# Lesson (8)

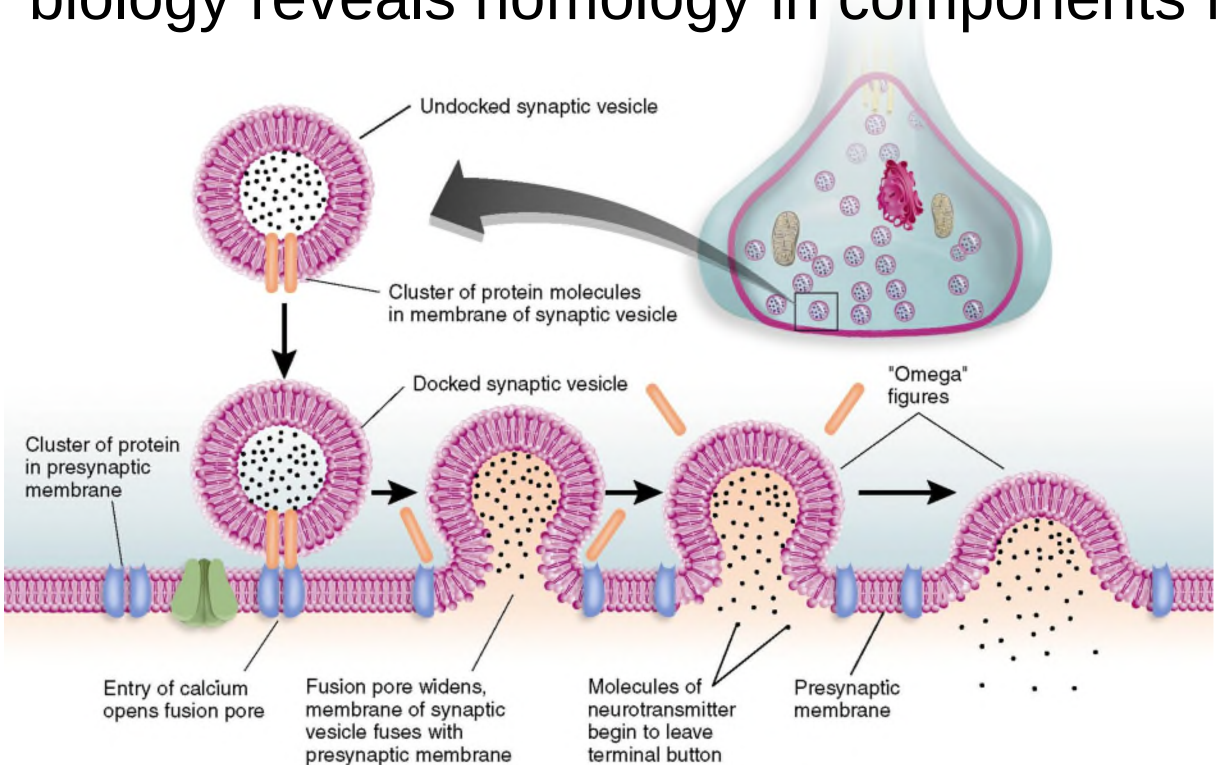
## Inside the neuron IV:

## Presynaptic secretion

# What is the molecular machinery of synaptic vescicles fusion ?

## Some observations:

- Synaptic transmission, a specialized form of normal exocytosis.
- Specific proteins on vesicles and targets guide the process
- Molecular biology reveals homology in components from yeast to man



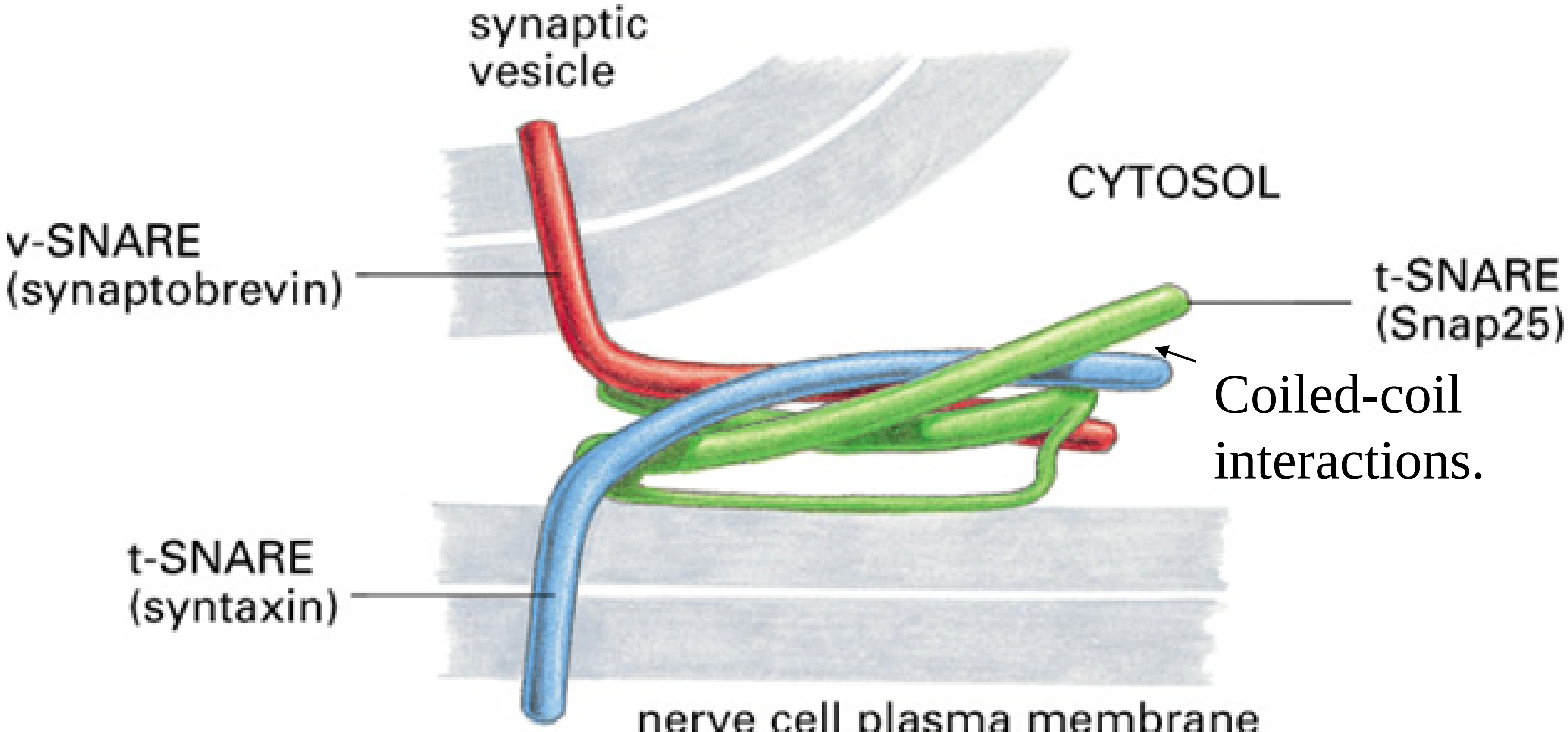
# The SNARE hypothesis

- Synaptic vesicles have a specific protein that directs them to a receptor on the plasma membrane (with the aid of other proteins SNAPs etc.).

- The vesicle associated SNAP receptor (v-SNARE) in neurons is believed to be synaptobrevin (VAMP).

- Target sites such as the plasma membrane (nerve terminal) would have a corresponding t-SNARE (syntaxin and SNAP-25).

- At sites other than the nerve terminal specific vesicle and target SNAREs would function to target vesicles to specific compartments.



Coiled-coil interactions.

Figure 13–12. Molecular Biology of the Cell, 4th Edition.

# The SNARE mechanism is shared among many membrane fusion events

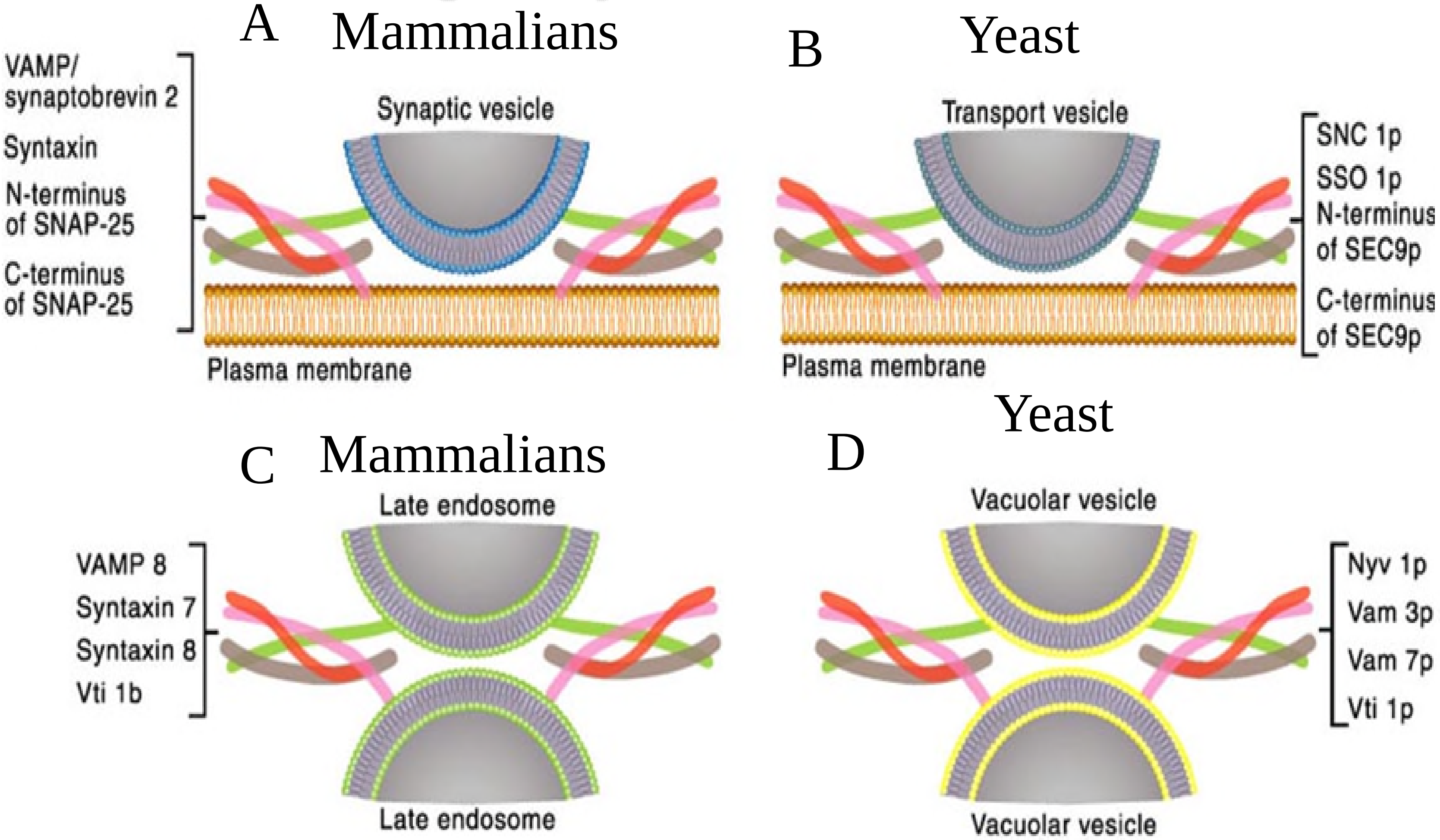


**FIGURE 7** Neurotransmitter release shares a core mechanism with many membrane fusion events within eukaryotic cells. The fusion of synaptic vesicles (A) is driven by a particular complex of four coiled-coil domains contributed by three different proteins. Exocytosis in yeast (B), the fusion of late endosomes in mammalian cells (C), and the fusion of vacuolar vesicles in yeast (D) exemplify the closely related four-stranded coiled–coil complexes required to drive fusion in other membrane-trafficking steps.

# Generic fusion mechanism involves a tightening of the SNAREs to push out $H_20$

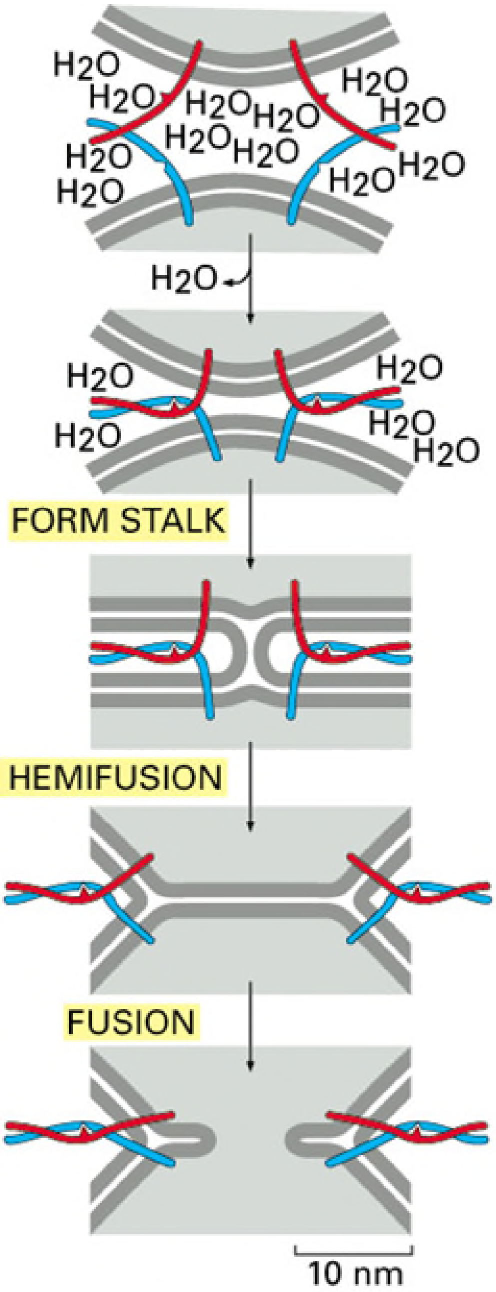


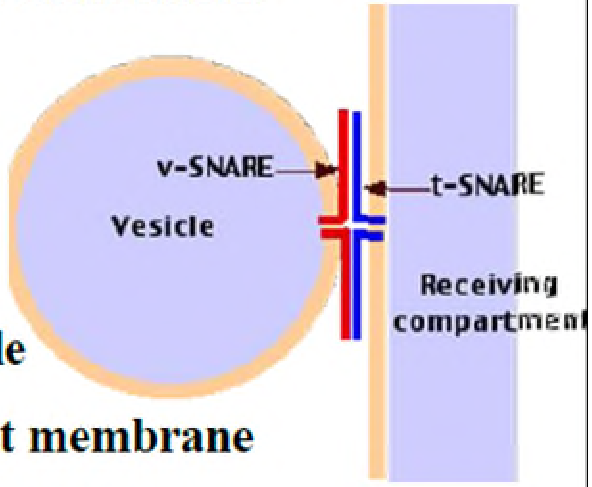
Figure 13–15. Molecular Biology of the Cell, 4th Edition.

# Specificity of vesicle fusion

Need mechanism for selective vesicle trafficking -controlled by SNAREs and Rab proteins

SNARE hypothesis proposes specific interactions between v- SNAREs and t-SNAREs govern vesicle docking and fusion

Each organelle has specific SNAREs leading to specific vesicle fusion



# Vesicle docking controlled by Rab proteins

Monomeric GTPases attach to surface of budding vesicle

Rab-GTP on vesicle interacts with Rabeffector on target membrane

After vesicle fusion GTP hydrolysed, triggering release of Rab-GDP

Different Rab proteins found associated with different membrane-bound organelles

# Comparison between intra-organelles and synaptic vescicle trafficking

Different Rab GTPases & Rab effectors control docking of different vesicles on target membranes: vesicle docking controlled by Rab protein.

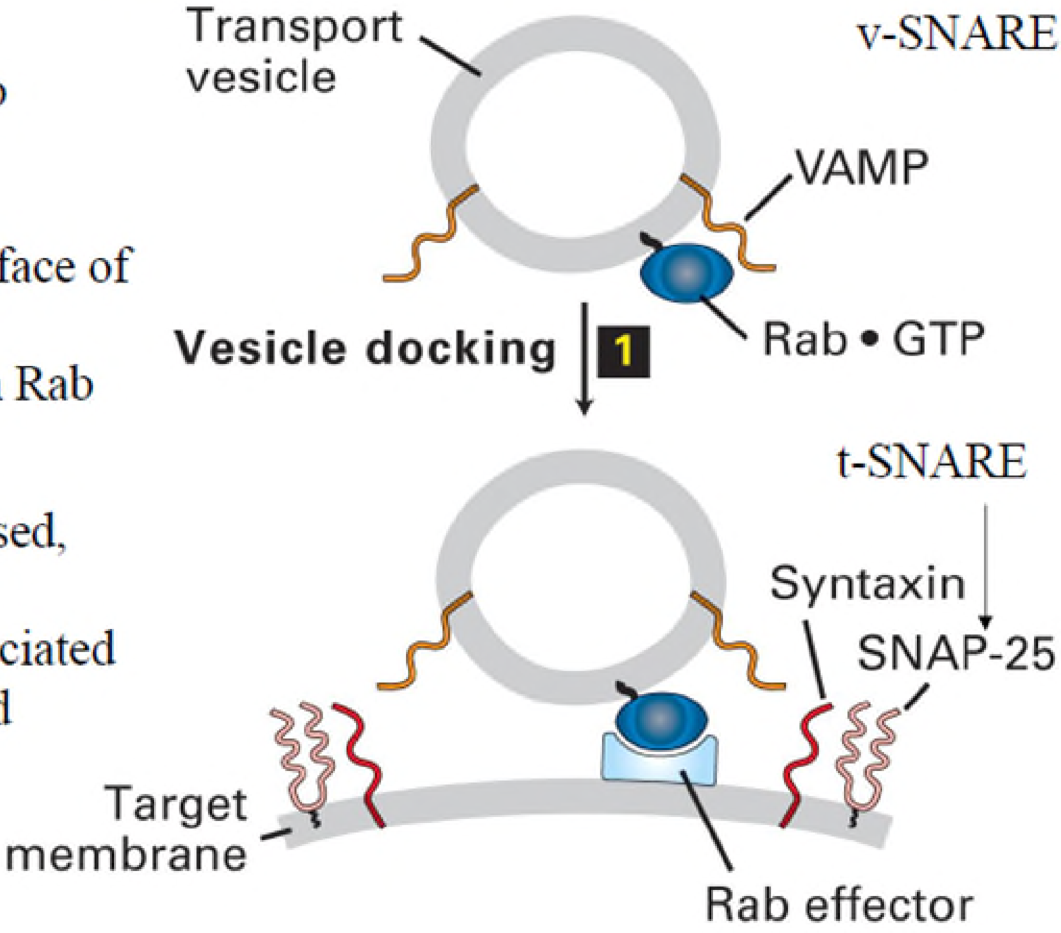
Vesicle docking controlled by Rab proteins

Monomeric GTPases attach to surface of budding vesicle

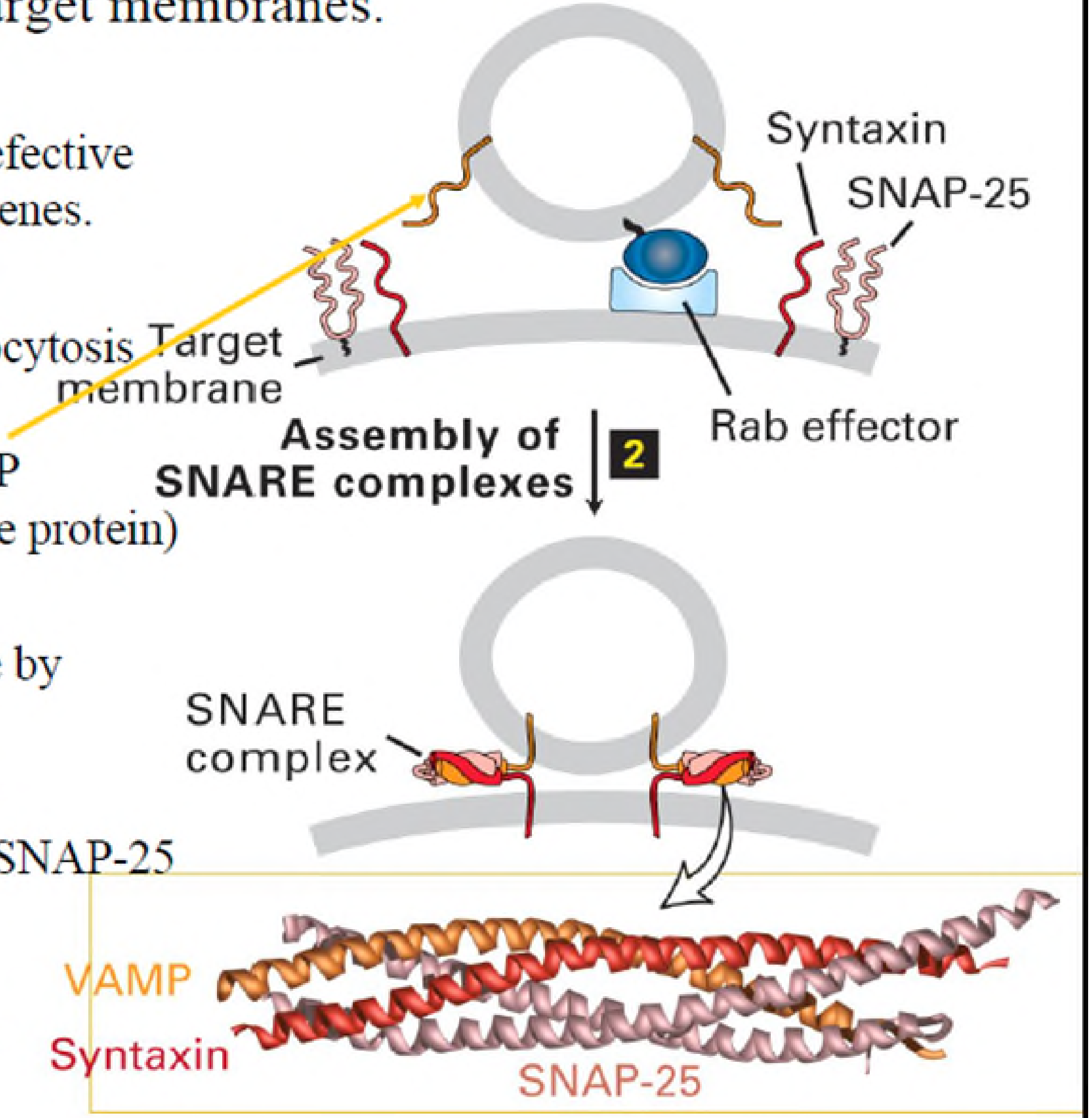
Rab-GTP on vesicle interacts with Rab effector on target membrane

After vesicle fusion GTP hydrolysed, triggering release of Rab-GDP

Different Rab proteins found associated with different membrane-bound organelles



# Paired sets of SNARE proteins mediates fusion of vesicles with target membranes.



Analysis of yeast *sec* mutants defective in each of the >20 SNARE genes.

In vitro liposome fusion assay.

SNARE-mediated fusion → exocytosis → secretory protein

In this case, v-SNARE as VAMP (vesicle associated membrane protein)

t-SNAREs are syntaxin

SNAP-25 attached to membrane by hydorphobic anchor.

Formation of four-helix bundle:

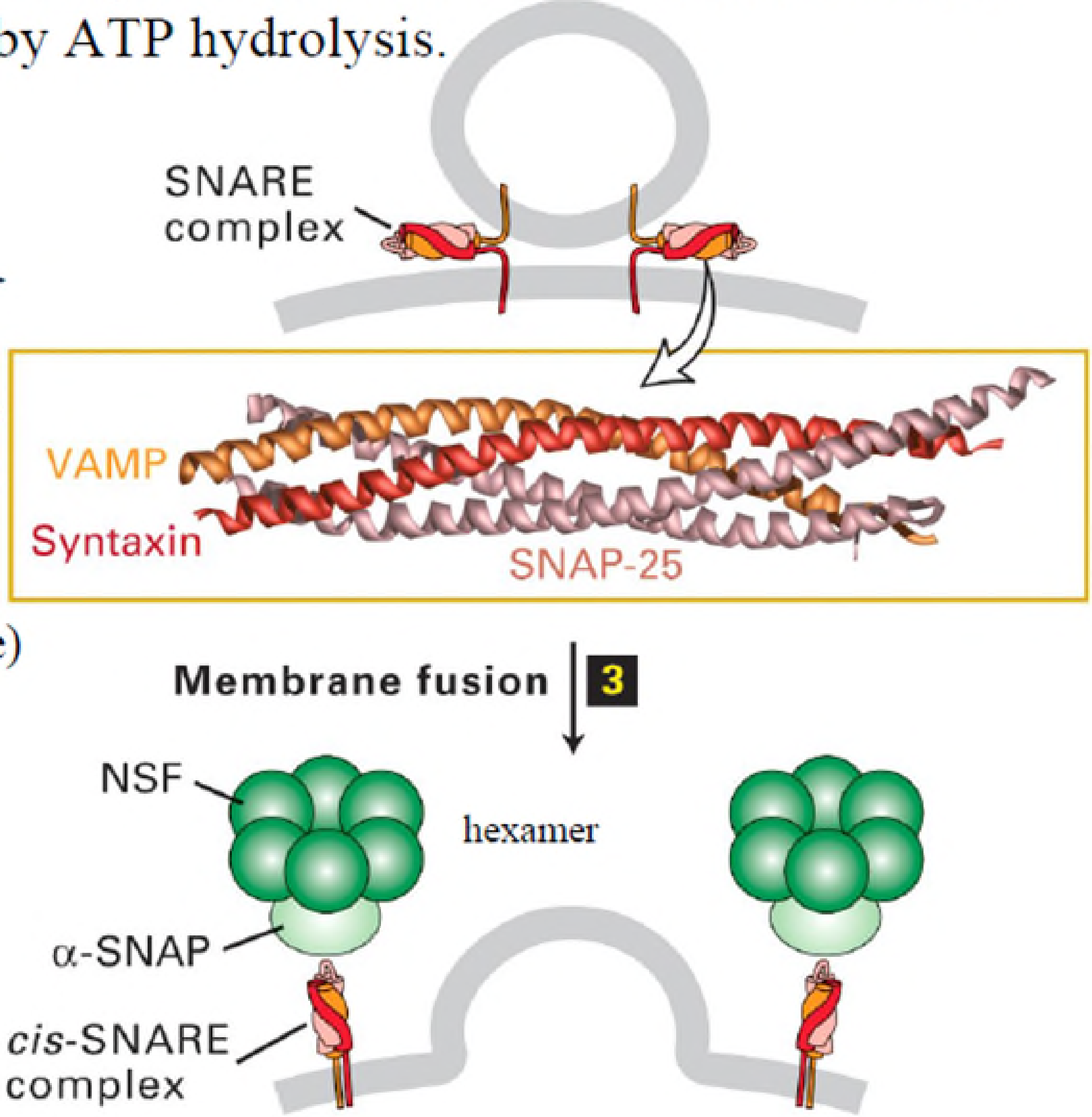
VAMP (1), Syntaxin (1) and SNAP-25 (2)

But, in COPII with cis, each SNARE has provide one helix

SNARE complex had specificity

# Dissociation of SNARE complexes after membrane fusion is driven by ATP hydrolysis.

- SNARE complex formation by non-covalent interaction. Dissociate → free SNARE → can fuse next time
- Two protein play important role of dissociation or fusion with a target membrane: NSF (NEM-sensitive factor, blocked by N-ethylmaleimide) & α-SNAP (soluble NSF attachment protein).



Soluble (i.e. cytoplasmic) Factors

**NSF** or n-ethylmaleimide (**N**EM) **S**ensitive **F**actor

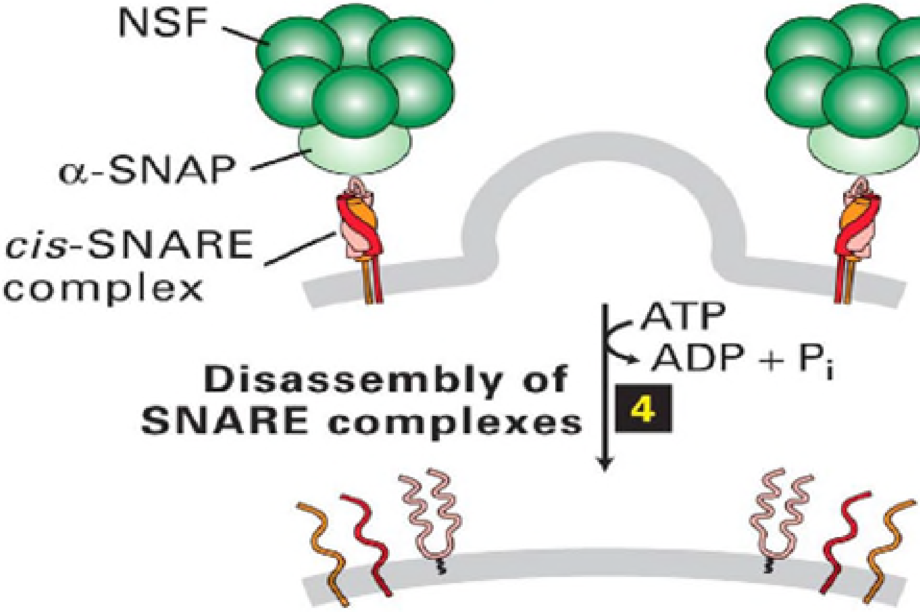
**SNAP**- **S**oluble **N**SF **A**ttachment **P**roteins
NSF + SNAP bind to target membranes (synaptic vesicle & plasma membrane)

Receptors for NSF and SNAP are synaptobrevin (vesicle), SNAP-25 (plasma membrane) and syntaxin (plasma membrane)

Membrane targets are called **SNARE**s (v- and t-) **S**oluble **N**SF **A**ttachment protein **RE**ceptors

SNAP-25- **Syn**aptosome **A**ssociated **P**rotein of **25** kDa
- Over-expression of truncated SNAP-25 blocks release
- Syntaxin, 15 kDa protein
- Sensitive to botulinum toxin A cleavage - release prevented

# Dissociation of SNARE complexes after membrane fusion is driven by ATP hydrolysis.



ATP is not actually required for release once vesicles are docked, but is thought to break down the SNARE complexes to promote recycling.

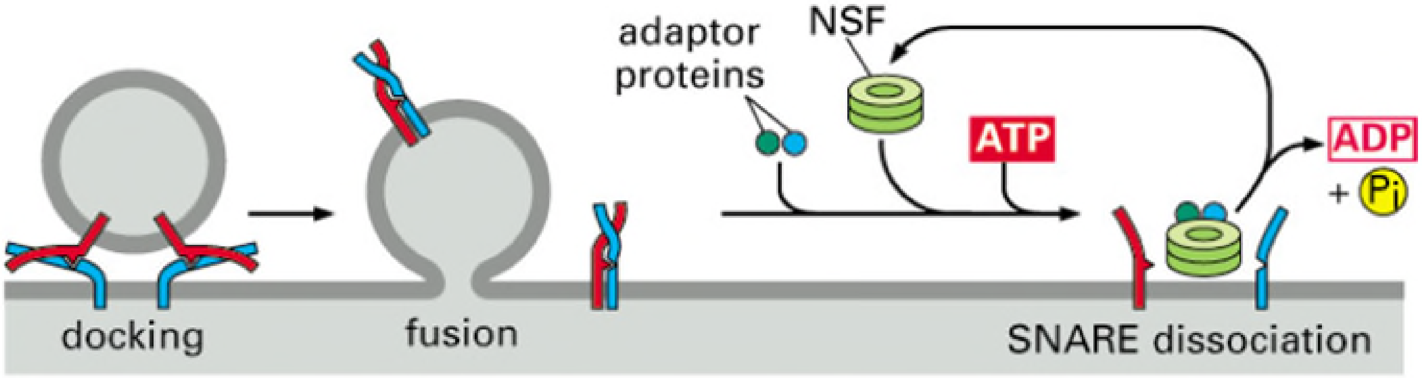
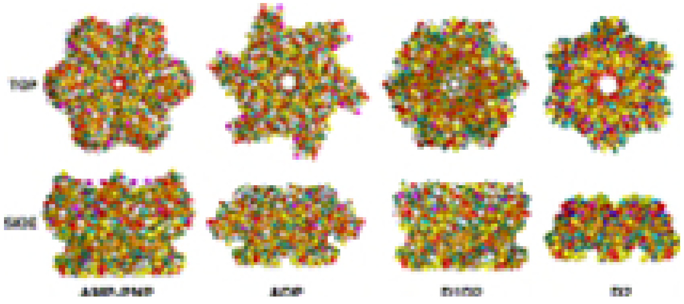
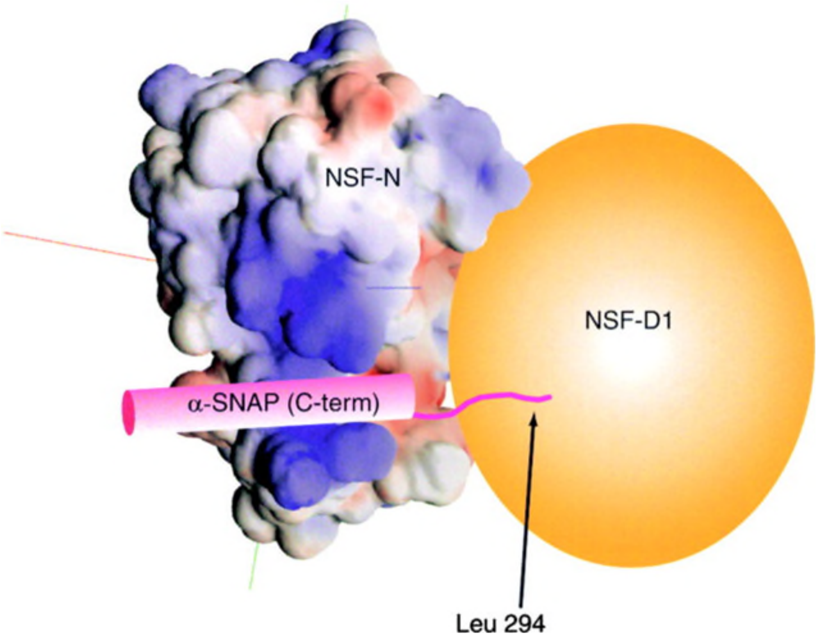


Figure 13–13. Molecular Biology of the Cell, 4th Edition.





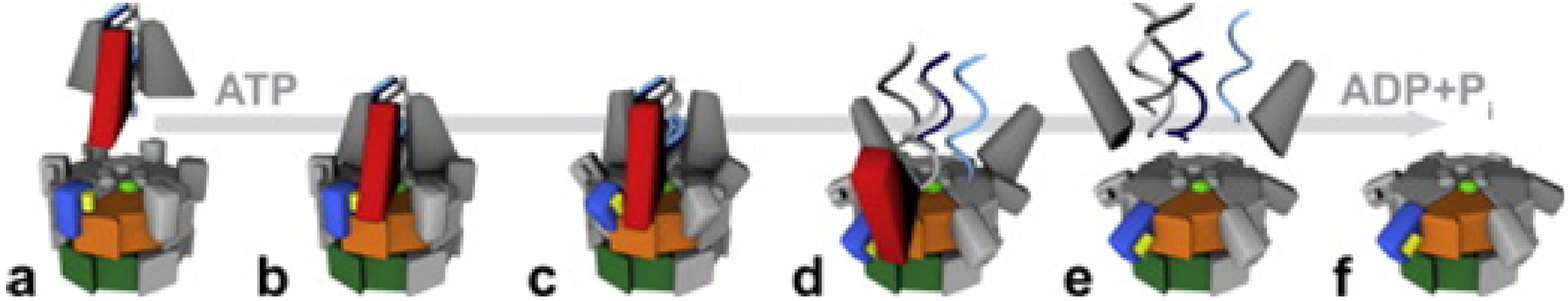
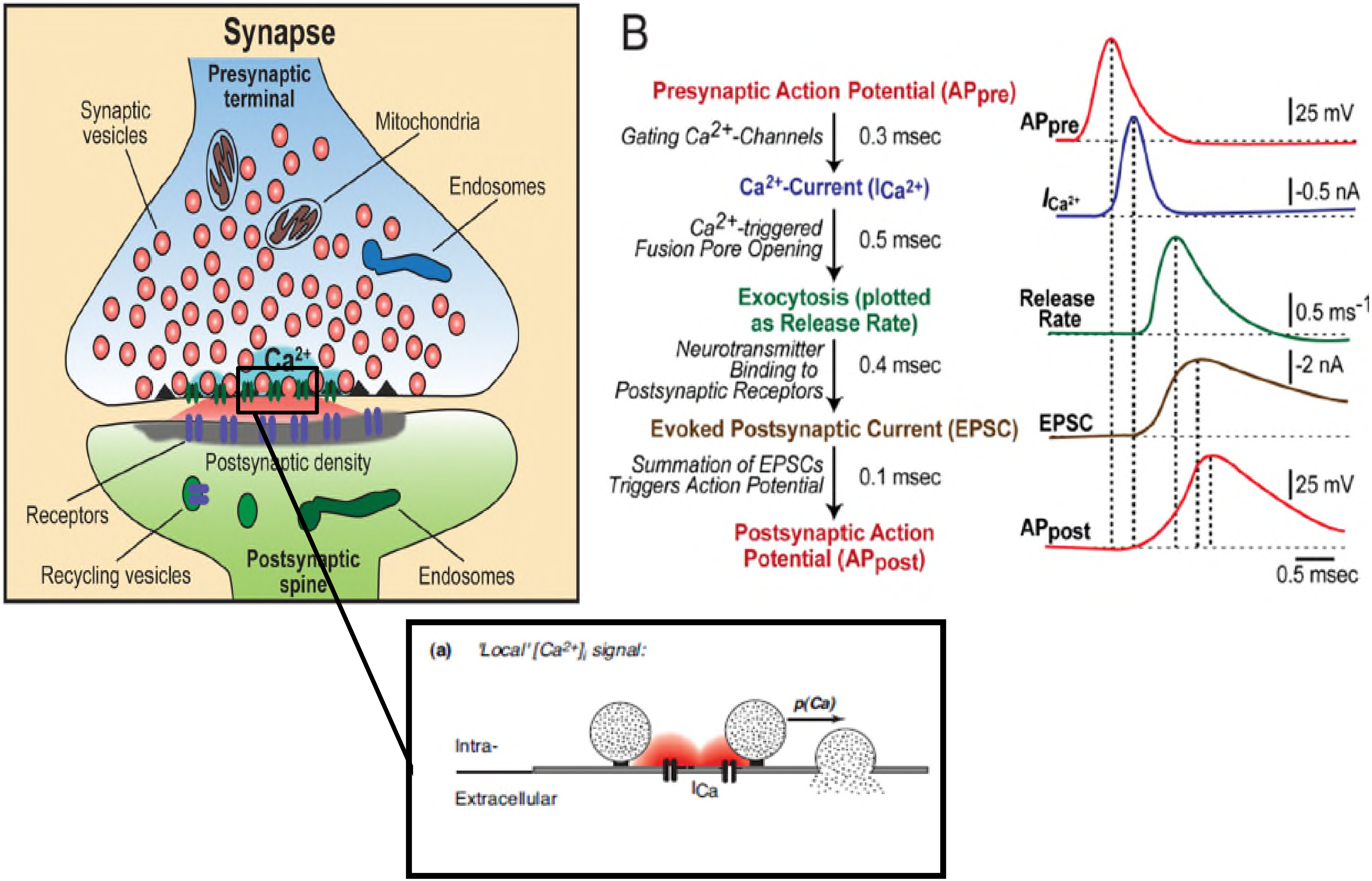


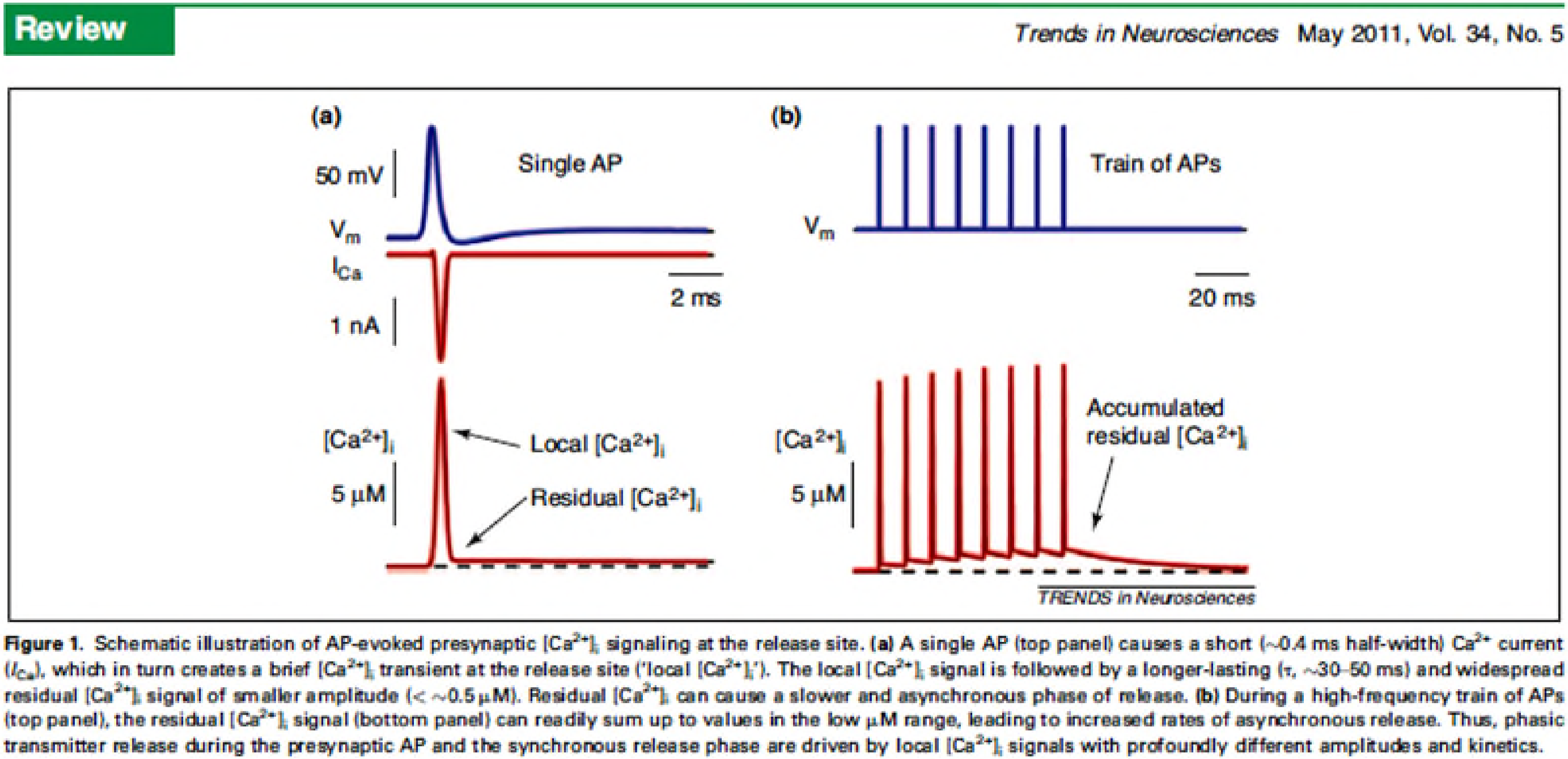
Fig.5. Potential mechanism of NSF. NSF-ATP binds to the C-terminus of α-SNAP (red wedge), which is bound to the coiled coil of the SNARE complex (a and b). Upon ATP hydrolysis the N-domains (blue block) pivot around the D1-domain, away from the center pulling, the α-SNAP and SNARE complex (c and d). This radial force serves to separate the SNAREs and thus disassemble the complex (e and f). The positively charged surface of NSF-N, which is important for SNAP–SNARE binding, is depicted as a yellow bar. The pore residues in NSF-D1 are depicted as green spheres. NSF-D1 and NSF-D2 are depicted as orange and green blocks, respectively.

**But....what about Calcium??**

# Ca2+ signalling at presynaptic sites



# Ca2+ signalling at presynaptic sites



**Review**

*Trends in Neurosciences* May 2011, Vol. 34, No. 5

**Figure 1.** Schematic illustration of AP-evoked presynaptic $[Ca^{2+}]_i$ signaling at the release site. **(a)** A single AP (top panel) causes a short (~0.4 ms half-width) $Ca^{2+}$ current ($I_{Ca}$), which in turn creates a brief $[Ca^{2+}]_i$ transient at the release site ('local $[Ca^{2+}]_i$'). The local $[Ca^{2+}]_i$ signal is followed by a longer-lasting (τ, ~30–50 ms) and widespread residual $[Ca^{2+}]_i$ signal of smaller amplitude (< ~0.5 µM). Residual $[Ca^{2+}]_i$ can cause a slower and asynchronous phase of release. **(b)** During a high-frequency train of APs (top panel), the residual $[Ca^{2+}]_i$ signal (bottom panel) can readily sum up to values in the low µM range, leading to increased rates of asynchronous release. Thus, phasic transmitter release during the presynaptic AP and the synchronous release phase are driven by local $[Ca^{2+}]_i$ signals with profoundly different amplitudes and kinetics.

**Olexiy Kochubey[1], Xuelin Lou[2] and Ralf Schneggenburger[1]**

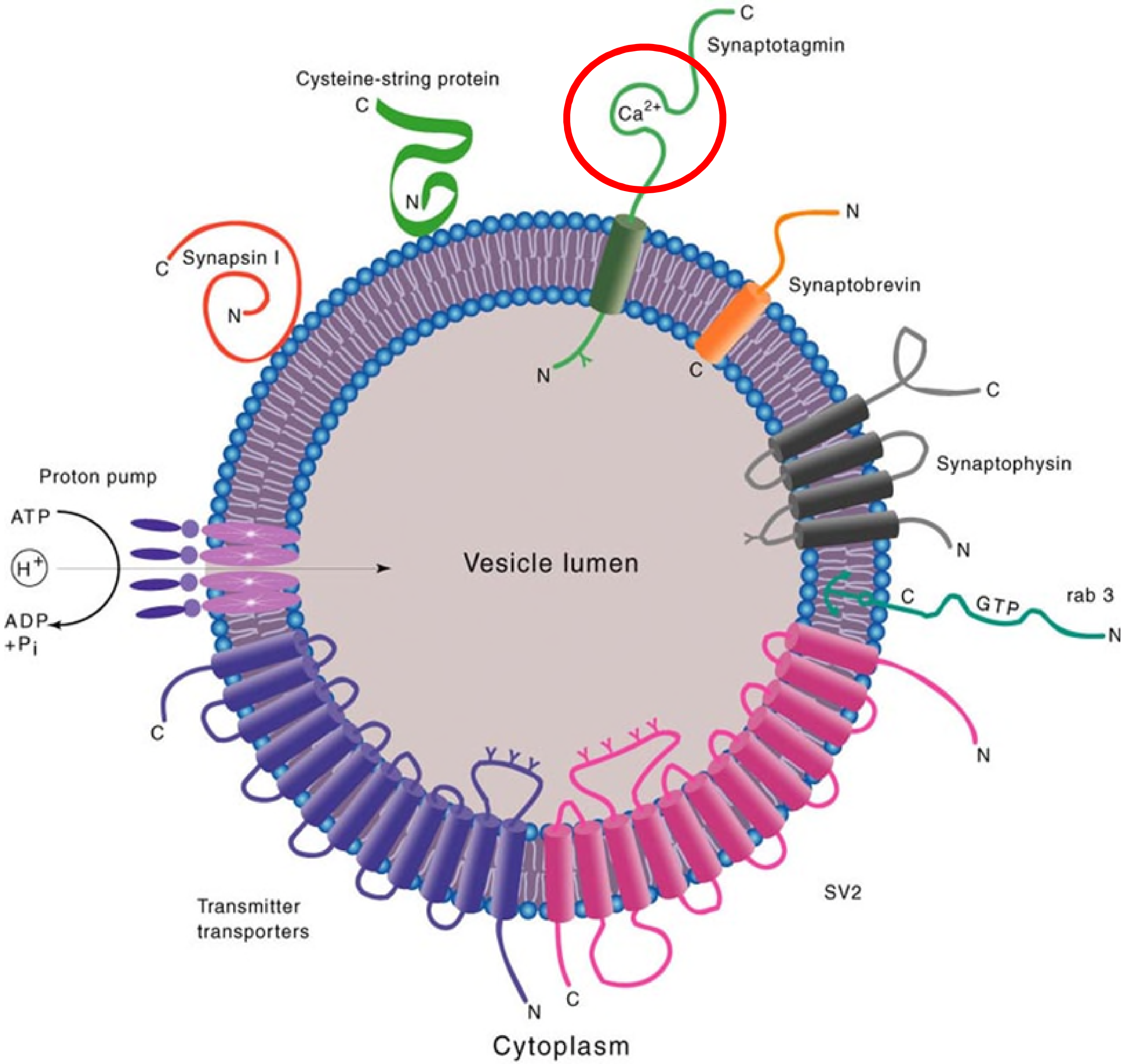
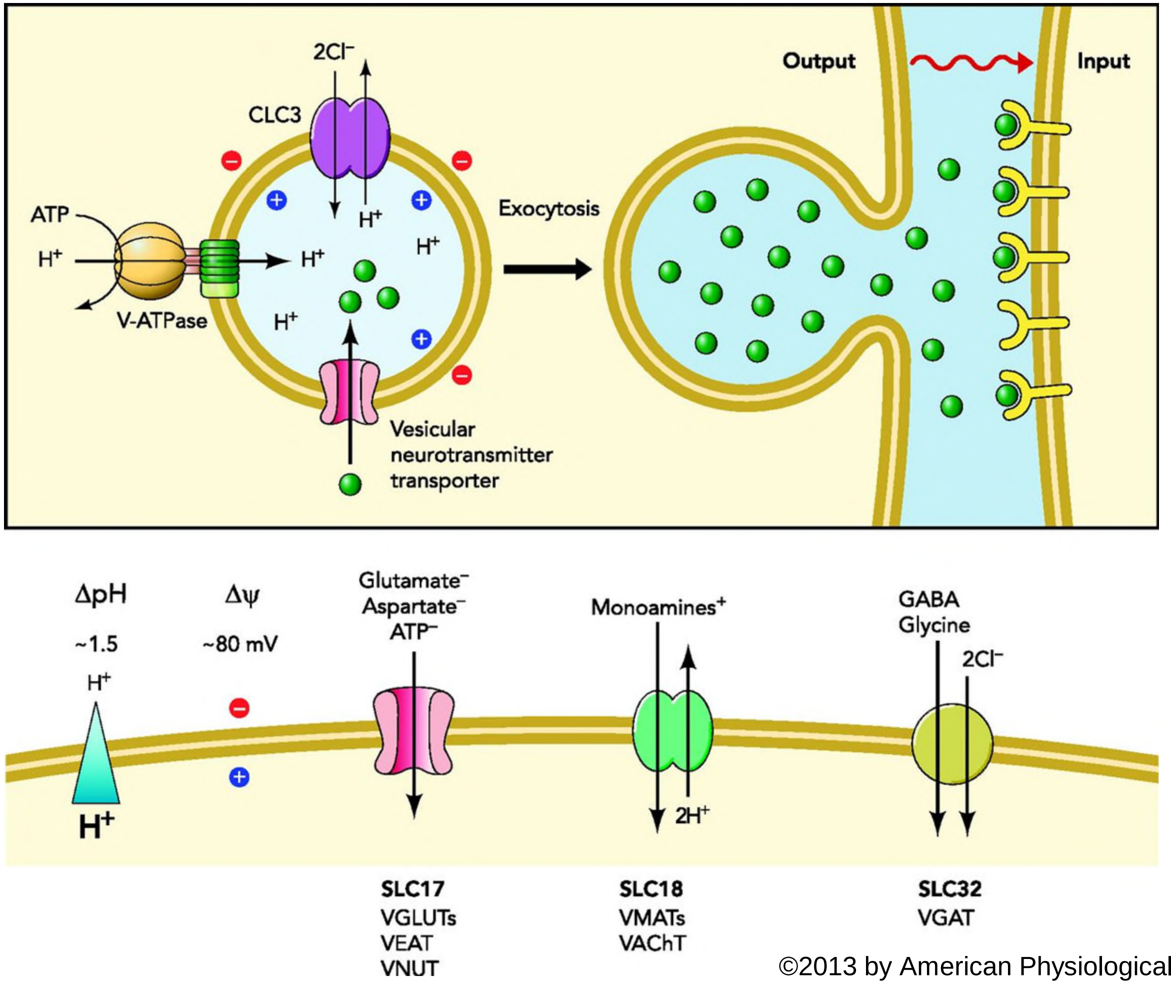


**FIGURE 4** Schematic representation of the structure and topology of major synaptic vesicle membrane proteins (see also Table 8.1).

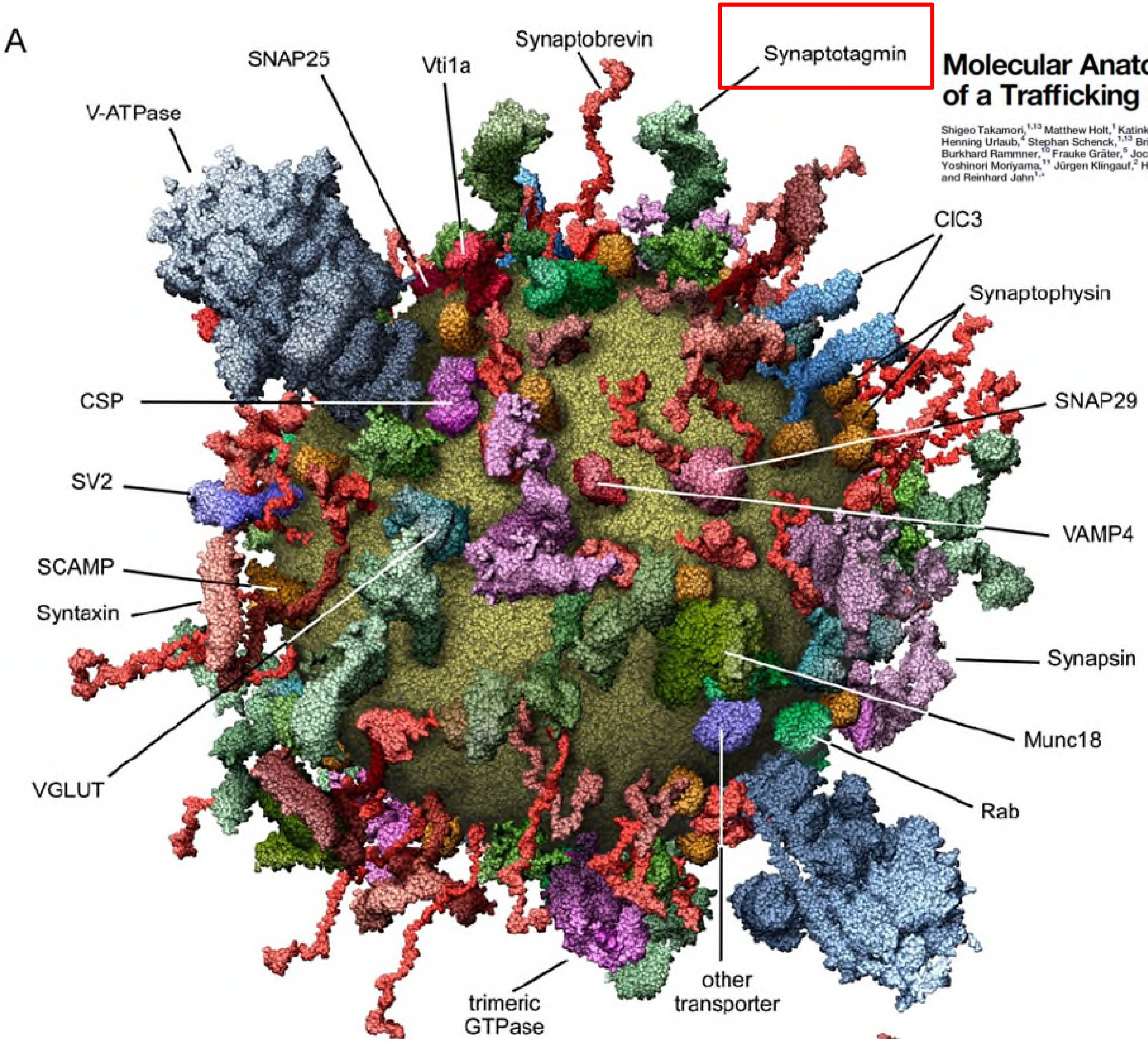
# V-ATPase-coupled neurotransmitter accumulation
# Vacuolar-type H+-ATPase (V-ATPase) pumps H+ into secretory vesicles.



Physiology

V-ATPase-coupled neurotransmitter accumulationVacuolar-type $H^+$-ATPase (V-ATPase) pumps $H^+$ into secretory vesicles. It generates an electrochemical $H^+$ gradient composed of a $H^+$ gradient and an inside-positive membrane potential. Typical $H^+$ gradient (ΔpH) and membrane potential (ΔΨ) of synaptic vesicles are 1.5 and 80 mV, respectively. Vesicular neurotransmitter transporters use such energy as their driving force. VGLUTs and other SLC17 type transporters transport anionic substrates using membrane potential as the driving force. VMATs and VAChT transport cationic monoamines with a counterflow of 2 $H^+$ per substrate molecule. On the other hand, VGAT is a cotransporter of GABA and 2 $Cl^-$. CLC3 $Cl^-$ channel performs the exchange of $H^+$ and $Cl^-$ and thereby controls internal $H^+$ concentration and membrane potential.

**Hiroshi Omote, and Yoshinori Moriyama Physiology 2013;28:39-50**



## Molecular Anatomy of a Trafficking Organelle

Shigeo Takamori,[1,13] Matthew Holt,[1] Katinka Stenius,[7,14] Edward A. Lemke,[2] Mads Grønborg,[1,4] Dietmar Riedel,[3] Henning Urlaub,[4] Stephan Schenck,[1,13] Britta Brügger,[8] Philippe Ringler,[9] Shirley A. Müller,[9] Burkhard Rammner,[10] Frauke Gräter,[5] Jochen S. Hub,[5] Bert L. De Groot,[5] Gottfried Mieskes,[1] Yoshinori Moriyama,[11] Jürgen Klingauf,[2] Helmut Grubmüller,[6] John Heuser,[12] Felix Wieland,[8] and Reinhard Jahn[1,*]

Cell *127*, 831–846, November 17, 2006 ©2006 Elsevier

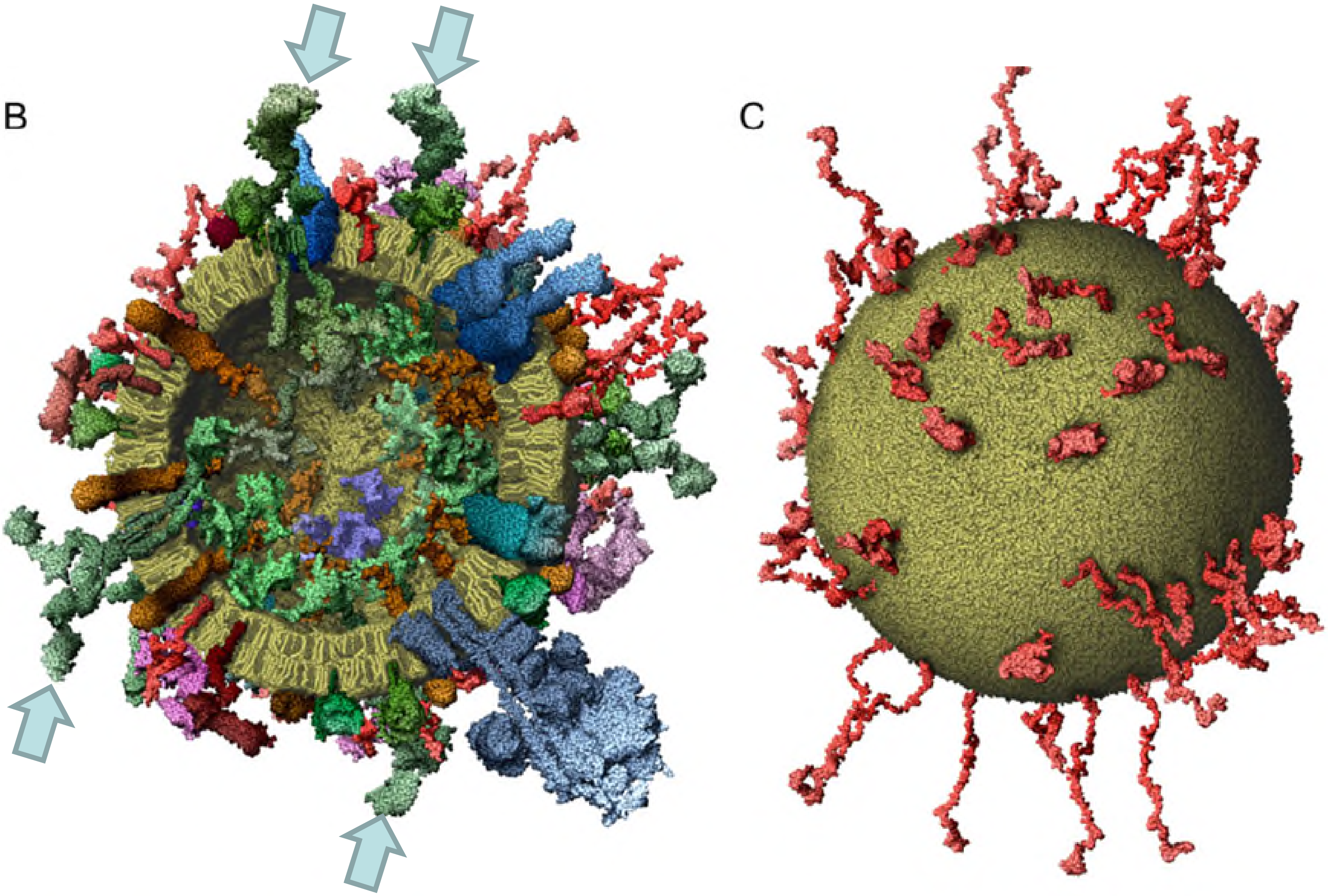


**Figure 4. Molecular Model of an Average SV**

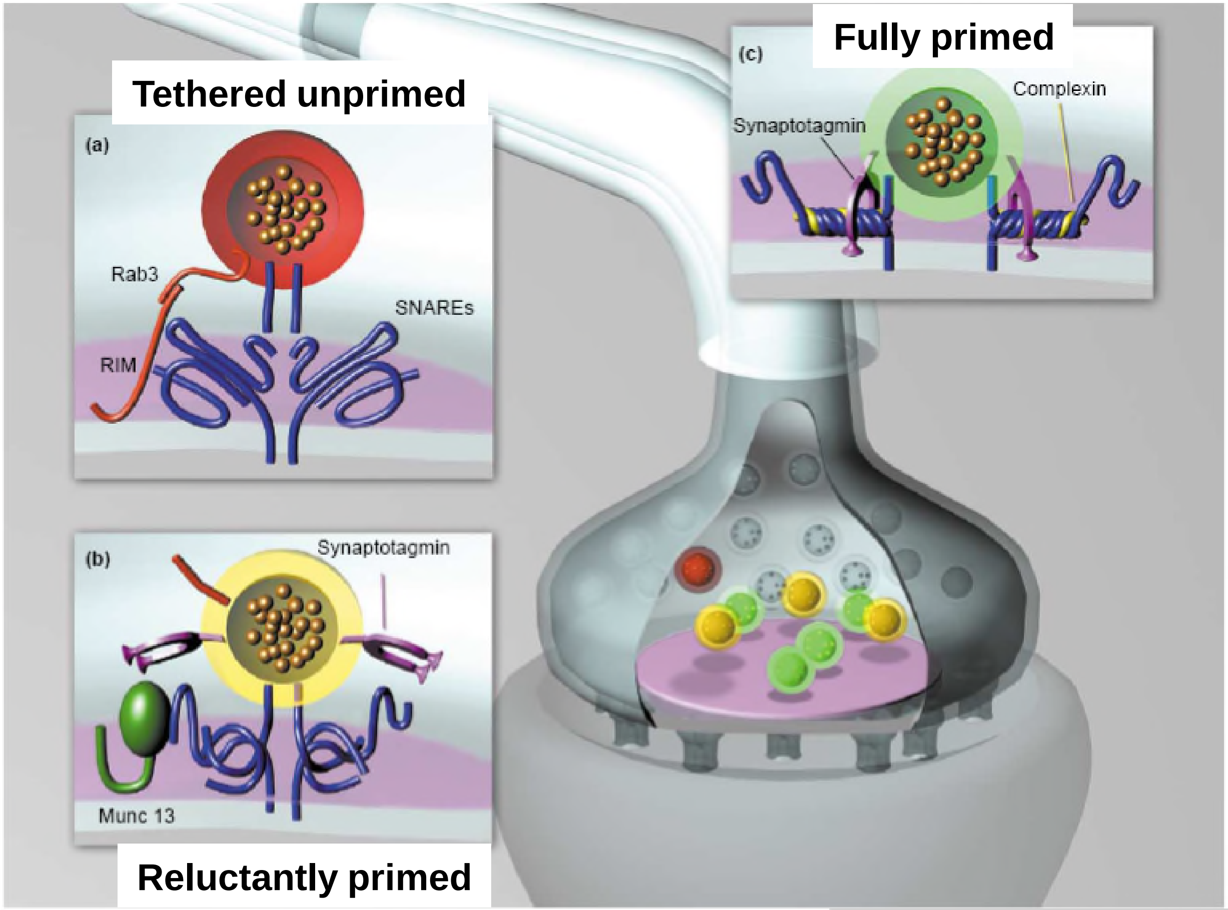
The model is based on space-filling models of all macromolecules at near atomic resolution.

(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.

In addition to the proteins listed in Table 2, the following proteins were included (copy number in parentheses): VAMP4 (2), SNAP-29 (1), vti1a (2) syntaxin 6 (2), other syntaxins (4), other synaptotagmins (5), other Rab proteins (15), Munc-18 (2), other transporters (2), chloride channels (2), and trimeric GTPases (2) (see Table S2 for more details).



Current Opinion in Neurobiology 2003, 13:509–519

Different stages of vesicle priming in central nerve terminals. The presynaptic terminal contains various neurotransmitter-filled vesicles, which are functionally defined as reserve vesicles (grey opaque), tethered unprimed vesicles (red), reluctantly primed (yellow), and fully primed (green). The reluctant and fully primed vesicles constitute the readily releasable vesicle pool. At each active zone (lilac circle), this pool consists of about 5-10 vesicles [24•]. The inserts document the current view of how presynaptic proteins regulate the priming and maturation status of vesicles and how this affects their properties. **(a)** The interaction of active zone specific RIM and the vesicular Rab3 is thought to be involved in vesicle docking and the initiation of vesicle priming. **(b)** During priming, a 'loose' form of the SNARE complex, consisting of synaptobrevin, SNAP-25 and Syntaxin 1 (SNAREs are the blue rods on the diagram) is formed with the aid of Munc13. The vesicles mature to this stage relatively quickly, but they exocytose only reluctantly in response to single action potentials. Their release probability is in the range of 1–2% and they are released rather asynchronously (time course 20 msec). The release probability of these vesicles is potentiated two to five-fold by Munc13s interacting with DAG/phorbol esters [31••]. **(c)** Fully primed vesicles require additional interactions with complexin and synaptotagmin 1 to increase vesicle release probability (to 12–14%) and to speed up the release time course (2 msec). It is thought that fully primed vesicles obtain their release properties either through the formation of a tightened SNARE complex structure as shown here, a defined interaction of synaptotagmin 1 with the plasma membrane and the SNARE complex, and/or their proximity to voltage-activated $Ca^{2+}$ channels.

Rizo and Sudhof 2002
Nature Rev. Neurosci.

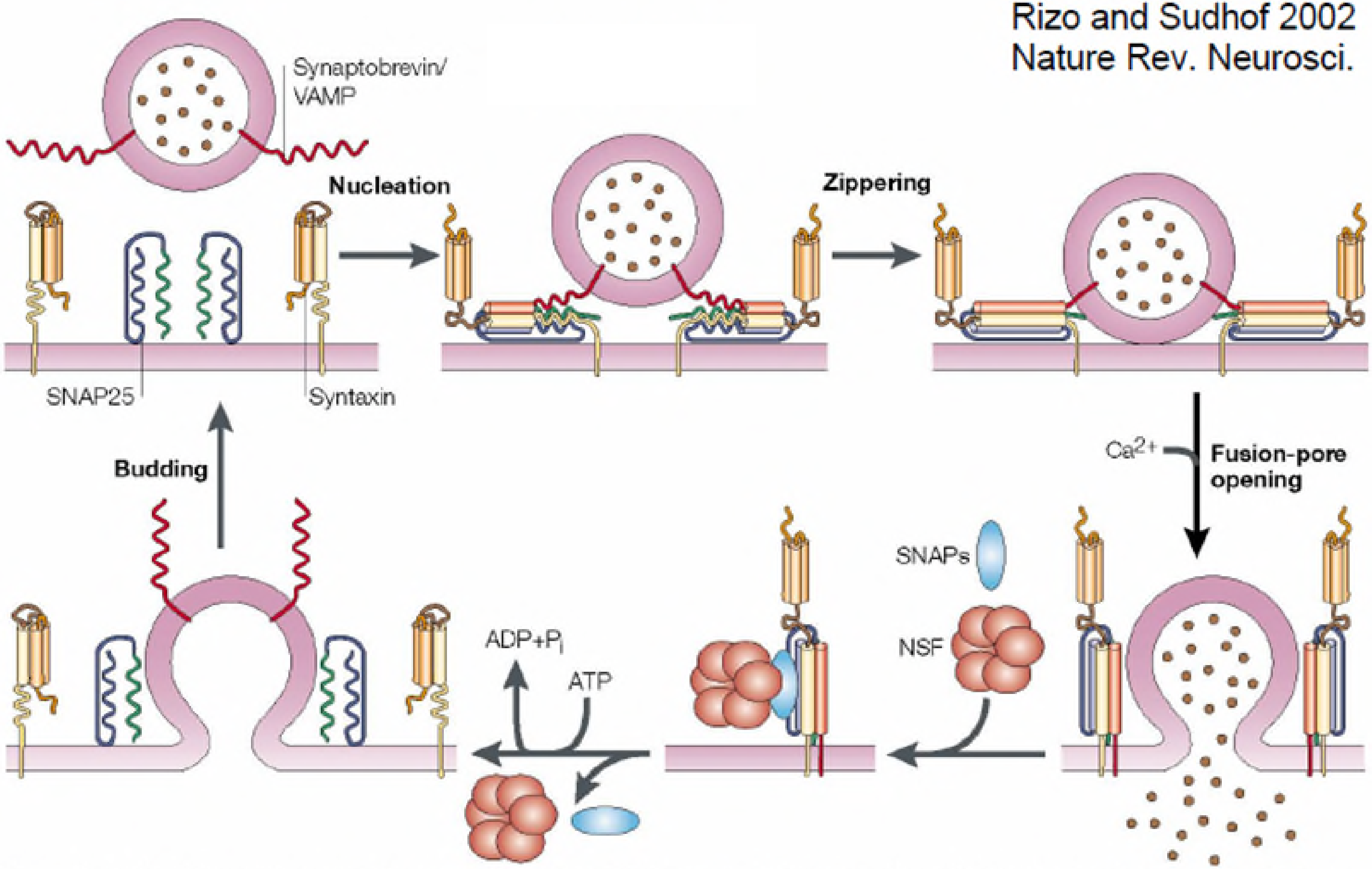


Figure 1 | **Cycle of assembly and disassembly of the SNARE complex in synaptic vesicle exocytosis.** Syntaxin exists in a closed conformation that needs to open to initiate core-complex assembly (nucleation). ‘Zippering’ of the four-helix bundle towards the carboxyl terminus brings the synaptic vesicle and plasma membranes towards each other, which might lead to membrane fusion. After fusion, *N*-ethylmaleimide-sensitive fusion protein (NSF) and soluble NSF-attachment proteins (SNAPs) disassemble the *cis*-core complexes that remain on the same membrane to recycle them for another round of fusion. SNAP25, synaptosomal-associated protein of 25 kDa; SNARE, SNAP receptor.

Rizo and Sudhof 2002
Nature Rev. Neurosci.

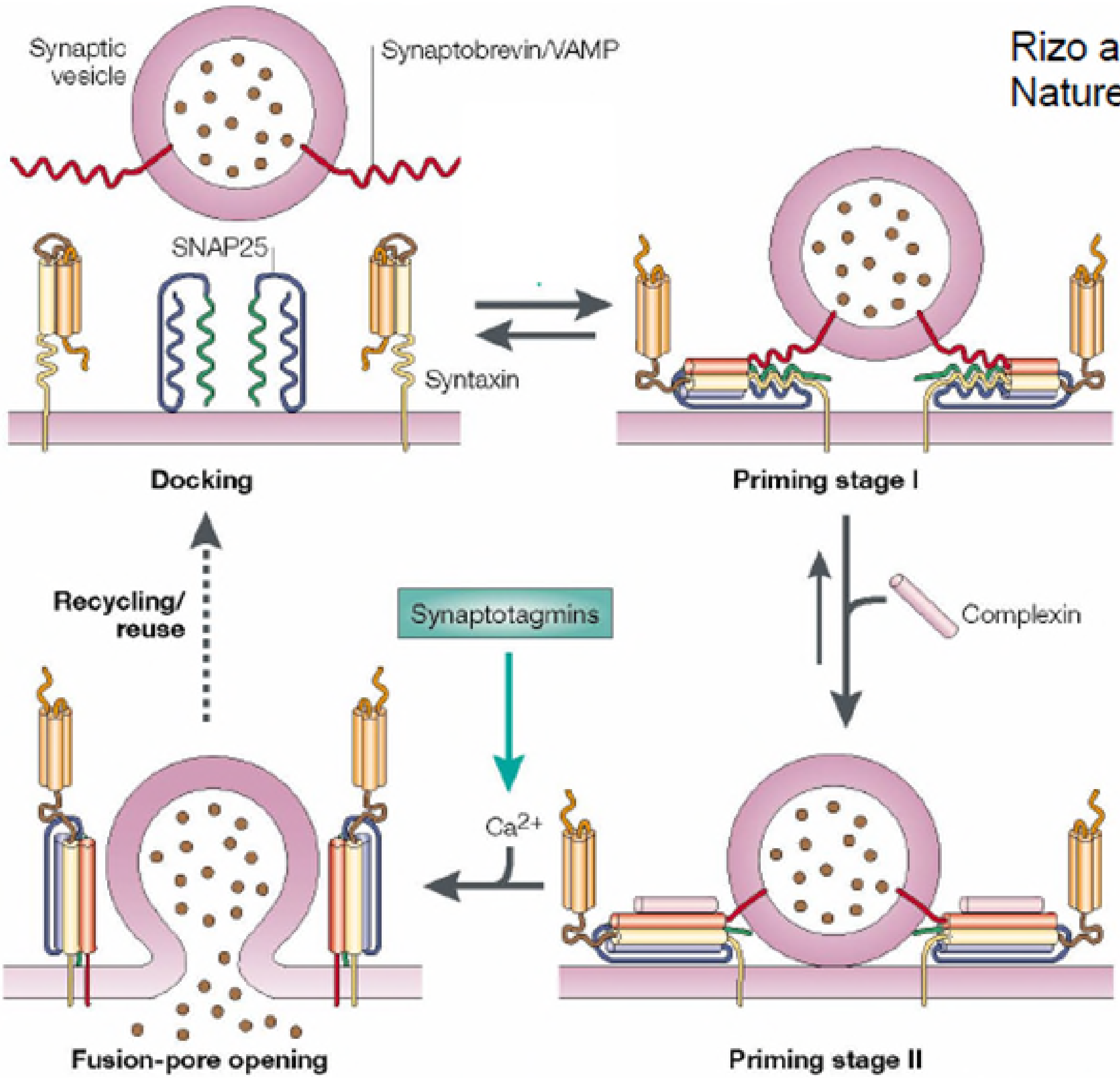
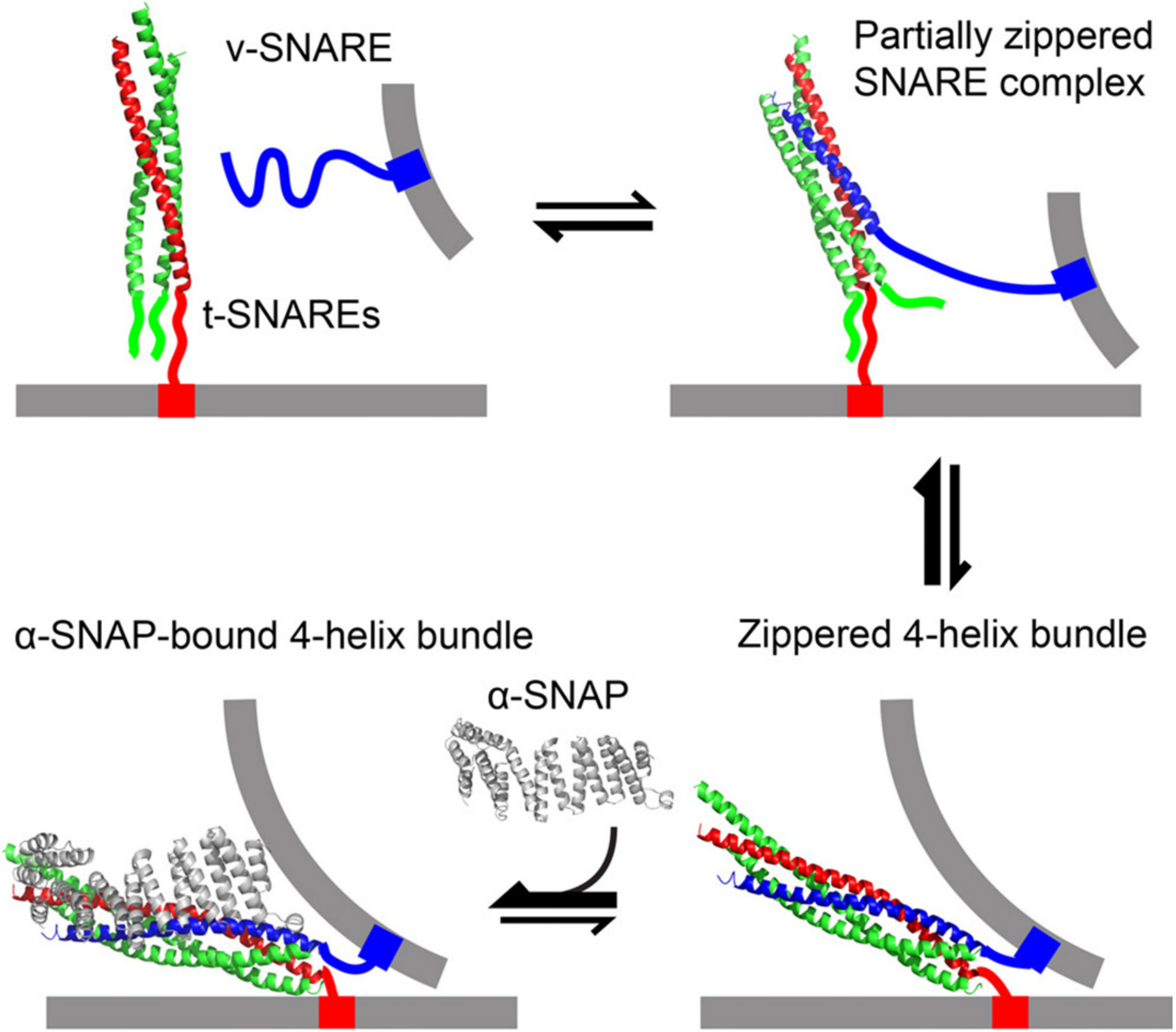
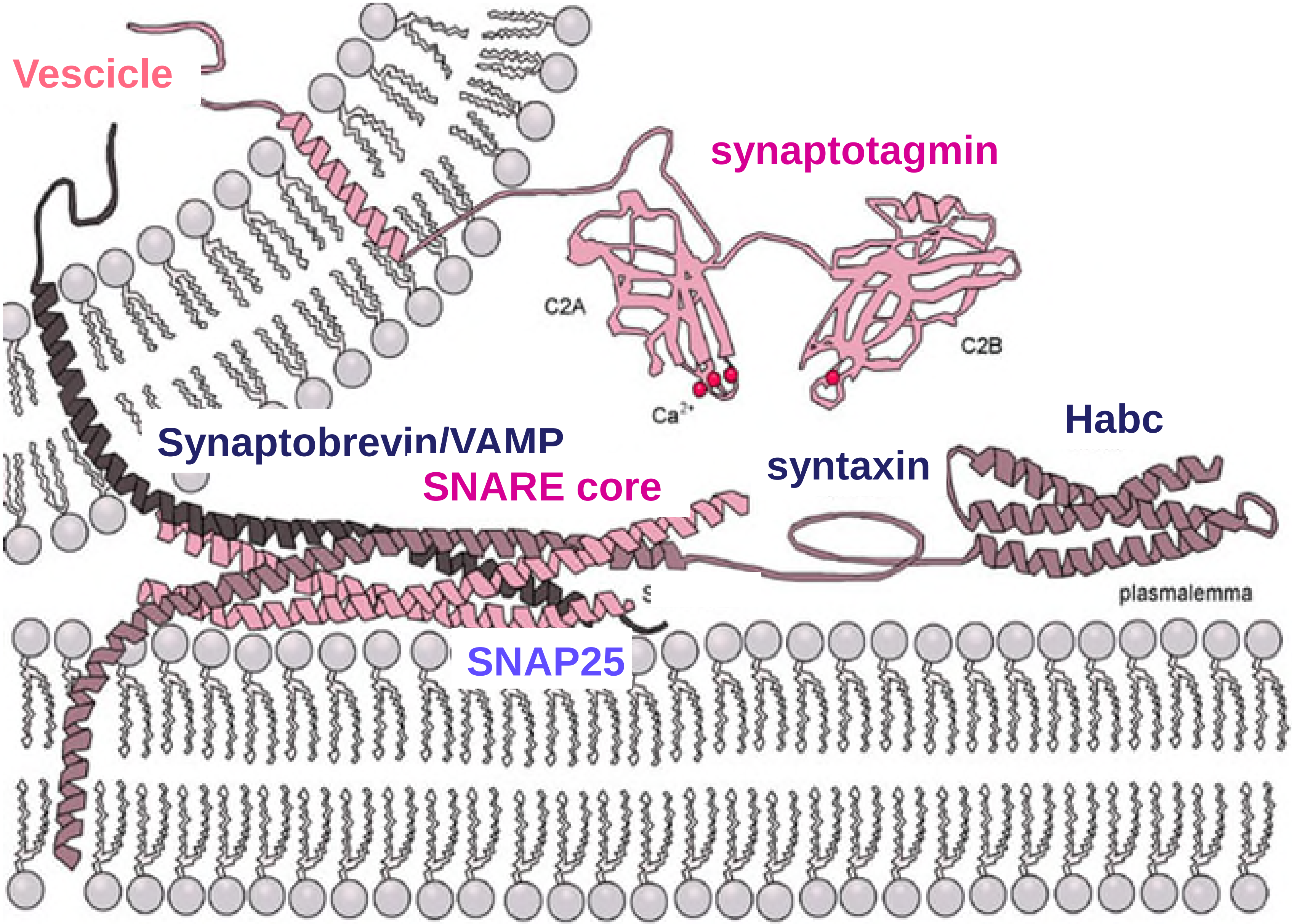


Figure 7 | **Model of complexin function.** The crucial aspect of this model is that priming might occur in two steps that involve partial and full assembly of the core complex. Only the fully assembled state, which is stabilized by complexin, can support fast, $Ca^{2+}$-triggered neurotransmitter release. SNAP25, synaptosomal-associated protein of 25 kDa.


v-SNARE
t-SNAREs
Partially zippered SNARE complex
Zippered 4-helix bundle
α-SNAP
α-SNAP-bound 4-helix bundle

# Molecular machinery driving vesicle fusion



# Synaptotagmin (P65) $Ca^{2+}$ binding.

- Single polypeptide, one transmembrane spanning region and large cytoplasmic domain.

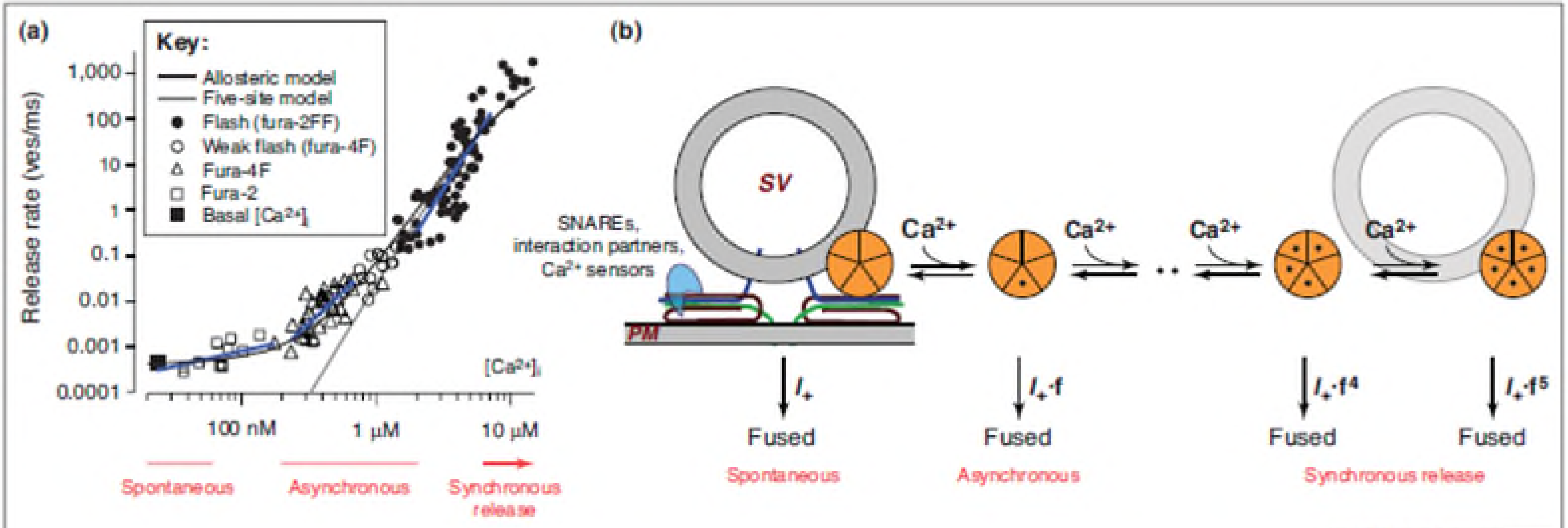
- Protein kinase C like homology in carboxyl terminal (C2 domain), involved in calcium and phospholipid binding. Injection of C2 peptide can block release after the vesicle is docked (squid; Augustine lab).

- Forms tetramers with each binding calcium (possible) cooperatively consistent with 3-4 power of Ca dependence of release (Sudhof and others).

- In vitro studies it binds Ca cooperatively at concentrations in the physiological range (10-100 $\mu$M) for release.

- In the absence of $Ca^{2+}$ synaptotagmin may serve as a brake for release. $Ca^{2+}$ removes the brake. May explain how release in non-neuronal cells occurs without synaptotagmin.

# Synchronous and Asynchronous release of synaptic vescicles



*TRENDS in Neurosciences*

**Figure 2.** The intracellular $Ca^{2+}$-sensitivity of transmitter release over a wide range of $[Ca^{2+}]_i$, and the allosteric model of $Ca^{2+}$ binding and vesicle fusion. **(a)** Dose–response curve of the intracellular $Ca^{2+}$-sensitivity of release determined over a wide range of $[Ca^{2+}]_i$ in young rat calyces of Held (postnatal days P8–P11) [18]. For the different concentration ranges, $[Ca^{2+}]_i$ was either controlled by presynaptic $Ca^{2+}$-buffer loading together with the indicated $Ca^{2+}$ indicators (i.e. fura-4F or fura-2), or instead by using weak or strong flashes in $Ca^{2+}$ uncaging experiments as indicated. The blue fit lines show linear fits in double-logarithmic coordinates (slope values 0.66 at $[Ca^{2+}]_i$ <0.2 μM, 2.77 at intermediate $[Ca^{2+}]_i$ <1 μM, and 4.2 at high $[Ca^{2+}]_i$ >2 μM). Note that the five-site model (grey line) failed to predict release rates at low $[Ca^{2+}]_i$ <2 μM, whereas the allosteric model could fit the data well (black line). The $[Ca^{2+}]_i$ ranges relevant for spontaneous, asynchronous and phasic $Ca^{2+}$-evoked release are highlighted. Reproduced, with permission, from [18]. **(b)** Graphical representation of the allosteric model of $Ca^{2+}$ binding and synaptic vesicle (SV) fusion [18]. The model allows low rates of vesicle fusion from the $Ca^{2+}$-free state (spontaneous fusion; rate constant $l_+$) and from partially $Ca^{2+}$-bound states of the vesicle fusion machinery. Fusion rates rise in geometric progression upon further $Ca^{2+}$-binding to a general $Ca^{2+}$ sensor complex with maximally five $Ca^{2+}$ ion-binding sites (shown in orange). The states with four and five bound $Ca^{2+}$ ions largely carry the synchronous release component evoked by $[Ca^{2+}]_i$ >2 μM (the complete state diagram of the allosteric model is presented in Figure S1b in the supplementary material online).

The temporal resolution between Ca2+ influx induced by action potentials and SV fusion coupled to Ca2+ influx differs in synchronous and asynchronous release. The temporal precision largely depends on the distance between SV and VGCC, which matters for the timing of SV fusion. Fast synchronous neurotransmission is mediated by SV coupled to VGCC, while recruitment of new vesicles to the proximity of VGCC seems to contribute to the delayed asynchronous release.

# Structure and C2+ properties of synaptotagmins

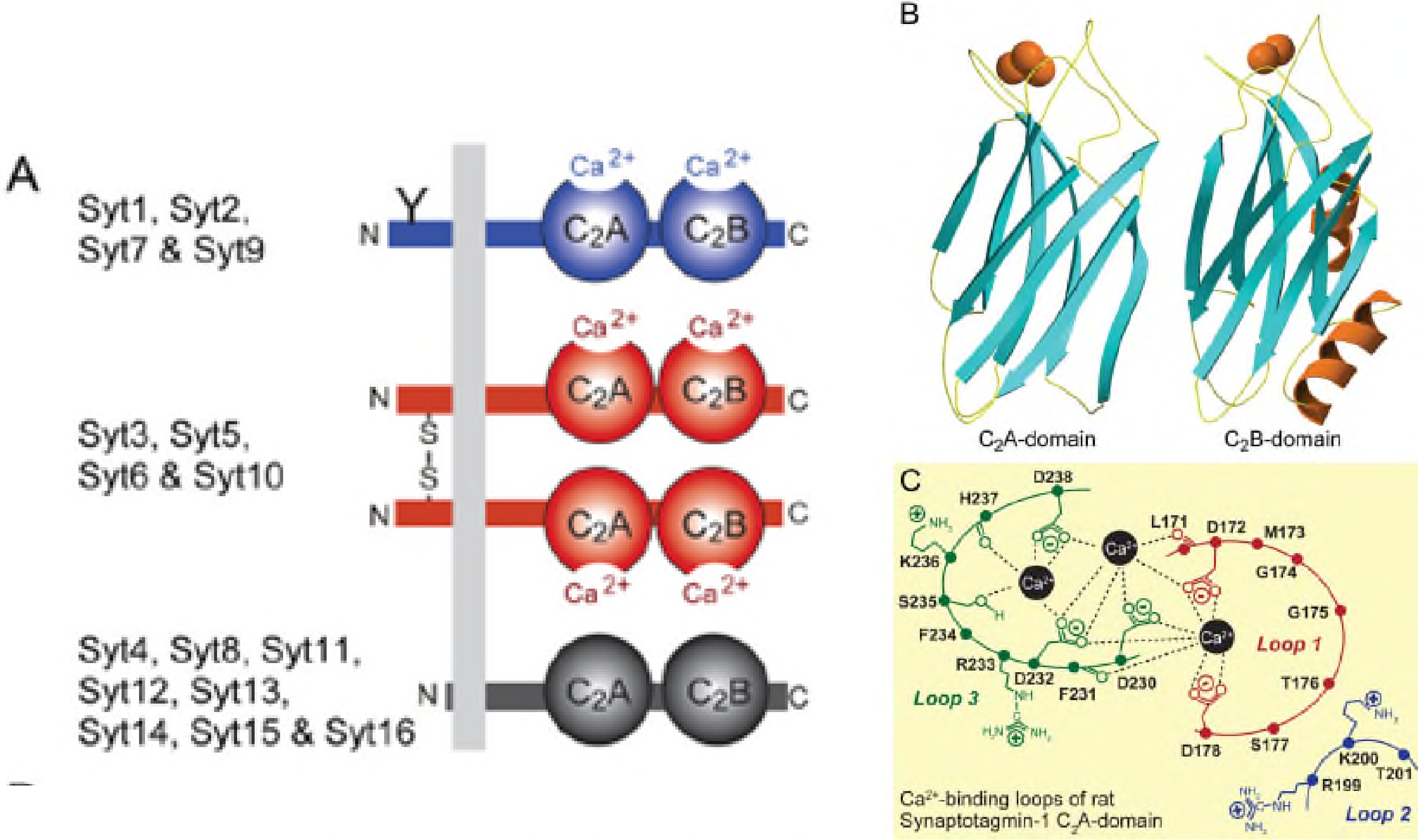


**Figure 2. Structures and Ca$^{2+}$-binding properties of synaptotagmins**

*A*. Canonical domain structures and classification of synaptotagmins. Mammals express 16 synaptotagmins composed of an N-terminal transmembrane region preceded by a short non-cytoplasmic sequence and followed by a variable linker sequence and two C2-domains; in addition, a 17th related protein called B/K-protein contains the same domain structure but an N-terminal lipid anchor instead of the transmembrane region (see Pang and Südhof, 2010, for a discussion of additional synaptotagmin-related proteins). Eight synaptotagmins bind Ca$^{2+}$ (Syt1, 2, 3, 5, 6, 7, 9, and 10; blue and red); the remaining synaptotagmins do not (black). The eight Ca$^{2+}$-binding synaptotagmins fall into two broad classes that differ in the absence (Syt1, 2, 7 and 9; blue) or presence (Syt3, 5, 6, and 10; red) of disulfide-bonded cysteine residues in their N-terminal sequences. Note that Syt1 and 2 include an N-glycosylated sequence at the N-terminus (indicated by a 'Y'), and that Syt7 is extensively alternatively spliced in the linker sequence (Han et al., 2004).

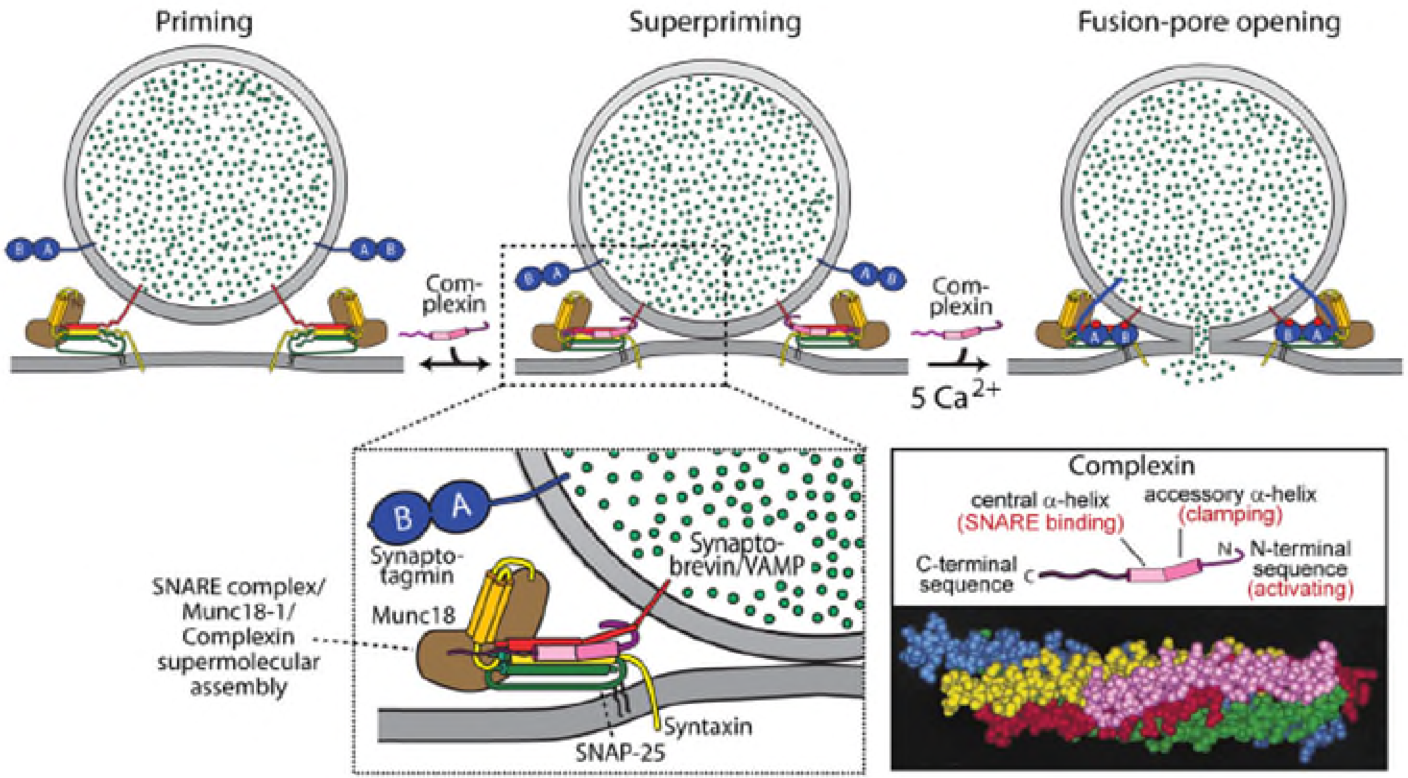


**Figure 5. Schematic diagram of the mechanism of action of synaptotagmin and its complexin cofactor in $Ca^{2+}$-triggered exocytosis**

The top diagram displays the sequential priming of synaptic vesicles by partial SNARE/SM protein complex assembly, superpriming by binding of complexin to partially assembled SNARE complexes, and $Ca^{2+}$-triggering of fusion-pore opening by $Ca^{2+}$-binding to synaptotagmin. The objects in the diagram approximate real size relationships. The diagram on the bottom left depicts a cartoon of a partially assembled SNARE/SM protein complex with complexin bound. The functional domain structure of complexin is shown on the right, with a space-filling model of complexin bound to a SNARE complex at the bottom (pink, the two α-helices of complexin; yellow, syntaxin-1; red, synaptobrevin; green and blue, SNAP-25). The model and the functional assignment of complexin sequences are modified from Maximov et al. (2009); the complexin/SNARE complex crystal structure is from Chen

# Does synaptic vescicles fusion occur at specific places ?

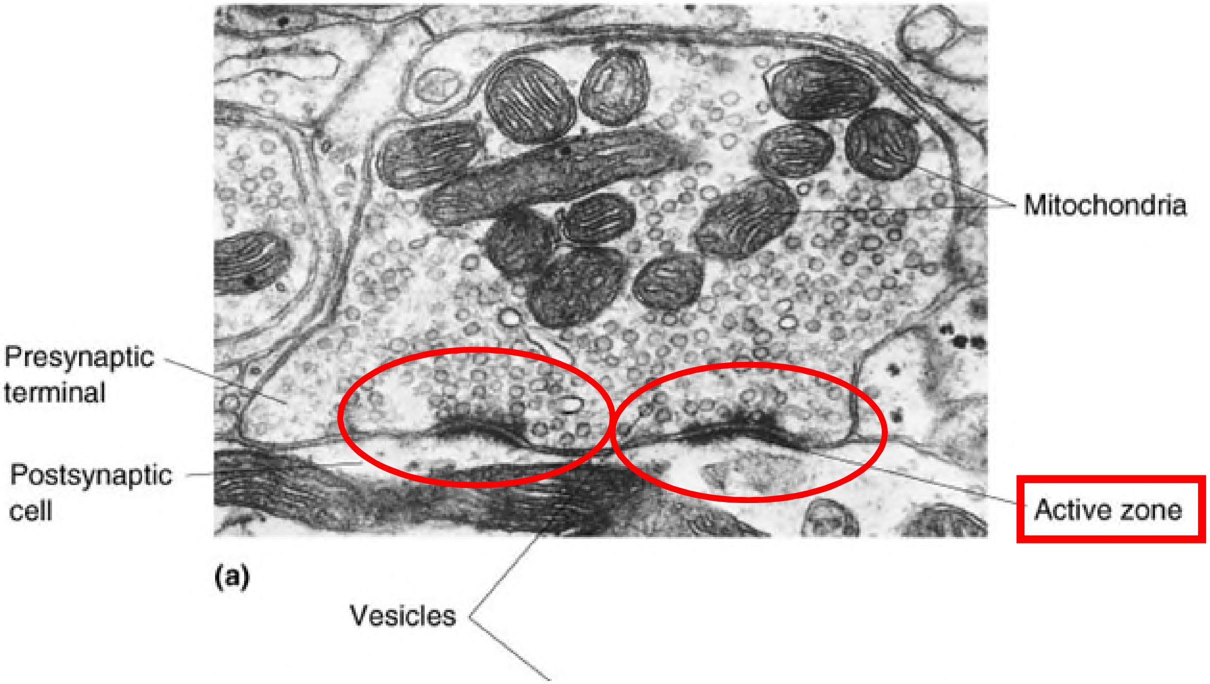


Figure 5.3
Chemical synapses, as seen with the electron microscope. (a) Fast excitatory synapse in the CNS. (Source: Adapted from Heuser and Reese, 1977, p. 262.) (b) A synapse in the PNS, with numerous dense-core vesicles. (Source: Adapted from Heuser and Reese, 1977, p. 278.)

# The active zone: definition and structure

Morphologically, active zones are defined as sites of synaptic vesicle docking and fusion, and physiologically they are defined as sites of neurotransmitter release.

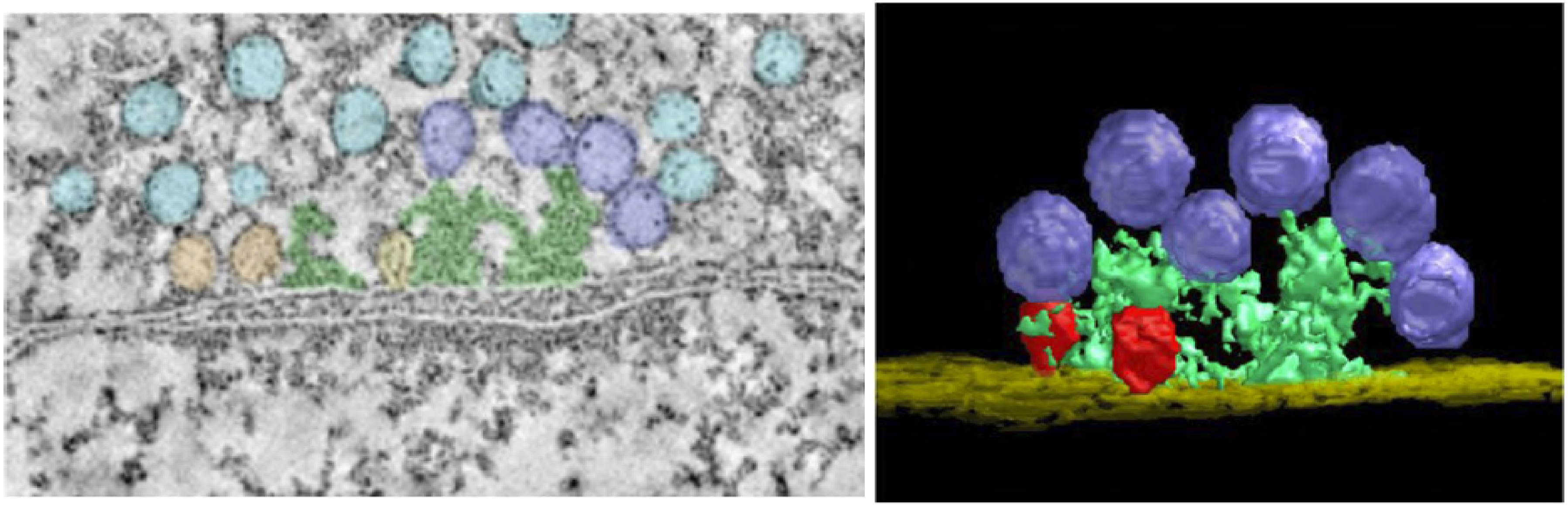
Active zone have three morphologically and functionally distinct components:

1. the plasma membrane juxtaposed to the PSD where synaptic vesicle fusion occurs,

2. the cytomatrix immediately internal to the plasma membrane where synaptic vesicles dock, and

3. the electron-dense projections extending from the cytomatrix into the cytoplasm on which synaptic vesicles are tethered

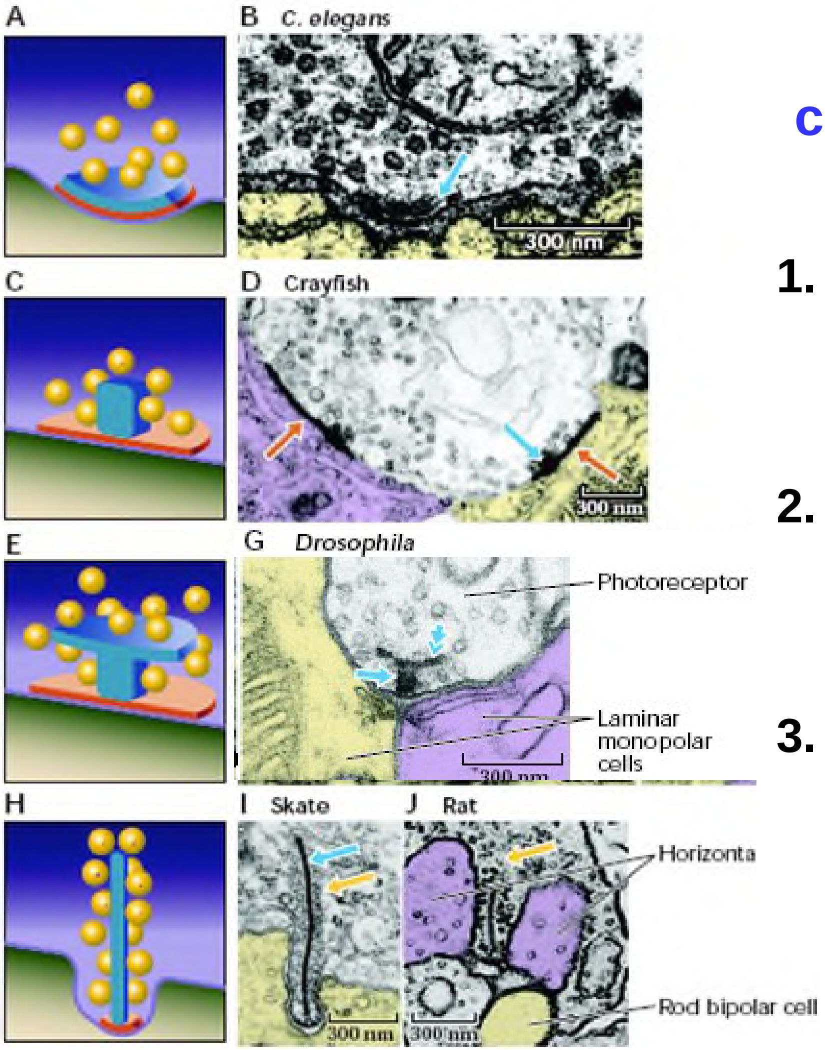
# Architecture of the presynaptic Active Zone



Reconstruction of two dense projections (green) showing their relationship with six synaptic vesicles (blue) and two fusing vesicles (red).

Burette et al., Microsc Microanal 13(Suppl 2), 2007

# Architecture of the presynaptic Active Zone



**All active zones have three components, with variable size and shape of dense projections:**

1. **the plasma membrane juxtaposed to the PSD where synaptic vesicle fusion occurs,**

2. **the cytomatrix immediately internal to the plasma membrane where synaptic vesicles dock,**

3. **the electron-dense projections extending 0.5-1 µm from the cytomatrix into the cytoplasm on which synaptic vesicles are tethered (SYNAPTIC RIBBONS)**

Zhai & BellenPHYSIOLOGY 19: 262–270, 2004

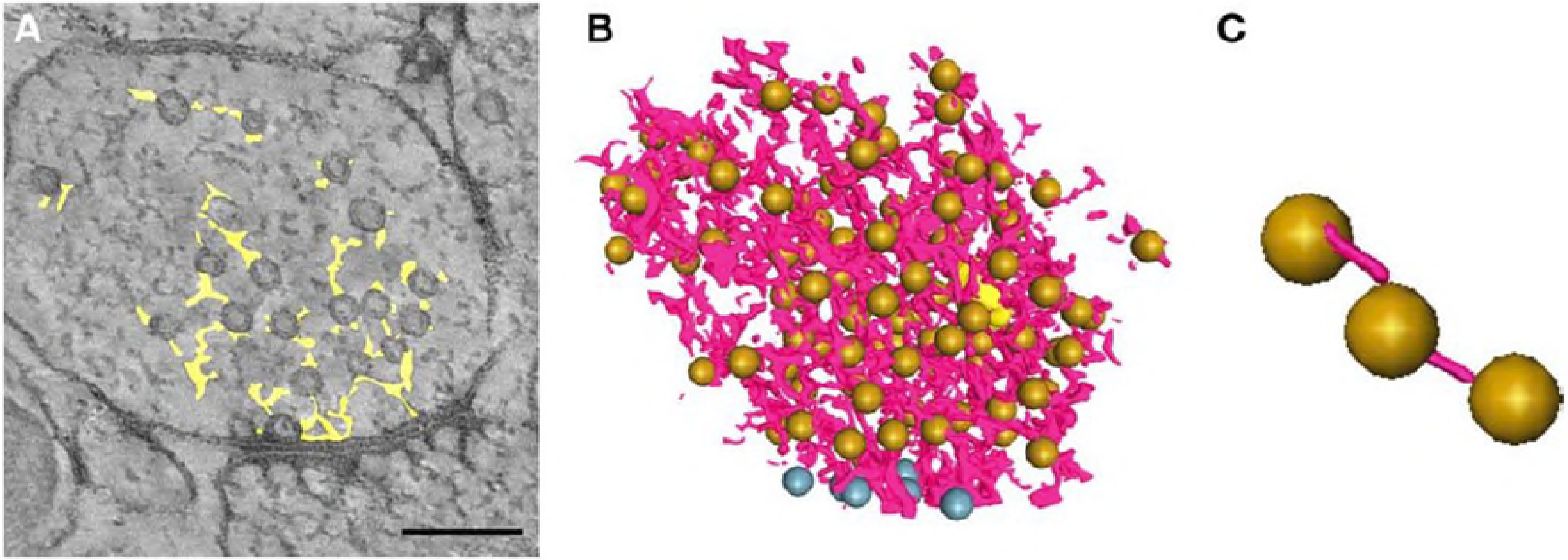


**Figure 2.** Network of filaments linking SVs in the presynaptic terminal. ***A***, The electron-dense materials surrounding the SVs (yellow) are superimposed on a virtual section. ***B***, 3D reconstruction emphasizing that docked (blue) and other (gold) SVs are embedded in a tight meshwork of filaments (pink). ***C***, Higher magnification of a partial reconstruction exemplifying the connections (pink) between neighboring SVs. Scale bar, 200 nm.

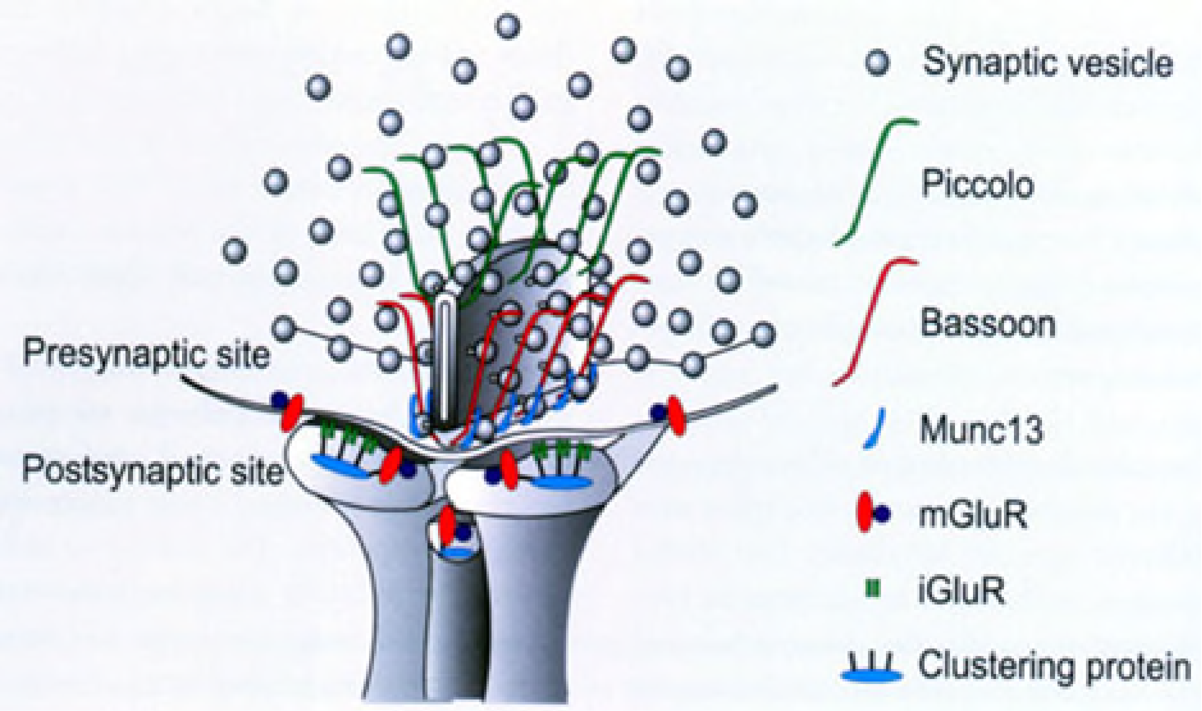
# Proteins identified in the active zone cytomatrix can be classified into 3 categories.

- **First,** the classical cytoskeletal proteins corresponding to actin, tubulin, myosin, spectrin-chain and -chain, and -catenin are the fundamental elements of the framework of active zone cytomatrix.

-**Second** are the known scaffolding proteins, including SAP97, and CASK/LIN-2

- **Third**, there are the active zone-specific proteins including RIM1, Munc13/unc13, Bassoon, Piccolo/Aczonin, and CAST/ERCs

# Molecular structure of a ribbon synapse



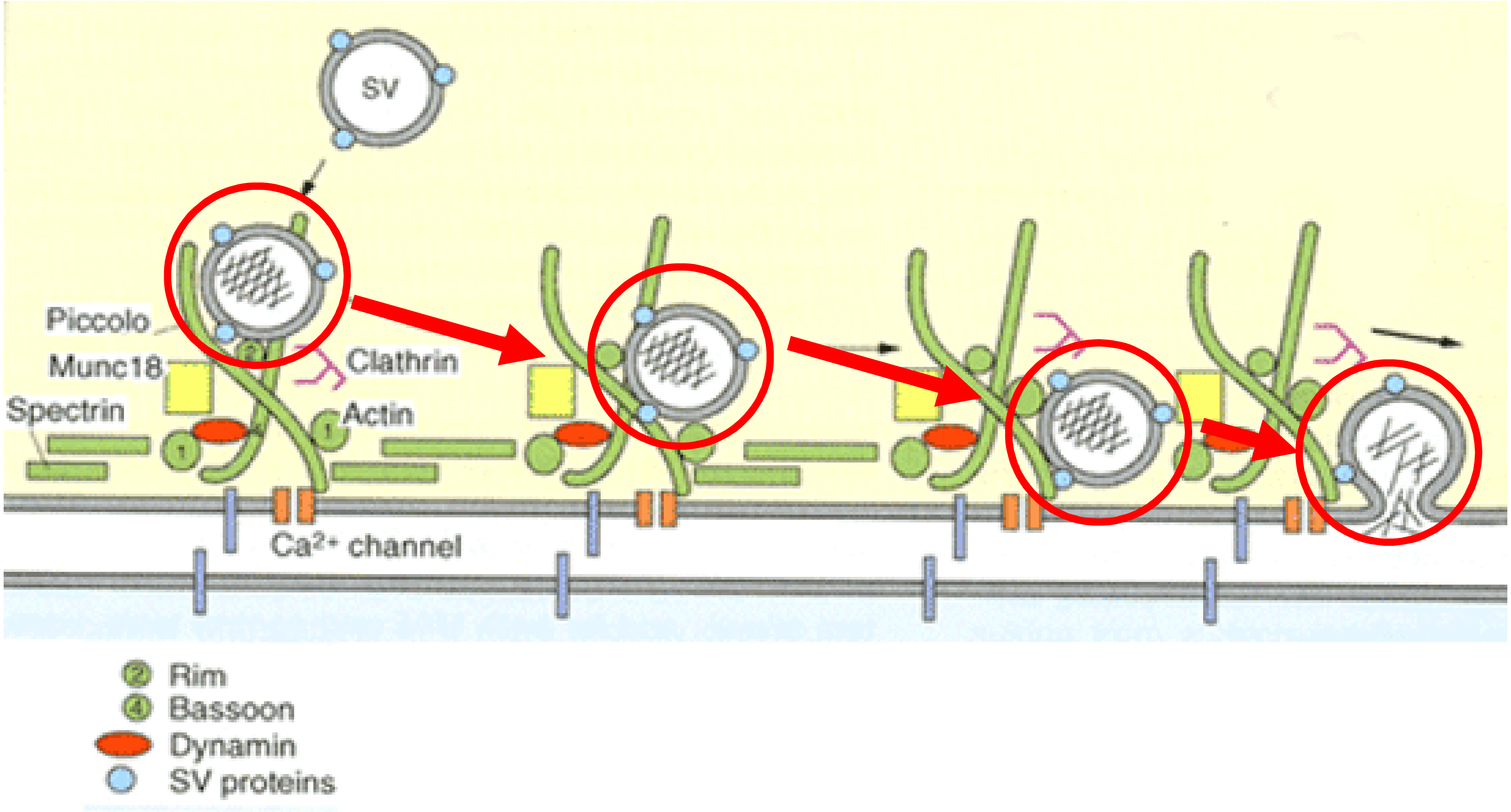
**Structural molecules piccolo e bassoon anchor the synaptic ribbon on the membrane**

# Proteins identified in the active zone cytomatrix can be classified into 3 categories.

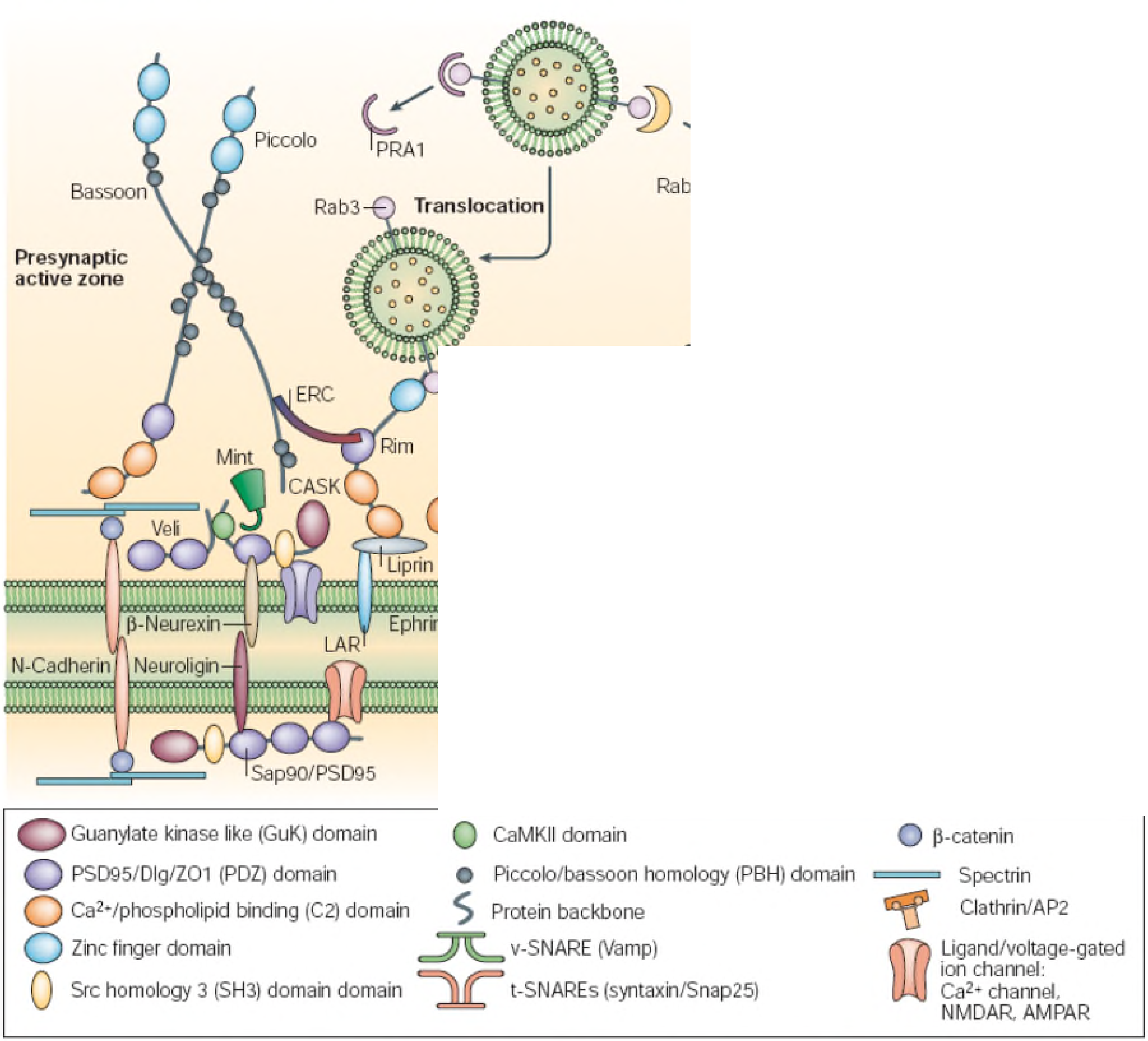
1. **cytoskeletal proteins** :
   actin, tubulin, myosin, spectrin, catenin
   .
2. **scaffolding proteins** :
   SAP90/PSD95/Dlg, SAP97, CASK/LIN-2
3. **active zone-specific proteins** :
   RIM1, Munc13/ unc13, Bassoon,
   Piccolo/Aczonin, and CAST/ERCs

Trafficking mediated by Dynein, Kinesin, microtubule associated protein (MAP)

# Progressive approach of the SV to the plasma membrane requires interactions with the cytoskeleton and Piccolo & Basson



## Box 1 | **Molecular structure of CNS glutamatergic synapses**



Current studies indicate that three distinct complexes help to define the active zone (see TABLE 1 for a list of key active

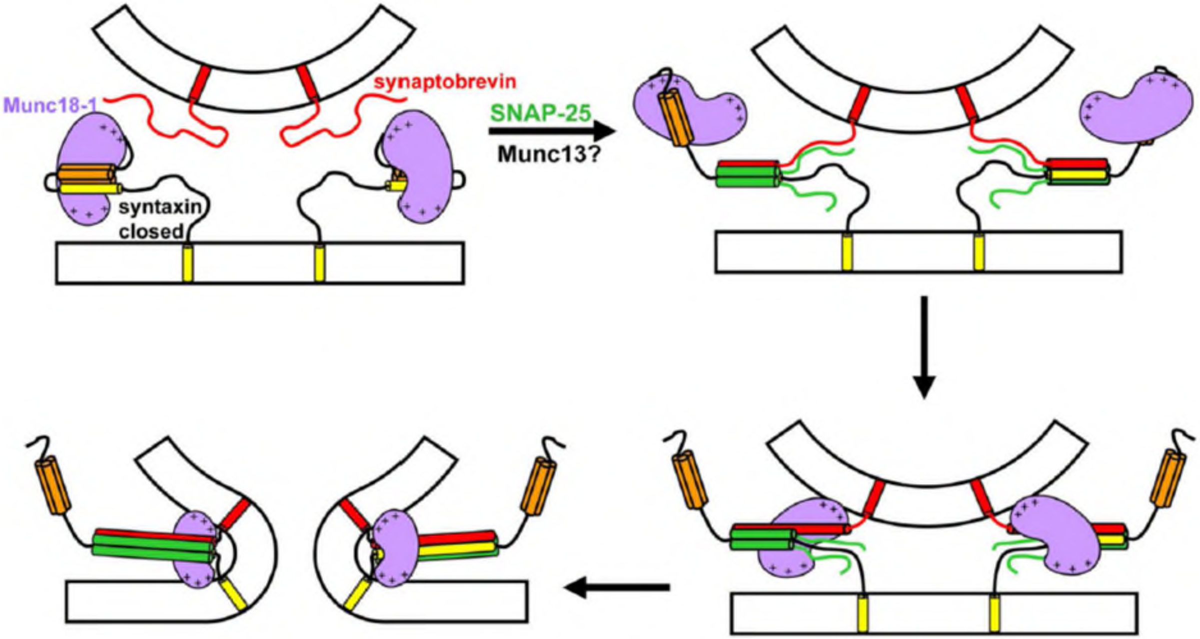
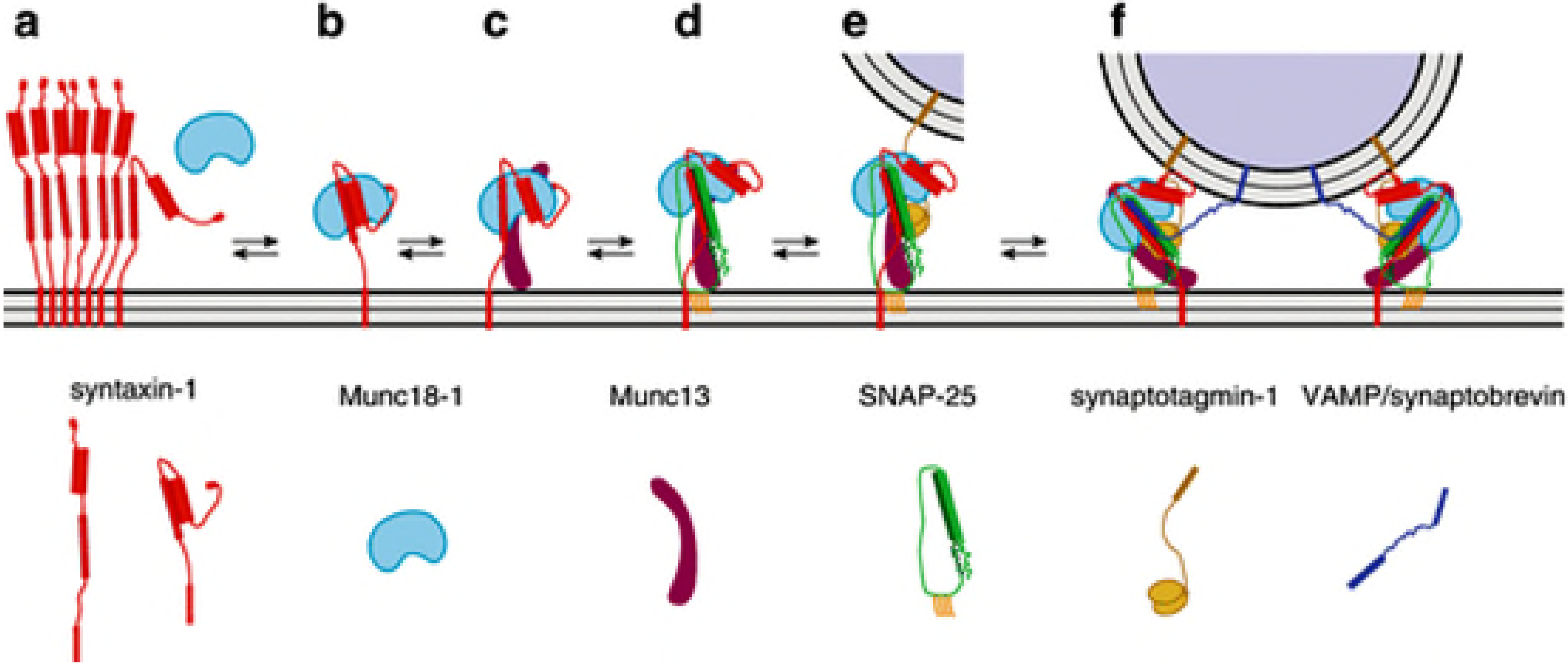


**Figure 6.**

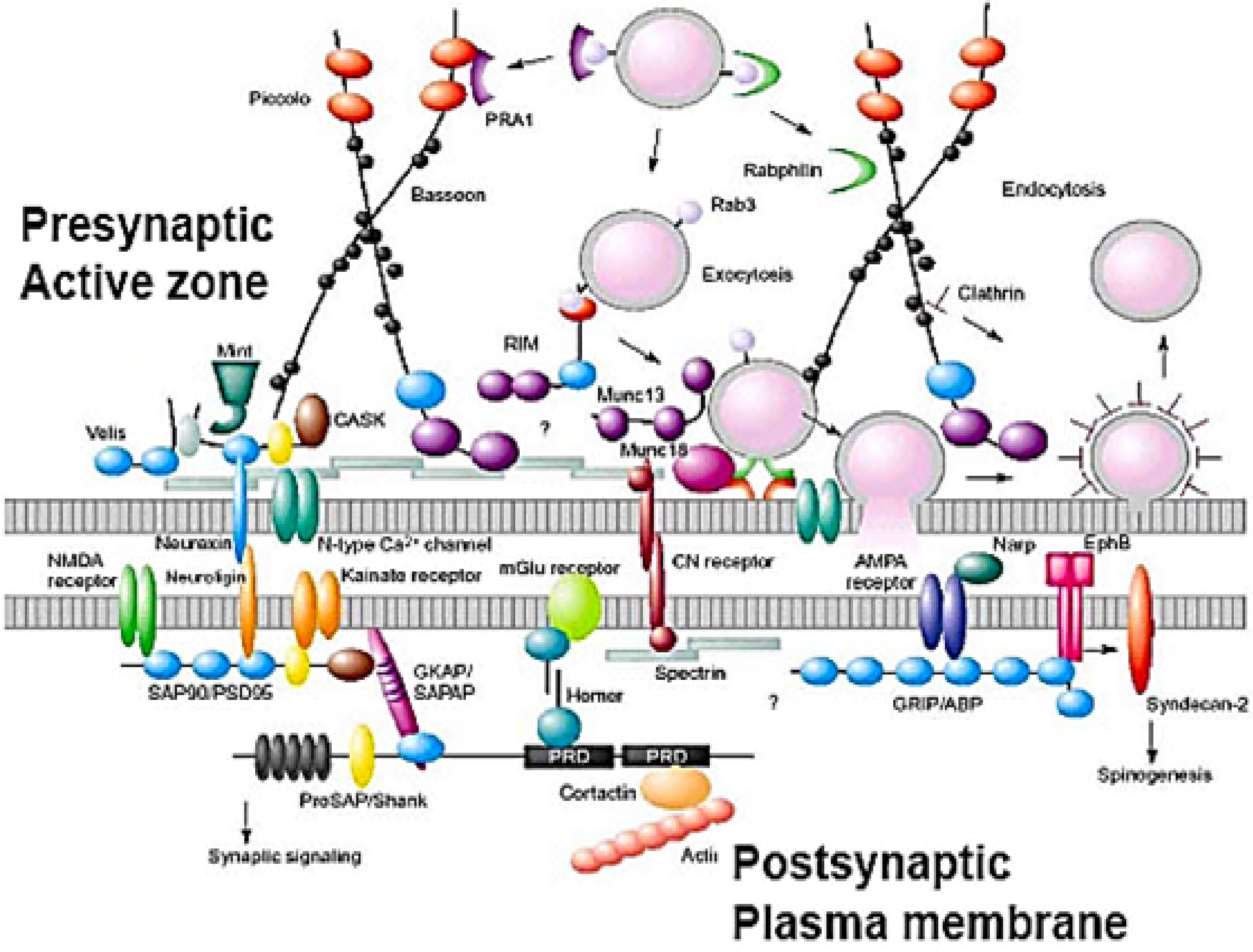
Proposed model of neurotransmitter release involving three types of Munc18-1/SNARE interactions. The model assumes that Munc18-1 (purple) is initially bound to closed syntaxin-1 (Habc domain, orange; SNARE motif, yellow) (upper left panel). Partial assembly of SNARE complexes of syntaxin-1 with synaptobrevin (red) and SNAP-25 (green) occurs by an unknown mechanism that likely involves Munc13 (not shown); here we propose that Munc18-1 is bound only to the N-terminal region of syntaxin-1 at this stage (upper right panel). The next step is proposed to involve transition of Munc18-1 from the syntaxin-1 N-terminal region to the C-terminus of the synaptobrevin SNARE motif, which could be favored by cooperativity with other interactions such as Munc18-1 binding to the vesicle membrane (bottom right panel). The central aspect of this model is that membrane fusion results from the cooperative action of Munc18-1 and the SNAREs, which would be favored by the binding of Munc18-1 to synaptobrevin and could involve interactions of basic residues of Munc18-1 (indicated by the + signs) with both membranes (bottom left panel; see text for further details). Other proteins involved in triggering release and conferring its $Ca^{2+}$ sensitivity are not represented for simplicity, but they are expected to cooperated with Munc18-1 and the SNAREs to trigger release.


a
b
c
d
e
f
syntaxin-1
Munc18-1
Munc13
SNAP-25
synaptotagmin-1
VAMP/synaptobrevin

**active zone cytomatrix**

# Proteins identified in the active zone cytomatrix can be classified into 3 categories.

**cytoskeleton, scaffolding, active zone proteins**



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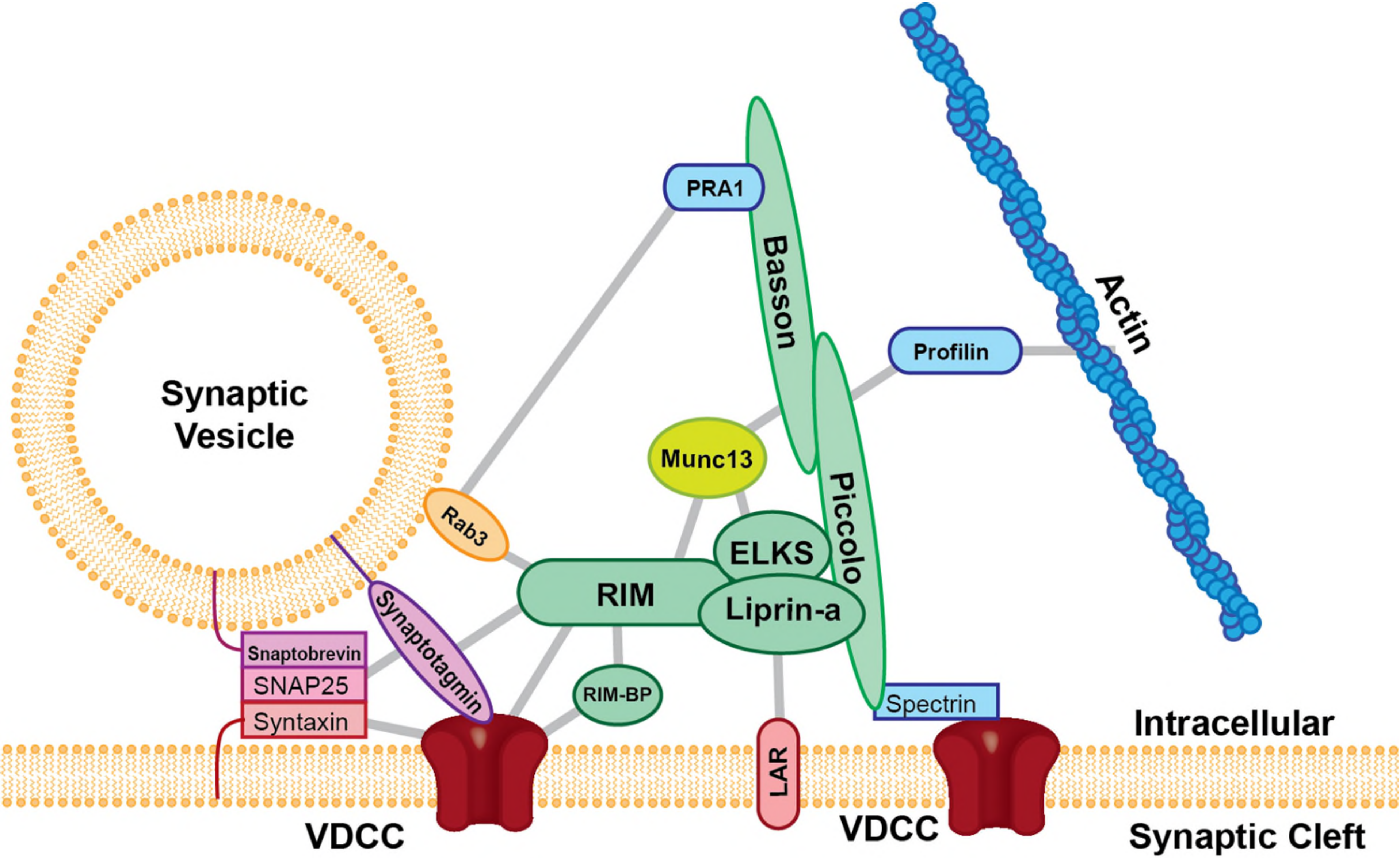
PRA1
Basson
Actin
Profilin
Synaptic
Vesicle
Munc13
Piccolo
Rab3
ELKS
RIM
Liprin-a
Synaptotagmin
Snaptobrevin
SNAP25
Syntaxin
RIM-BP
Spectrin
Intracellular
LAR
VDCC
VDCC
Synaptic Cleft