can we distinguish these mechanisms through the indirect experiments?

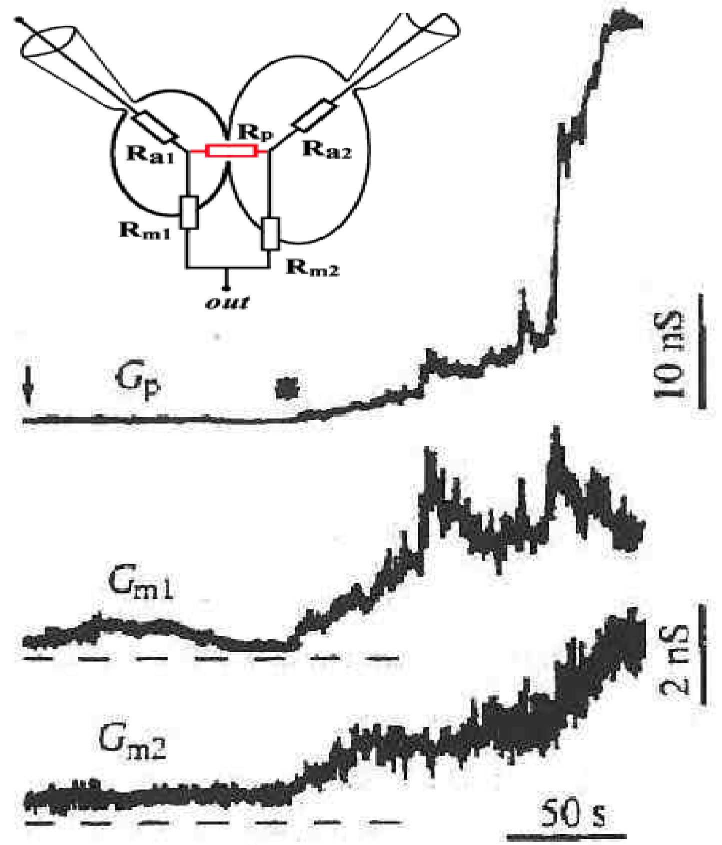
Consequences???

Fusion and Leakage

Simulation



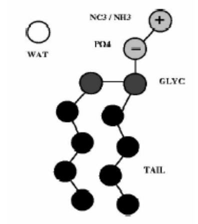
Experiment

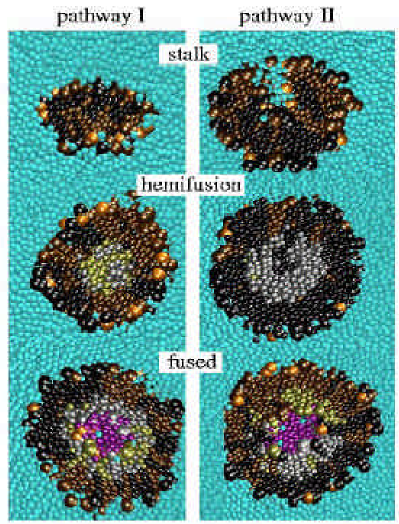


Dunina-Barkovskaya et al.
Memb.Cell.Biol. (2000)

Many models – same result…

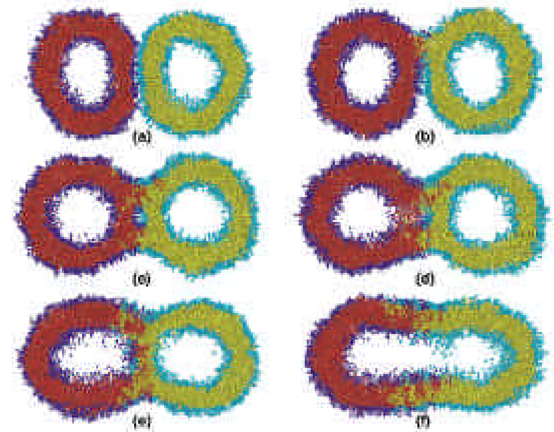
MD





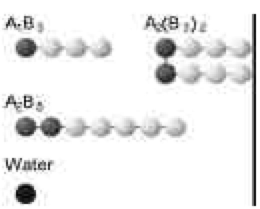
Marrink and Mark, JACS (2003)

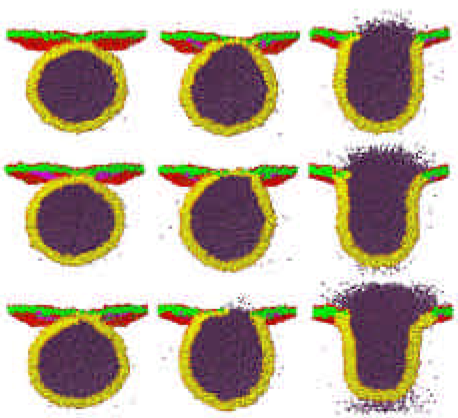
MD



Stevens, Hoh and Wolf, PRL(2003)

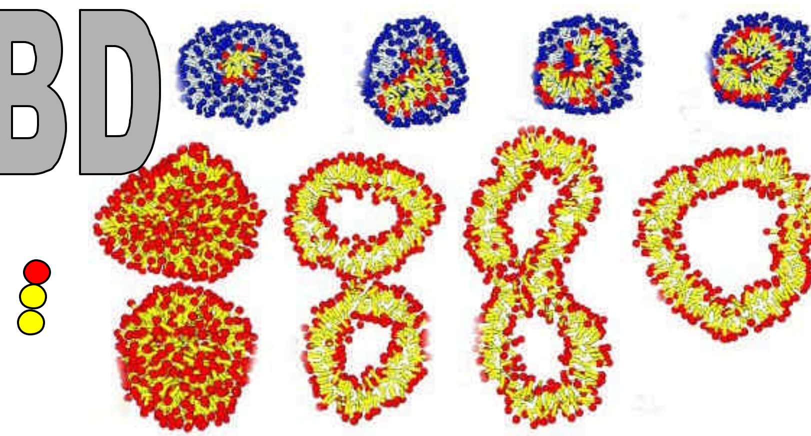
DPD





Shillcock, Nat.Mat. (2005)

BD



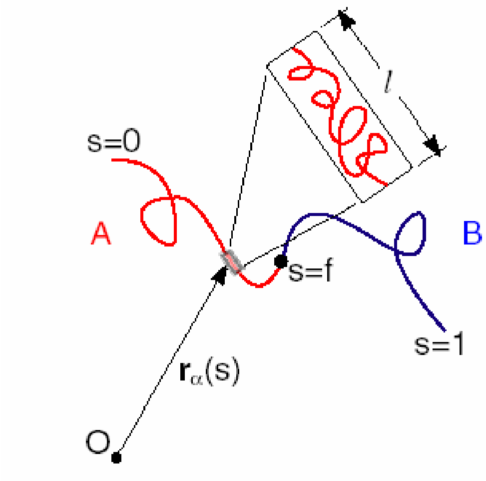
Noguchi and Takasu, JCP(2001)

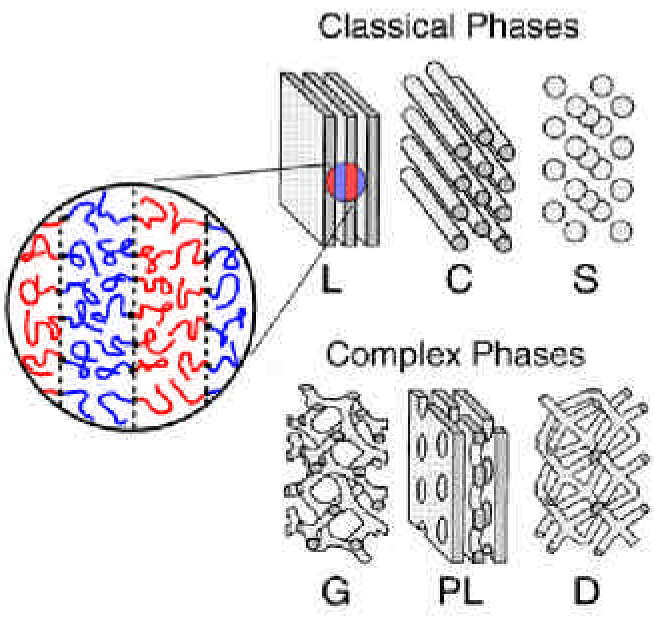
- **Why no stalk expansion?**
- **Why transient holes form?**
  (Isolated bilayer is very stable)

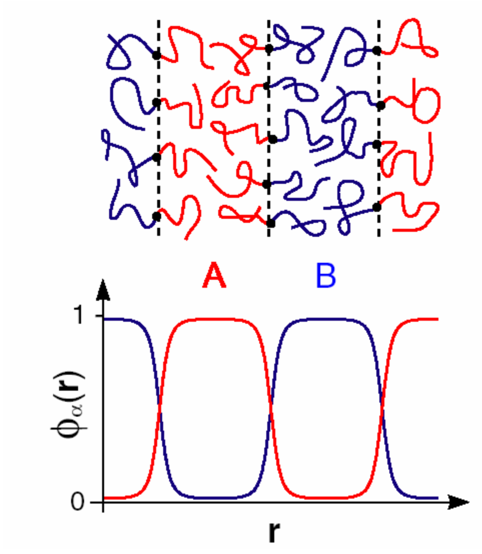
- **Need to compare Free Energy of possible intermediates and transition barriers**
- **Hard to get Free Energy from MC simulations**
- **The model is simple enough to be treated with microscopic theory (not phenomenological)**

Self-Consistent Field Theory

- Homopolymer-diblock blend
- Flory-Huggins interaction
- Incompressible
- Gaussian chain statistics







Matsen, JPCM(2002)

Why SCFT?

- Same **microscopic polymer model** as in MC
- Proven to be **reliable** (agrees with MC)
- Provides **structural** and **energetic** information
- **Intermediates** ([meta]stable configurations)
- **Transition states** (saddle points) can be found
- **Reaction coordinate constraint**

SCFT

$$\frac{\mathcal{H}[w_A, w_B, \phi_A, \phi_B, \xi]}{k_B T \Phi} = - \;\; Q_s[w_A] - z Q_a[w_A, w_B]$$

$$+ \;\; \chi N \int \mathrm{d}V \;\; \phi_A(\mathbf{r})\phi_B(\mathbf{r})$$

$$+ \;\; \int \mathrm{d}V \;\; (\phi_A(\mathbf{r}) w_A(\mathbf{r}) + \phi_B(\mathbf{r}) w_B(\mathbf{r}))$$

$$+ \;\; \int \mathrm{d}V \; \xi(\mathbf{r})(\phi_A(\mathbf{r}) + \phi_B(\mathbf{r}) - 1)$$

$$\bar{w} \ldots = \chi N \bar{\phi}_B(\mathbf{r}) + \ldots)$$

$$\ldots\mathbf{r}) = \chi N \bar{\phi}_A(\mathbf{r}) + \xi(\mathbf{r})$$

$$1 = \bar{\phi}_A(\mathbf{r}) + \bar{\phi}_B(\mathbf{r})$$

$$\ldots(\mathbf{r}) = \int_0^1 \mathrm{d}s \; q_s(\mathbf{r}, s) q_s(\mathbf{r}, 1 \ldots \int^f \ldots(\mathbf{r}, s) q_c^\dagger(\mathbf{r}, s)$$

$$\ldots) = z \int_f^1 \mathrm{d}s \; q_c(\mathbf{r}, s) q_c^\dagger(\mathbf{r}, \ldots$$

$$\frac{\ldots q_s(s)}{\partial s} = R_g^2 \nabla^2 q_s(\mathbf{r}, s) - w_A(\mathbf{r}) q_s(\mathbf{r}, s), \;\; \text{with } q_s(\mathbf{r}, s = 0) = 1$$

$$\Omega = \mathcal{H}[\bar{w}_A, \bar{w}_B, \bar{\phi}_A, \bar{\phi}_B, \bar{\xi}]$$

From Particles to Fields

- Partition function

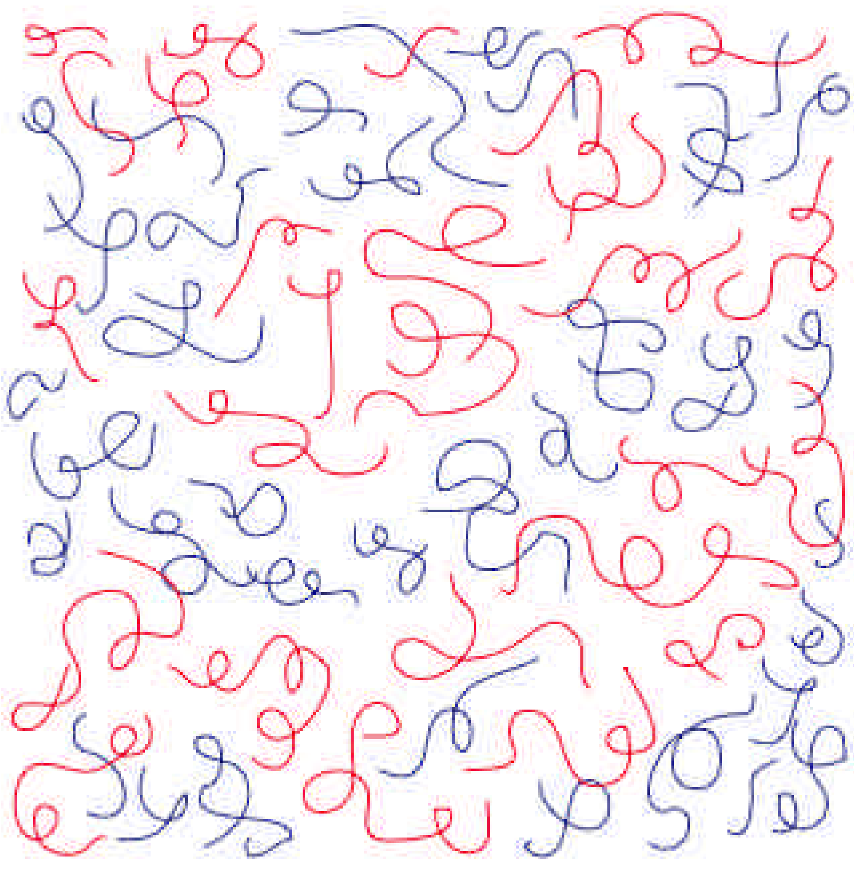
Particle-based

$$Z = \int D[\mathbf{R}] e^{-U_0 - U_1}$$

Hubbard-Stratonovich Transformation

Field-based

$$Z = \int D[W] e^{-H[W]}$$

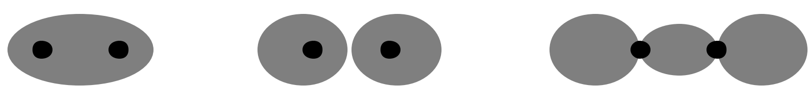


If you hold a cat by the tail you learn things you cannot learn any other way. --Mark Twain

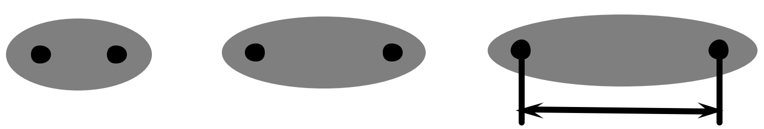
Reaction coordinate constraint

Quantum chemistry

- Born-Oppenheimer approx.
  (fast electrons/slow nuclei)
- Electronic wave function

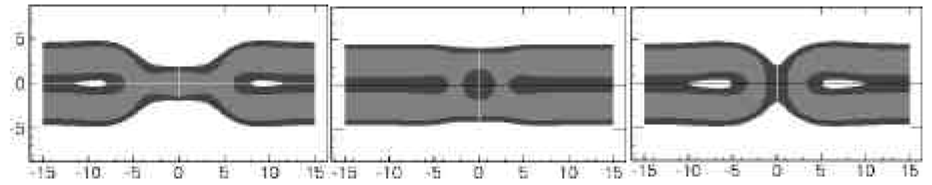


- Electronic ground state
- Inter-nuclear distance

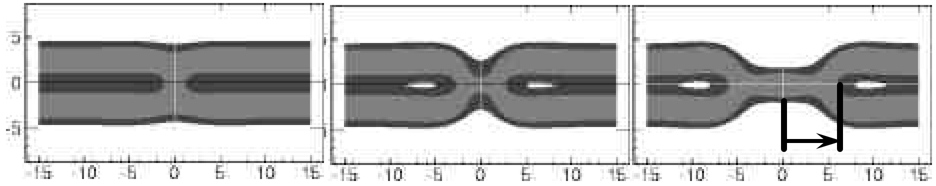


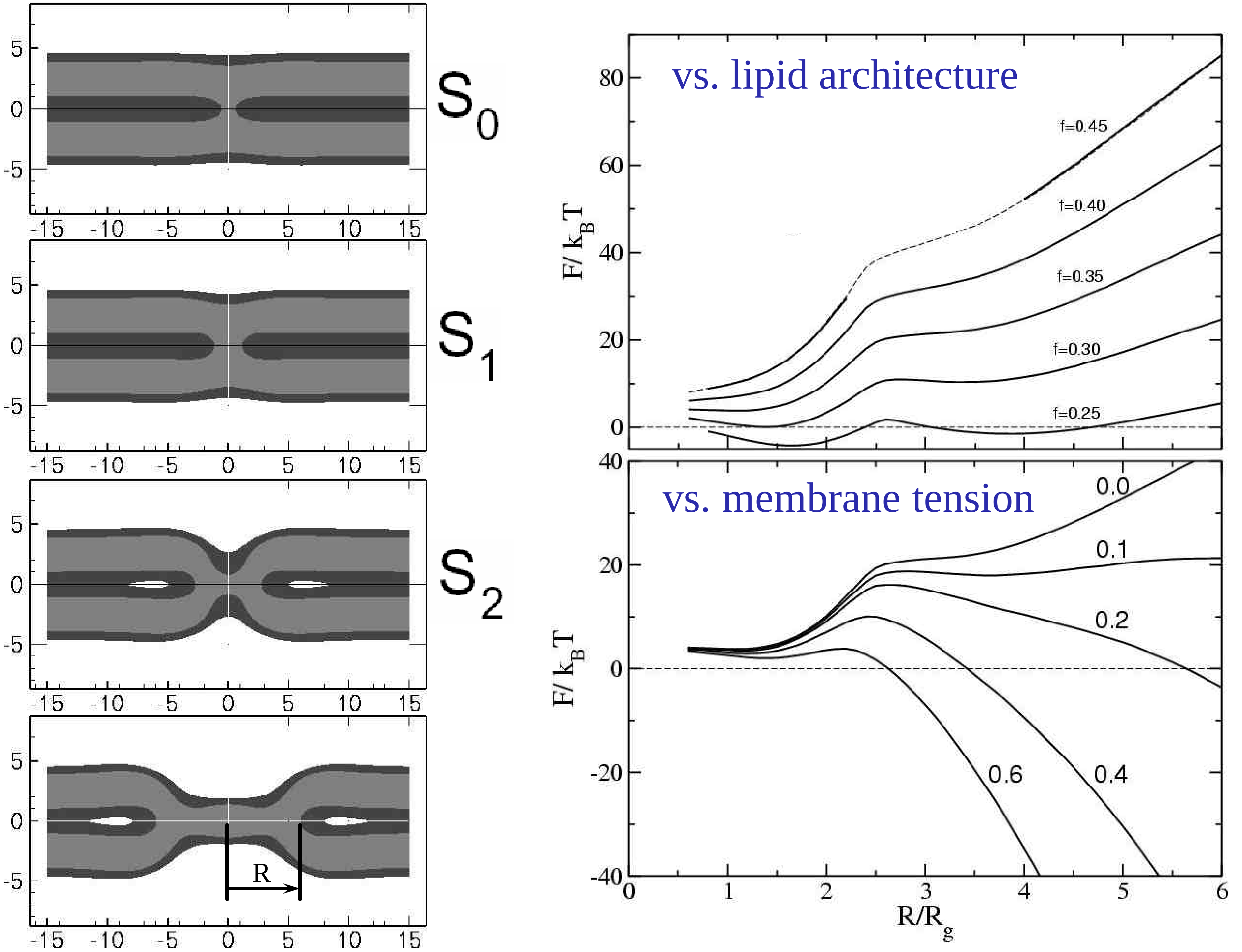
Membrane fusion

- Quasi-static approx.
  (fast individual/slow collective)
- Interface topology



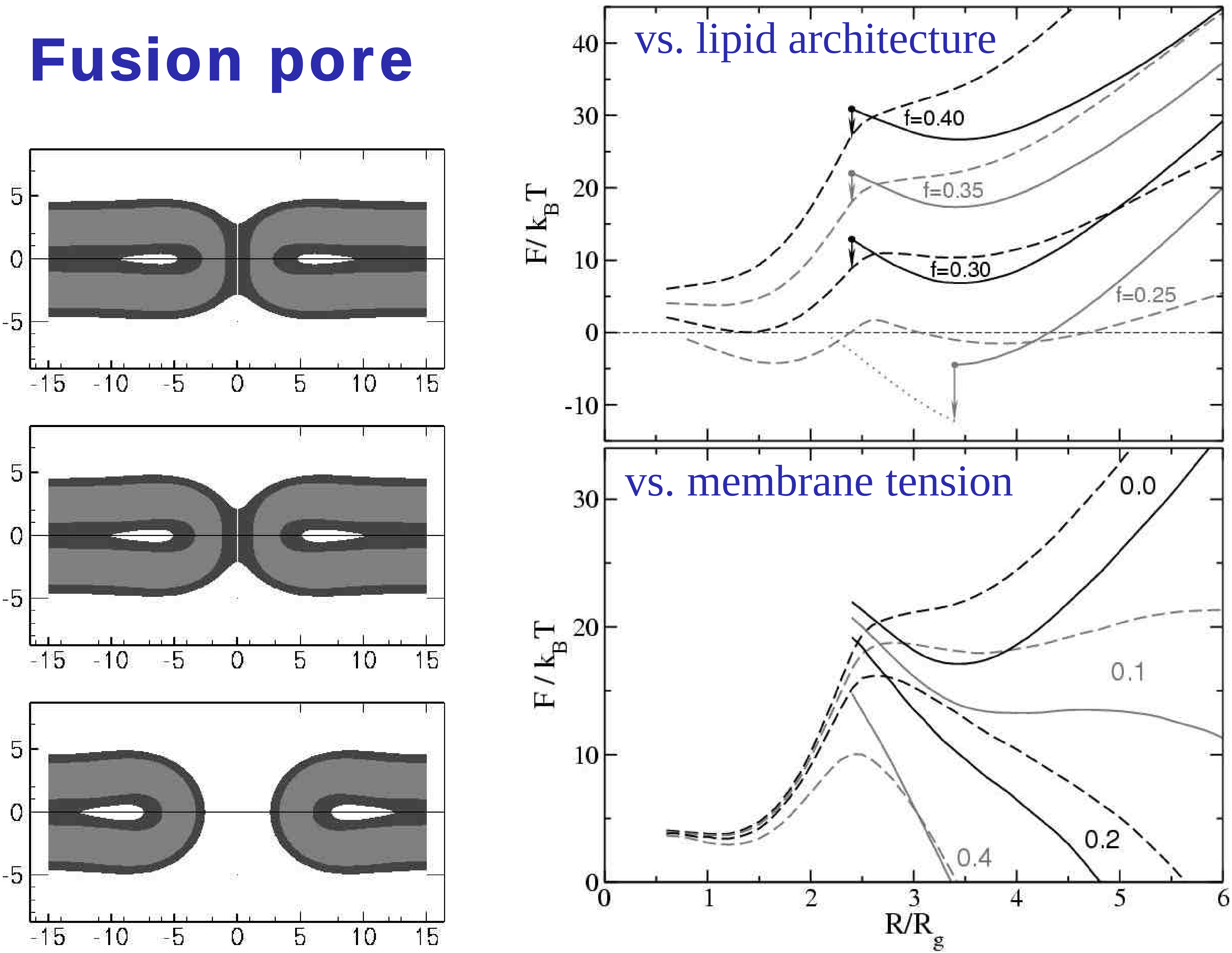
- Lowest free-energy state
- Structure size





S0
S1
S2
R
vs. lipid architecture
f=0.45
f=0.40
f=0.35
f=0.30
f=0.25
F/k_B T
vs. membrane tension
0.0
0.1
0.2
0.6
0.4
R/R_g

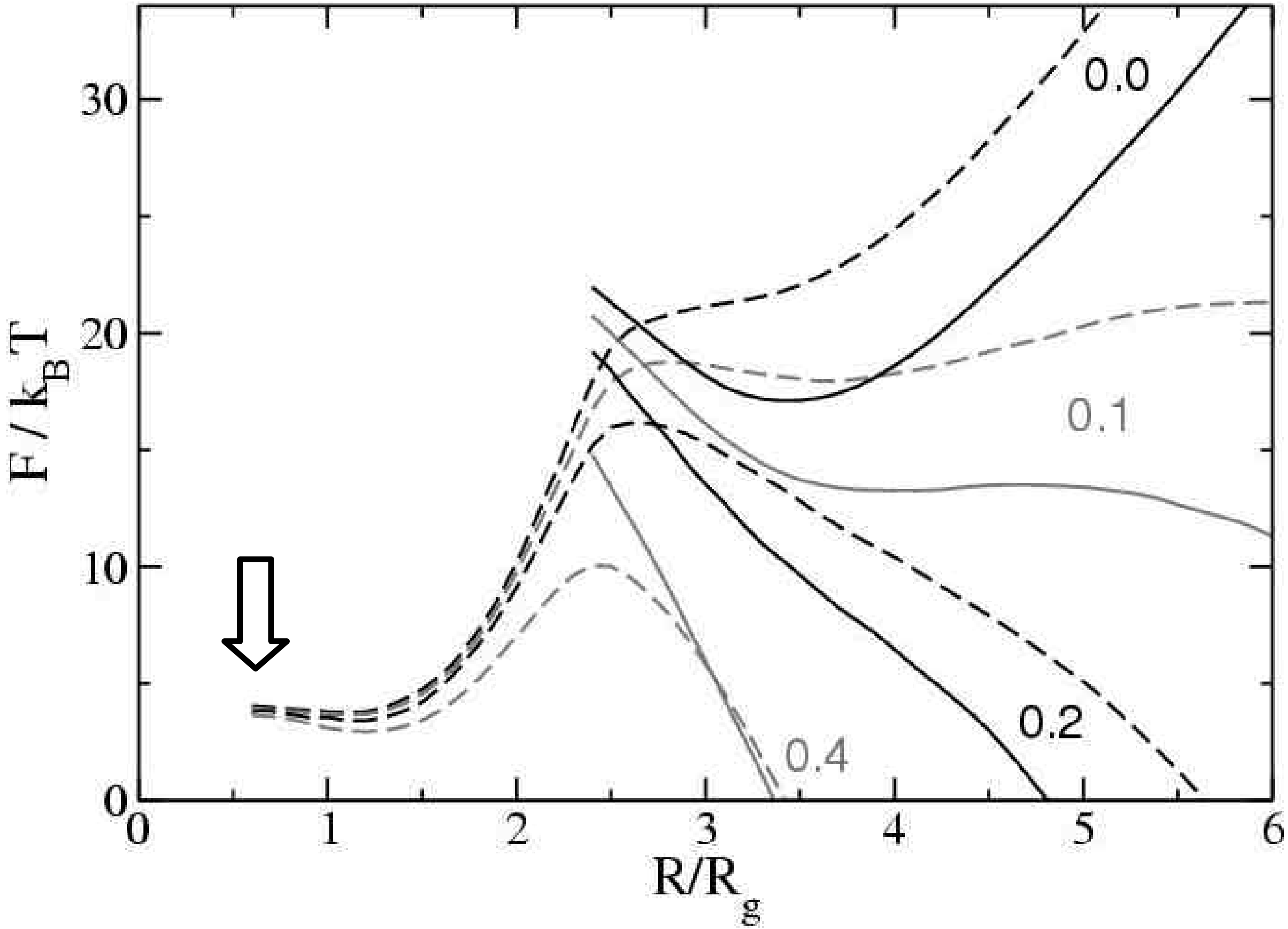
Fusion pore



vs. lipid architecture
f=0.40
f=0.35
f=0.30
f=0.25
vs. membrane tension
0.0
0.1
0.2
0.4
F / k_B T
R/R_g

Consequences

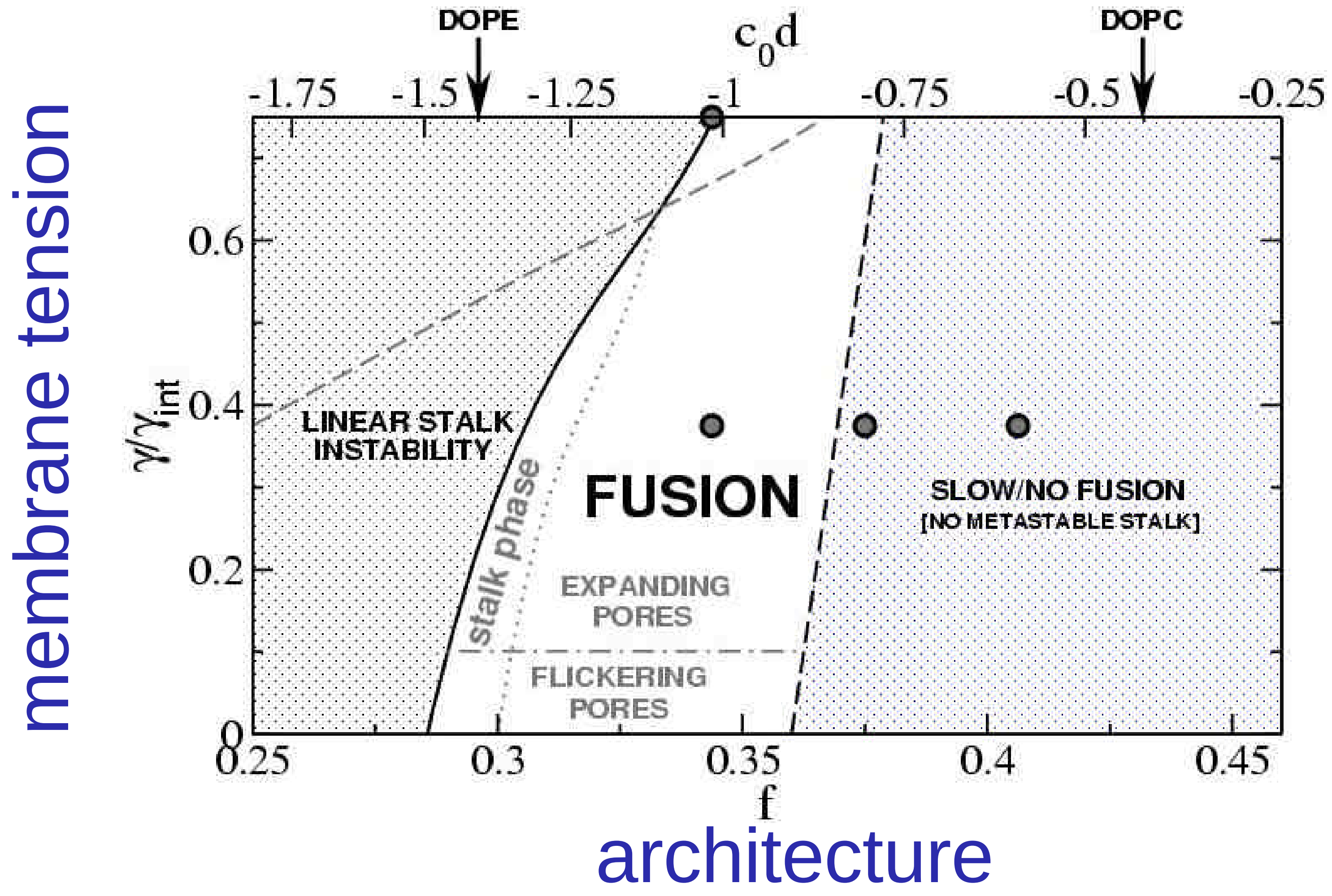
Major barrier: radial stalk expansion



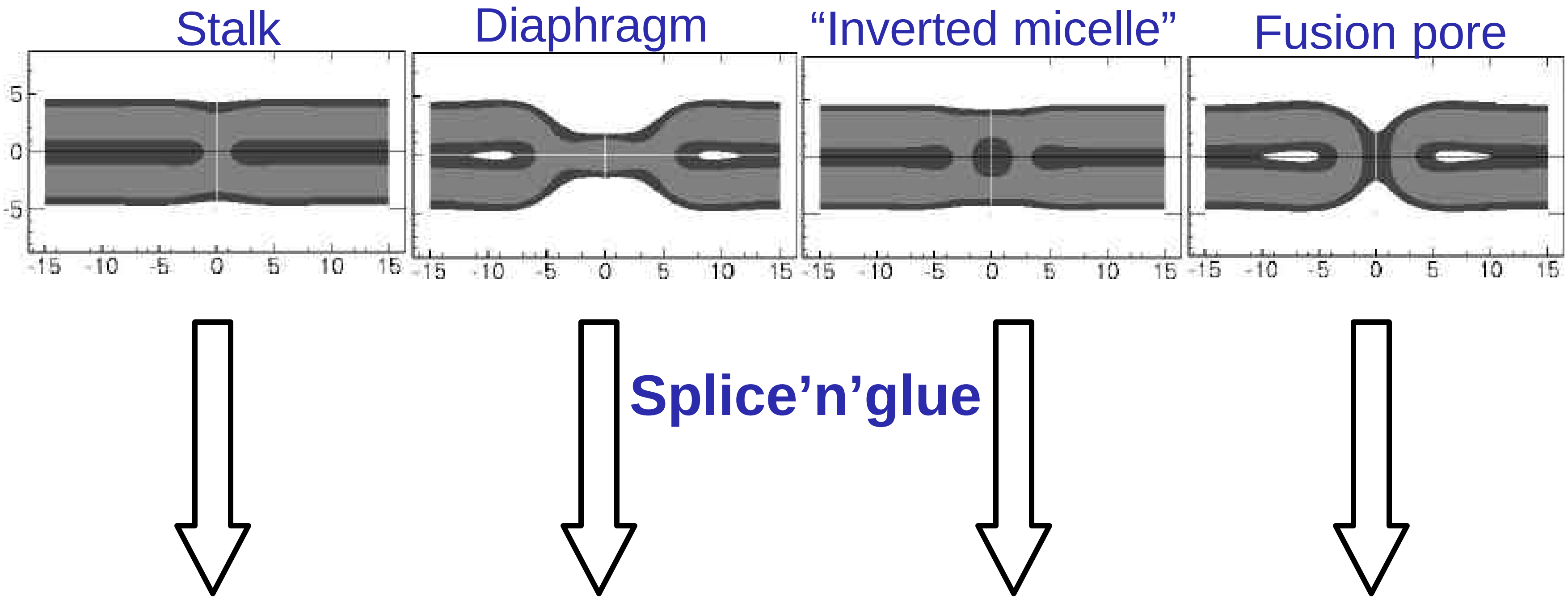
Consequences

- Major barrier is stalk expansion

- Fusion is limited (regulated)

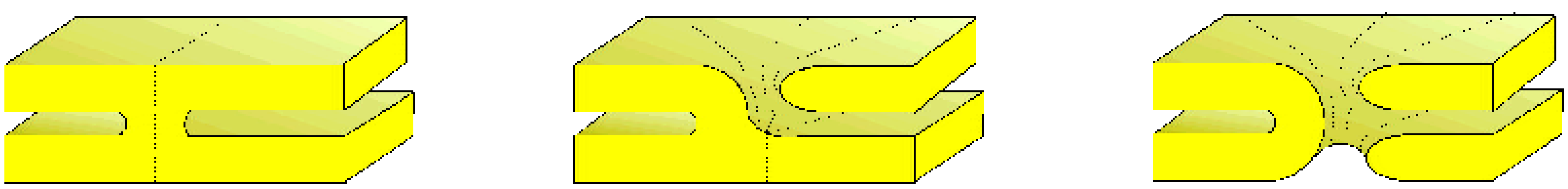


The alternative mechanism

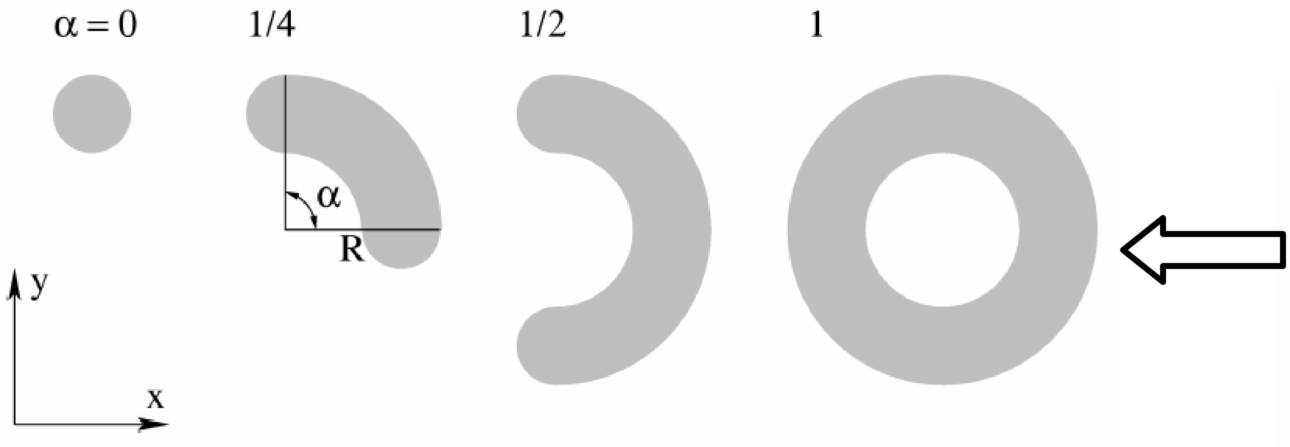


Splice'n'glue

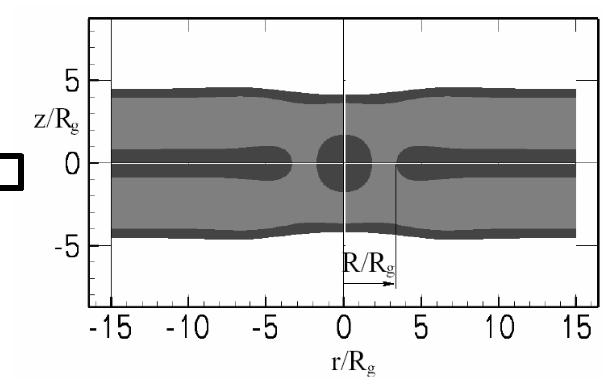
Non-axially symmetric intermediates



1: Extended stalk

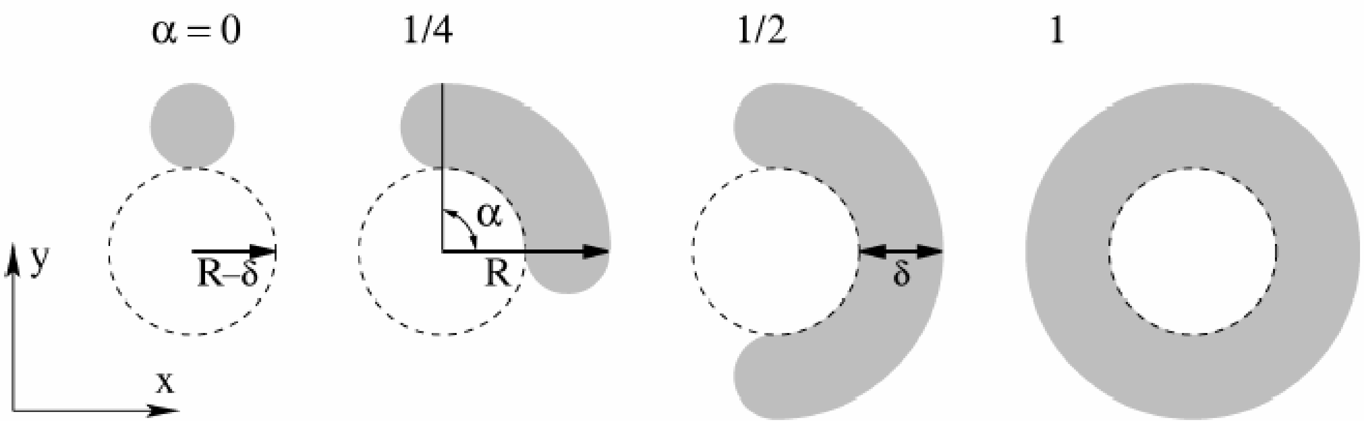


IMI



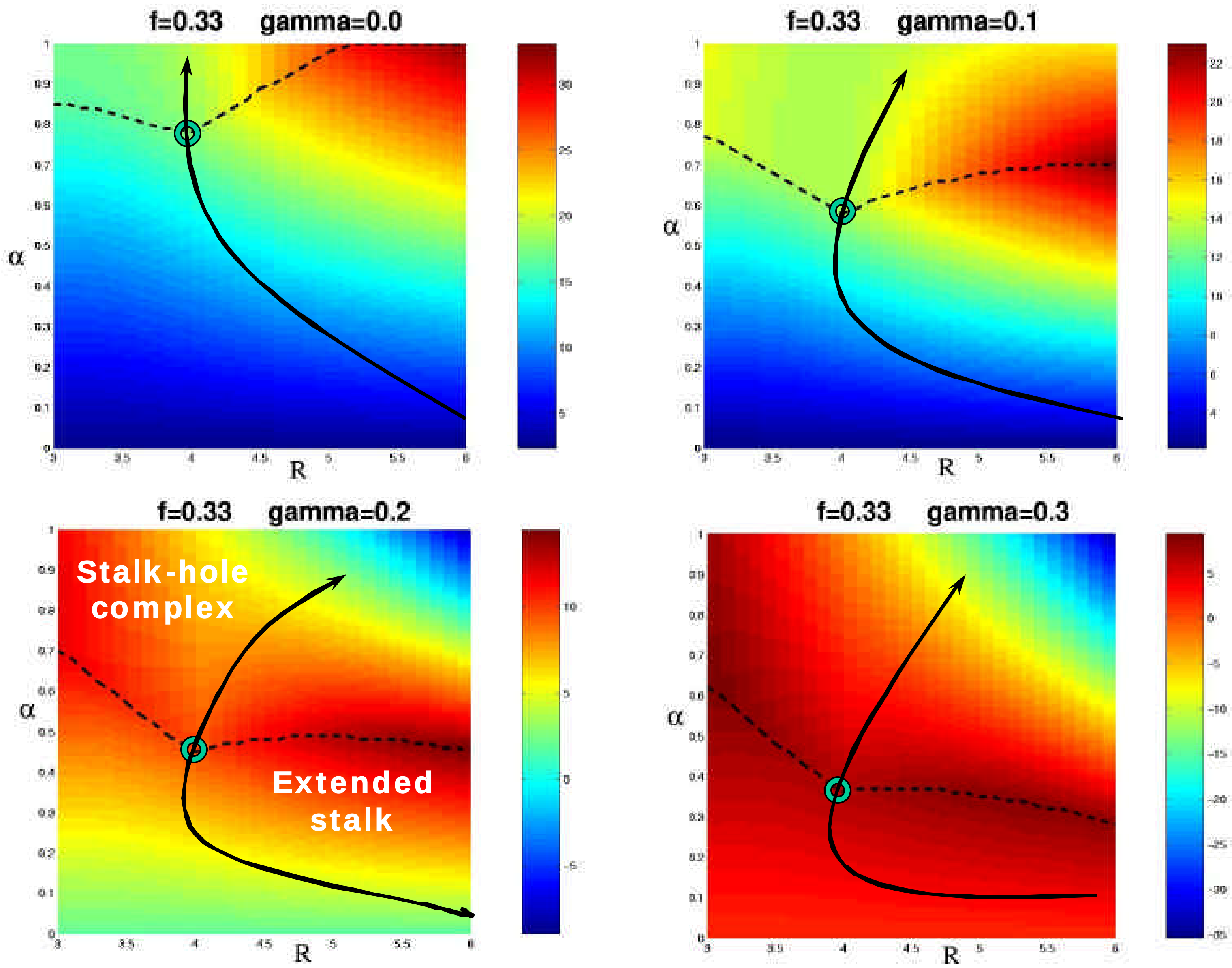
$$F_1(R, \alpha) = \alpha F_{\mathrm{IMI}}(R) + F_S$$

2: Stalk+hole complex

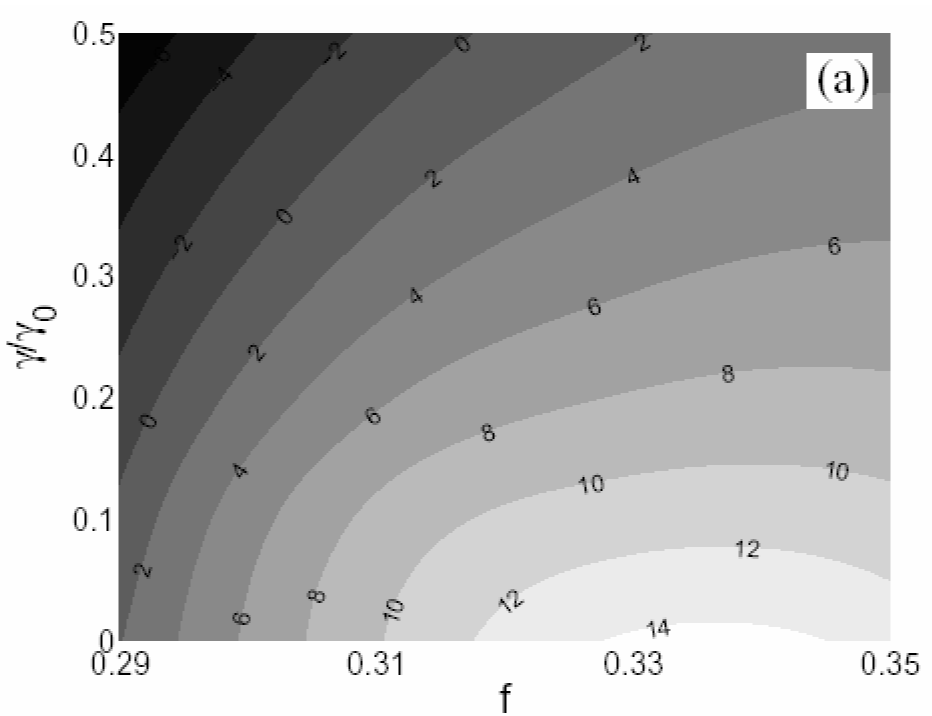


$$F_2(R, \alpha) = \alpha F_{HI}(R) + (1 - \alpha) F_H(R - \delta) + F_d$$

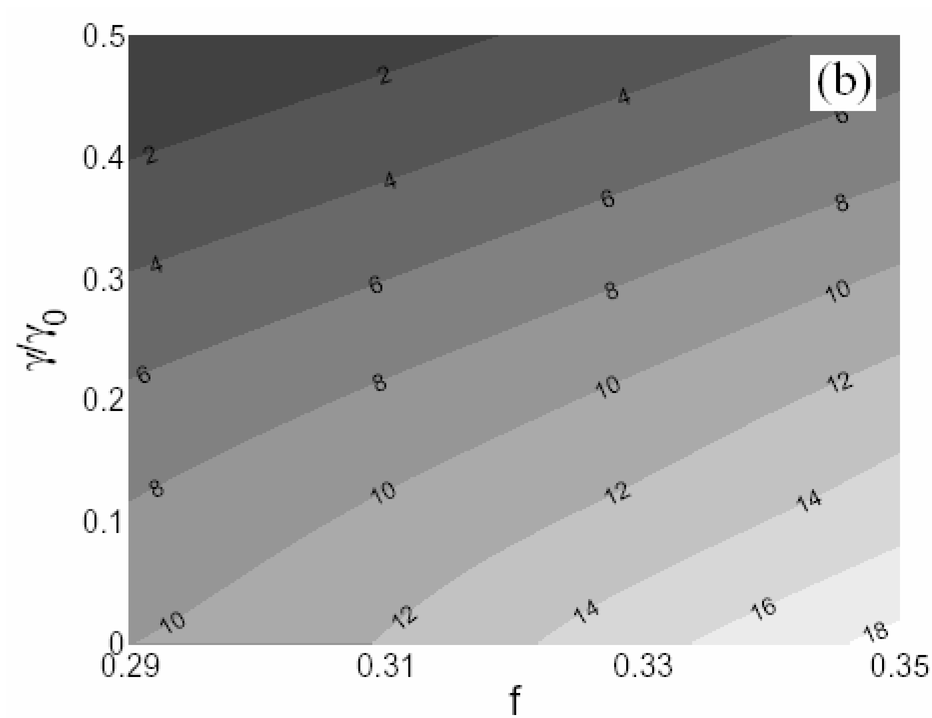
Free energy "landscape"



Free energy barrier

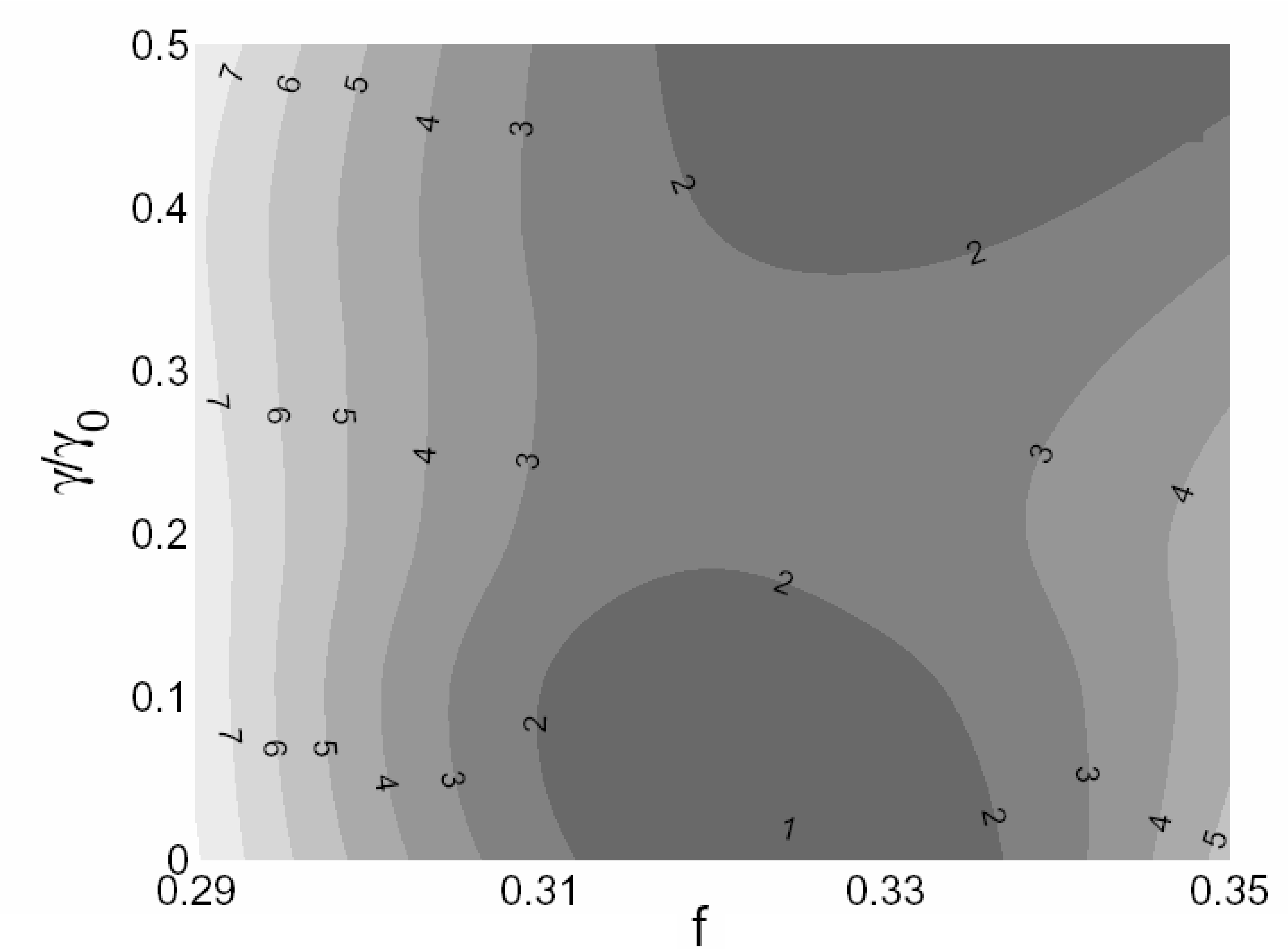


New mechanism

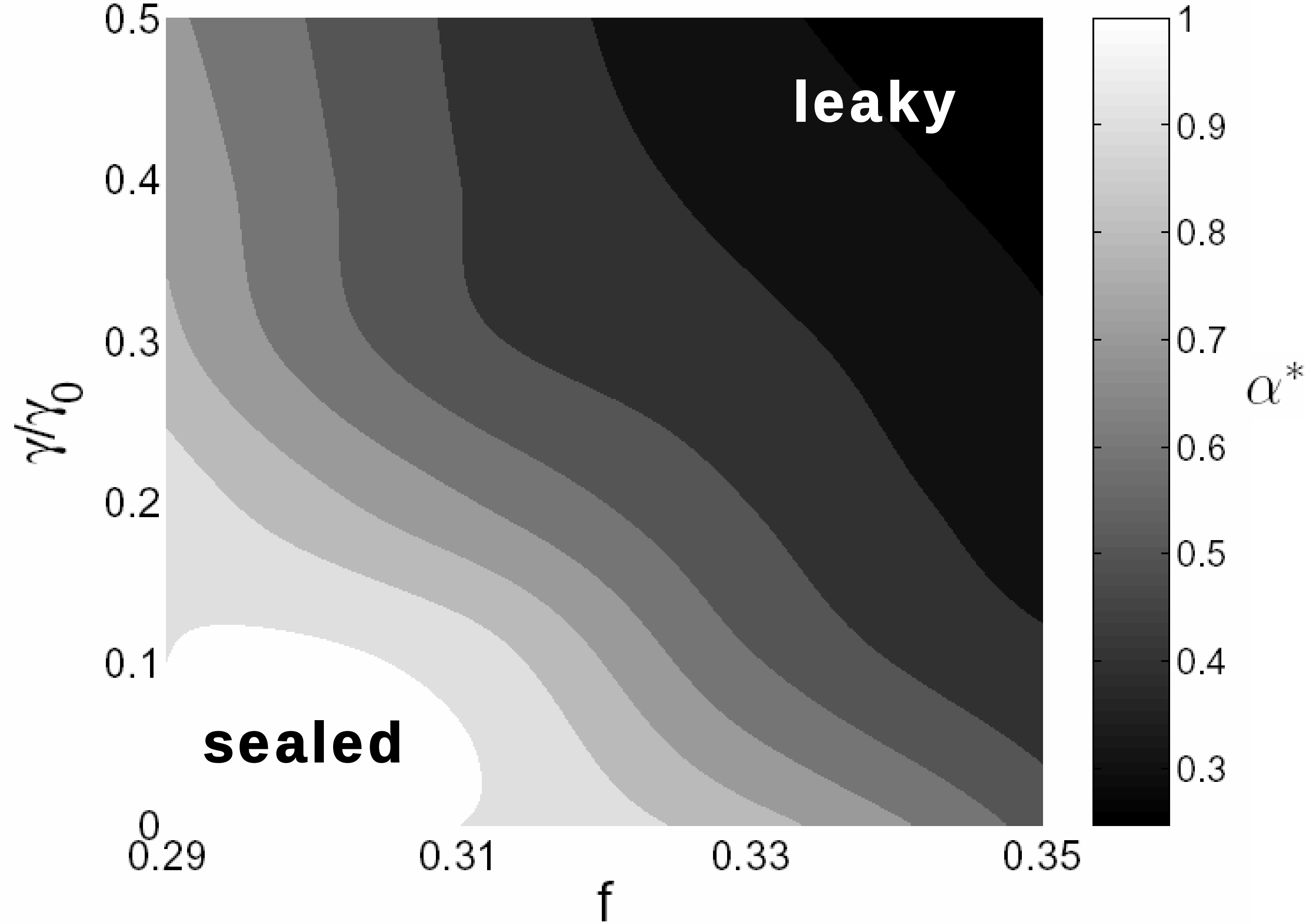


Old mechanism

Free energy barrier difference between old and new mechanisms



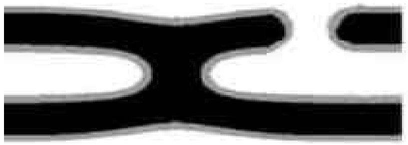
Leakiness of Transition State



Resolving stability vs. fusion paradox
or
bare vs. screened holes

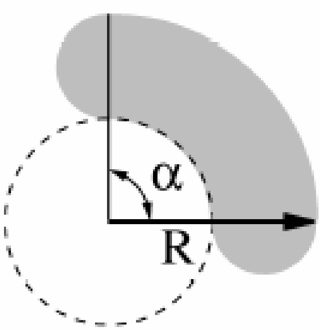
$$F_H = 2\pi\lambda_H R - \pi\gamma R^2$$

Probability to nucleate supercritical hole



$$P_H \;=\; \frac{A_H}{\ell^2}\exp(-\pi\lambda_H^2/\gamma k_B T)$$

$$P_{SH} = \frac{N_S a_S}{\ell^2}\exp\left(-\pi\bar{\lambda}_\alpha^2/\gamma k_B T\right)$$



Effective line tension

$$\lambda_H \to \bar{\lambda}_\alpha \equiv \alpha\lambda_{SH} + (1-\alpha)\lambda_H$$

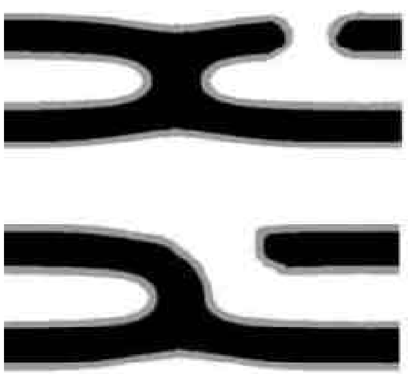
Resolving stability vs. fusion paradox
or
bare vs. dressed holes

Under simulation conditions

Under experimental conditions

$P_{SH}/P_H \sim 14$

Resolving stability vs. fusion paradox
or
bare vs. dressed holes

	Under simulation conditions	Under experimental conditions
	$P_{SH}/P_H \sim 14$	1×10^4

Membrane can both be stable and undergo fusion

What is really going on in biological fusion?

-Multi component membranes

[lipid mixtures, cholesterol, asymmetry]

-External constraints

[vesicle size, volume]

-Role of fusion peptides

-More experiments!

Thanks to:

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B.Lentz (UNC)
D.Siegel
J.Zimmerberg (NIH)

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Vadim Frolov (Inst.of Electrochemistry, Moscow/NIH)

For listening, asking, suggesting and doing the experiments

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DFG: Bi 314/17
Computer time: NIC Jülich
HLR Stuttgart and Mainz
Office/Home desktop