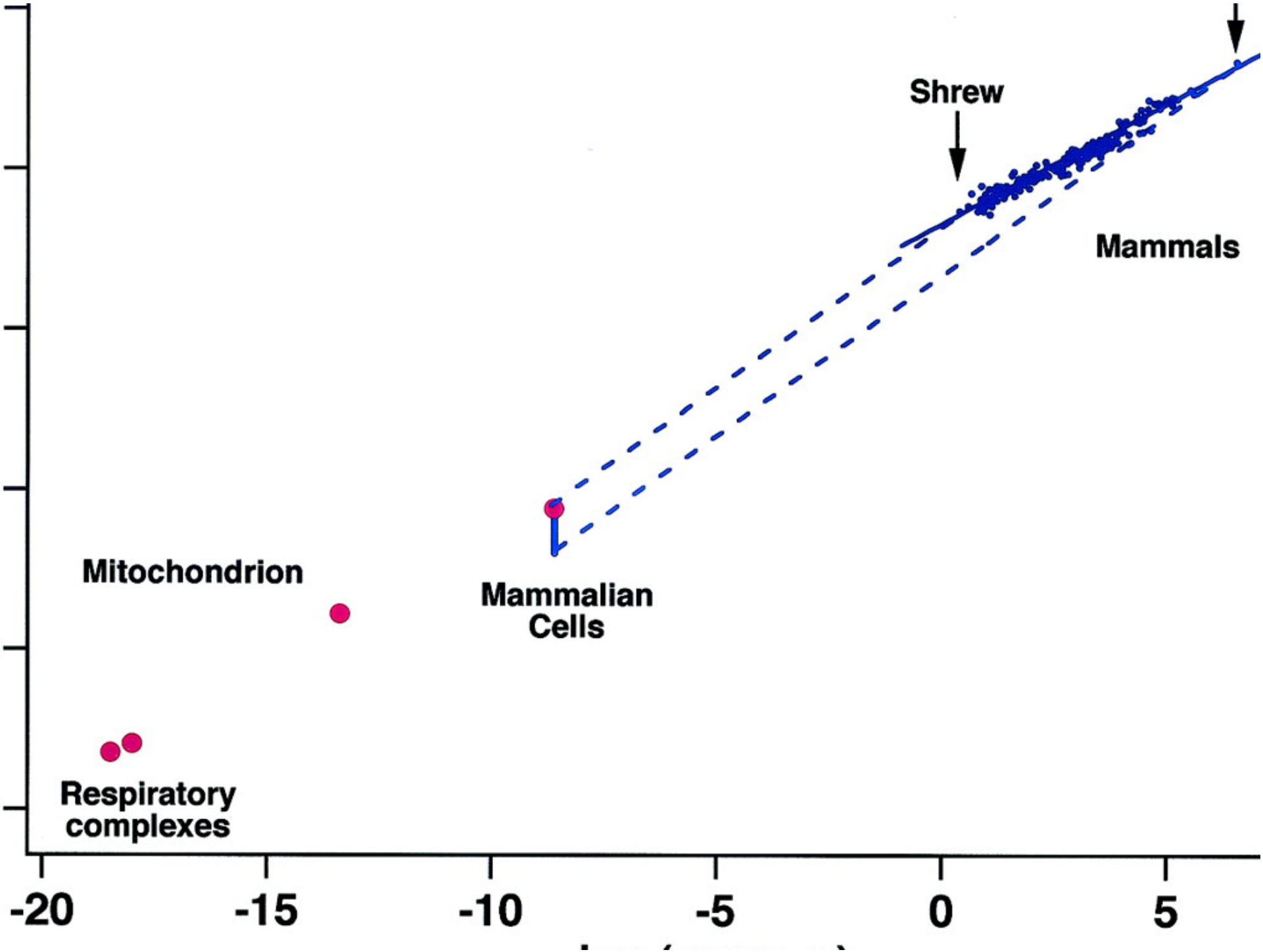
Biological Physics

Biological Physics

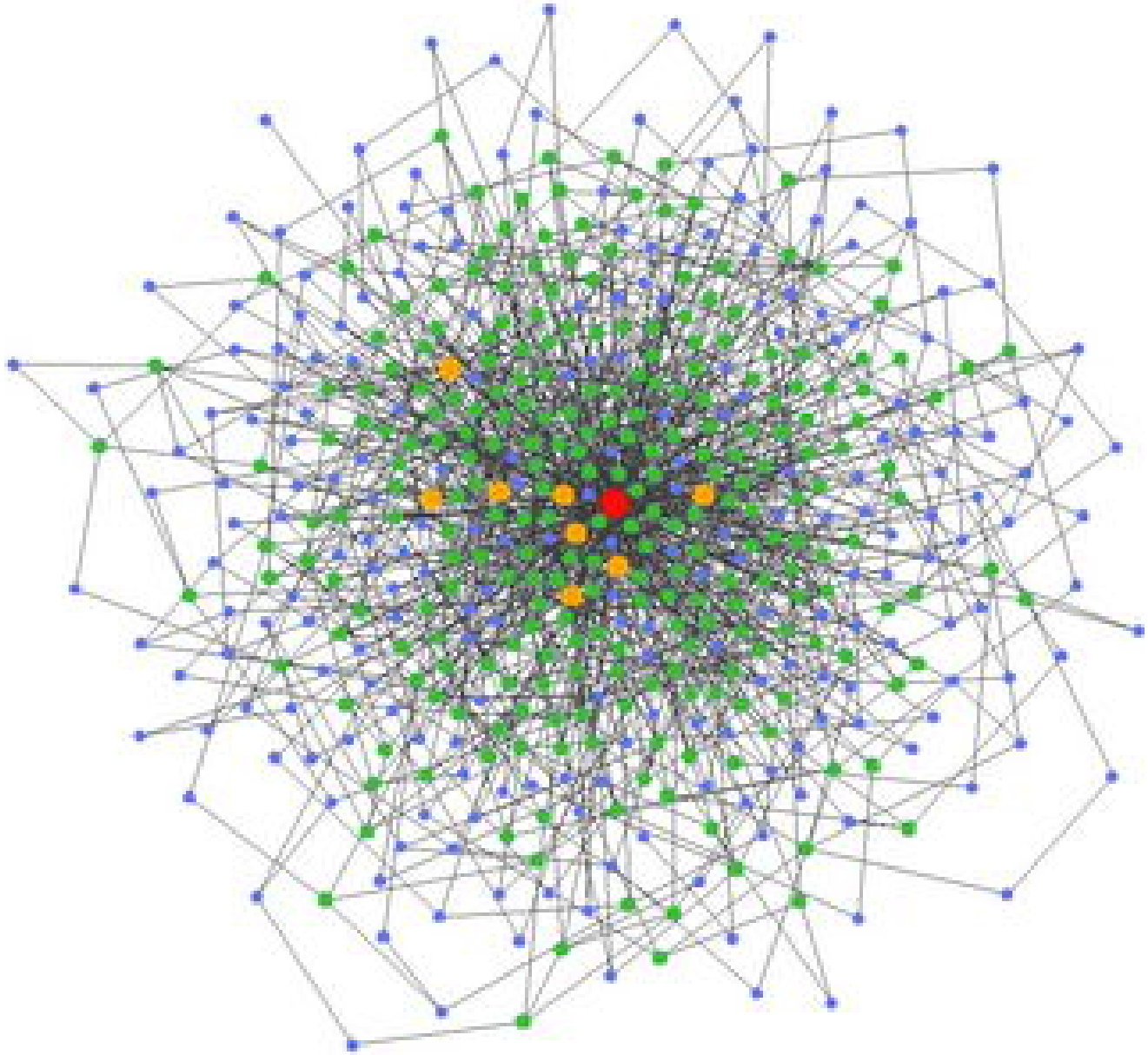
Grand Scale: Allometric scaling


Shrew
Mammals
Mitochondrion
Mammalian
Cells
Respiratory
complexes
-20
-15
-10
-5
0
5

Biological Physics

Grand Scale: Allometric scaling

Networks



Biological Physics

Grand Scale: Allometric scaling

Networks

Systems Biology

Biological Physics

Intermediate Scale: Biopolymers

Sequencing

FIGURE 3 | Nanopore sequencing has been proposed as an alternative to conventional sequencing.

FROM THE FOLLOWING ARTICLE:

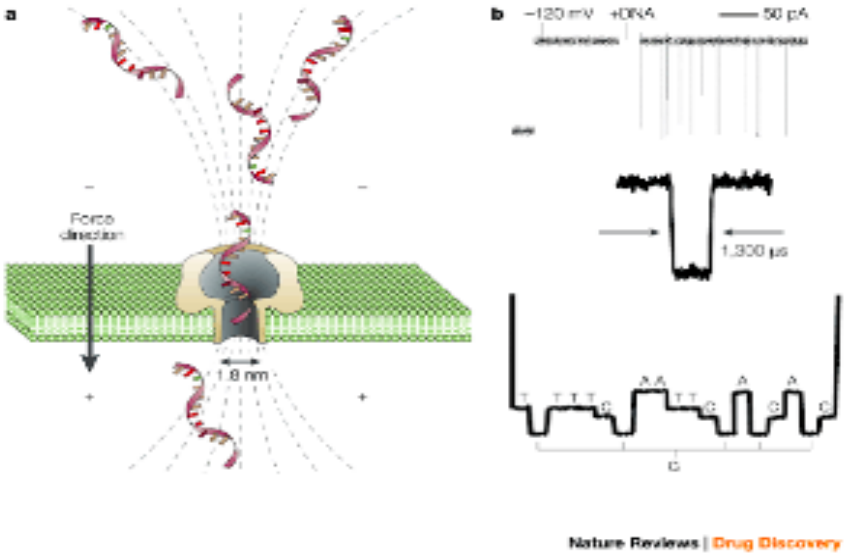
Moving smaller in drug discovery and delivery

David A. LaVan, David M. Lynn & Robert Langer

Nature Reviews Drug Discovery **1**, 77-84 (January 2002)

doi:10.1038/nrd707

Back to article | **Back to figures and tables** | **Previous figure** | **Next figure**



Biological Physics

Intermediate Scale: Biopolymers

Sequencing

viruses

vesicles

drug delivery

cells

Biological Physics

Small Scale: New molecules

Motors

Single Molecule techniques

Fusion and Lysis

two outcomes, one mechanism

Michael Schick

She knows there’s no success like failure,
And failure’s no success at all.

Bob Dylan

Question:

What can we learn about how fusion works when we observe that it doesn’t?

Outline

- Basics
- A new mechanism of membrane fusion which can also lead to leakage and lysis
- Evidence for leakage and lysis in several systems

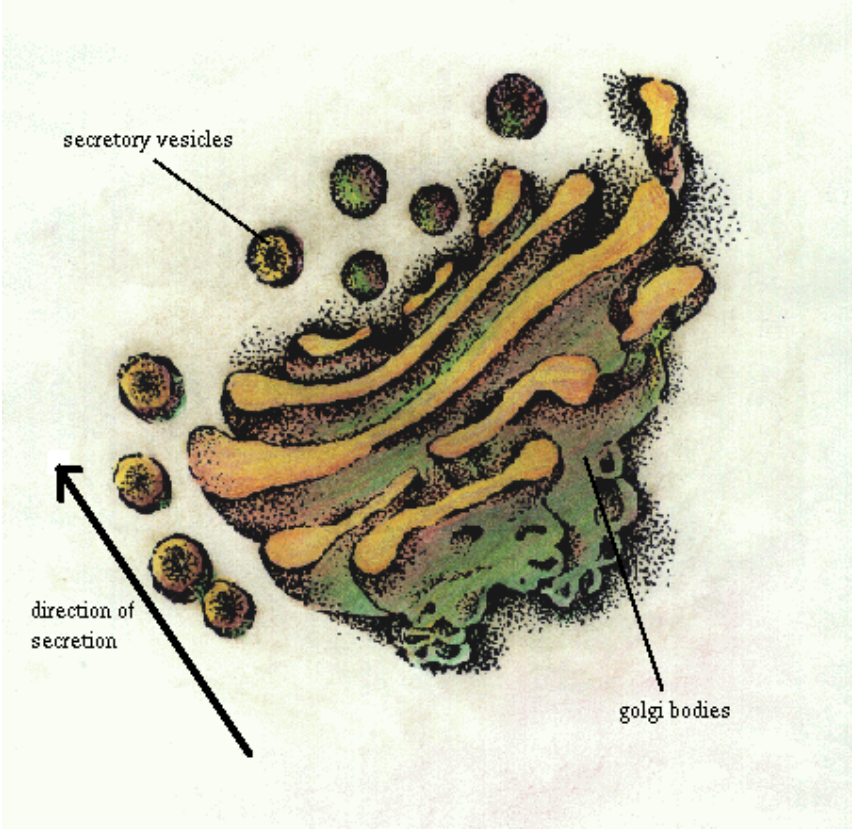
Why is Fusion Important?

Cell Trafficking

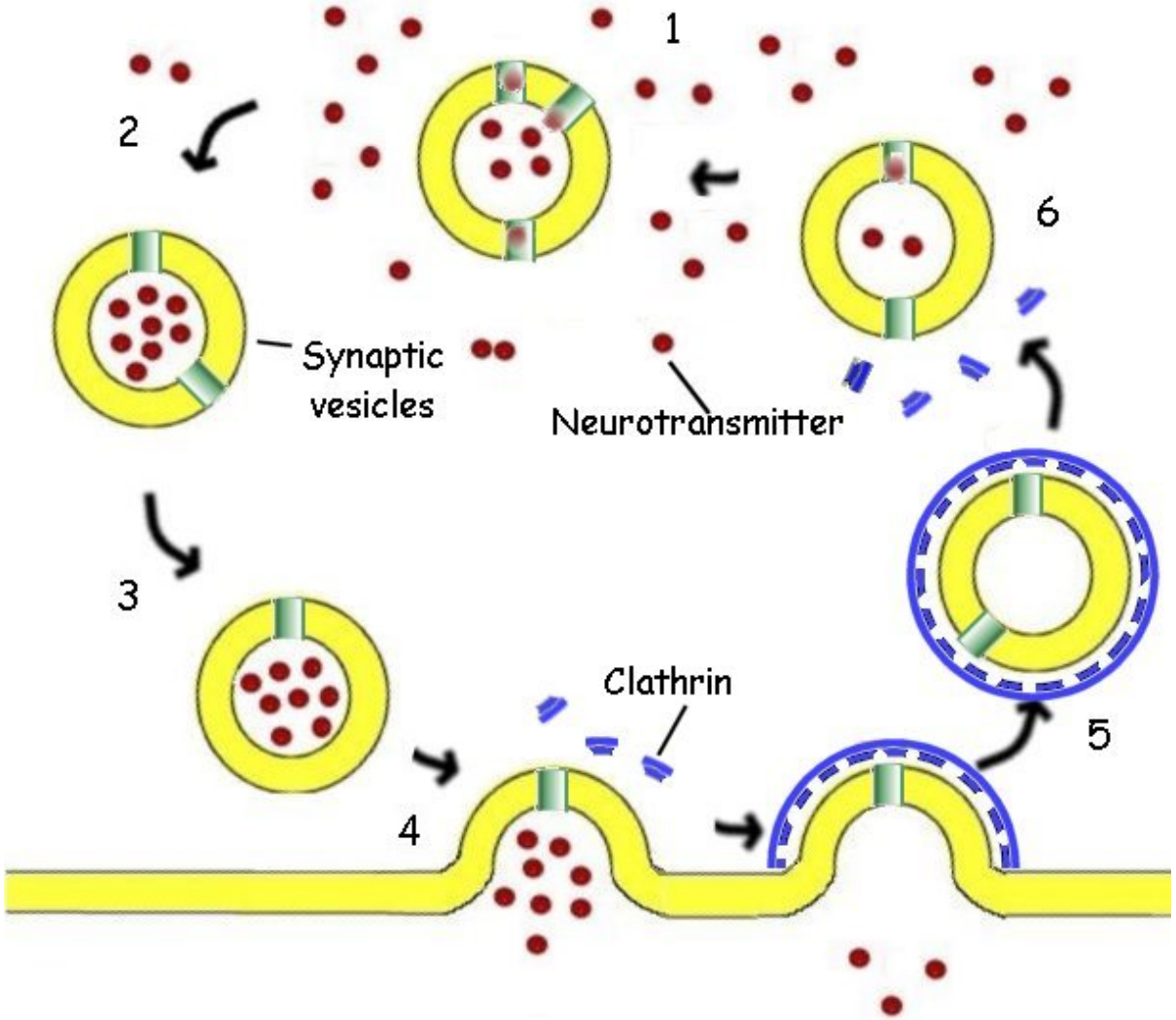
Excocytosis/Endocytosis

Viral Entry

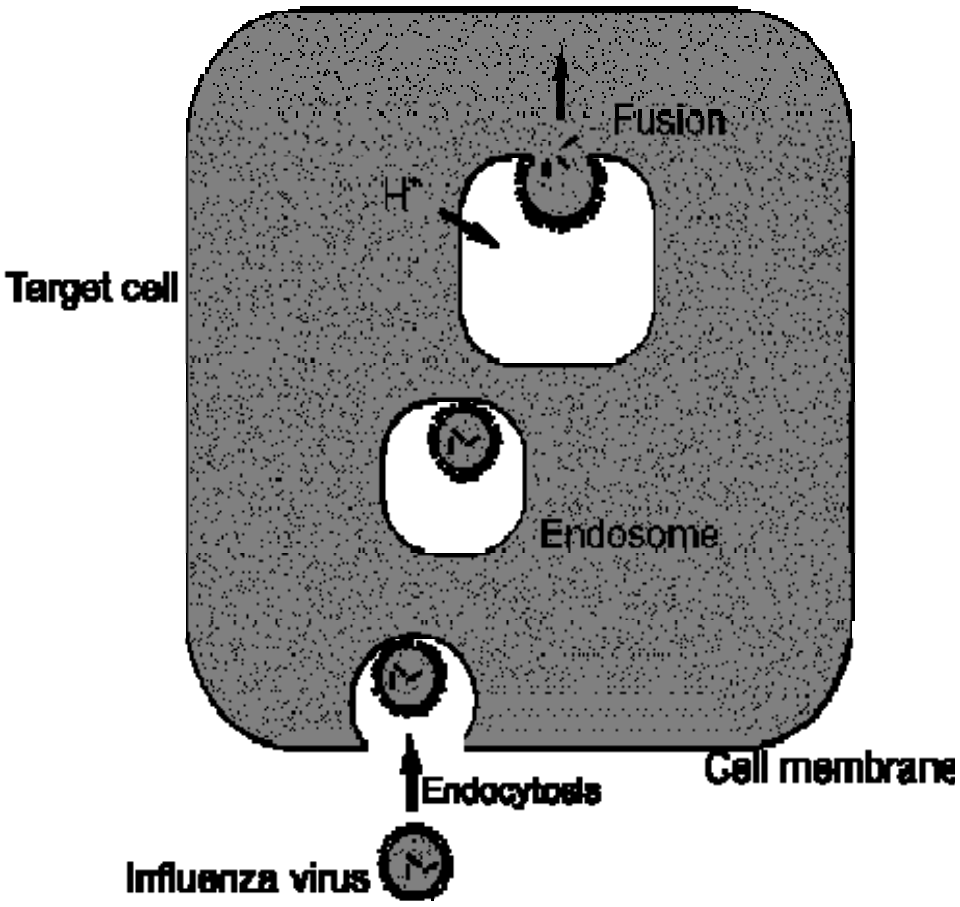
Trafficking



Exocytosis



Viral Entry


Fusion
H+
Target cell
Endosome
Cell membrane
Endocytosis
Influenza virus

Why is Fusion Difficult to Understand?

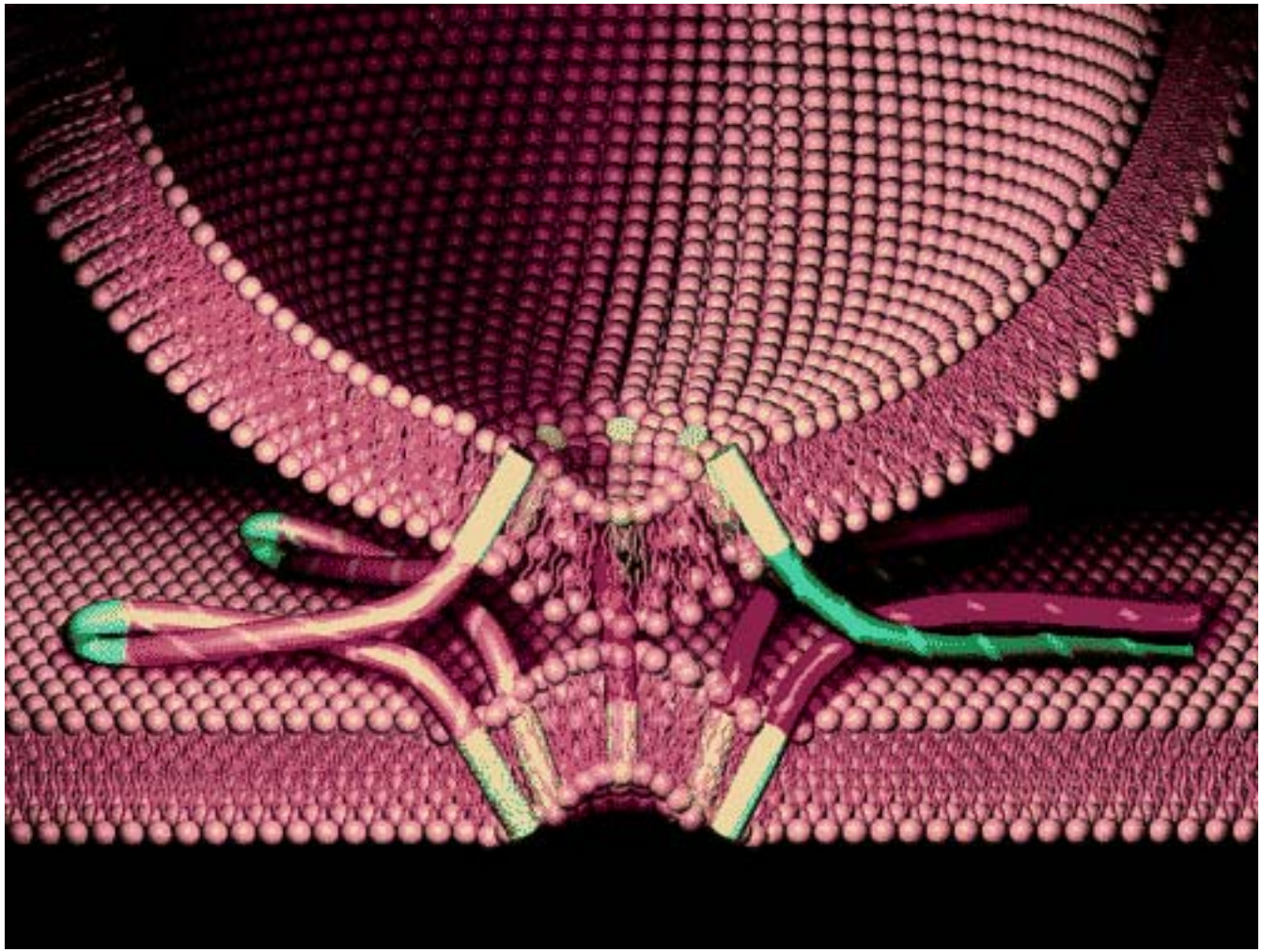
1. Stability: long-lived holes must be **difficult** to form

2. Fusion: long-lived holes must be **easy** to form

Basics

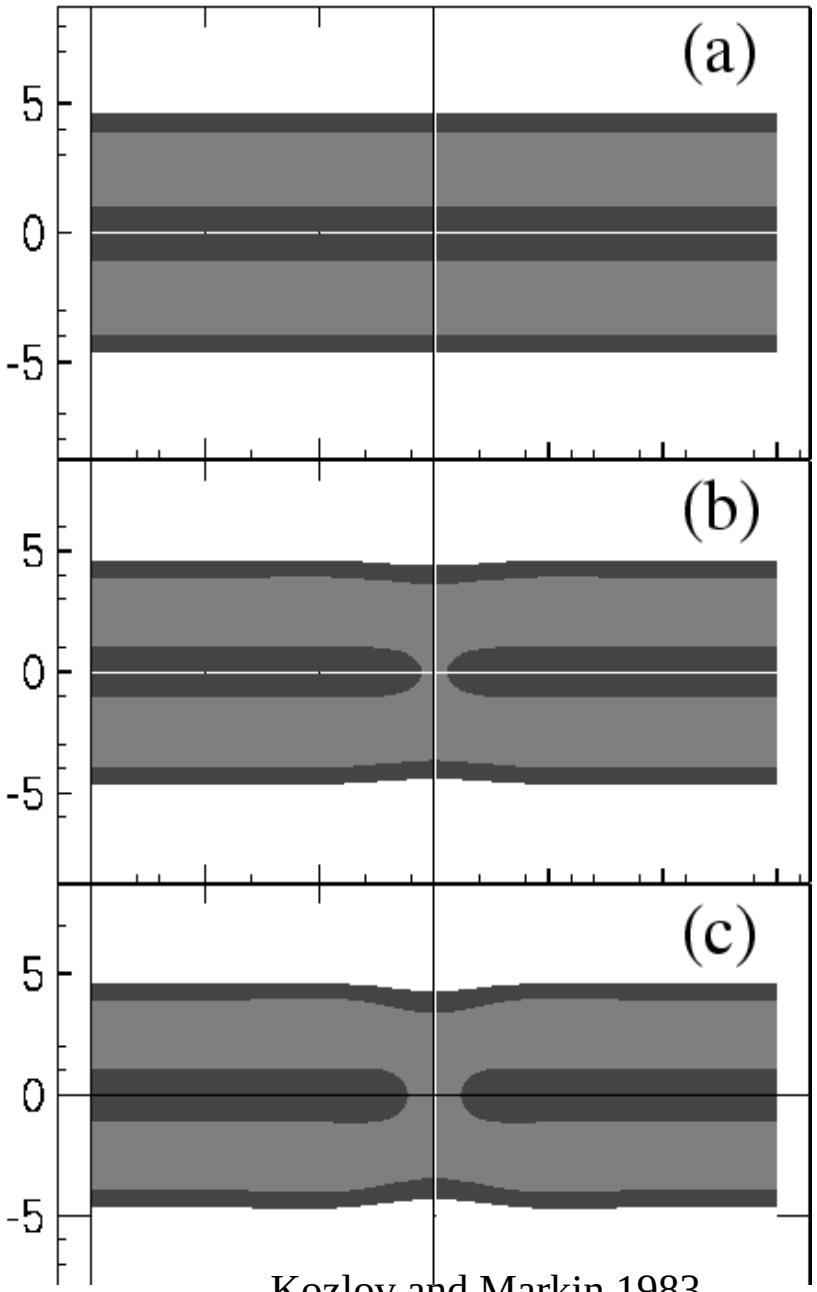
- Bringing membranes together costs energy (function of fusogen)

The Biologist's View of Fusion

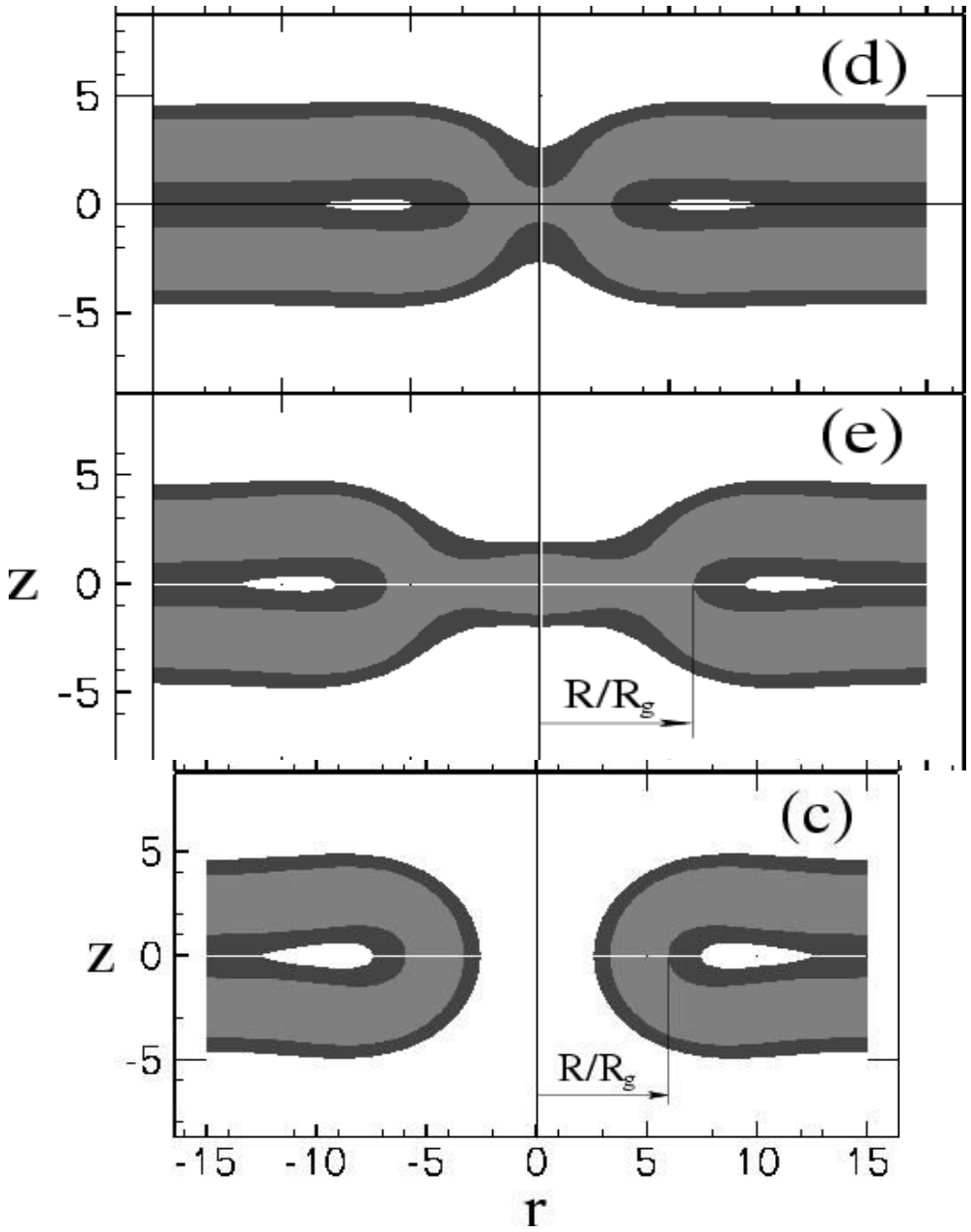


J.E. Rothman 1998

The Physicist's View



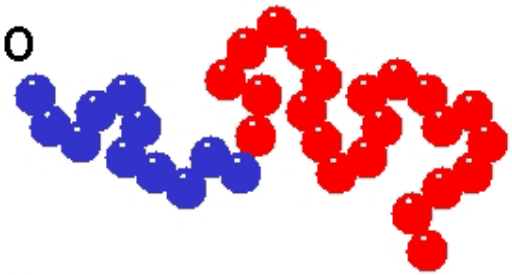
Kozlov and Markin 1983



SIMULATING FUSION

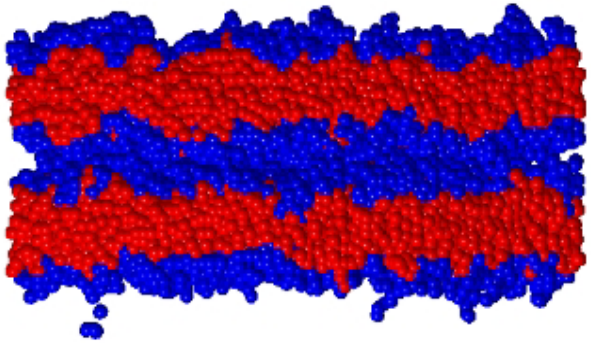
System: diblock co-polymer + solvent

Algorithm: Bond Fluctuation Model
Lattice Monte Carlo

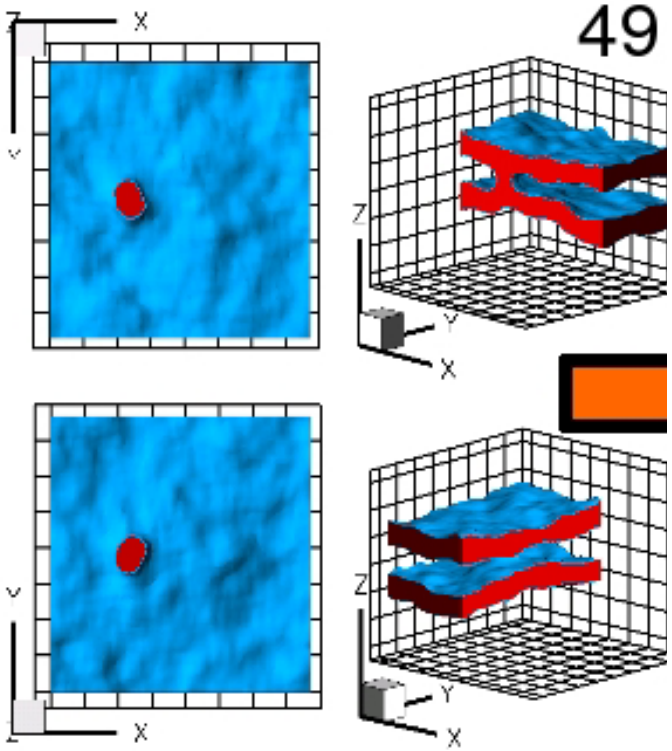


Advantages:

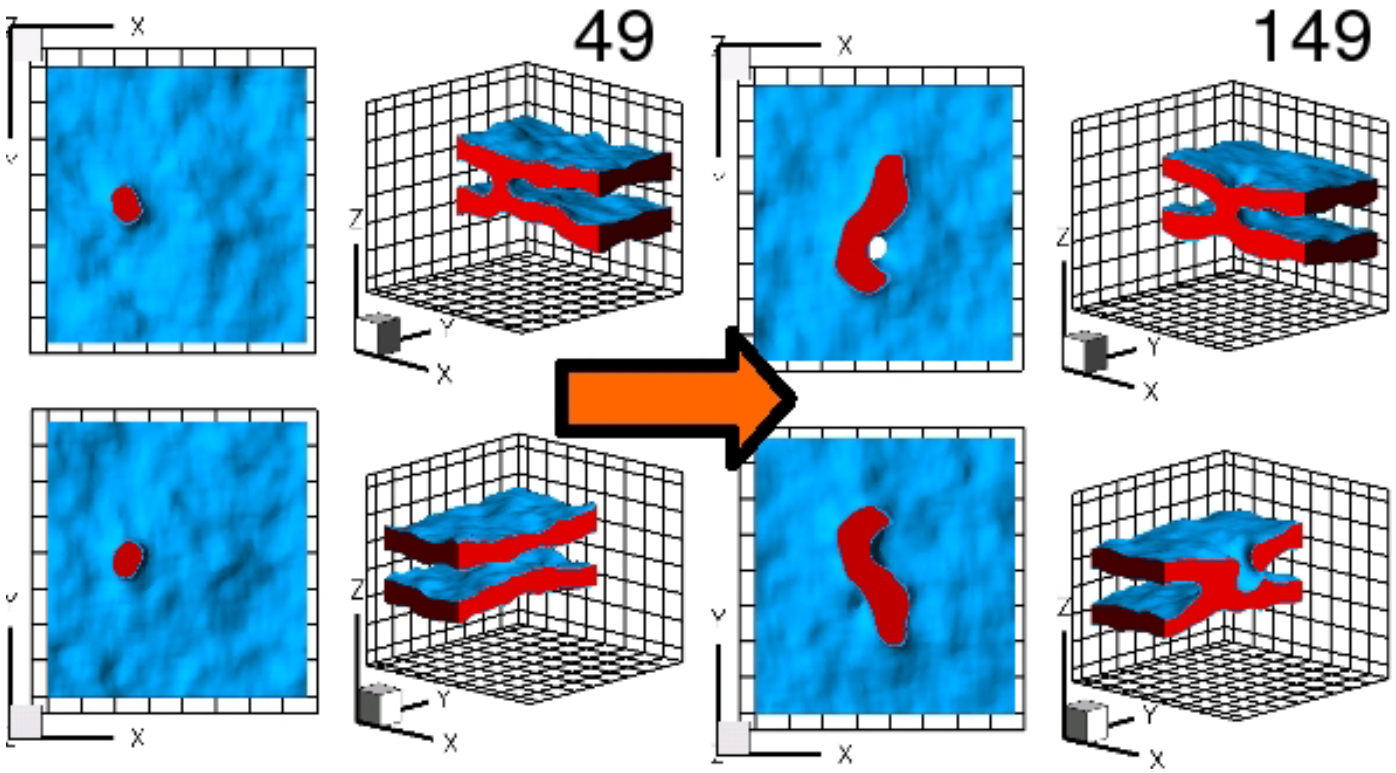
- **fast** (fusion does happen)
- **large** system size (~ 250 nm^2, 2500 "lipids")
- **diffusive** dynamics (local moves)



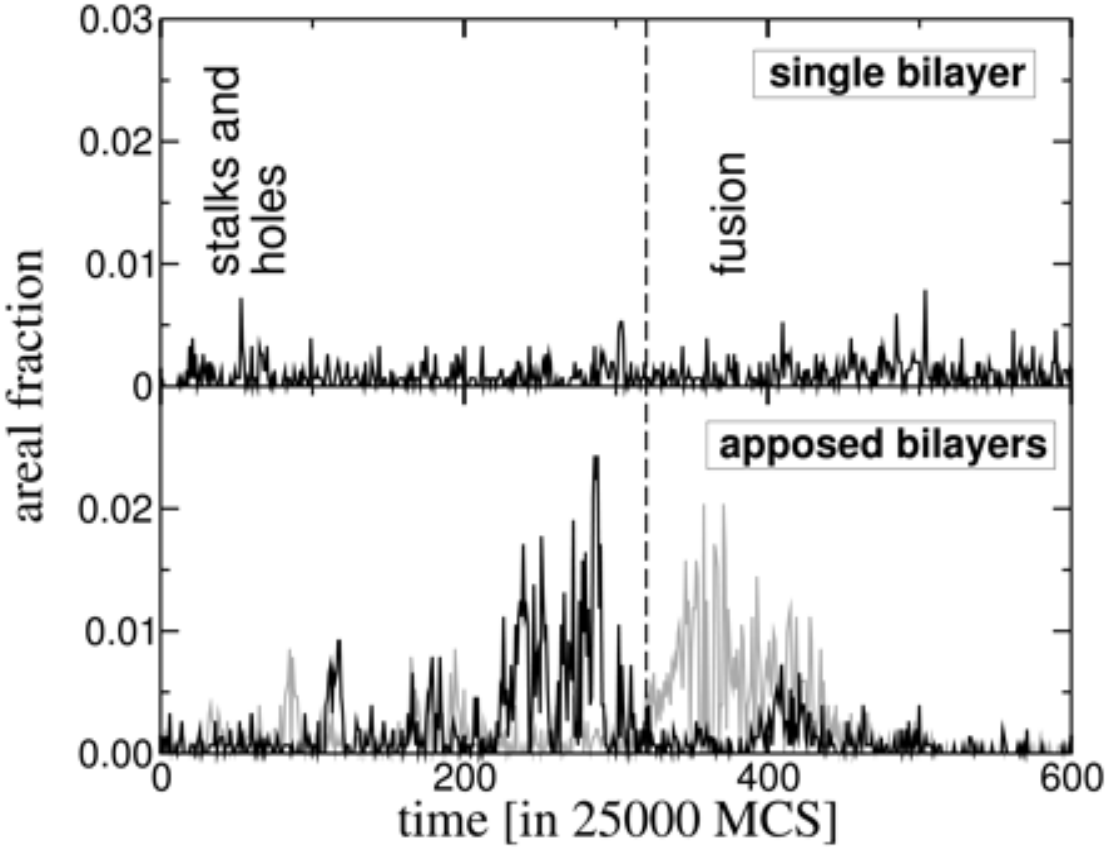
Stalk Formation



Stalk Formation and Expansion



Stalks increase rate of hole formation



Why does rate of hole formation go up?

Presumably, due to reduced line tension

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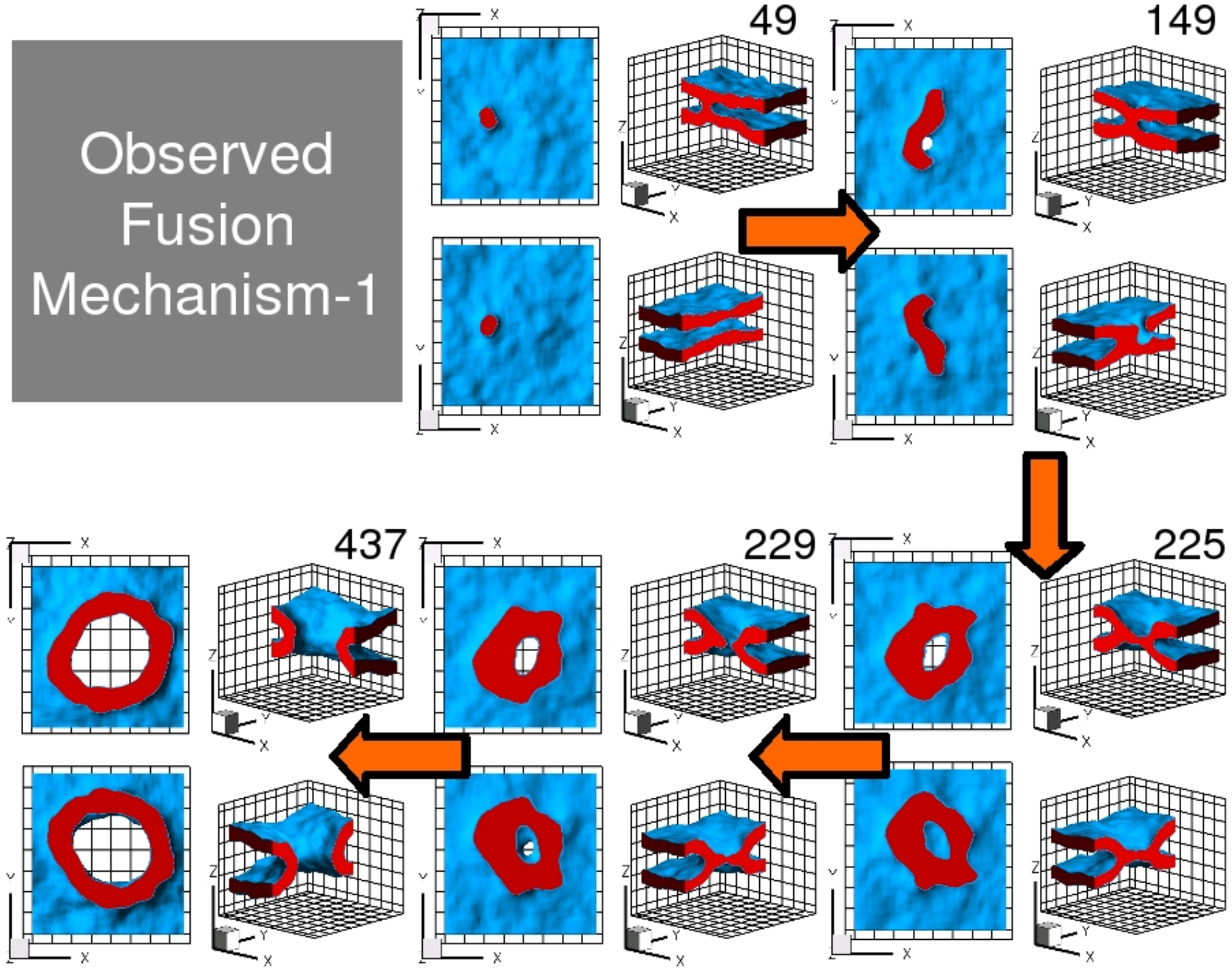
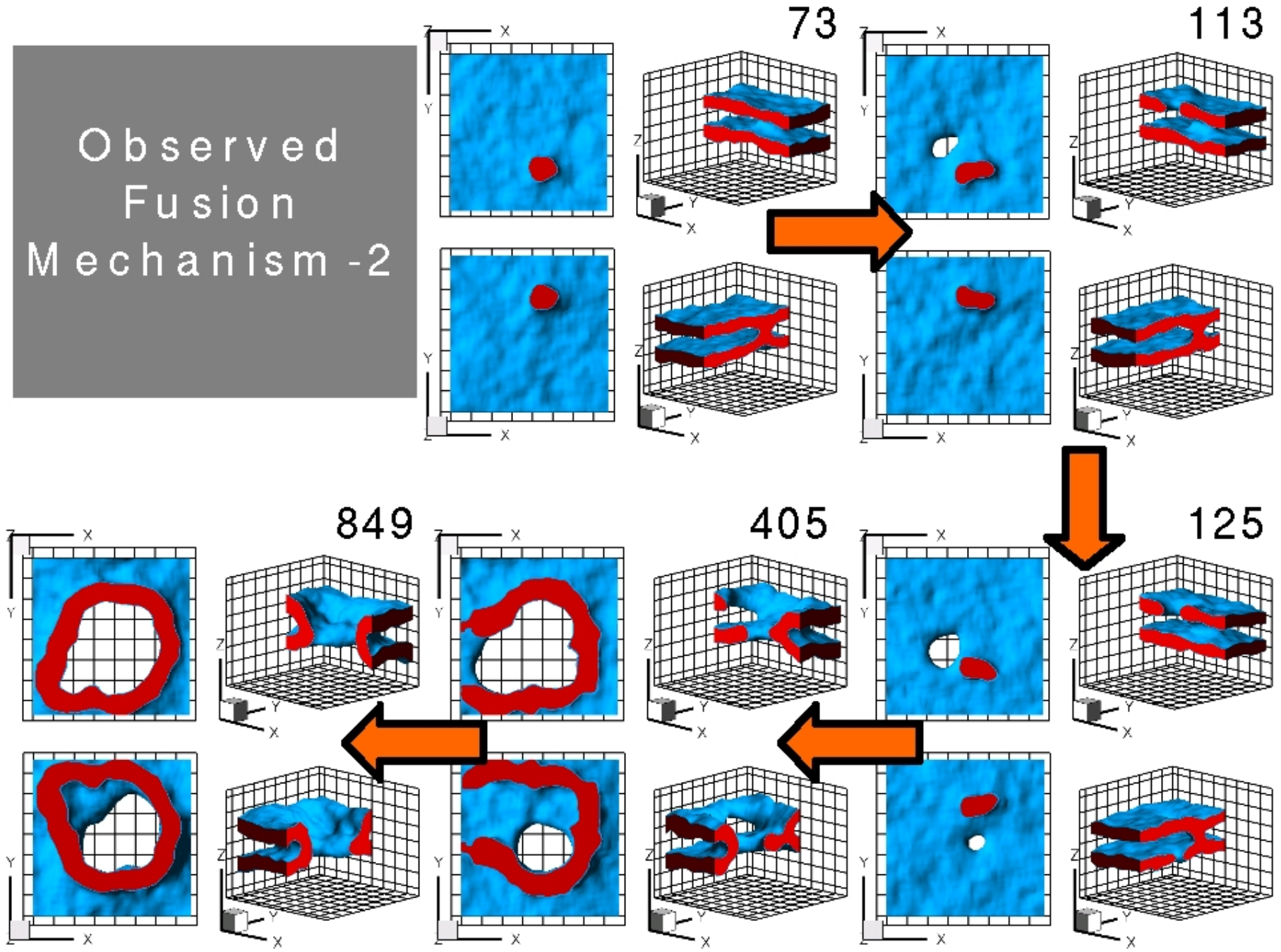
Presumably, due to reduced line tension

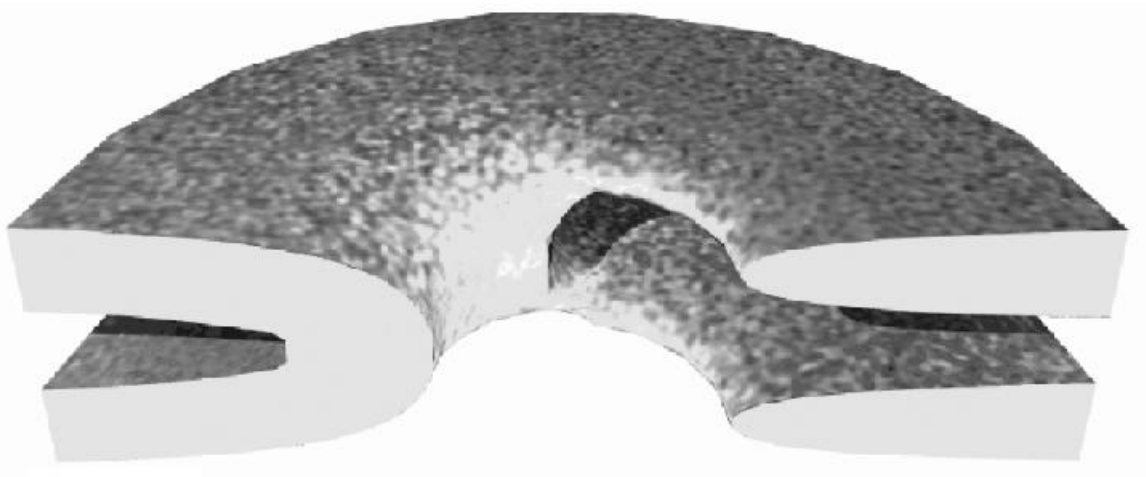
Observed Fusion Mechanism-1



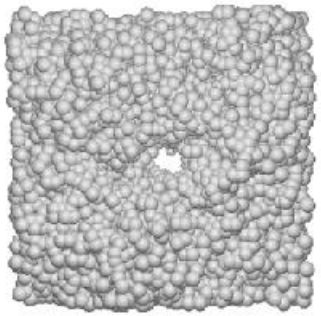
Observed Fusion Mechanism -2



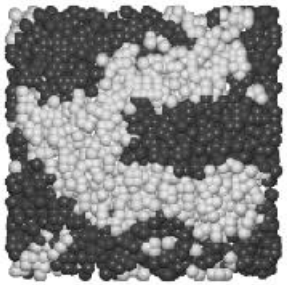
The intermediate in this second scenario



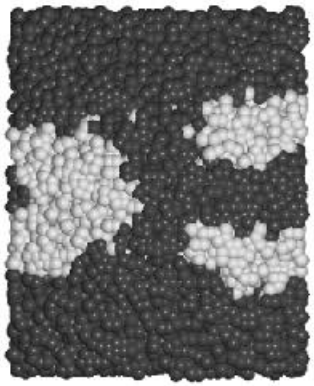
(a)



(b)

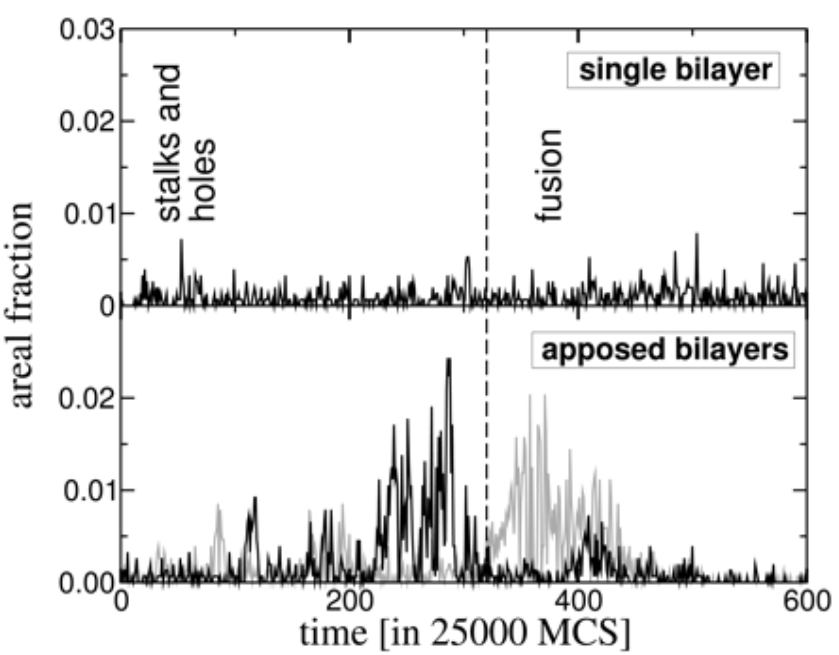


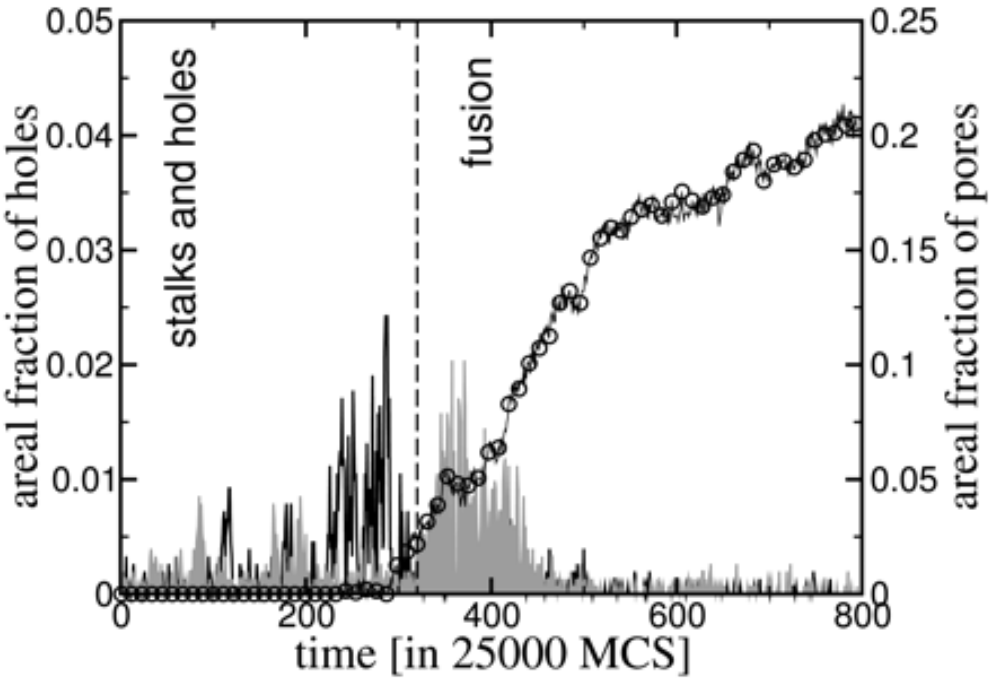
(c)



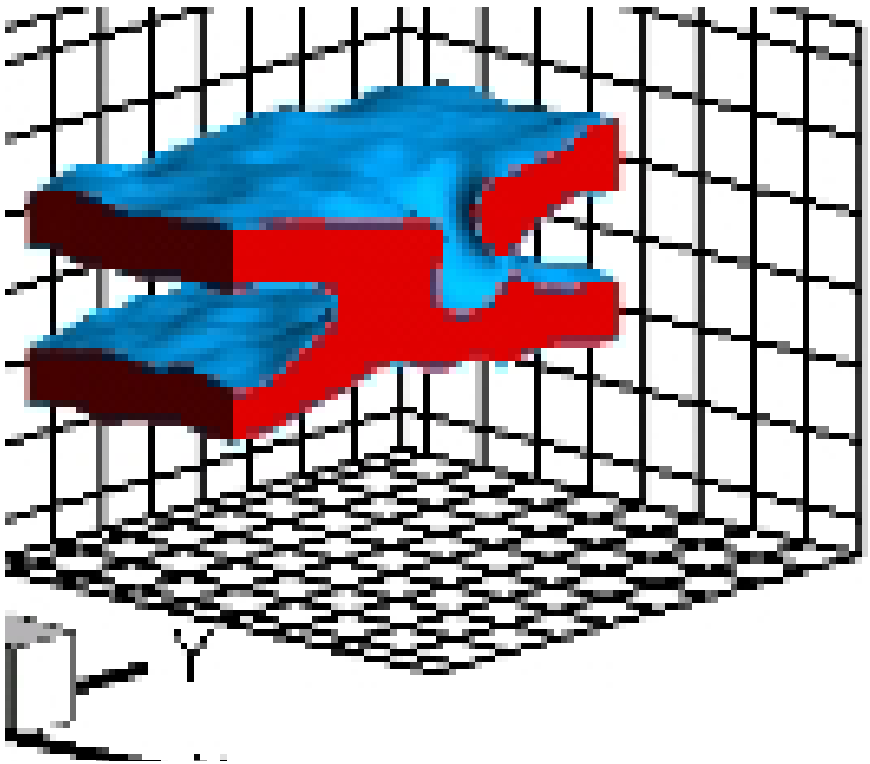
(d)

Hole Formation and Fusion are Correlated





Consequence for Experiment: Leakage



Some of the Evidence for Leakage and Lysis in Three Different Systems

1. HA induced fusion and lysis

HA-mediated virus-liposome fusion and lysis

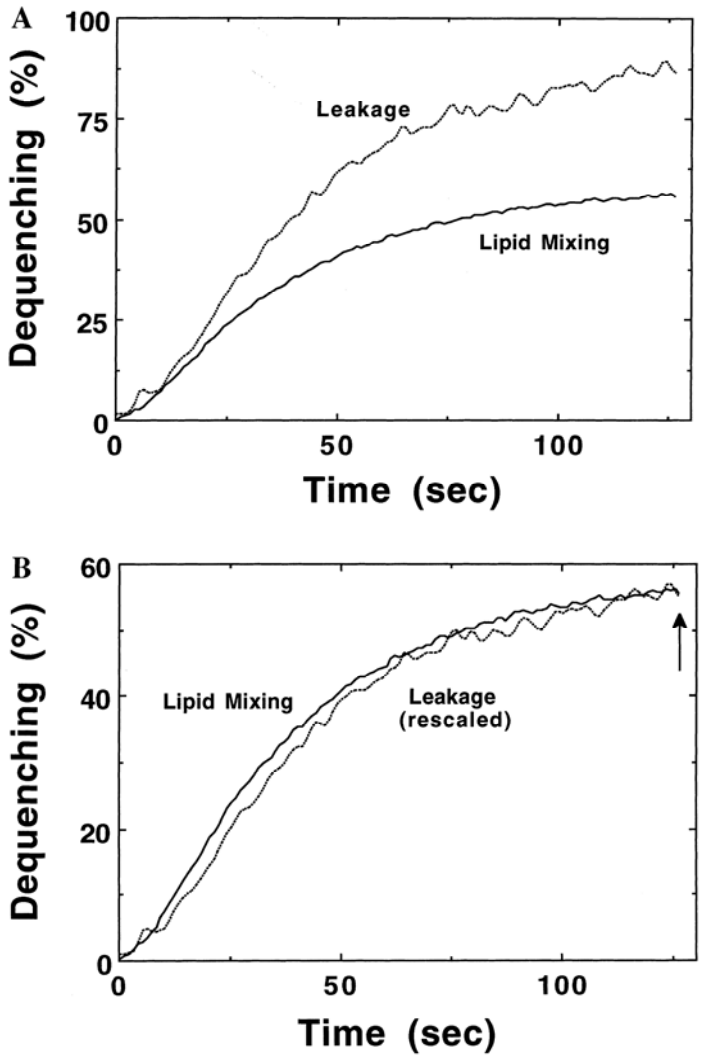
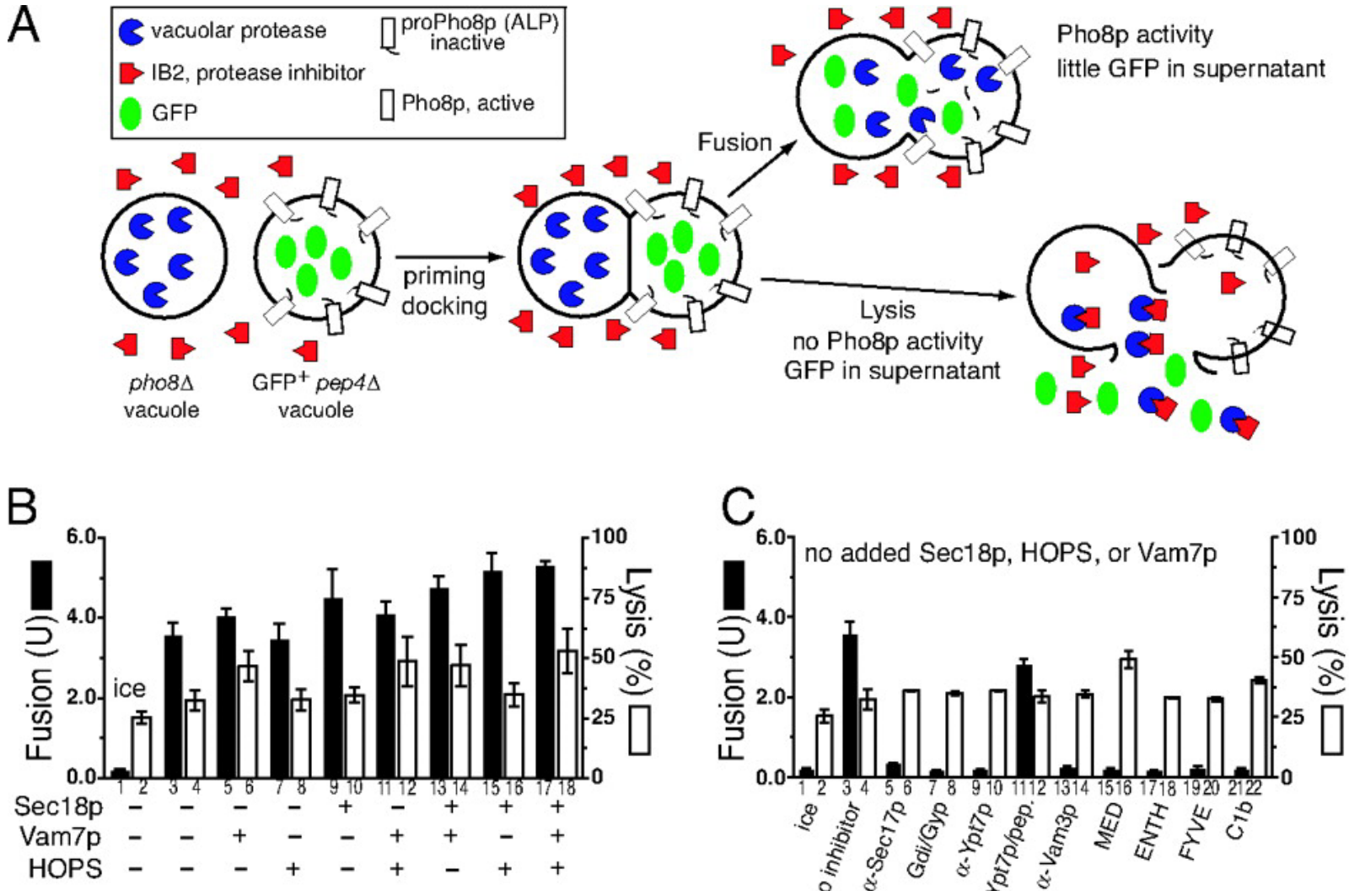


Figure 7 (A) Leakage (dotted line) and lipid mixing (solid line) kinetics of DOPC/GD1a (90:10) liposomes during fusion with PR/8 virus at 37 C. Leakage is measured by ANTS/DPX assay, whereas lipid mixing is measured by CPT/DABS assay. (B) The leakage curve in A is rescaled (dotted line) to the same final extent as the lipid mixing curve (solid line) at time 125 s (arrow). The lipid mixing curve is not changed from panel A.

Shangguan, Alford, and Bentz (1996)

2. Snare-driven vacuole fusion and lysis

The soluble vacuolar SNARE Vam7p promotes lysis during vacuole fusion



Starai V. J. et.al. PNAS;2007;104:13551

3. Fusion and lysis of yeast mating pairs

The Plasma Membrane Proteins Prm1 and Fig1 Ascertain Fidelity of Membrane Fusion during Yeast Mating

Pablo S. Aguilar, Alex Engel, and Peter Walter

Mol. Biol. Of the Cell 2007

The Plasma Membrane Proteins Prm1 and Fig1 Ascertain Fidelity of Membrane Fusion during Yeast Mating

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Mol. Biol. Of the Cell 2007

Our results support in multiple ways a functional coupling of lysis to the engagement of the fusion machine:

First, by removing Ca^{2+} to favor lysis, we observe that the timing of lysis events is the same as the timing of fusion.

Second, we demonstrate that the two cells of a mating pair lyse synchronously, as expected for events at the interface between both cells in a mating pair.

Third, mixing of cytoplasmic contents occurs concomitant with the initiation of lysis.

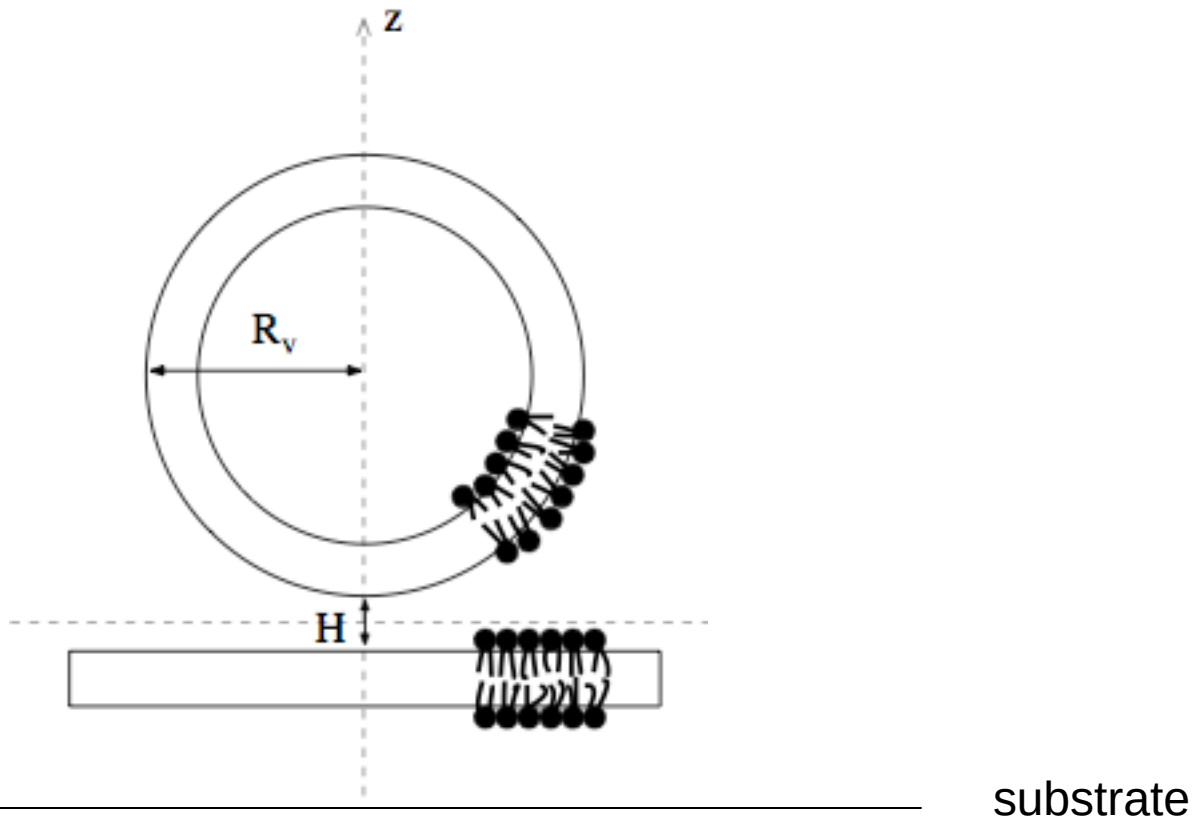
This implies that lysis is initiated as fusion is catalyzed, most simply explained by hypothesizing a common machinery for the two outcomes.

Most recently:

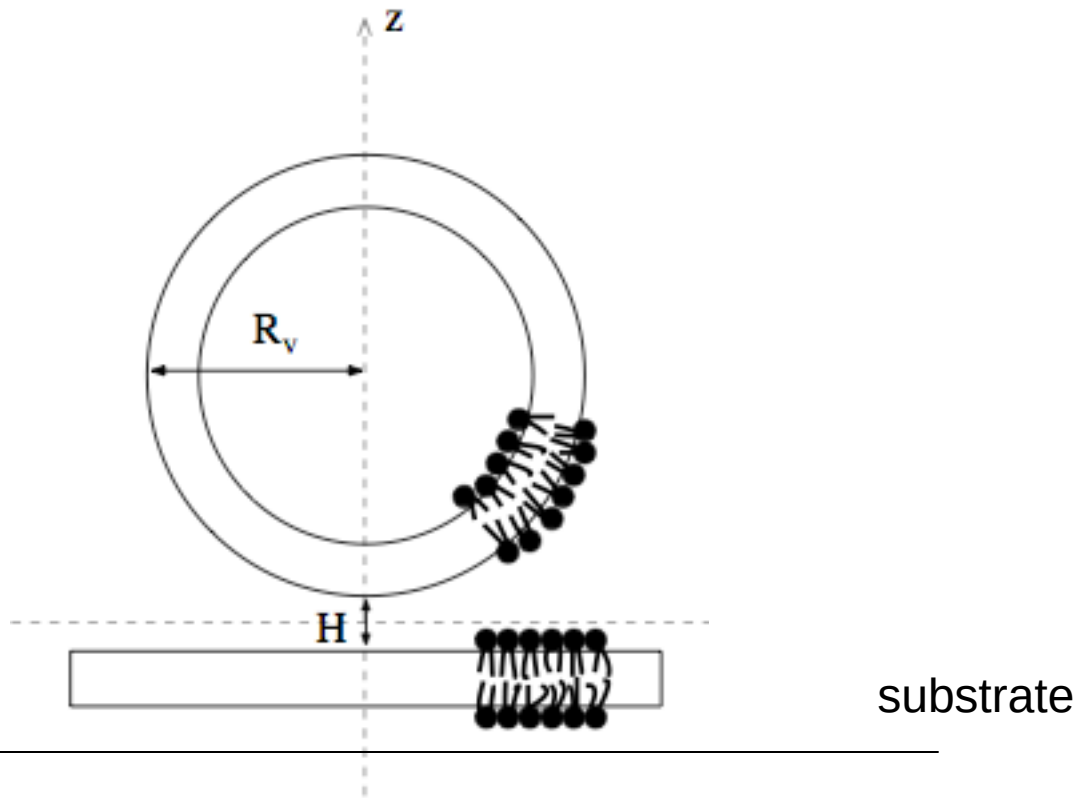
SNARE-driven, single-vesicle fusion: vesicle plus planar bilayer geometry

Lipid Mixing and Content Release in Single-Vesicle, SNARE-Driven Fusion Assay with 15 ms Resolution

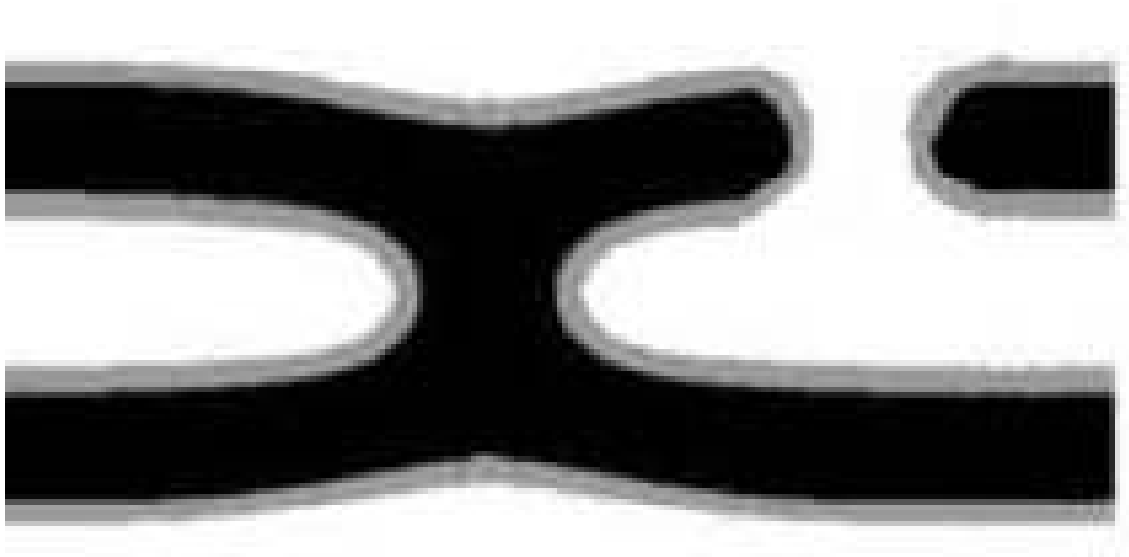
Tingting Wang,Elizabeth A. Smith,
Edwin R. Chapmanand, James C. Weisshaar 2009



…it is disappointing that the content is evidently released abruptly into the 3D space above the planar bilayer rather than into the 2 nm thin, watery space between the glass and the planar bilayer.

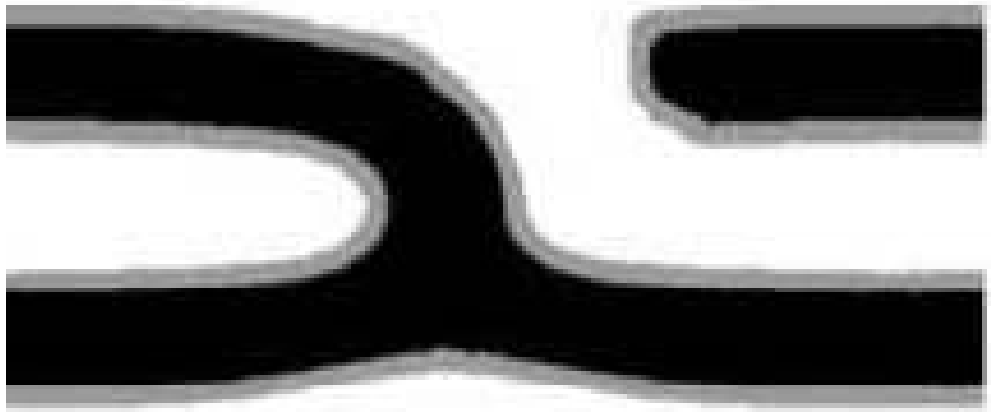


This is precisely what we expect from the alternate mechanism



substrate

Conclusion



FUSION

LYSIS

If possible failure is an option, fusion or lysis can be tolerated.

Case of viral fusion?

If failure is not an option, can conclude that fusion machinery either

a) ensures that fusion proceeds predominantly by standard mechanism or
b) if it proceeds predominantly, or at all, by the new mechanism, that hole formation is directed to fusion, not lysis.