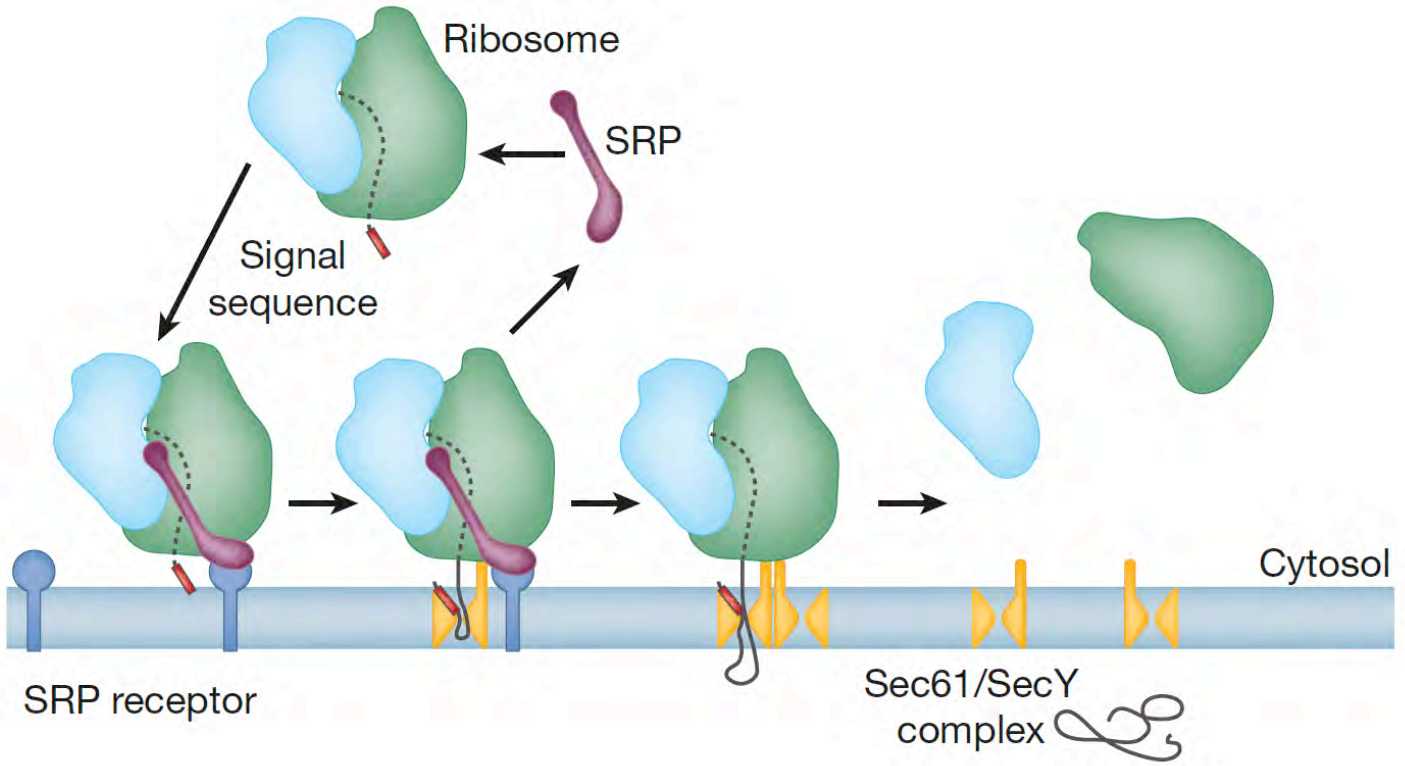
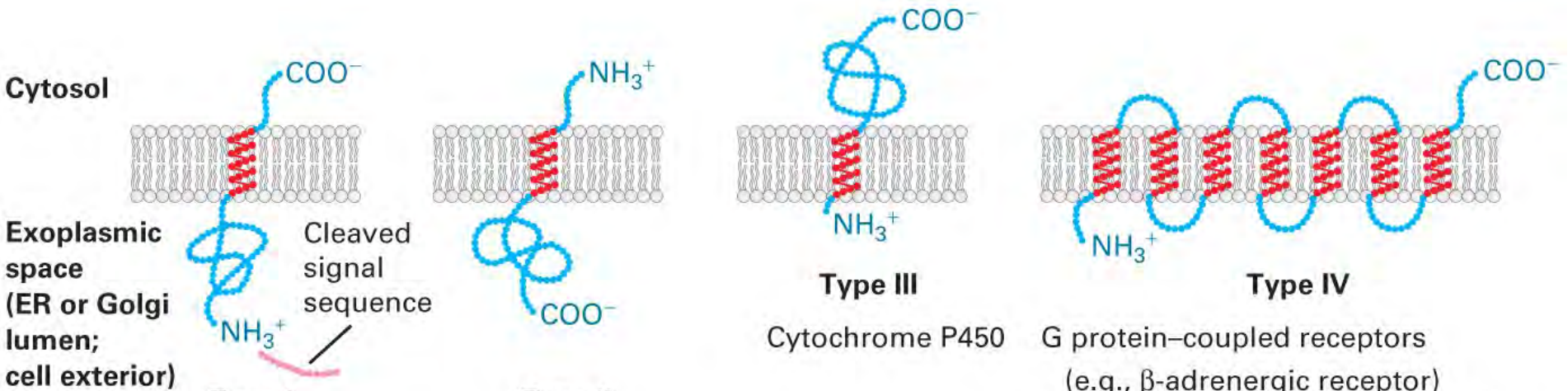
Co-translational import into the ER

- signal sequence recognized by SRP
- translational arrest
- targeting to membrane: SRP-SRP receptor; then ribosome and Sec61
- translational elongation: exiting peptide goes through channel



Rapoport (2007) Nature

Topologies of some integral membrane proteins synthesized on the rough ER



Type I

Glycophorin
LDL receptor
Influenza HA protein
Insulin receptor
Growth hormone receptor

N-out
C-in

Type II

Asialoglycoprotein receptor
Transferrin receptor
Sucrase–isomaltase precursor
Golgi galactosyltransferase
Golgi sialyltransferase
Influenza HN protein

N-in
C-out
single signal-anchor

Type III

Cytochrome P450

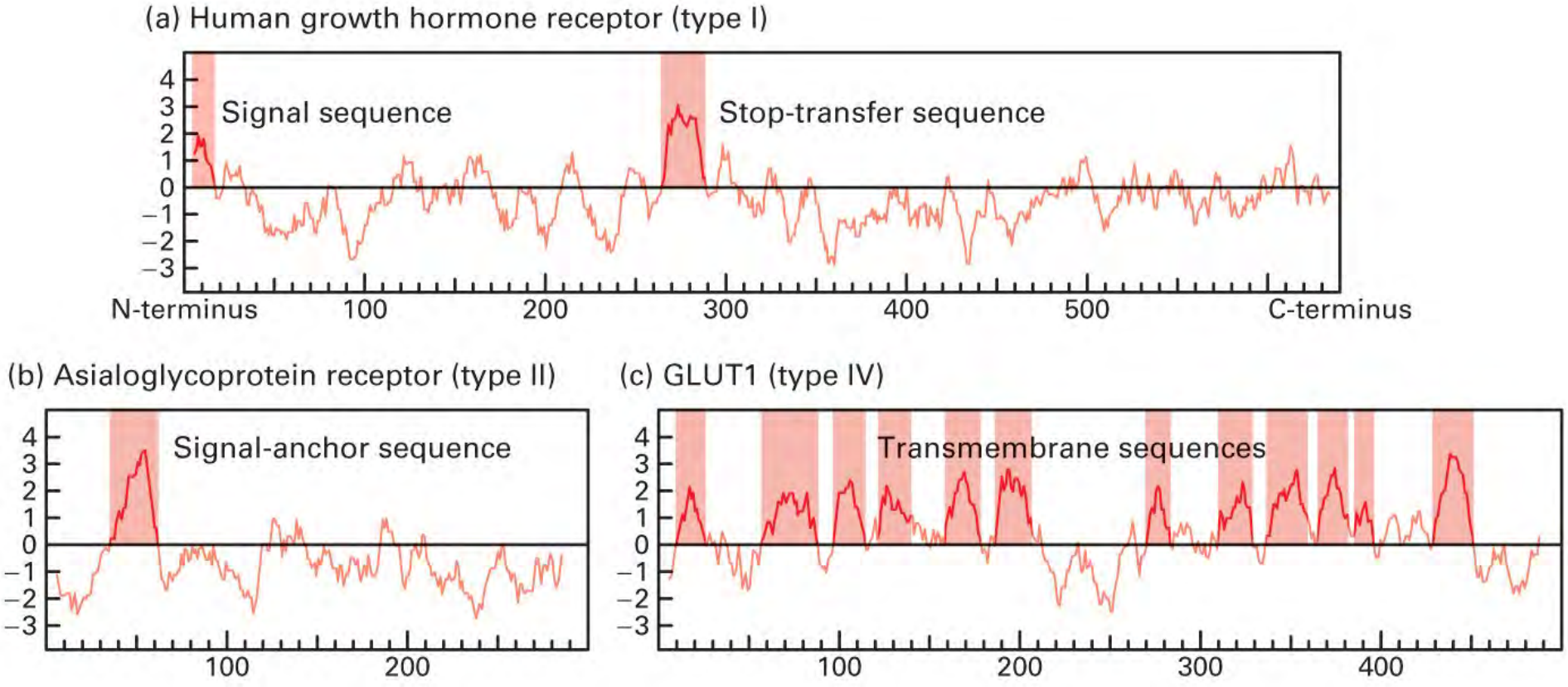
N-out
C-in
single signal-anchor

Type IV

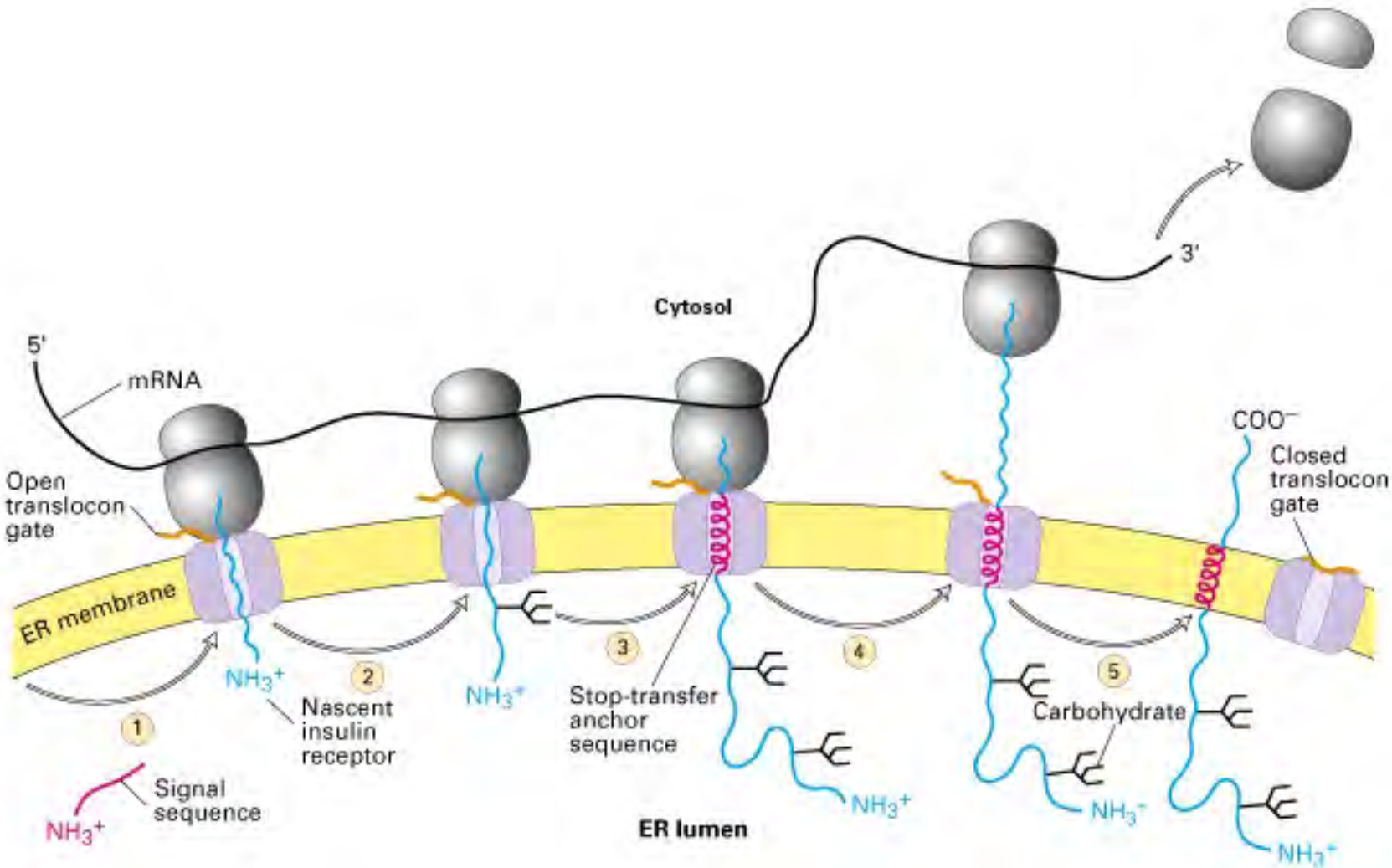
G protein–coupled receptors (e.g., β-adrenergic receptor)
Glucose transporters (e.g., GLUT1)
Voltage-gated Ca_2^+ channels
ABC small molecule pumps
CFTR (Cl^-) channel
Sec61
Connexin

N-term start-transfer
internal stop-transfer anchor

Lodish et al, 2004

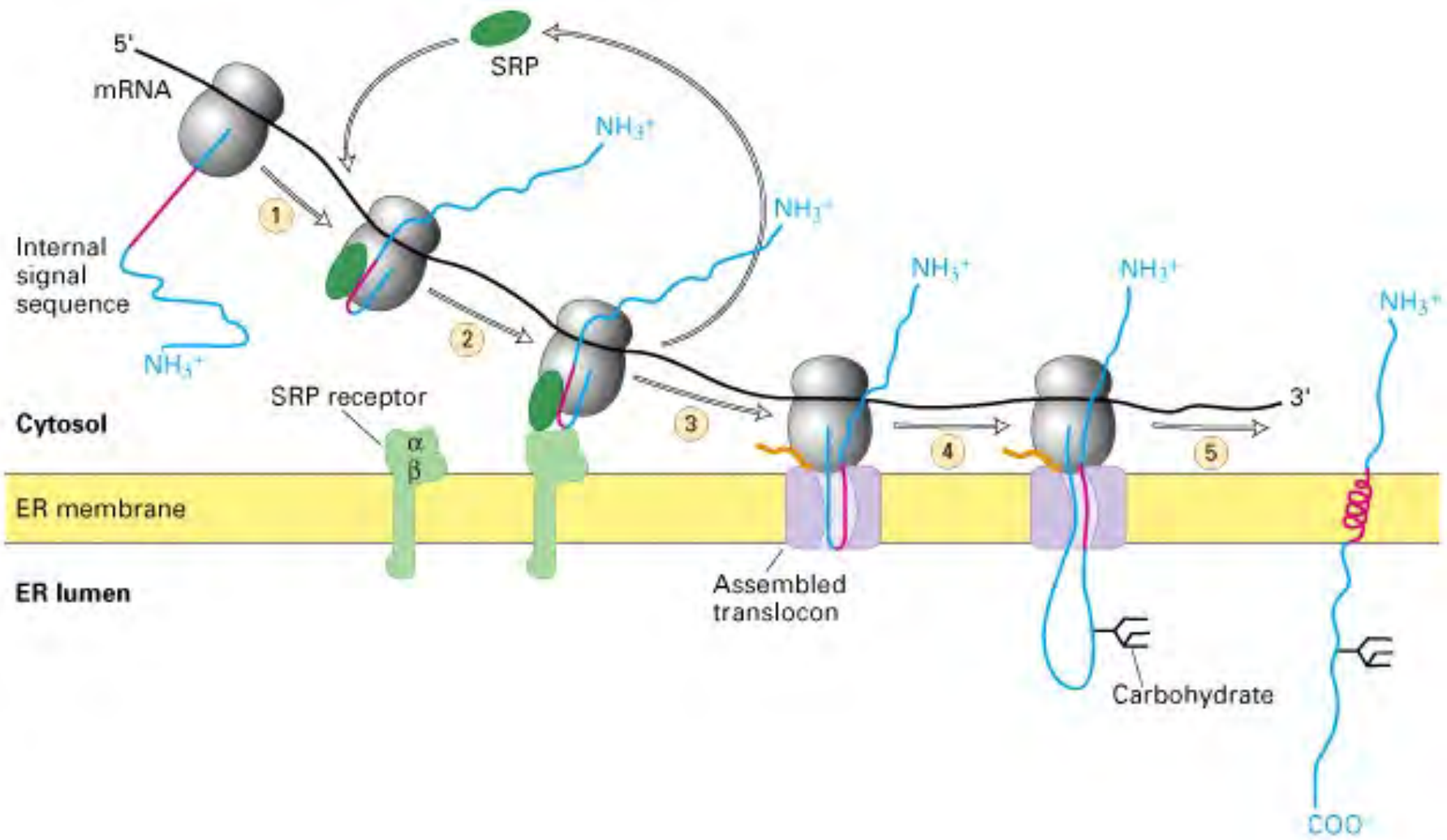

(a) Human growth hormone receptor (type I)
Signal sequence
Stop-transfer sequence
4
3
2
1
0
−1
−2
−3
N-terminus
100
200
300
400
500
C-terminus
(b) Asialoglycoprotein receptor (type II)
Signal-anchor sequence
100
200
(c) GLUT1 (type IV)
Transmembrane sequences
100
200
300
400

Most transmembrane proteins (Type I) have an N-terminal signal sequence and an internal topogenic sequence



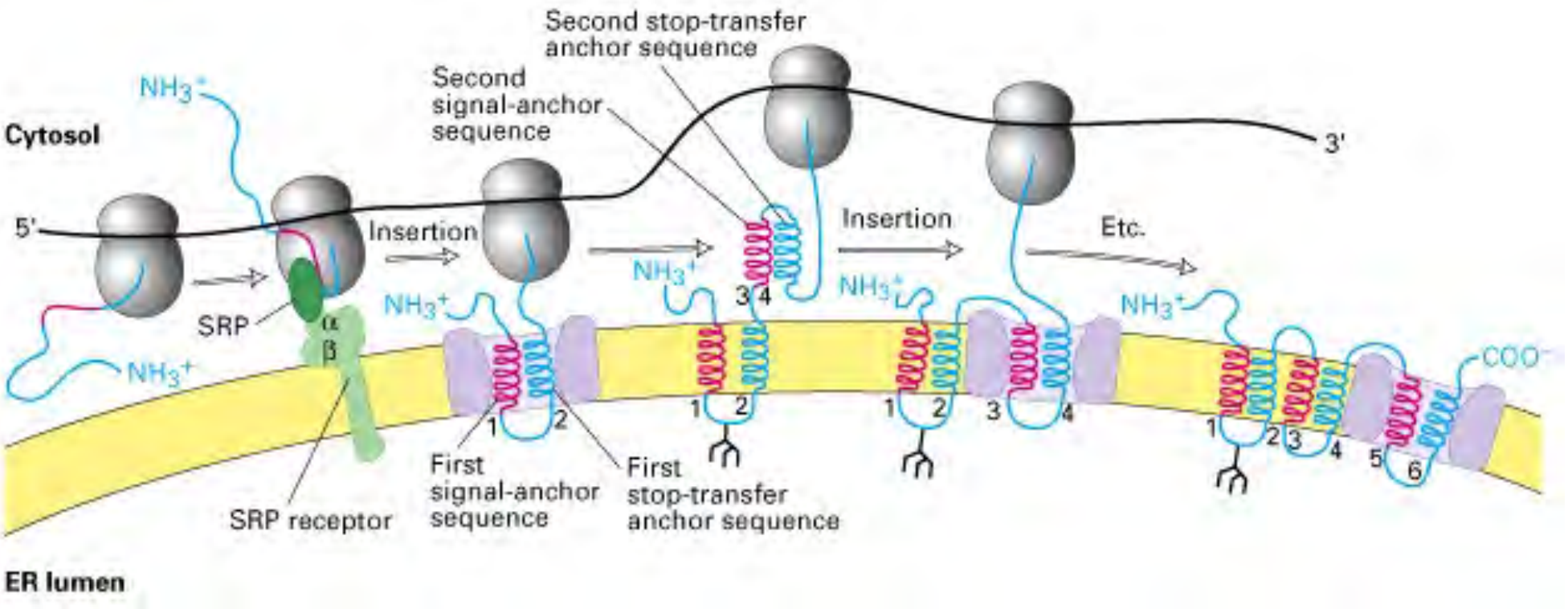
Lodish et al, 2000

A single internal topogenic sequence directs insertion of some single-pass transmembrane proteins (Type II)



Lodish et al, 2000

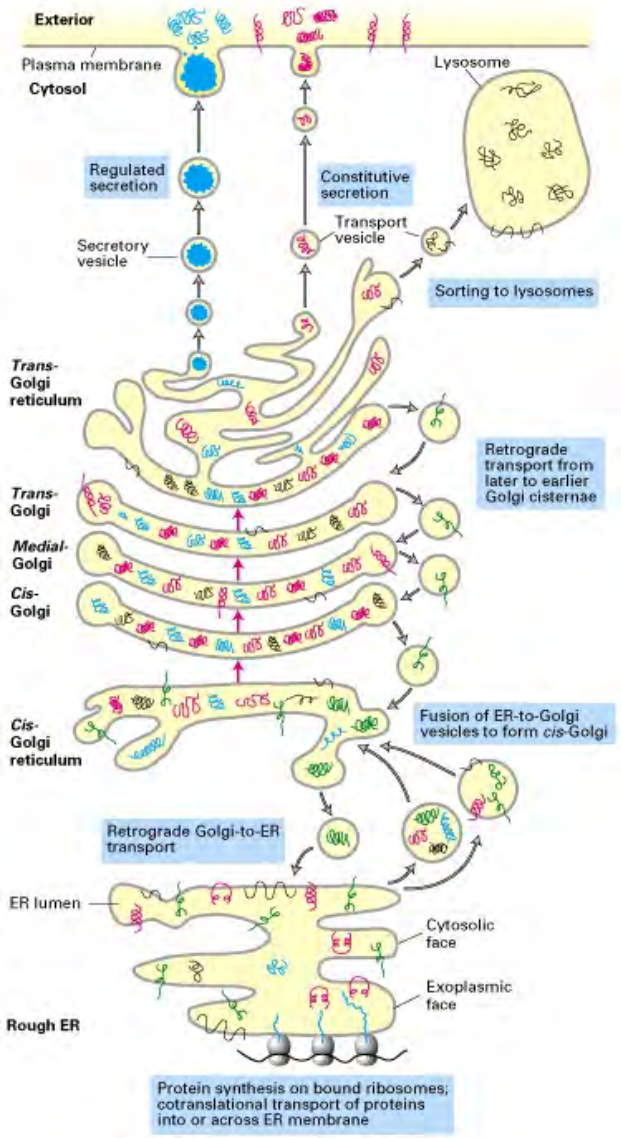
Multipass transmembrane proteins have multiple topogenic sequences



Lodish et al, 2000

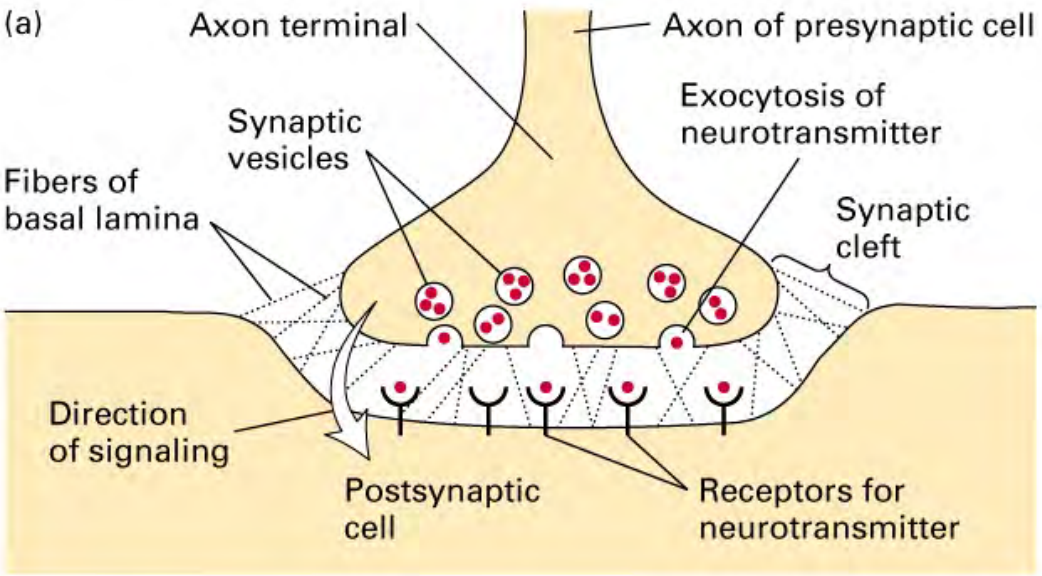
Overview of the secretory pathway

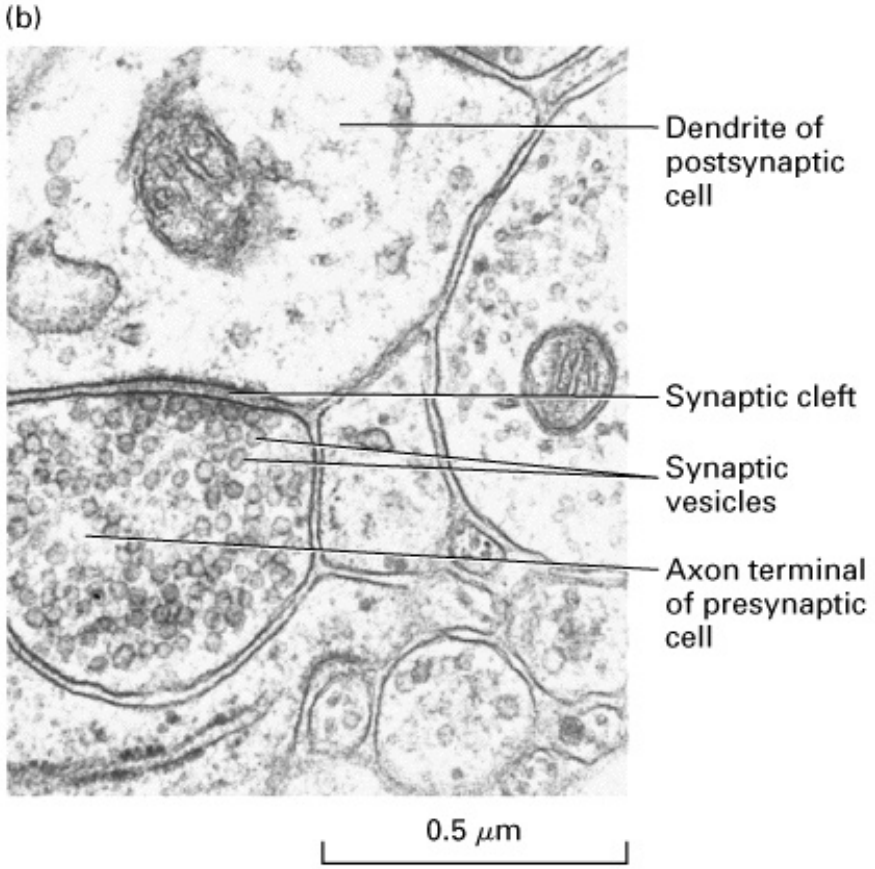
- How are vesicles bud from donor membranes?
- What is the core fusion machinery?
- What is the mechanism of vesicle fusion?



Lodish et al, 2000

Regulated exocytosis underlies neural communication





Lodish et al, 2000

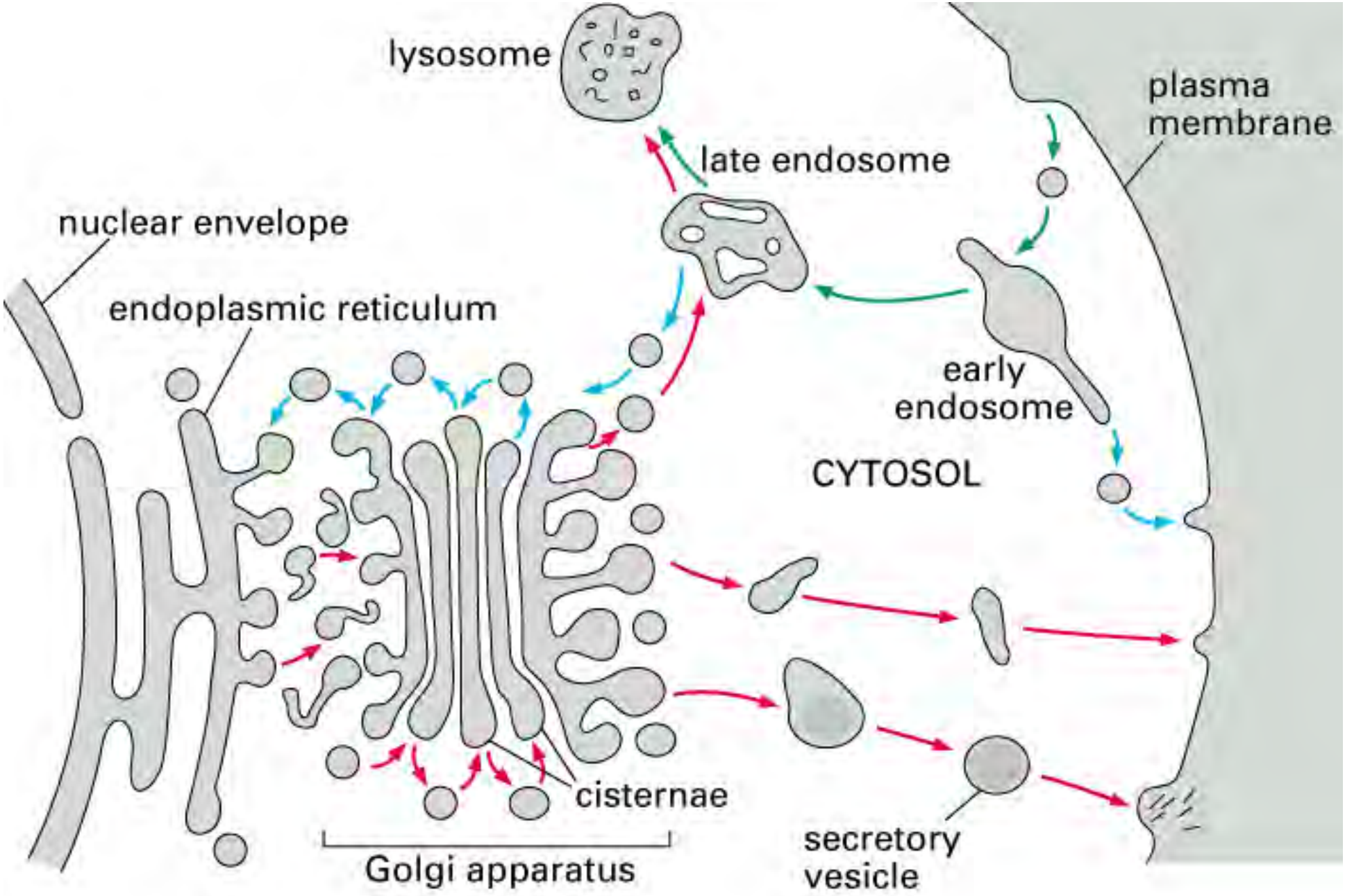
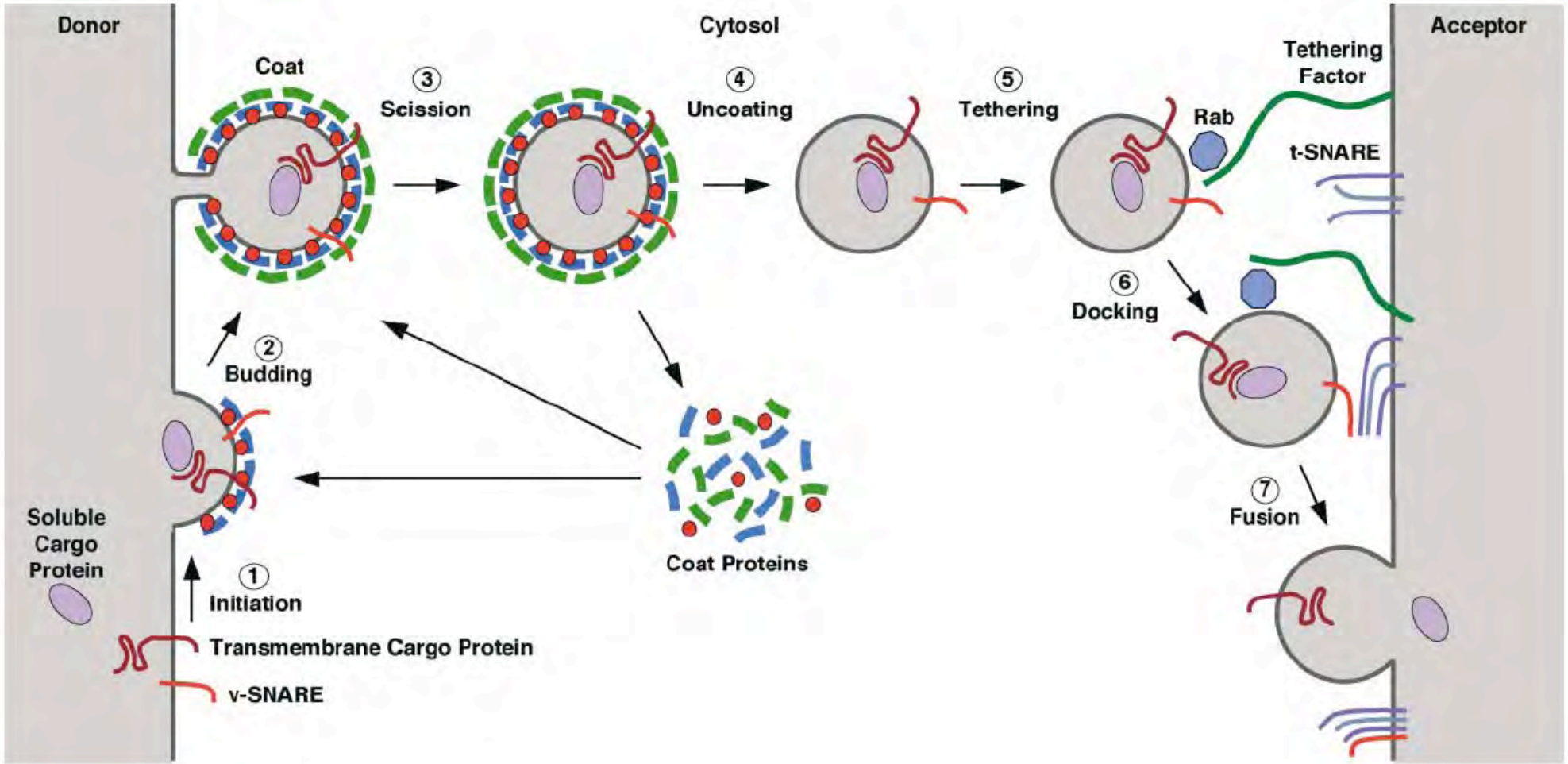


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

Vesicle budding and fusion



Bonifacino and Glick (2004) Cell

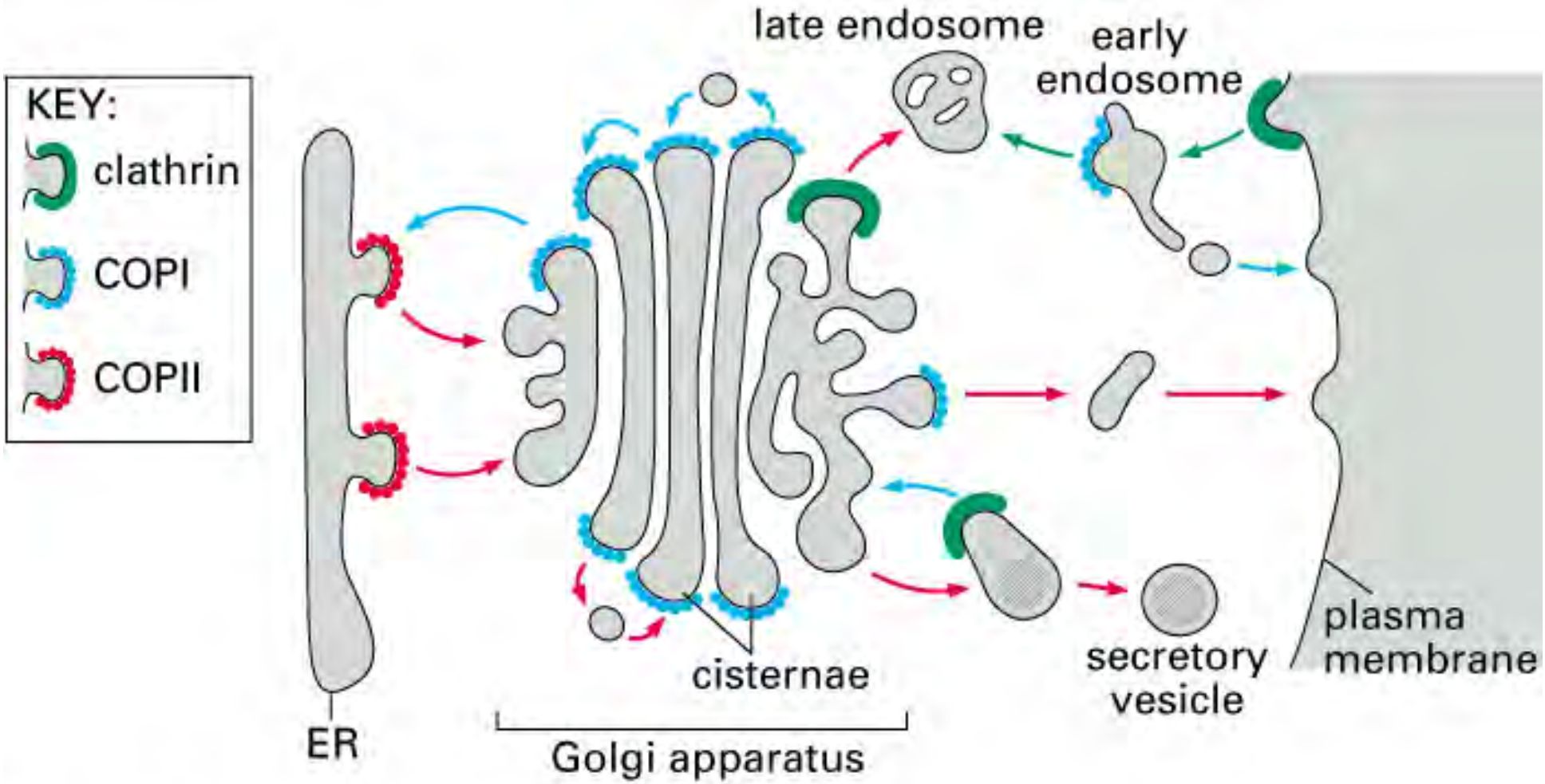


Figure 13–5. Molecular Biology of the Cell, 4th Edition.

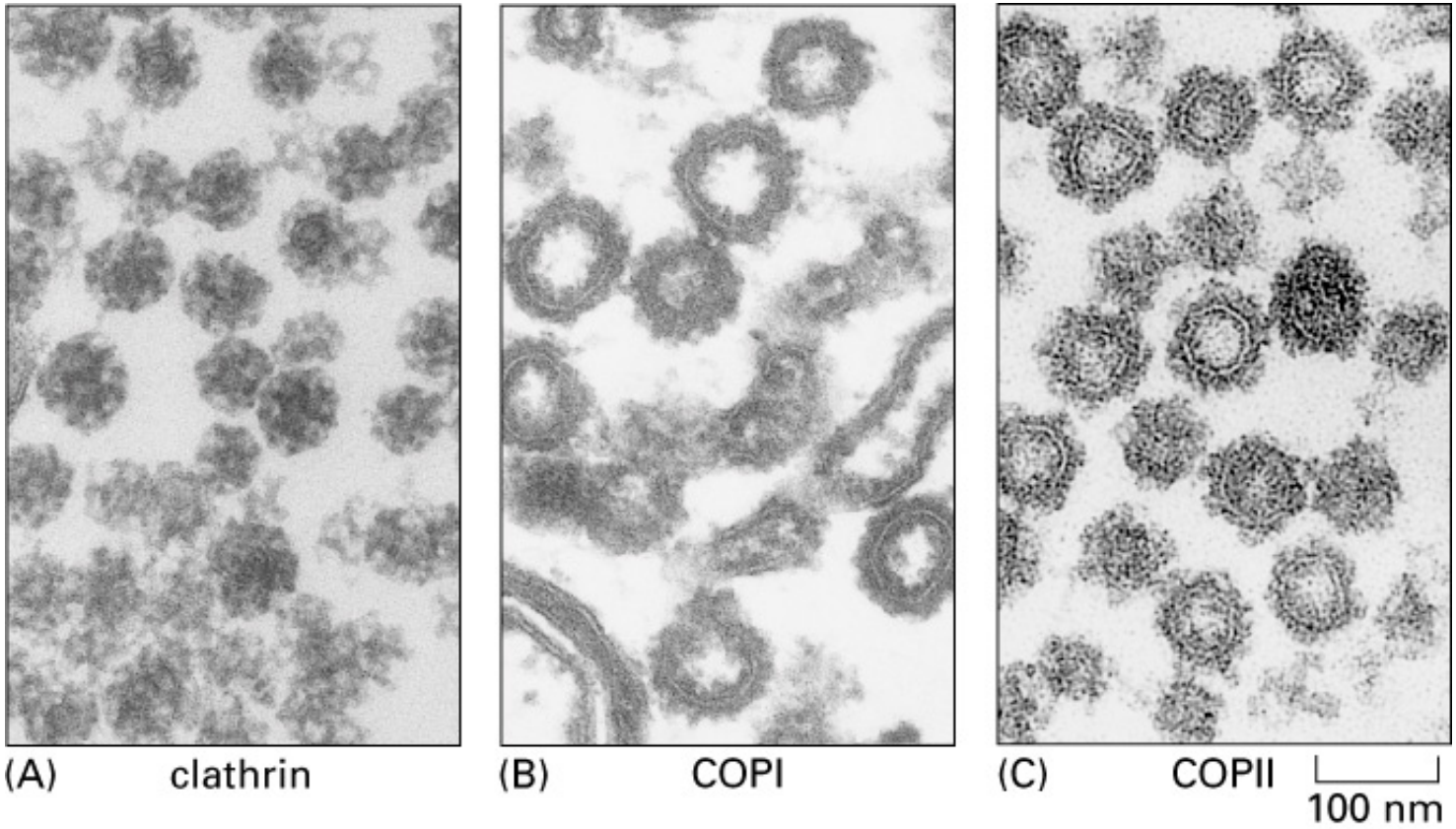
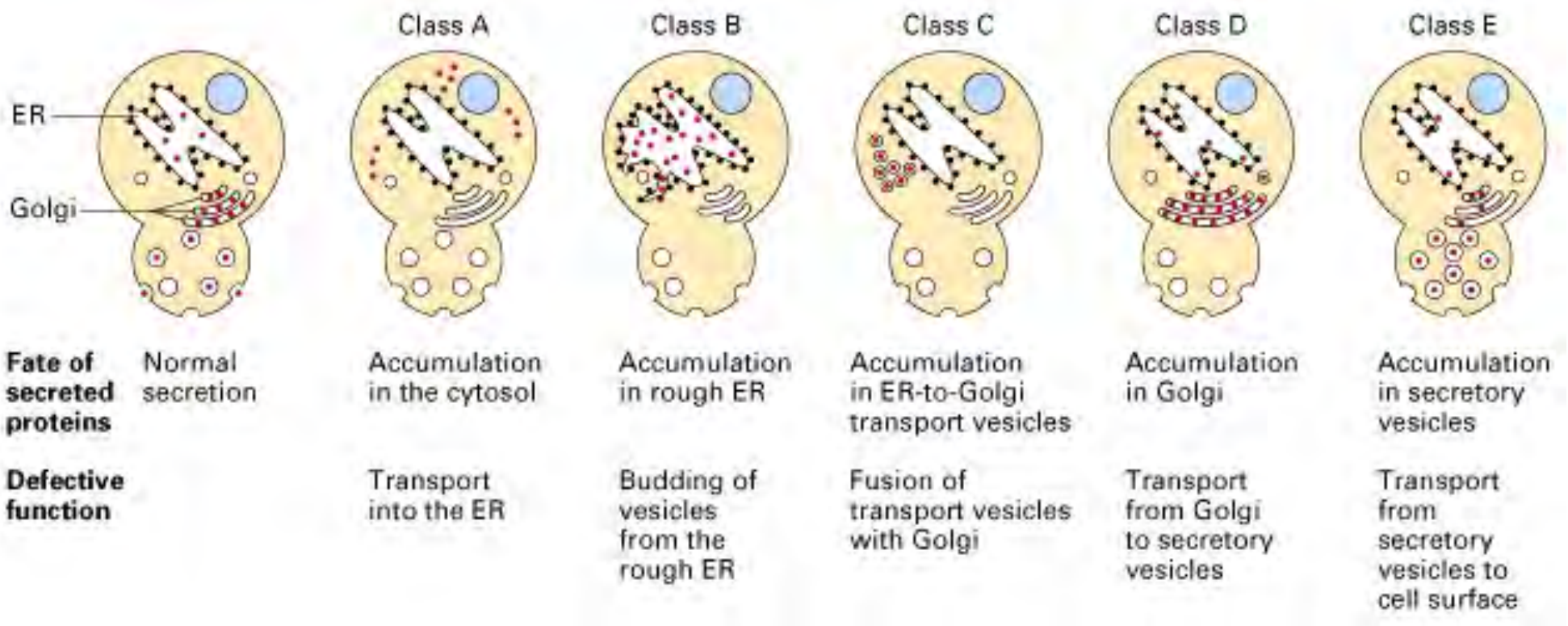


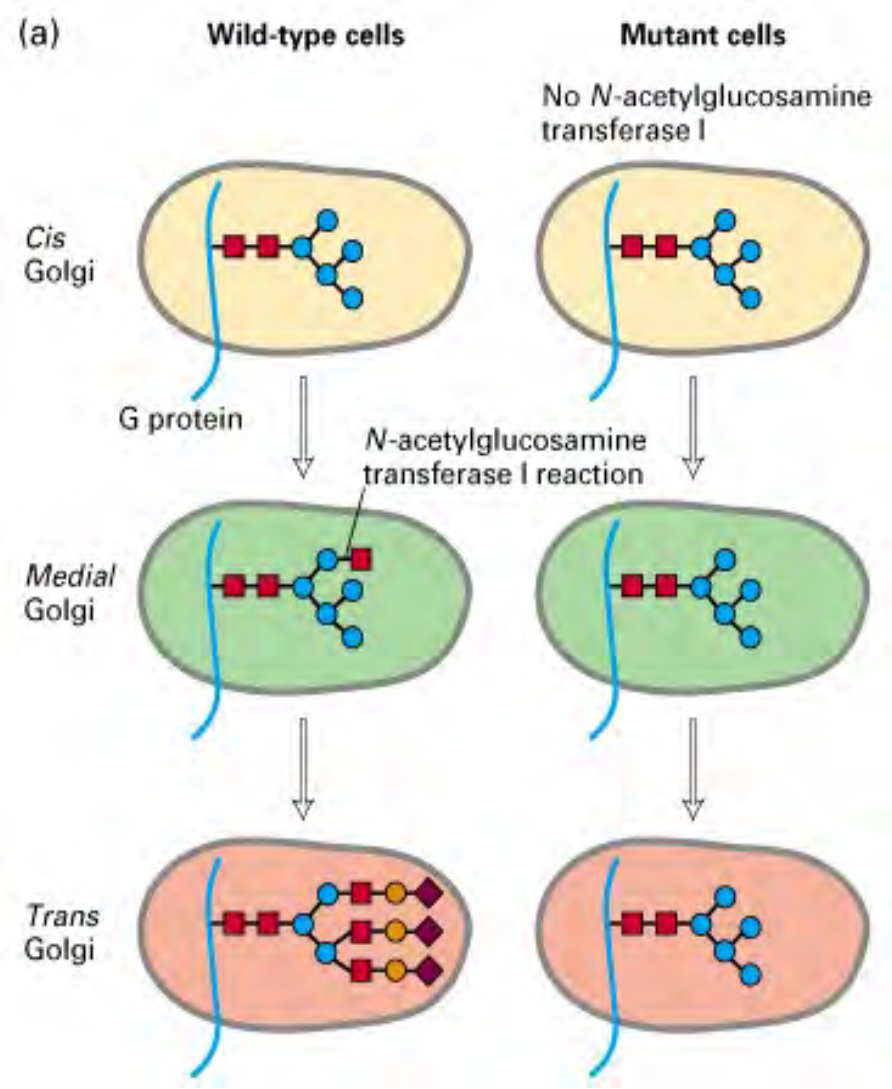
Figure 13–4. Molecular Biology of the Cell, 4th Edition.

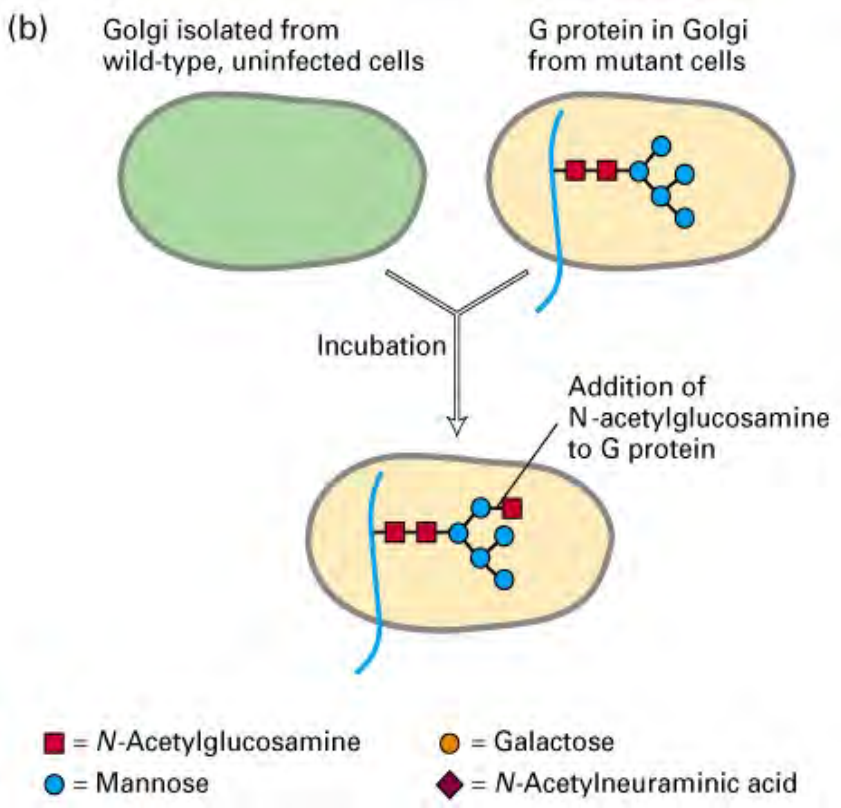
Analysis of yeast mutants defined the major steps in the secretory pathway



Lodish et al, 2000

A cell-free assay for fusion between Golgi cisternae



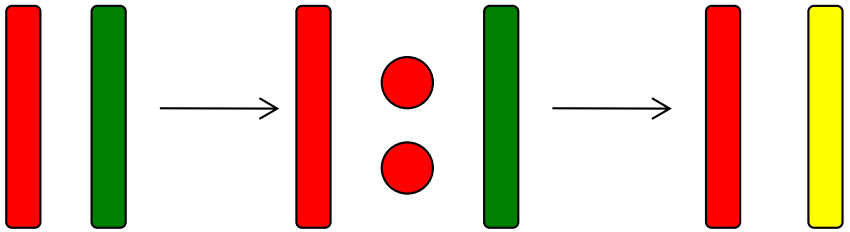


mix wt Golgi + mutant Golgi + cytosol + ^{3}H-GlcNac +ATP = ^{3}H GlcNac incorporated into G protein

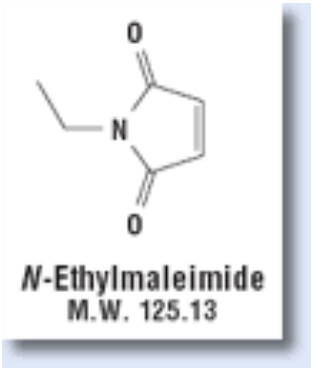
GlcNAc=N=Acetylglucosamine

Lodish et al, 2000

Identification of NSF as a fusion factor for inter-cisternal Golgi trafficking

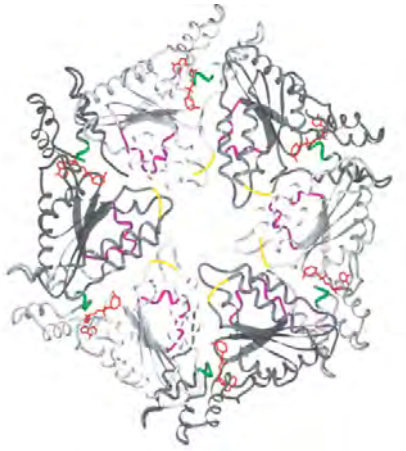


donor compartment
acceptor compartment



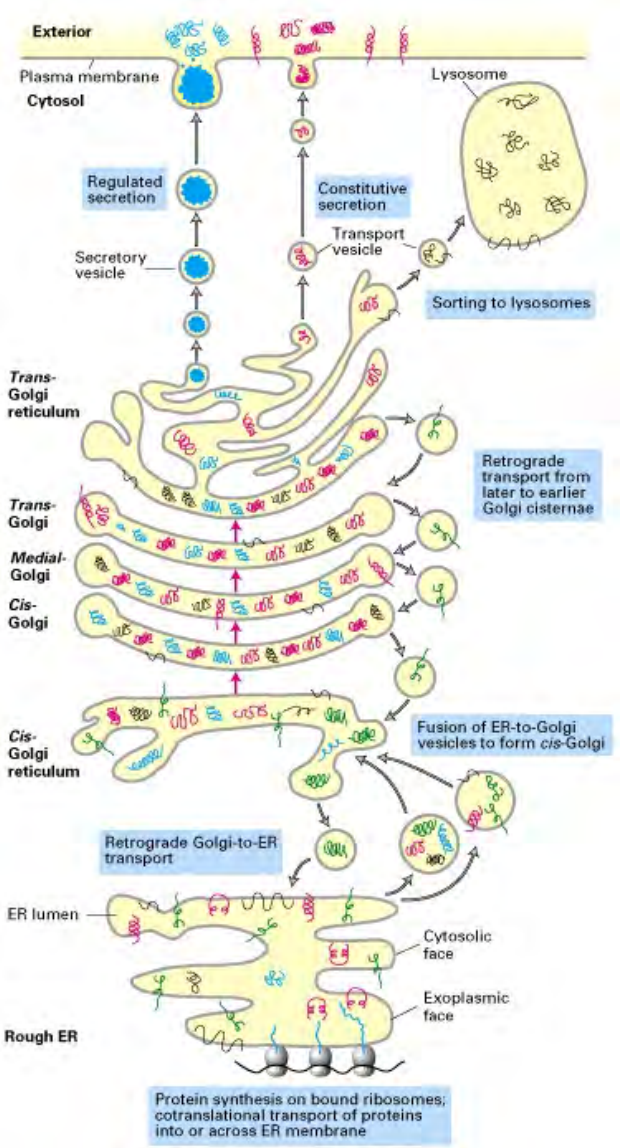
1. Reaction blocked when cytosol treated with N-ethylmaleimide (NEM). NEM covalently bonds sulfhydryl group on cysteine.
    - •NEM-sensitive factor (NSF) required for Golgi fusion
    - •Accumulate uncoated vesicles on acceptor Golgi

2. Fusion is restored by adding fresh cytosol
    - •bioassay for purification of NSF
    - •protein identified by peptide sequencing

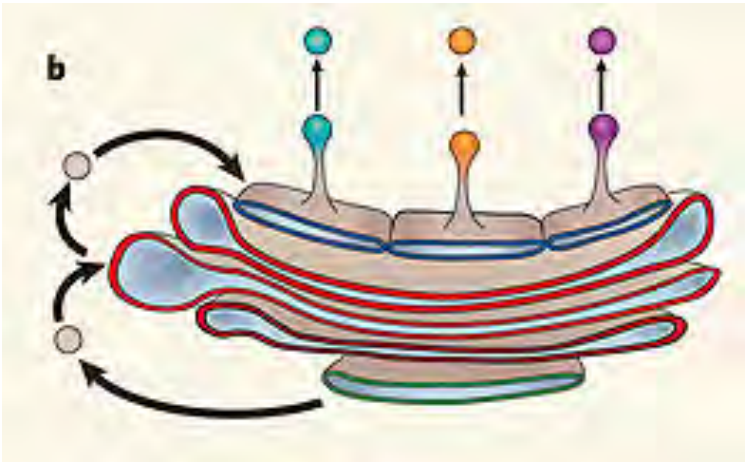


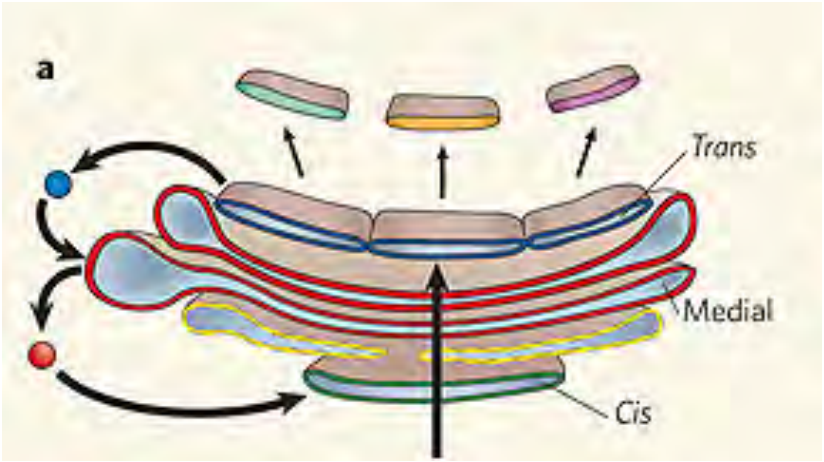
3. NSF is an AAA ATPase (**A**TPases **A**ssociated with various cellular **A**ctivities)
    - •homologous to Sec18 (yeast gene required for ER to Golgi transport)
    - •hexameric ATPase (6x 76 kD)
    - •Original idea: ATP hydrolysis provides the energy to drive membrane fusion?

Models of Golgi trafficking



Lodish et al, 2000





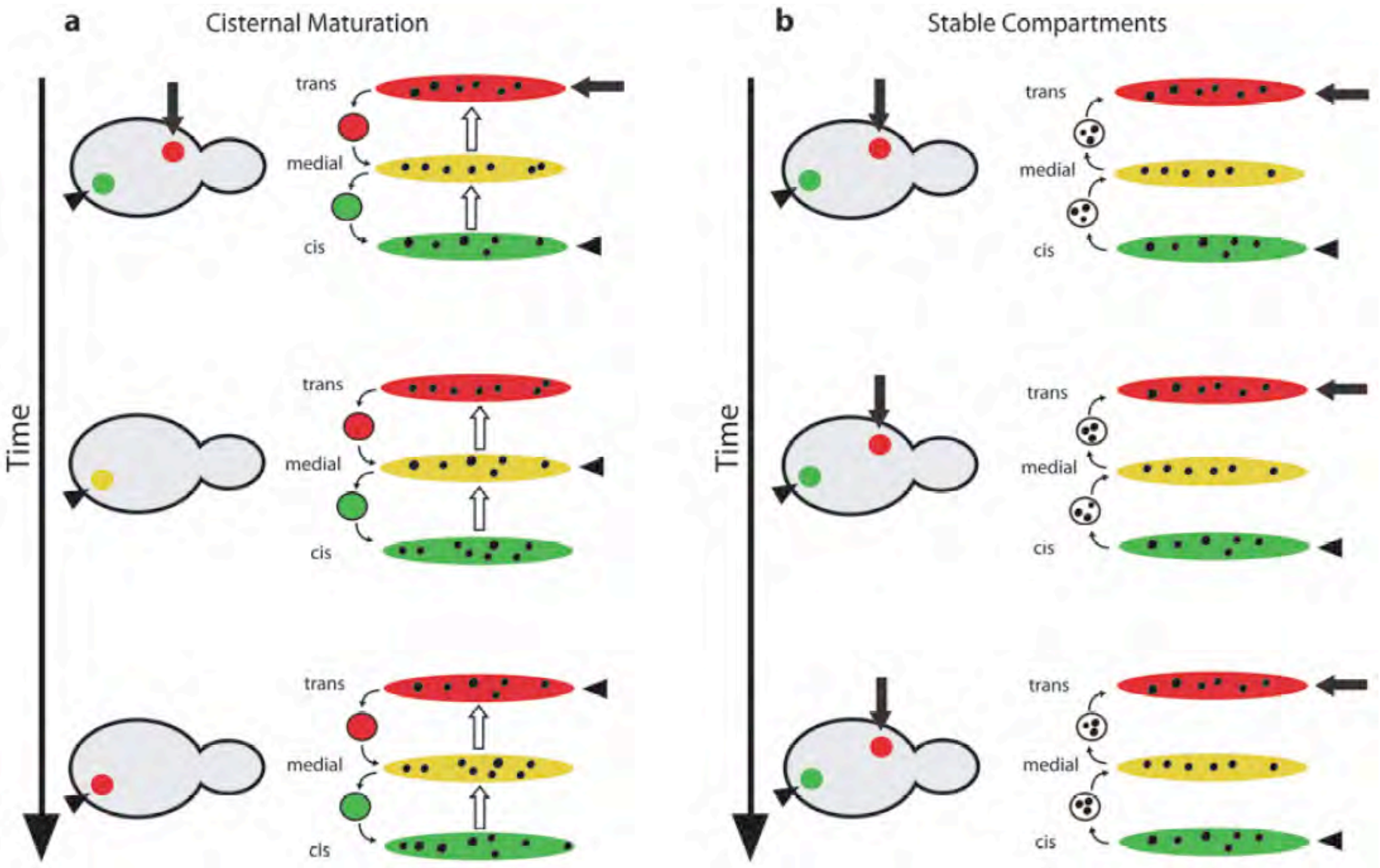
<u>vesicle-shuttling/stable compartments</u>

- Compartments are distinct
- Anterograde movement of secretory cargo

<u>cisternal maturation</u>

- Identity of cisternae change
- Retrograde transport involved in sorting components

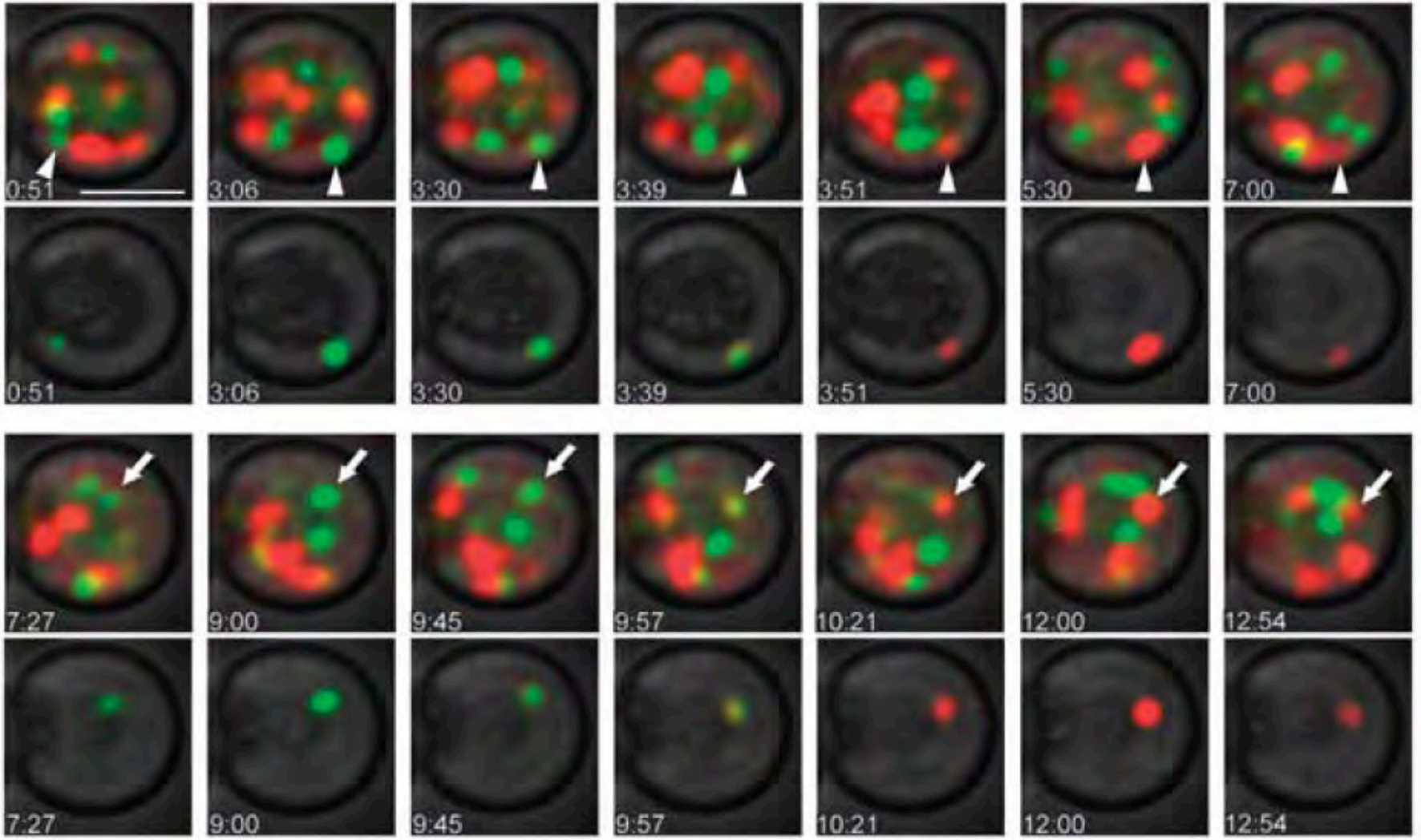
Models of Golgi dynamics



budding yeast do not have stacked Golgi

Losev et al. (2006) Nature

Evidence for the cisternal maturation model



GFP-Vrg4 (early Golgi)
Sec7-DsRed (late Golgi)

Losev et al. (2006) Nature

SNAP cooperates with NSF

1. NSF alone cannot bind Golgi membranes.
    - a crude cytosolic fraction is required
    - an assay for "soluble NSF attachment proteins" (SNAPs)

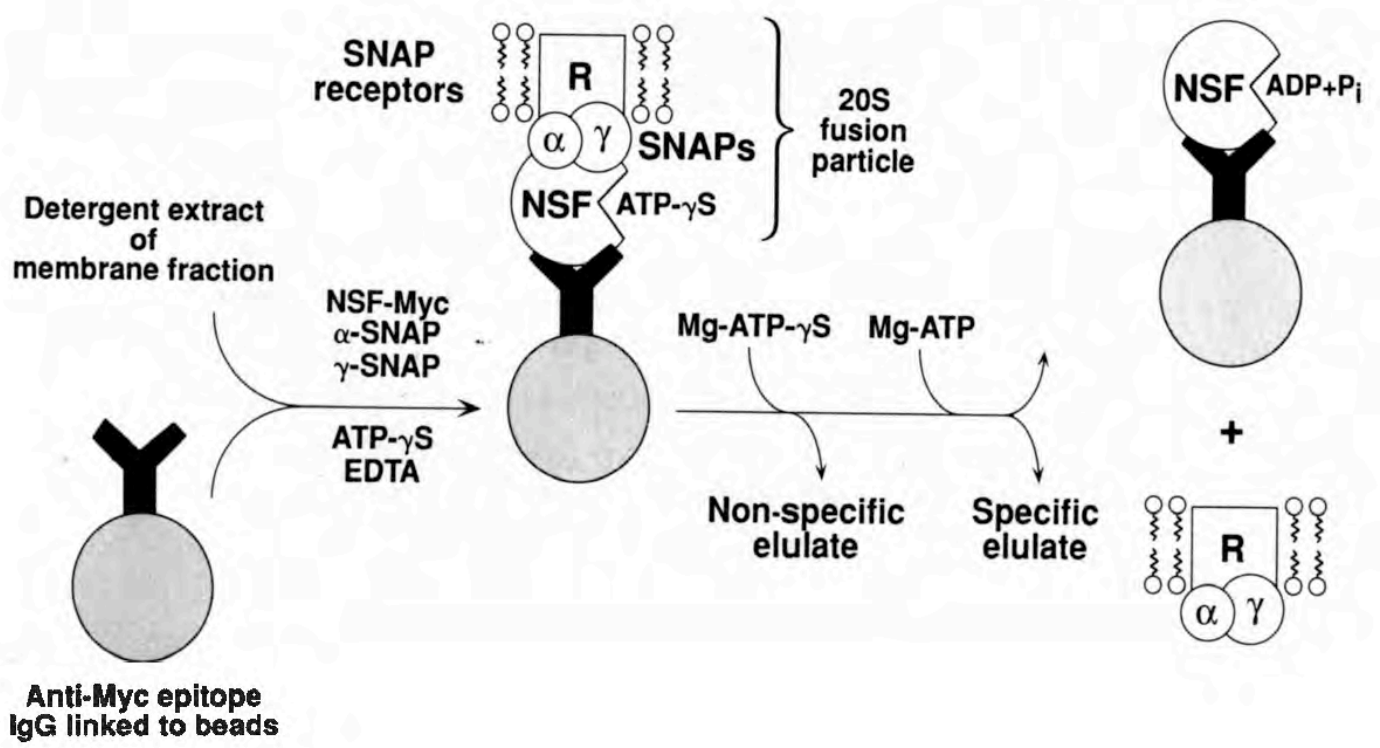
2. Purification yielded:
    - α-SNAP
    - β-SNAP
    - γ-SNAP

3. SNAP does not bind NSF in solution; it forms a complex with NSF on Golgi membranes.

4. Homologous to yeast Sec17.

Identification of SNAREs (SNAP receptors)

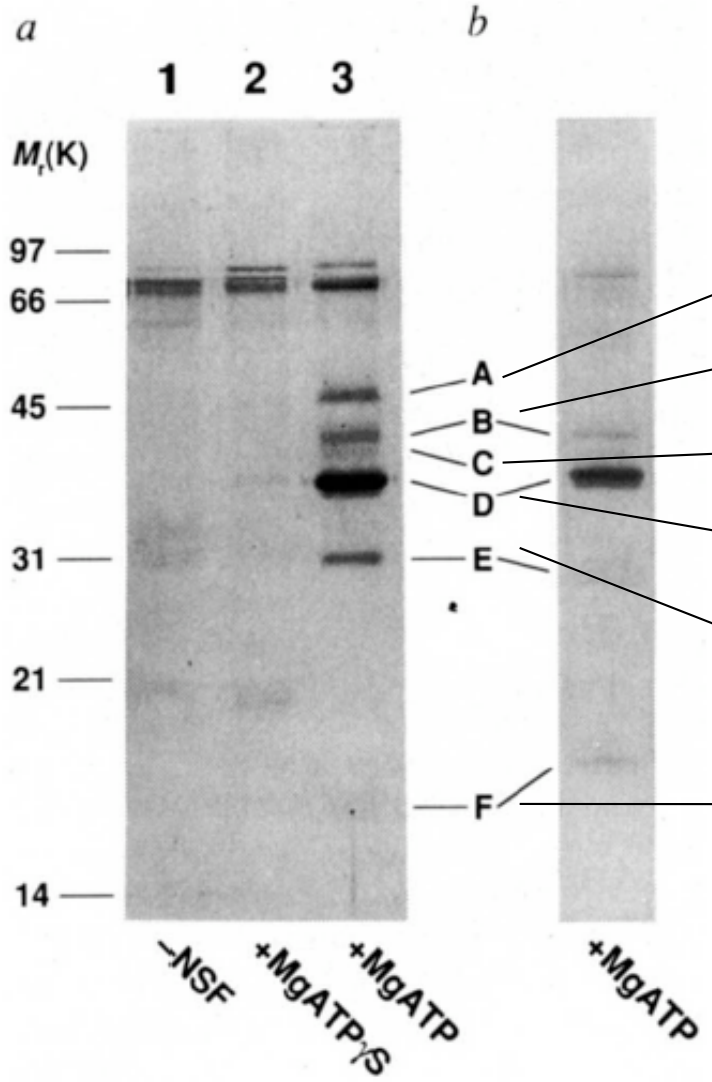
1. NSF required for fusion.
2. NSF requires SNAP to bind Golgi vesicles.
3. NSF + SNAP + ATPγS + detergent extract ➔ 20S "fusion" particle.
4. NSF + SNAP + Mg-ATP + detergent extract ➔ 20S particle dissociates (NSF released)



Specificity provided by Myc monoclonal and ATP hydrolysis

Sollner et al (1993) Nature

Identification of SNAREs (SNAP receptors)



a. Coomassie-stained
1. Control
2. Non-specific
3. Specific eluate

b. Silver-stained

SNAP-25: synaptosome-associated protein 25
VAMP: vesicle-associated membrane protein

Sollner et al (1993) Nature

SNAREs are the target of Clostridial neurotoxins

1. Clostridium: gram-positive anaerobic bacteria

2. *C. tetani*
   - •contamination of wounds with spores, which lead to infection
   - •neurological disorder (increased muscle tone, spasm)
   - •due to release of tetanus toxin, which blocks inhibitory interneuron
   - • vaccine= tetanus toxin inactivated with formaldehyde

3. *C. botulinum*
   - •eating improperly prepared foods
   - •paralytic disease due to blockage of nerve transmission
   - •due to preformed botulinum toxin
   - • from Latin *botulus* (sausage) for sausage poison
   - • lethal in ng amounts

4. Tetanus and botulinum toxin are metalloproteases that cleave synaptobrevin, sytaxin, or SNAP-25

http://www.botoxcosmetic.com

Evolution of models for vesicular fusion

1. NSF/SNAP use ATP hydrolysis to drive the energetics of membrane fusion.

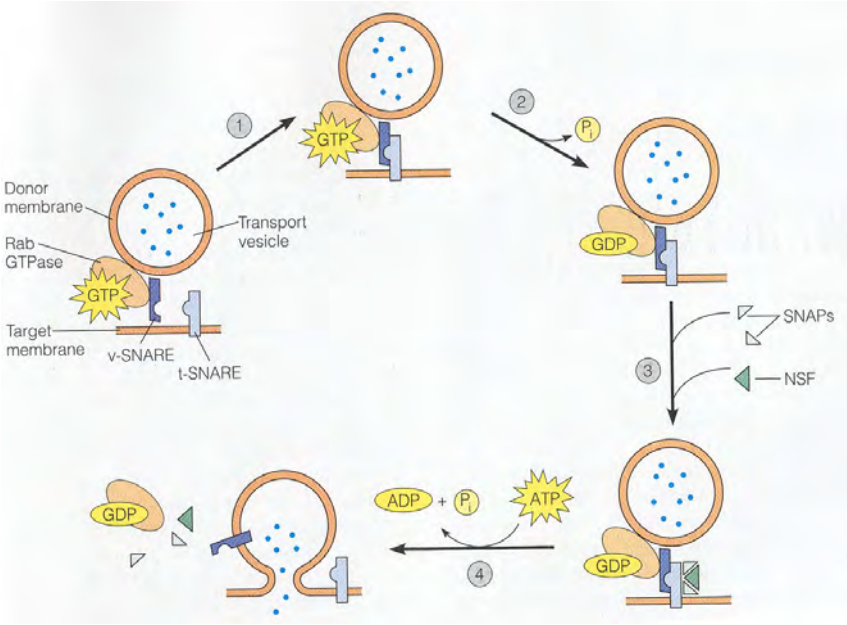
2. SNARE complex does not proceed to fusion until disassembly by NSF/NAP

3. SNAREs are an address code.

4. NSF/SNAP disassemble SNAREs to replenish free SNAREs and enable multiple cycles of fusion.

5. SNAREs are the core machinery for membrane fusion.

6. SM (Sec1/Munc18-like) proteins play critical roles in SNARE complex formation and therefore fusion.

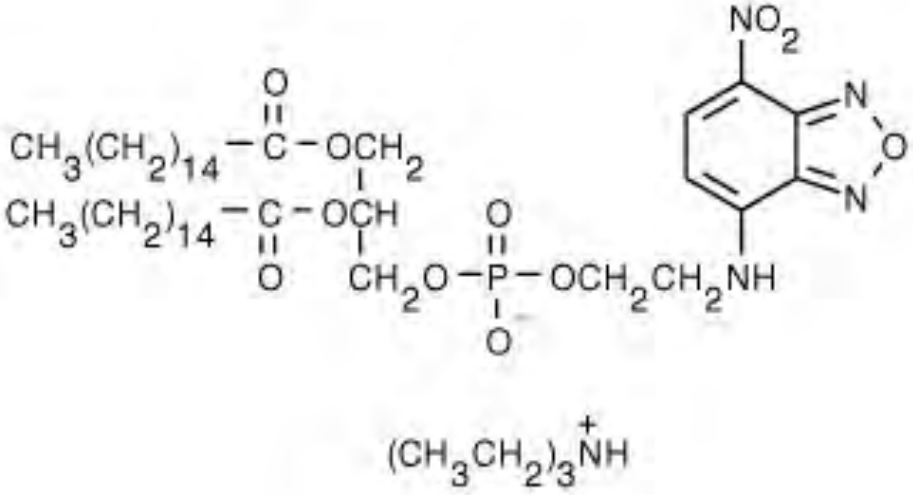


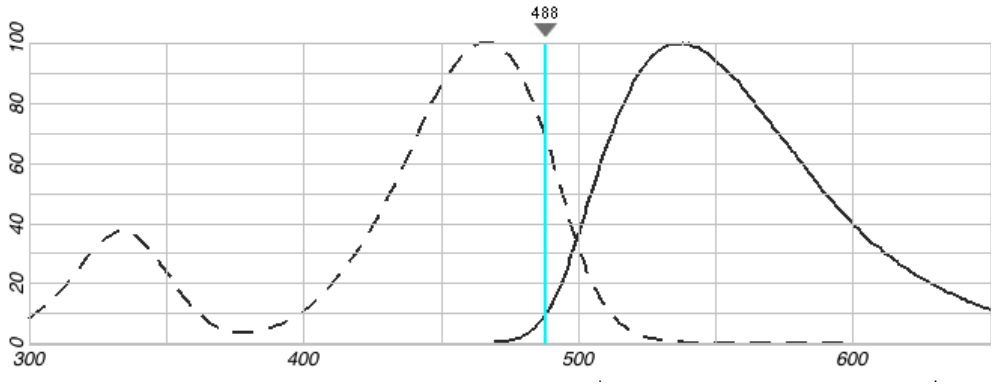
Early model

N-(7-nitrobenz-2-oxa-1,3-diazol- 4-yl)-1,2-dihexadecanoyl-*sn*- glycero-3-phosphoethanolamine, triethylammonium salt (NBD-PE)

Molecular Formula: $C_{49}H_{90}N_5O_{11}P$

Molecular Weight: 956.25



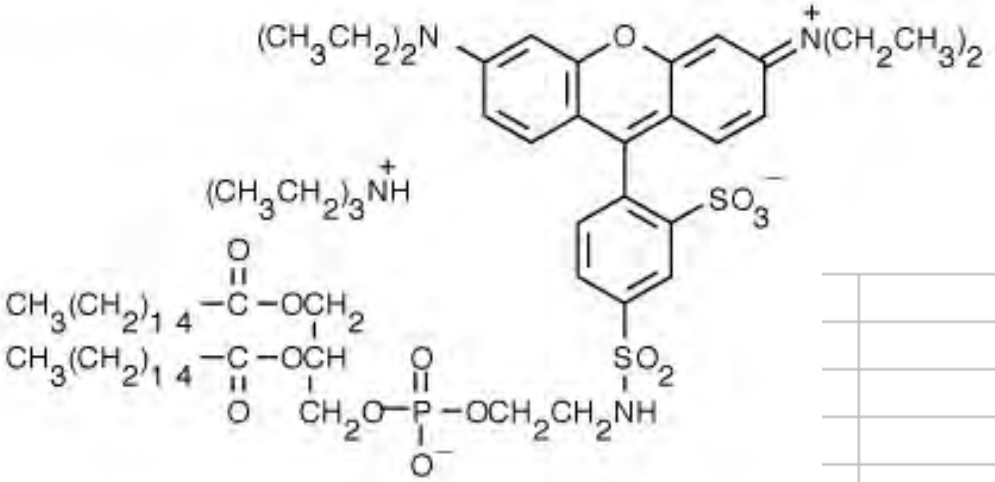


excitation: 460 nm
emission: 538 nm

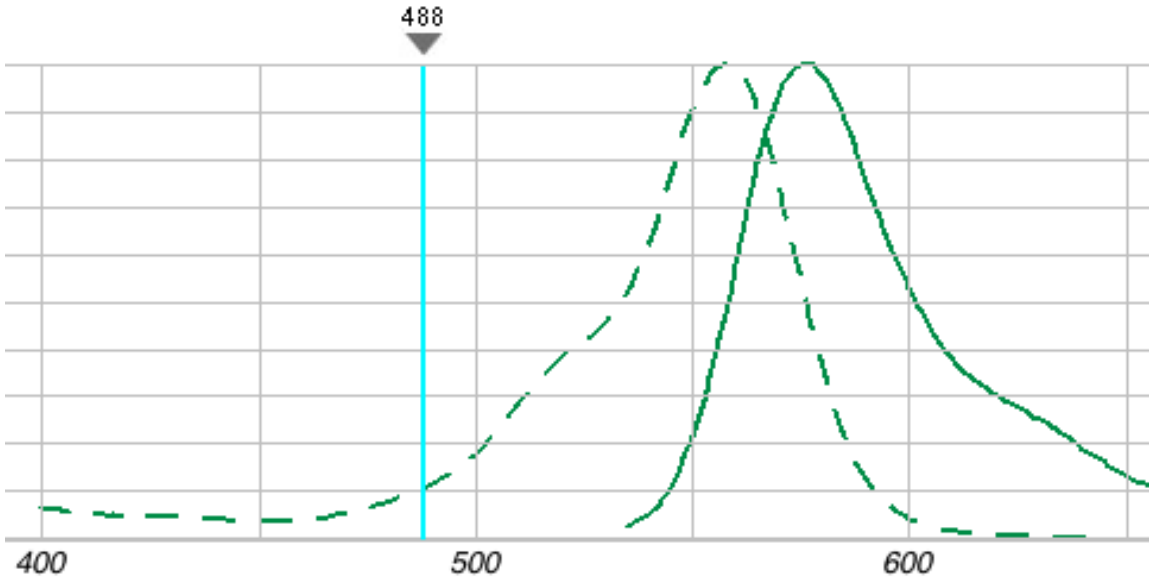
Lissamine™ rhodamine B 1,2-dihexadecanoyl-*sn*-glycero- 3-phosphoethanolamine, triethylammonium salt (rhodamine DHPE)

Molecular Formula: $C_{70}H_{117}N_4O_{14}PS_2$

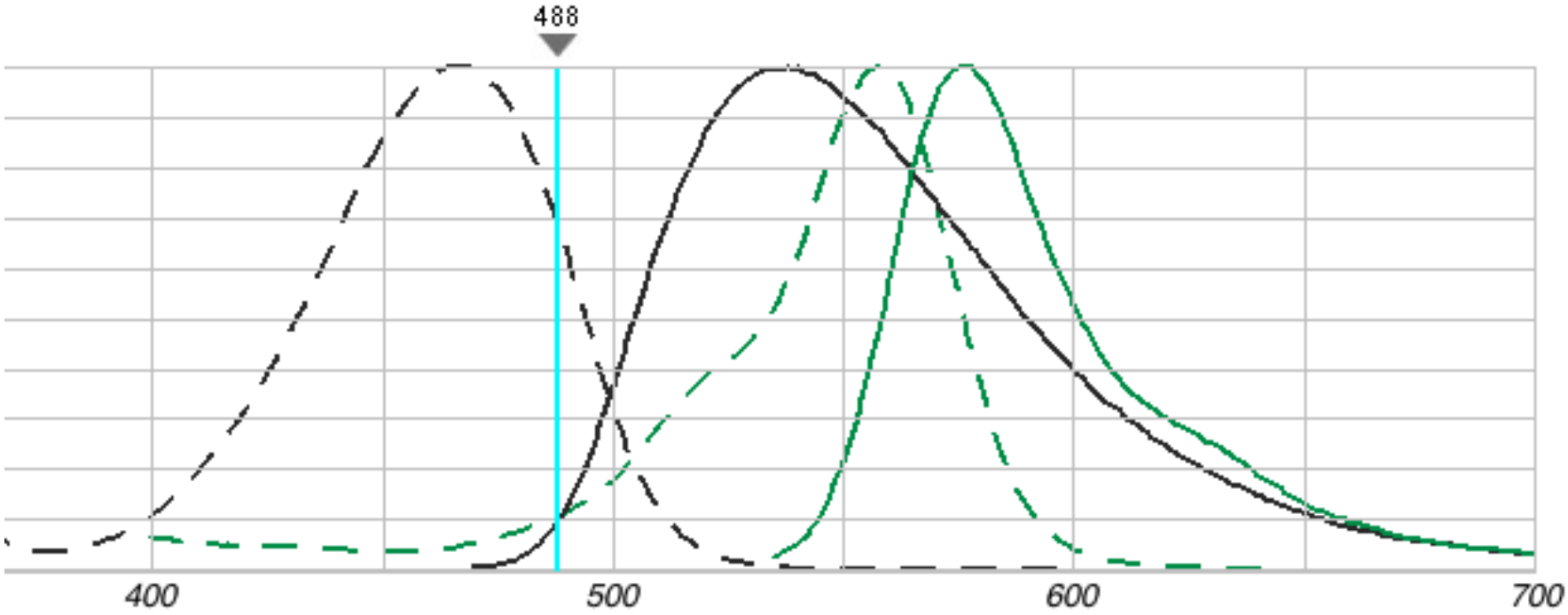
Molecular Weight: 1333.81



excitation: 560 nm
emission: 590 nm



Fluorescent measurement of vesicle fusion



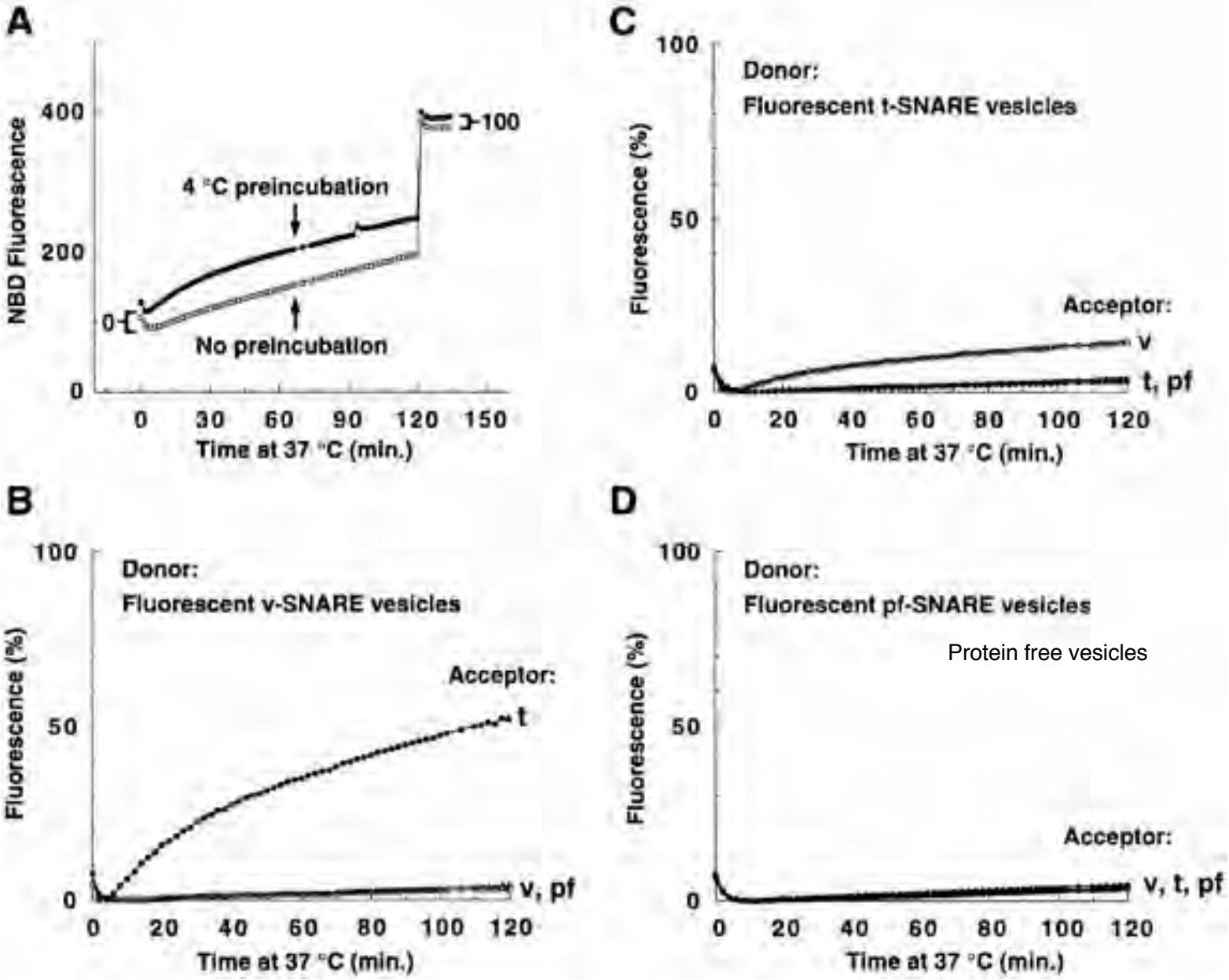
Donor vesicle contains NBD-PE and rhodamine-PE.

To measure donor and acceptor vesicle fusion, measure de-quenching of NBD fluorescence.

NBD:
excitation: 460 nm
emission: 538 nm

Rhodamine:
excitation: 560 nm
emission: 590 nm

SNARE complex formation can mix lipids



A. Donor v-SNARE (VAMP) vesicles containing NBD/Rhod mixed with t-SNARE (syntaxin 1/SNAP25) vesicles.

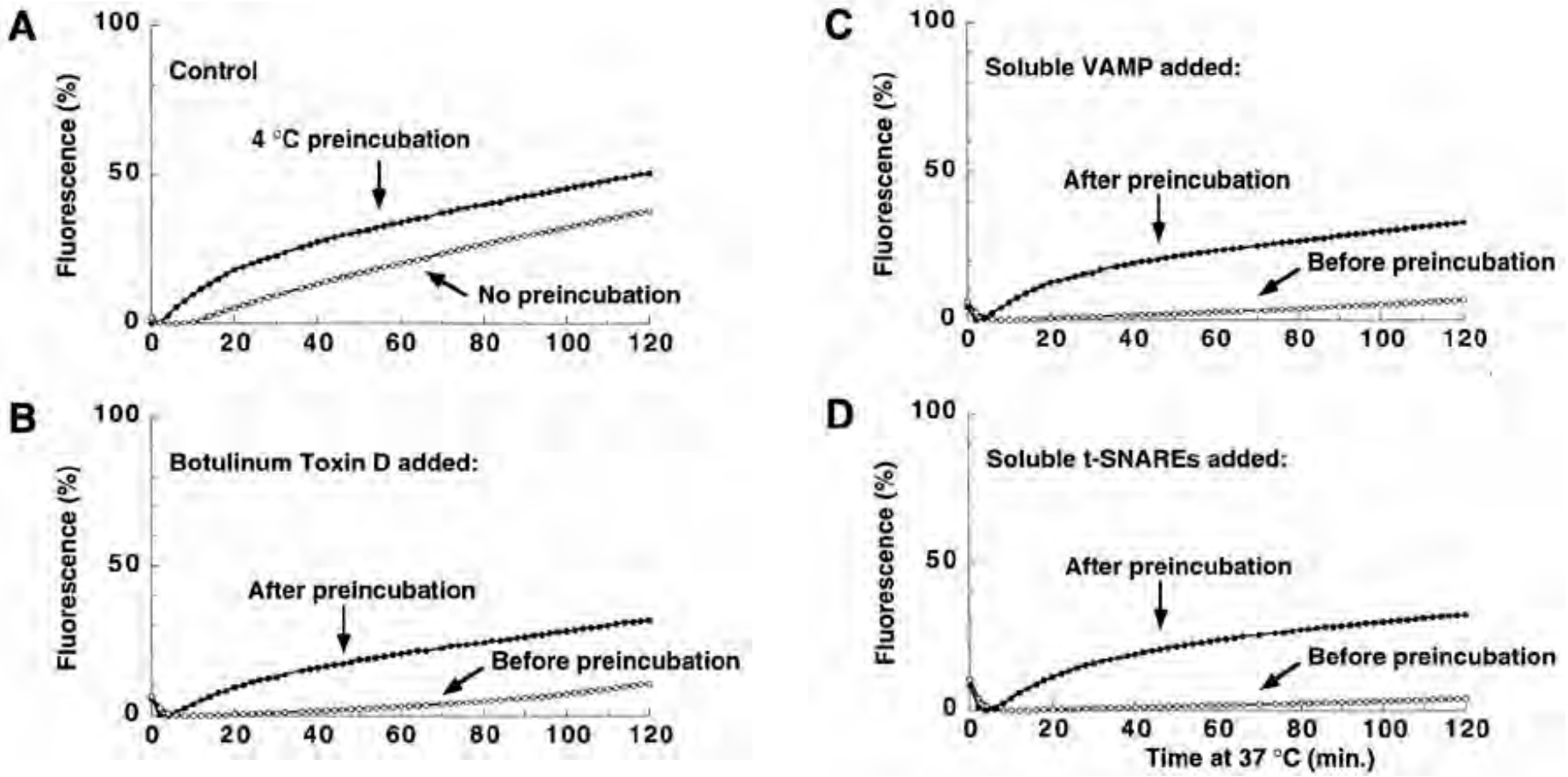
B. Donor v-SNARE vesicles mixed with t-, protein-free, or v-SNARE vesicles.

C. Donor t-SNARE vesicles mixed with v-SNARE, protein-free, or t-SNARE vesicles.

D. Protein-free donor vesicles incubated with v-, t-, or protein-free vesicles.

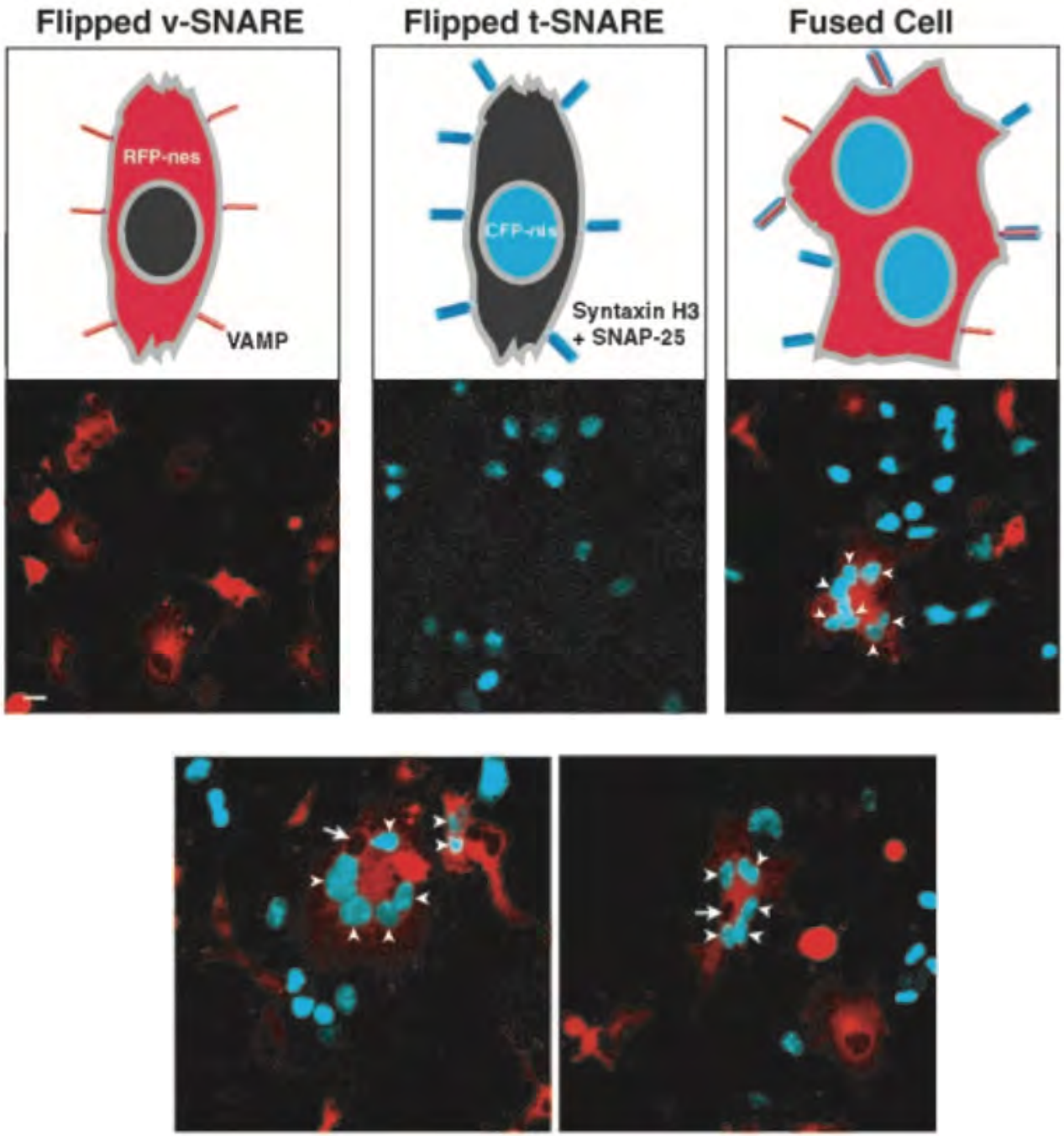
Weber et al (1998). Cell 92: 759

Pre-assembled SNARE complexes are resistant to inhibitors



Weber et al (1998). Cell 92: 759

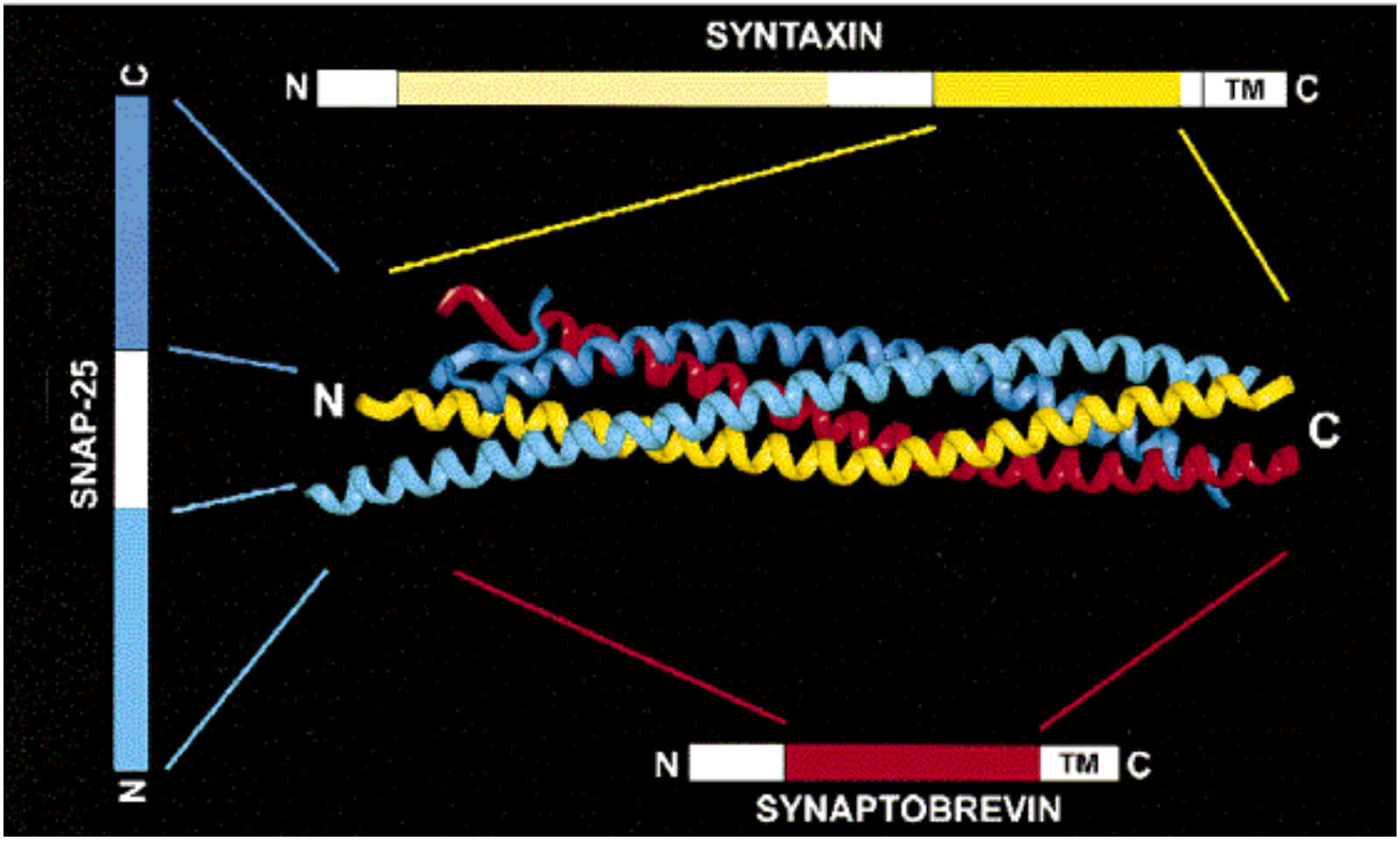
Cell fusion by “flipped” SNAREs



- Signal sequences place at N-terminus of v- and t-SNARE proteins.
- SNAREs expressed on cell surface.
- Cells expressing such “flipped” v-SNARE could fuse with cells expressing “flipped” t-SNAREs to form multinucleated cells.

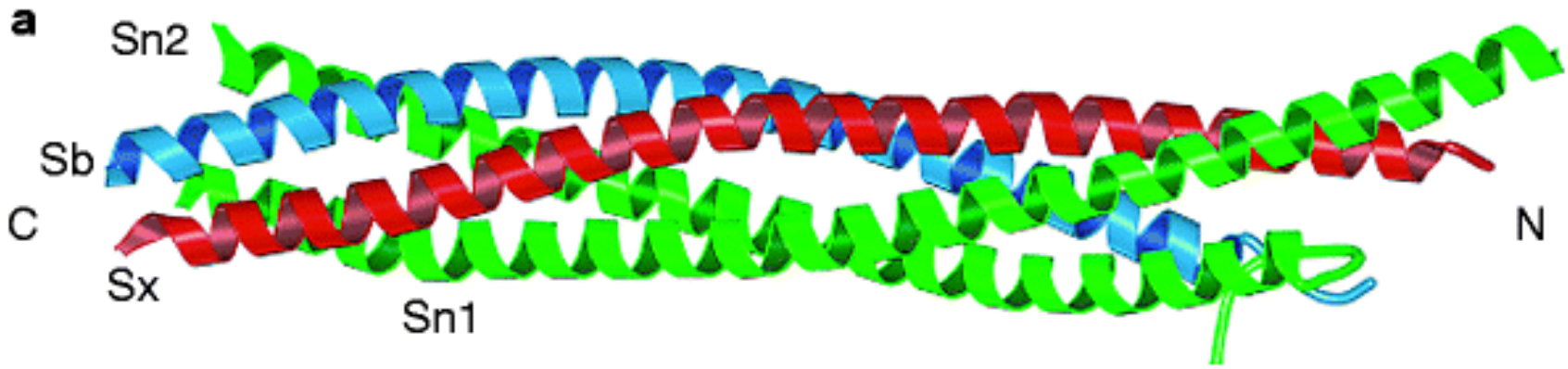
Hu et al (2003) Science

Crystal structure of SNARE complex



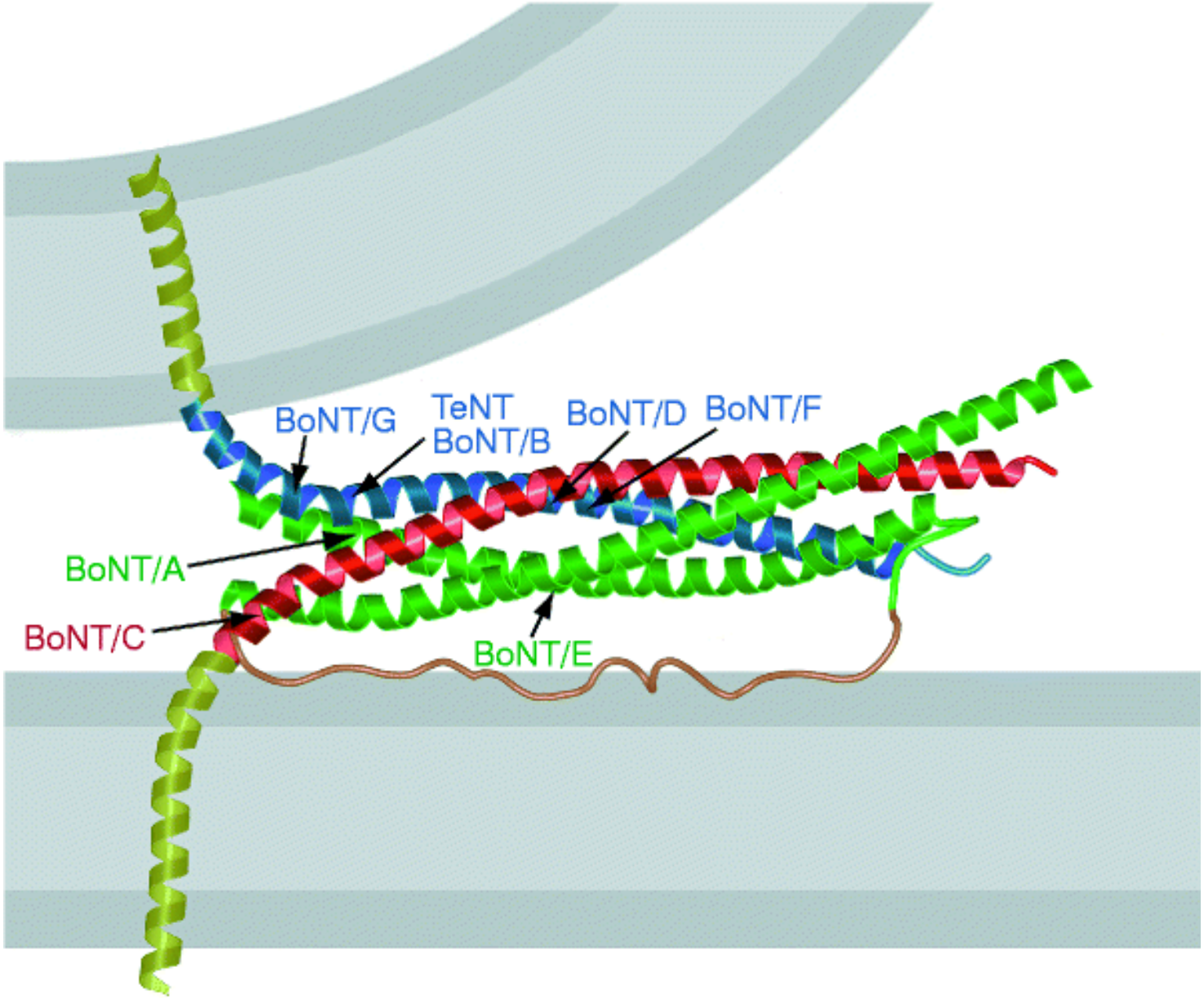
Rizo & Sudhof (1998) Nat Struct Biol 5, 839

Crystal structure of SNARE complex



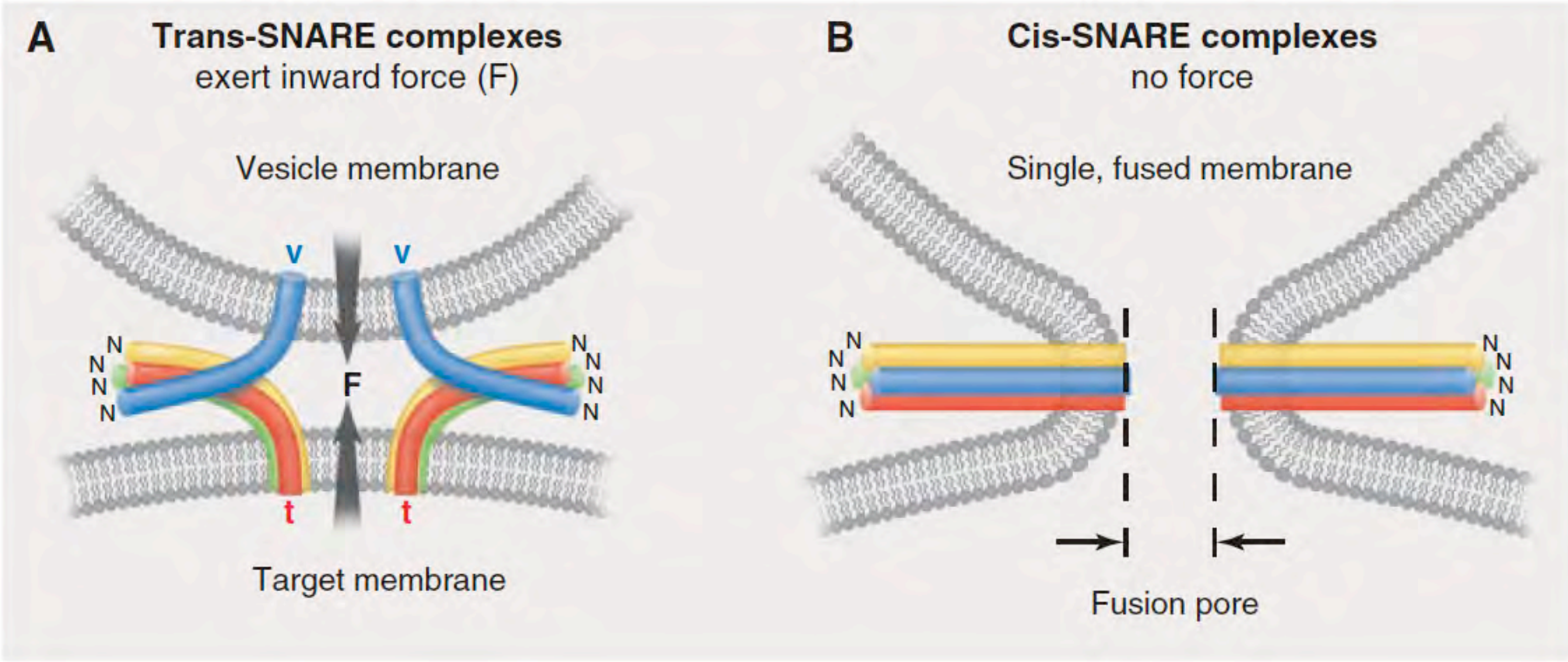
Sutton et al (1998) Nature 395:347

Model of SNARE complex in membrane fusion



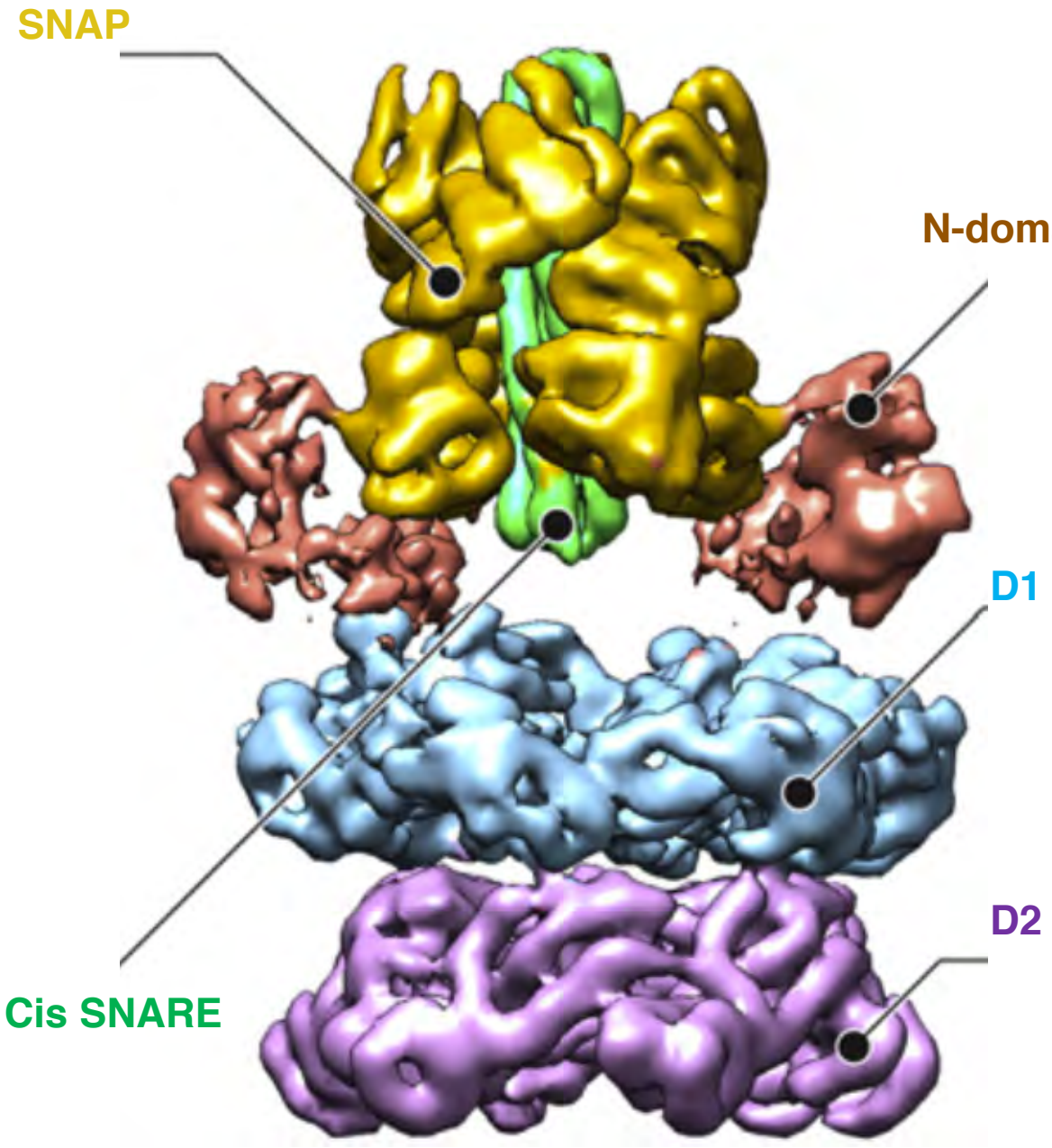
Sutton et al (1998) Nature 395:347

Model for SNARE-mediated membrane fusion



Sudhof and Rothman (2009) Science

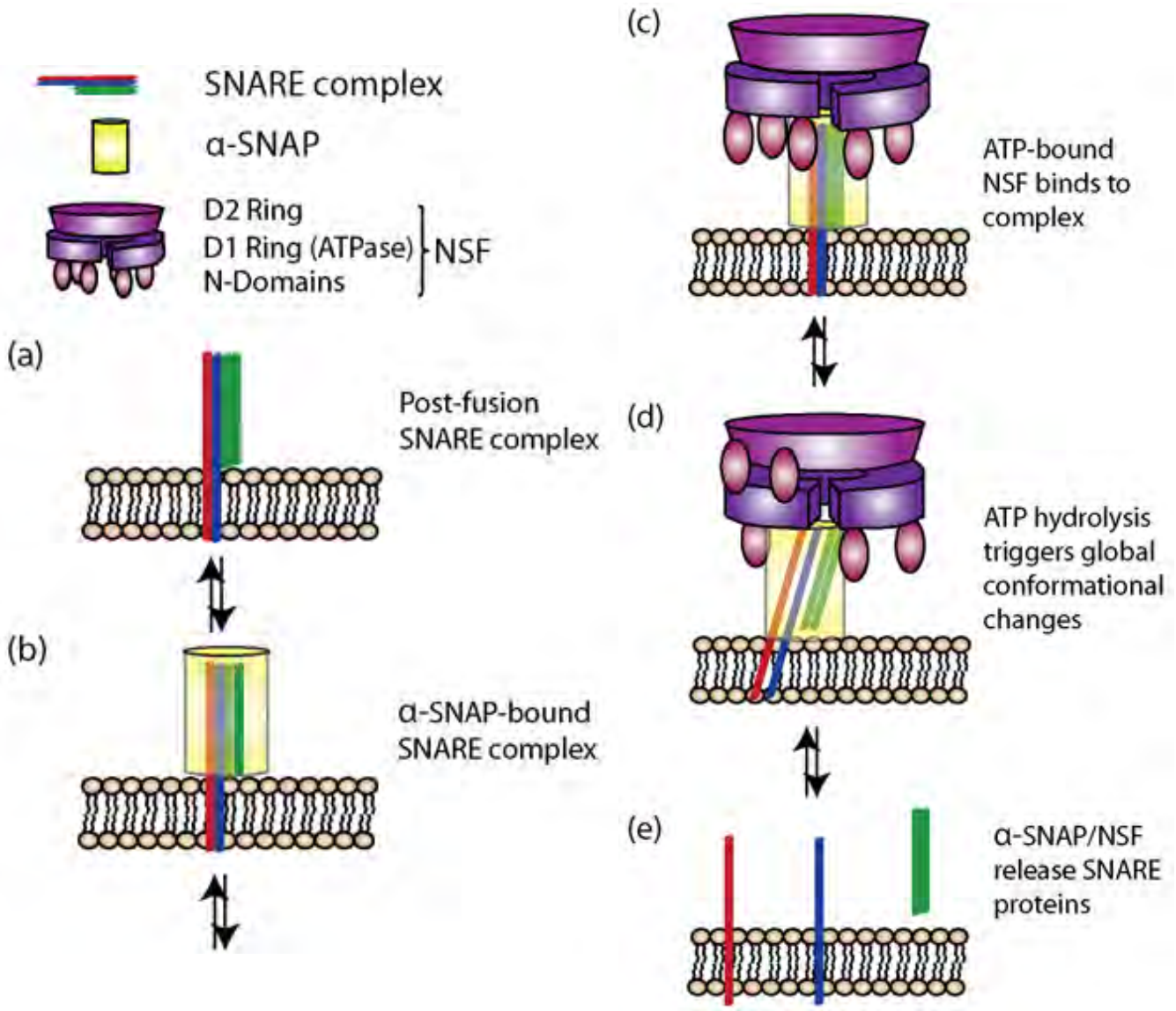
Structure of 20S supercomplex in presence of AMPPNP



- NSF is aa AAA+ ATPase with double ATPase rings (hexameric), D1 and D2.
- SNAREs mediate fusion; NSF/SNAP act as chaperone to disassembly them and replenish free SNARE pools for further fusion.
- D1 has higher ATPase activity; D2 may function more in oligomerization.
- Cryo-EM structure shows large conformational changes upon ATP hydrolysis.
- Evidence on stoichiometry of SNAP to NSF conflicting; this structure has 4 SNAPs.

Zhao & Brunger (2015) JMB

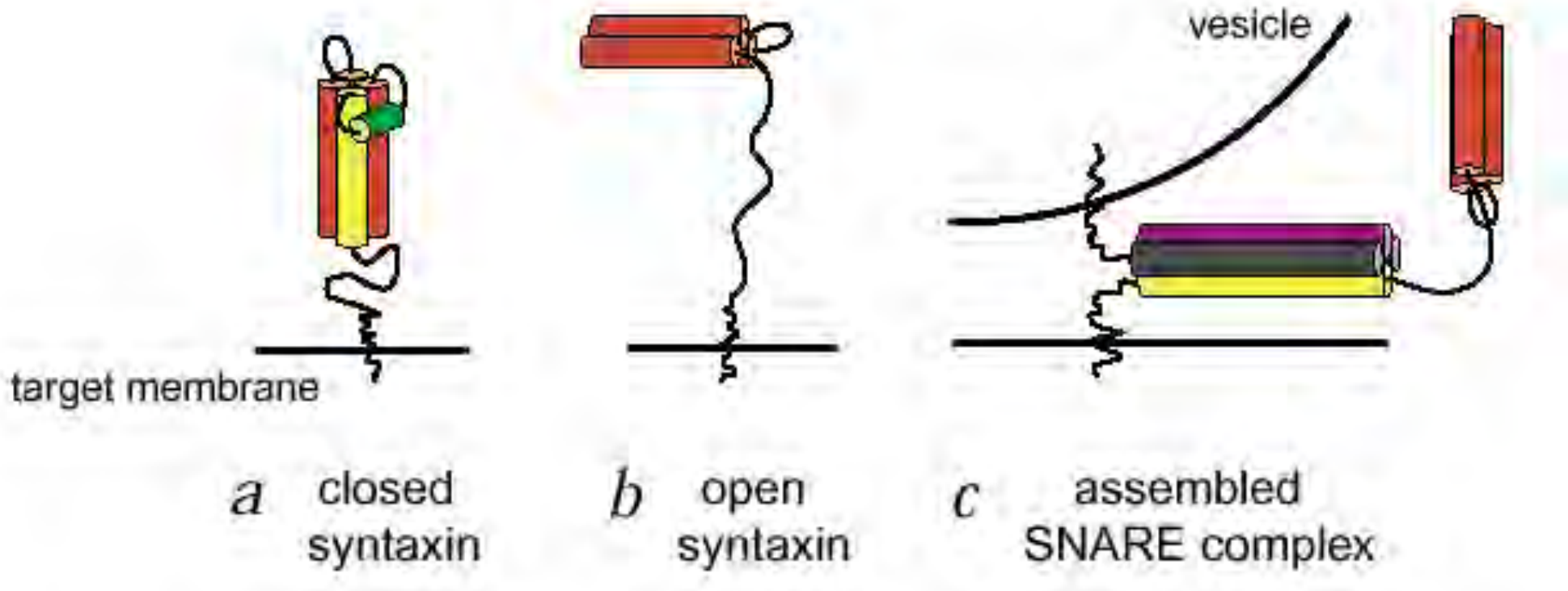
Model of NSF/SNAP action



- Cis SNARE complex is bound by SNAP.
- NSF-ATP binds to SNAP/SNARE complex.
- A single round of ATP hydrolysis is sufficient to disassemble complex.
- ATP hydrolysis causes large conformational changes, within NSF and between NSF and SNAP, that are transmitted to SNARE.
- Twisting forces pull 4 helix bundle apart.

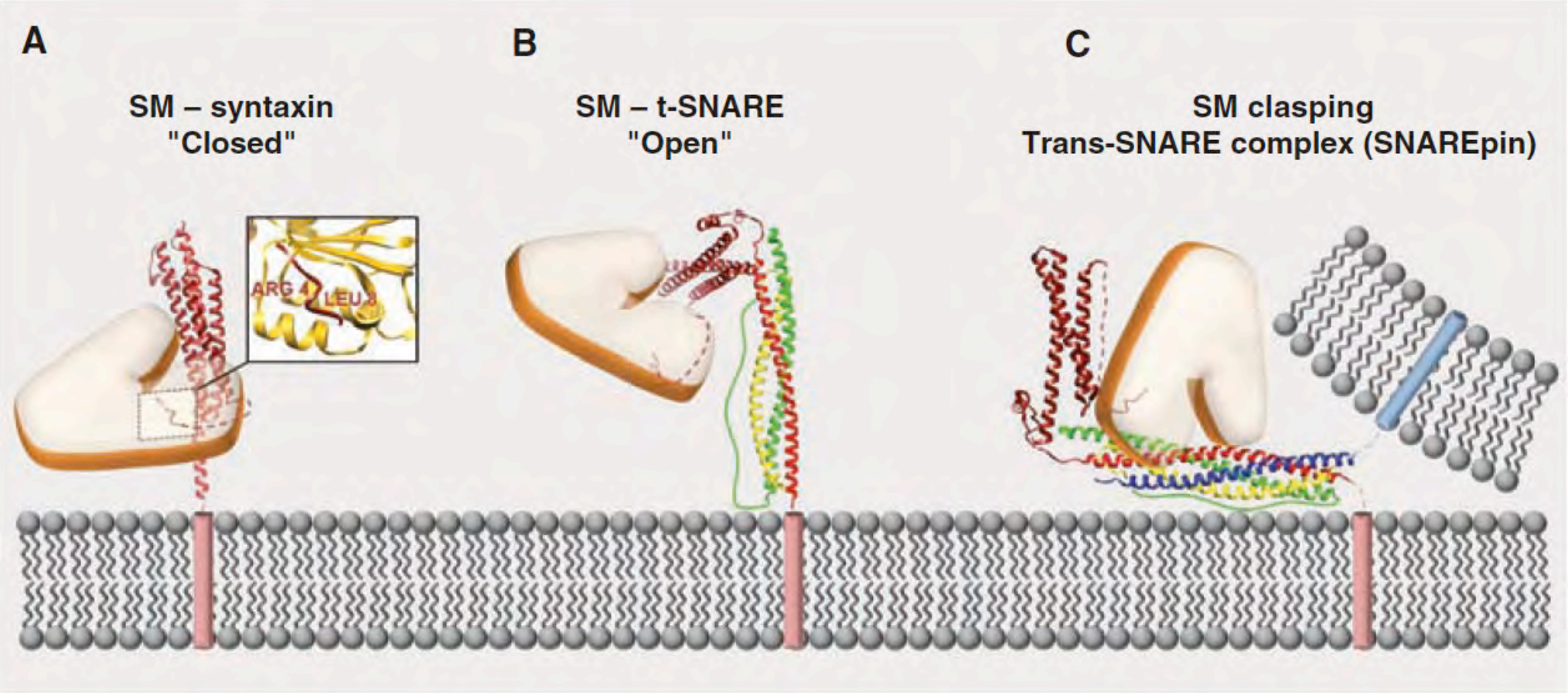
Bombardier & Munson (2015) Curr Opin in Chem Biol

Distinct conformations of a t-SNARE in crystal structures



Carr (2001). Nat Struct Biol 8:186

SM proteins regulate SNARE structure



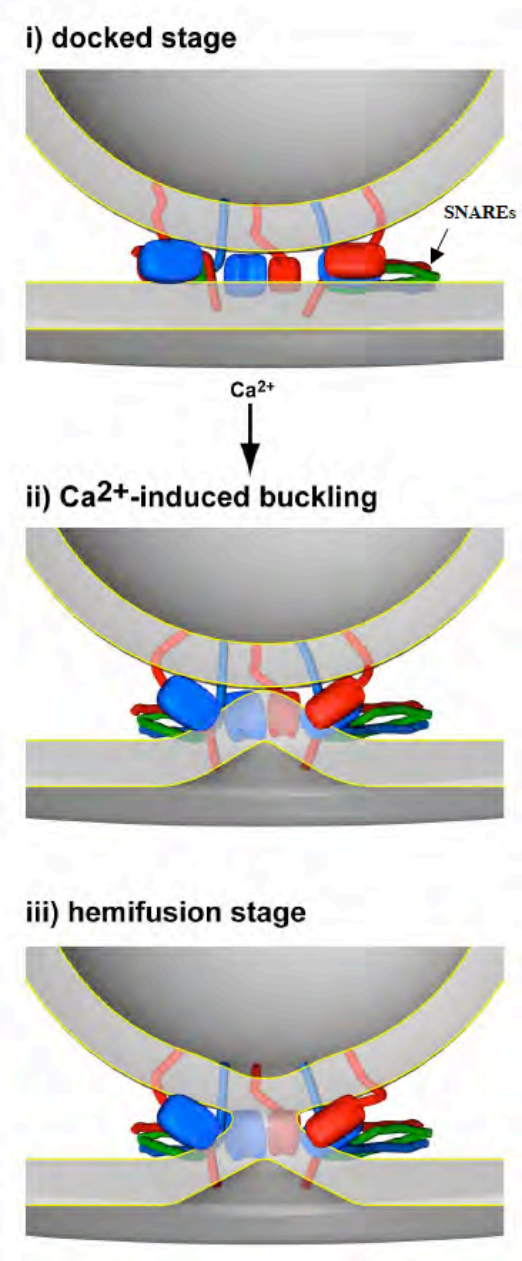
SM proteins bind to t-SNARE syntaxin-1's 4-helix bundle (closed conformation). Later they somehow regulate formation of the trans-SNARE complex.
In addition, synaptotagmin is a calcium sensor that triggers SNARE-mediated fusion in synaptic vesicle fusion.

Sudhof and Rothman (2009) Science

Possible role of synaptotagmin

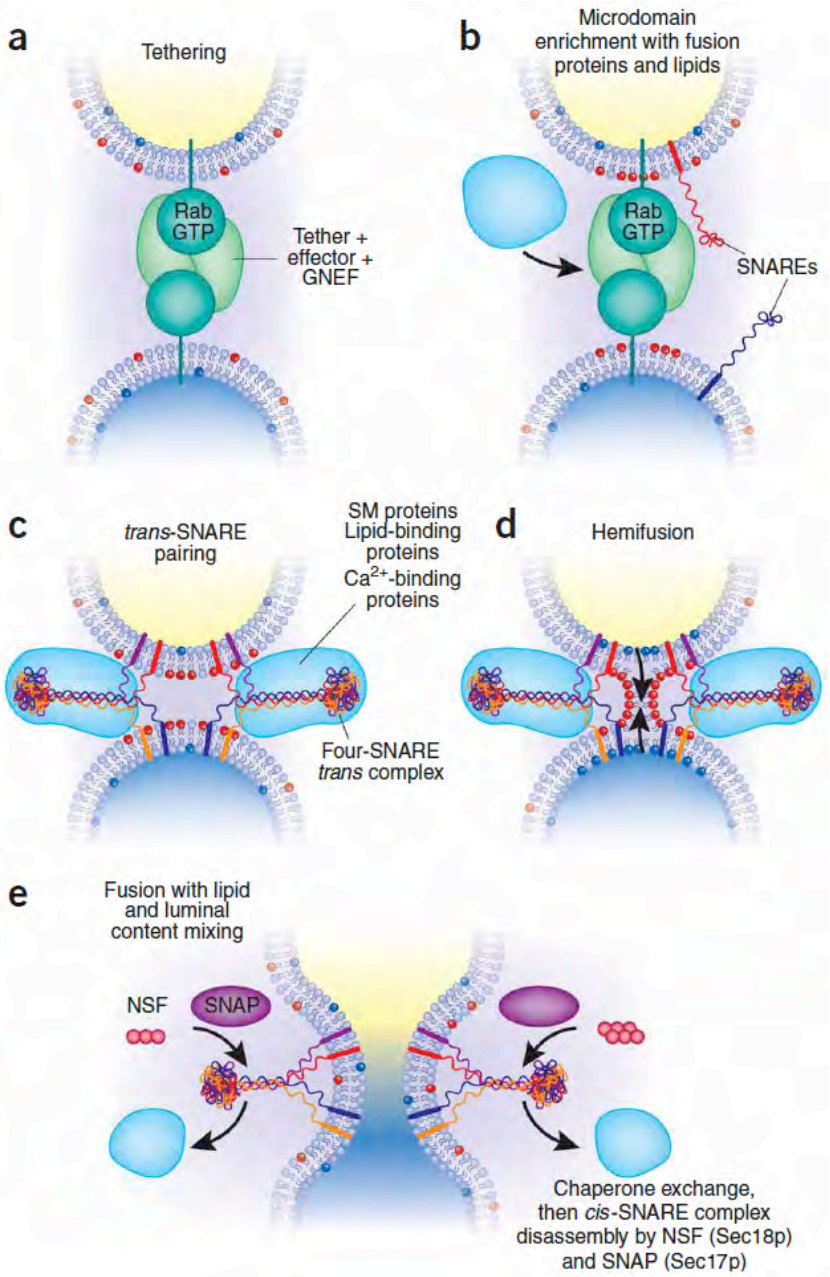
Synaptotagmin regulates Ca++-dependent exocytosis.

C2 domains of synaptotagmin tubulate membranes.



Martens et al (2007) Science

Summary of membrane fusion cycles



Wickner and Schekman (2008) Nat Struct Mol Biol