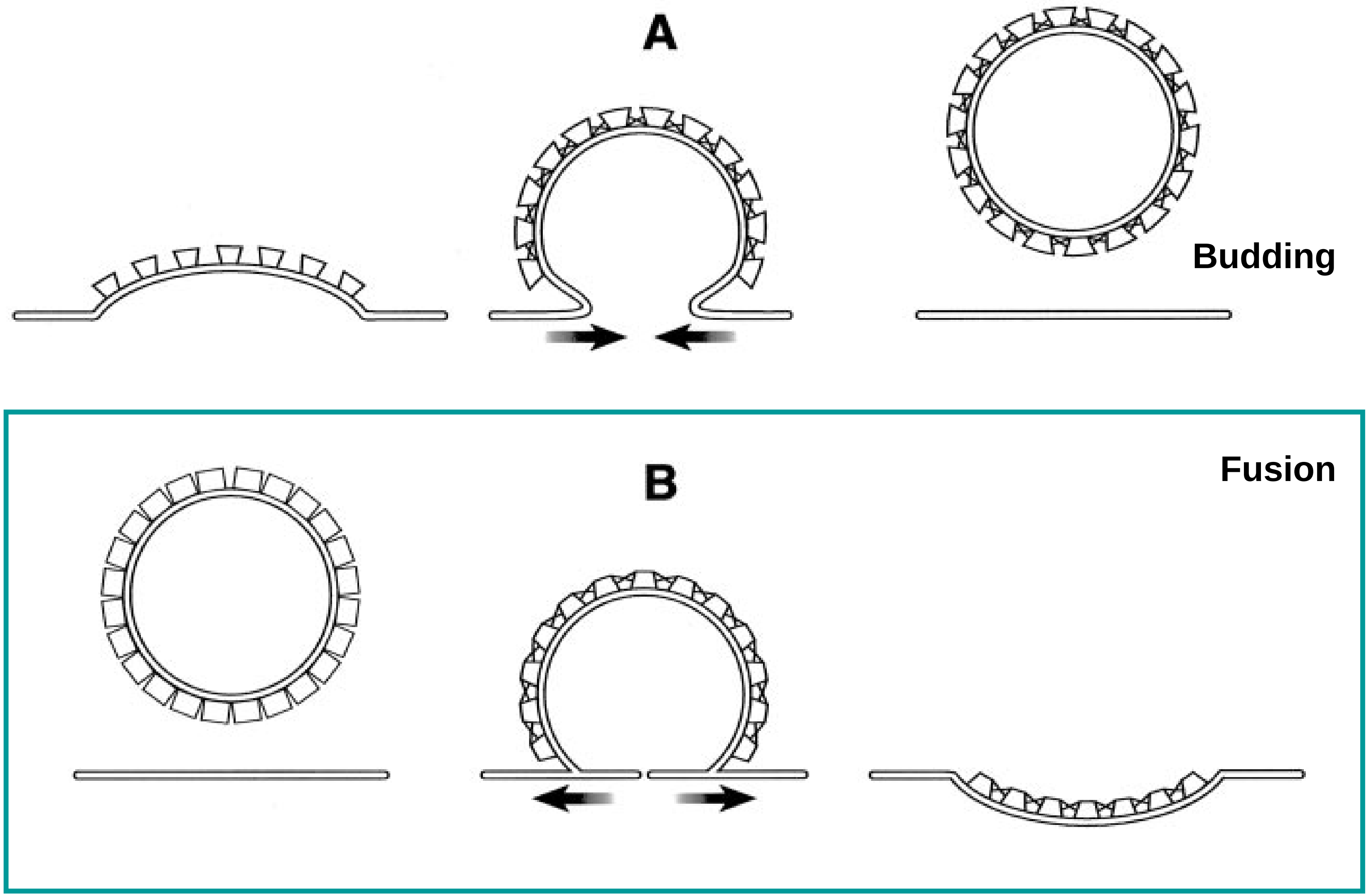
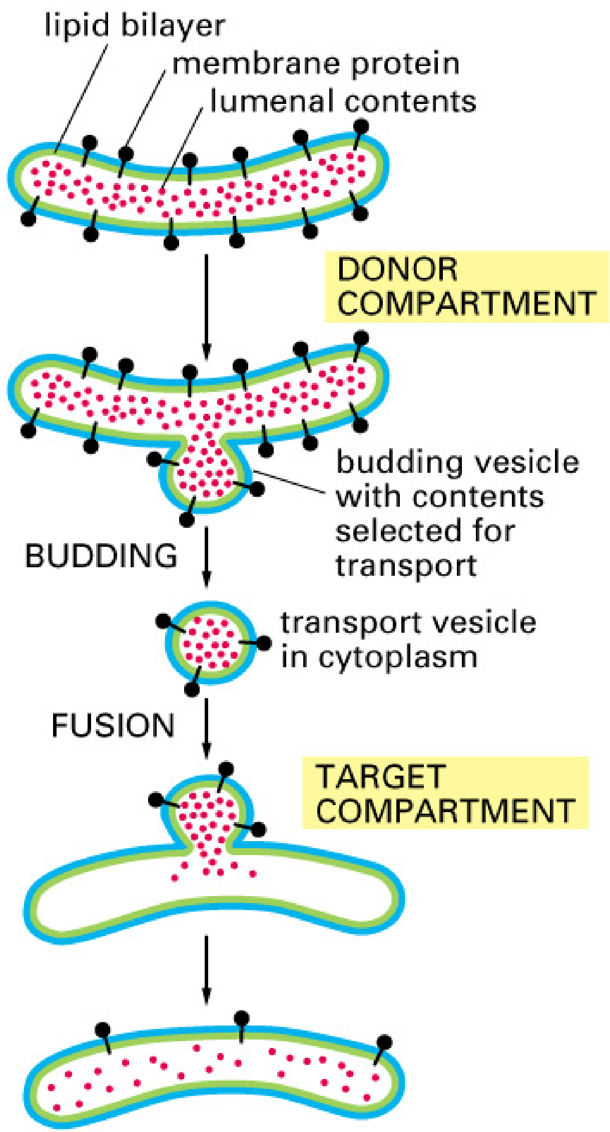
Membrane Fusion

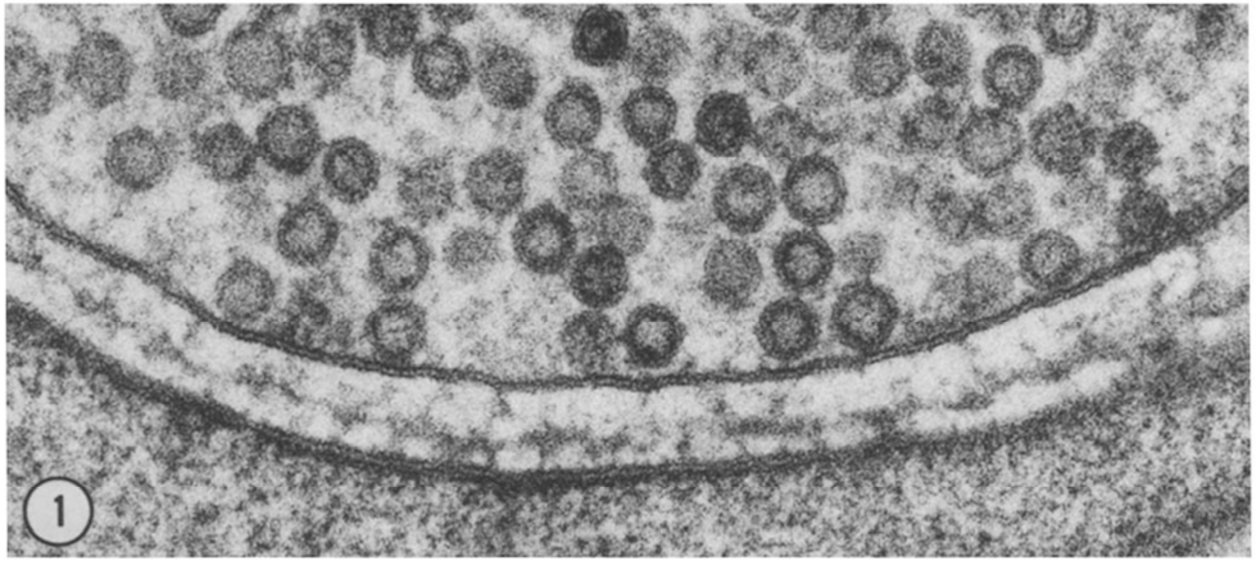
Mary Munson, University of Massachusetts Medical School



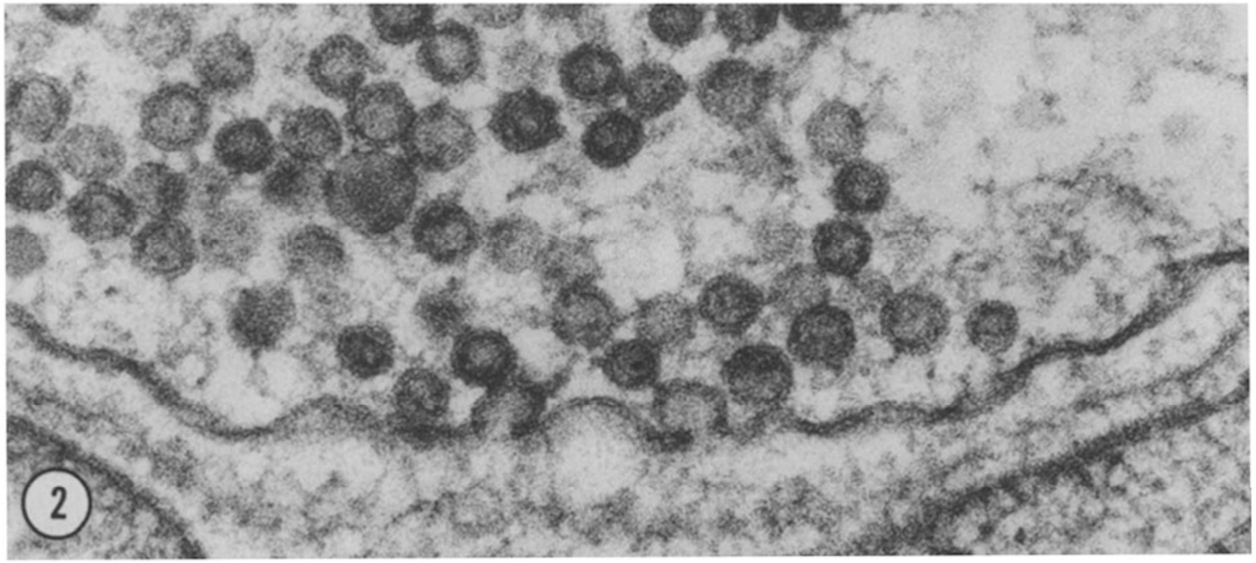
Membrane fusion is used for delivery of intracellular cargo



Synaptic vesicles fuse with the plasma membrane



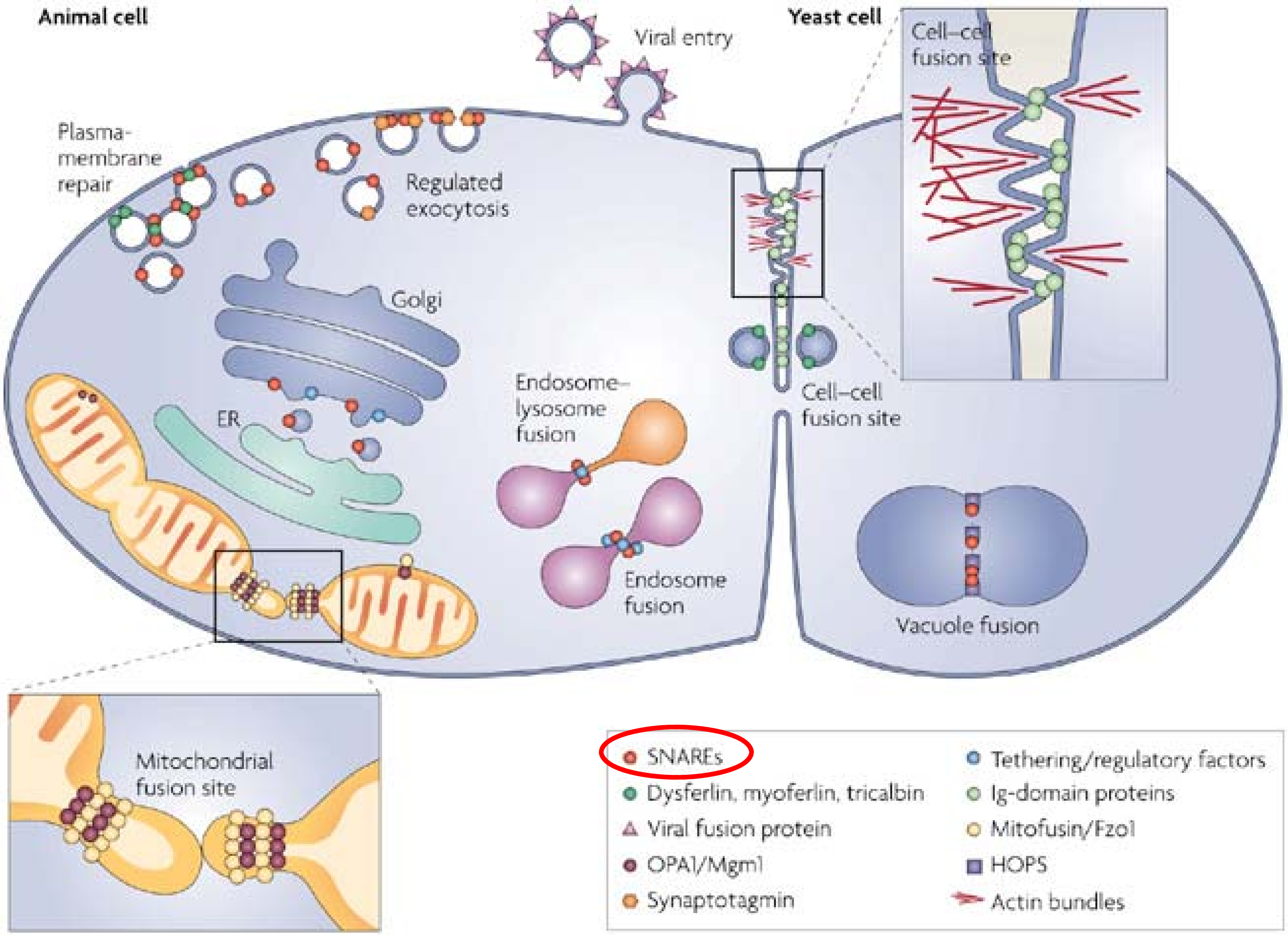
Fixed at rest



Fixed 5ms after stimulation

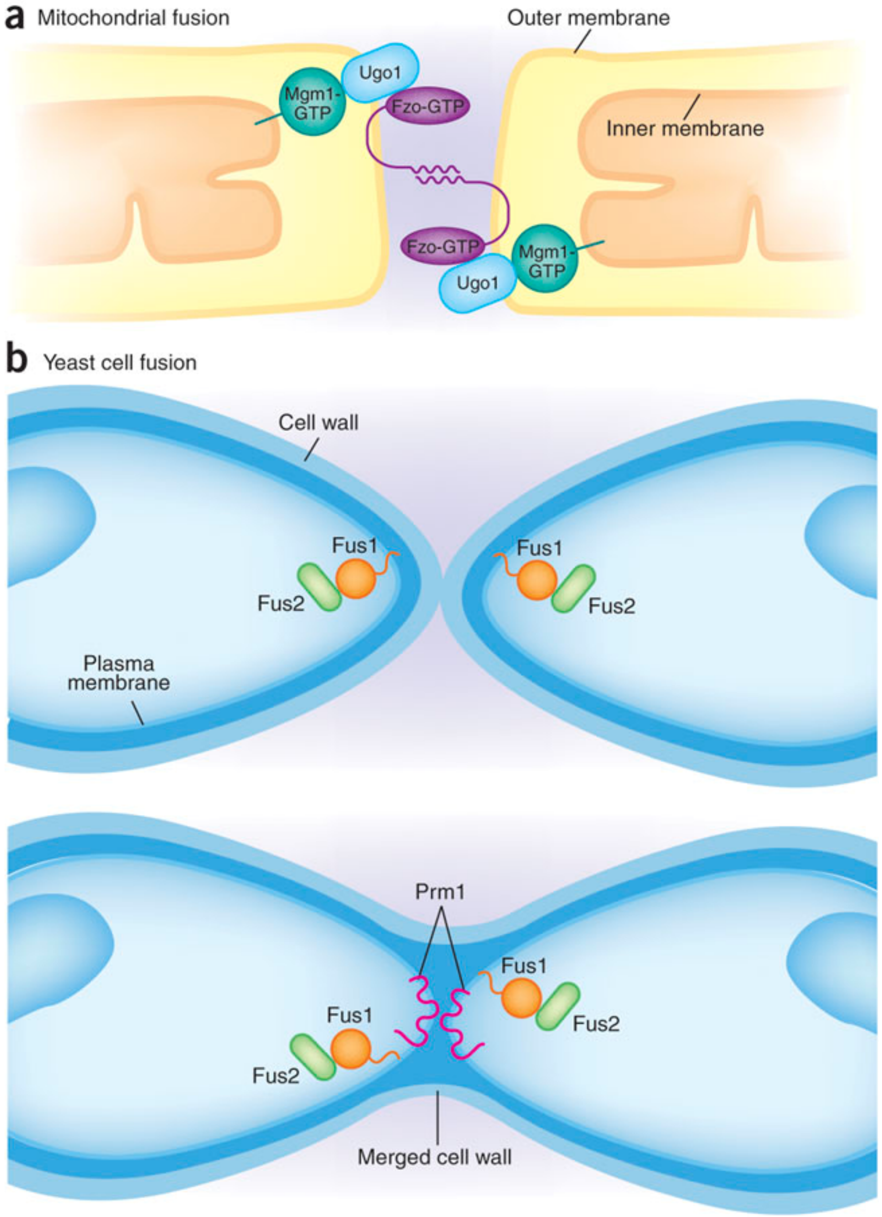
(Heuser & Reese, *J Cell Biol* 1981)

Membrane fusion is used for a variety of other processes



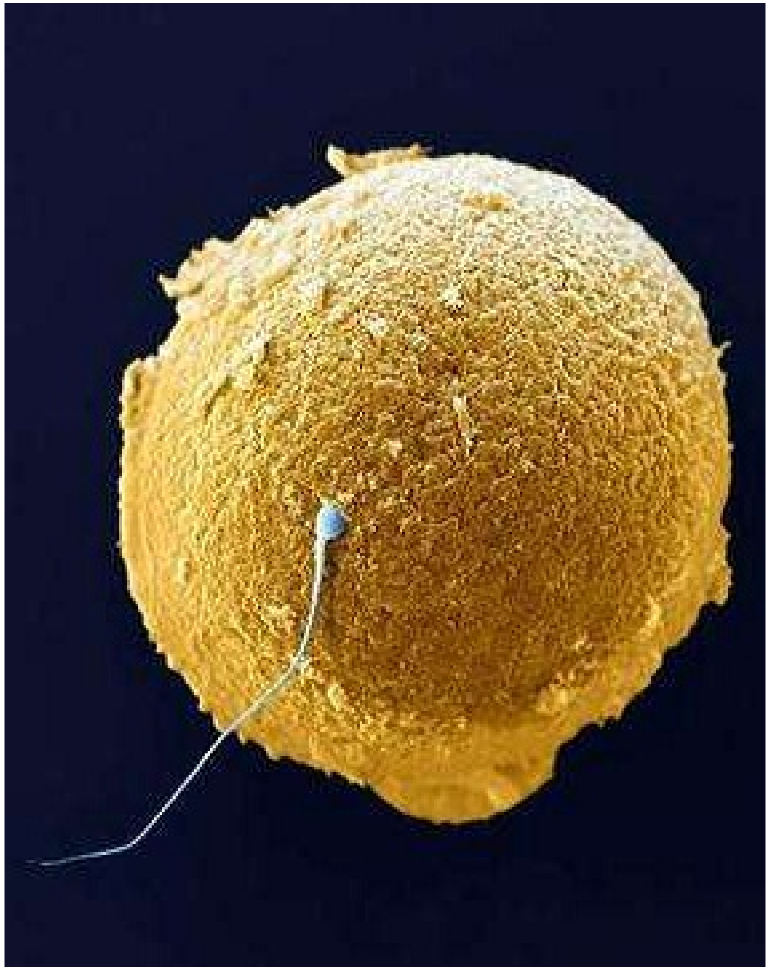
Nature Reviews | Molecular Cell Biology

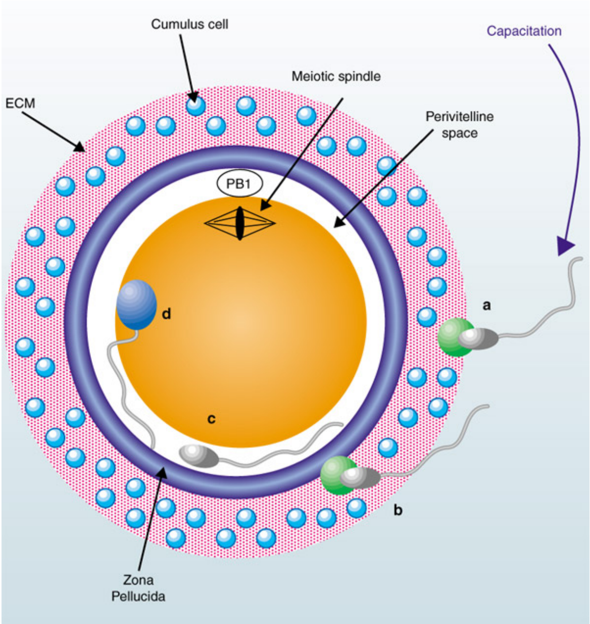
Membrane fusion is used for a variety of other processes



(Wickner & Schekman, *Nat Str Mol Biol* 2008)

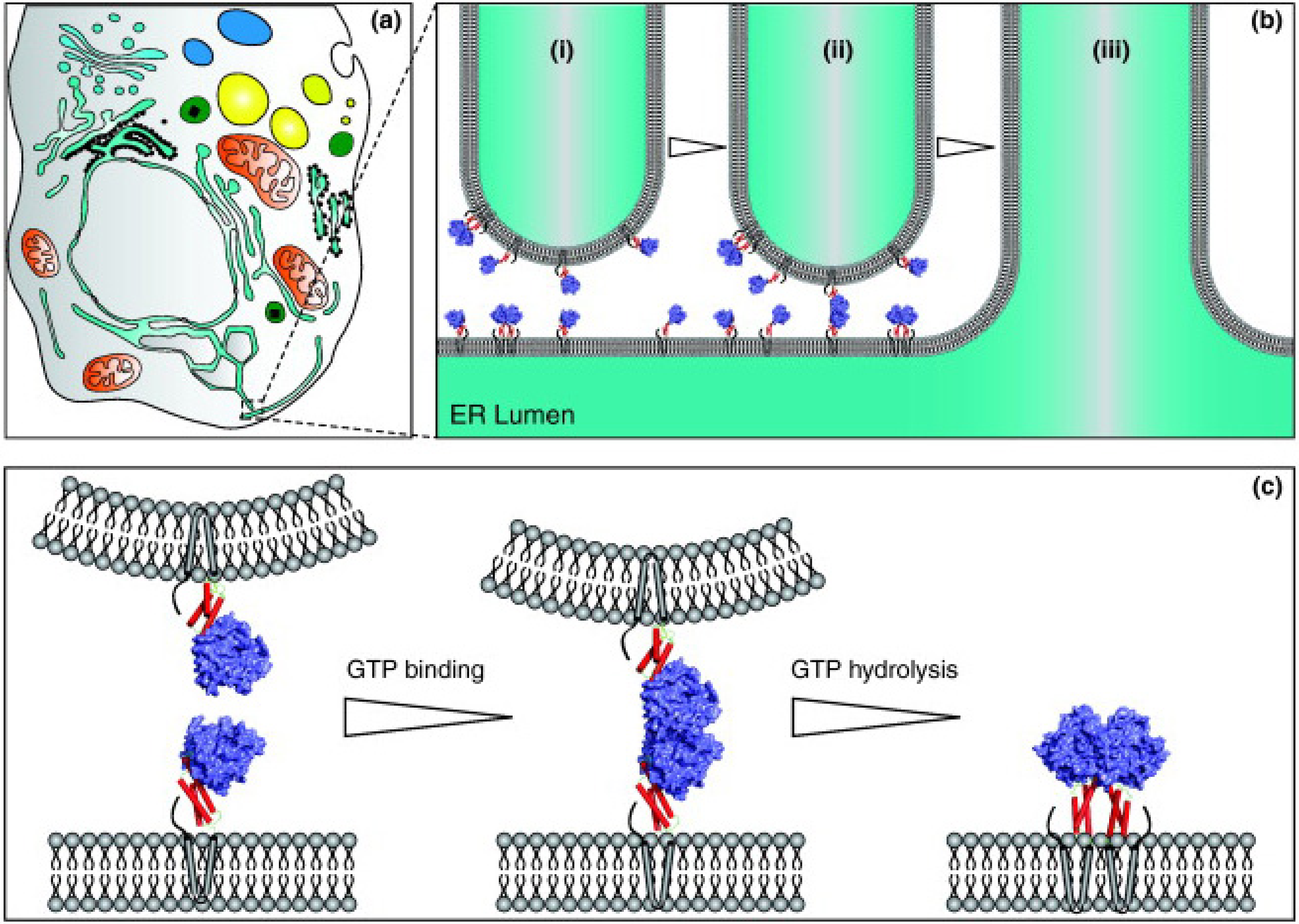
Membrane fusion is used for a variety of other processes





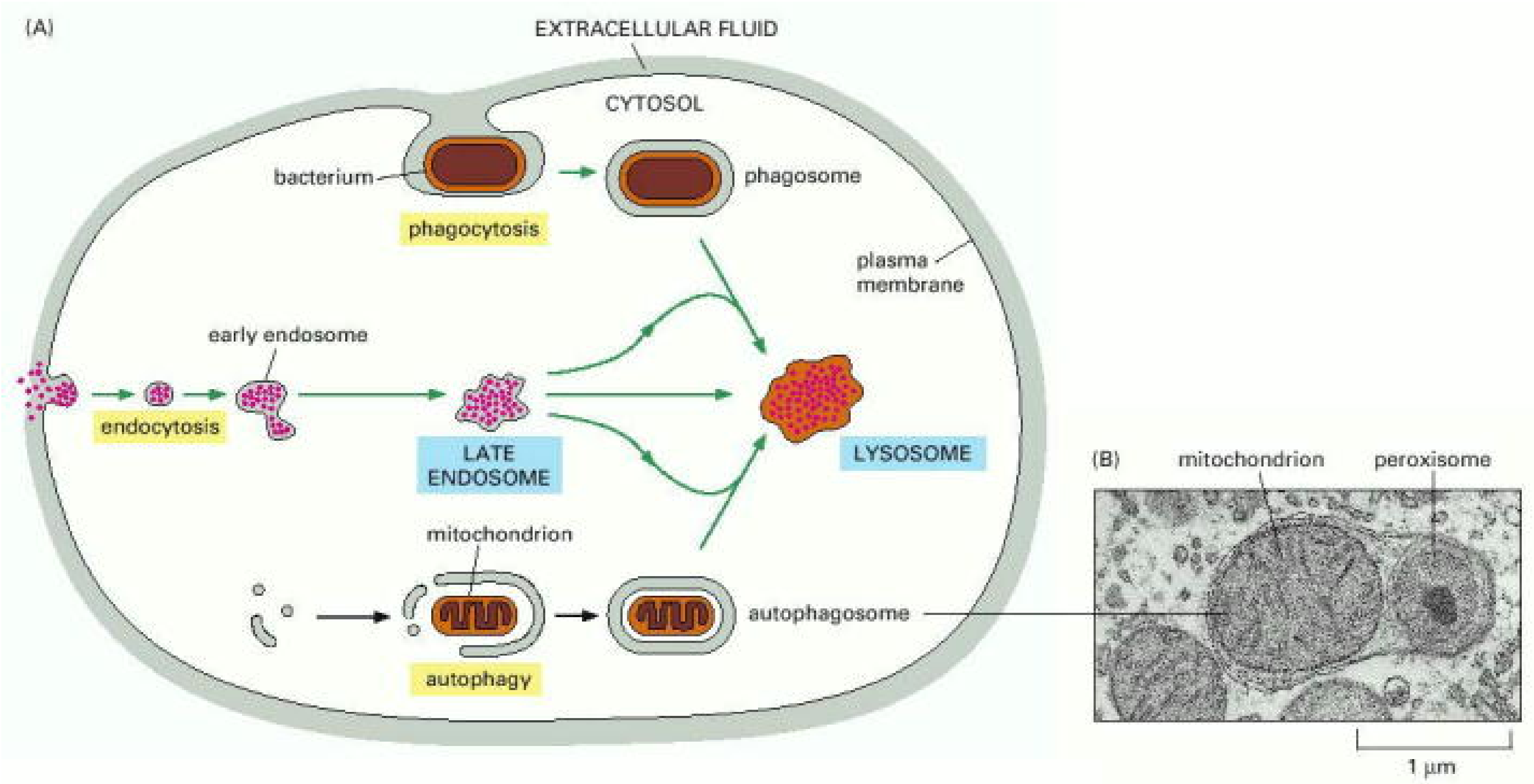
Nature Cell Biology 4 (S1), S57�S63 (2002)

Atlastin is used for ER-ER fusion



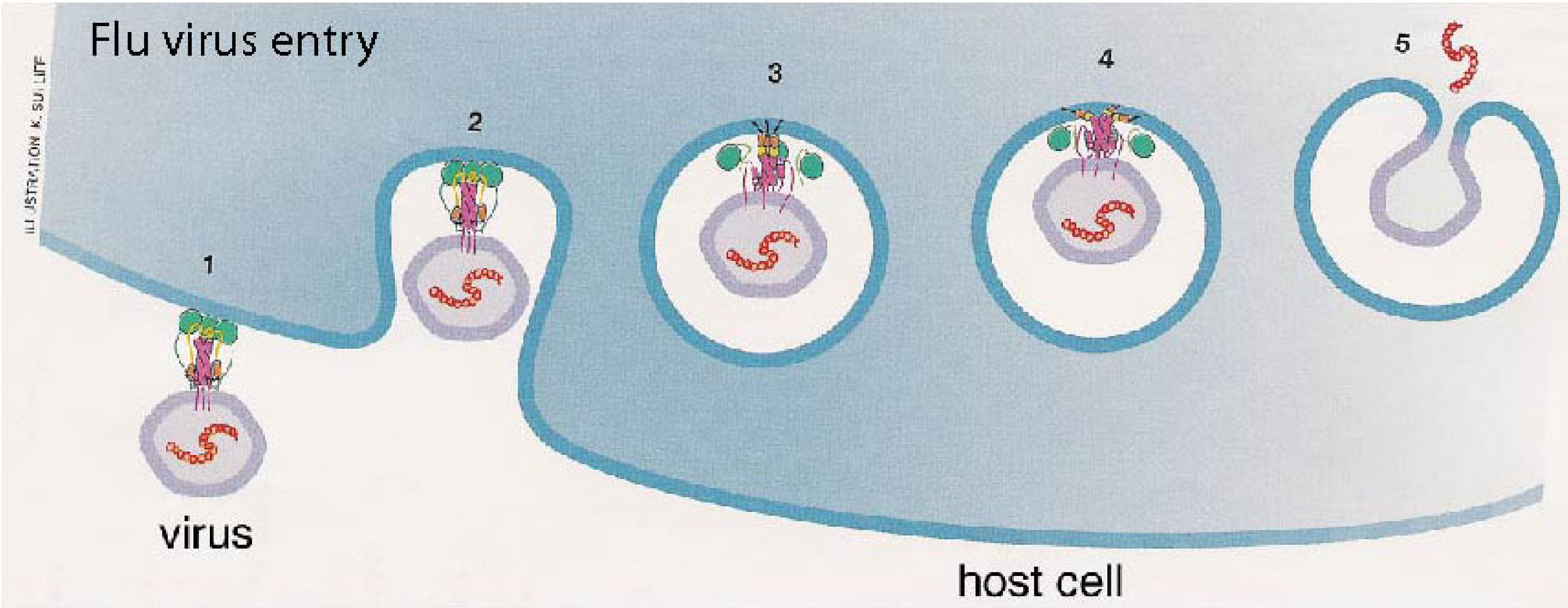
(Moss, Daga & McNew, *Trends Cell Bio* 2011)

Autophagy requires several membrane fusion events



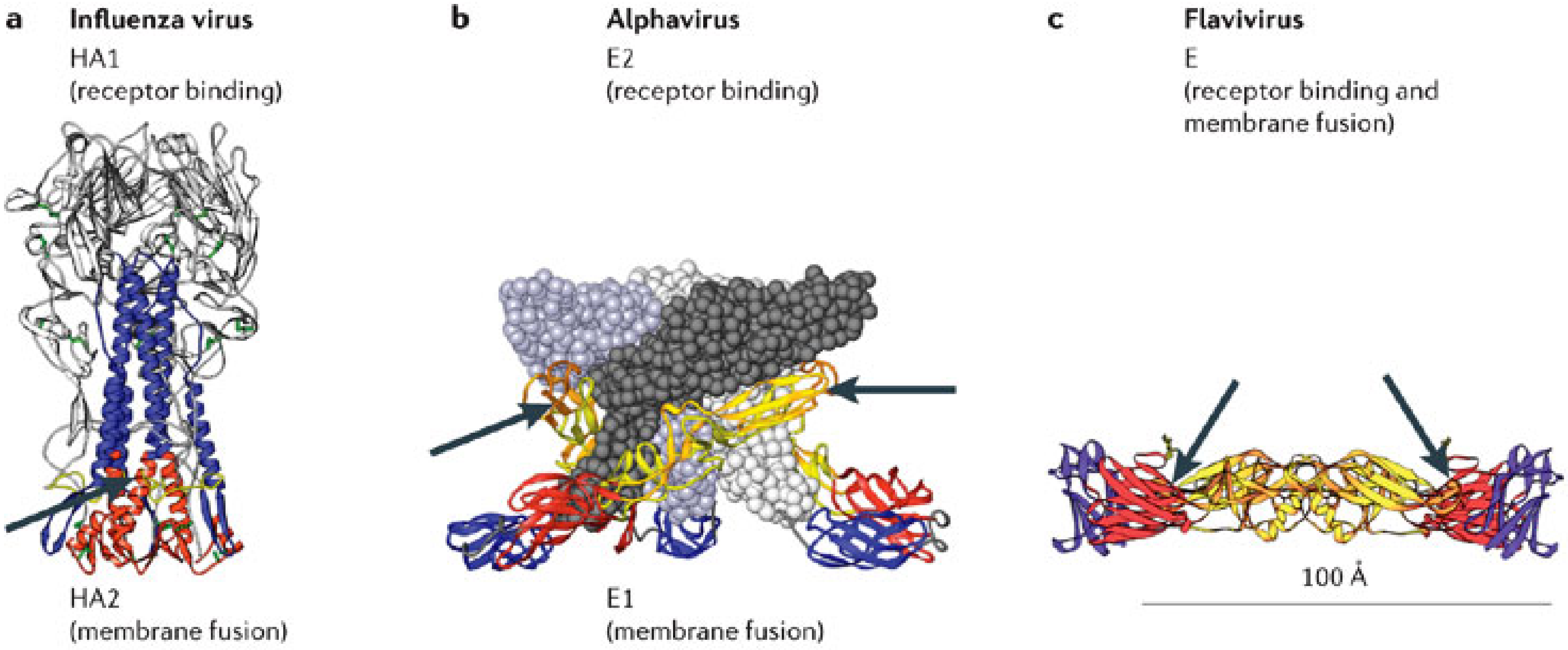
Membrane fusion is also used for entry of enveloped viruses in the cell

Influenza virus enters the cell though endocytosis and membrane fusion by HA is triggered by the low pH of the endosome.



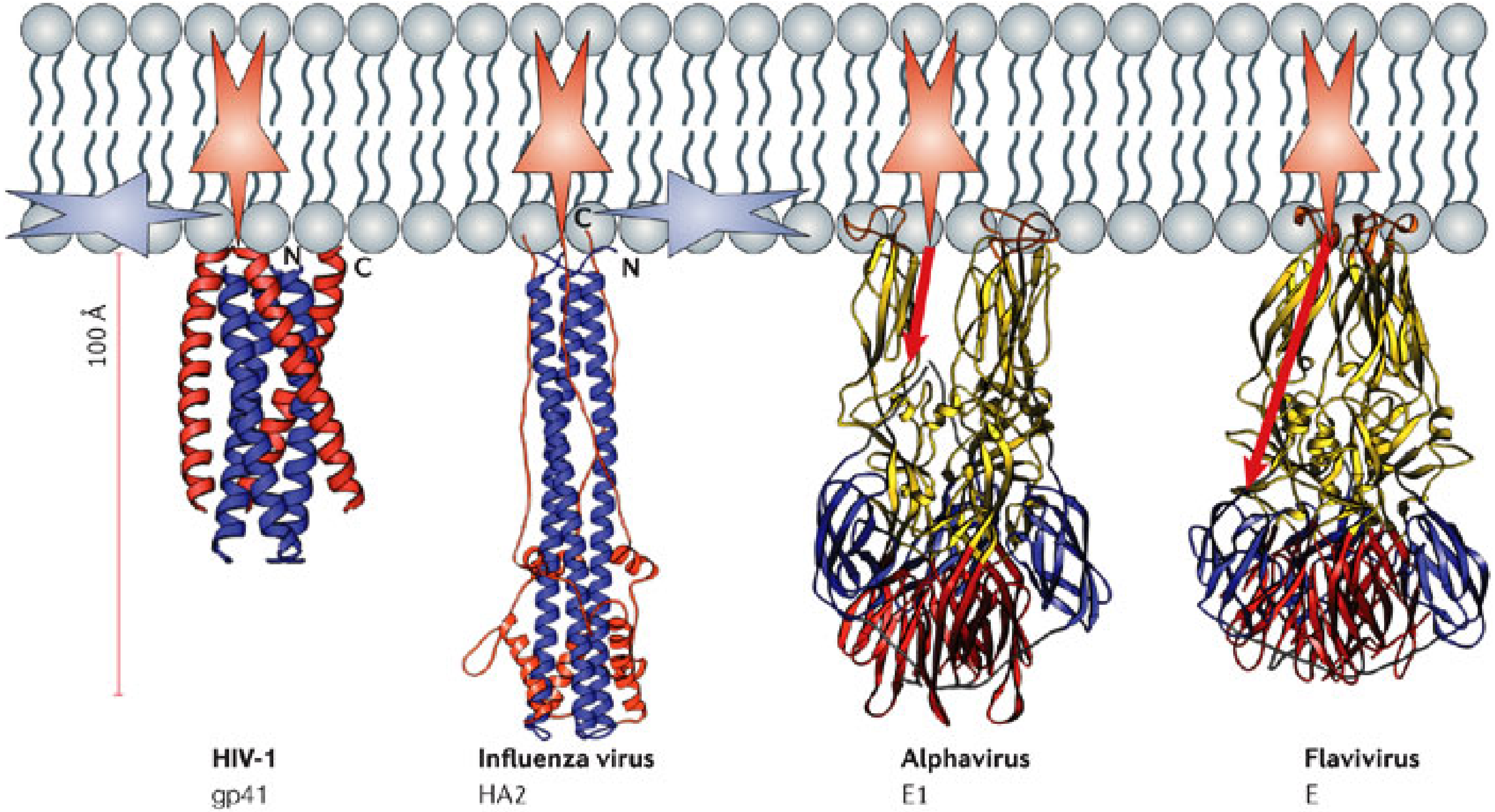
Carr and Kim, 1994

Viral membrane fusion proteins are structurally different… (pre-fusion structures)



Kielian M and Rey FA (2006) Virus membrane-fusion proteins: more than one way to make a hairpin
Nat Rev Microbiol. **4**: 67–76 doi:10.1038/nrmicro1326

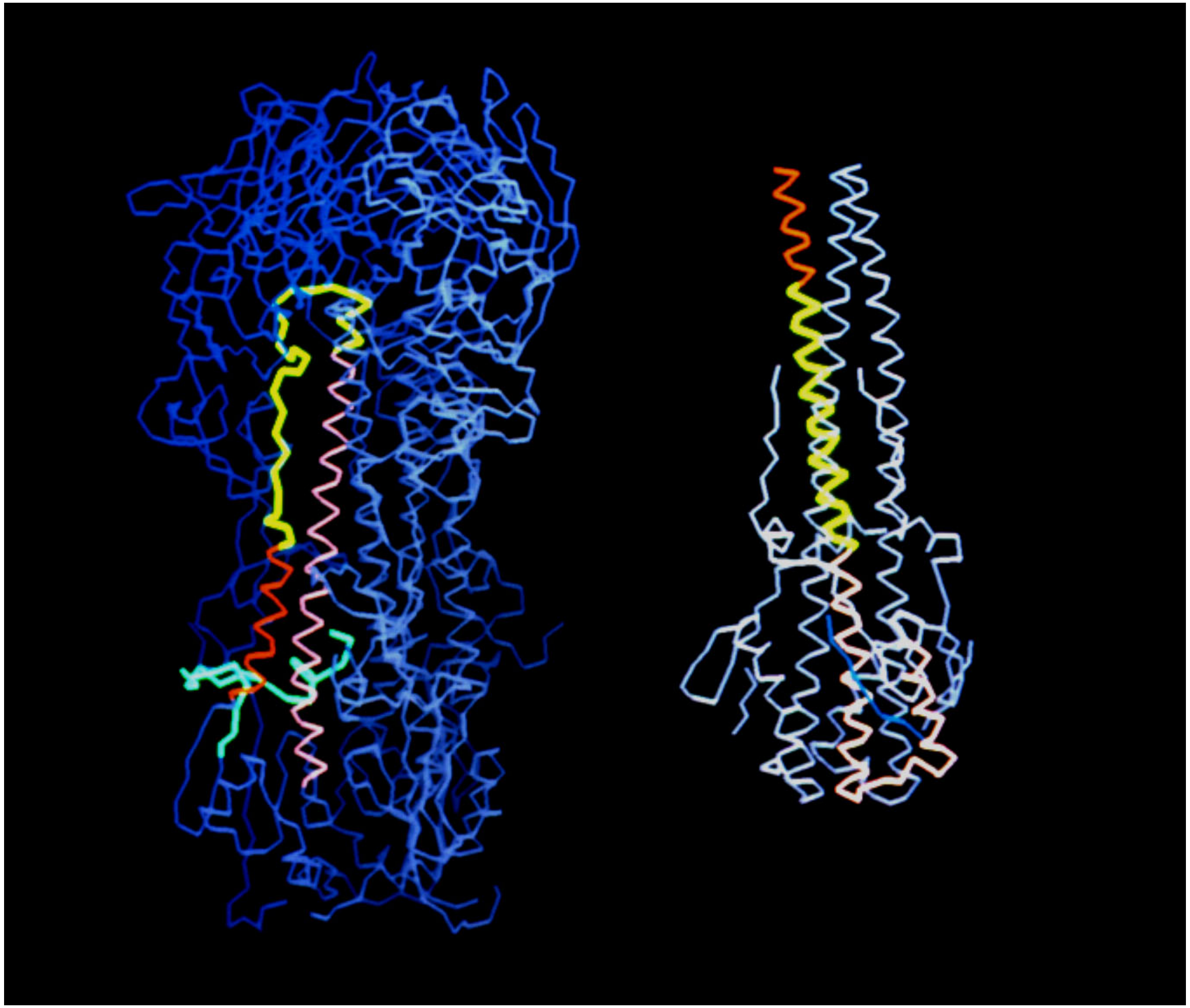
But functionally similar (post-fusion structures)



Kielian M and Rey FA (2006) Virus membrane-fusion proteins: more than one way to make a hairpin
Nat Rev Microbiol. **4**: 67–76 doi:10.1038/nrmicro1326

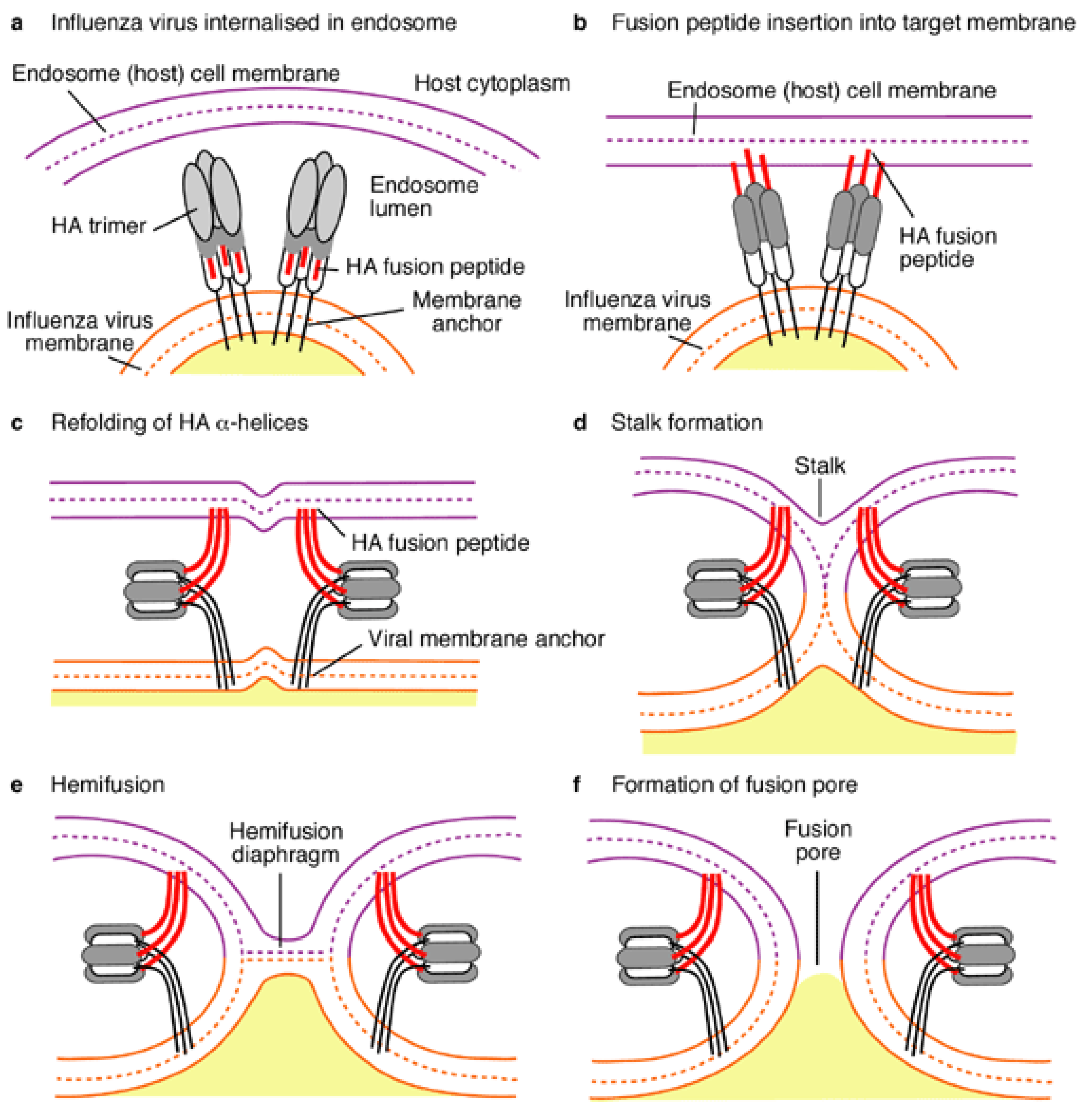
nature REVIEWS MICROBIOLOGY

X-ray crystal structures show the HA conformational changes



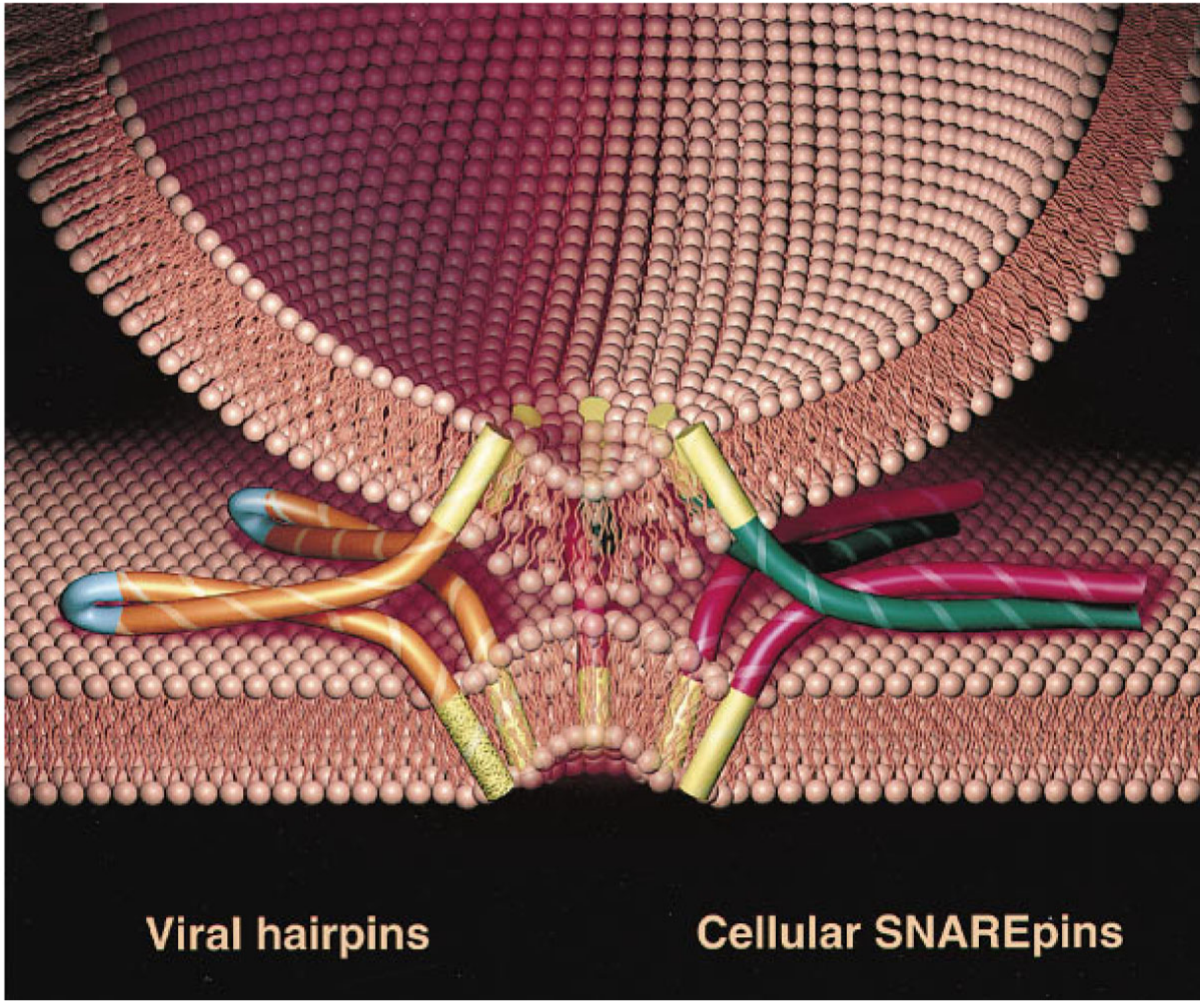
Wilson et al., 1981

Bullough, Hughson et al., 1994



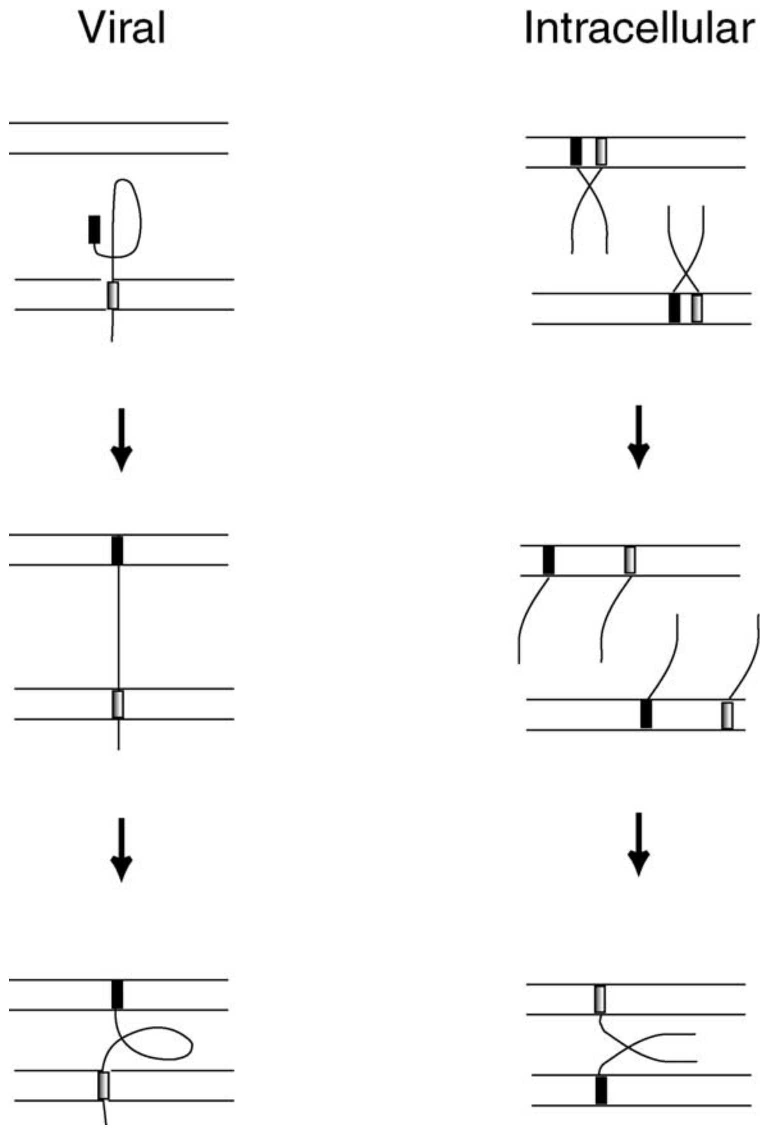
Stalk-pore hypothesis for membrane fusion mediated by influenza haemagglutinin (HA)

Cartoon shows a hypothetical fusion intermediate

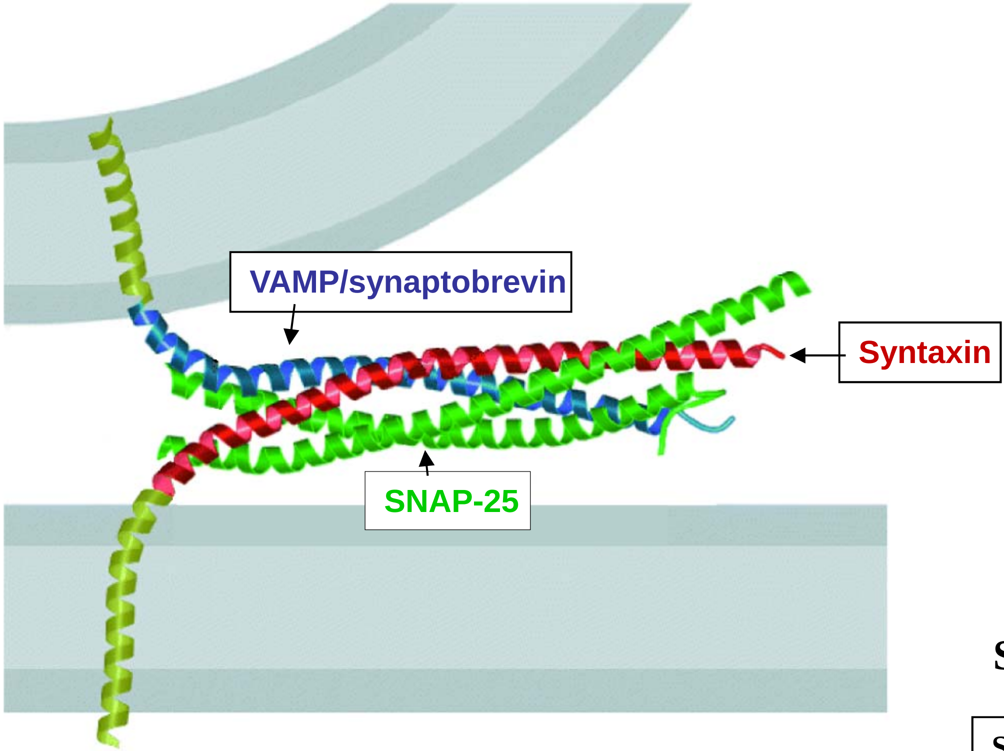


Weber et al., 1998

Viral and intracellular membrane fusion reactions use similar structures to pin membranes together



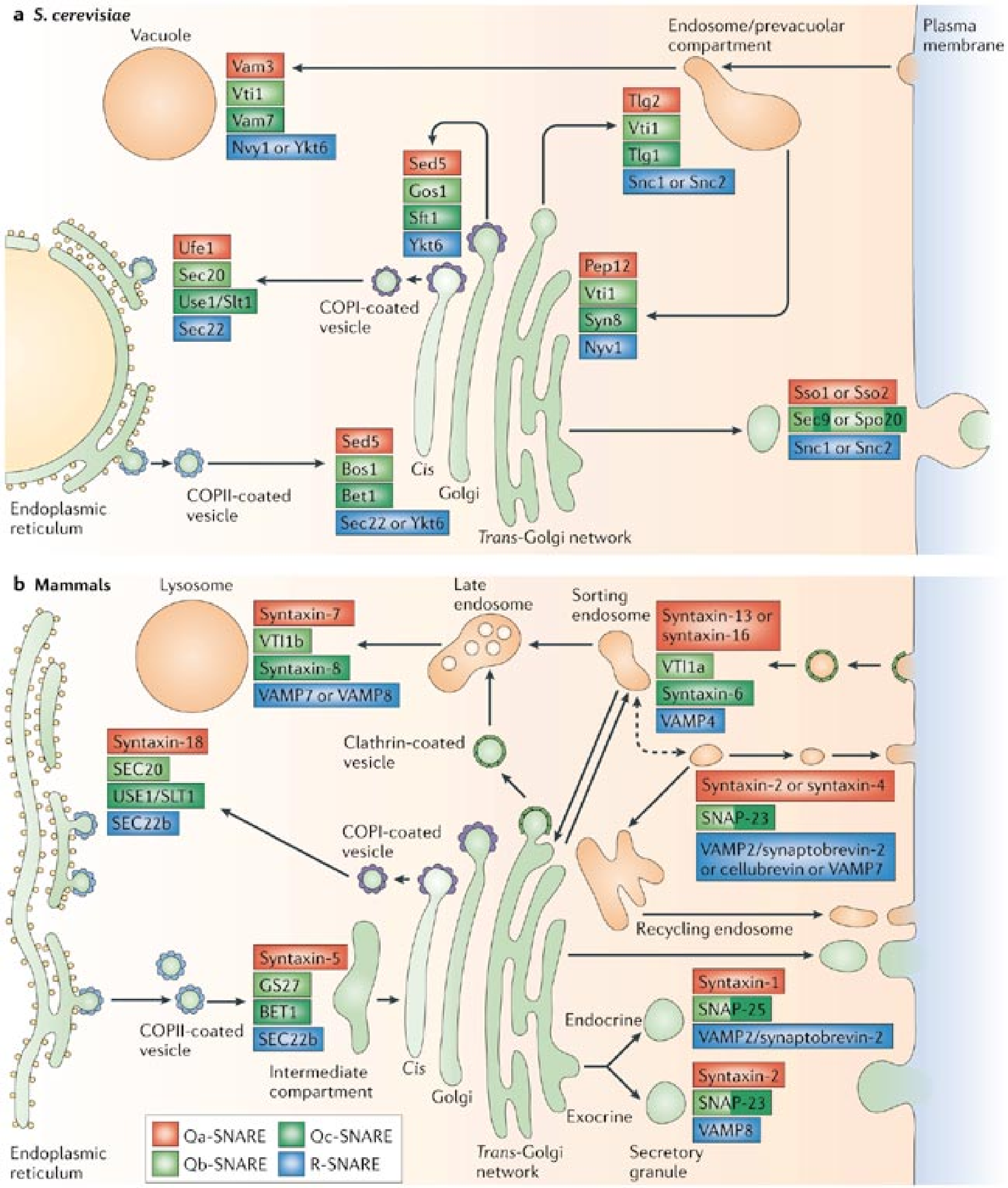
SNARE complex



Neuronal
SNARE complex

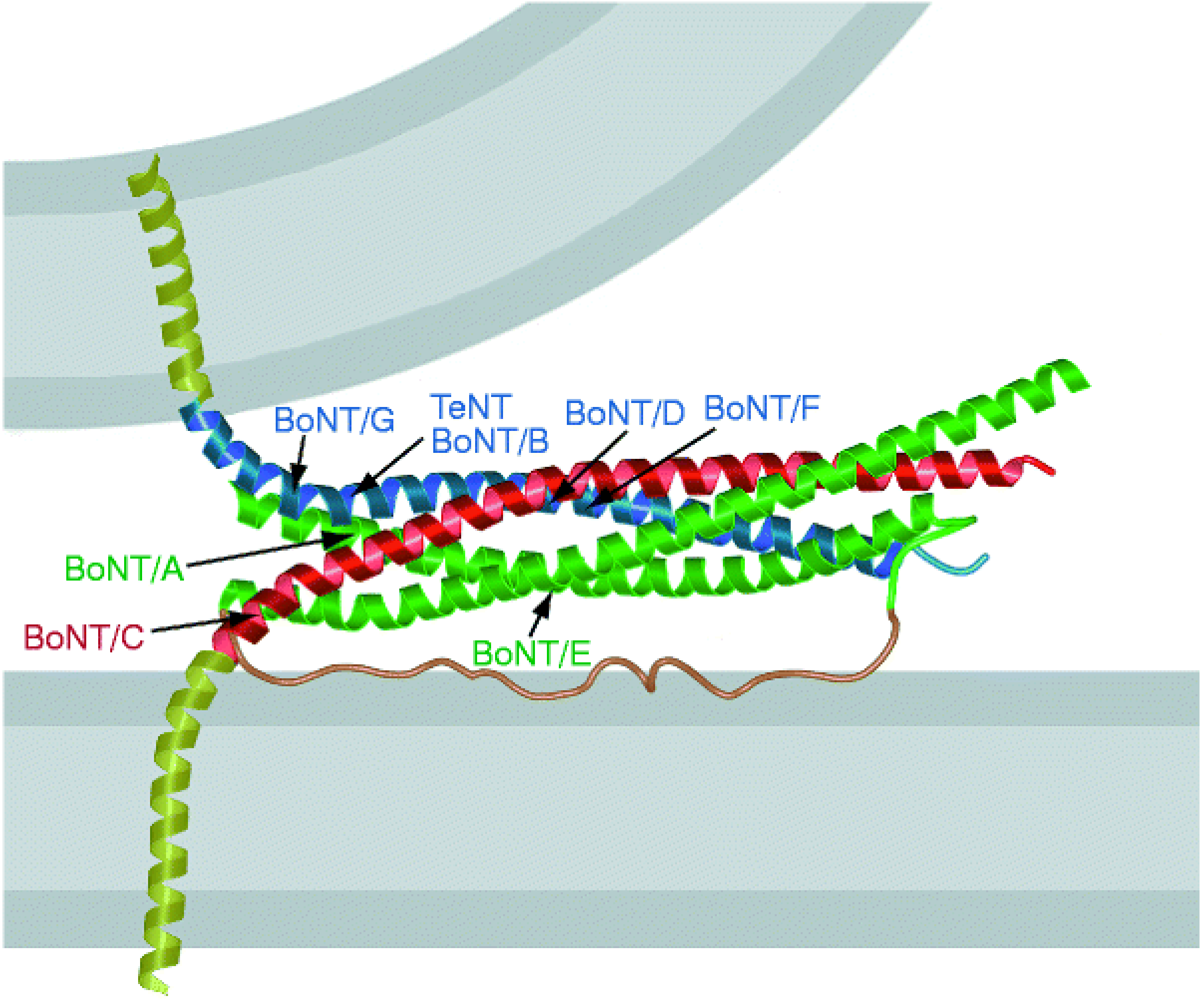
Sutton, et al. *Nature* (1998)

Present on the vesicle and target membranes
Form a parallel four-helix bundle
Have a central role in the membrane fusion machinery



Jahn &Scheller
Nat Rev Mol Cell Biol **(2006)**

SNAREs are cleaved by botulinum and tetanus toxins

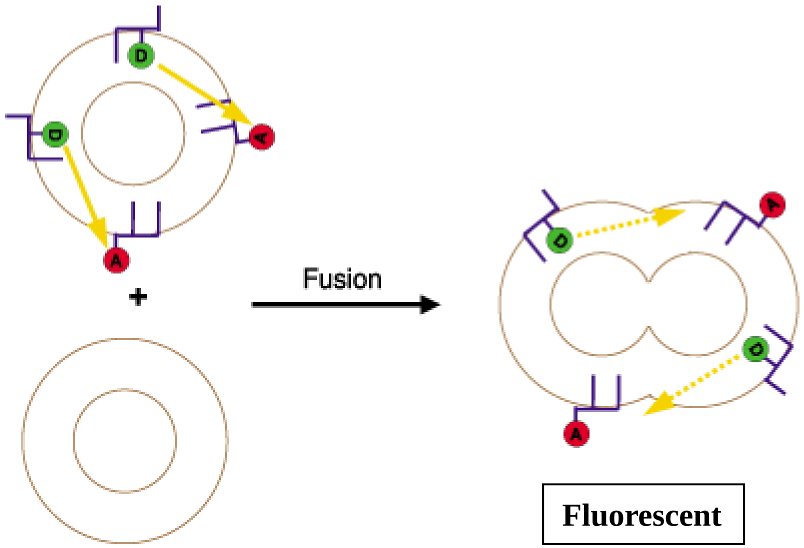


SNARE liposome fusion assay

v-SNARE liposome
NBD phospholipid (D)
Rhodamine phospholipid (A)

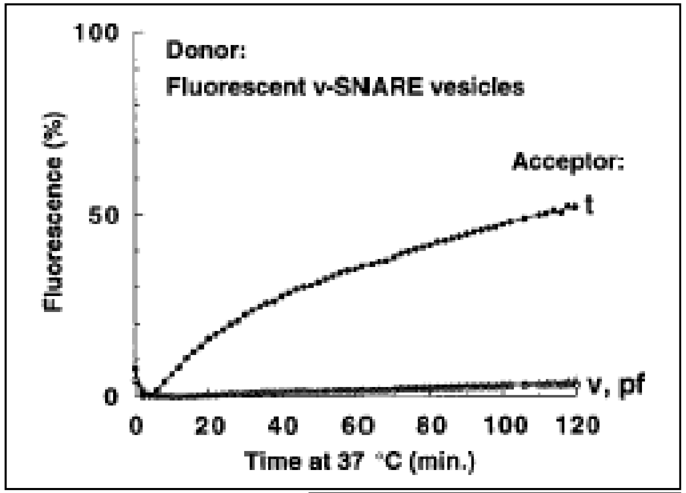
Quenched, not fluorescent

t-SNARE liposome



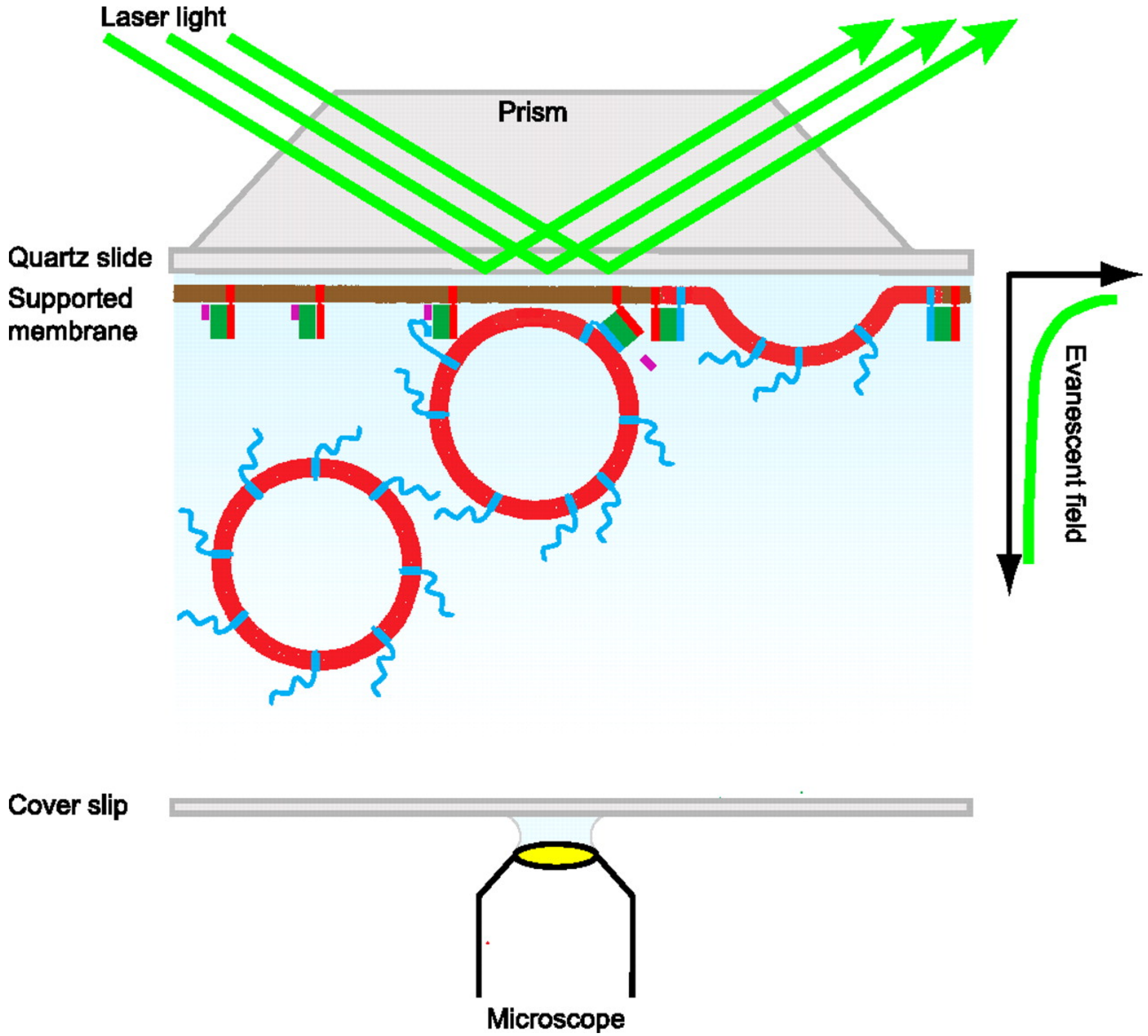
Fluorescent

Molecular Probes:
www.probes.com



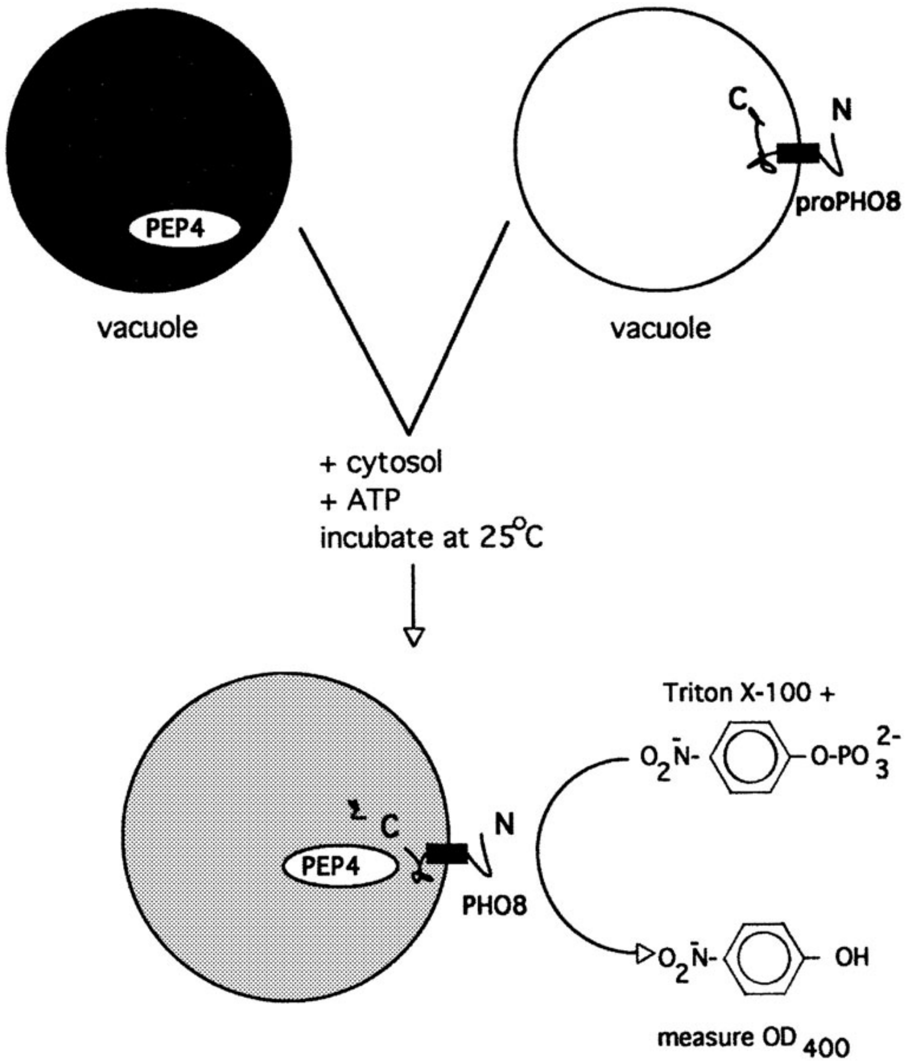
Weber et al., *Cell* (1998)

Diagram of the experimental configuration of the single vesicle fusion assay in supported membranes

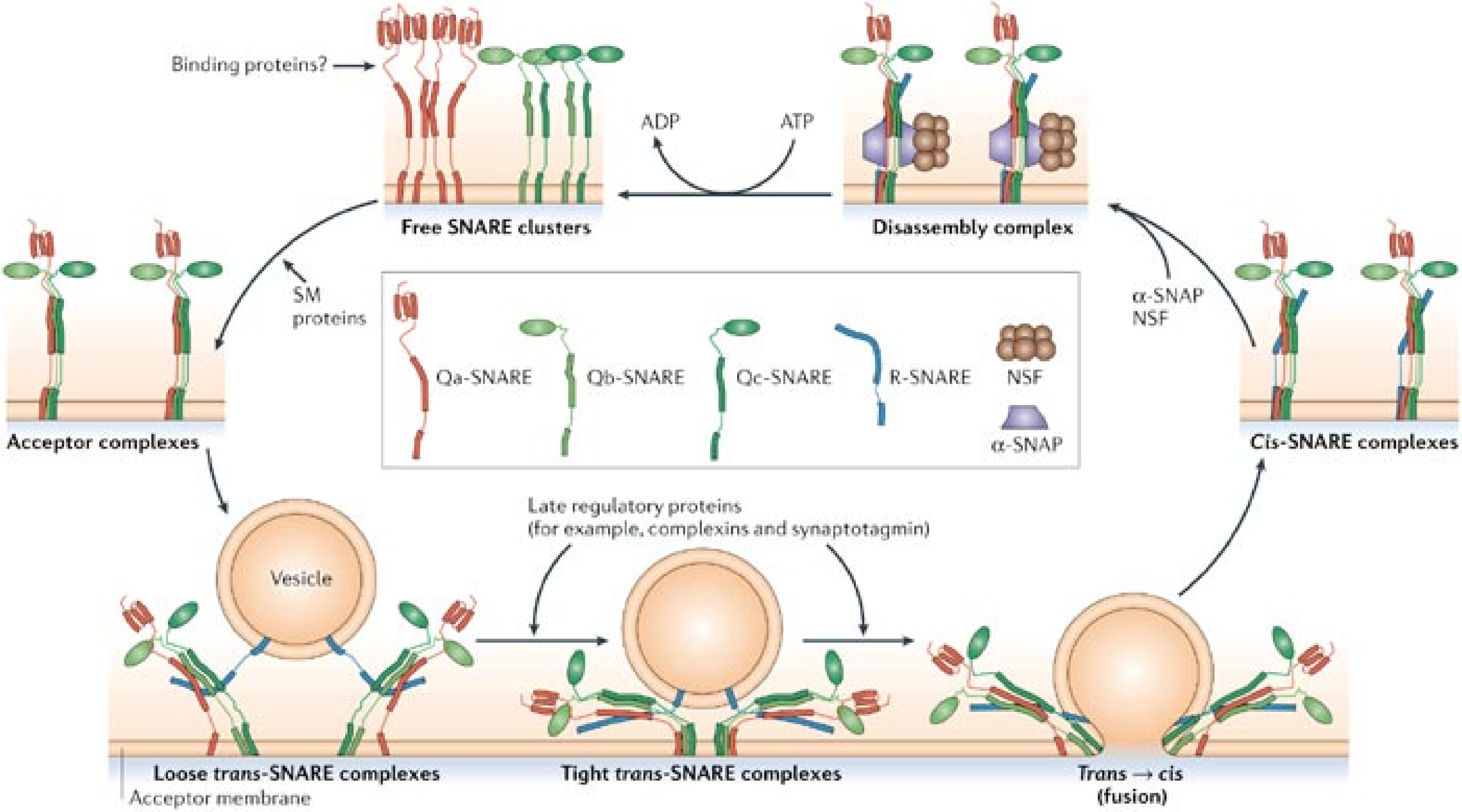


(Domanska M K et al. *J Biol Chem* 2009)

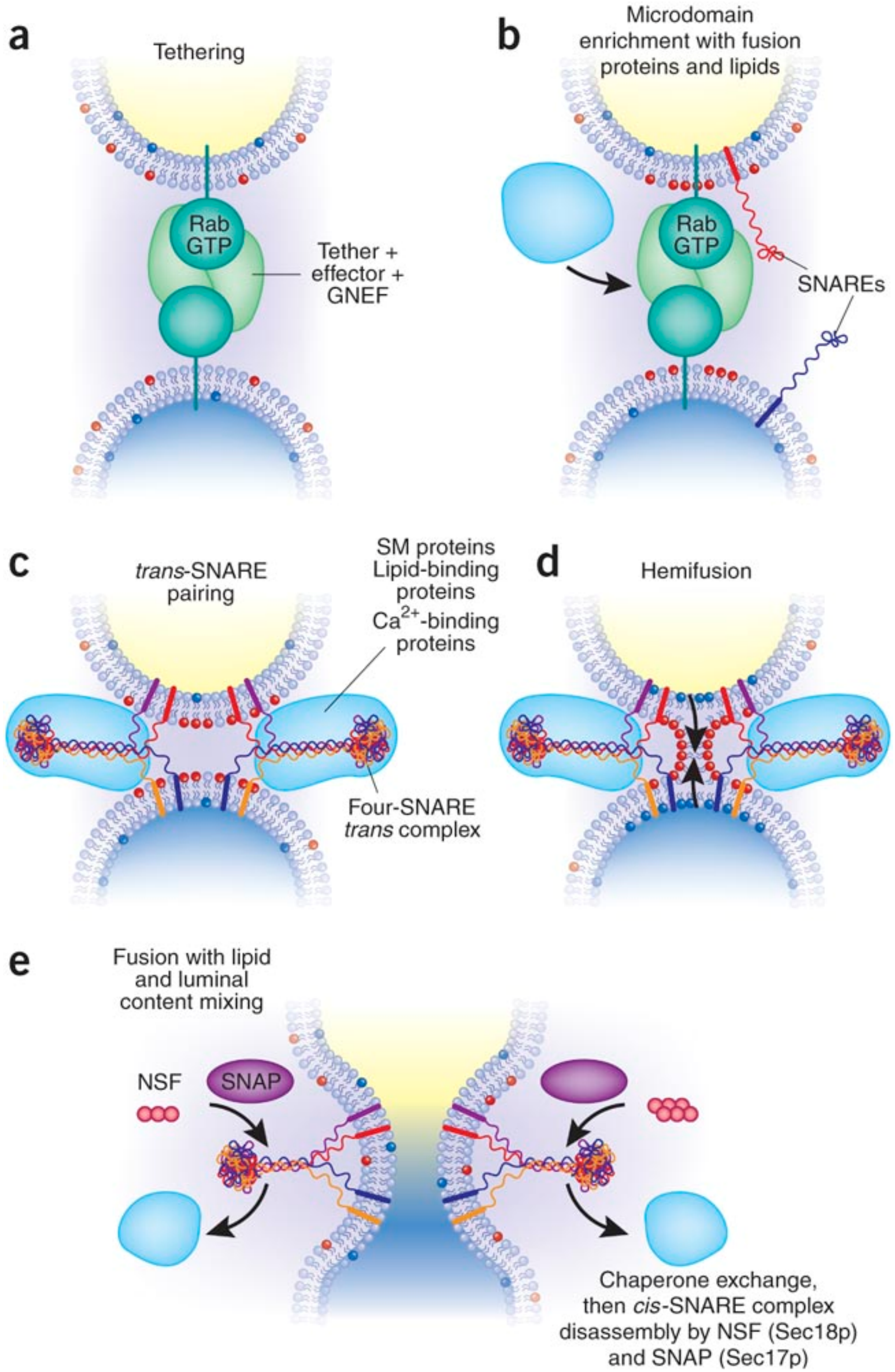
In vitro yeast vacuole homotypic fusion assay



Wickner, *EMBO J* (2002)



Jahn and Scheller *Nat Rev Mol Cell Biol* (2006)



(Wickner & Schekman,
***Nat Str Mol Biol* 2008)**

Proteins essential for vesicle transport

NSF (N-ethylmaleimide Sensitive Factor)

SNAPs (Soluble NSF Attachment Protein)

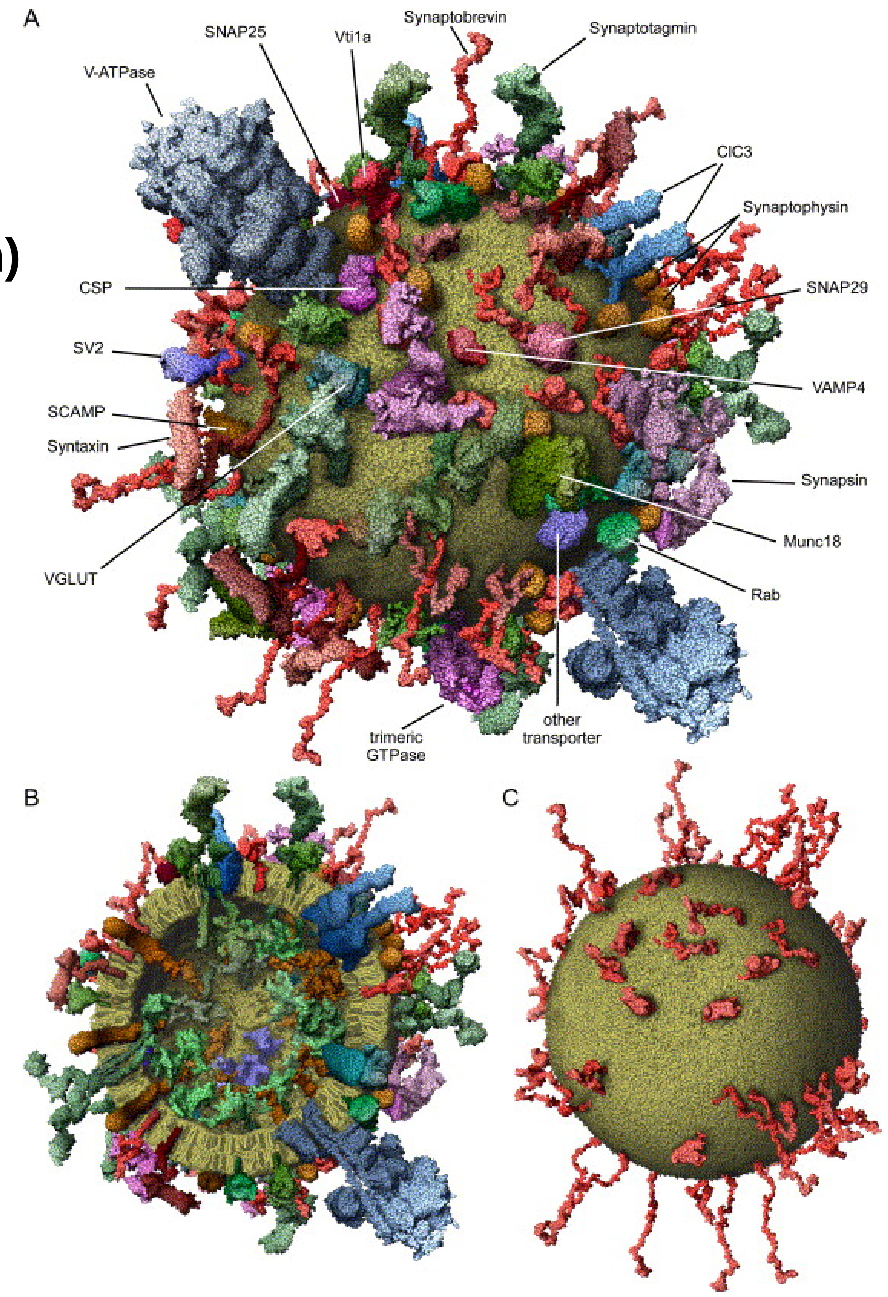
SNAREs (SNAP Receptors)

Coat proteins and adaptors

Sec1p/Munc18 proteins

Rabs and regulatory factors

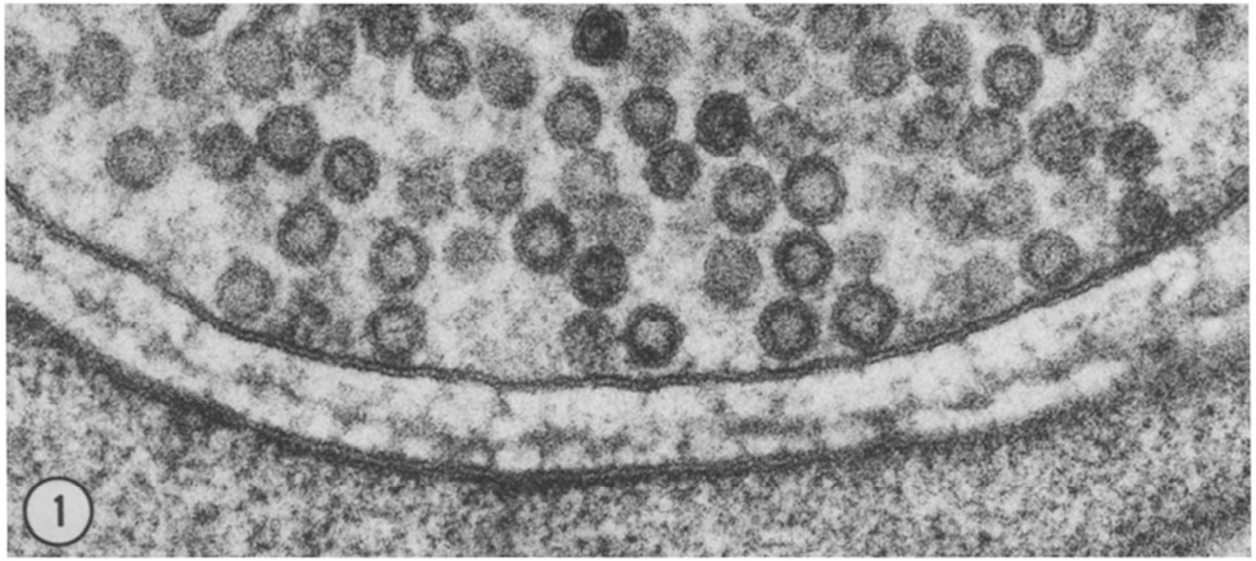
Tethers



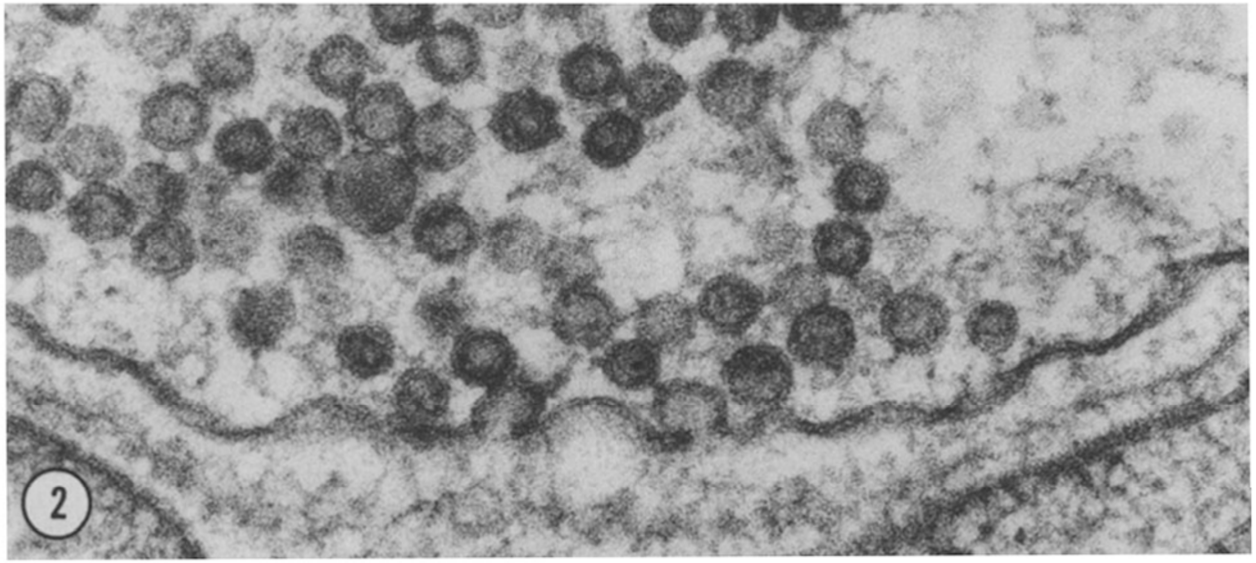
Molecular Model of an Average Synaptic Vesicle
The model is based on space-filling models of all macromolecules at near atomic resolution.
(A) Outside view of a vesicle.
(B) View of a vesicle sectioned in the middle (the dark-colored membrane components represent cholesterol).
(C) Model containing only synaptobrevin to show the surface density of the most abundant vesicle component.

Takamori, et al., *Cell* 2006

Synaptic vesicles fuse with the plasma membrane



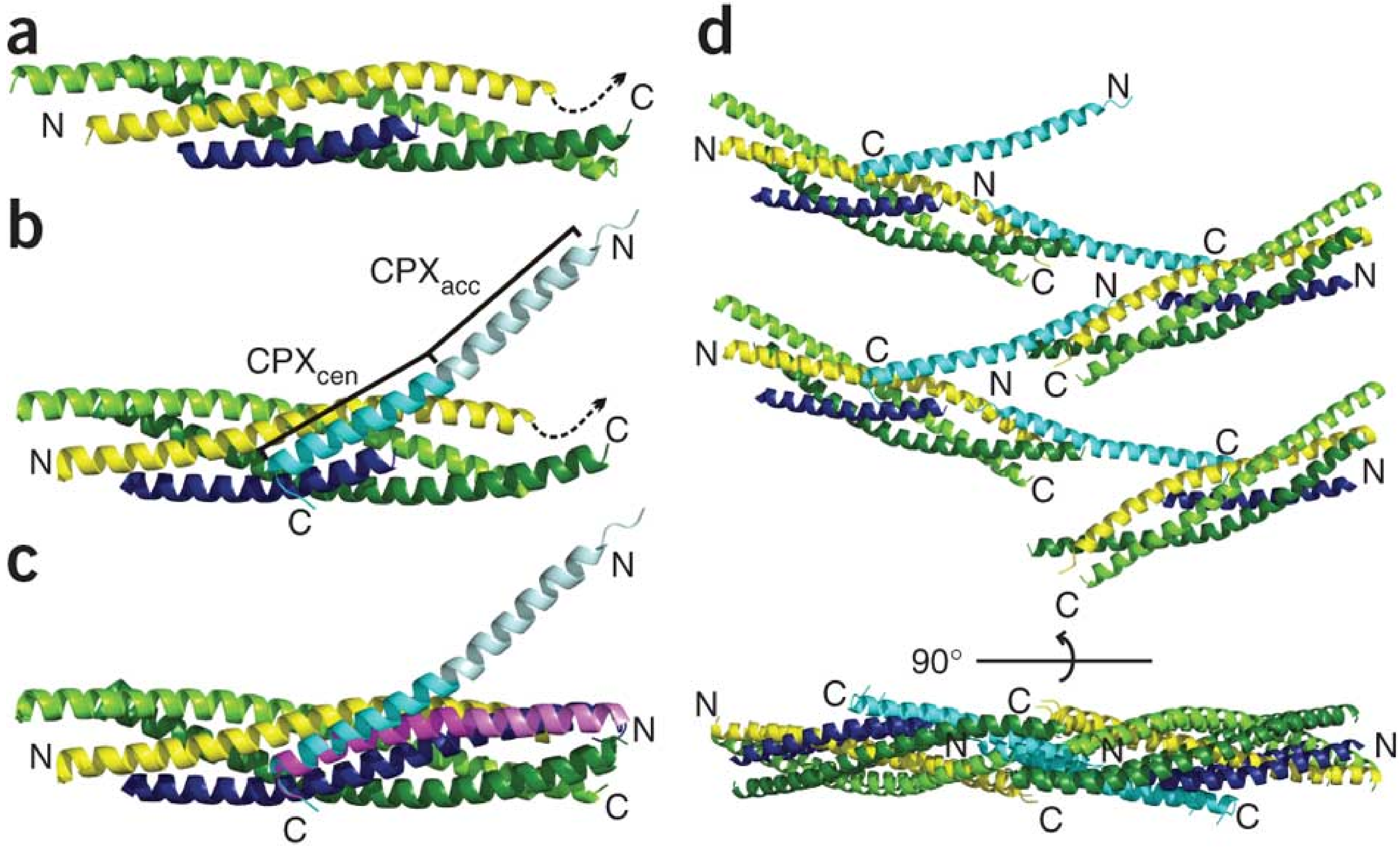
Fixed at rest



Fixed 5ms after stimulation

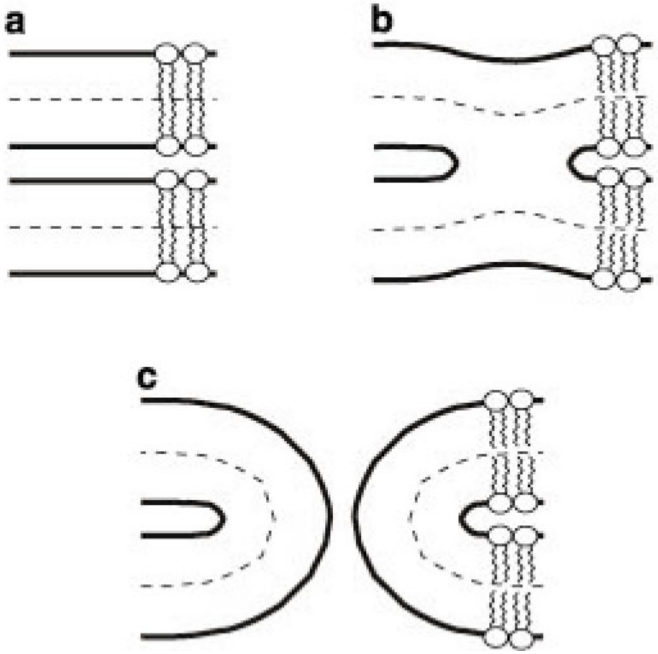
Heuser and Reese, 1981, J. Cell Biol. 88, 564-580

Complexin and Synaptotagmin "clamp and release" to drive *fast* membrane fusion upon Ca^{2+} entry



Nature Struct & Mol Biol **18, 927–933 (2011)**

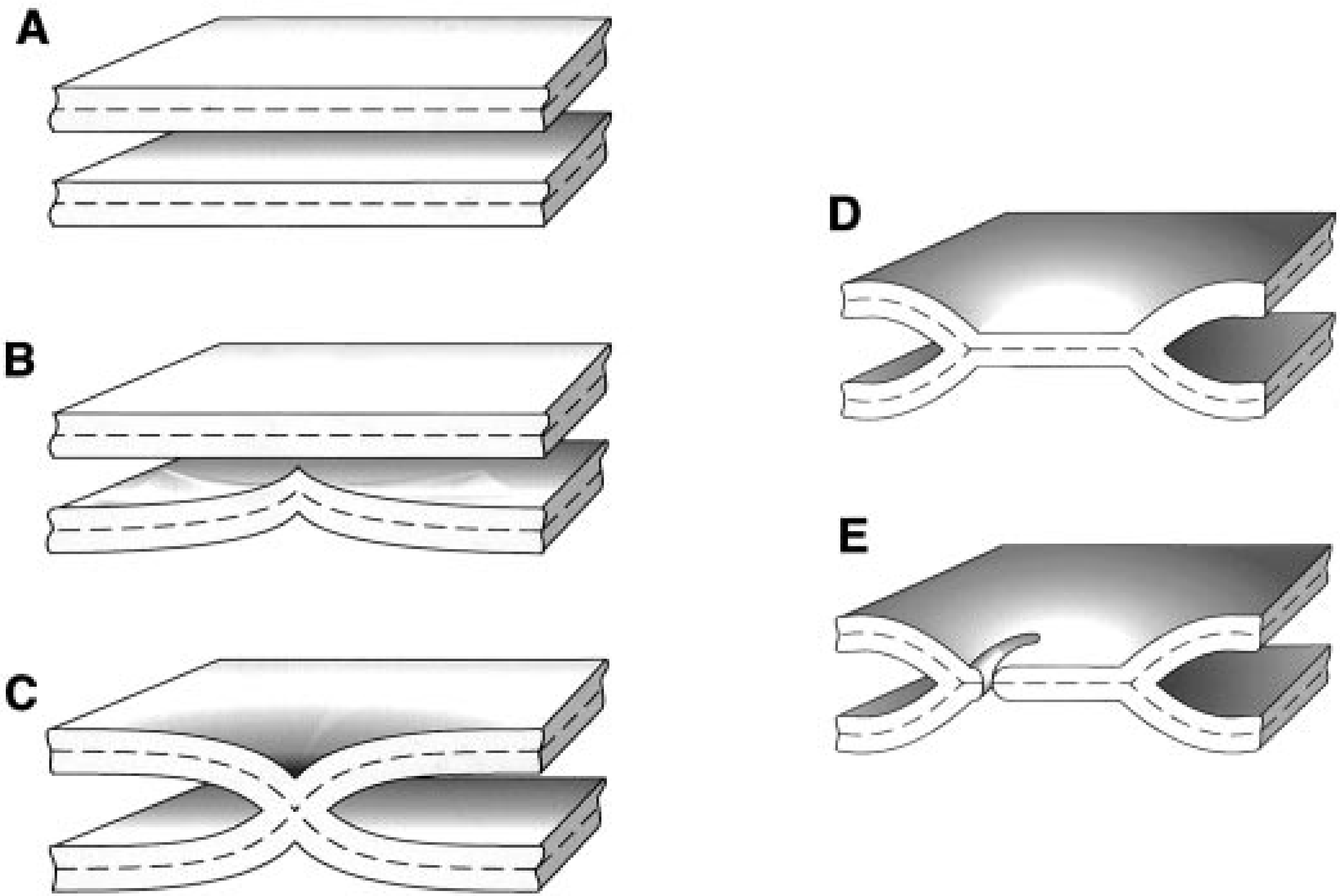
Is close apposition enough to induce membrane fusion?

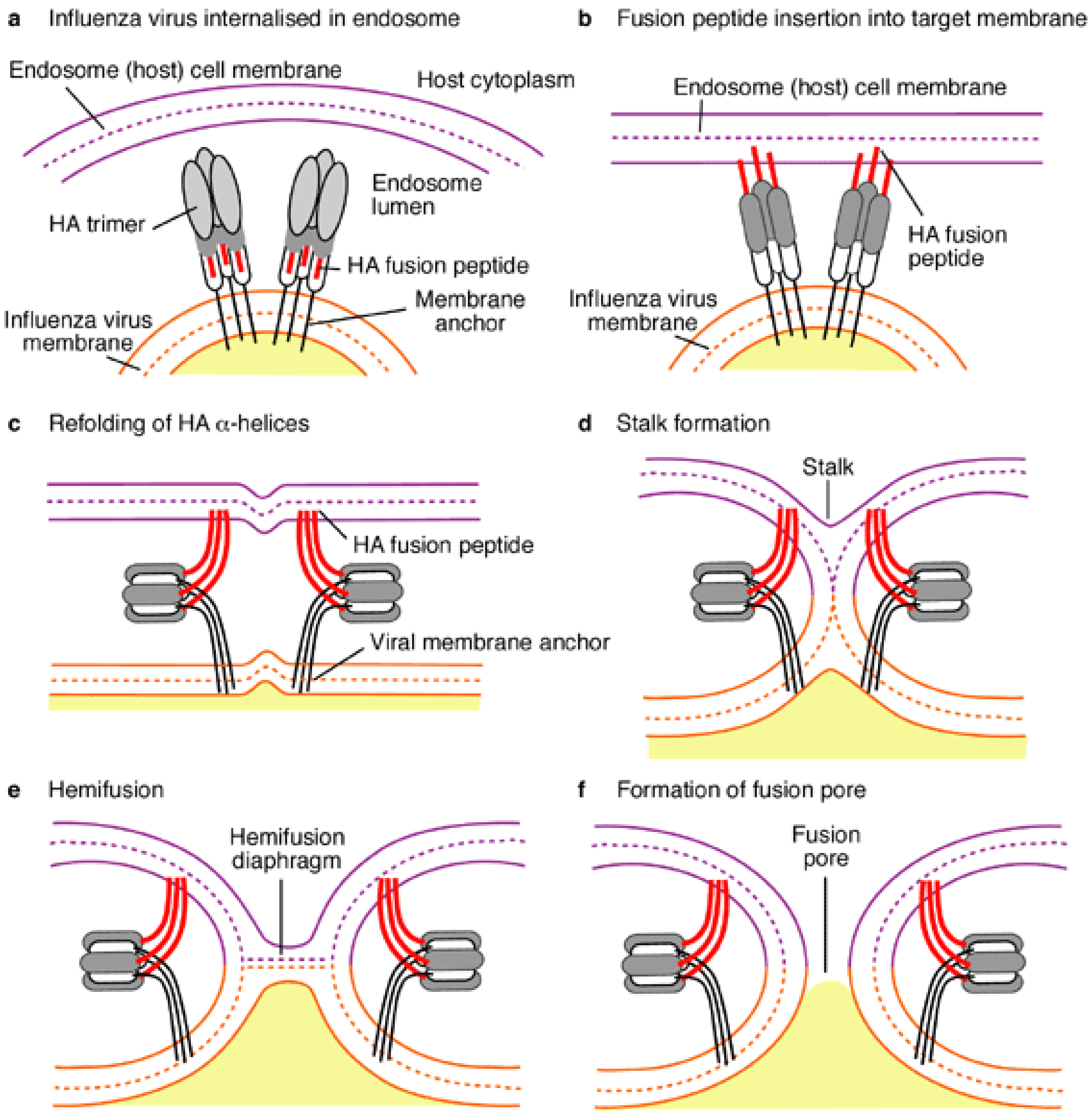


NO

1) Lipid-anchored fusion proteins get stuck at the hemifusion intermediate
2) Insertion of additional residues N-terminal to the transmembrane domains inhibits fusion

Hemifusion: Theory behind the fusion of lipid bilayers





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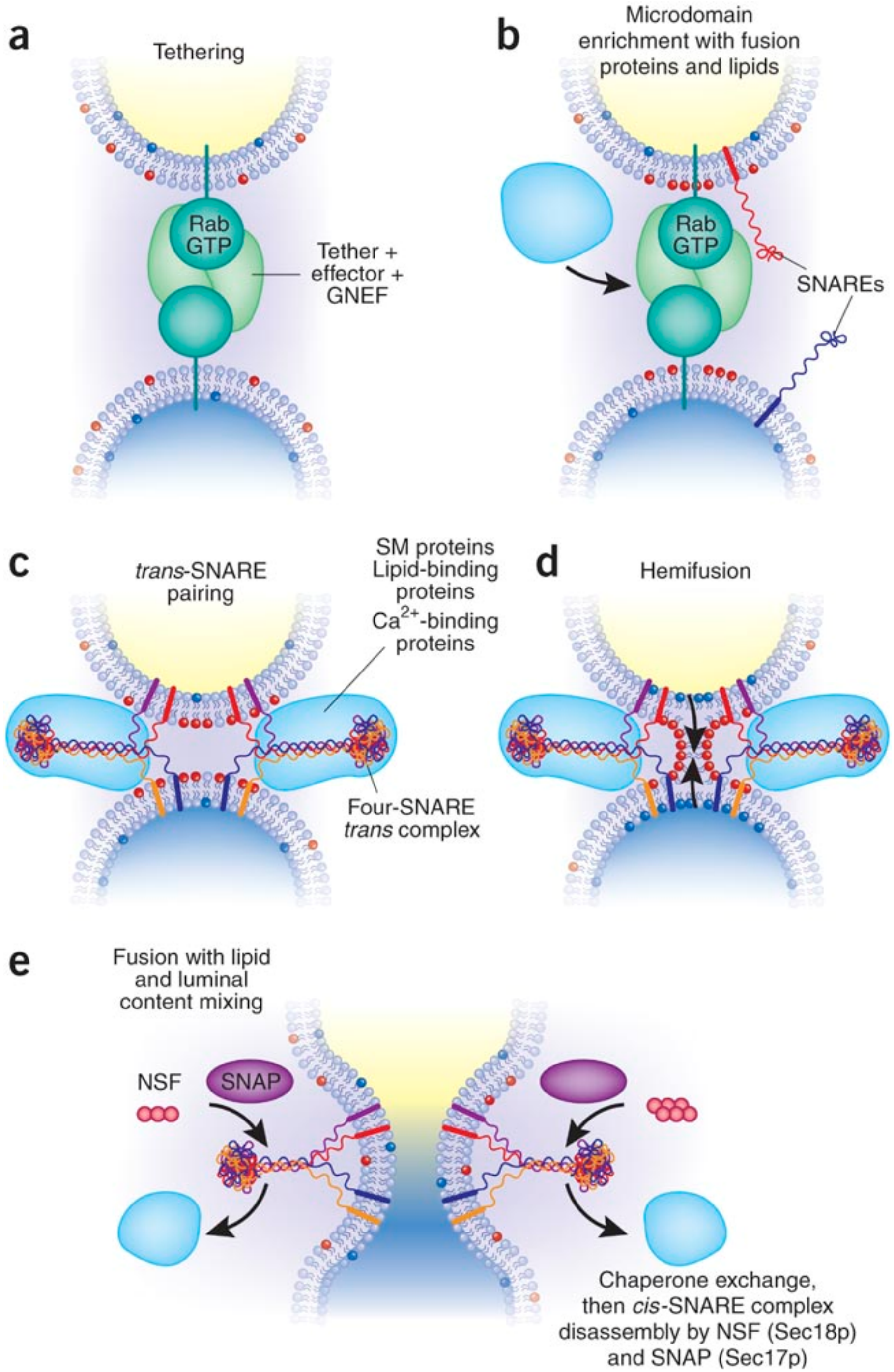
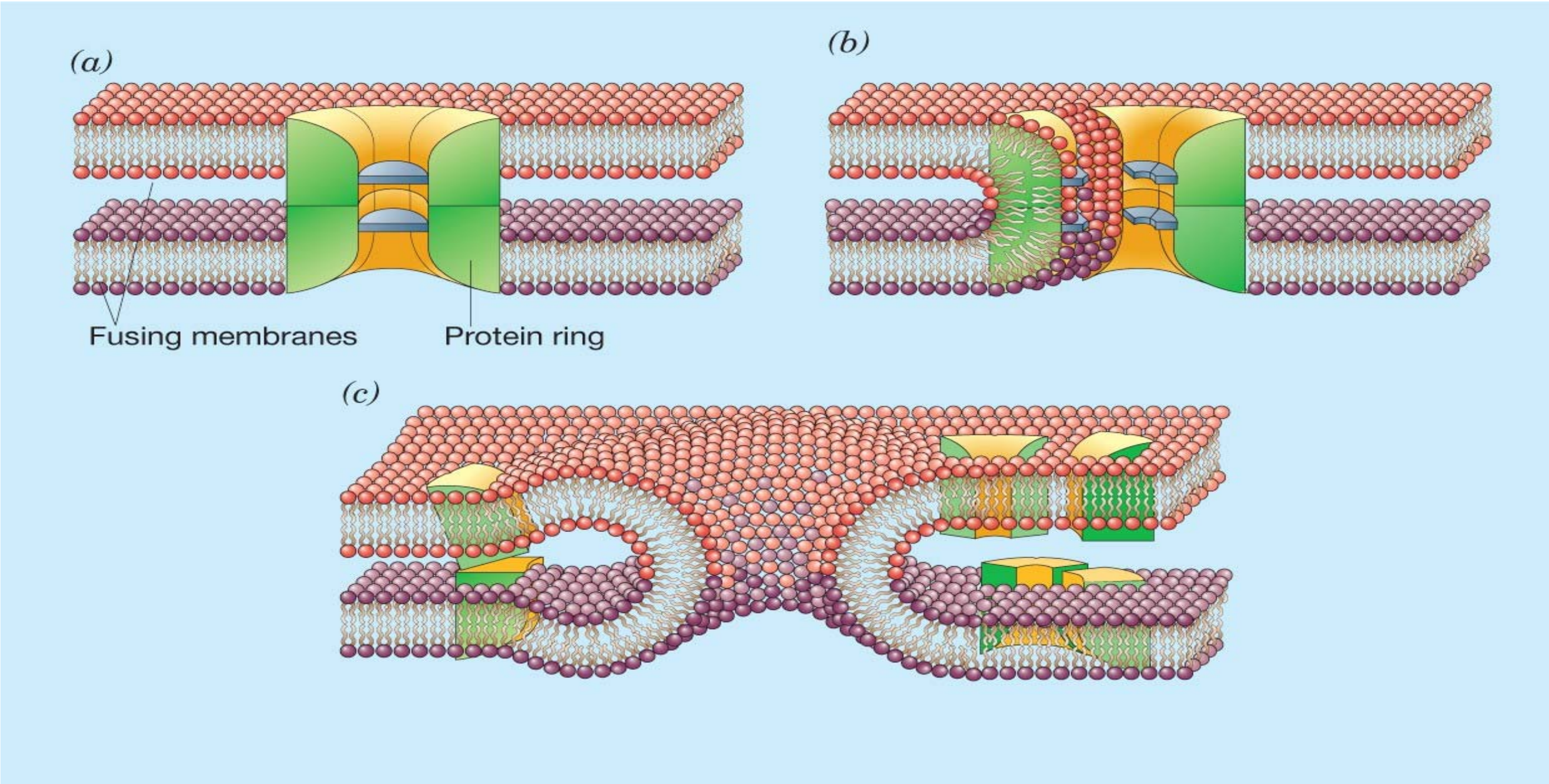
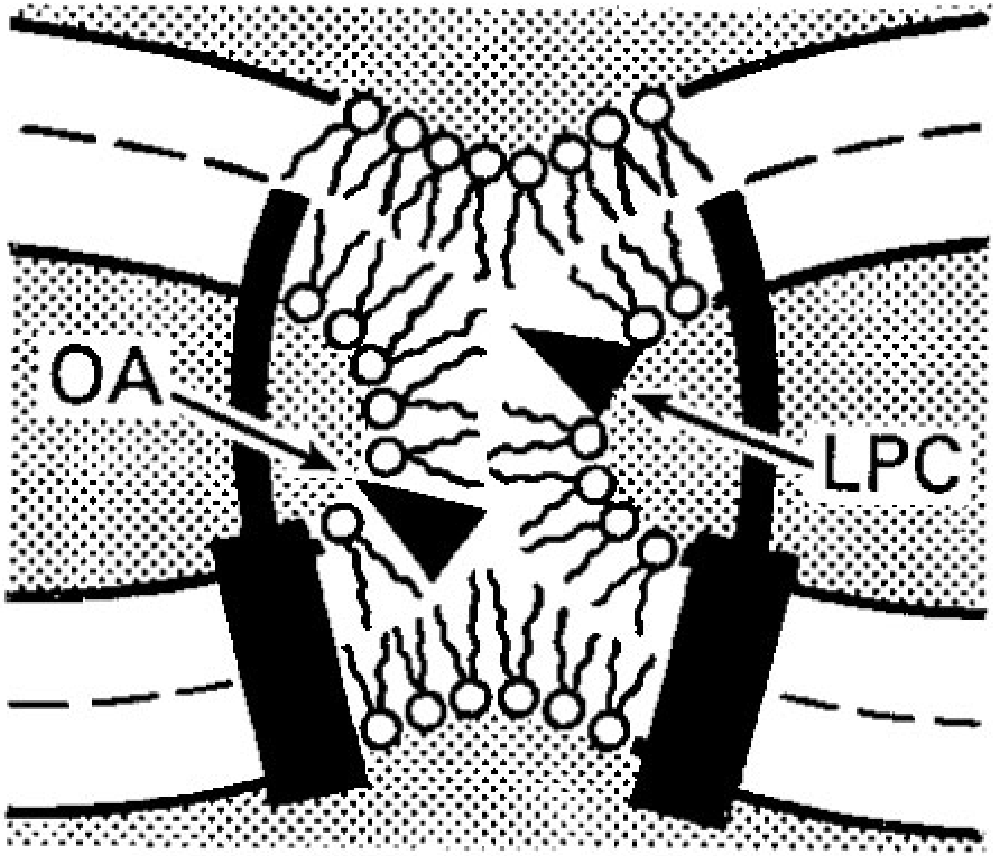


(Wickner & Schekman, *Nat Str Mol Biol* 2008)

Is the fusion intermediate a pore surrounded by fusion proteins, like an ion channel?



The stalk intermediate is sensitive to lipids of a specific shape



Leaflet:

cytoplasmic

extracellular

extraviral

viral lumen

Oleic Acid (cone)

Lysophosphatidyl Choline (inverted cone)

(Chernomordik et al., 1997)

Packing preference is determined by lipid shape

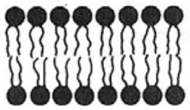
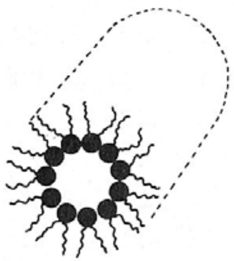
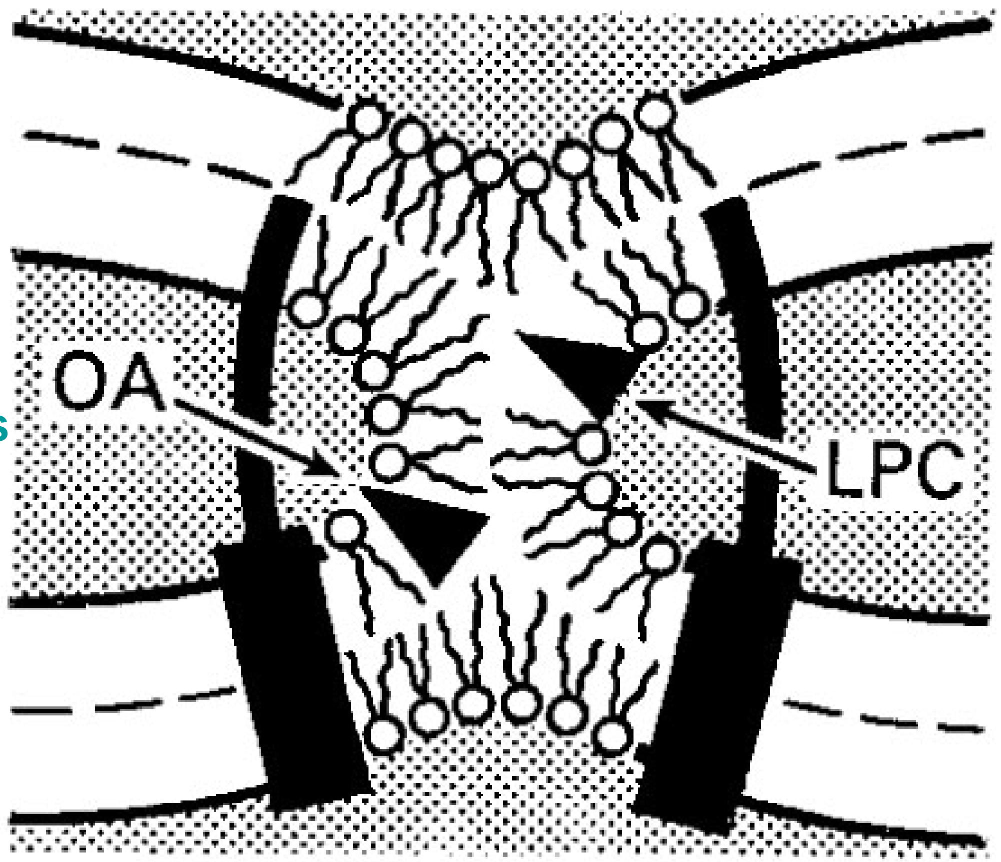
LIPID	PHASE	MOLECULAR SHAPE	CRITICAL PACKING PARAMETER (v/ℓ S.)
Lysophospholipids Detergents	 Micellar	 Inverted Cone	<⅓ (Sphere) LPC ⅓ to ½ (Globular Shapes; Rods)
Phosphatidylcholine Sphingomyelin Phosphatidylserine Phosphatidylinositol Phosphatidylglycerol Phosphatidic Acid Cardiolipin Digalactosyldiglyceride	 Bilayer	 Cylindrical	½ to 1
Phosphatidylethanolamine (Unsaturated) Cardiolipin - Ca^{2+} Phosphatidic Acid - Ca^{2+} (pH < 6.0) Phosphatidic Acid (pH < 3.0) Phosphatidylserine (pH < 4.0) Monogalactosyldiglyceride	 Hexagonal (H_{II})	 Cone	OA >1

Figure 2.18. Polymorphic phases, molecular shapes, and the critical packing parameter for some membrane lipids. Adapted from ref. 263. Drawing kindly provided by Dr. P. Cullis and Dr. M. Hope.

The stalk intermediate is sensitive to lipids of a specific shape



Summary of membrane fusion:

1. **Remodeling membranes during fusion requires a stalk-like lipid intermediate that is sensitive to lipid shape**

2. **Membrane-fusion proteins undergo a conformational change that results in transmembrane domains inserting into both of the fusing membranes**

3. **In their fusogenic conformation, viral and cellular fusion proteins pierce and pull membranes together as the proteins refold into α-helical bundles between membranes**

4. **The fusion pore is not a proteinaceous channel, but more likely a tear around the perimeter of the attached membranes**