Молекулярная организация нервной системы

Лекция 6: Молекулярная организация пресинаптического окончания

Казанский медицинский университет

Казань

Лекция

ноябрь 2015

П.Д. Брежестовский

Институт динамики мозга

Факультет медицины

Университет Aix-Marseille

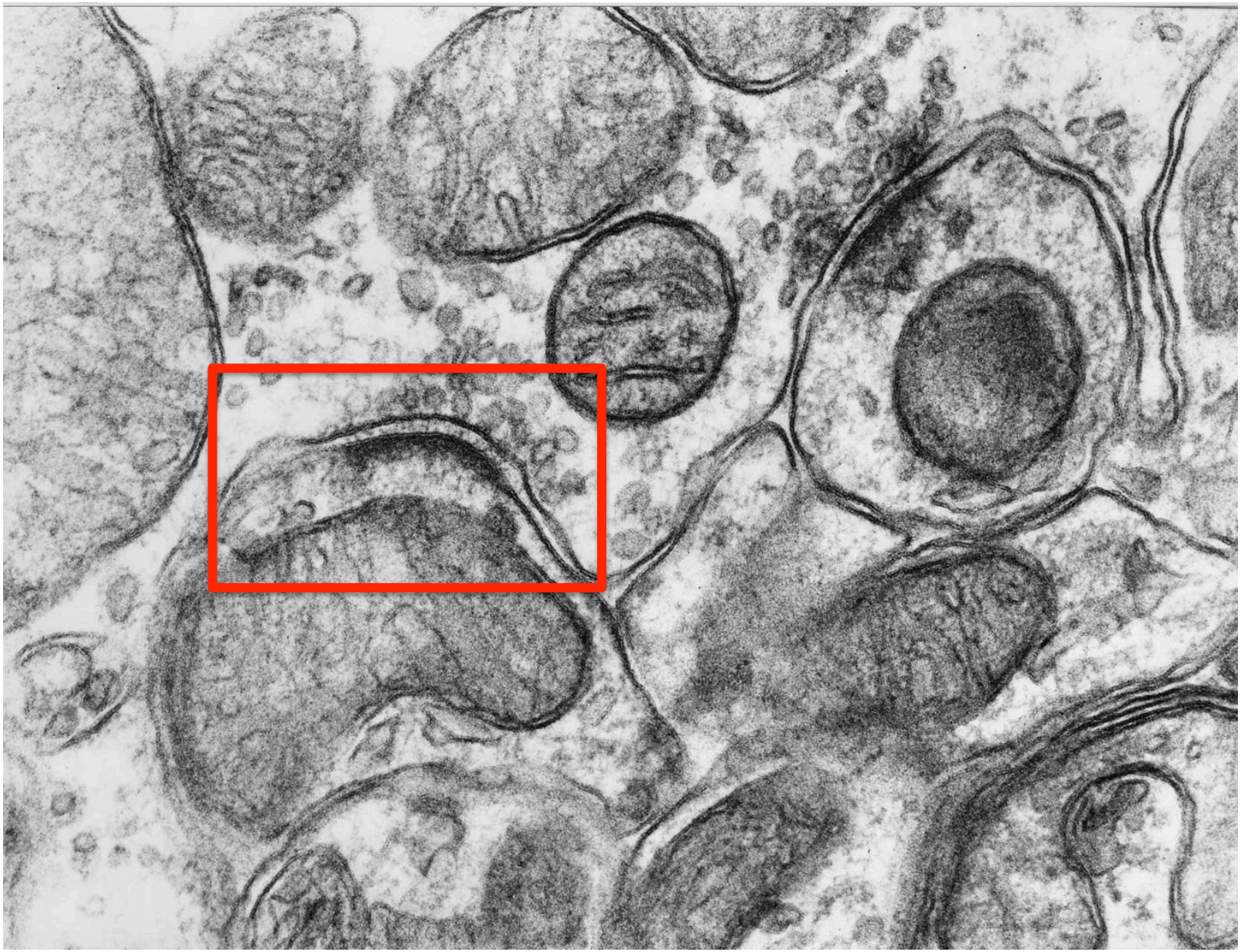
Марсель, Франция

piotr.bregestovski@univ-amu.fr pbreges@gmail.com

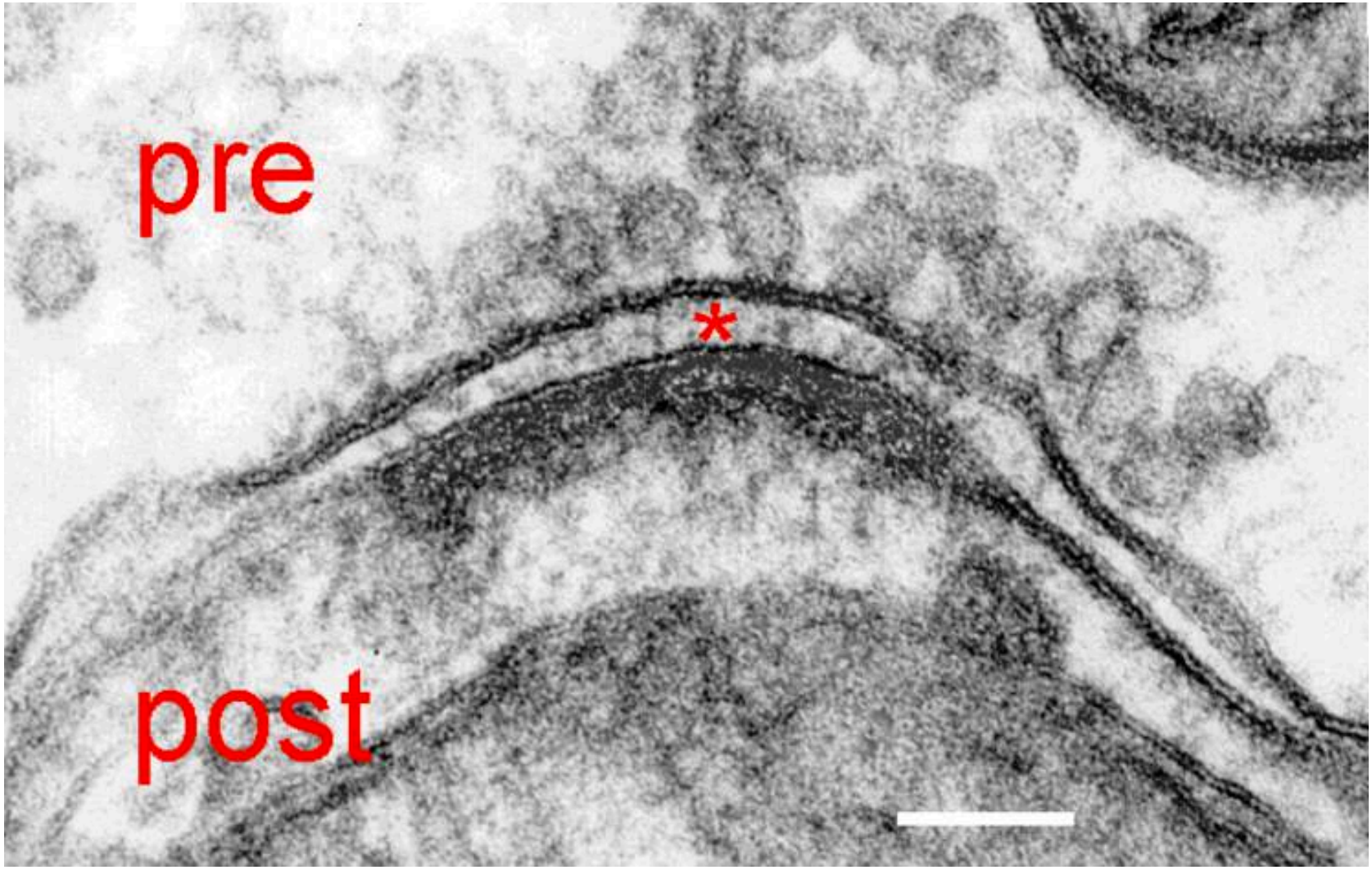
План

- Ключевые этапы выброса нейромедиатора
- Процесс синтеза нейромедиатора
- Транспорт нейромедиатора по микротрубочам
- Что представляет собой везикула?
- Загрузка нейромедиатора в везикулы
- Молекулярная организация основных белков, обеспечивающих слияние везикул с синаптической мембраной:
  - SNARE комлекс
  - Синаптотагмин
- Нейропатологии, связанные с нарушением выброса нейромедиаторов

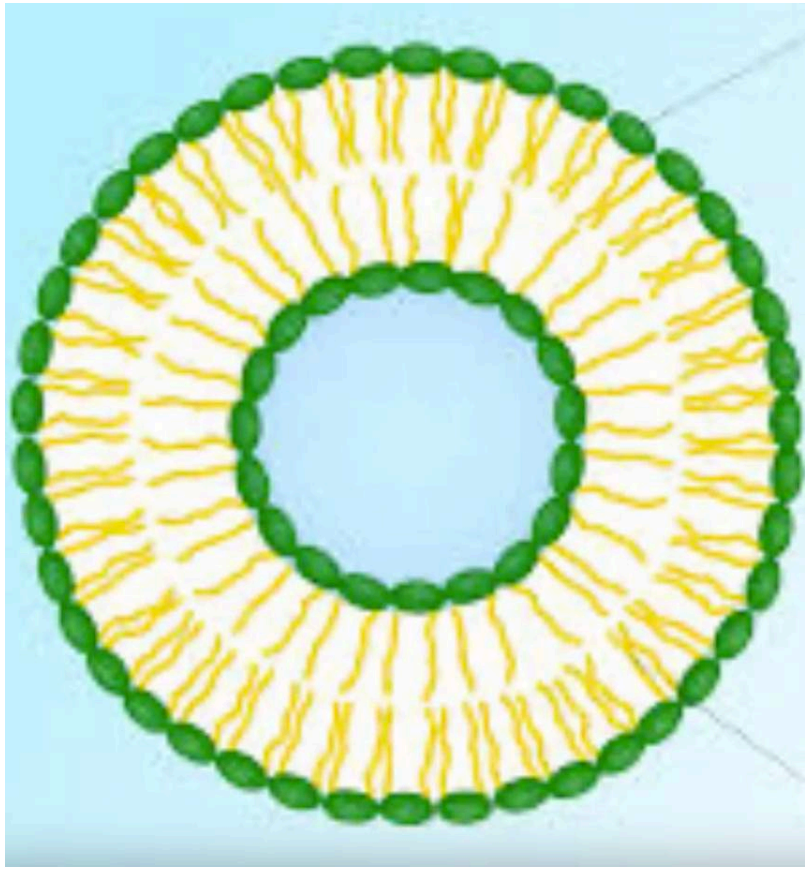
Синаптическая щель

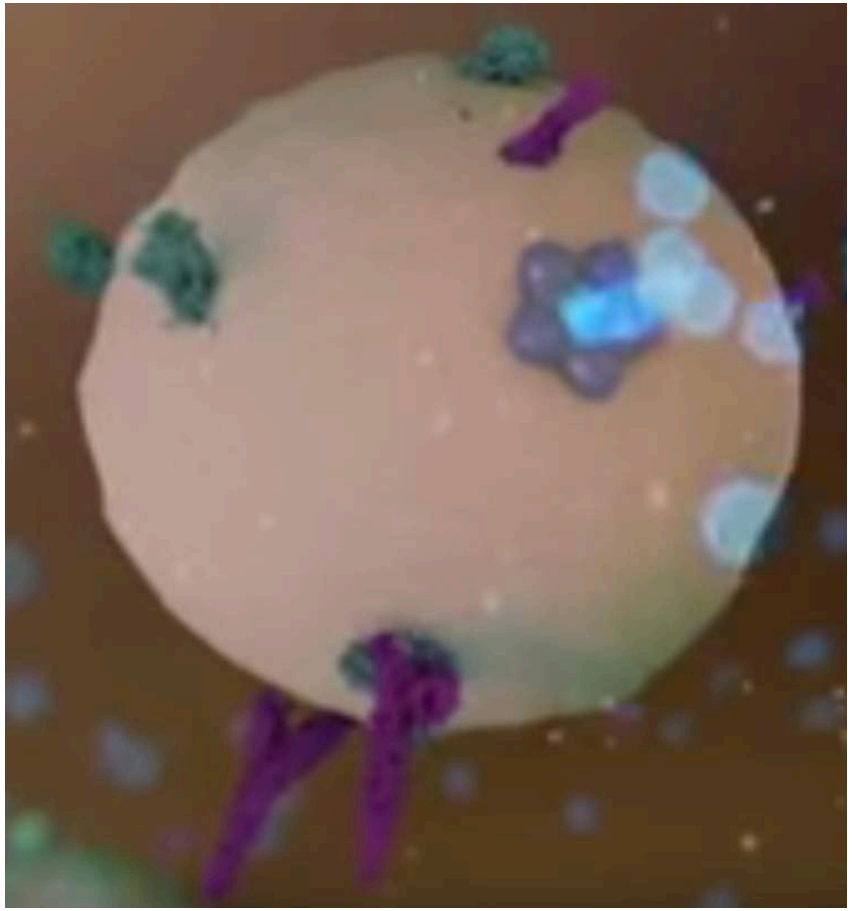


Синаптическая щель

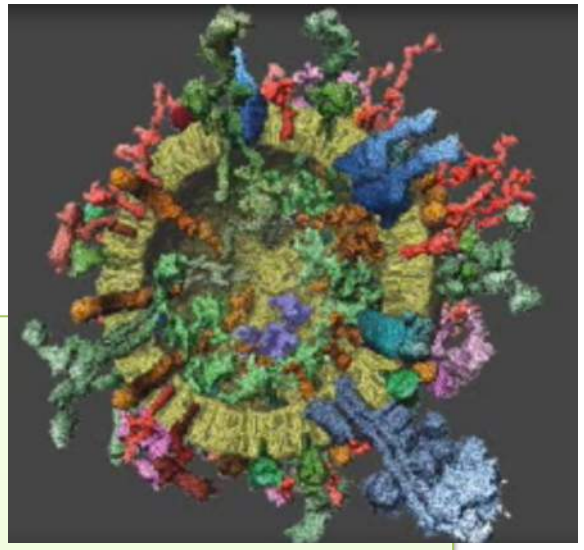


Что собой представляет везикула?

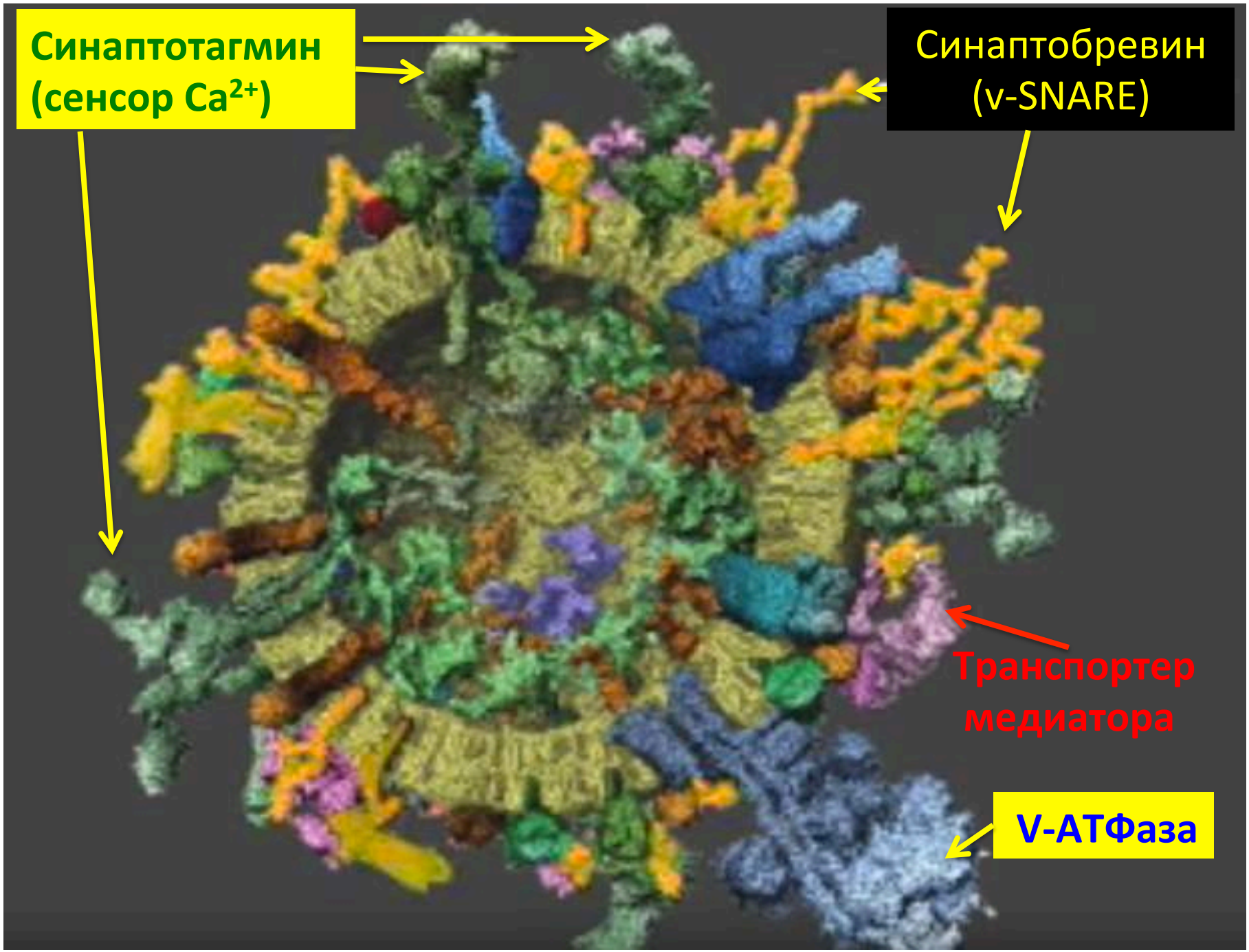


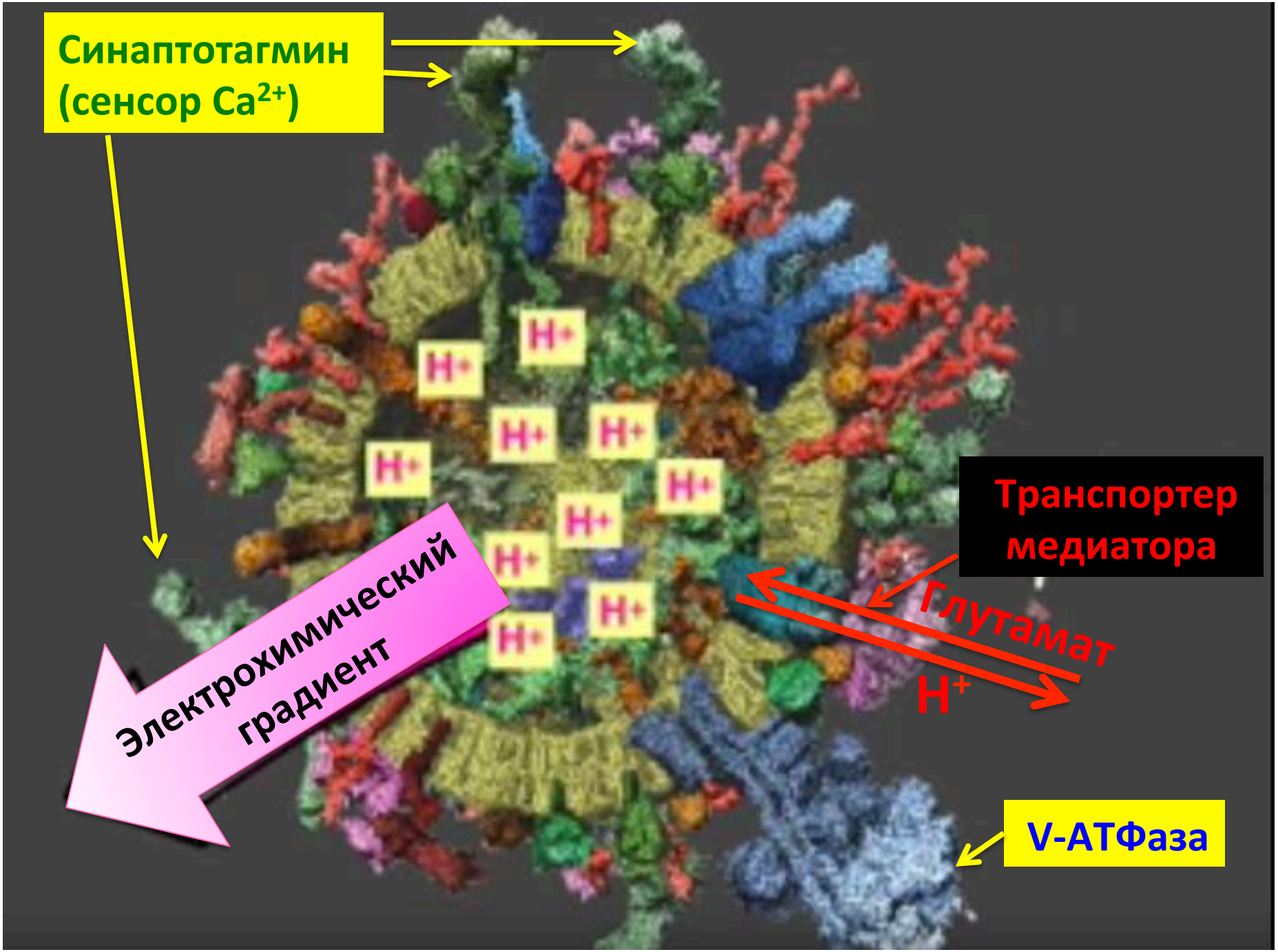


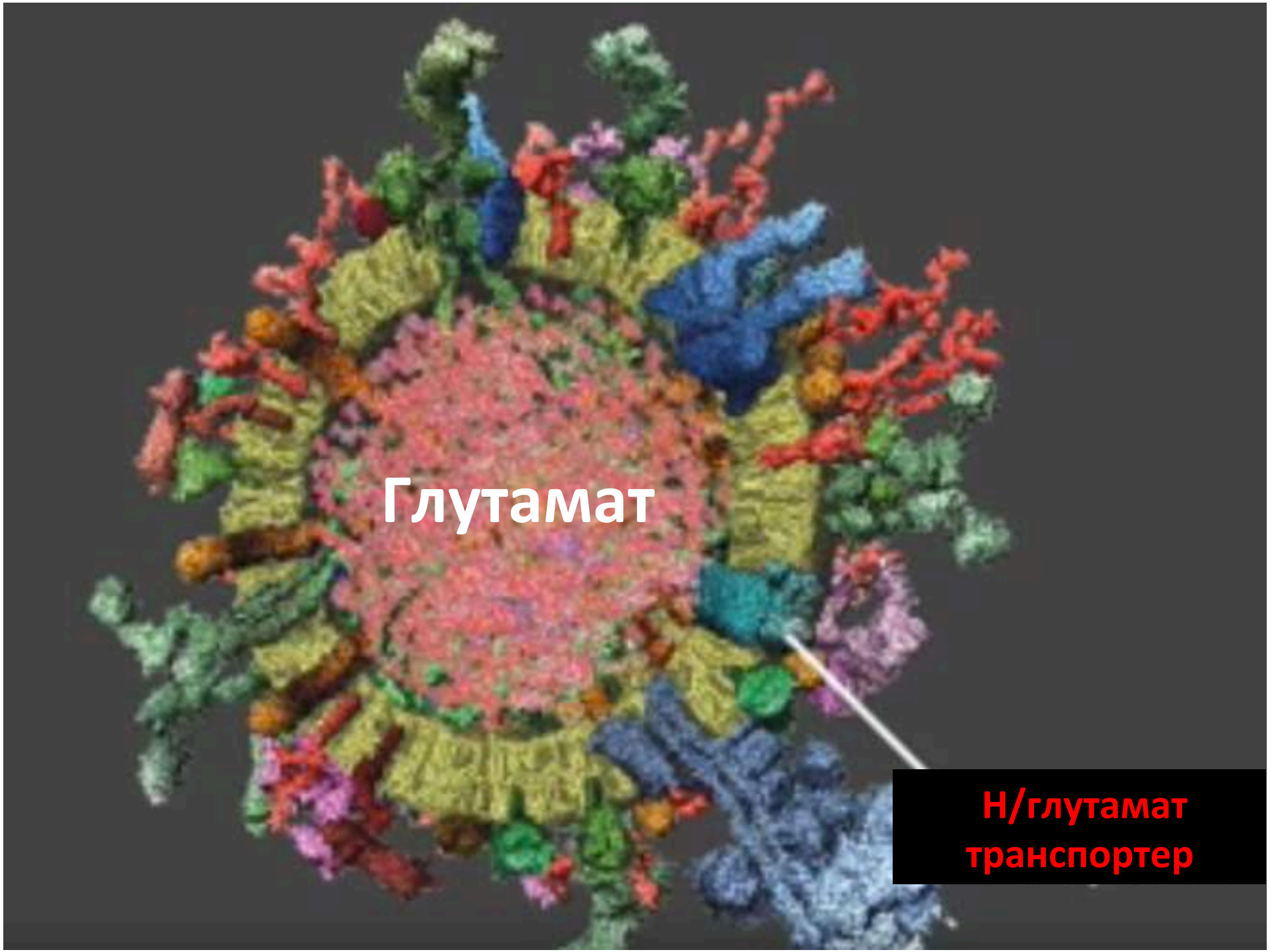
Везикула содержит около 50 разных белков



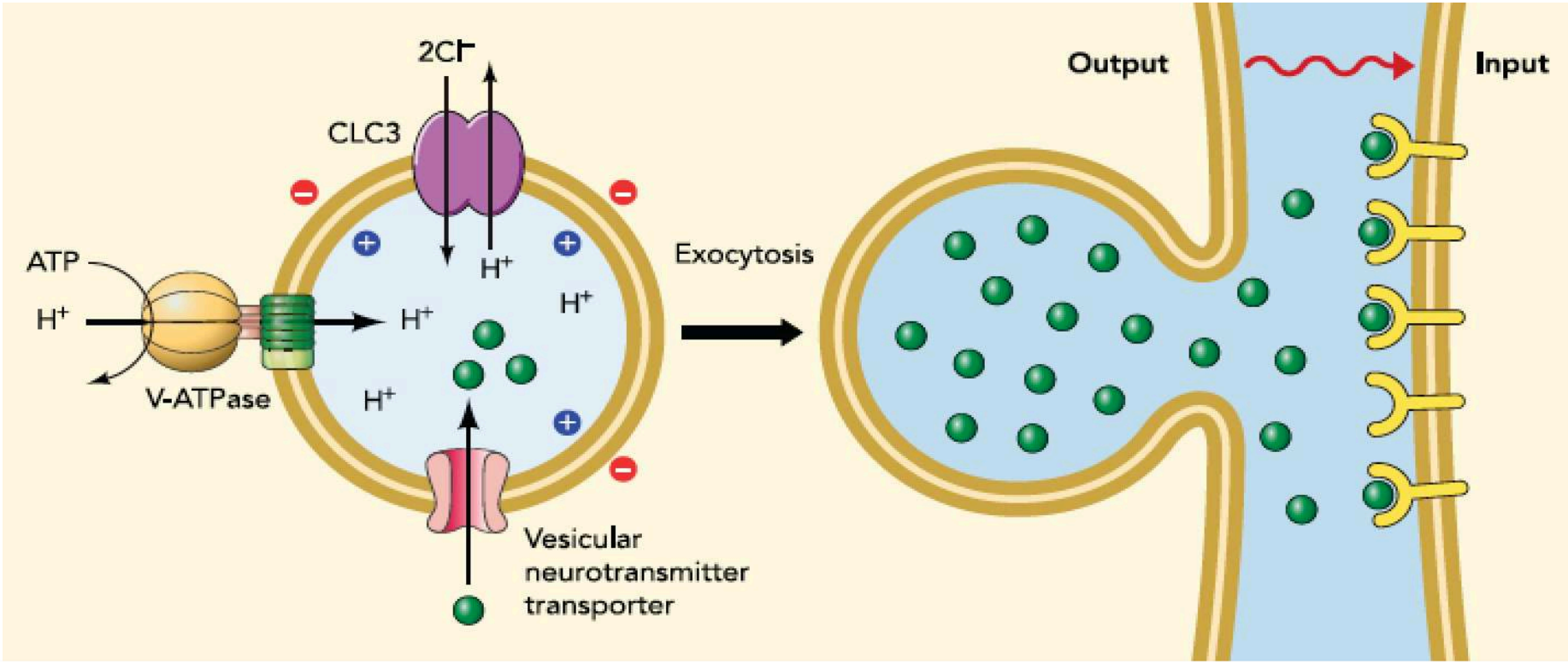
- 7 000 молекул фосфолипидов
- 5 700 молекул холестерина
- 70 копий **синаптобревина** (v-SNARE)
- 1-2 **V-АТФазы**, которая использует энергию гидролиза АТФ для закачки водорода внутрь
- **Транспортеры** нейромедиаторов
- **Синаптотагмин** - рецептор Ca^{2+}


Синаптотагмин
(сенсор Ca^{2+})
Синаптобревин
(v-SNARE)
Транспортер
медиатора
V-АТФаза


Синаптотагмин
(сенсор Ca^{2+})
H+
H+
H+
H+
H+
H+
H+
H+
H+
H+
Транспортер
медиатора
Глутамат
H^+
Электрохимический
градиент
V-АТФаза


Глутамат
H/глутамат
транспортер

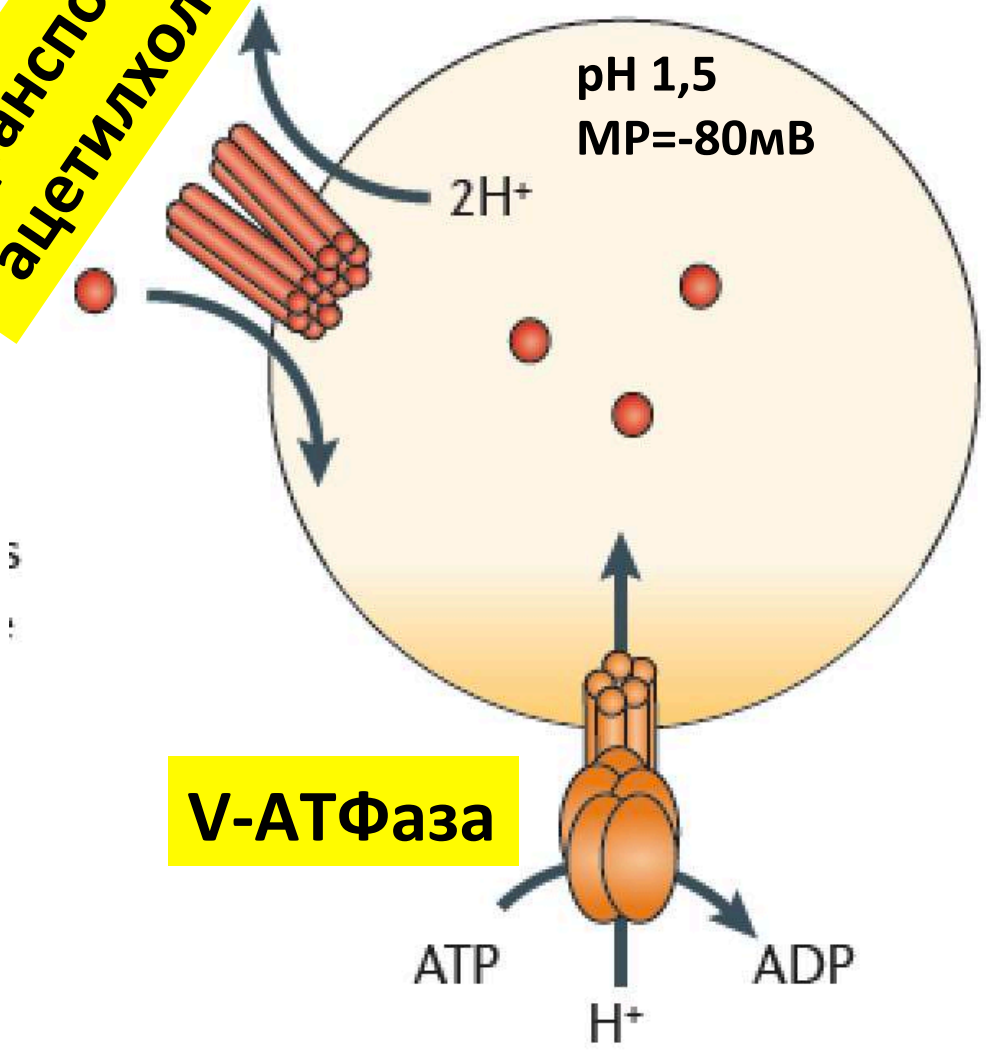
Транспортеры нейромедиаторов

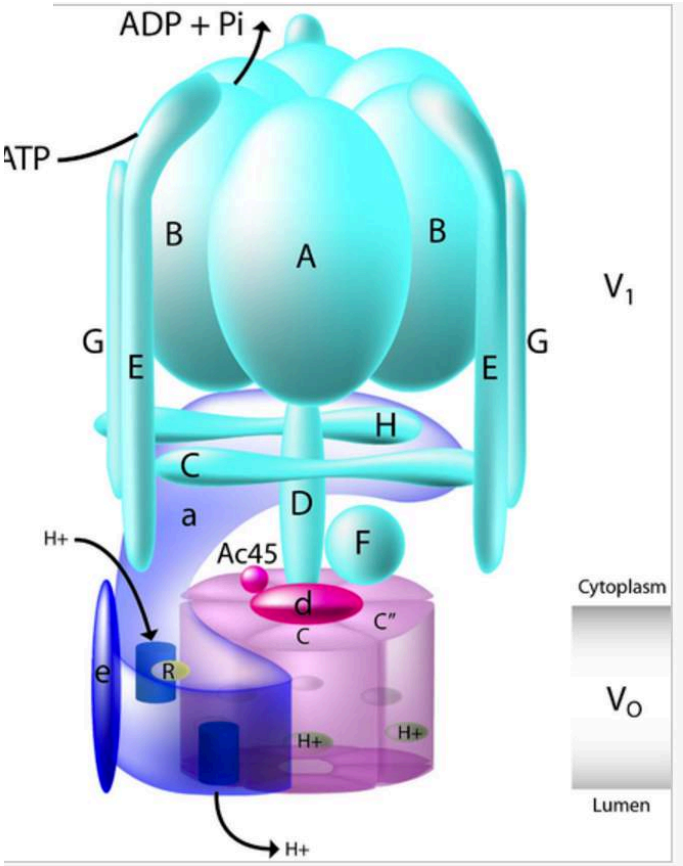


Закачка нейромедиатора

Транспортер ацетилхолина

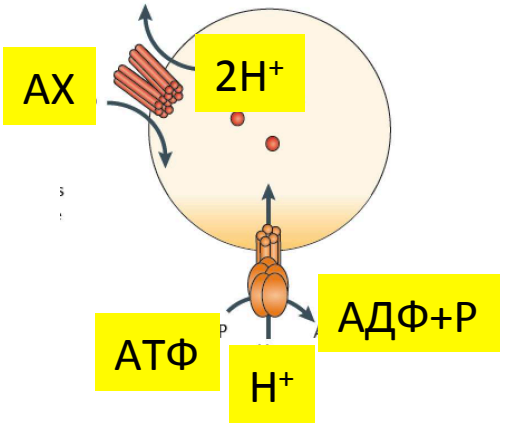
Везикула ацетилхолина



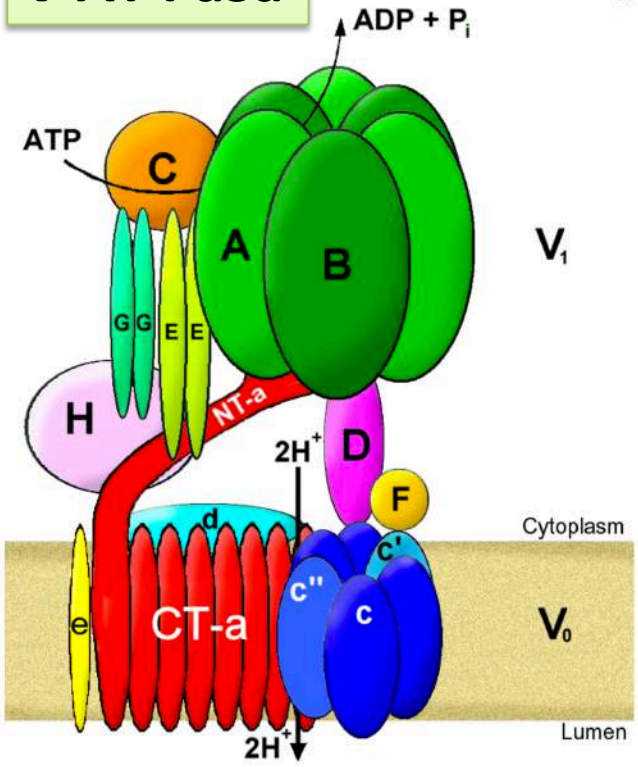


V-АТФаза

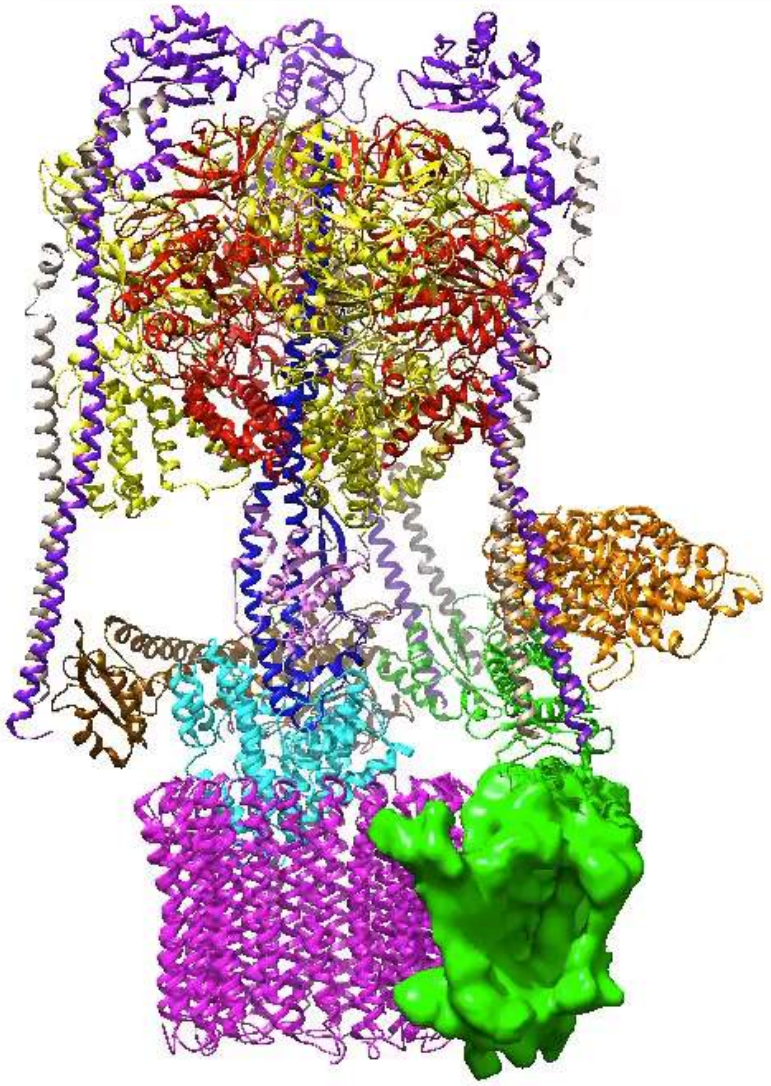
Везикула ацетилхолина



v-АТФаза

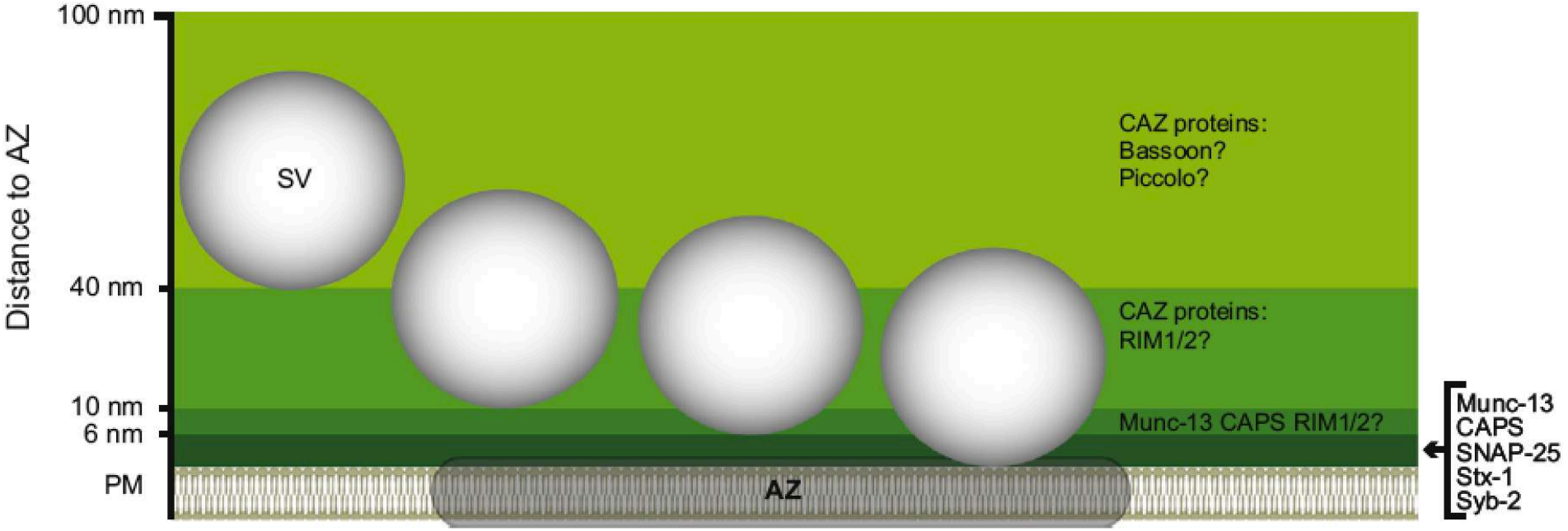


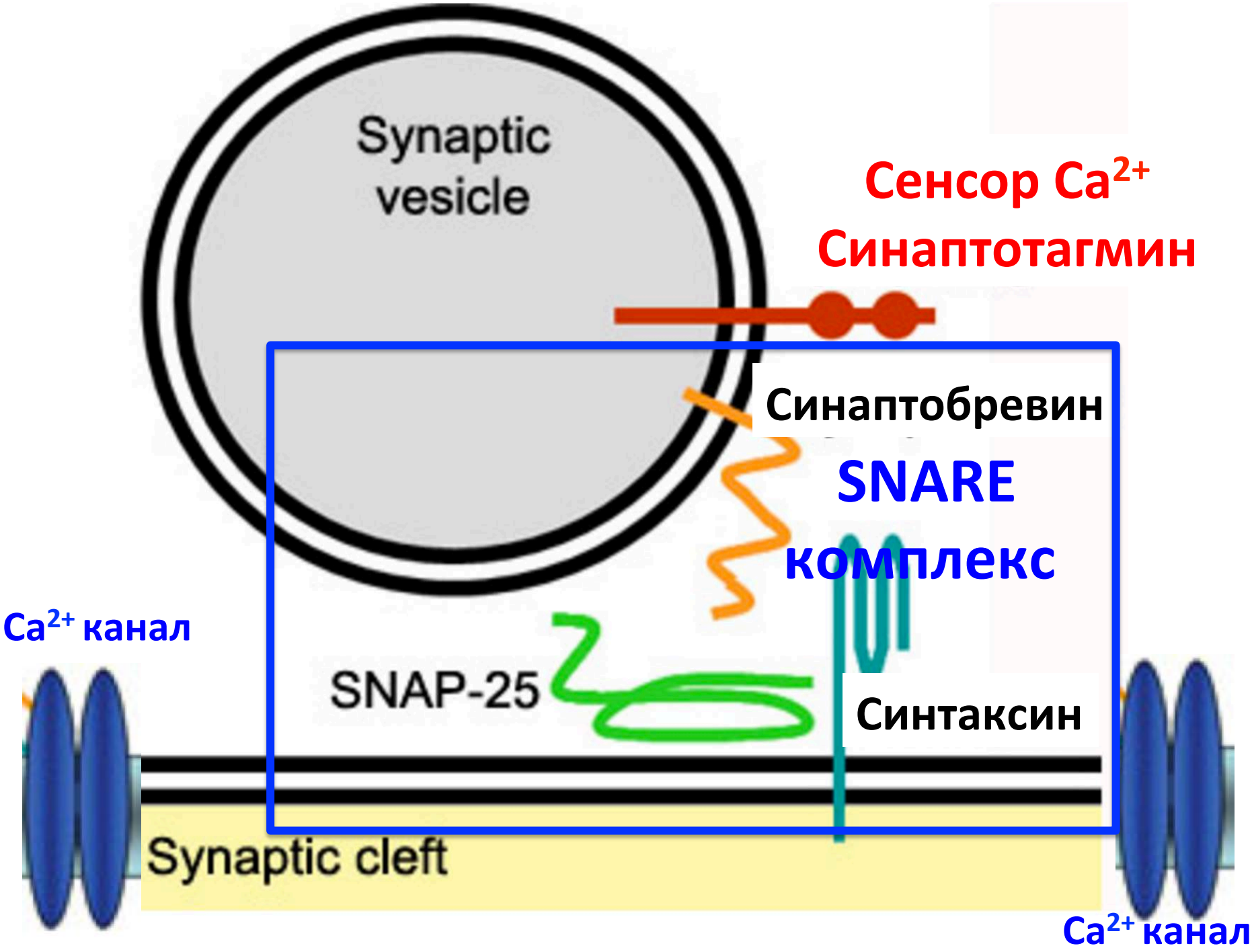
Модель v-АТФазы



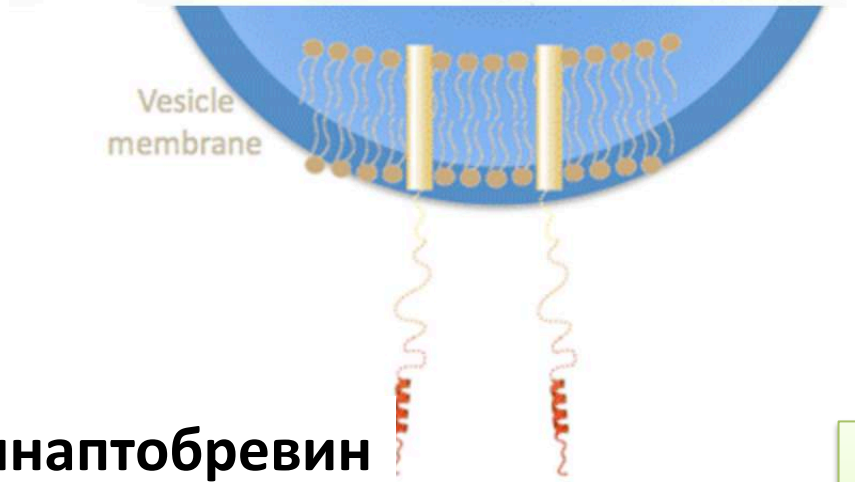
Zhao, Rubinstein et al., *Nature* **521**, 241–245, 2015

Как происходит выброс нейромедиатора?




Synaptic vesicle
Сенсор Ca^{2+}
Синаптотагмин
Синаптобревин
SNARE комплекс
Ca^{2+} канал
SNAP-25
Синтаксин
Synaptic cleft
Ca^{2+} канал

Везикула встречает родственный участок мембраны

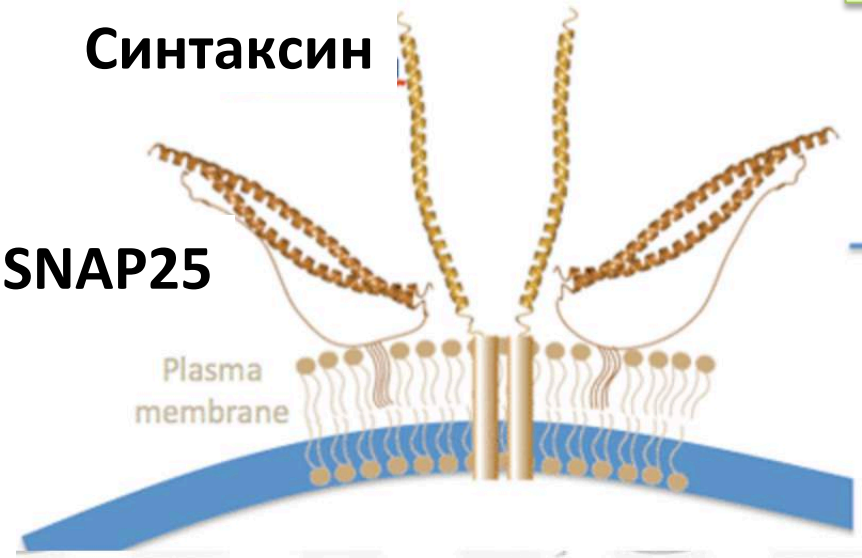


Синаптобревин

Образование SNARE комплекса

Синтаксин

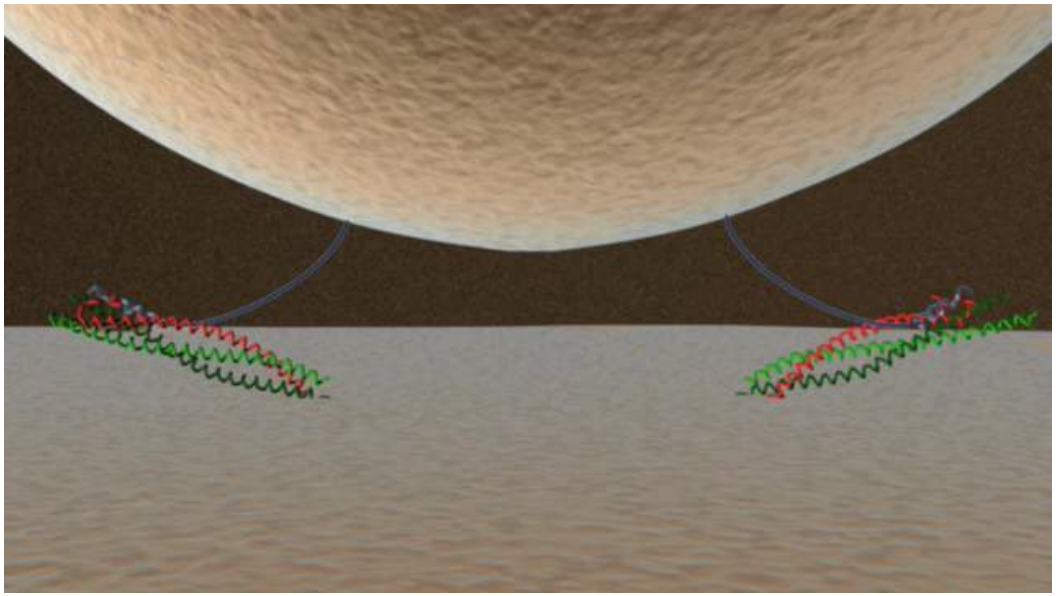
SNAP25

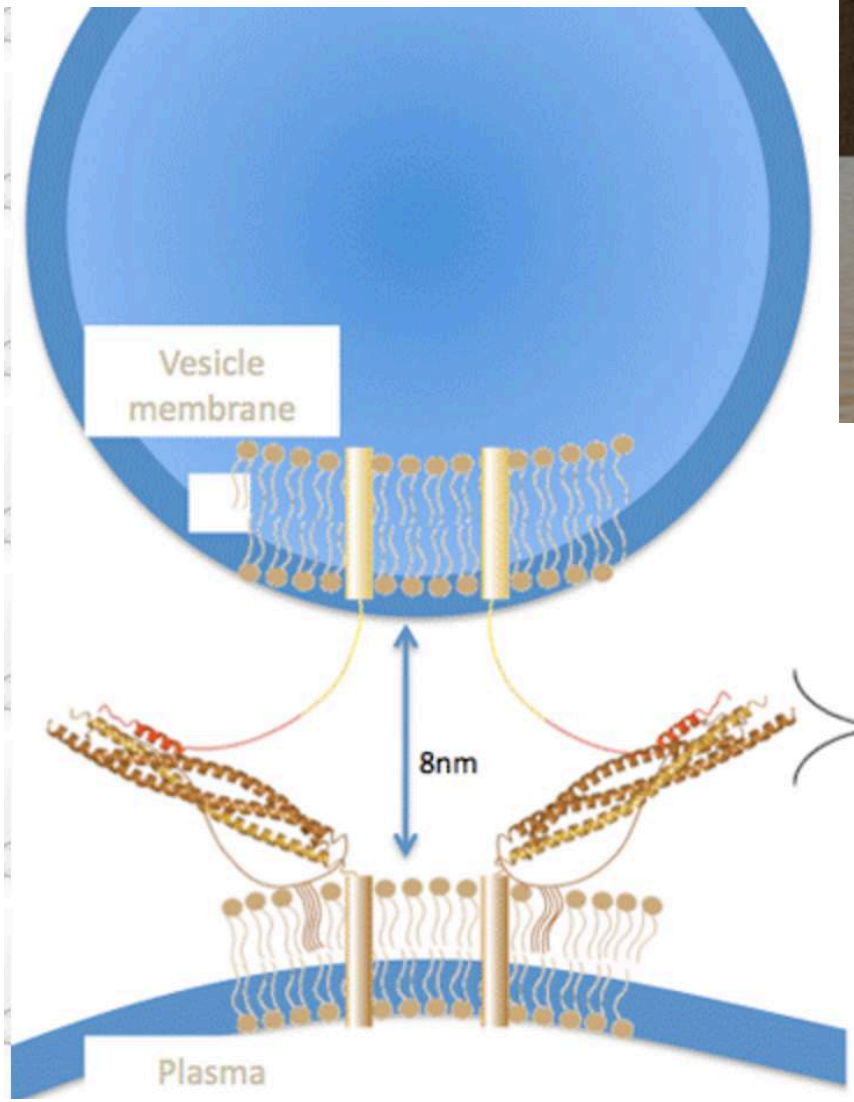


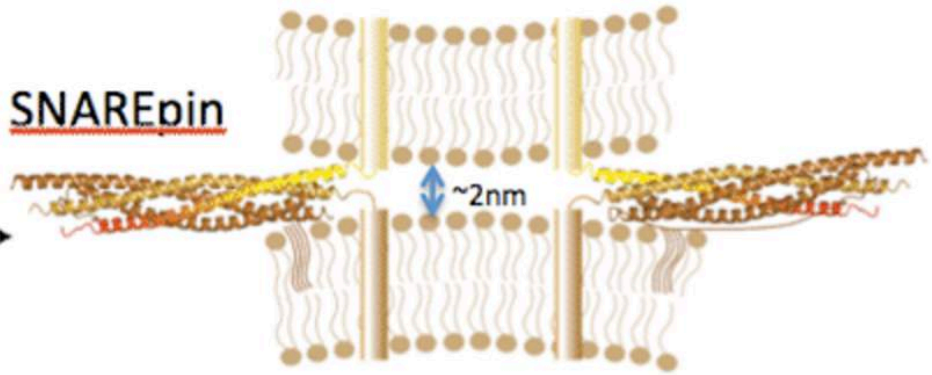
11 нм

3-спиральная цепь

Притягивание везикулы к мембране

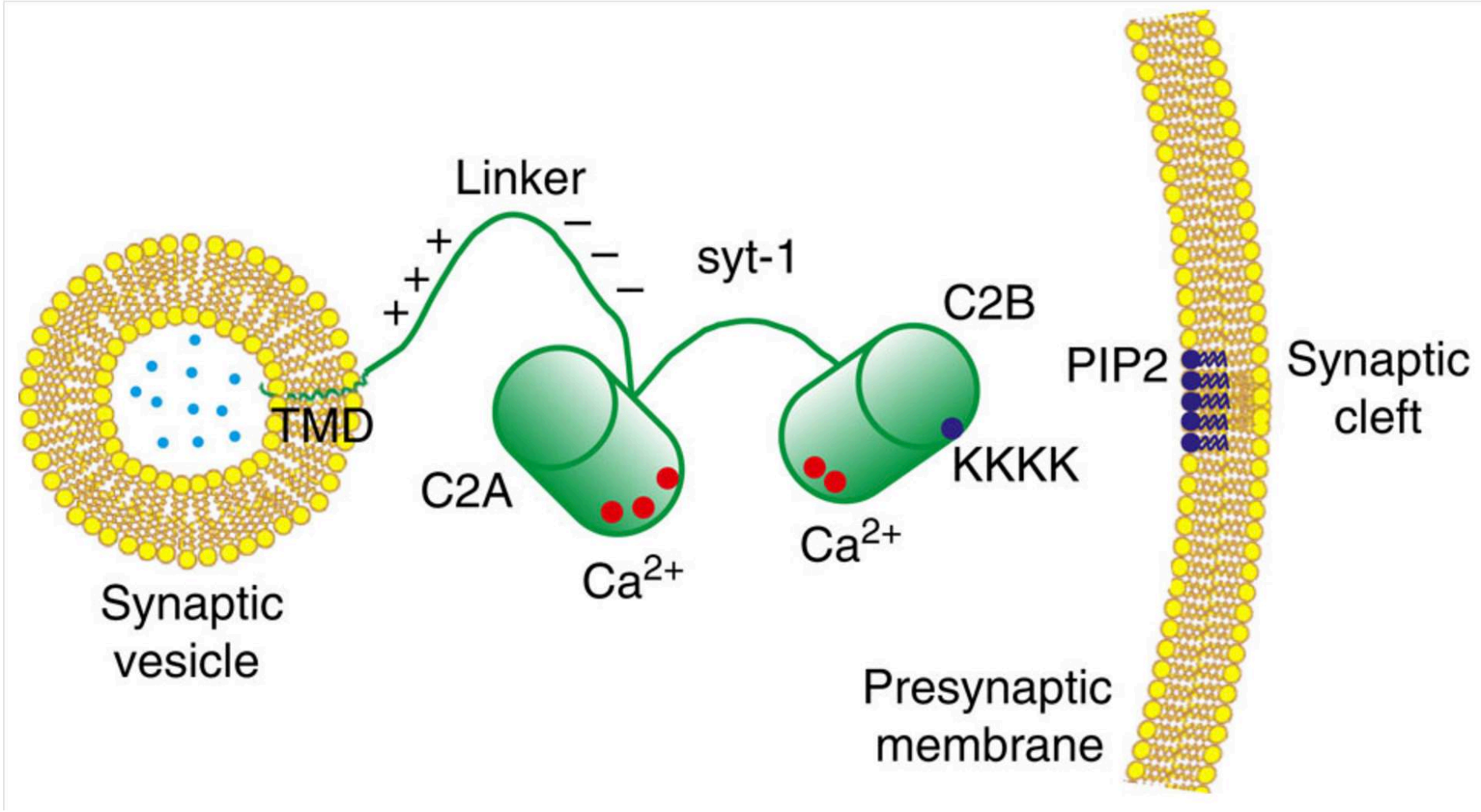




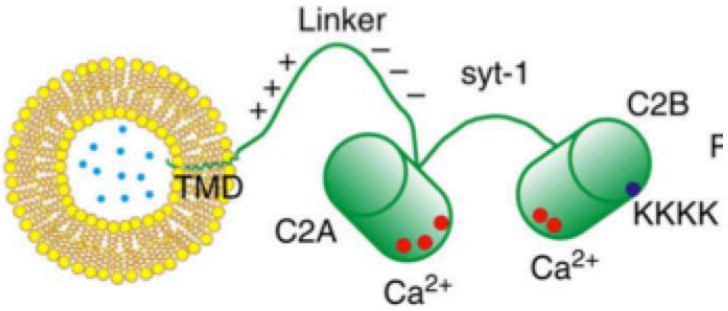


Образование SNARE комплекса

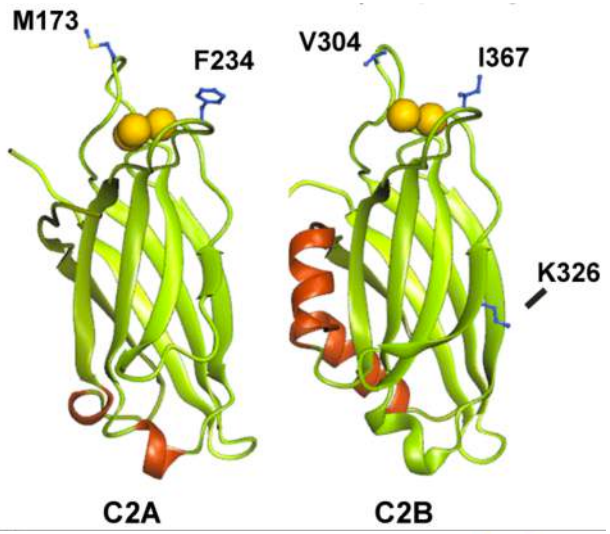
Синаптотагмин – сенсор кальция

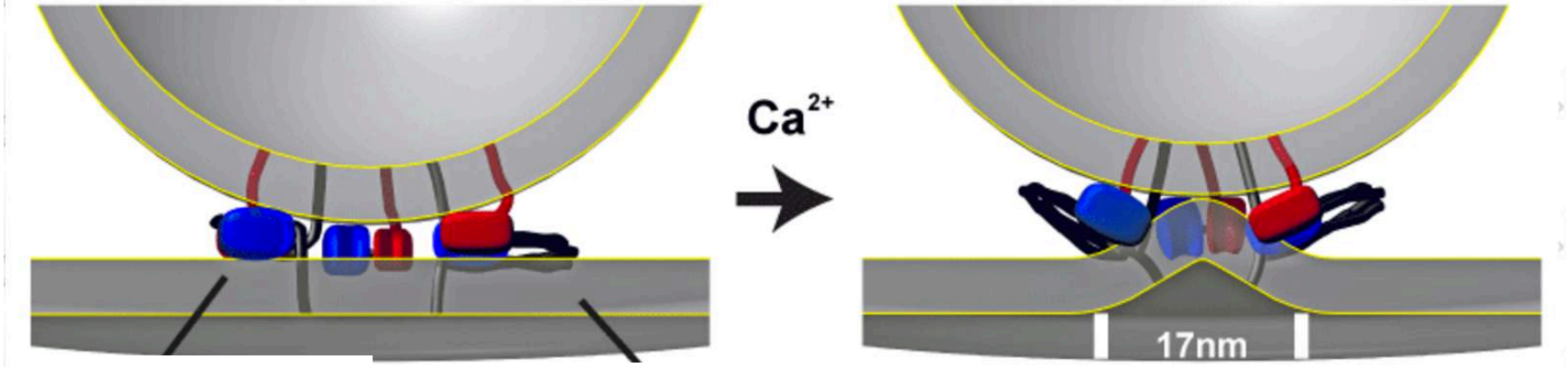


Синаптотагмин



Везикула





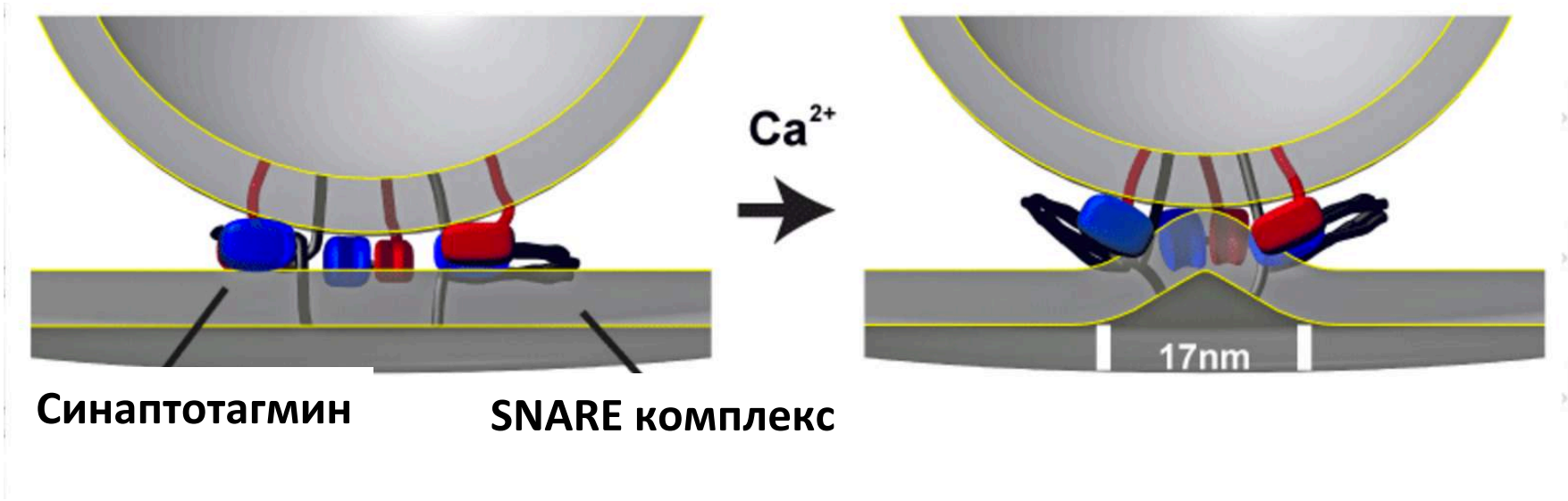
Синаптотагмин

C2A – красный

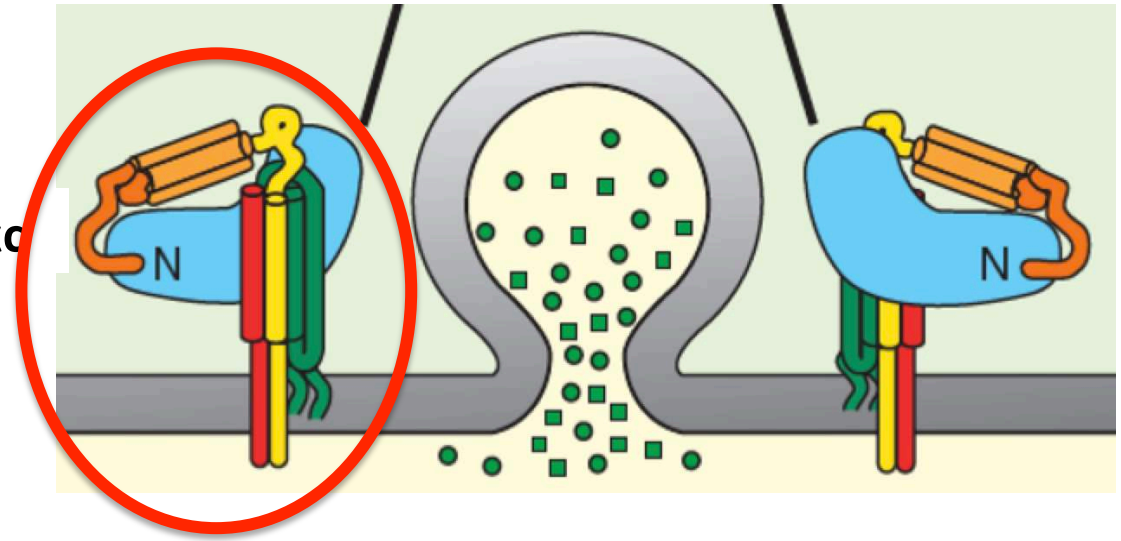
C2B - синий

SNARE комплекс

Выброс нейромедиатора

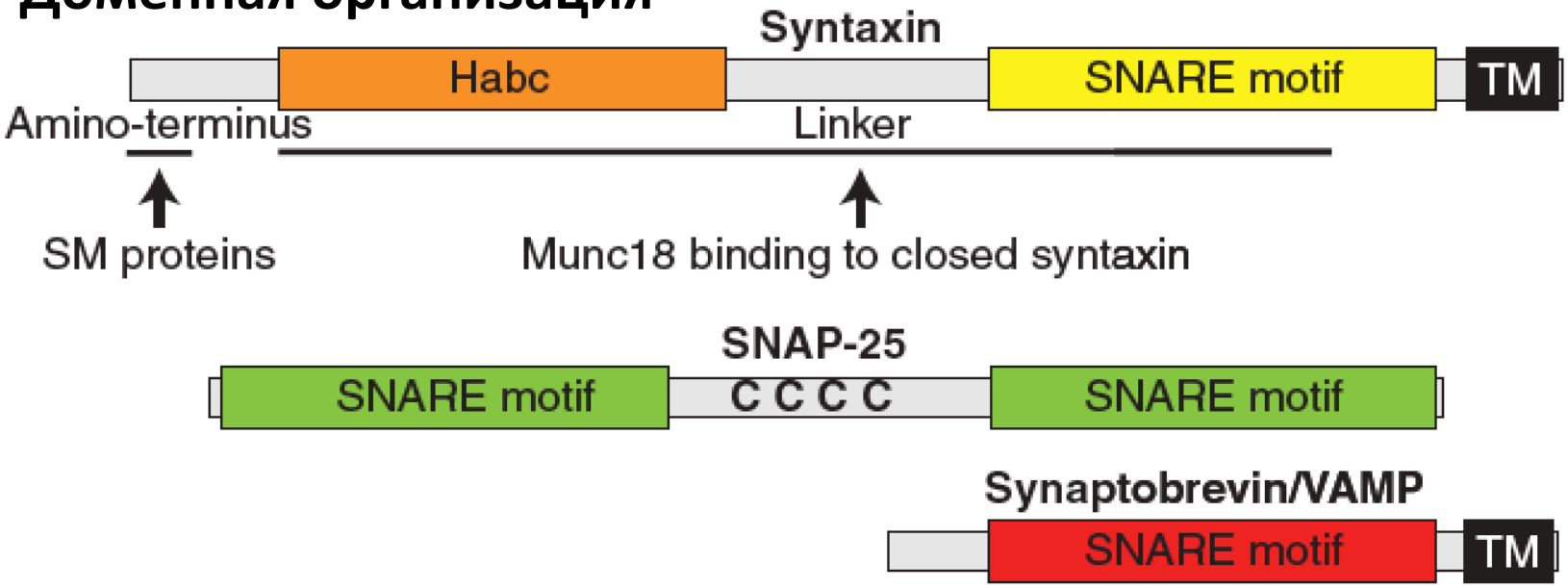


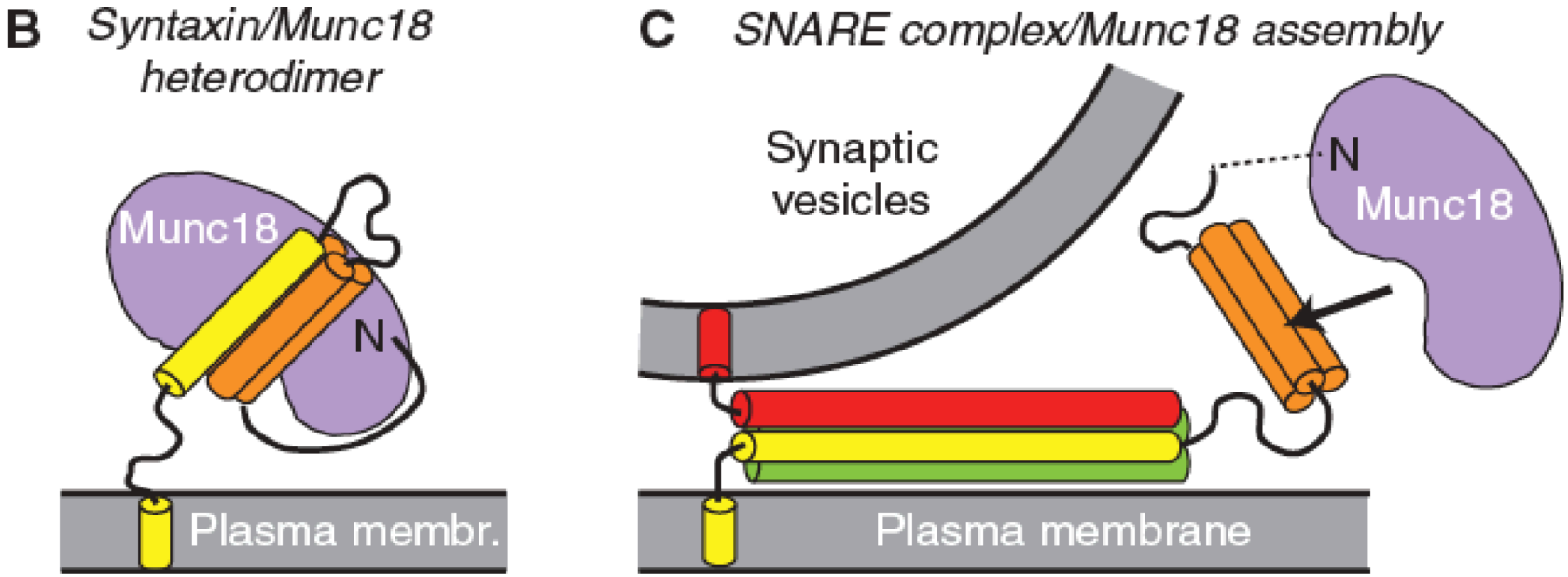
Синаптотагмин/SNARE комплекс



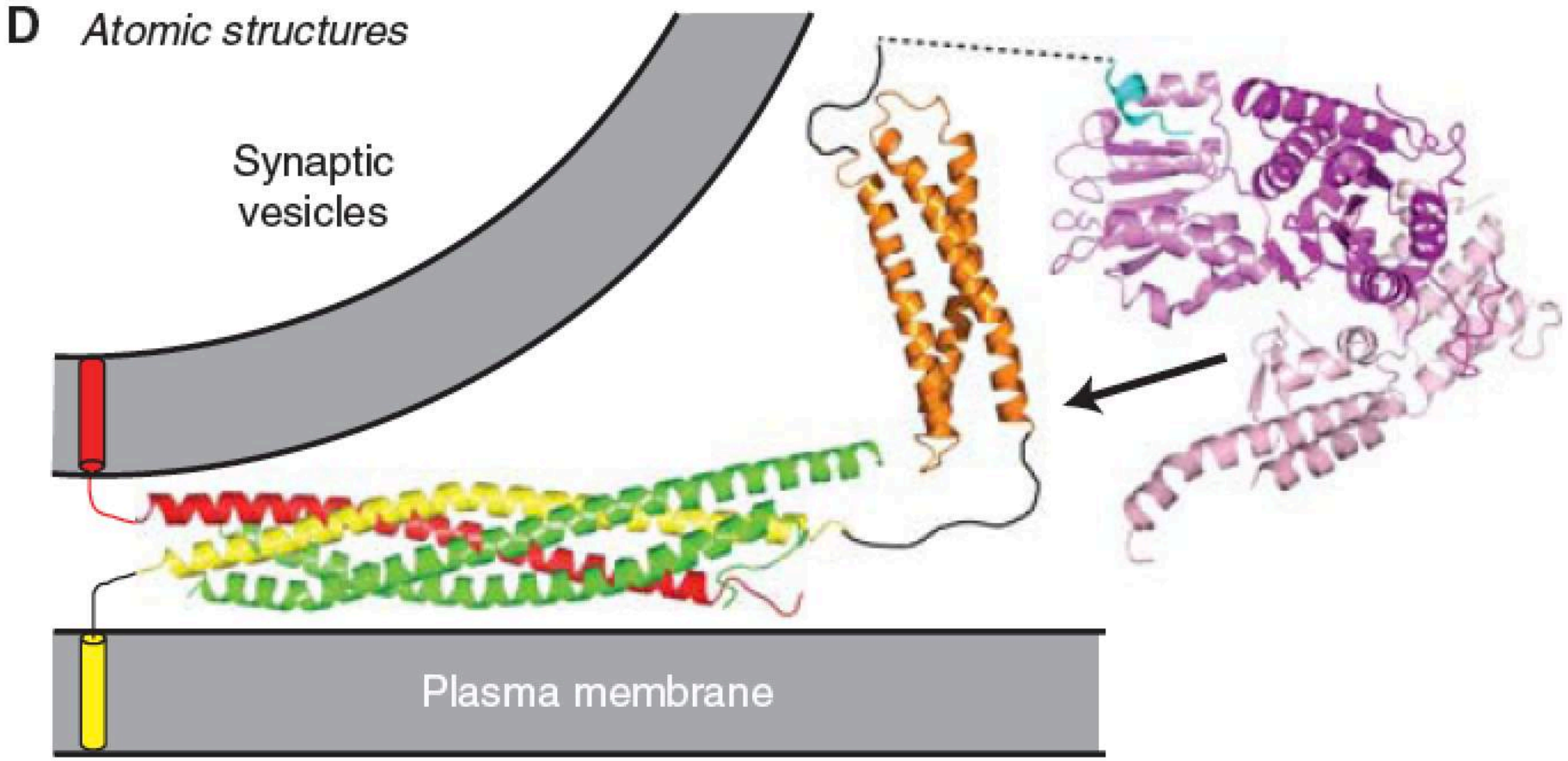
Организация SNARE белков

Доменная организация

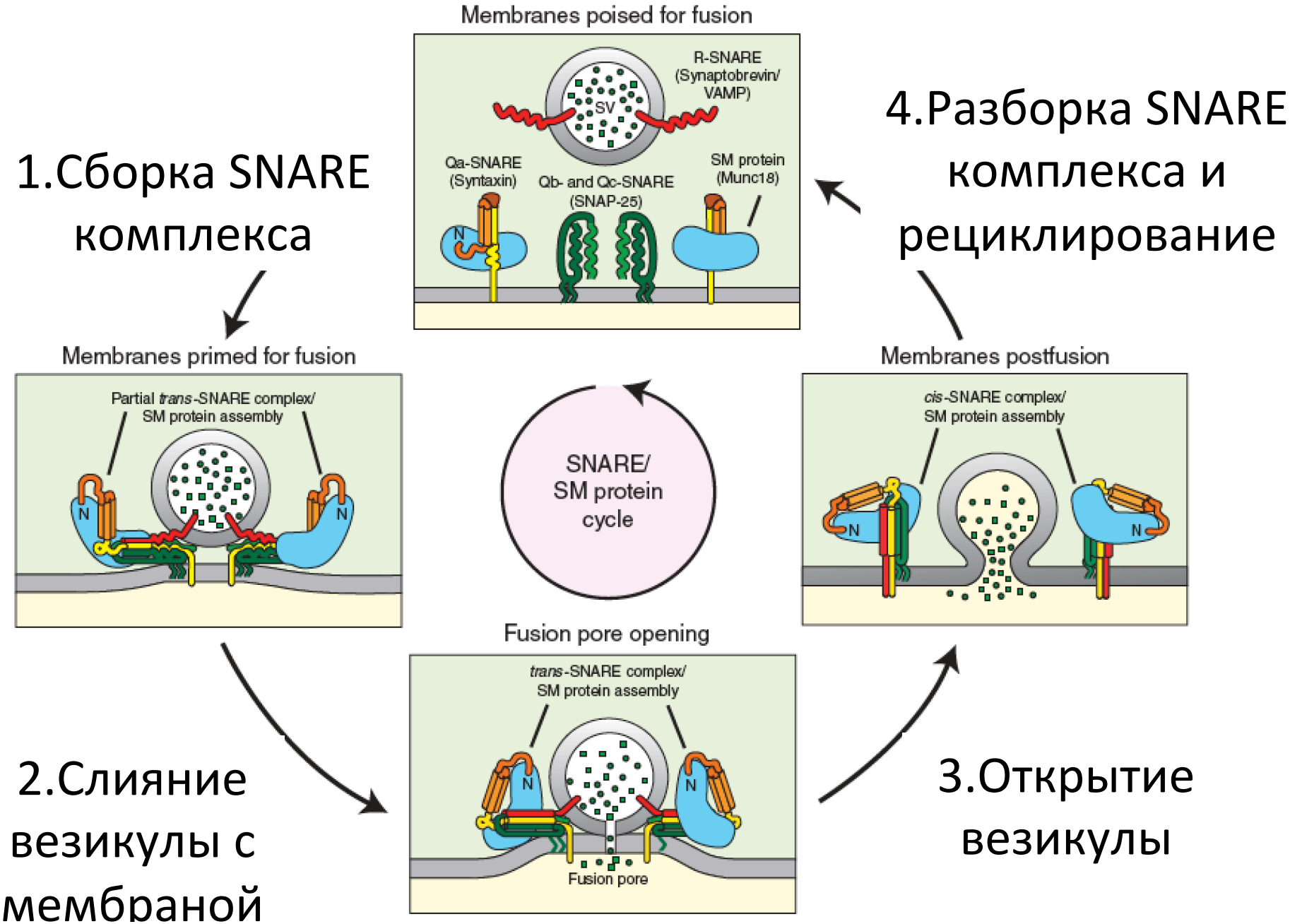




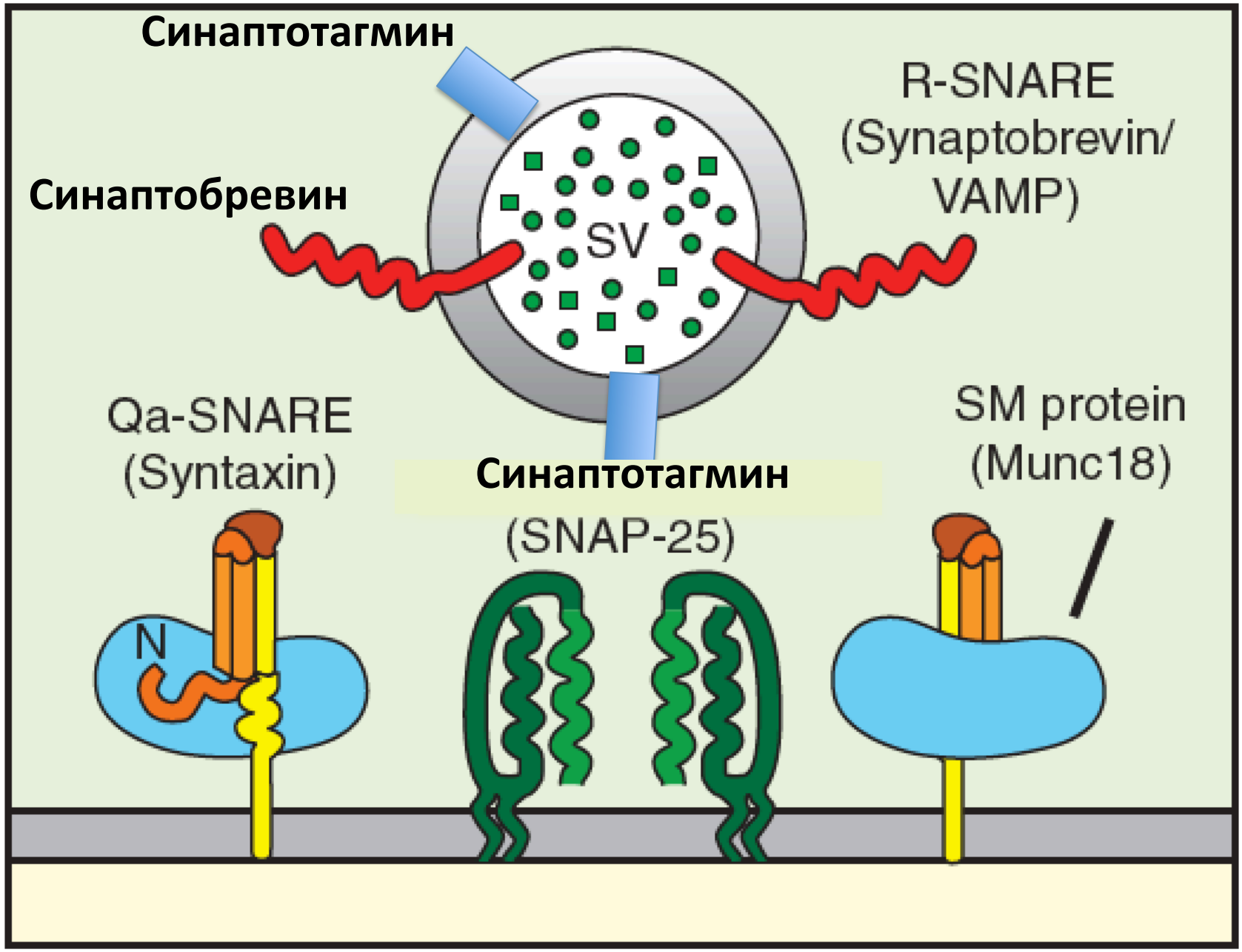
Атомная структура SNARE комплекса



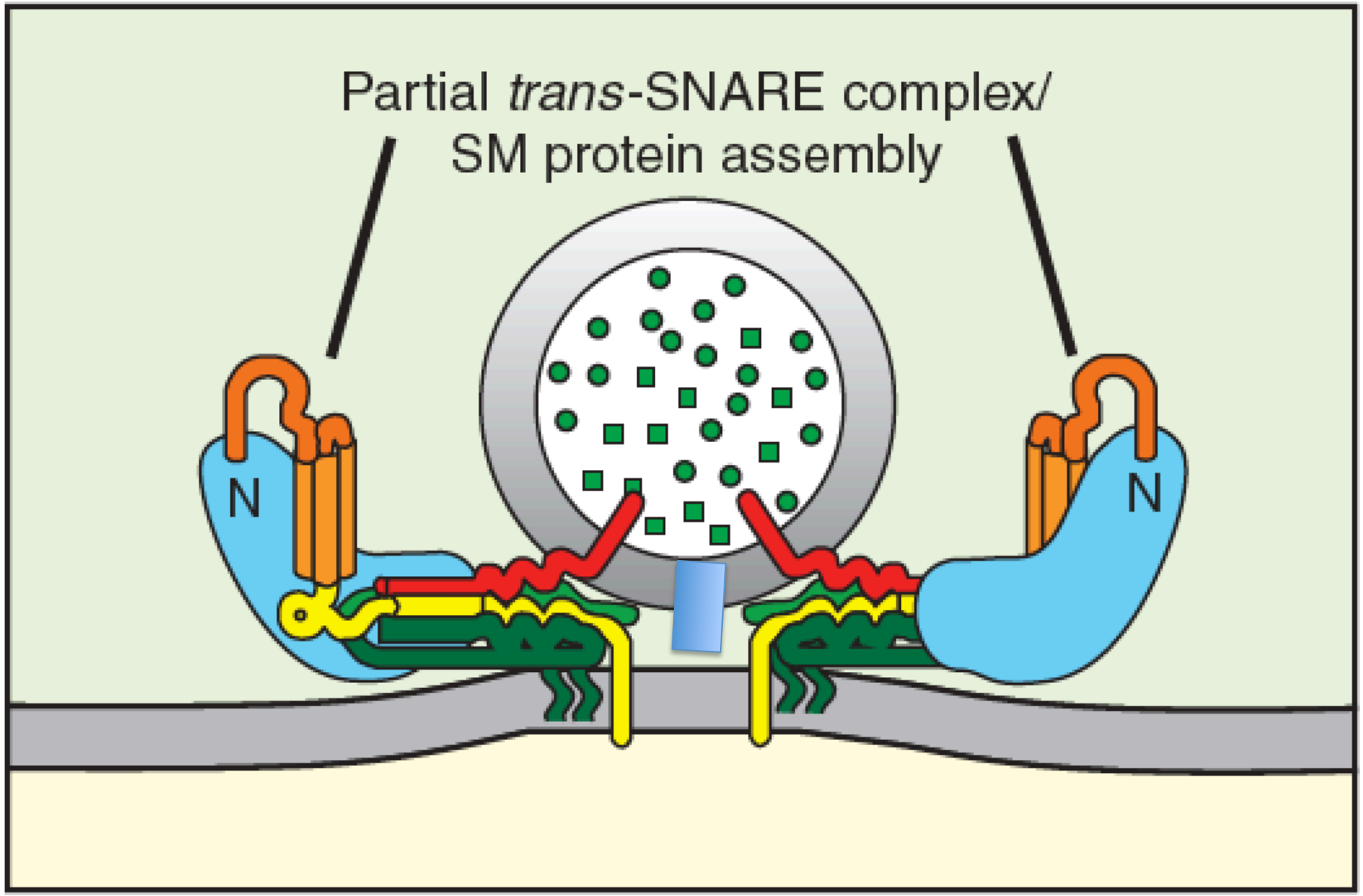
Цикл везикул нейромедиатора



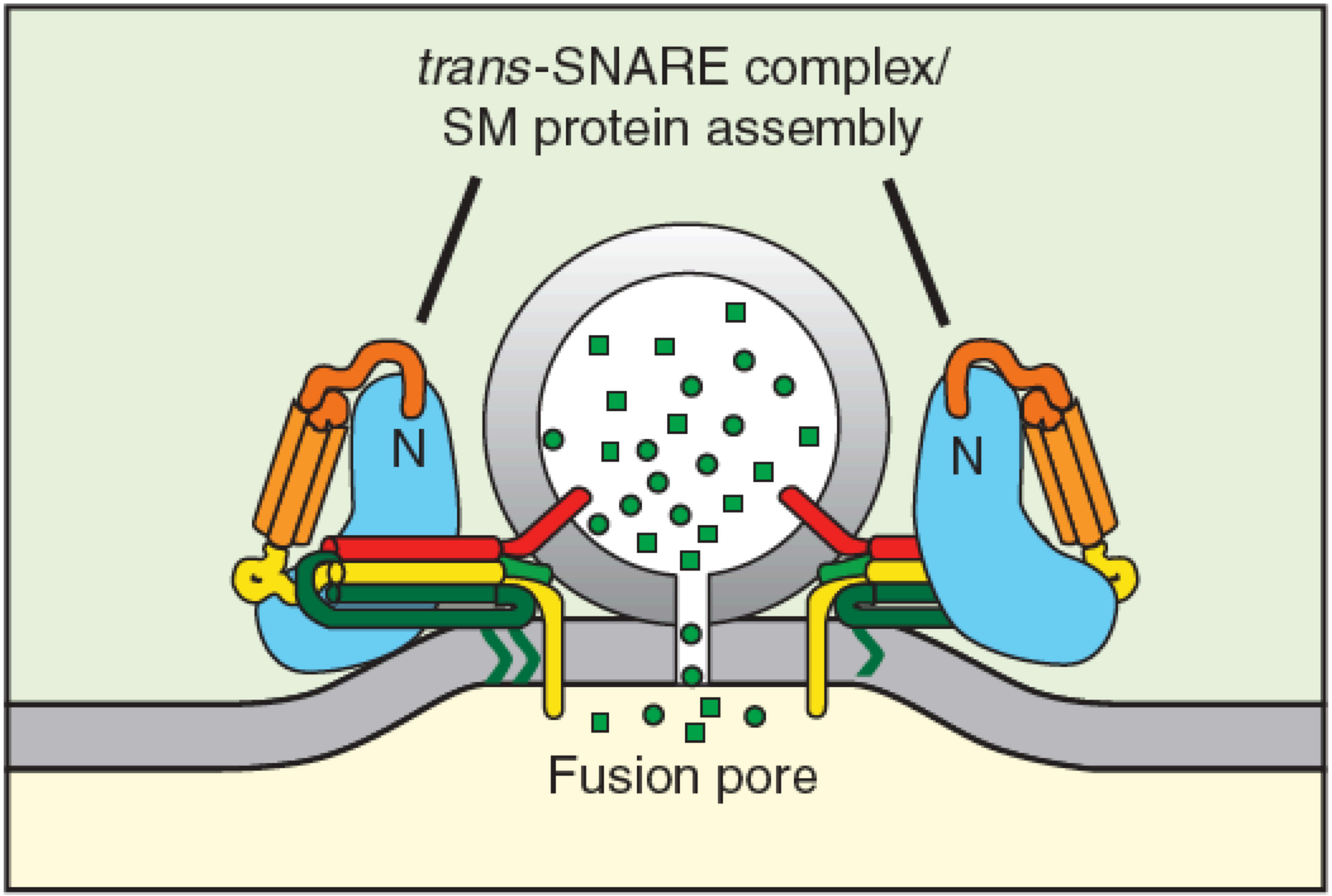
Везикула не может слиться с мембраной



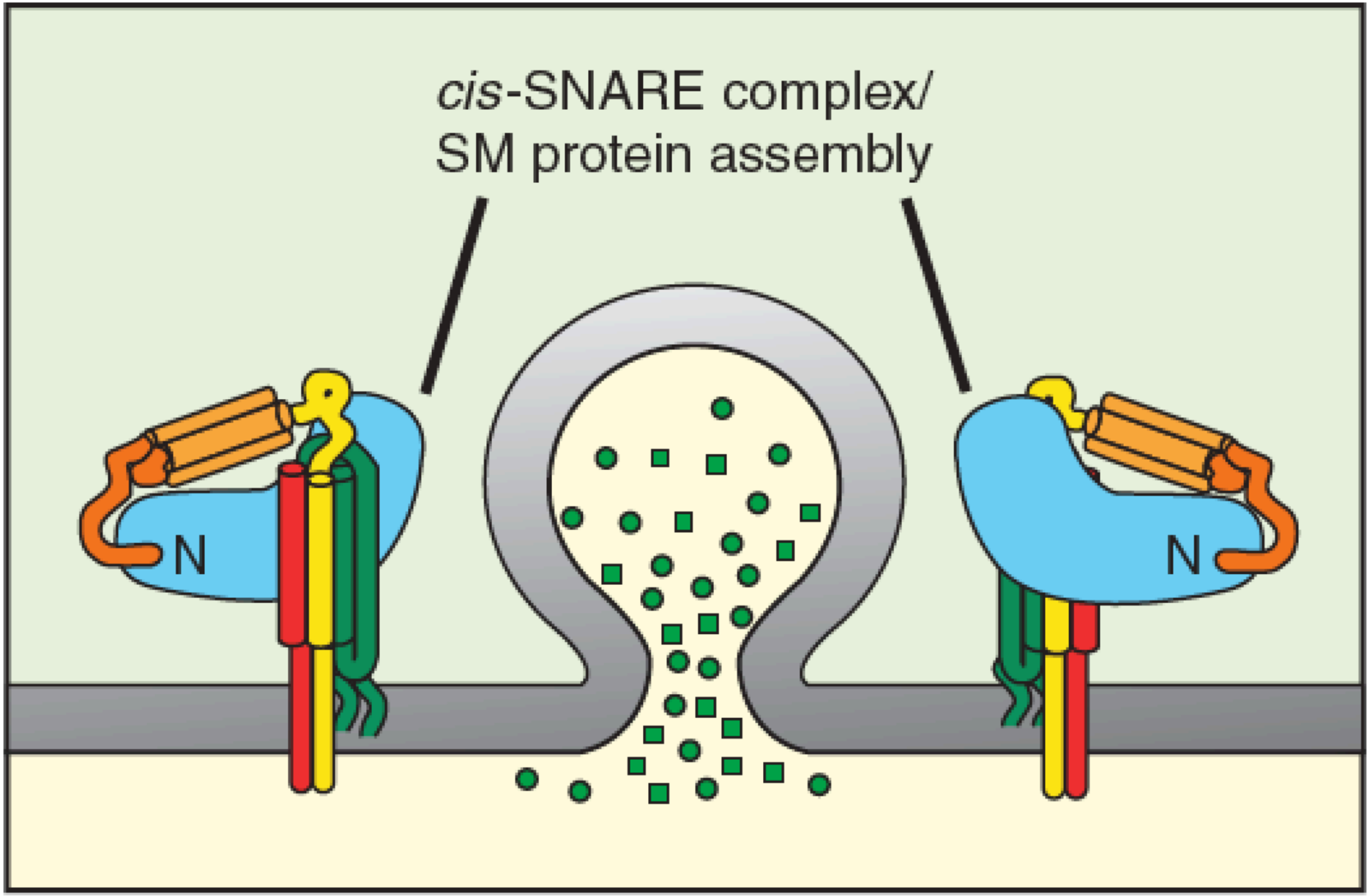
1.Сборка SNARE комплекса



2.Слияние везикулы с мембраной

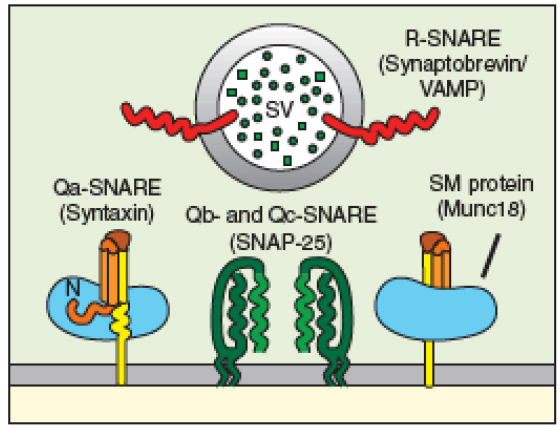


3.Выброс медиатора из везикулы

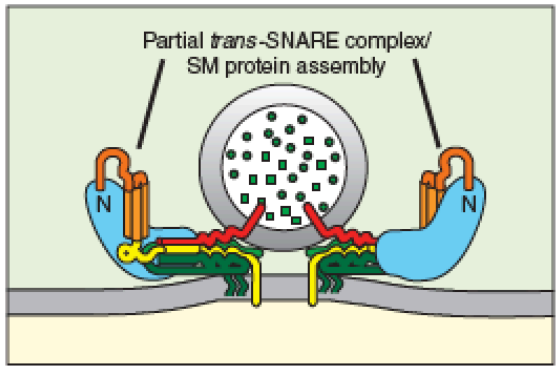


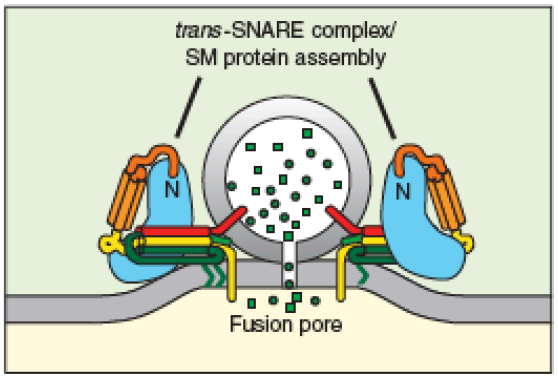
Везикула не может слиться с мембраной

1.Сборка SNARE комплекса

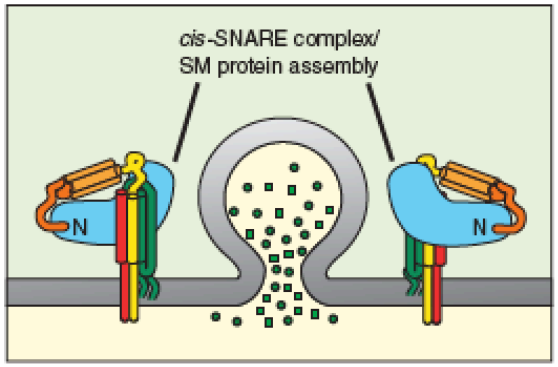


2.Слияние везикулы с мембраной





3.Открытие везикулы

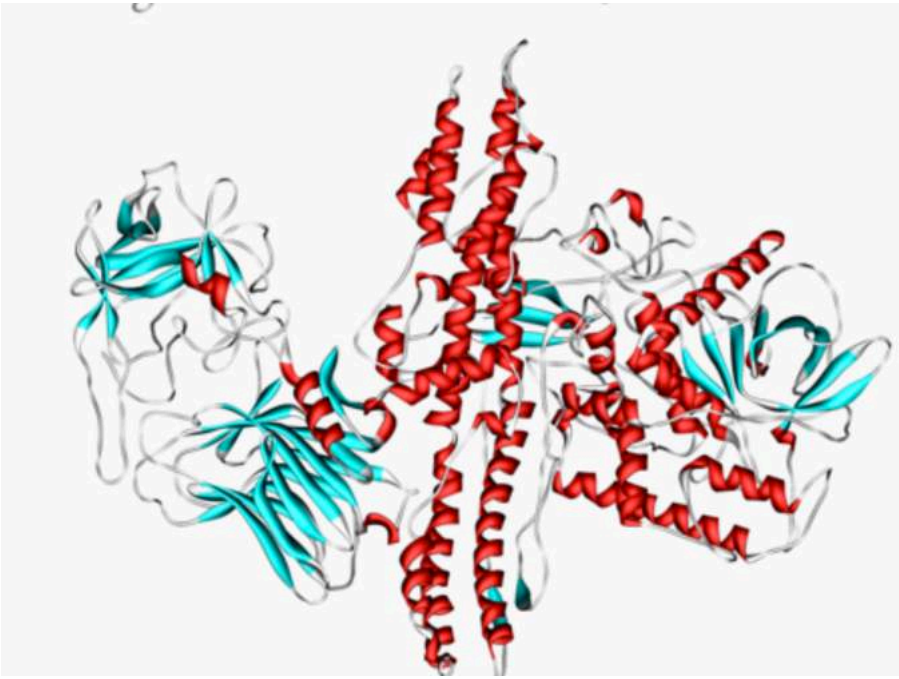


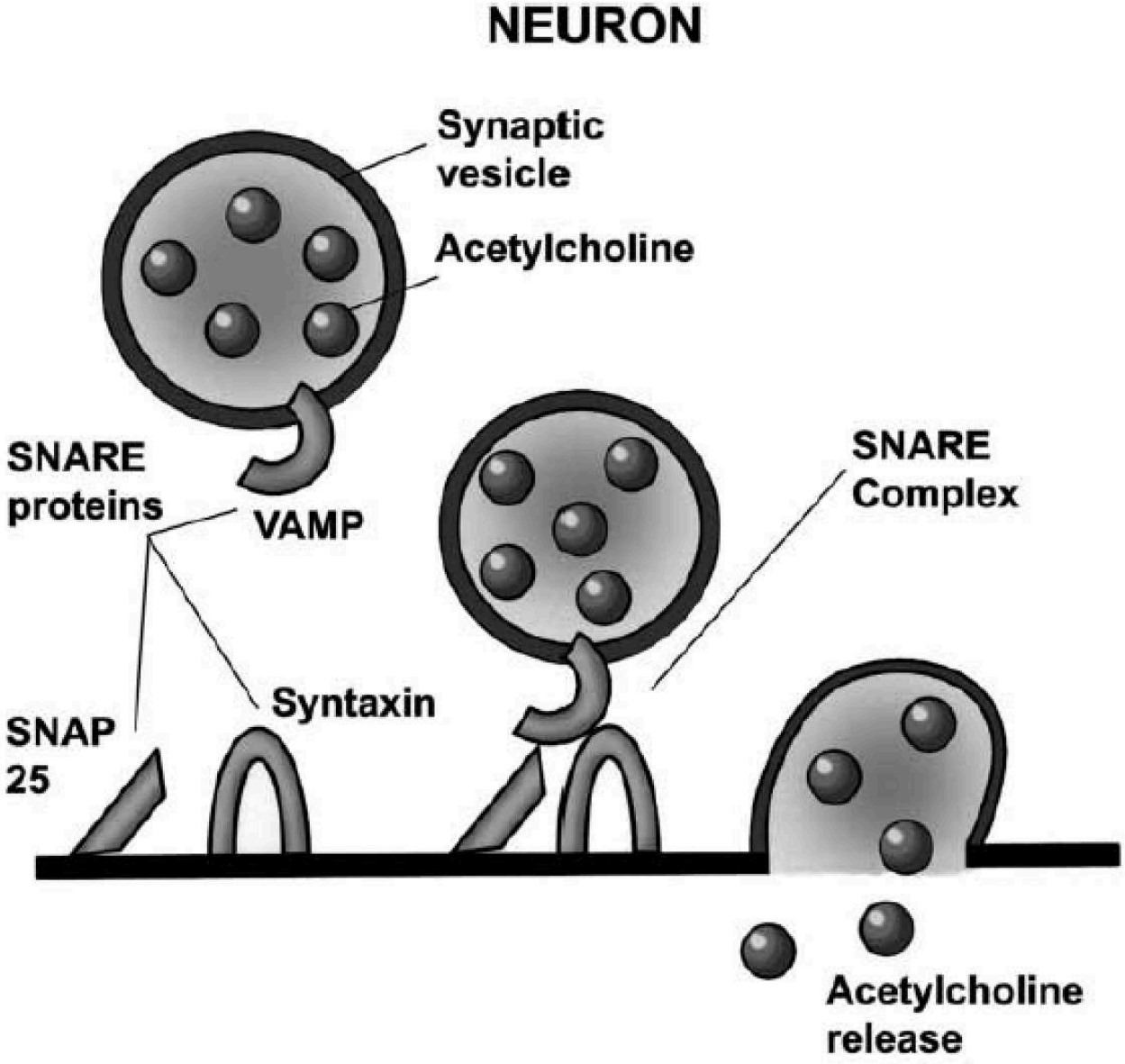
4.Разборка SNARE комплекса и рециклирование

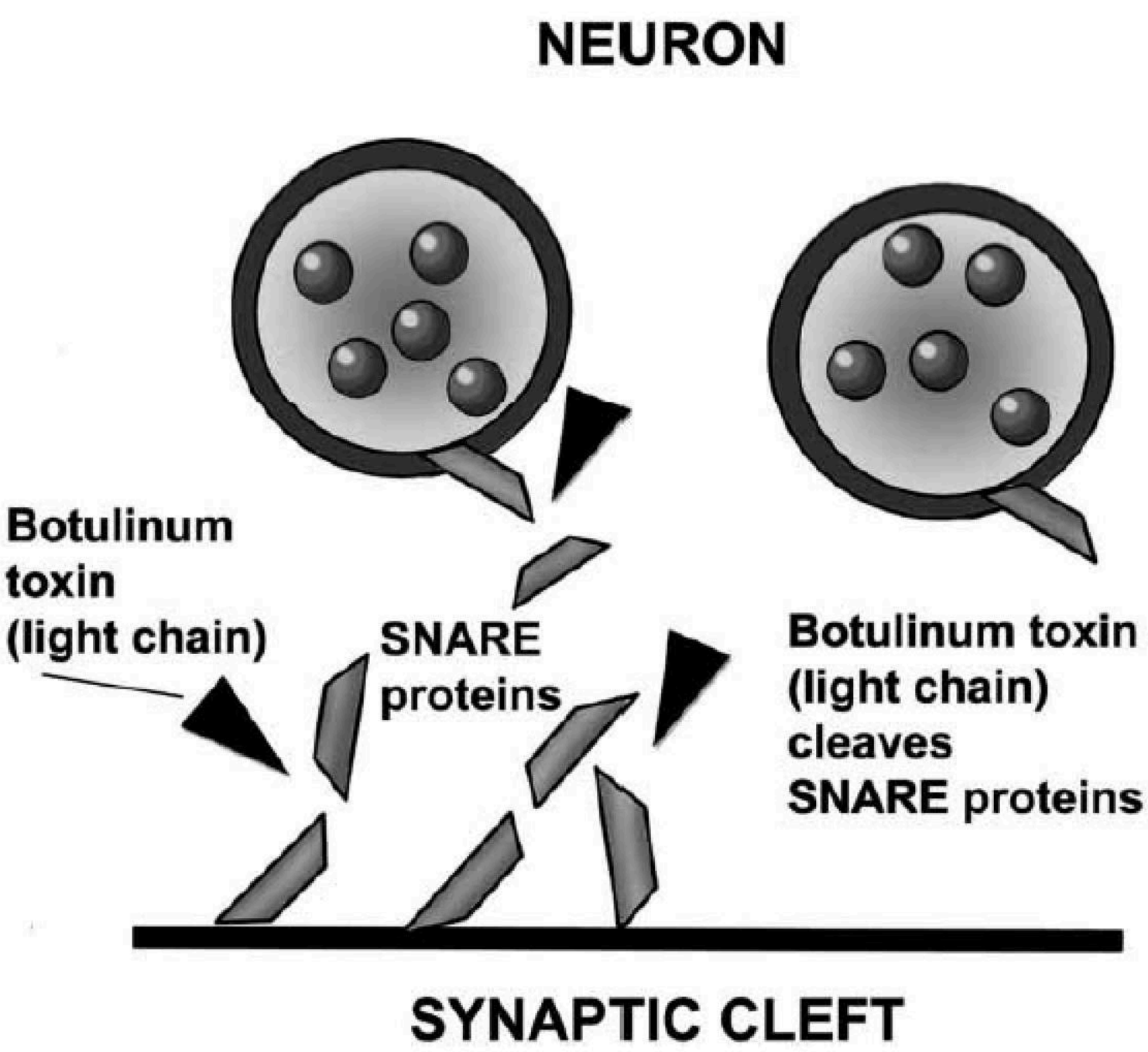
SNARE/
SM protein
cycle

Выброс нейромедиатора

Ботулиновый токсин - блокатор выброса нейромедиаторов



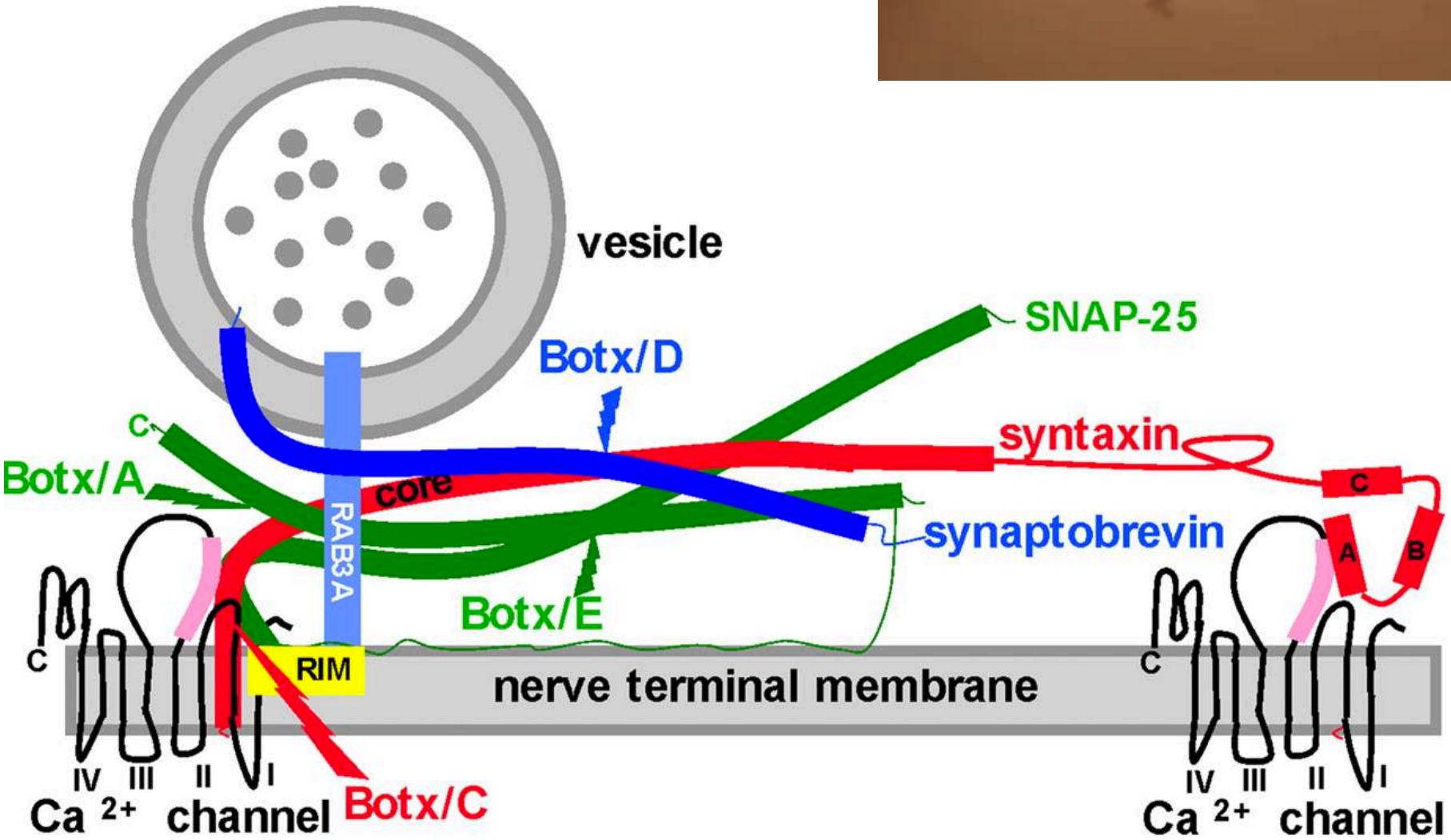

NEURON
Synaptic vesicle
Acetylcholine
SNARE proteins
VAMP
SNARE Complex
Syntaxin
SNAP 25
Acetylcholine release
SYNAPTIC CLEFT


NEURON
Botulinum
toxin
(light chain)
SNARE
proteins
Botulinum toxin
(light chain)
cleaves
SNARE proteins
SYNAPTIC CLEFT

Какие белки расщепляют ботулиновый токсины

Toxin	Synaptic Protein	Location
Botulinum toxins A & E	SNAP-25	Synaptic plasma membrane
Botulinum toxin C1	Syntaxins	Synaptic plasma membrane
Botulinum toxin B, D, F & G & tetanus toxin	Синаптобревин	Synaptic vesicle

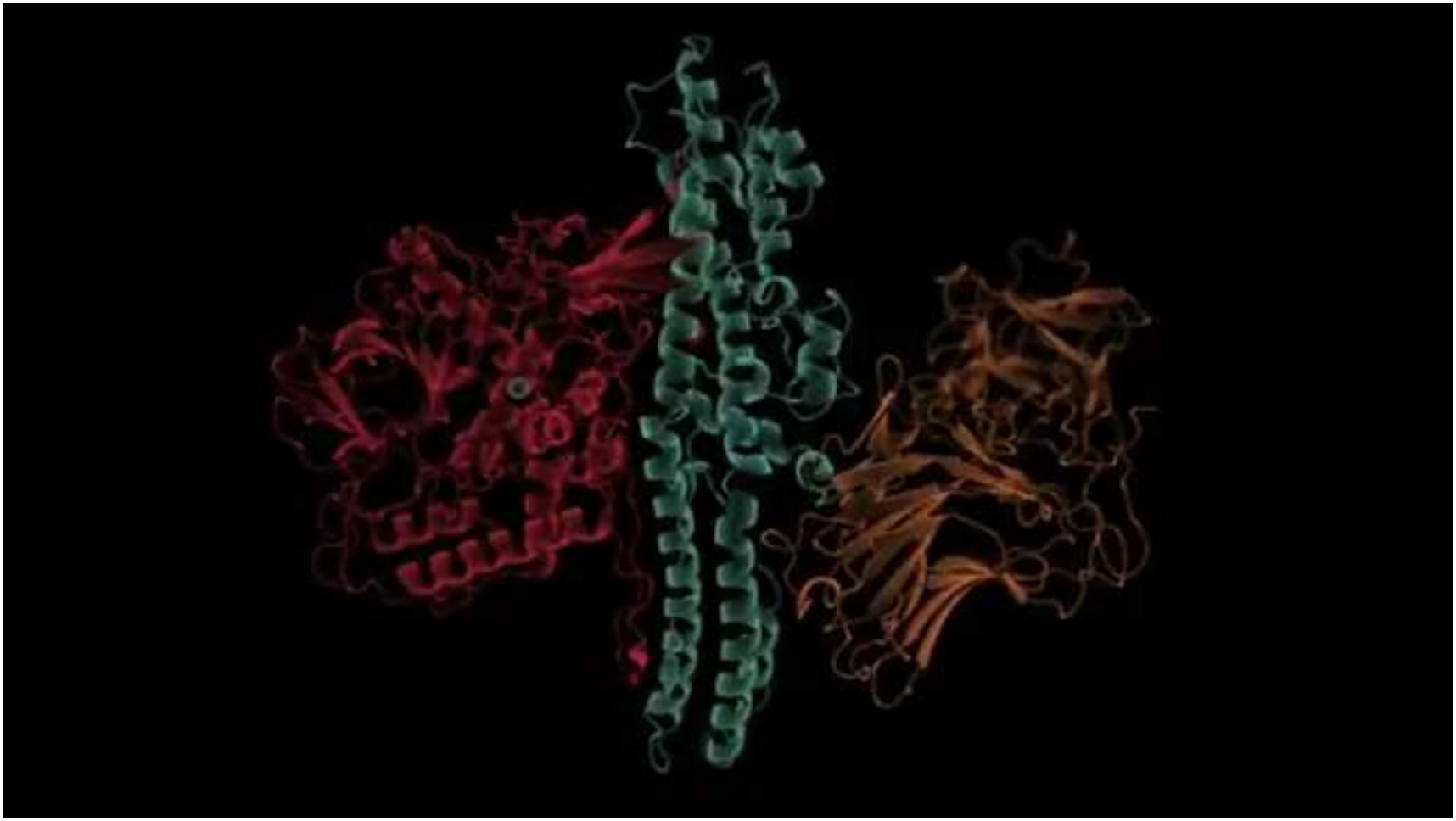
На память


vesicle
SNAP-25
Botx/D
syntaxin
C
Botx/A
core
RAB3A
synaptobrevin
C
A
B
Botx/E
RIM
nerve terminal membrane
C
C
IV III II I
Ca 2+ channel
Botx/C
IV III II I
Ca 2+ channel

Нейропатологии, связанные с нарушением выброса нейромедиаторов

- **Тонический блефароспазм** – неконтролируемые сокращения век глаз.
- **Цервикальная дистония** – болезненное состояние, когда мышцы шеи непроизвольно сокращаются, вызывая резкие движения головы в стороны или назад.
- **Мышечная спастичность** – измененная мышечная деятельность: частые судороги, повышенная рефлекторность сухожилий, гипертония.
- **Гиперактивность мочевого пузыря -** широко распространенное заболевание, 16 % населения США
- **Спастичность конечностей**

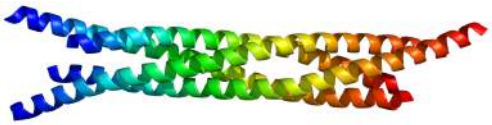
Болезни синтаксина

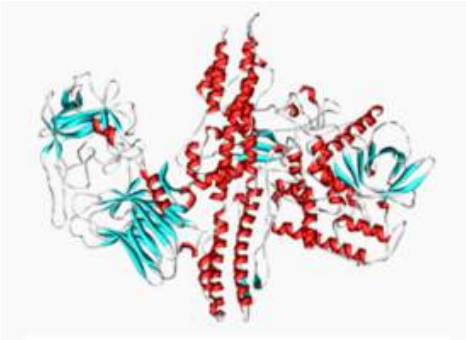


Словарь

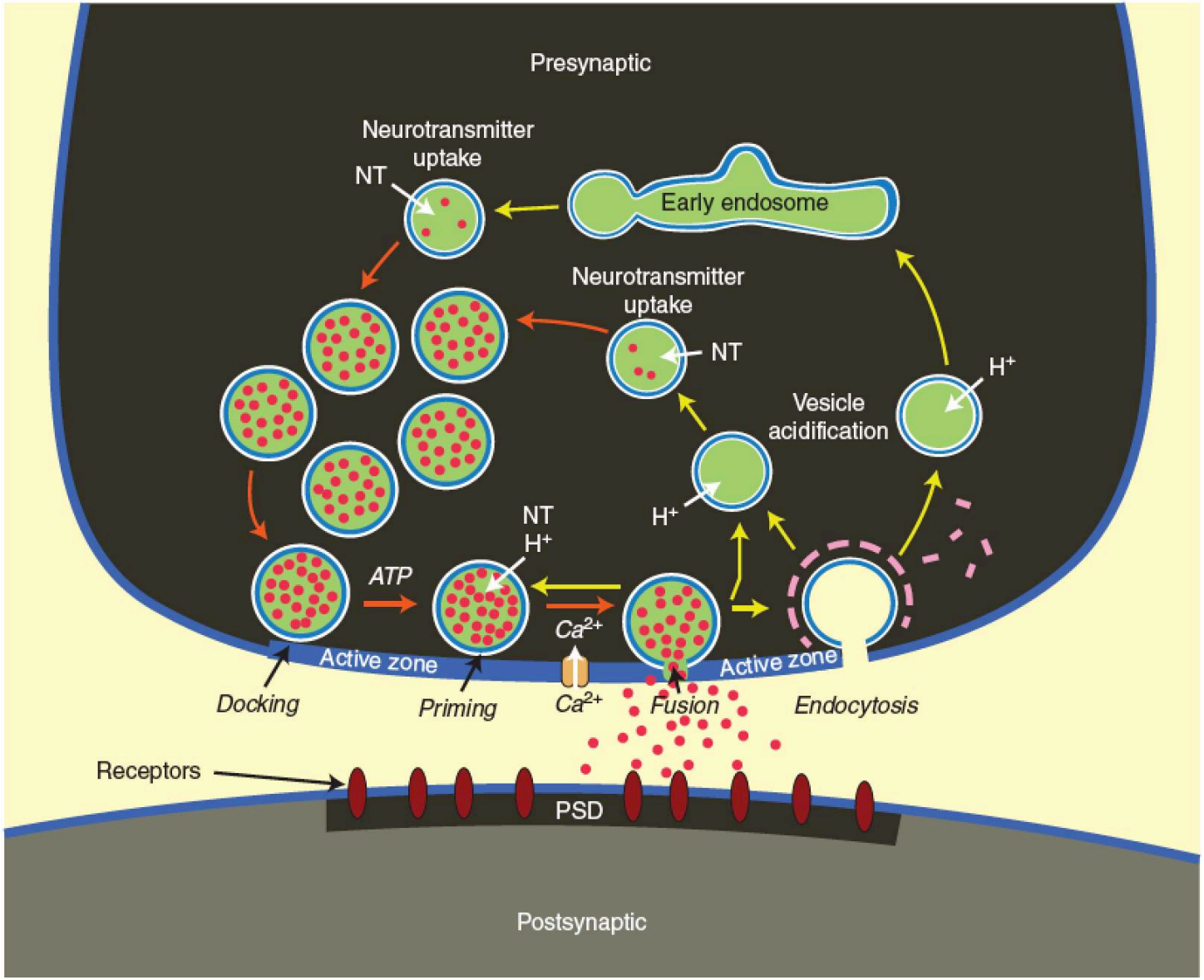
- **synaptobrevin/VAMP -** vesicle membrane protein, part of SNARE complex
- **Syntaxin** – presynaptic membrane protein, part of SNARE complex
- **SNAP-25** - presynaptic membrane protein, part of SNARE complex
- **synaptogagmin** – receptor of Ca^{2+}
- **RIMs** - Rab3-interacting molecules;
- **RIM-BPs** - RIM-binding proteins;
- **Rab3** - G protein family: four members, Rab3A, 3B, 3C, 3D
- **Rab3A -** regulates Ca^{2+}-dependent neurotransmitter release

- **SNAP25** (от англ. synaptosomal-associated protein, **25**-kD) — мембранный белок, компонент белкового комплекса SNARE, осуществляющего стыковку синаптической везикулы с пресинаптической мембраной нейрона и их слияние с последующим высвобождением нейромедиатора.
- **Ботулиновый токсин** типа A, C и E приводят к расщеплению SNAP25, что вызывает паралич.





На память


Presynaptic
Neurotransmitter uptake
NT
Early endosome
Neurotransmitter uptake
NT
H+
Vesicle acidification
H+
NT
H+
ATP
Ca2+
Active zone
Active zone
Docking
Priming
Ca2+
Fusion
Endocytosis
Receptors
PSD
Postsynaptic

На память:

Основные этапы передачи синаптического сигнала

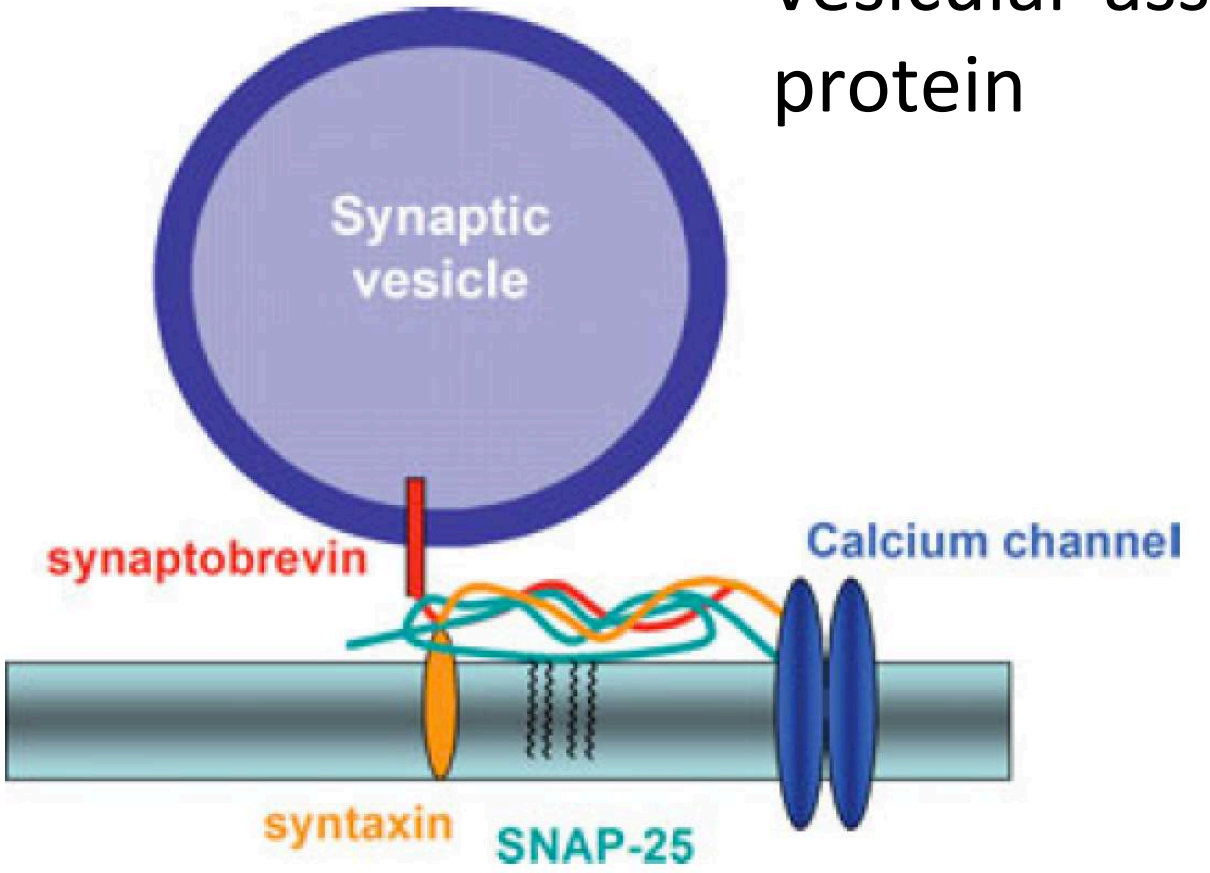
- Синапс – специализированный контакт между клетками, обеспечивающий передачу нервного импульса с пресинаптического окончания на постсинаптическую мембрану
- Нервный импульс (потенциал действия) стимулирует выброс химического вещества (нейромедиатора) в синаптическую щель
- Нейромедиатор пересекает синаптическую щель и взаимодействует с рецепторами на постсинаптической мембране

На память

- Везикула содержит:
  - фосфолипиды и холестерин (≈ 13 000 молекул)
  - **Синаптобревин (**≈70 копий**):** член SNARE комплекса
  - **Синаптотагмин** - рецептор Ca^{2+}
  - **V-АТФазы:** используют энергию гидролиза АТФ для закачки водорода внутрь
  - **Транспортеры** нейромедиаторов
- Неропатологии
- SNARE комплекс:
  - Синтаксин/SNAP25/Синаптобревин

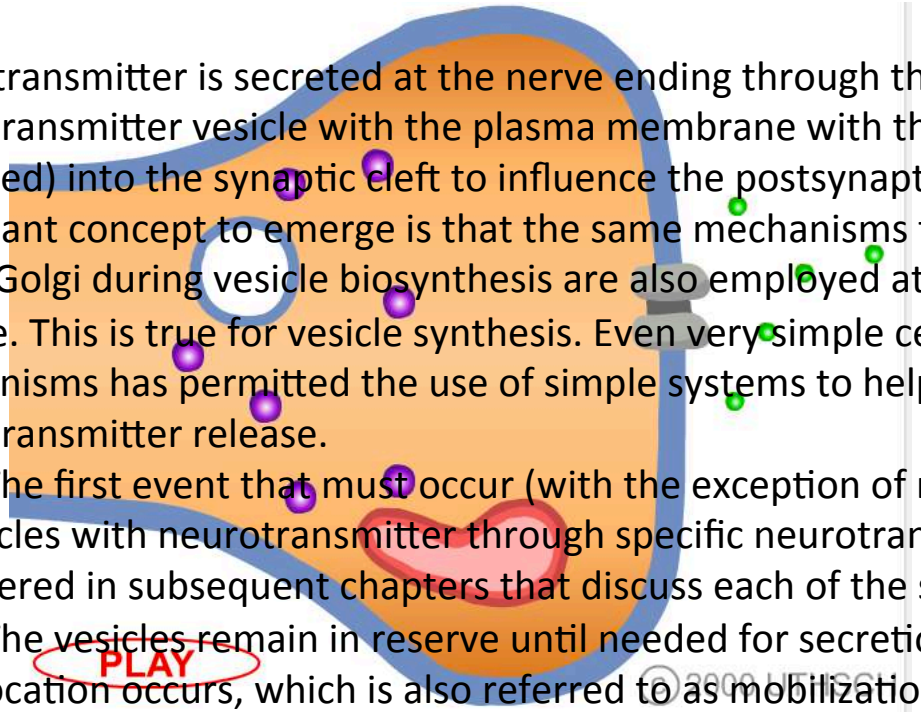
SNAP-25 - synaptosomal-associated protein 25 kDa

- SNAP-25 - synaptosomal-associated protein 25 kDa
- VAMP/synaptobrevin - vesicular-associated membrane protein



Словарик

- SNAP-25 - synaptosomal-associated protein 25 kDa
- VAMP/synaptobrevin - vesicular-associated membrane protein
- **SNARE -** Soluble NSF Attachment Protein)**RE**ceptor") суперсемейство из > 60 белков. Роль – слияние везикул с мембраной (лизосомы, пресинаптическая мембрана)



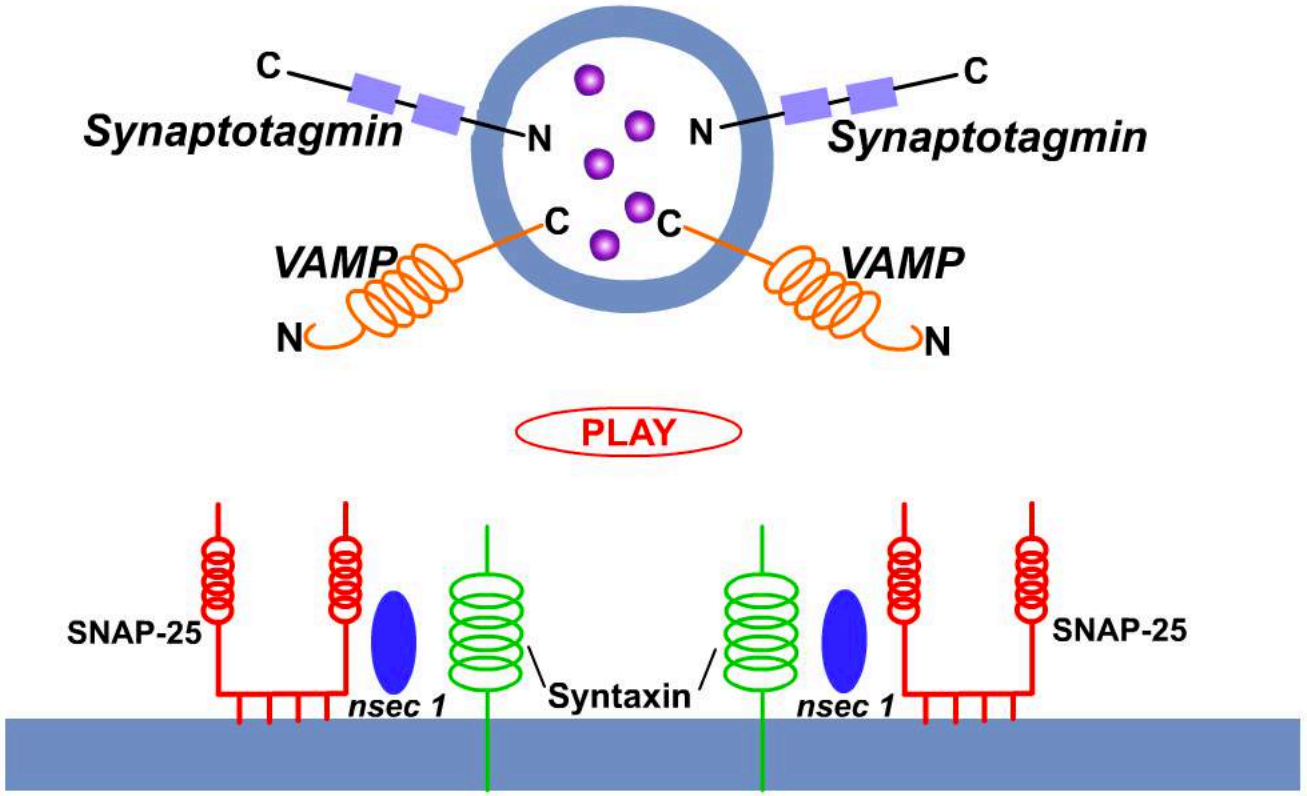
Neurotransmitter is secreted at the nerve ending through the Ca2+-dependent fusion of neurotransmitter vesicle with the plasma membrane with the neurotransmitter being secreted (released) into the synaptic cleft to influence the postsynaptic cell. This process is termed exocytosis. An important concept to emerge is that the same mechanisms that occur in vesicle fusion with membranes in the Golgi during vesicle biosynthesis are also employed at the nerve ending for neurotransmitter release. This is true for vesicle synthesis. Even very simple cells like yeast. This conservation of mechanisms has permitted the use of simple systems to help understand the molecular mechanisms of neurotransmitter release.

The first event that must occur (with the exception of neuropeptide neurotransmitters) is the filling of vesicles with neurotransmitter through specific neurotransmitter uptake (NT Uptake). This uptake will be covered in subsequent chapters that discuss each of the specific neurotransmitters.

The vesicles remain in reserve until needed for secretion. When needed for secretion, a translocation occurs, which is also referred to as mobilization. The vesicles move to a region of plasma membrane called the active zone. The active zone is the release site and is characterized by the appearance of dense material adjacent to the plasma membrane. The influx of Ca2+ is believed to increase translocation by increasing the Ca2+ dependent phosphorylation of a vesicle binding protein termed synapsin. The theory is that Ca2+ dependent phosphorylation of synapsin frees the vesicles from binding to actin microfilaments. The vesicles then bind to the active zone of the plasma membrane, where they are in position to undergo release of their neurotransmitter.

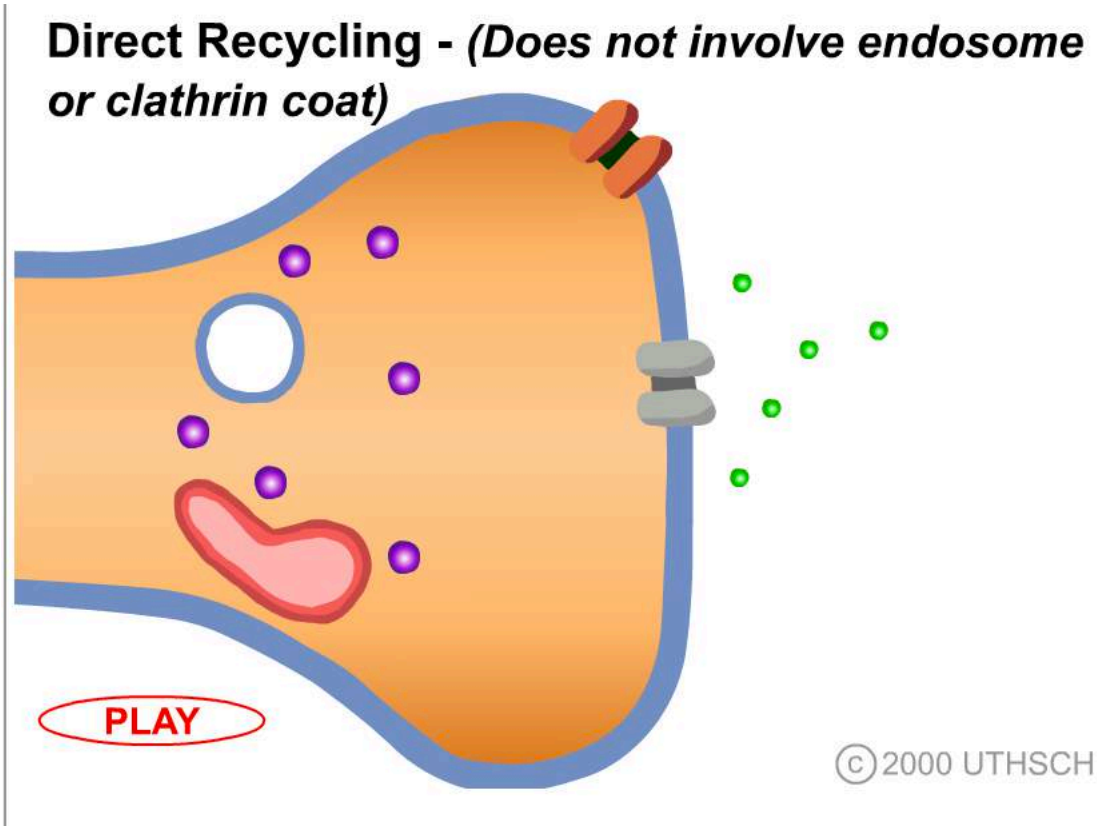
The association of the vesicle with the plasma membrane is termed docking and serves to prime the vesicle for secretion. The docking is believed to occur through the binding of proteins in the vesicle membrane to proteins in the plasma membrane. Several of these proteins have been discovered because they are targets for clostridia bacterial toxins that block synaptic transanimation. Several of these toxins and the proteins they detect are shown in Table I. The toxins are so toxic that a single molecule can poison a whole nerve terminal. One of the synaptic vesicle proteins is VAMP, and two of the synaptic plasmal membrane proteins are syntaxin and SNAP-25.

Toxin	Synaptic Protein	Location
Botulinum toxins A & E	SNAP-25	Synaptic plasma membrane
Botulinum toxin C1	Syntaxins	Synaptic plasma membrane
Botulinum toxin B, D, F & G & tetanus toxin	VAMPs	Synaptic vesicle



A third plasma membrane protein, n-sec-1, is important because its loose association with the plasma membrane prevents the binding of the neurotransmitter vesicle proteins until n-sec-1 is displaced (the mechanism of n-sec-1 displacement is currently not understood). This and subsequent steps in the secretory process are shown in Figure 10.8. The vesicle and plasma membrane proteins are hypothesized to complex with one another upon the displacement of n-sec-1 to form a "trimeric complex" (SNAP-25, syntaxin and VAMP). This three-member complex has been isolated, intact, from the nerve endings of animals. This association of the proteins initiates fusion. Vesicles at this stage are primed for release.

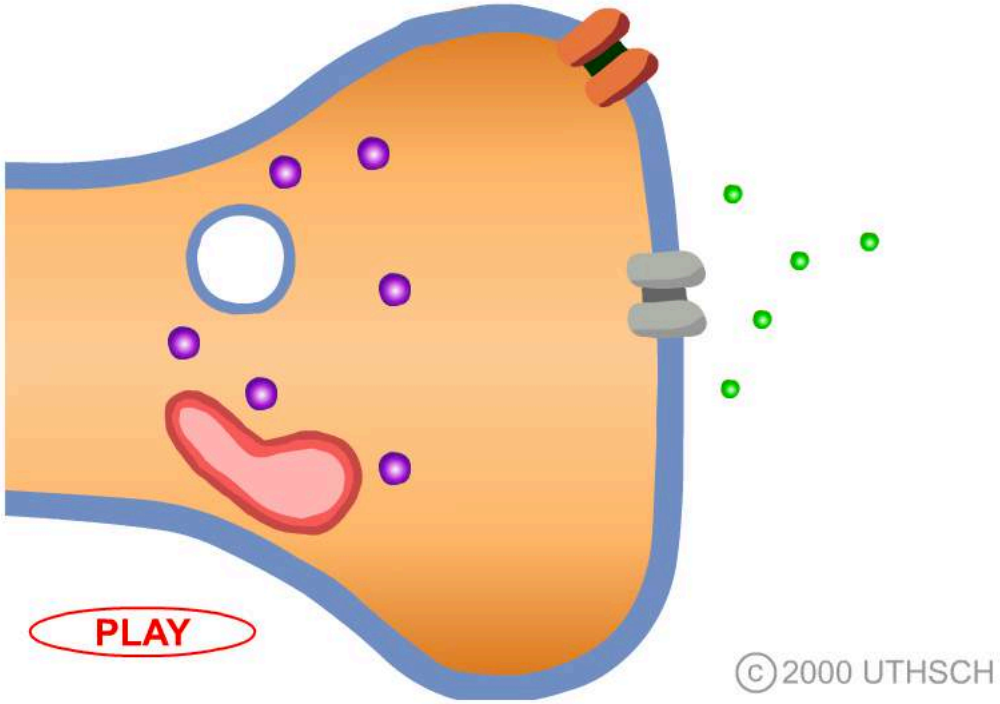
The final stage of release, also shown in Figure 10.8, is the fission of the membrane at the point of contact between the vesicle and the plasma membrane. Exocytosis of neurotransmitter into the synaptic cleft occurs when this fission takes place. This step is Ca2+ stimulated, but the mechanism of the Ca2+ trigger is unknown. One hypothesis is that a vesicle protein called synaptotagmin binds Ca2+ to initiate fission. Support for synaptotagmin, as the Ca2+ sensor, is that it possesses two binding sites for Ca2+. Additional evidence comes from studies of mice in which synaptotagmin has been knocked out. In these mice fast Ca2+-triggered synaptic vesicle exocytosis is severely limited. Many aspects of the fusion-fission mechanism remain to be understood, including: what causes the dissociation of n-sec-1 from the complex, how Ca2+ functions in the release process and how all the proteins that are involved in release become reassociated with the proper membrane following release as the vesicle membrane is recycled.



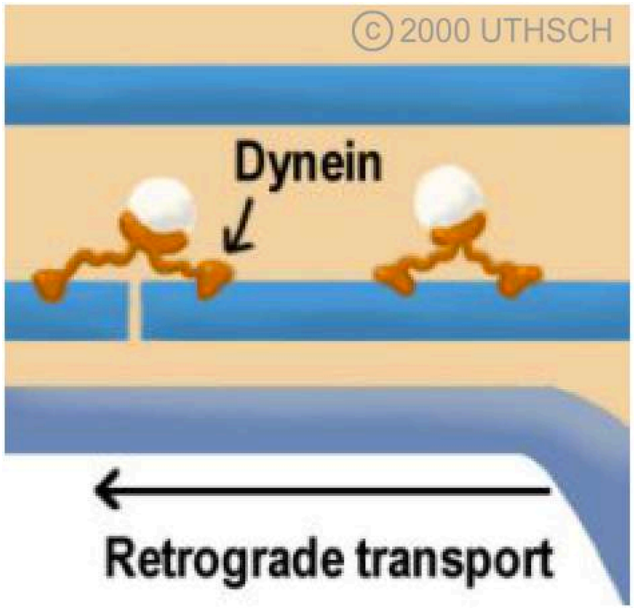
зезикул

Although the source of vesicles for neurotransmitter secretion comes from biosynthesis in the Golgi apparatus at the cell body, evidence indicates that local re-synthesis of synaptic vesicle is an important aspect of neurotransmitter secretion. Figures 10.9 and 10.10 provides two schematic summaries of how vesicles are locally reused. Both utilize the recapture of vesicle membrane from the nerve ending. In one, vesicles bud off the plasma membrane through the formation of pits that migrate directly to become a neurotransmitter vesicle as soon as it can be refilled with neurotransmitter through the neurotransmitter uptake process. This is shown in Figure 10.9. This mechanism is referred to as the "kiss and run" hypothesis.

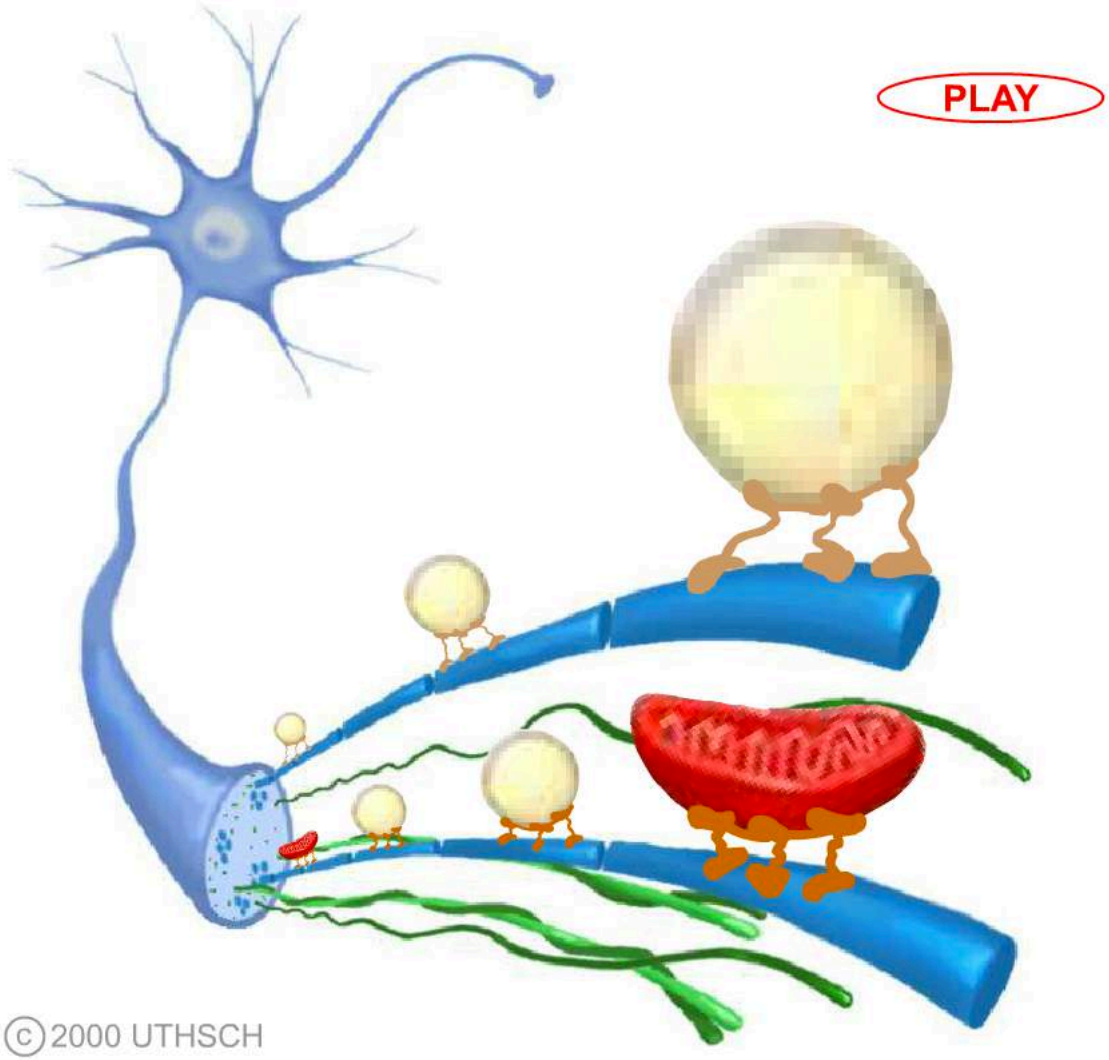
Indirect Recycling - *(Does involve endosome)*



The second mechanism involves the formation of clathrin coated pits to recapture vesicle membrane, and the vesicles cycle through the endosomal compartment in the nerve ending before becoming functional synaptic vesicles. The vesicles then bud off the endosome to form the neurotransmitter vesicle. This is shown in Figure 10.10. It is believed that both mechanisms can exist in the same nerve ending or only one of the two can be present. Both are important in that they recover vesicle protein to permit a plentiful supply for synaptic transmission. This mechanism also prevents the enlargement of the nerve ending that would occur if vesicle membrane were not recaptured. No matter which mechanism is involved, the supply and resupply of vesicles can only keep pace with a high rate of synaptic transmission for a few minutes.



Eventually the proteins utilized for synaptic transmission in the nerve ending are targeted and returned to the cell body of the neuron to be recycled to make new protein and vesicles. The proteins are returned to the soma through a retrograde axoplasmic transport that is analogous to anterograde transport but uses a different motor protein, dynein. Transport is mediated by the interaction of dynein with microtubules and proceeds at a rate somewhat slower than that of fast anterograde transport (0.2-1 cm per day). In addition to returning proteins to the soma, retrograde transport serves as a means of communication between the nerve endings and the cell soma. This is a mechanism to transport signaling molecules to regulate the development and maintenance of axonal contacts with postsynaptic cells



summary of retrograde and anterograde axoplasmic transport. It shows the motor proteins, kinesin and dynein, mediating the movement of vesicles and mitochondria anterogradely and vesicles retrogradely along microtubules. The animation shows the motor proteins as a part of the organelle that is transported. The other possible relationship between the motor protein and microtubules is that the motor proteins are a part of the microtubule and pass the vesicles along the microtubule.

Question:

Which of the following processes dictate the amount of neurotransmitter released from a nerve ending on a short-term, minute-to-minute, basis? (NOTE: there is more than one correct answer.)

A. Neurotransmitter synthesis

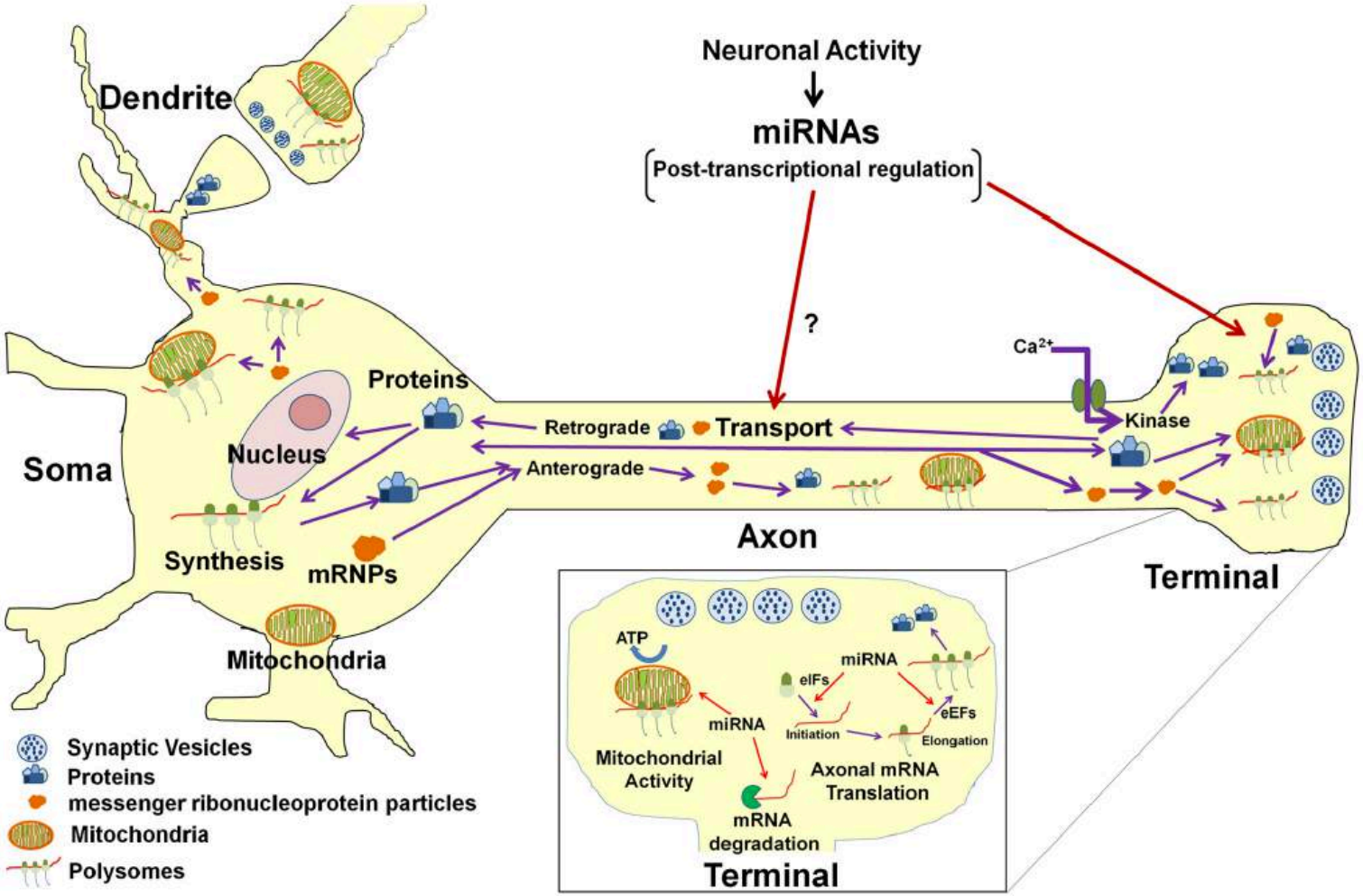
B. Vesicle synthesis in the cell soma

C. Vesicle recycling in the nerve ending

D. Axoplasmic transport

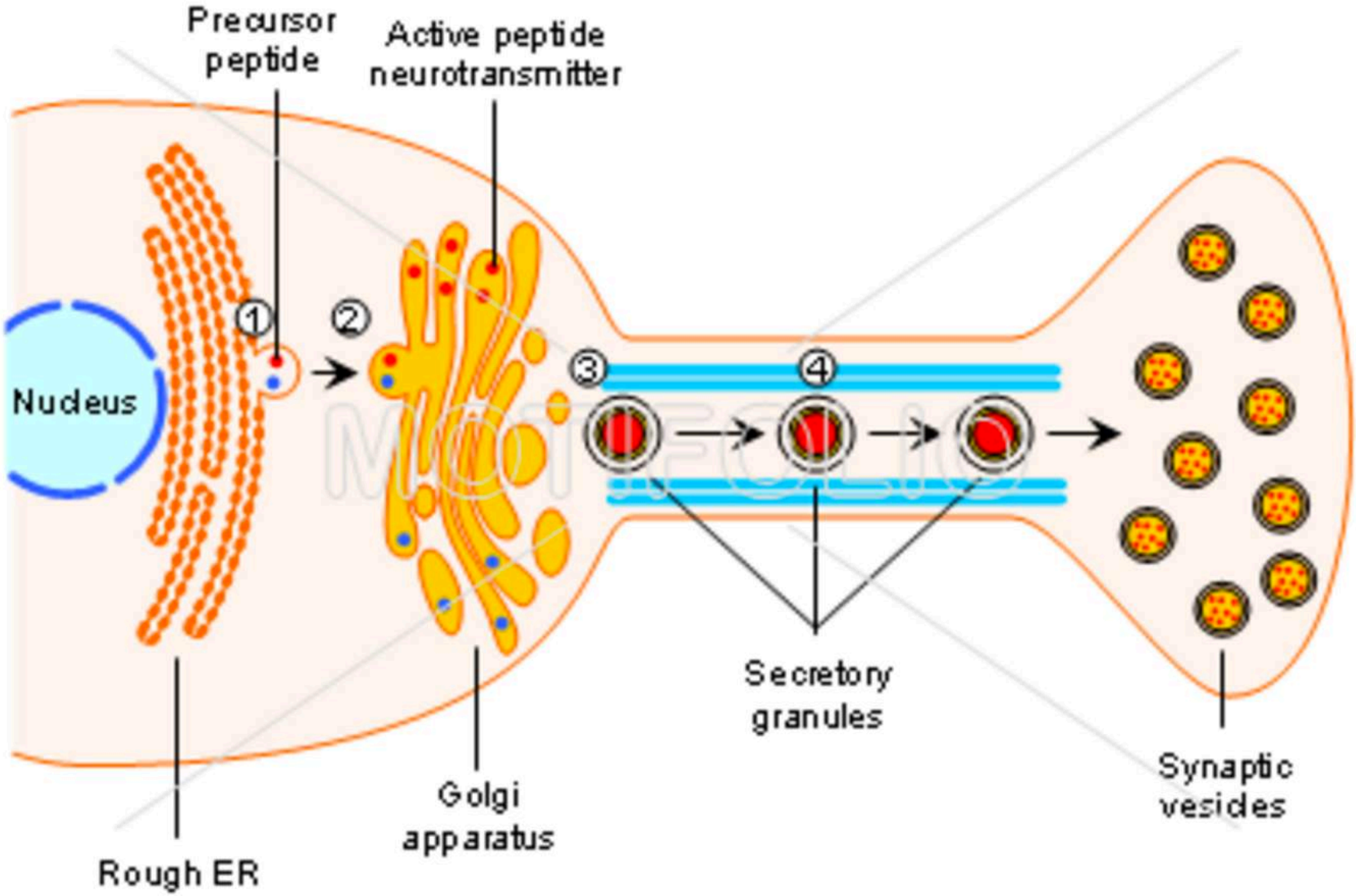
E. Calcium availability

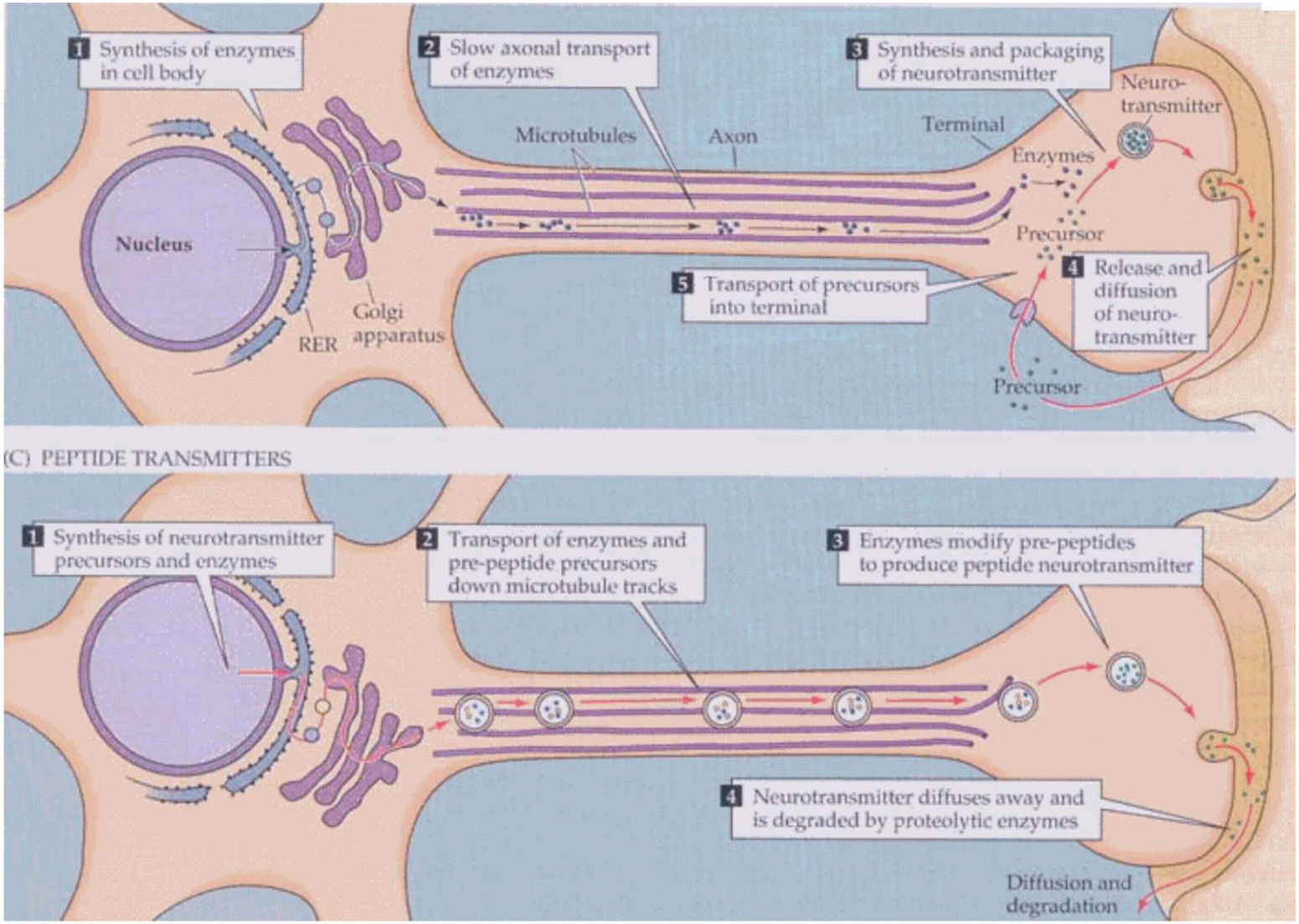
Синтез и транспорт нейромедиаторов

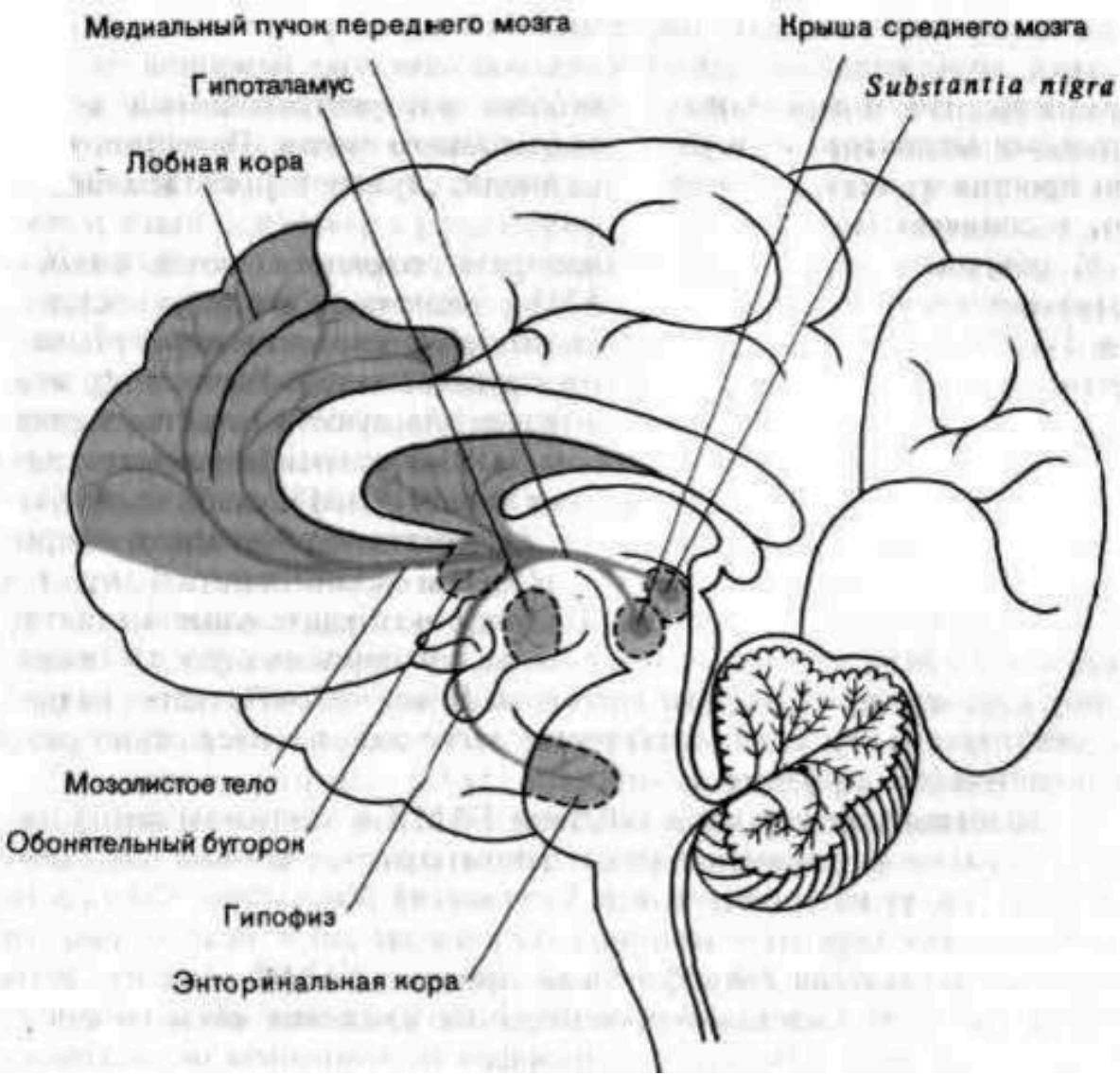


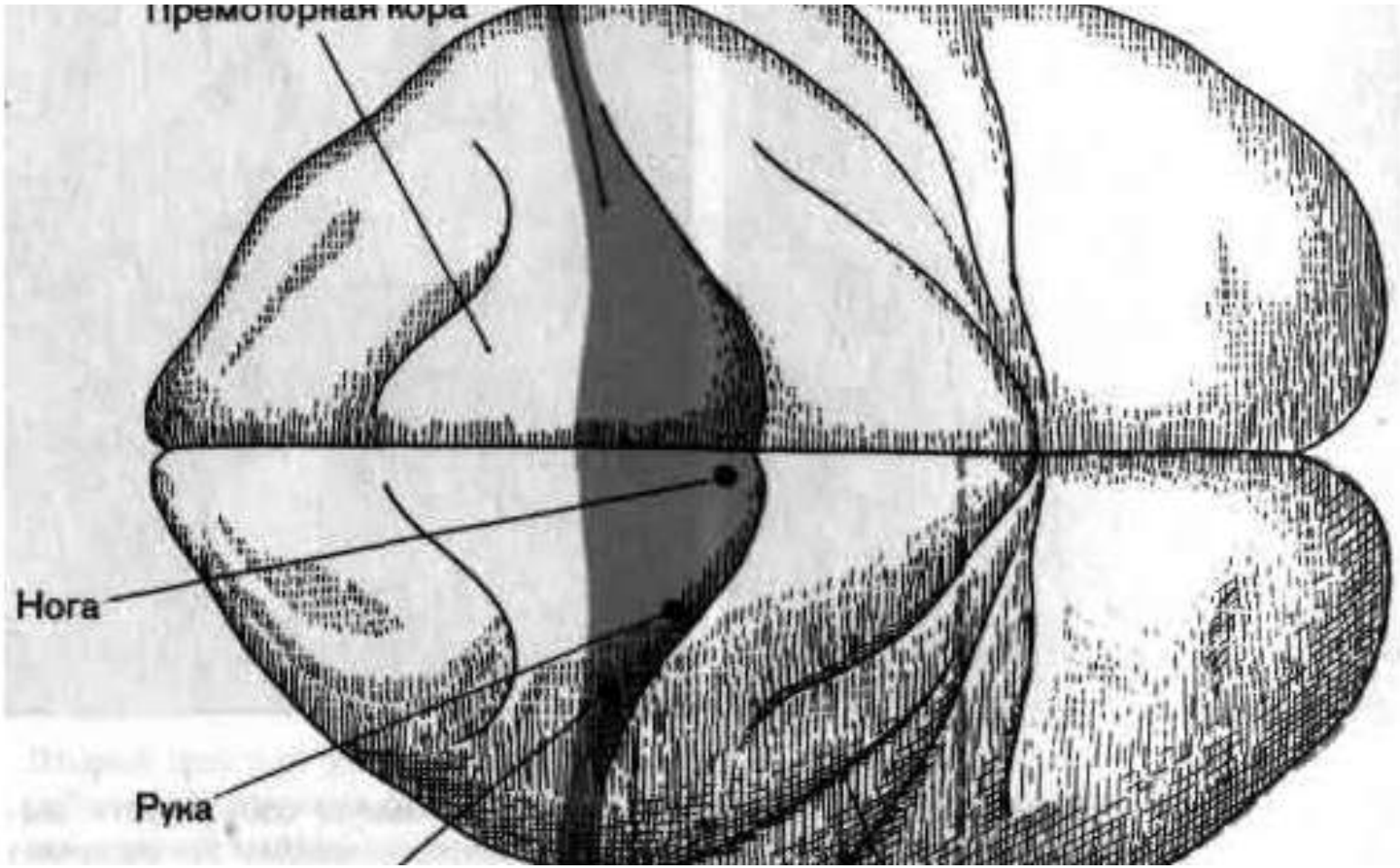
Kaplan et al., 2013

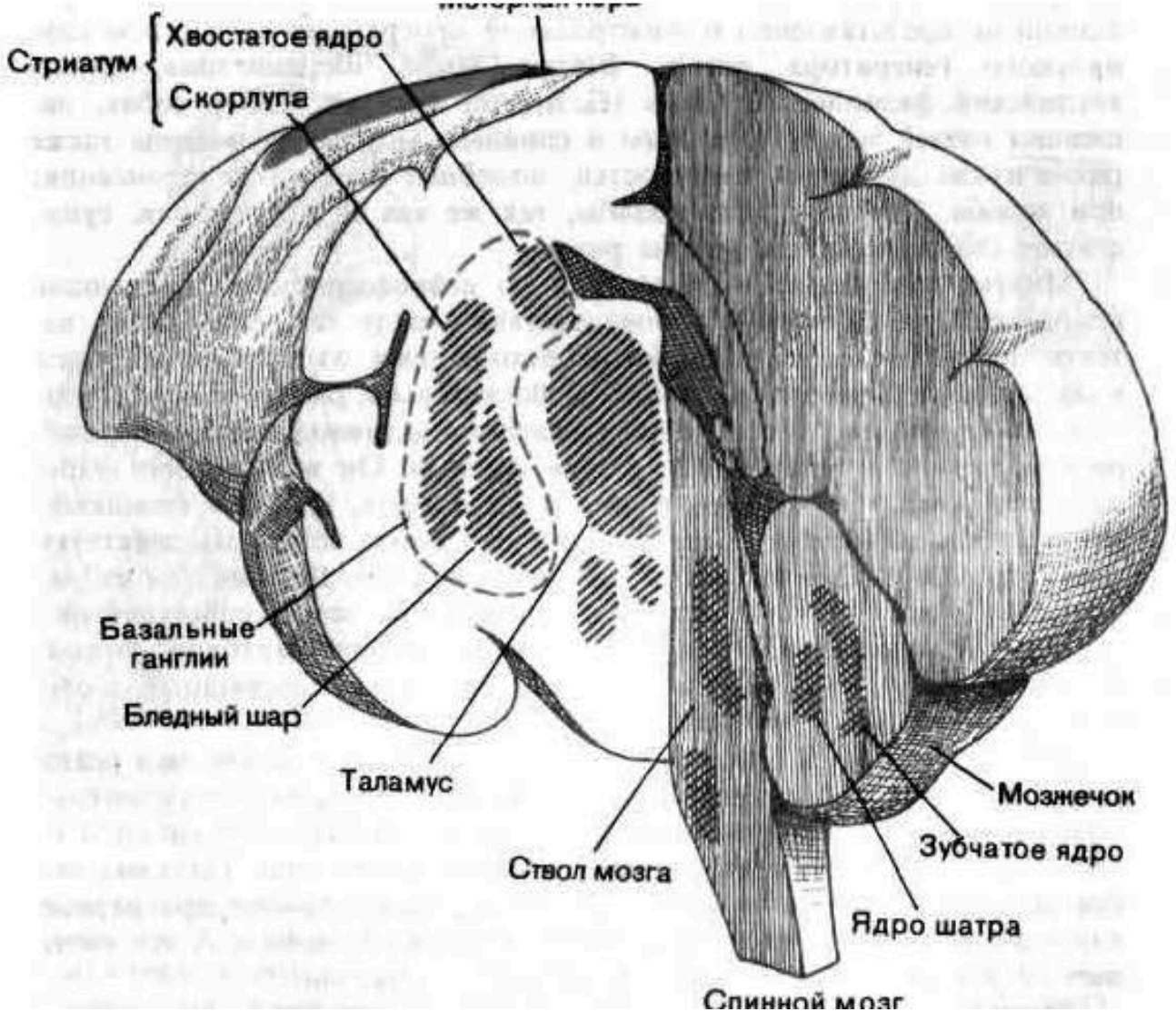
Synthesis and storage of neurotransmitter (peptides)

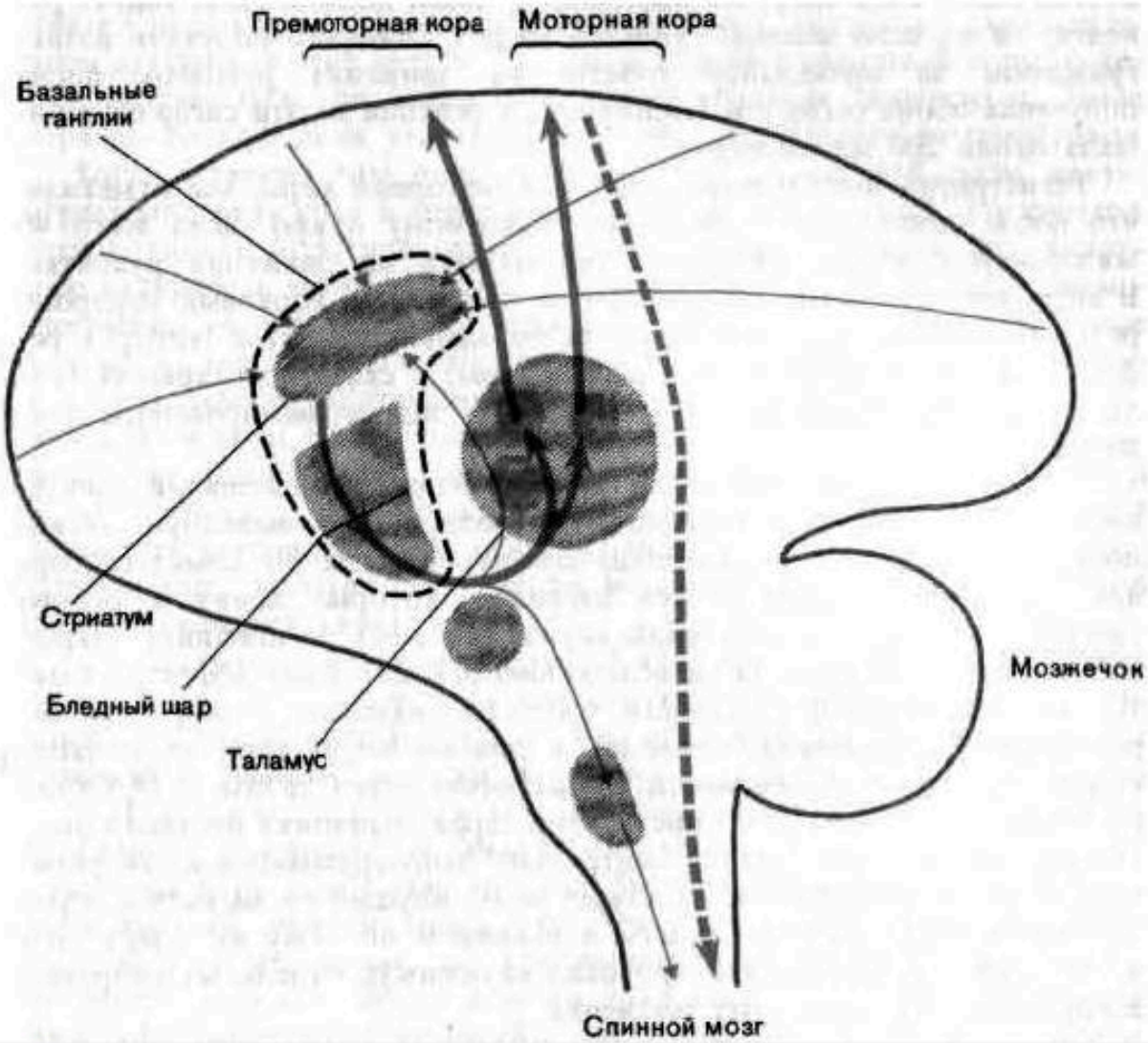


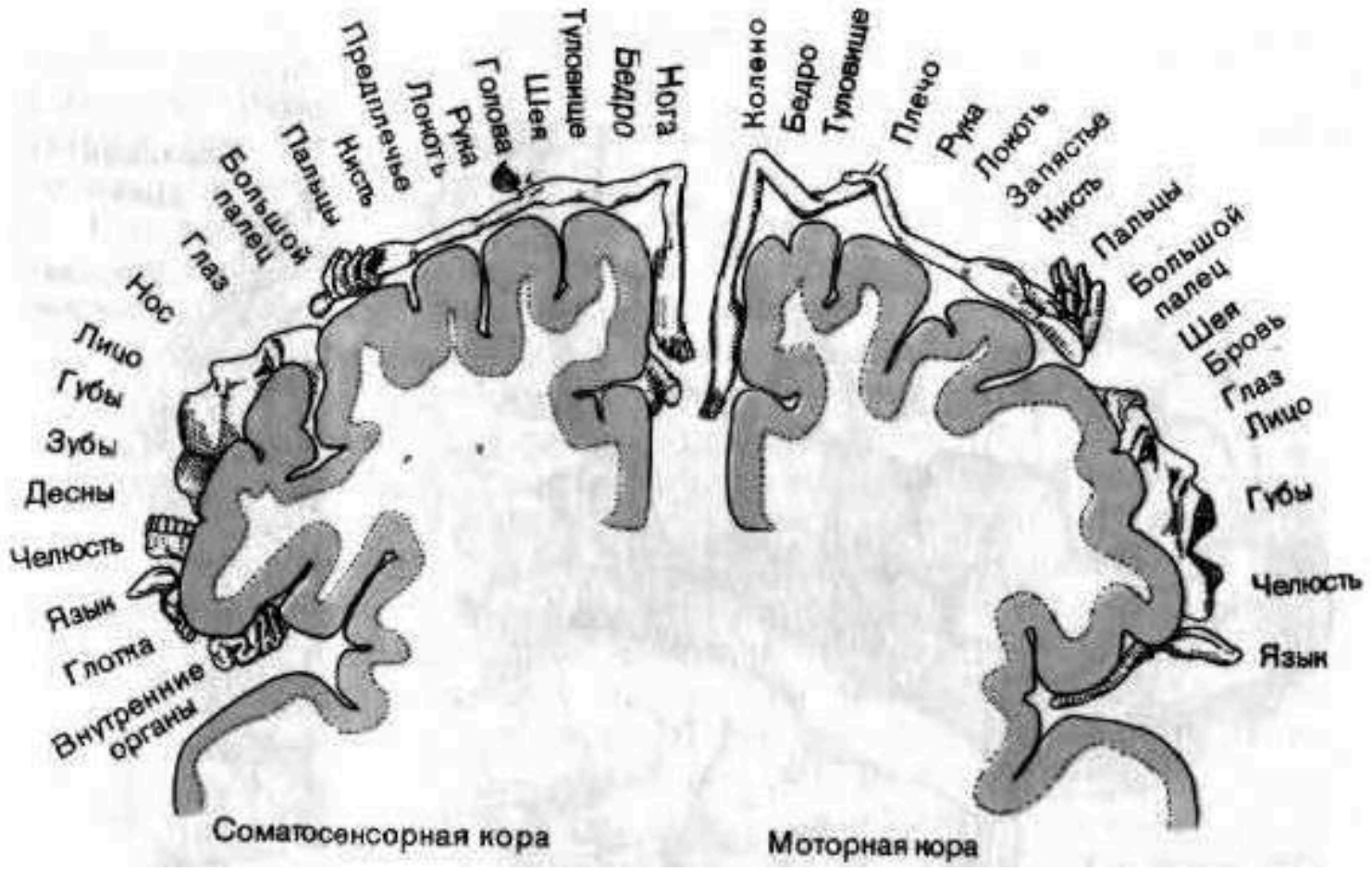

1 Synthesis of enzymes in cell body
2 Slow axonal transport of enzymes
3 Synthesis and packaging of neurotransmitter
Neuro-transmitter
Terminal
Microtubules
Axon
Enzymes
Nucleus
Precursor
4 Release and diffusion of neuro-transmitter
5 Transport of precursors into terminal
Golgi apparatus
RER
Precursor
(C) PEPTIDE TRANSMITTERS
1 Synthesis of neurotransmitter precursors and enzymes
2 Transport of enzymes and pre-peptide precursors down microtubule tracks
3 Enzymes modify pre-peptides to produce peptide neurotransmitter
4 Neurotransmitter diffuses away and is degraded by proteolytic enzymes
Diffusion and degradation

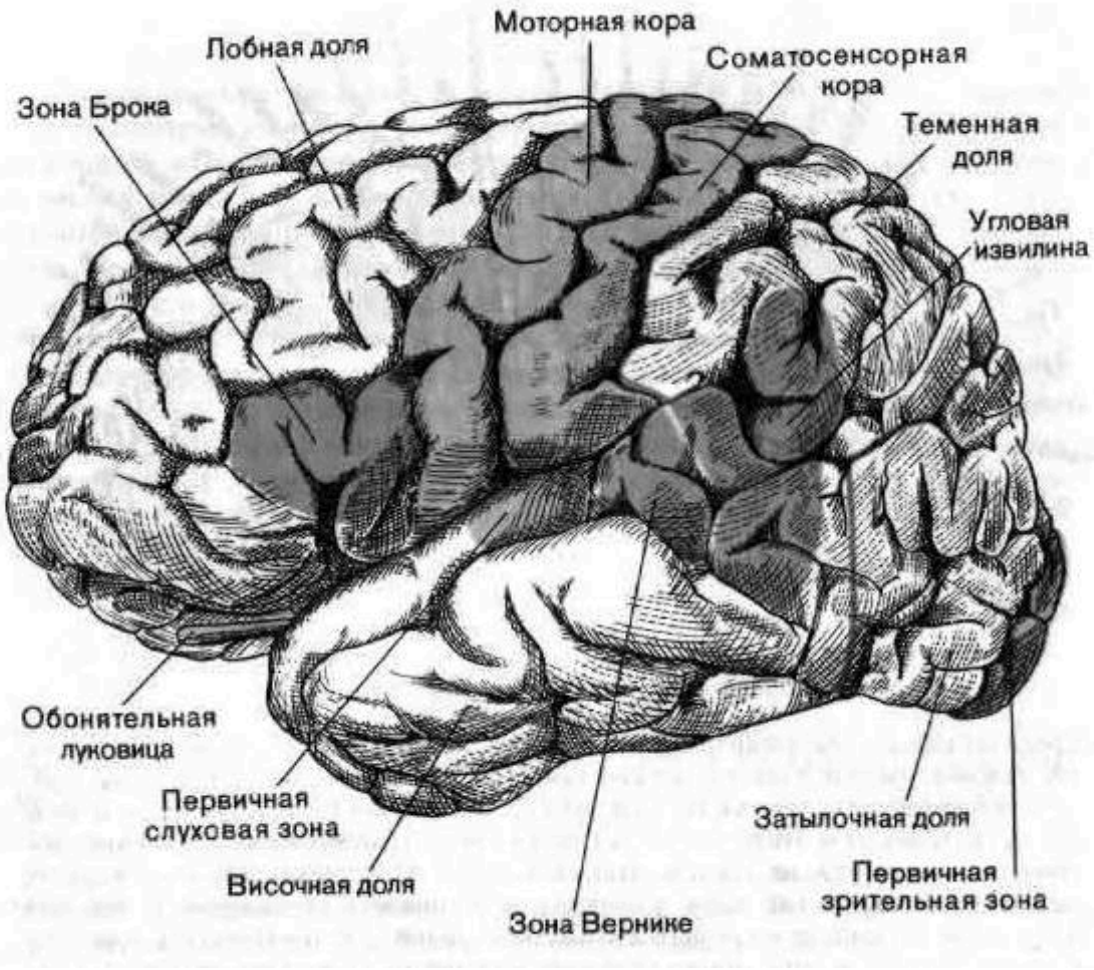

Медиальный пучок переднего мозга
Крыша среднего мозга
Гипоталамус
Substantia nigra
Лобная кора
Мозолистое тело
Обонятельный бугорок
Гипофиз
Энторинальная кора

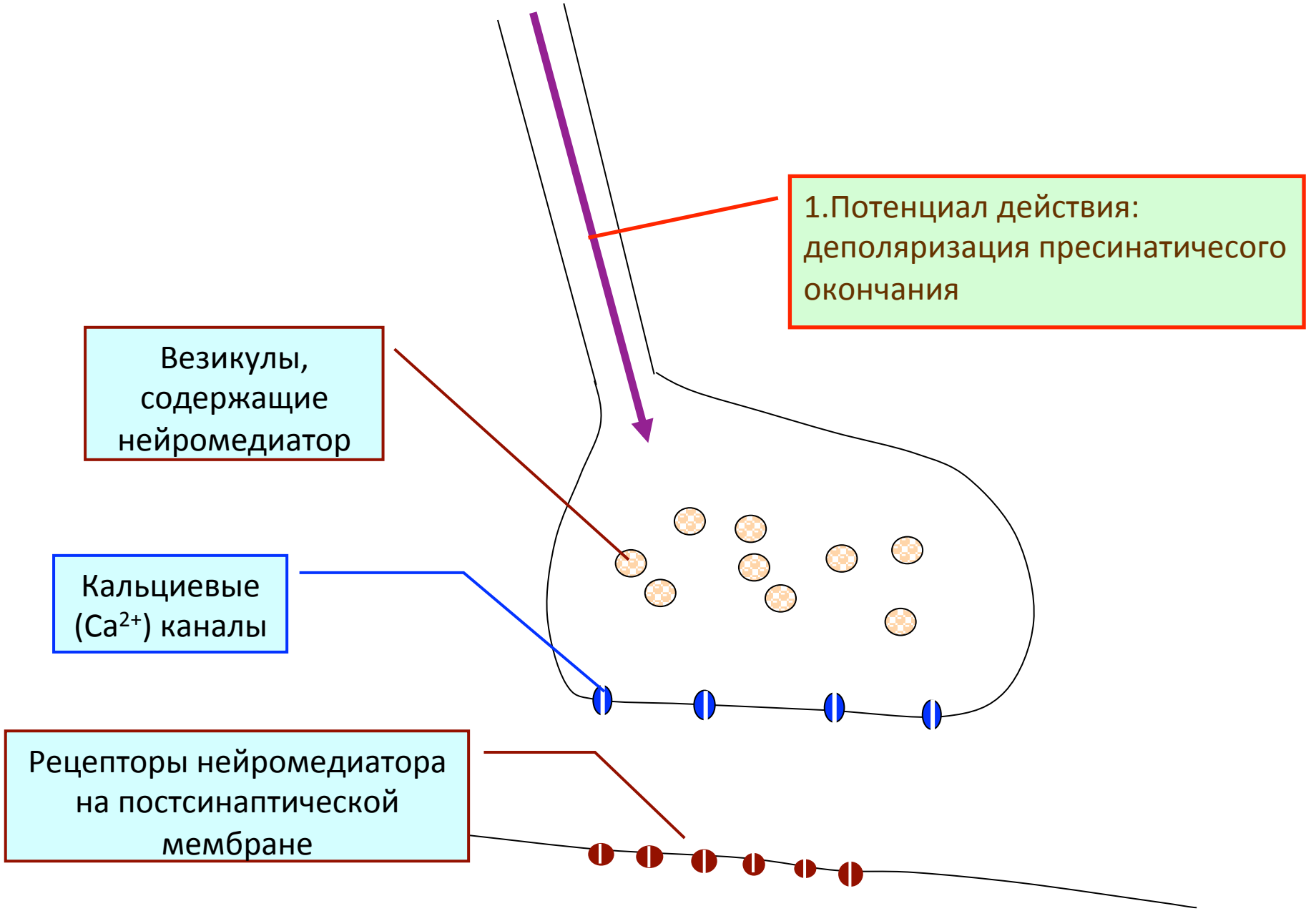

Премоторная кора
Нога
Рука

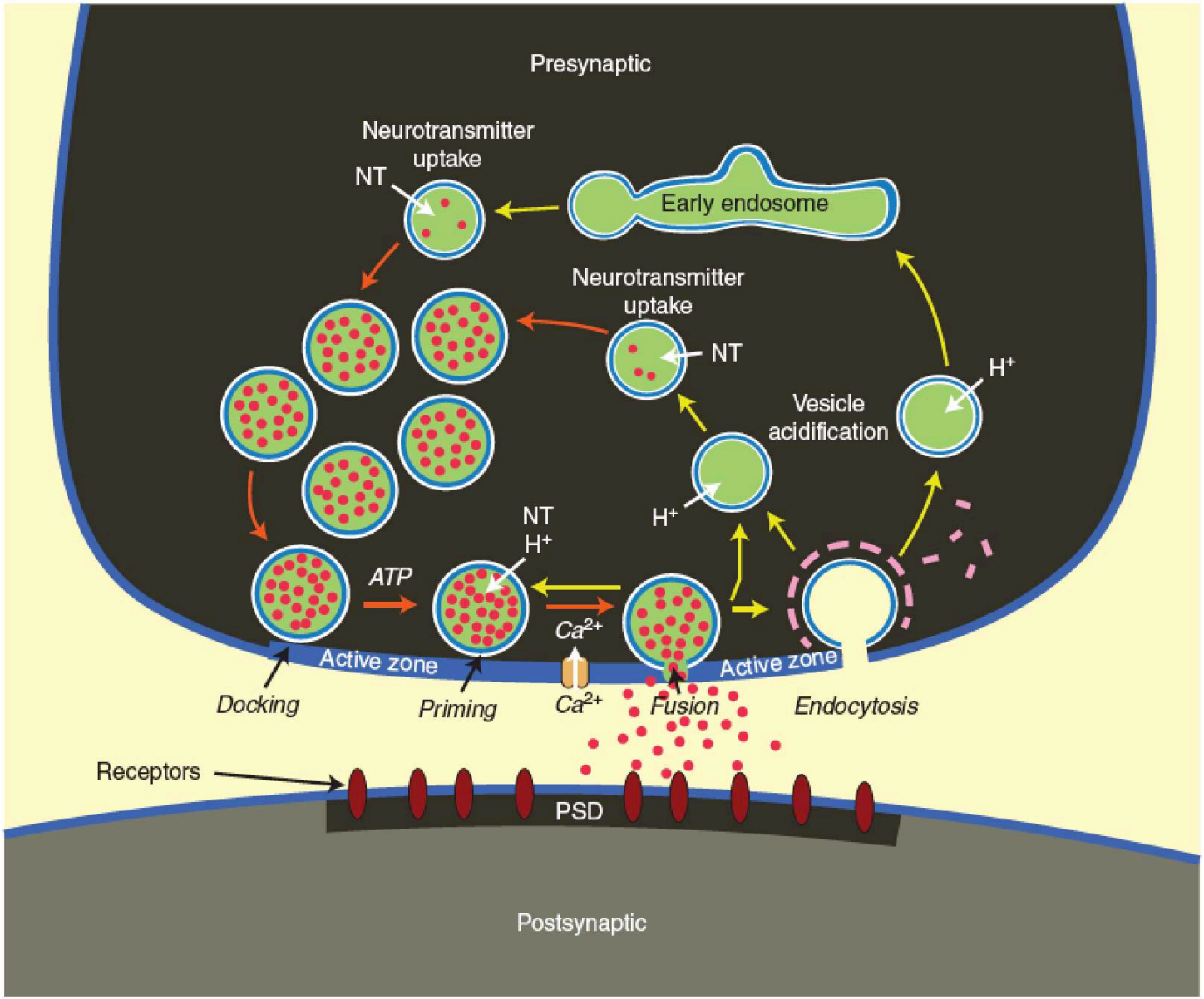

Стриатум
Хвостатое ядро
Скорлупа
Базальные ганглии
Бледный шар
Таламус
Ствол мозга
Мозжечок
Зубчатое ядро
Ядро шатра
Спинной мозг


Премоторная кора
Моторная кора
Базальные ганглии
Стриатум
Бледный шар
Таламус
Мозжечок
Спинной мозг


Нога
Бедро
Туловище
Шея
Голова
Рука
Локоть
Предплечье
Кисть
Пальцы
Большой палец
Глаз
Нос
Лицо
Губы
Зубы
Десны
Челюсть
Язык
Глотка
Внутренние органы
Соматосенсорная кора
Колено
Бедро
Туловище
Плечо
Рука
Локоть
Запястье
Кисть
Пальцы
Большой палец
Шея
Бровь
Глаз
Лицо
Губы
Челюсть
Язык
Моторная кора

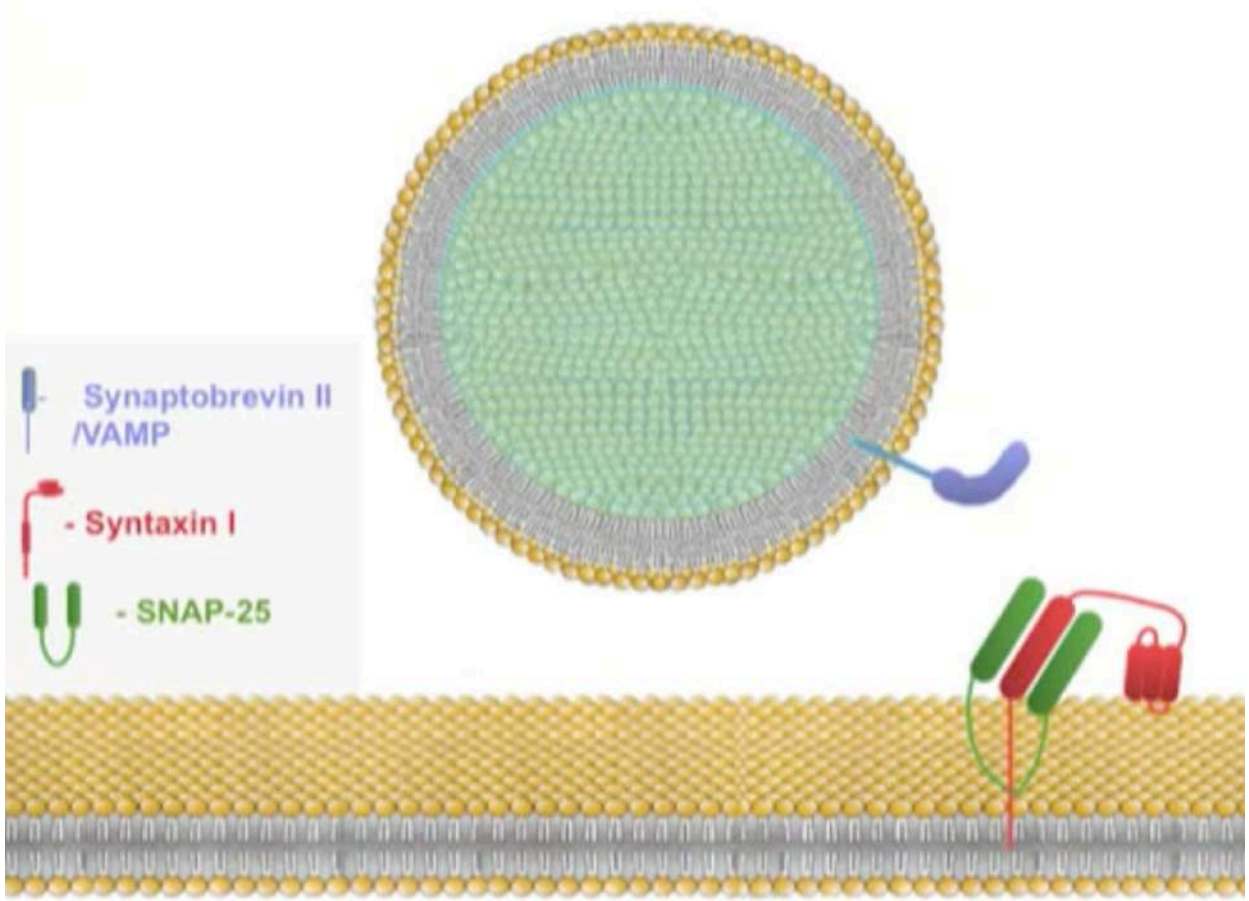

Моторная кора
Лобная доля
Соматосенсорная кора
Зона Брока
Теменная доля
Угловая извилина
Обонятельная луковица
Первичная слуховая зона
Височная доля
Зона Вернике
Затылочная доля
Первичная зрительная зона

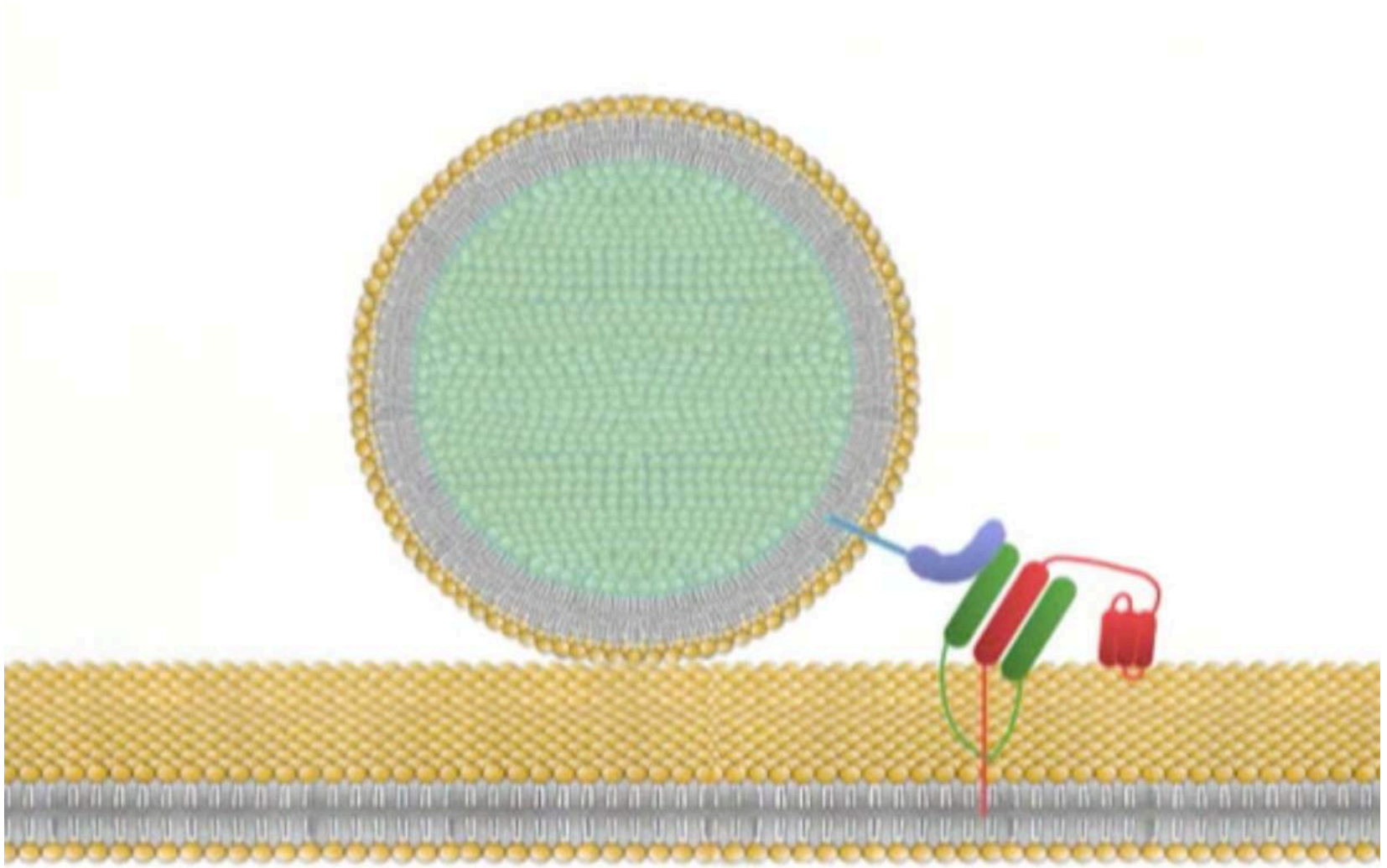

1.Потенциал действия: деполяризация пресинатичесого окончания
Везикулы, содержащие нейромедиатор
Кальциевые (Ca^{2+}) каналы
Рецепторы нейромедиатора на постсинаптической мембране

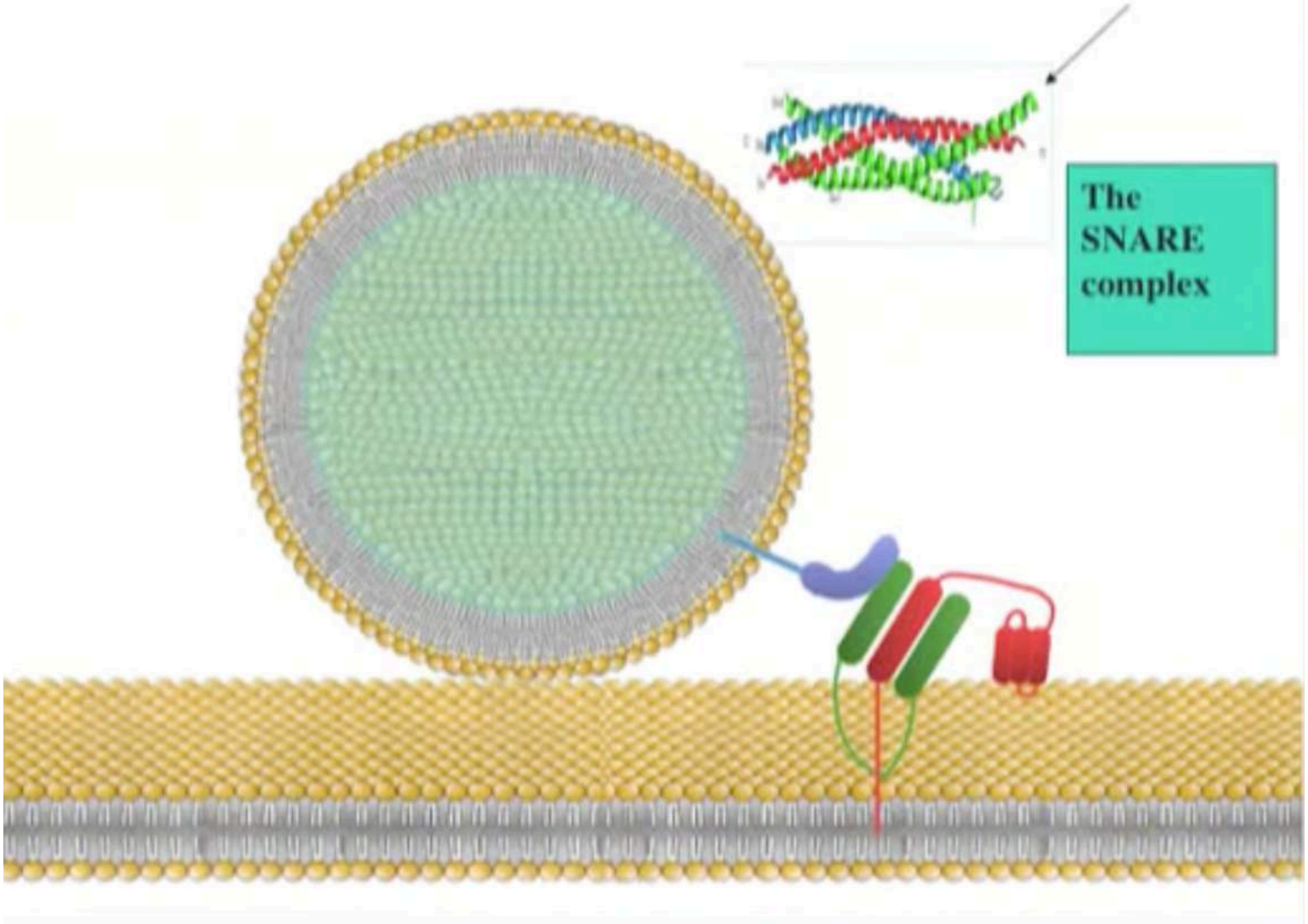

Presynaptic
Neurotransmitter uptake
NT
Early endosome
Neurotransmitter uptake
NT
H+
Vesicle acidification
H+
NT
H+
ATP
Ca2+
Active zone
Active zone
Docking
Priming
Ca2+
Fusion
Endocytosis
Receptors
PSD
Postsynaptic

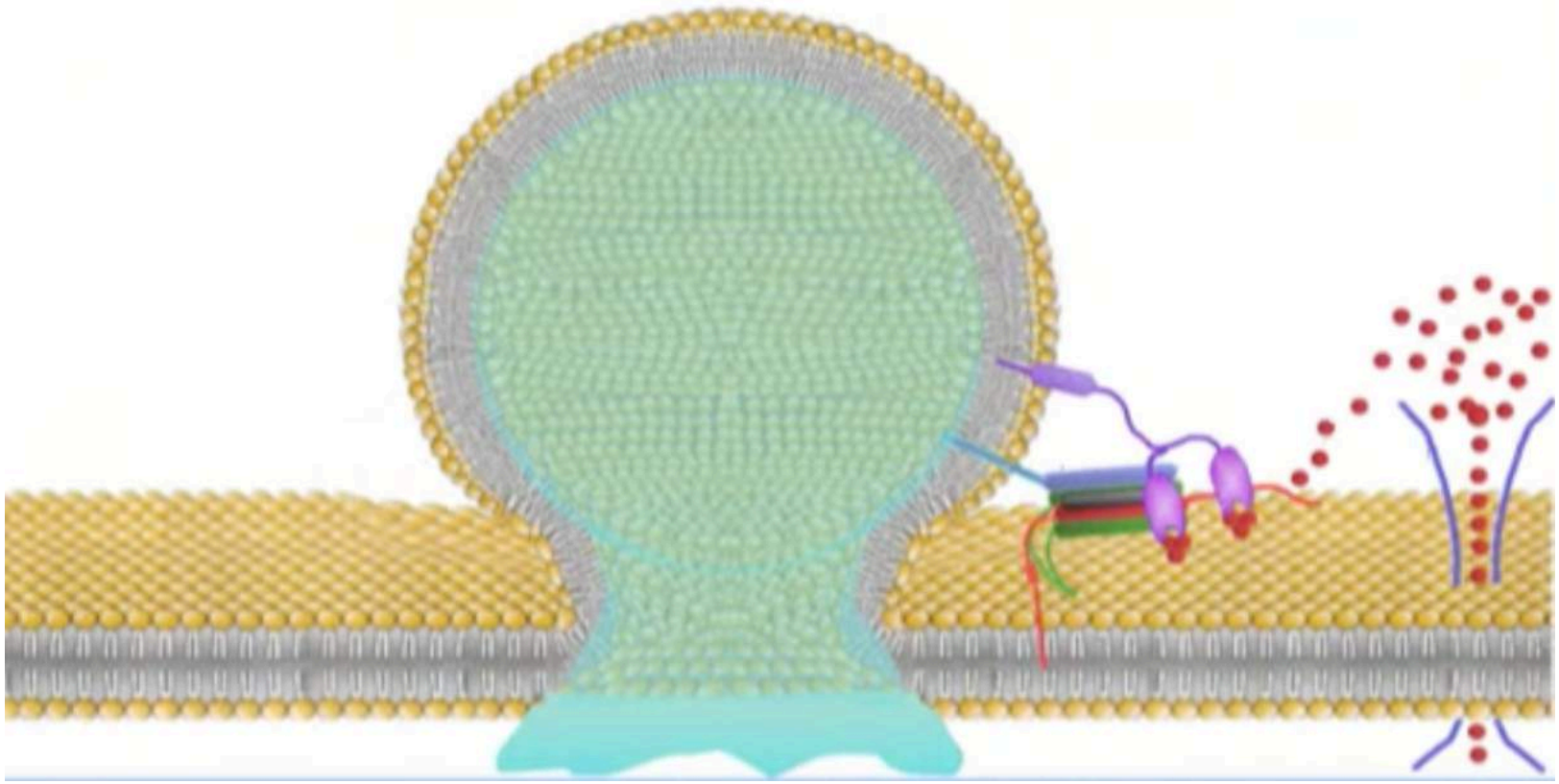
На память

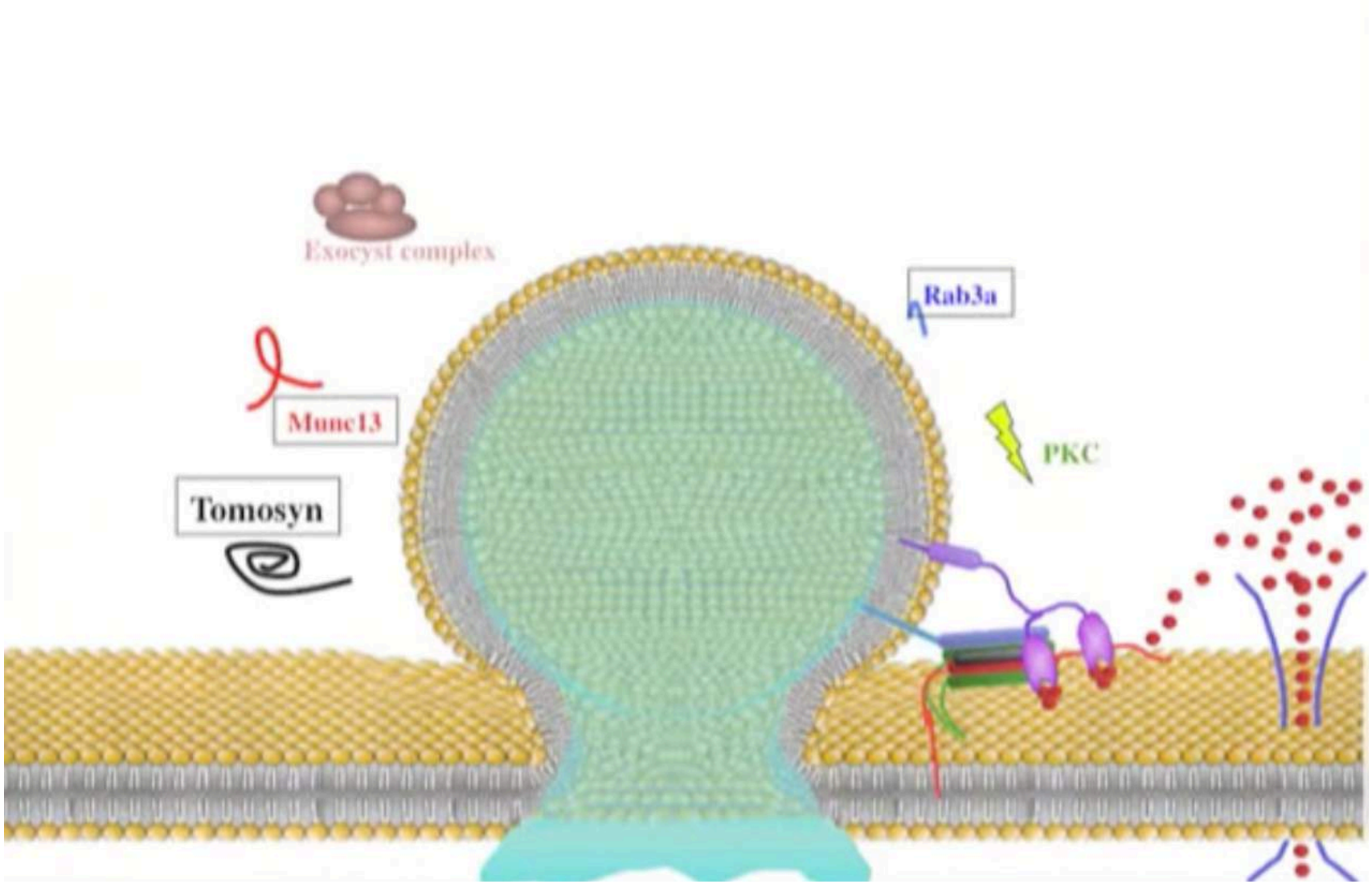
- Кальций связывается с везикулярным белком Синаптотагмином (synaptotagmin), который стимулирует взаимодействие трех белков комплекса SNARE и взаимодействие везикулы с синаптической мембраной, что приводит к её разрыву и выбросу нейромедиатора.

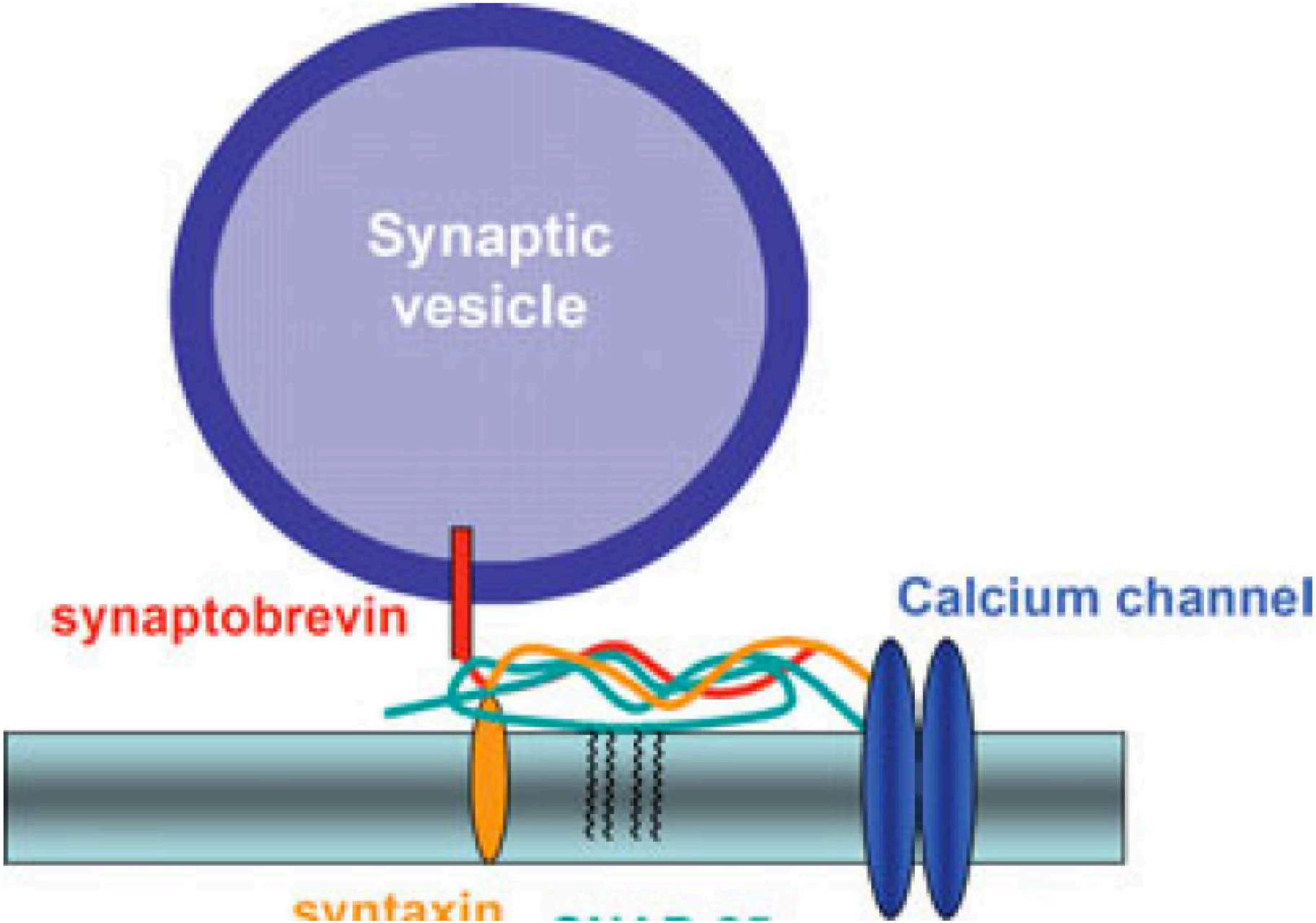

Synaptobrevin II
/VAMP
- Syntaxin I
- SNAP-25



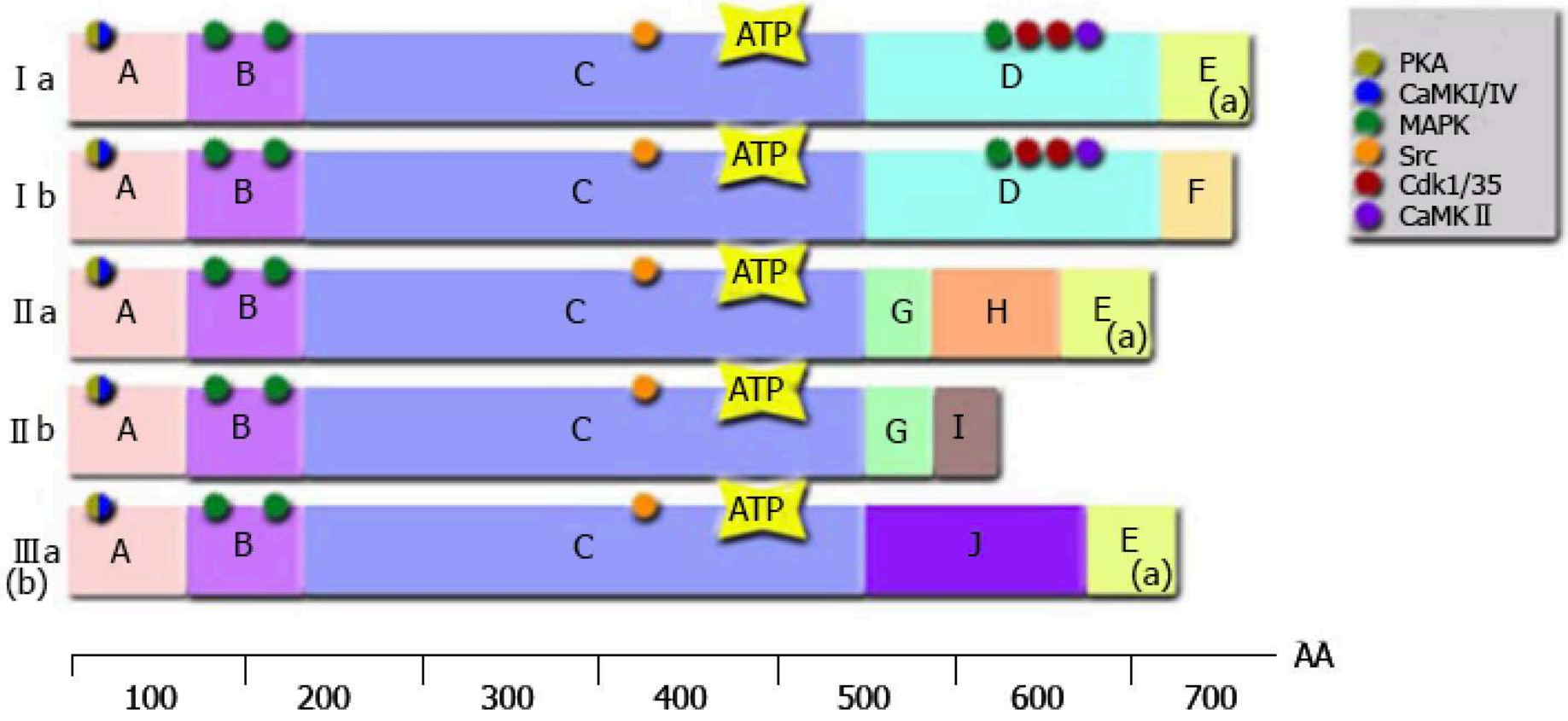

The
SNARE
complex




Exocyst complex
Rab3a
Munc13
PKC
Tomosyn


Synaptic vesicle
synaptobrevin
Calcium channel
syntaxin

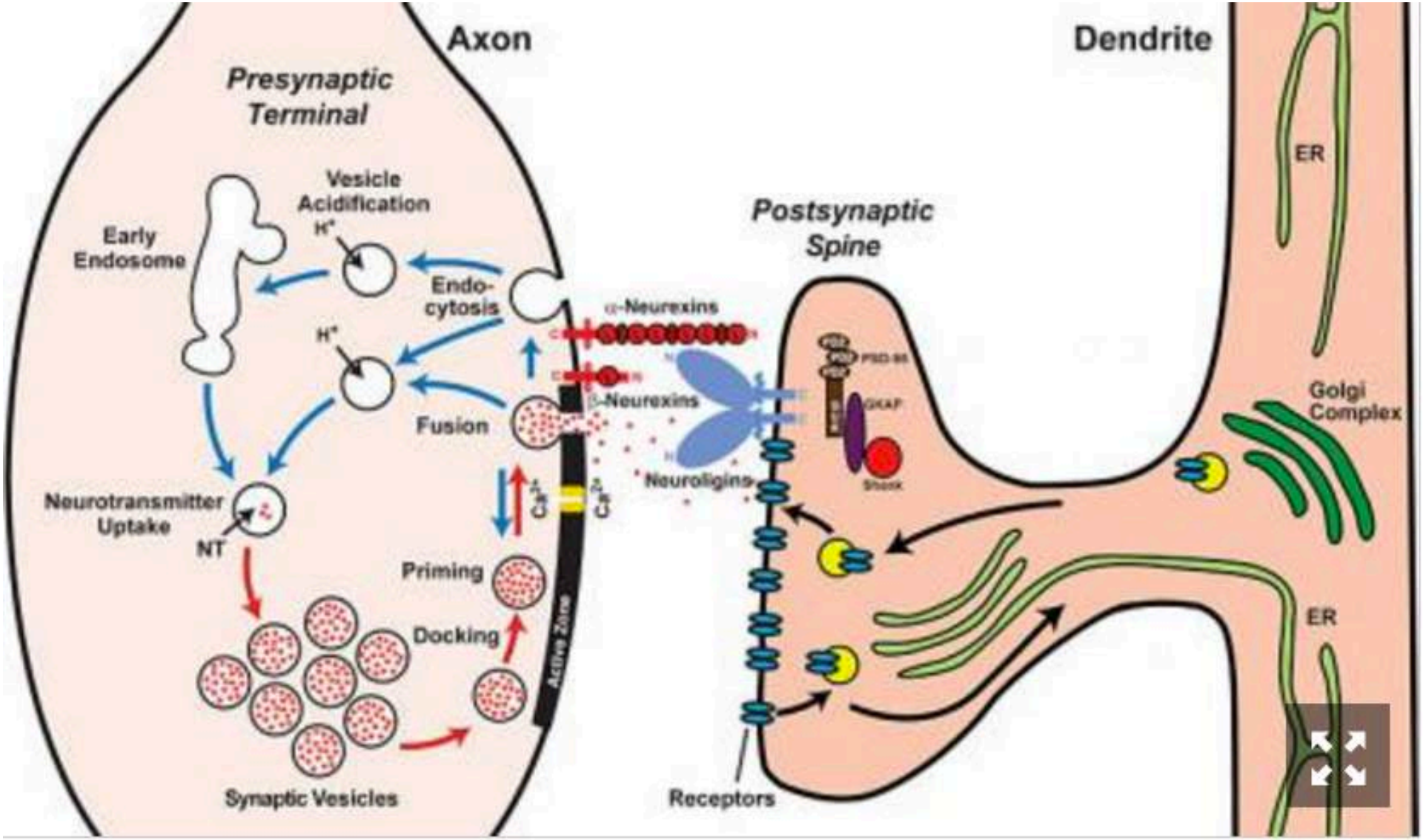
Синапсины: доменная организация



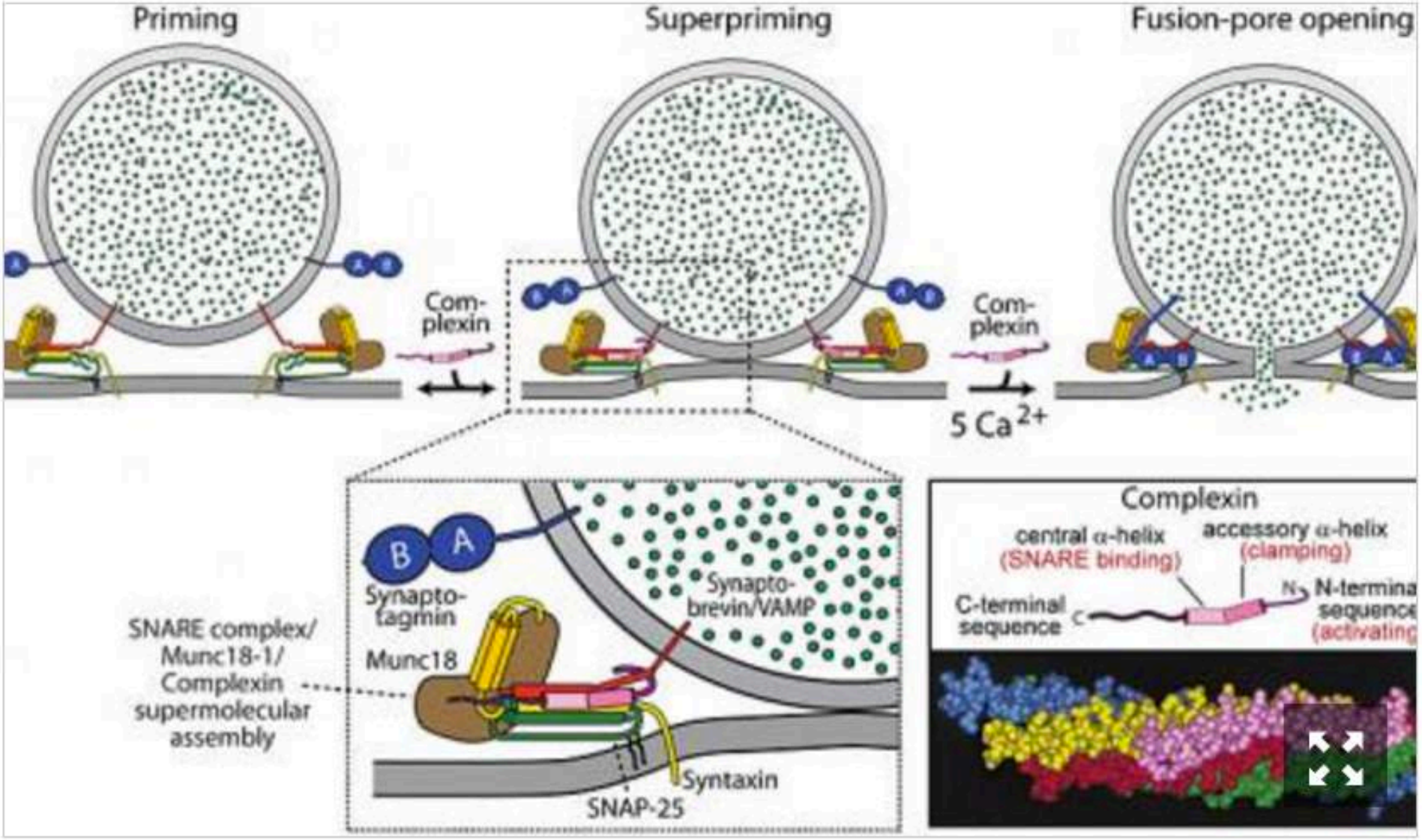
Синапсины

- 9% of the total amount of all vesicle proteins
- presynaptic proteins
- integral for many functional roles, including: synaptogenesis, synapse function, synapse maintenance and synaptic plasticity
- 3 genes:*synapsin* I , *synapsin* II , and *synapsin* III
- synapsins I and II – in mature synapses, while synapsin III - developing synapses
- at least 10 different isoforms of synapsin

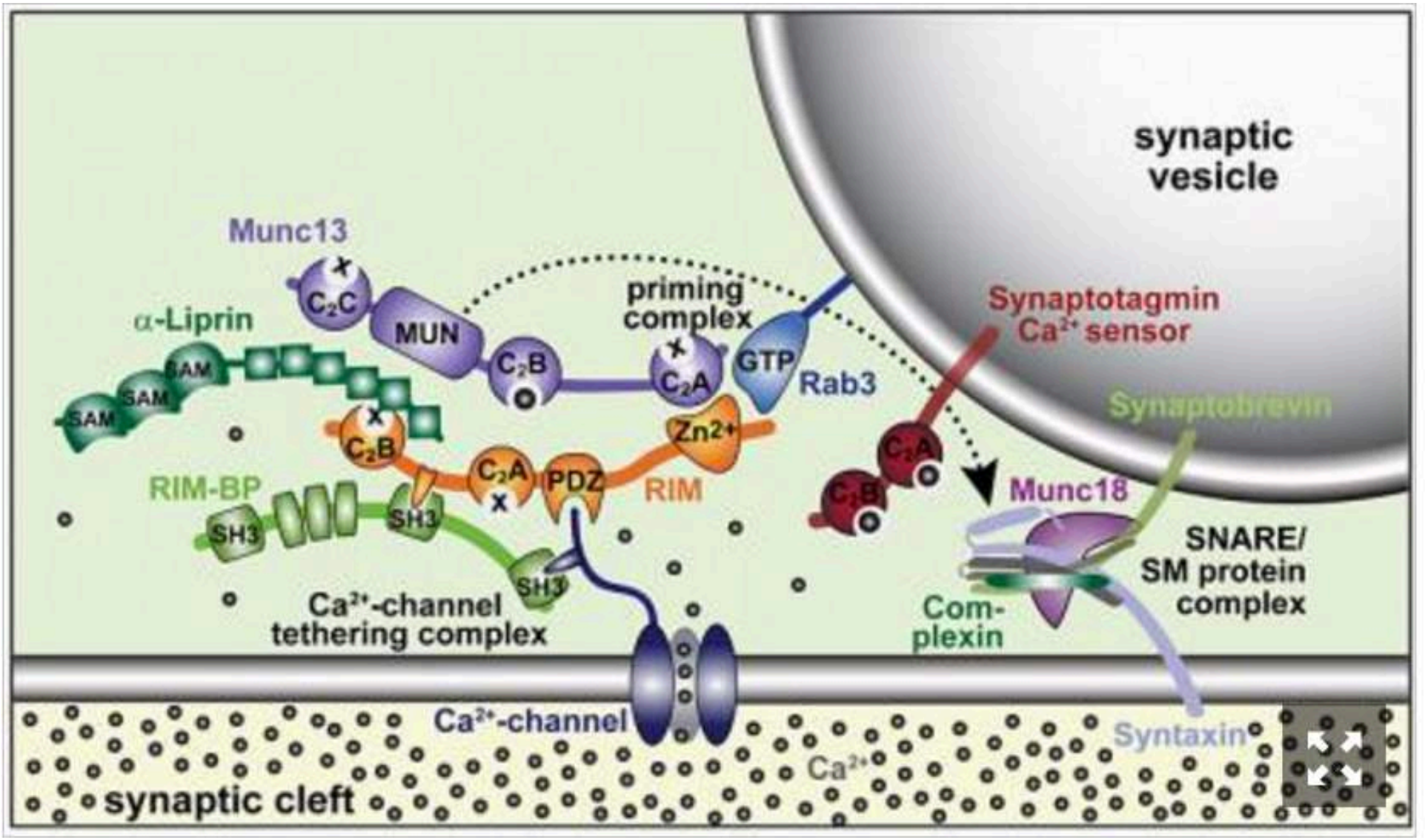
- Domain A contains a conserved phosphorylation site for protein kinase A
- (PKA) and Ca2+/calmodulin-dependent protein kinaseI (CaM kinase I)[1].
- Domain B functions to link the N-terminus to the large central C domain (approximately 300 residues)
- Functionally, synapsins bind to the lipid surface of vesicles *via* the N-terminus, while the variable, hydrophilic C-terminus often facilitates the stabilization of synapsin on phospholipid bilayers and cytoskeletal elements *via* domain E



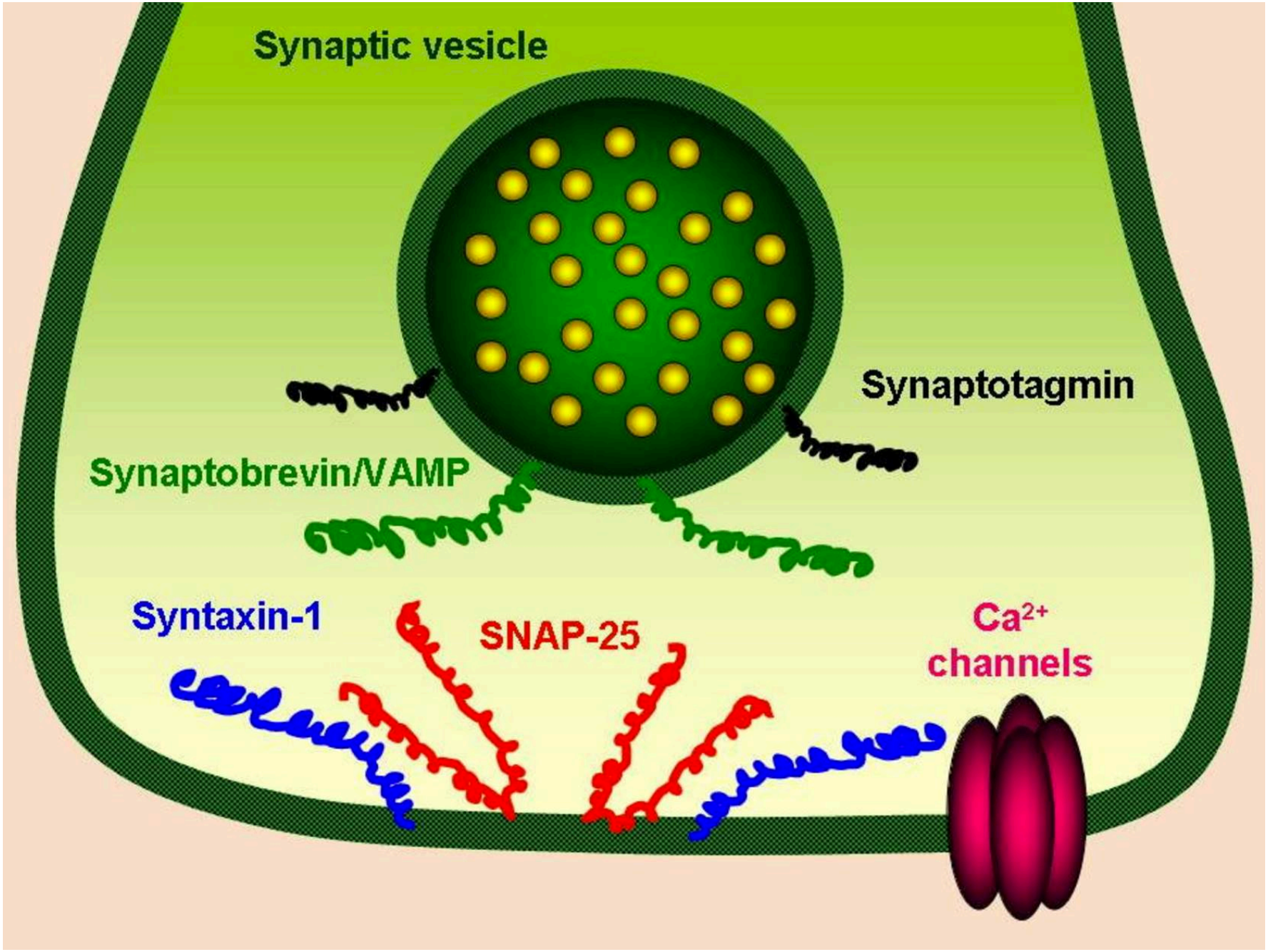
Südhof, T.C., Nature 2008



Gerber, S.H., et al. 2008. *Science* 321:1507–1510



Kaeser, P.S., et al. 2011. *Cell* 144:282-295


Synaptic vesicle
Synaptotagmin
Synaptobrevin/VAMP
Syntaxin-1
SNAP-25
Ca2+
channels

Выброс нейромедиатора:

Строго регулируемый процесс слияния везикул с пресинаптической мембраной под контролем:

- Потенциал-управляемых **Ca^{2+} каналов**, которые активируются в ответ на деполяризацию нервного окончания, вызванную нервным импульсом (активация Na^{+} каналов)
- **Синаптотагмина (synaptotagmin)** – сенсора Ca^{2+}
- Трех белков с общим названием **SNAREs**:
  - **синаптобревин/VAMP** (расположен в мембране везикулы)
  - **синтаксин (syntaxin-1** - в пресинаптической мембране)
  - **SNAP-25** (в пресинаптической мембране)

Выброс нейромедиатора:

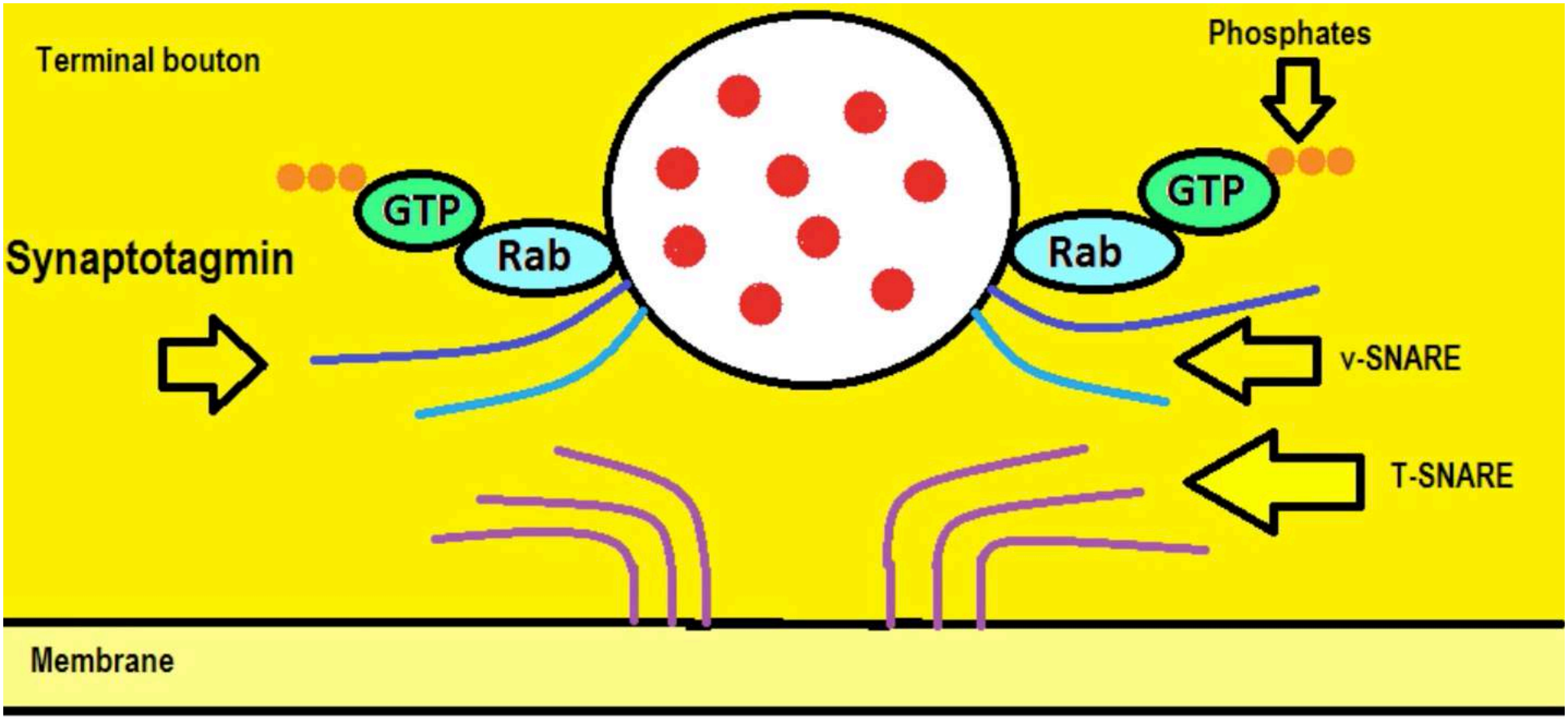
- **Строго регулируемый процесс слияния везикулы с пресинаптической мембраной под контролем:**
  - **потенциал-управляемых Ca^{2+} каналов, которые активируются в ответ на деполяризацию нервного окончания, вызванную нервным импульсом (активация Na^{+} каналов);**
  - **трех белков с общим названием SNAREs:**
    - **синаптобревин/VAMP (расположен в мембране везикулы)**
    - **синтаксин (syntaxin-1 - в пресинаптической мембране)**
    - **SNAP-25 (в пресинаптической мембране)**

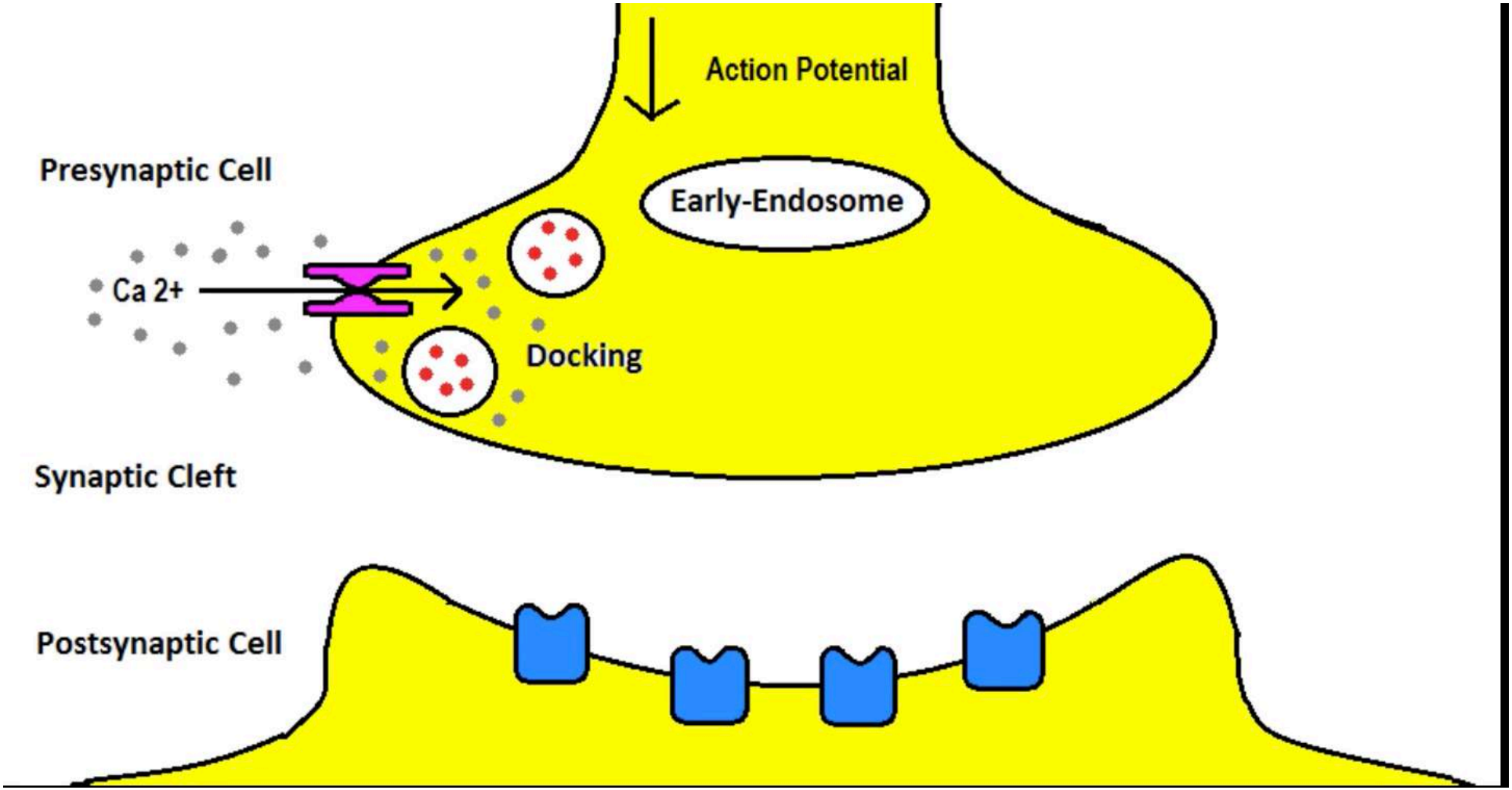
Болезни из-за нарушений экспрессии SNAREs белков

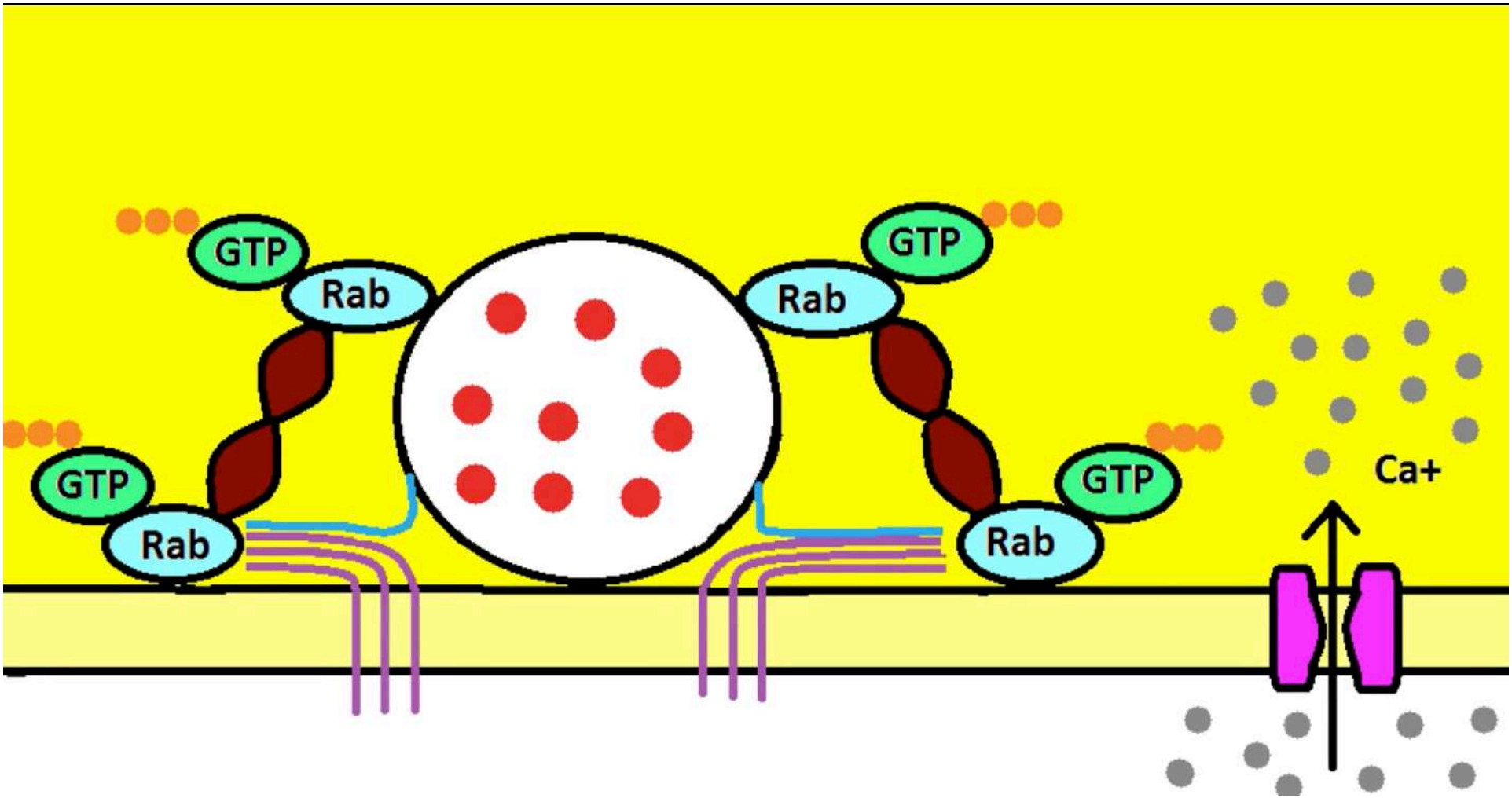
- **Болезнь Хантингтона Huntington disease (HD),**
- **Шизофрения Schizophrenia**
- Биполярное расстойство (маникально-депресси́вный психо́з, **МДП)**
- **Болезнь Альцгеймера**
- **Синдром дефицита внимания и гиперактивности (Attention deficit hyperactive disorders(ADHD))**

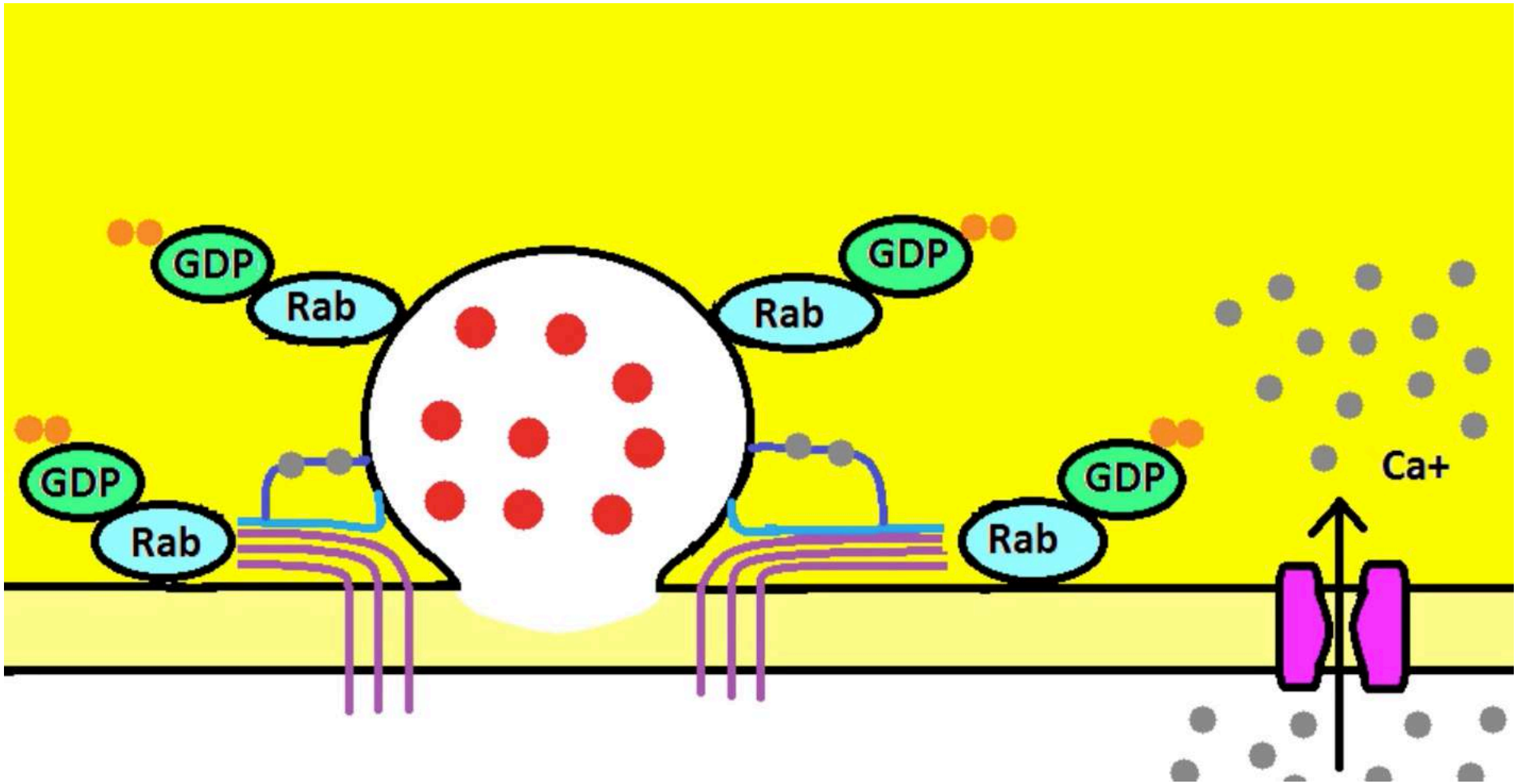
– **трех белков с общим названием SNAREs:**

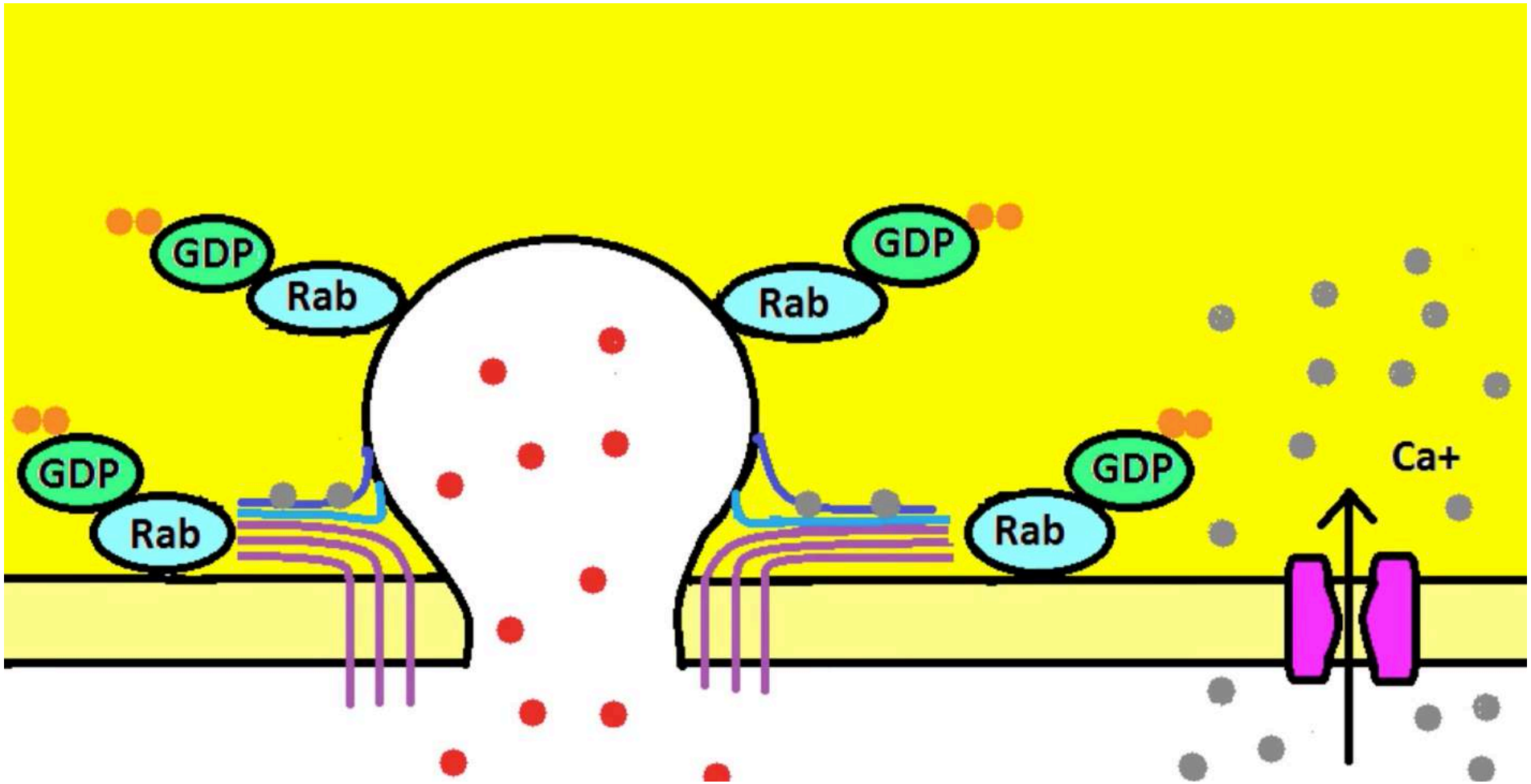
- **синаптобревин/VAMP (расположен в мембране везикулы)**
- **синтаксин (syntaxin-1 - в пресинаптической мембране)**
- **SNAP-25 (в пресинаптической мембране)**

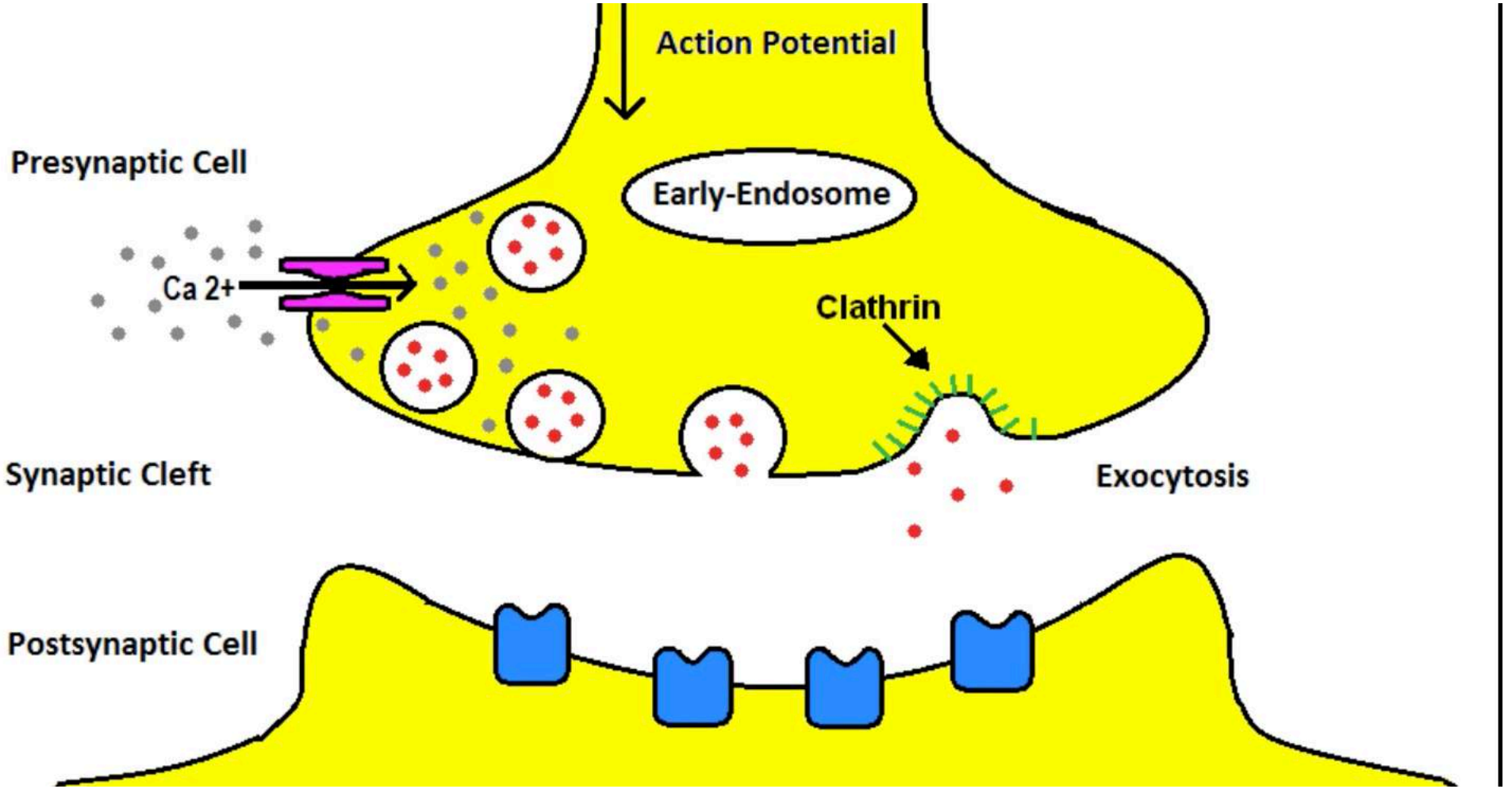

Terminal bouton
Phosphates
GTP
Rab
GTP
Rab
Synaptotagmin
v-SNARE
T-SNARE
Membrane
Synaptic Cleft

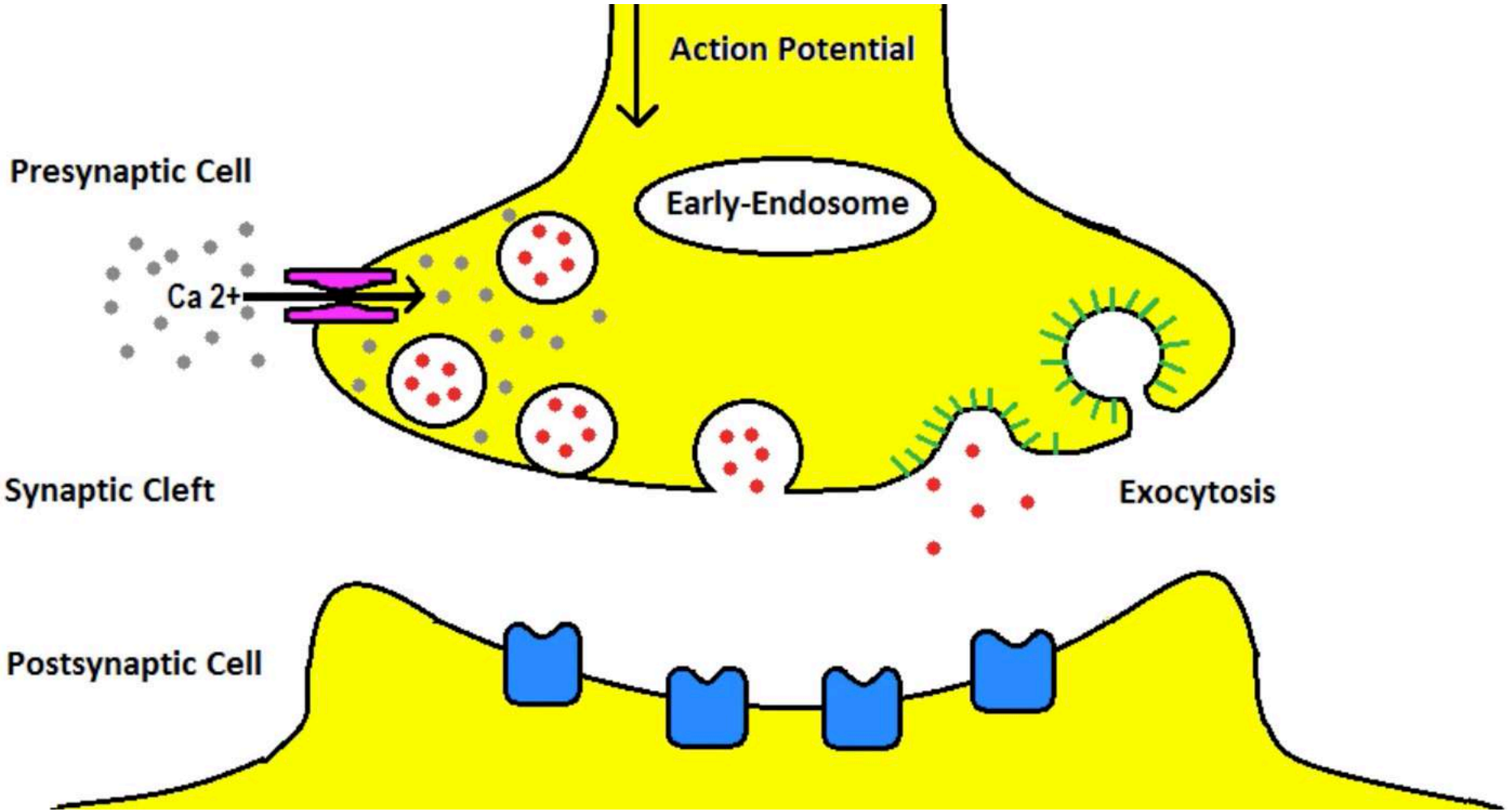

Action Potential
Presynaptic Cell
Early-Endosome
Ca 2+
Docking
Synaptic Cleft
Postsynaptic Cell

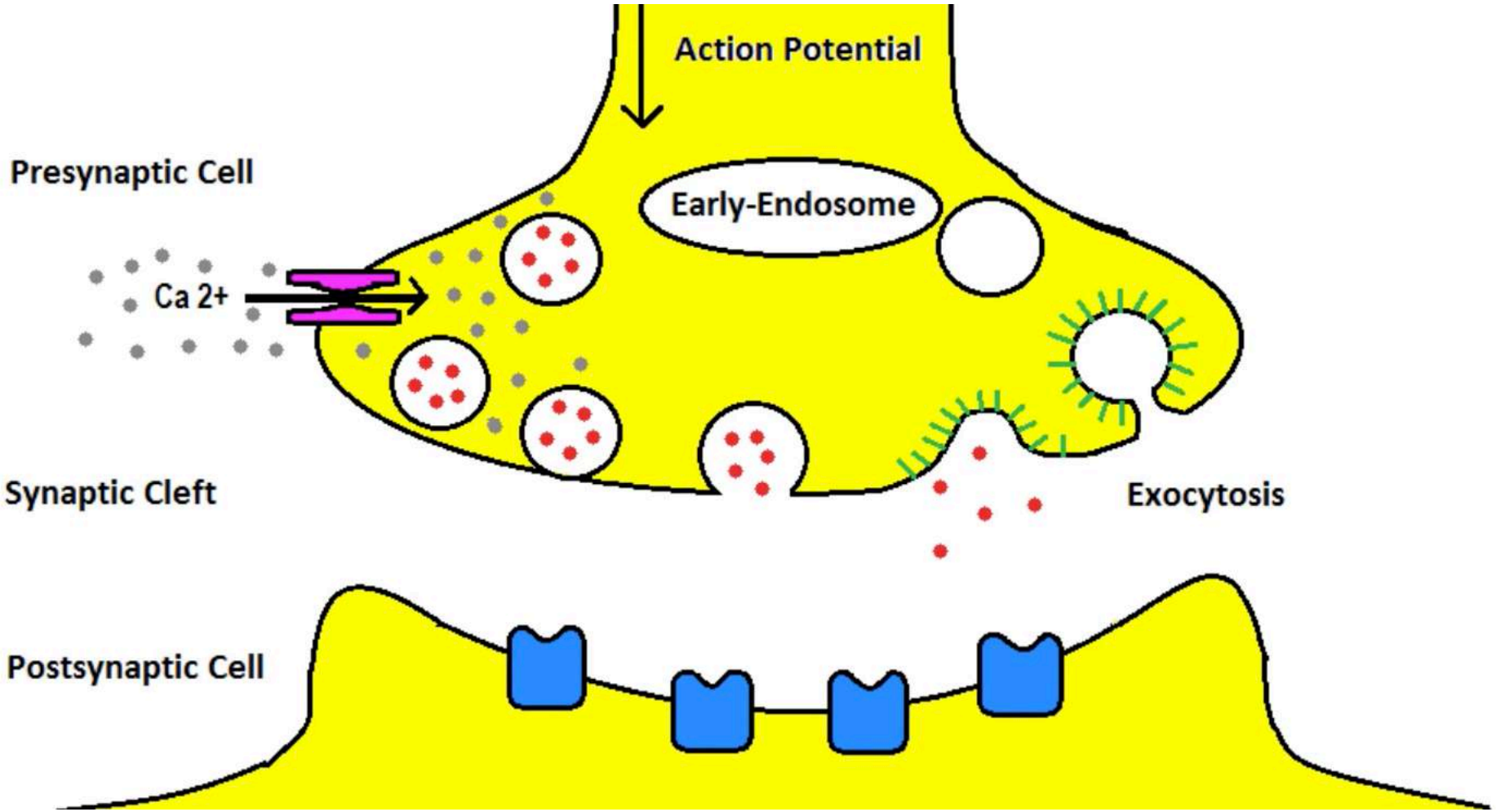

GTP
Rab
GTP
Rab
GTP
Rab
GTP
Rab
Ca+


GDP
Rab
GDP
Rab
GDP
Rab
GDP
Rab
Ca+


GDP
Rab
GDP
Rab
GDP
Rab
GDP
Rab
Ca+


Action Potential
Presynaptic Cell
Early-Endosome
Ca 2+
Clathrin
Synaptic Cleft
Exocytosis
Postsynaptic Cell


Action Potential
Presynaptic Cell
Early-Endosome
Ca 2+
Synaptic Cleft
Exocytosis
Postsynaptic Cell


Action Potential
Presynaptic Cell
Early-Endosome
Ca 2+
Synaptic Cleft
Exocytosis
Postsynaptic Cell

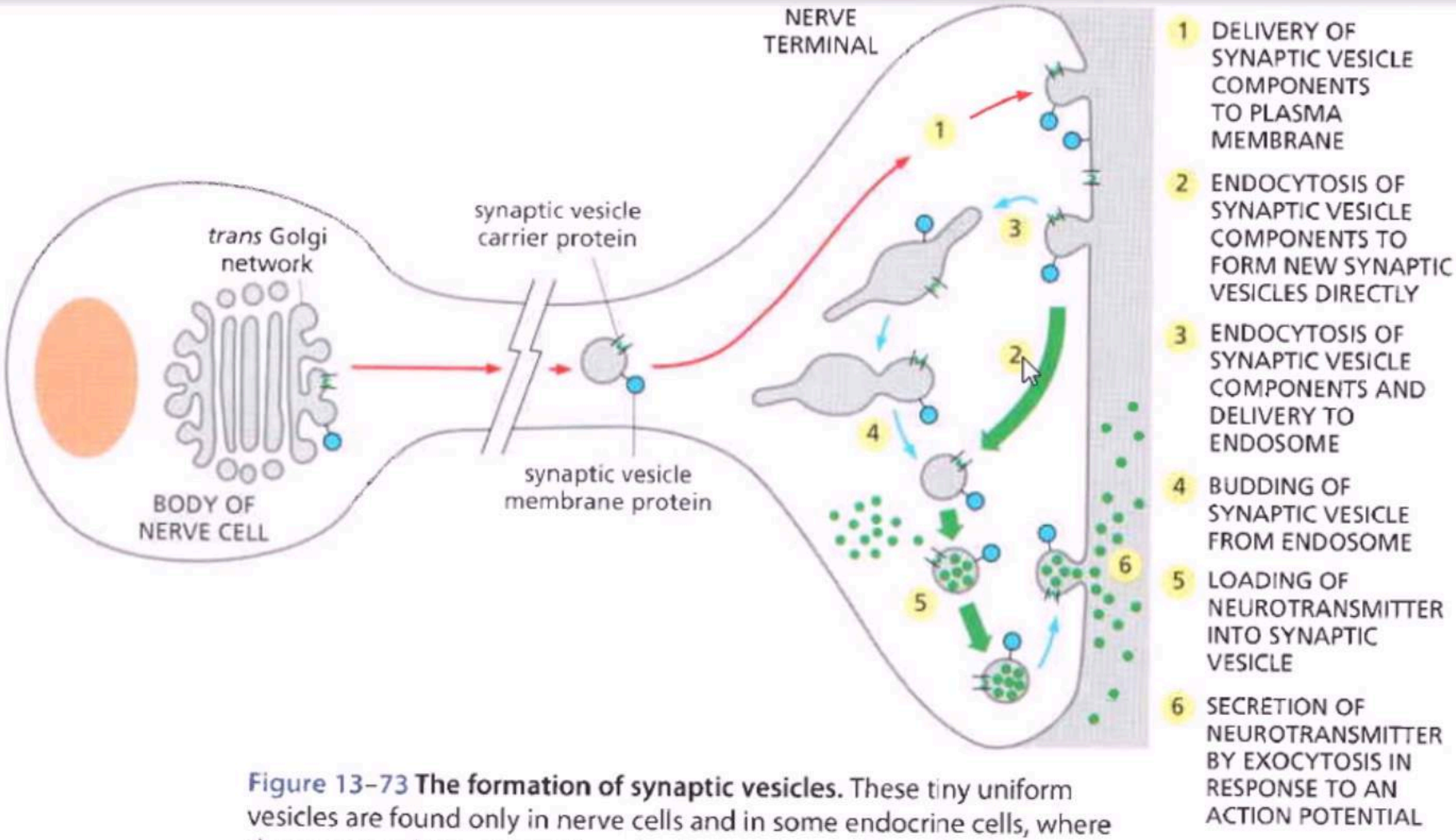
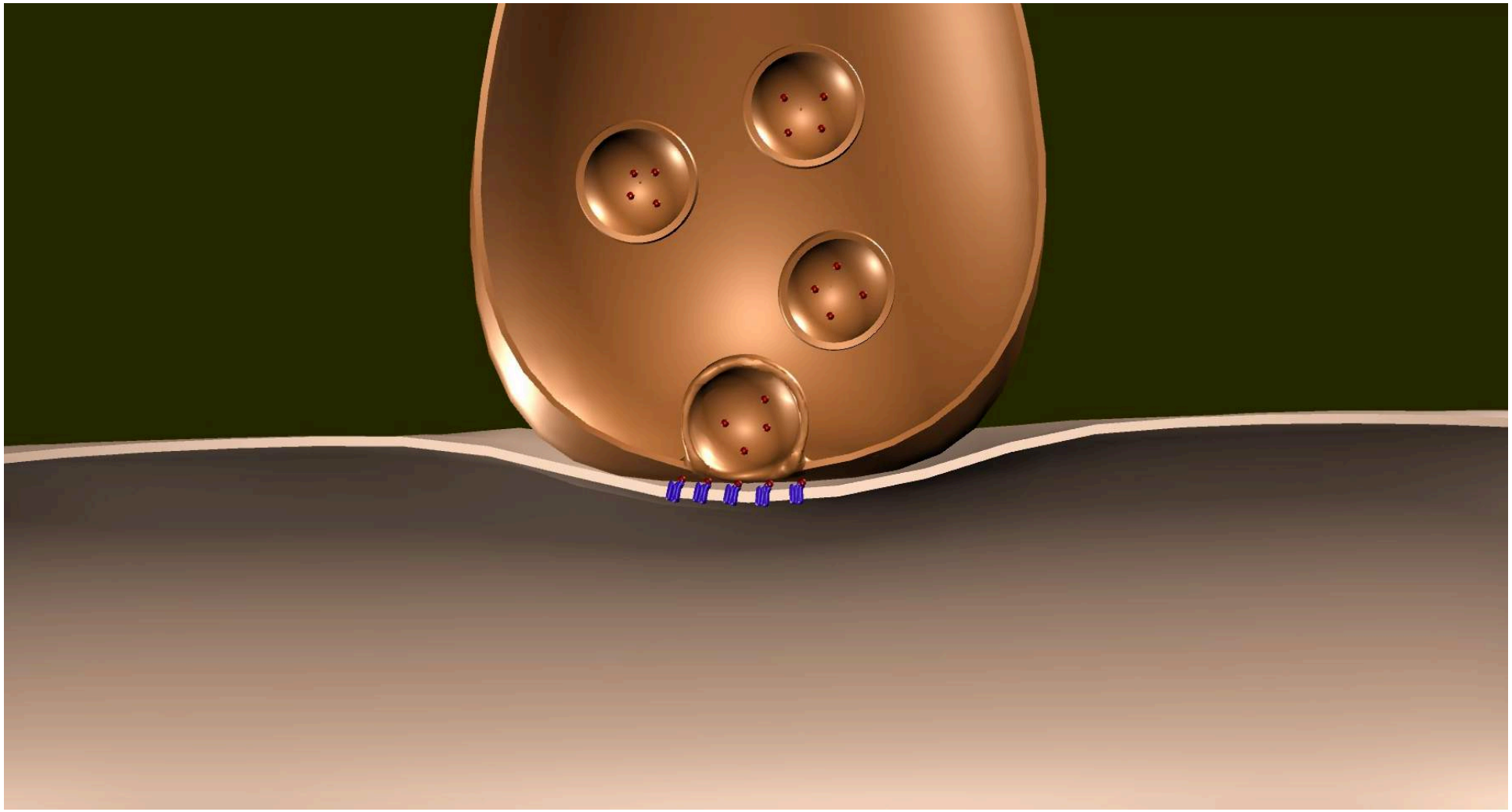
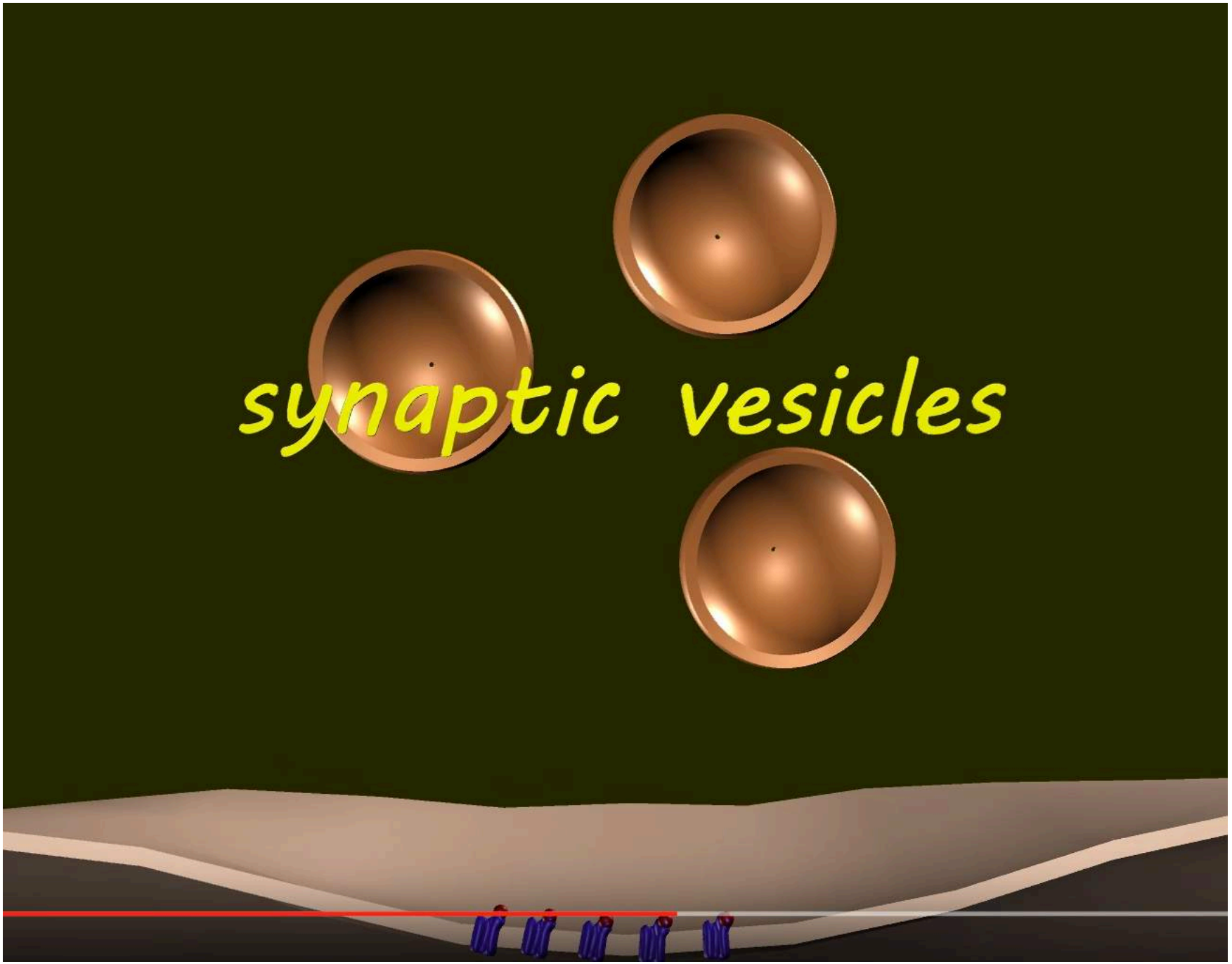
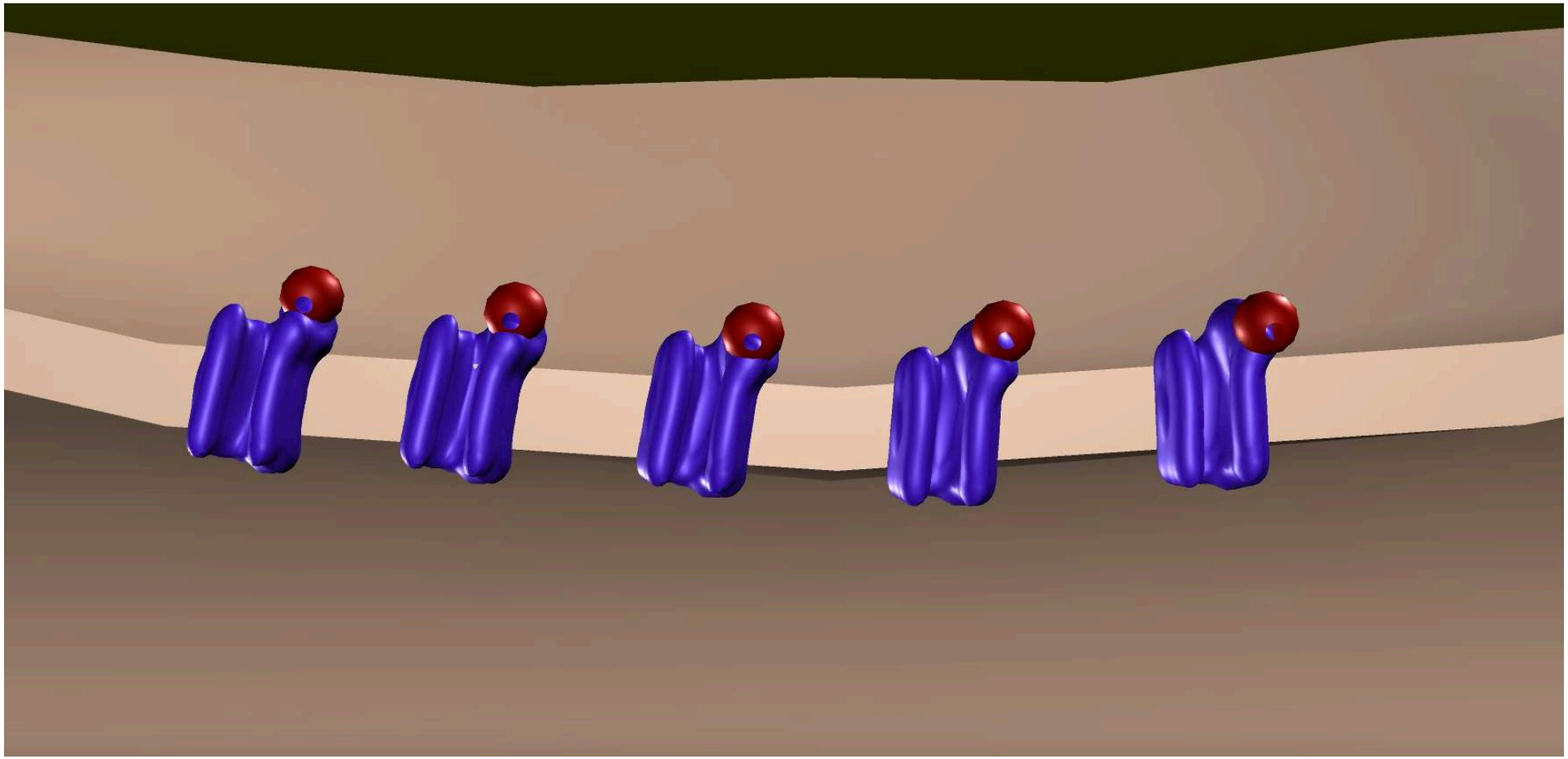
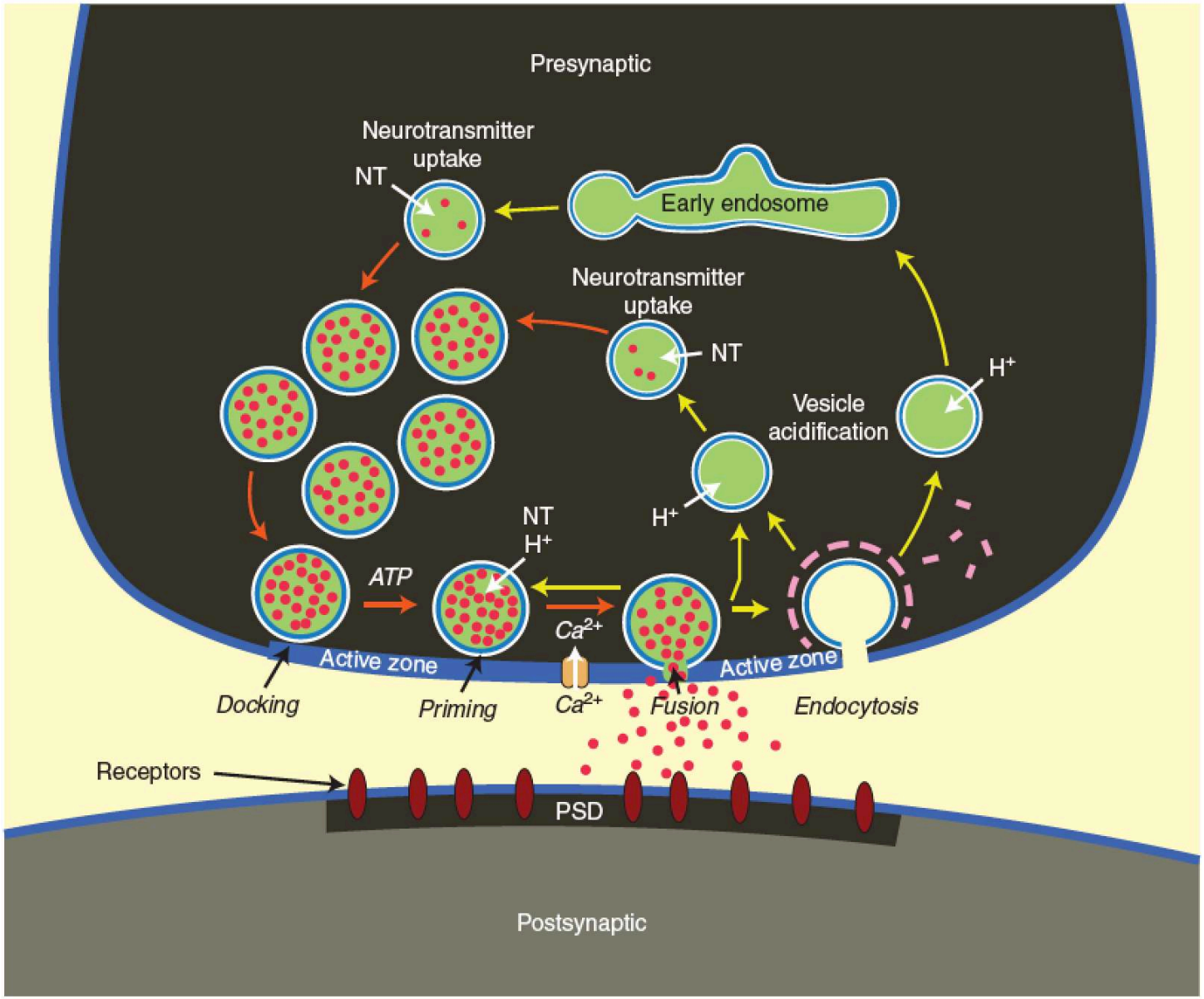


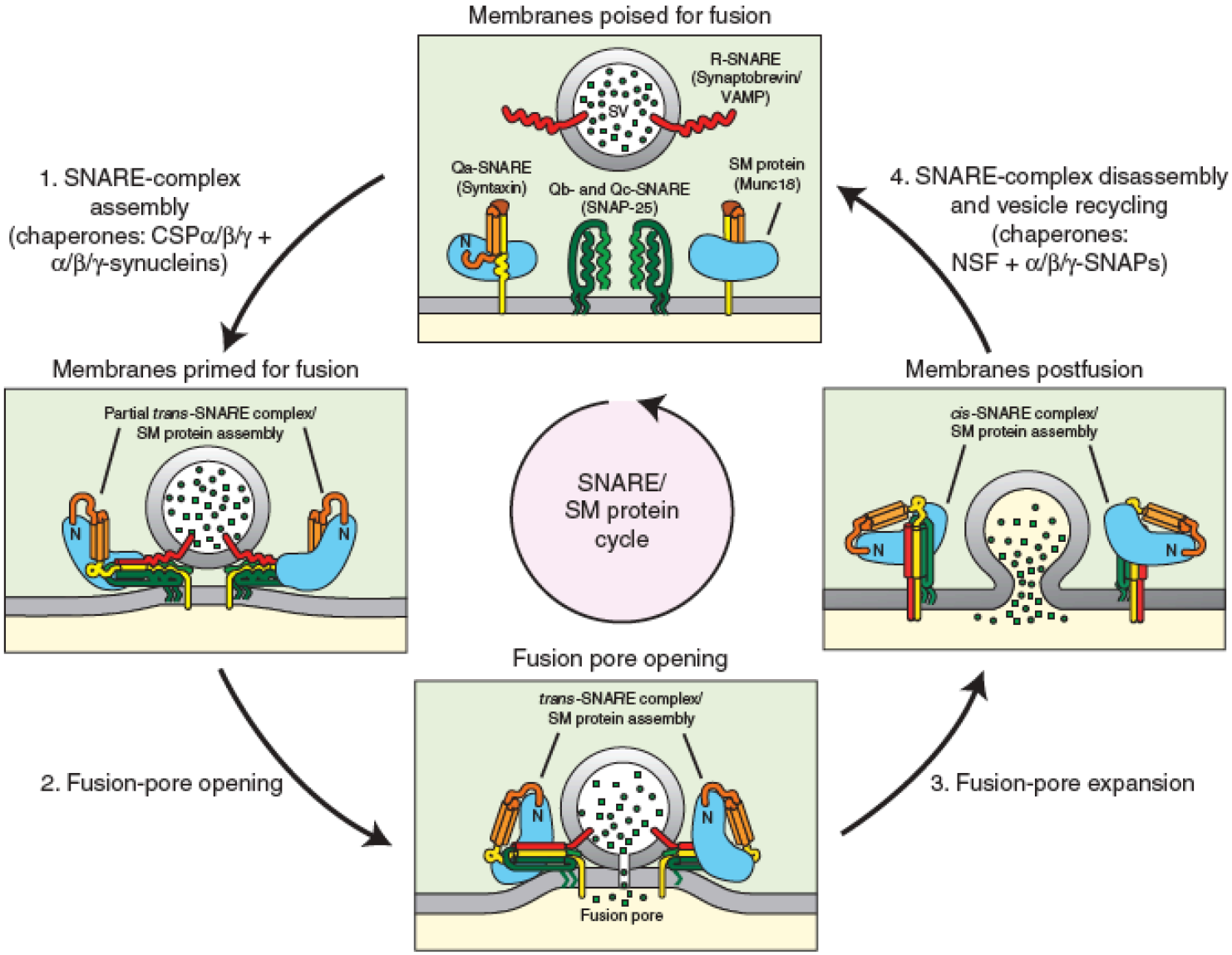
Figure 13–73 **The formation of synaptic vesicles.** These tiny uniform vesicles are found only in nerve cells and in some endocrine cells, where they store and secrete small-molecule neurotransmitters. The import of neurotransmitter directly into the small endocytic vesicles that form from the plasma membrane is mediated by membrane carrier proteins that function as antiports, being driven by a H^+ gradient maintained by proton pumps in the vesicle membrane.

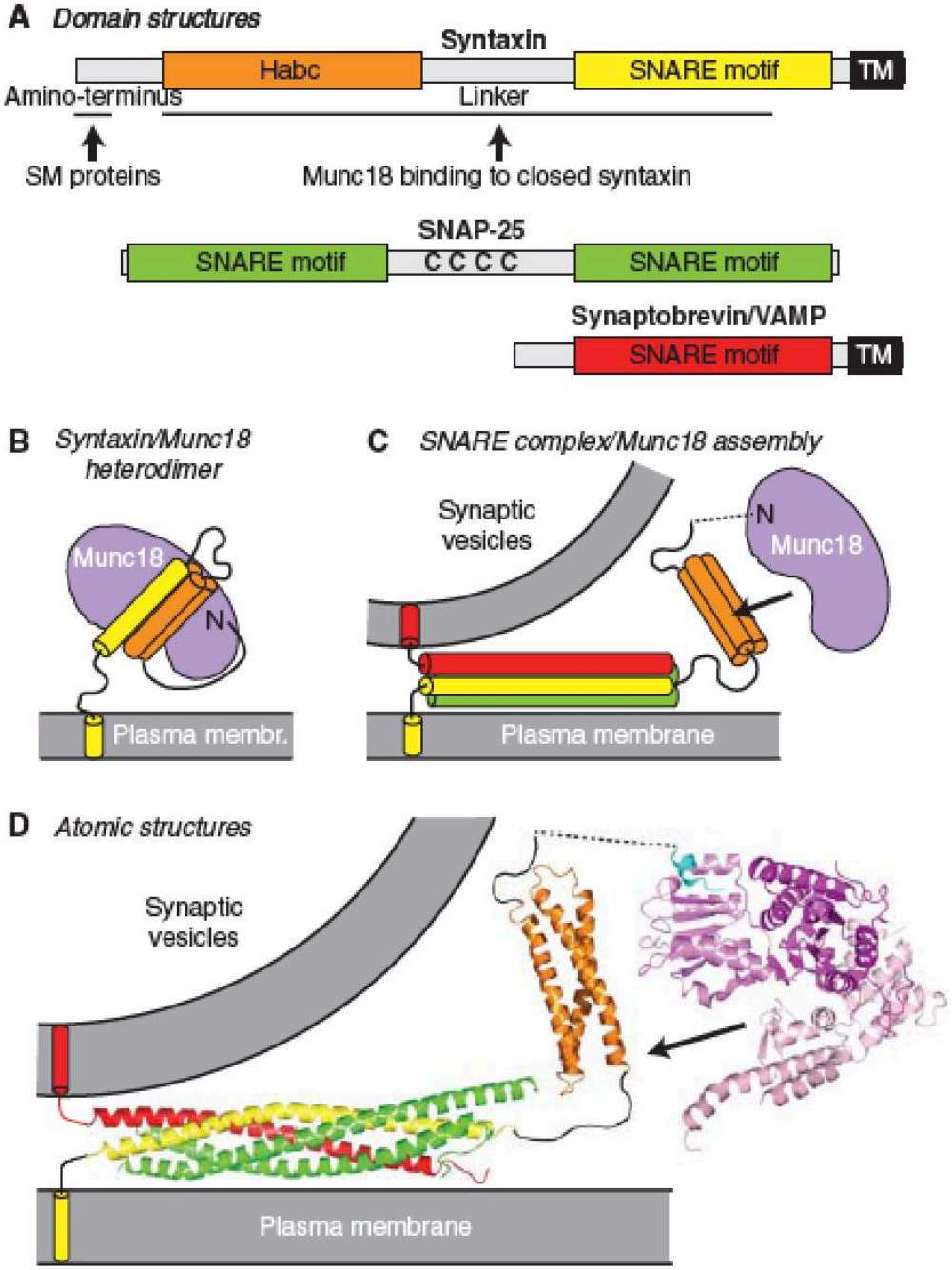


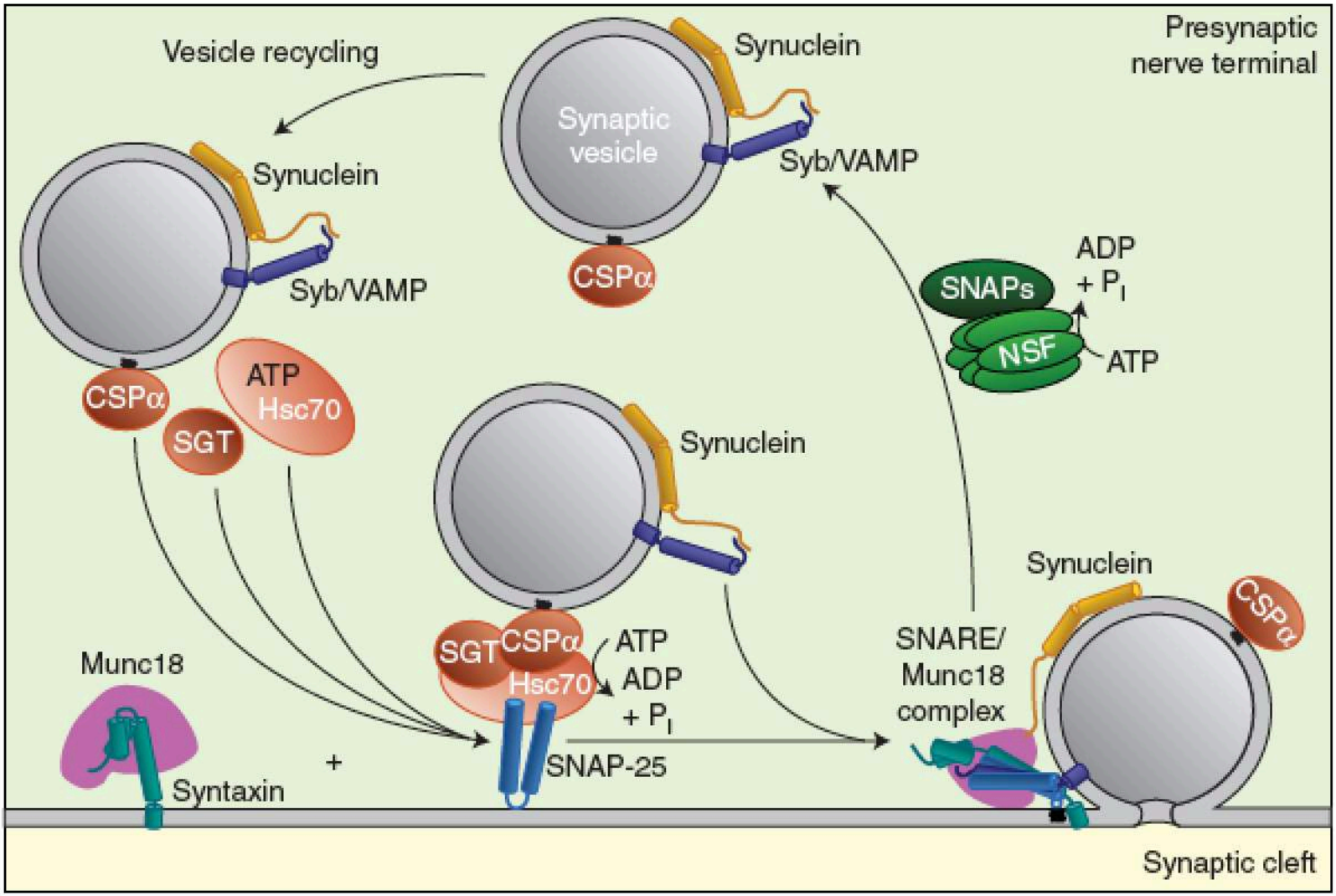

synaptic vesicles

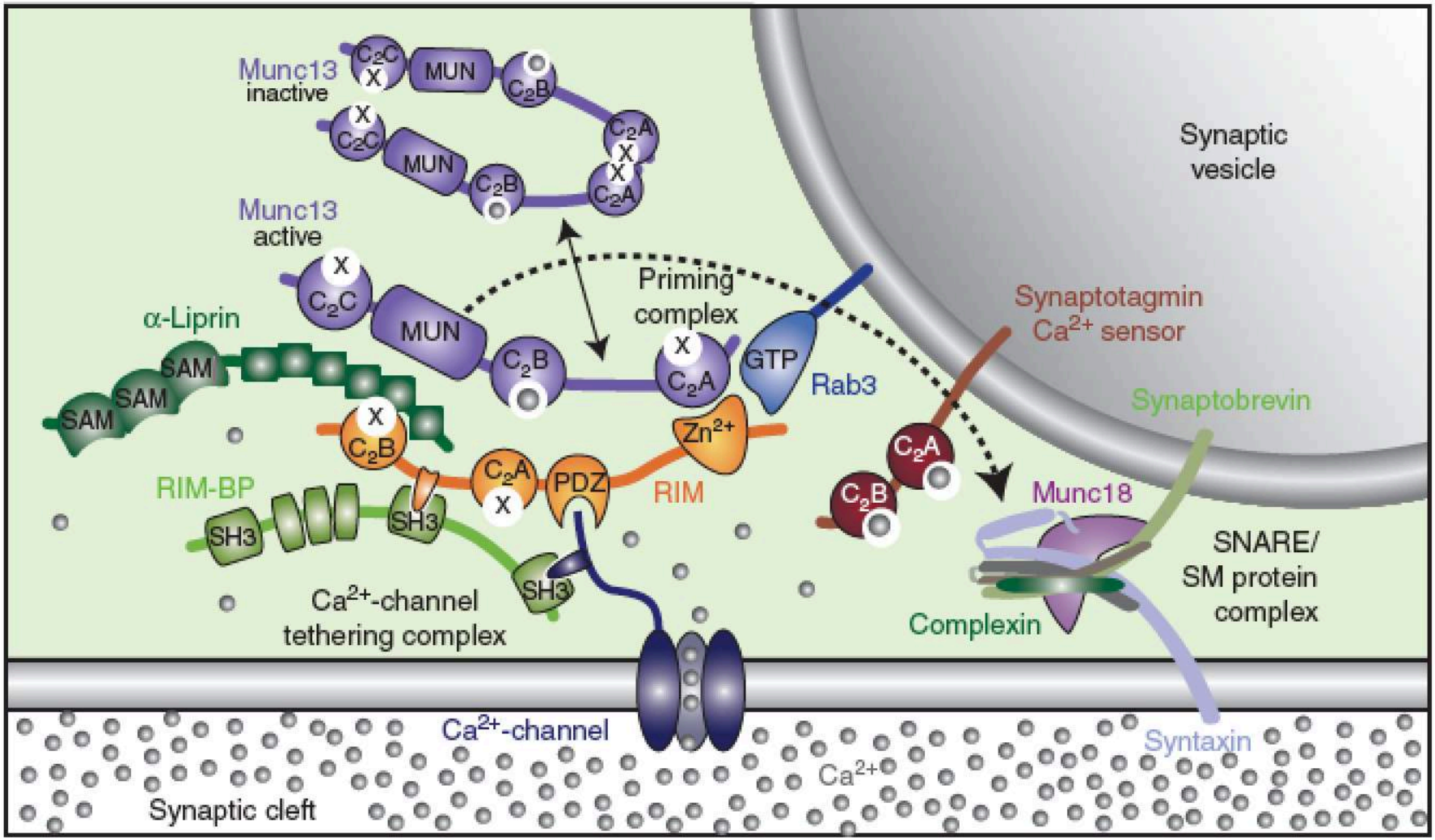


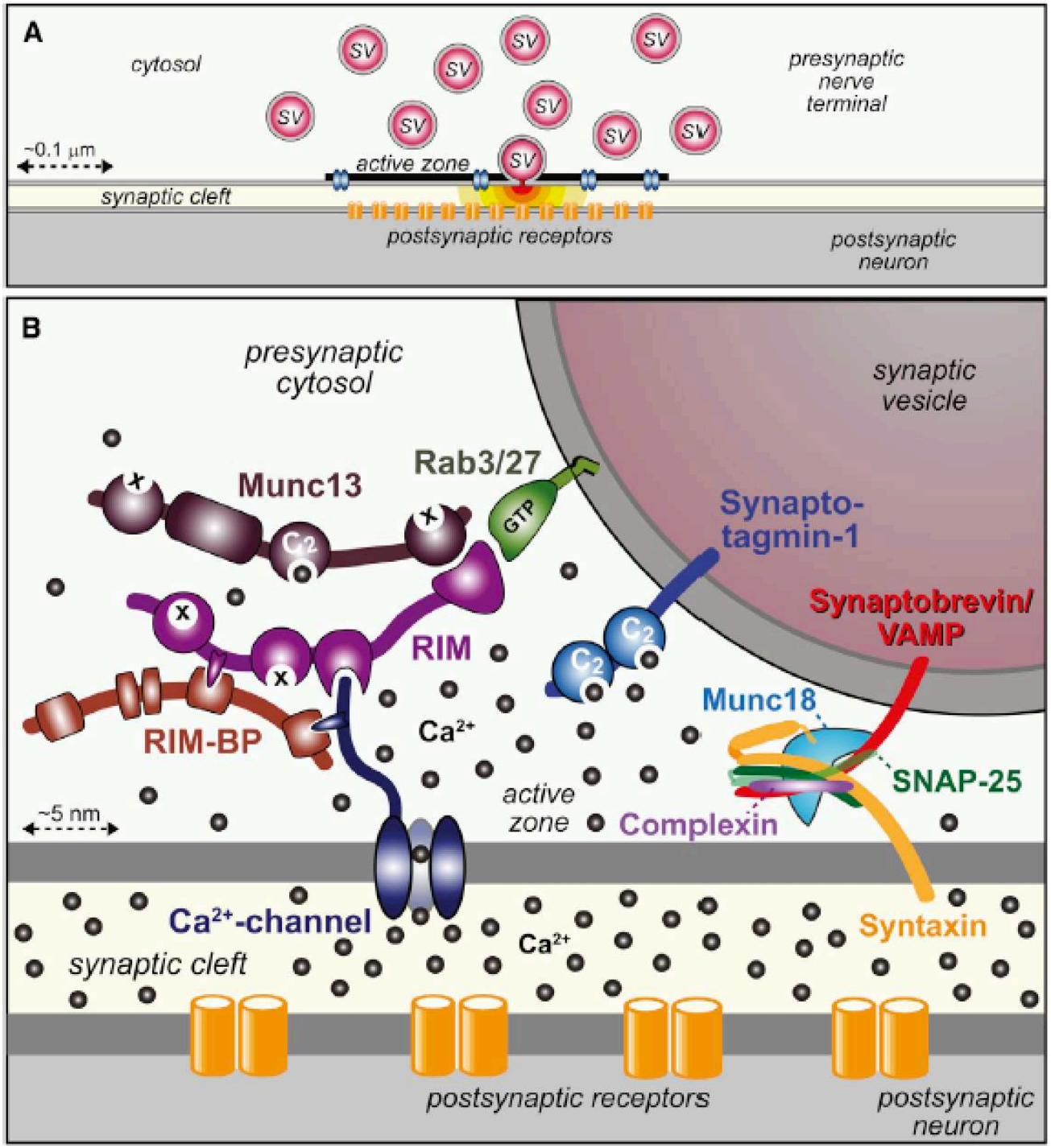

Presynaptic
Neurotransmitter uptake
NT
Early endosome
Neurotransmitter uptake
NT
H+
Vesicle acidification
H+
NT
H+
ATP
Ca2+
Active zone
Active zone
Docking
Priming
Ca2+
Fusion
Endocytosis
Receptors
PSD
Postsynaptic

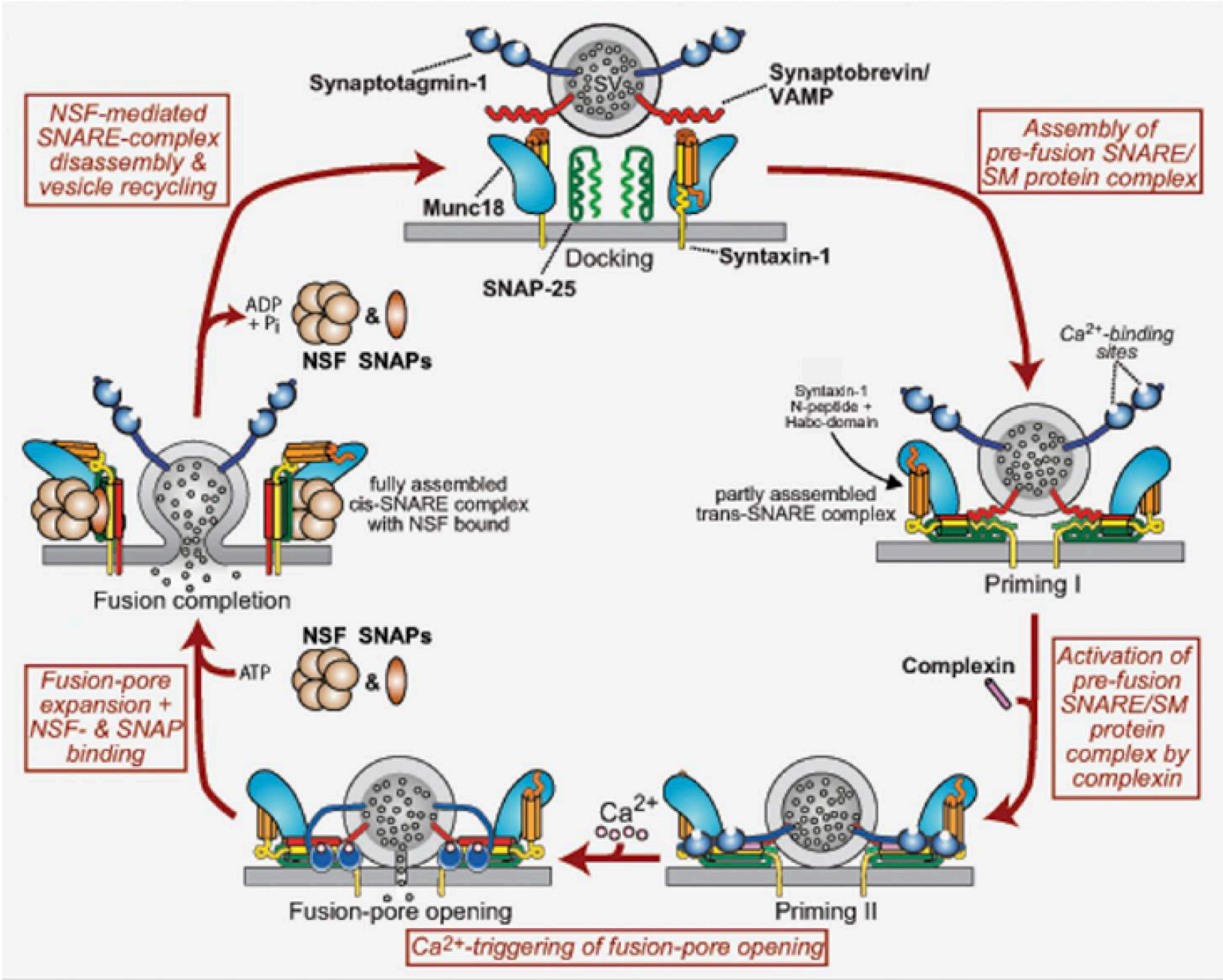

Membranes poised for fusion
R-SNARE
(Synaptobrevin/
VAMP)
SV
Qa-SNARE
(Syntaxin)
Qb- and Qc-SNARE
(SNAP-25)
SM protein
(Munc18)
N
1. SNARE-complex
assembly
(chaperones: CSPα/β/γ +
α/β/γ-synucleins)
4. SNARE-complex disassembly
and vesicle recycling
(chaperones:
NSF + α/β/γ-SNAPs)
Membranes primed for fusion
Partial trans-SNARE complex/
SM protein assembly
Membranes postfusion
cis-SNARE complex/
SM protein assembly
SNARE/
SM protein
cycle
Fusion pore opening
trans-SNARE complex/
SM protein assembly
Fusion pore
2. Fusion-pore opening
3. Fusion-pore expansion


A Domain structures
Syntaxin
Habc
SNARE motif
TM
Amino-terminus
Linker
SM proteins
Munc18 binding to closed syntaxin
SNAP-25
SNARE motif
C C C C
SNARE motif
Synaptobrevin/VAMP
SNARE motif
TM
B Syntaxin/Munc18 heterodimer
Munc18
N
Plasma membr.
C SNARE complex/Munc18 assembly
Synaptic vesicles
N
Munc18
Plasma membrane
D Atomic structures
Synaptic vesicles
Plasma membrane


Presynaptic
nerve terminal
Vesicle recycling
Synuclein
Synaptic
vesicle
Syb/VAMP
CSPα
Synuclein
Syb/VAMP
CSPα
ATP
Hsc70
SGT
SNAPs
NSF
ADP
+ P_I
ATP
Synuclein
Synuclein
CSPα
SGT
CSPα
Hsc70
ATP
ADP
+ P_I
SNARE/
Munc18
complex
Munc18
+
Syntaxin
SNAP-25
Synaptic cleft


Munc13
inactive
C2C
X
MUN
C2B
C2A
Munc13
active
Priming
complex
α-Liprin
SAM
SAM
SAM
GTP
Rab3
Synaptic
vesicle
Synaptotagmin
Ca2+ sensor
Synaptobrevin
Zn2+
PDZ
RIM
RIM-BP
SH3
Munc18
SNARE/
SM protein
complex
Complexin
Ca2+-channel
tethering complex
Ca2+-channel
Syntaxin
Ca2+
Synaptic cleft


A
cytosol
SV
presynaptic
nerve
terminal
~0.1 µm
active zone
synaptic cleft
postsynaptic receptors
postsynaptic
neuron
B
presynaptic
cytosol
synaptic
vesicle
Munc13
Rab3/27
GTP
Synapto-
tagmin-1
Synaptobrevin/
VAMP
RIM
RIM-BP
Munc18
SNAP-25
Ca2+
active
zone
Complexin
~5 nm
Ca2+-channel
Syntaxin
synaptic cleft
postsynaptic receptors
postsynaptic
neuron


Synaptotagmin-1
SV
Synaptobrevin/
VAMP
NSF-mediated
SNARE-complex
disassembly &
vesicle recycling
Assembly of
pre-fusion SNARE/
SM protein complex
Munc18
Docking
Syntaxin-1
SNAP-25
ADP
+ Pi
&
NSF SNAPs
Ca2+-binding
sites
Syntaxin-1
N-peptide +
Habc-domain
partly assembled
trans-SNARE complex
fully assembled
cis-SNARE complex
with NSF bound
Fusion completion
Priming I
NSF SNAPs
ATP
&
Complexin
Fusion-pore
expansion +
NSF- & SNAP
binding
Activation of
pre-fusion
SNARE/SM
protein
complex by
complexin
Ca2+
Fusion-pore opening
Priming II
Ca2+-triggering of fusion-pore opening

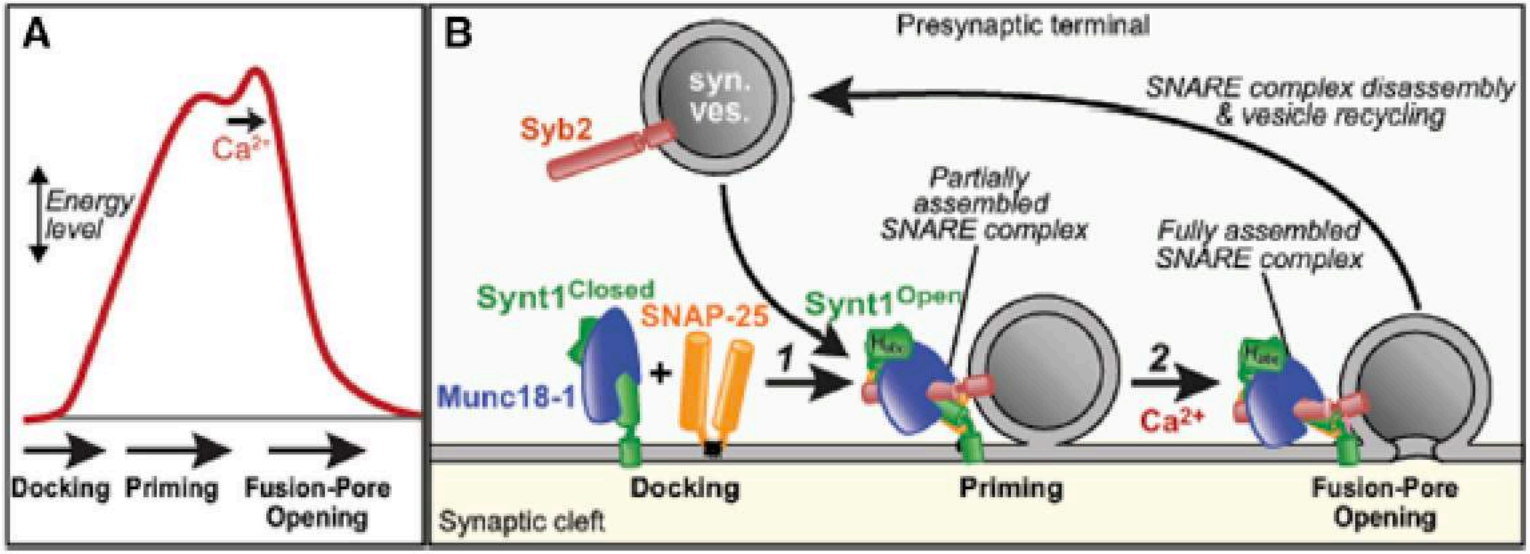
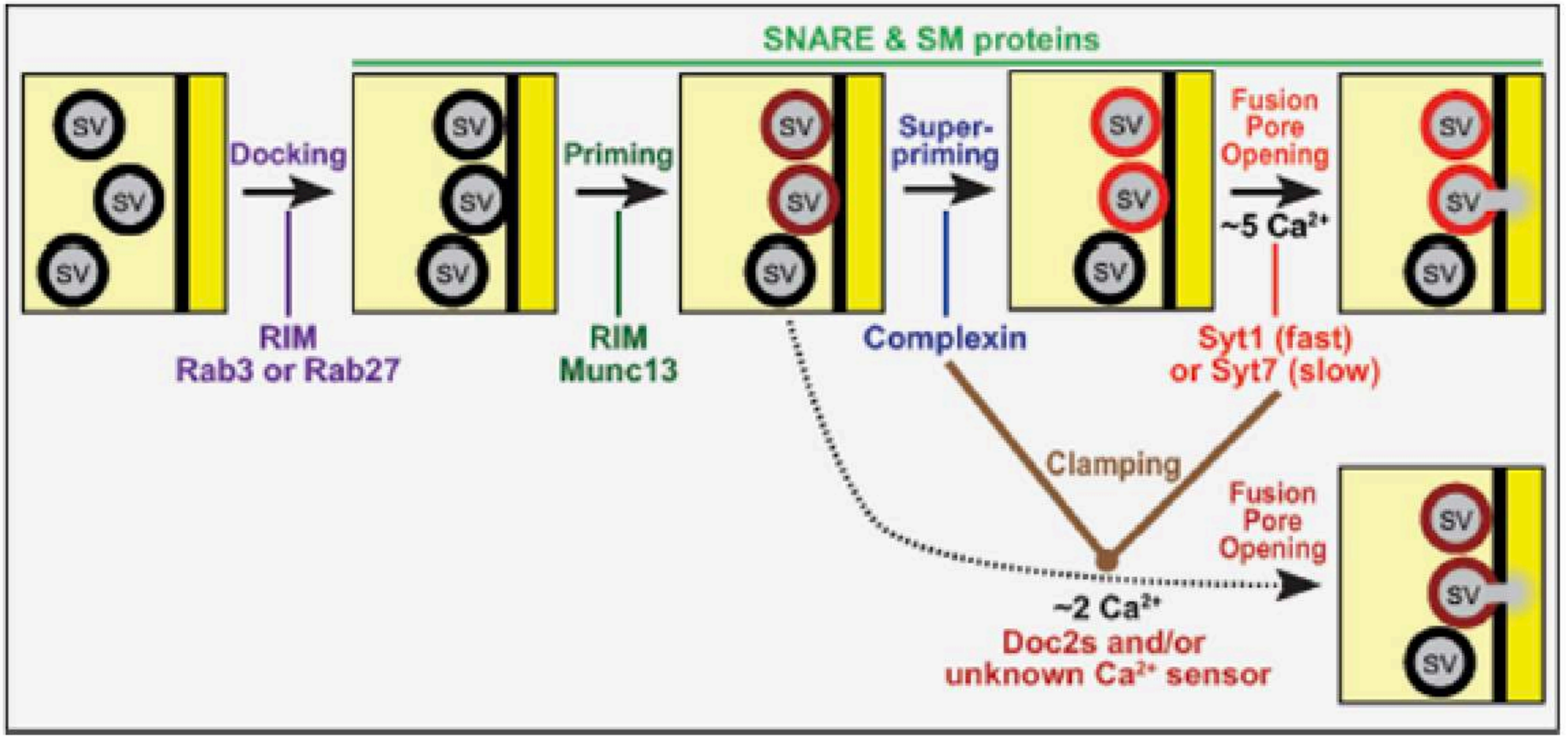


Figure 3. Energy Landscape of Fusion and Proposed Role of the SM Protein Munc18-1 in Promoting Fusion Pore Opening

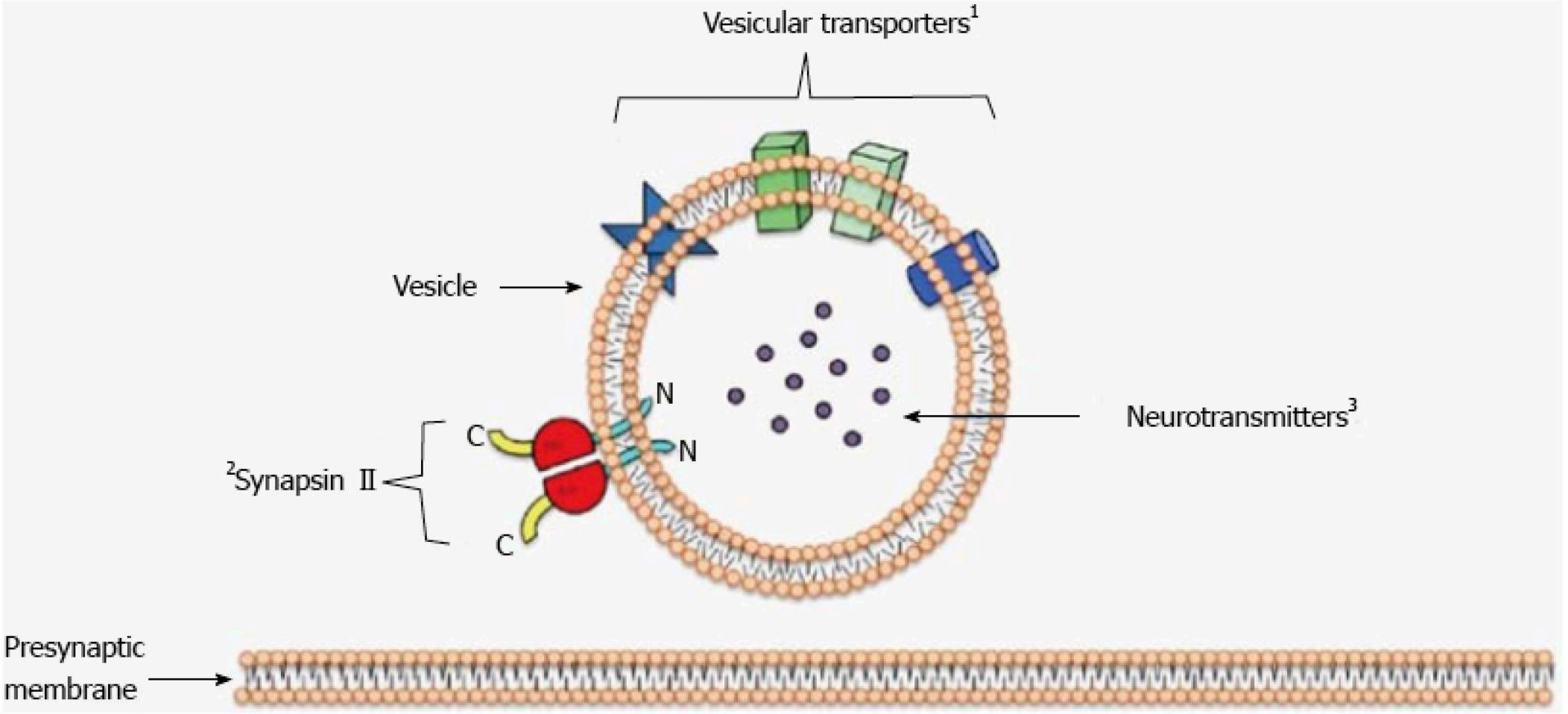
(A) Schematic diagram of the energy level of a vesicle that is docked, primed, and fused. The diagram illustrates that partial SNARE complex

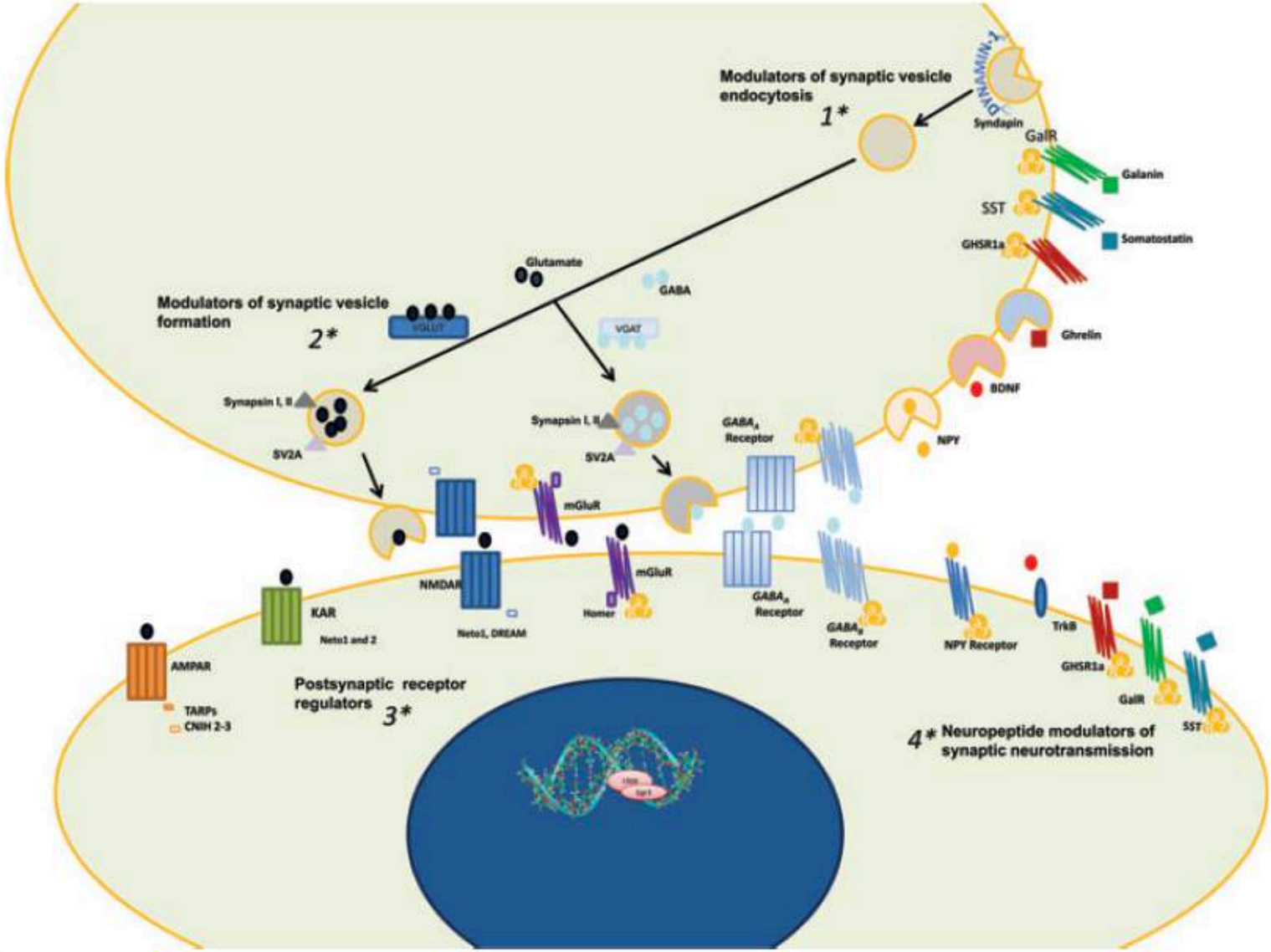
assembly during priming is proposed to provide most of the energy required for fusion, such that Ca2+ triggering only adds a small amount of additional energy to induce fusion pore opening.

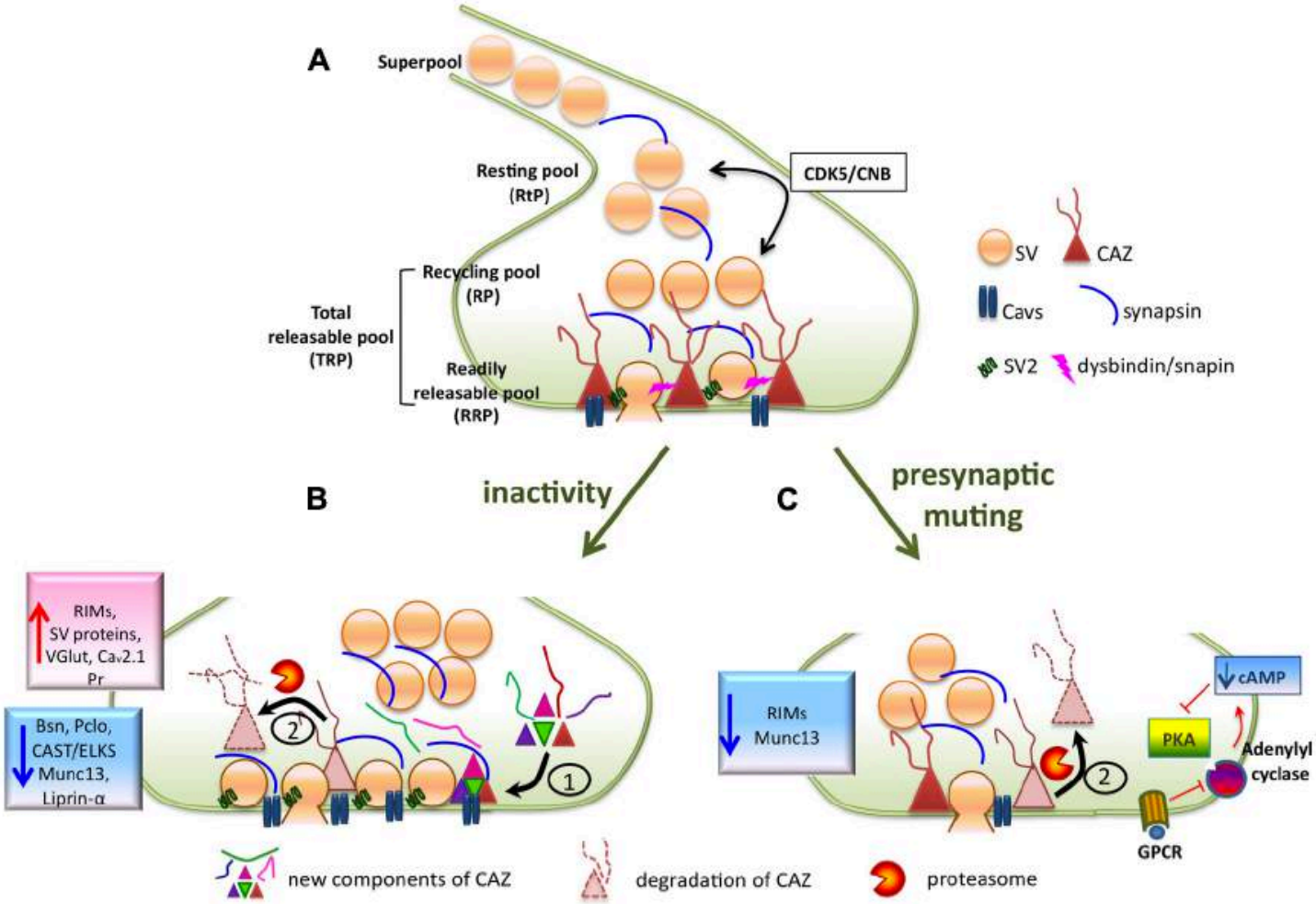
(B) Model of Munc18-1 function in fusion. Prior to priming of docked vesicles, Munc18-1 is bound to the closed conformation of syntaxin-1; this interaction is primarily regulatory to maintain a defined rate of entry into the fusion reaction and additionally serves for the mutual stabilization of Munc18-1 and syntaxin-1 for each other (Gerber et al., 2008; Zhou et al., 2013a). Partial SNARE complex assembly during priming (middle) is associated with a dramatic conformational change in syntaxin-1, which has to open, and in Munc18-1, whose binding changes from that to a closed syntaxin-1 conformation to binding to the open syntaxin-1


SNARE & SM proteins
SV
Docking
RIM
Rab3 or Rab27
Priming
RIM
Munc13
Super-
priming
Complexin
Fusion
Pore
Opening
~5 Ca2+
Syt1 (fast)
or Syt7 (slow)
Clamping
Fusion
Pore
Opening
~2 Ca2+
Doc2s and/or
unknown Ca2+ sensor

Sudhof, 2013

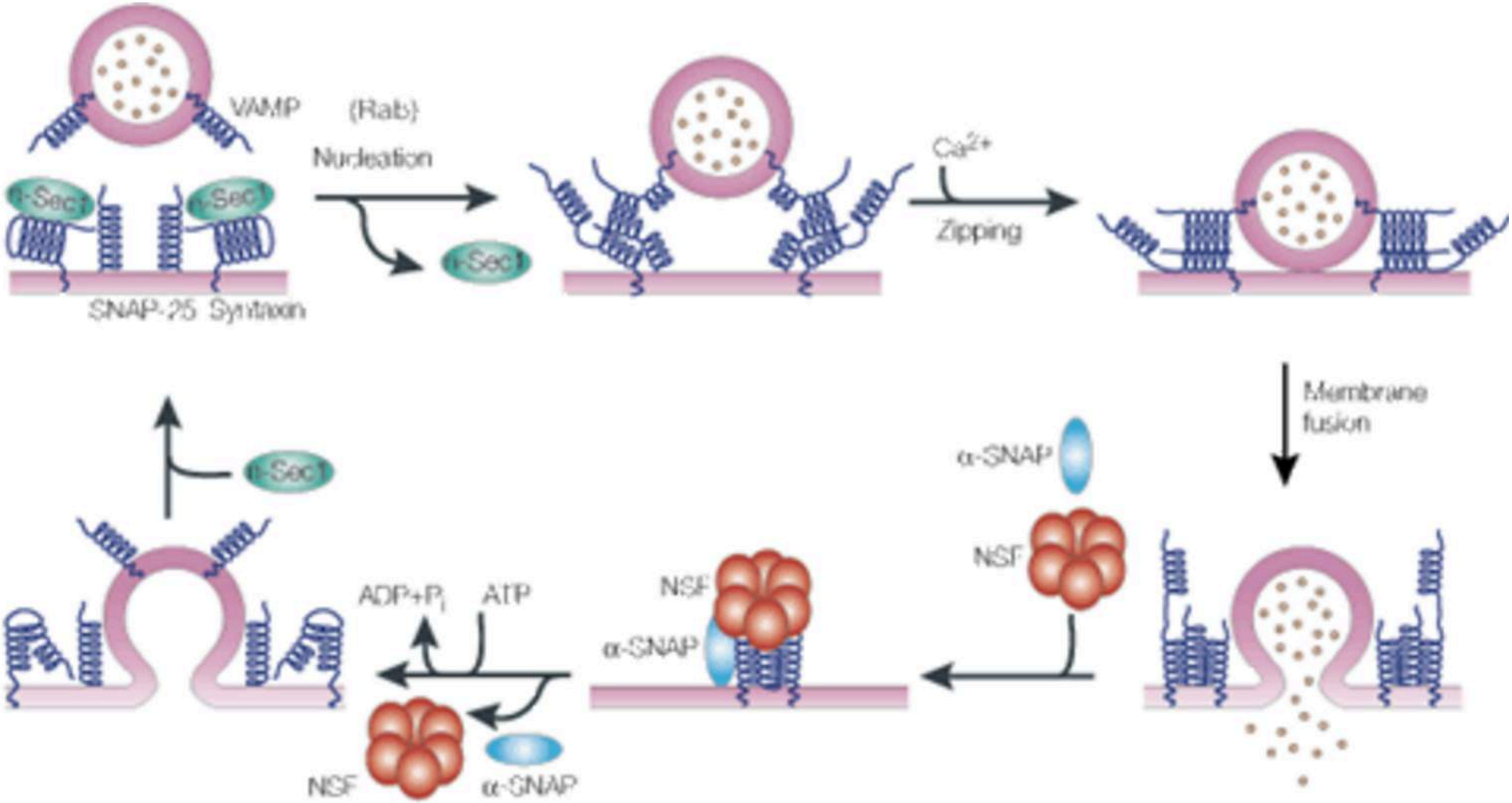

Vesicular transporters[1]
Vesicle
N
N
C
C
[2]Synapsin II
Neurotransmitters[3]
Presynaptic membrane

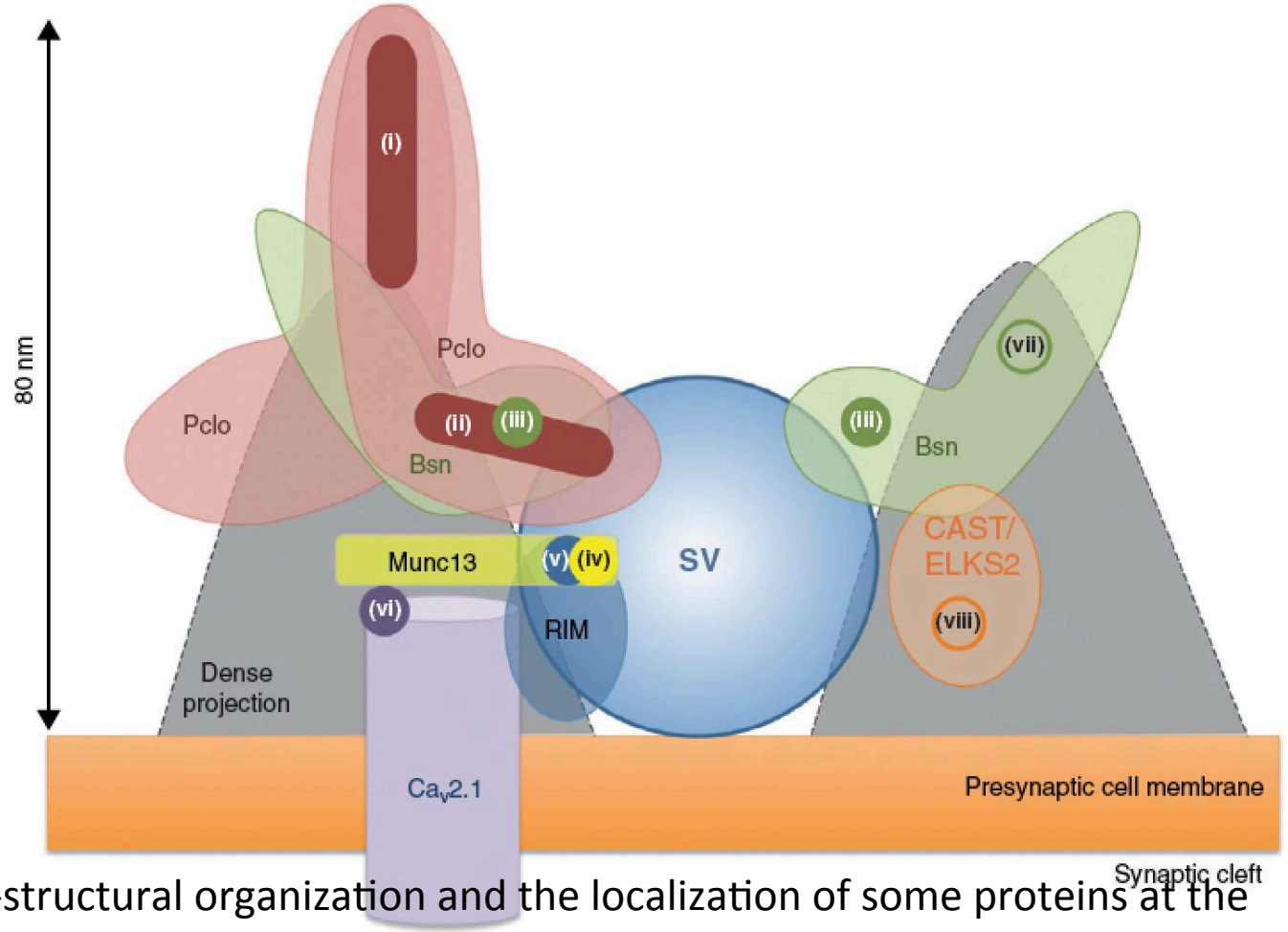

Modulators of synaptic vesicle endocytosis
1*
DYNAMIN-1
Syndapin
GalR
Galanin
SST
Somatostatin
GHSR1a
Ghrelin
BDNF
NPY
Glutamate
GABA
Modulators of synaptic vesicle formation
2*
VGLUT
VGAT
Synapsin I, II
SV2A
Synapsin I, II
SV2A
GABA$_A$ Receptor
mGluR
mGluR
NMDAR
Neto1, DREAM
Homer
KAR
Neto1 and 2
GABA$_A$ Receptor
GABA$_B$ Receptor
NPY Receptor
TrkB
GHSR1a
GalR
SST
AMPAR
TARPs
CNIH 2-3
Postsynaptic receptor regulators
3*
4* Neuropeptide modulators of synaptic neurotransmission


A
Superpool
Resting pool
(RtP)
CDK5/CNB
Recycling pool
(RP)
Total
releasable pool
(TRP)
Readily
releasable pool
(RRP)
SV
CAZ
Cavs
synapsin
SV2
dysbindin/snapin
B
inactivity
C
presynaptic
muting
RIMs,
SV proteins,
VGlut, Cav2.1
Pr
Bsn, Pclo,
CAST/ELKS
Munc13,
Liprin-α
2'
1
RIMs
Munc13
2
cAMP
PKA
Adenylyl
cyclase
GPCR
new components of CAZ
degradation of CAZ
proteasome

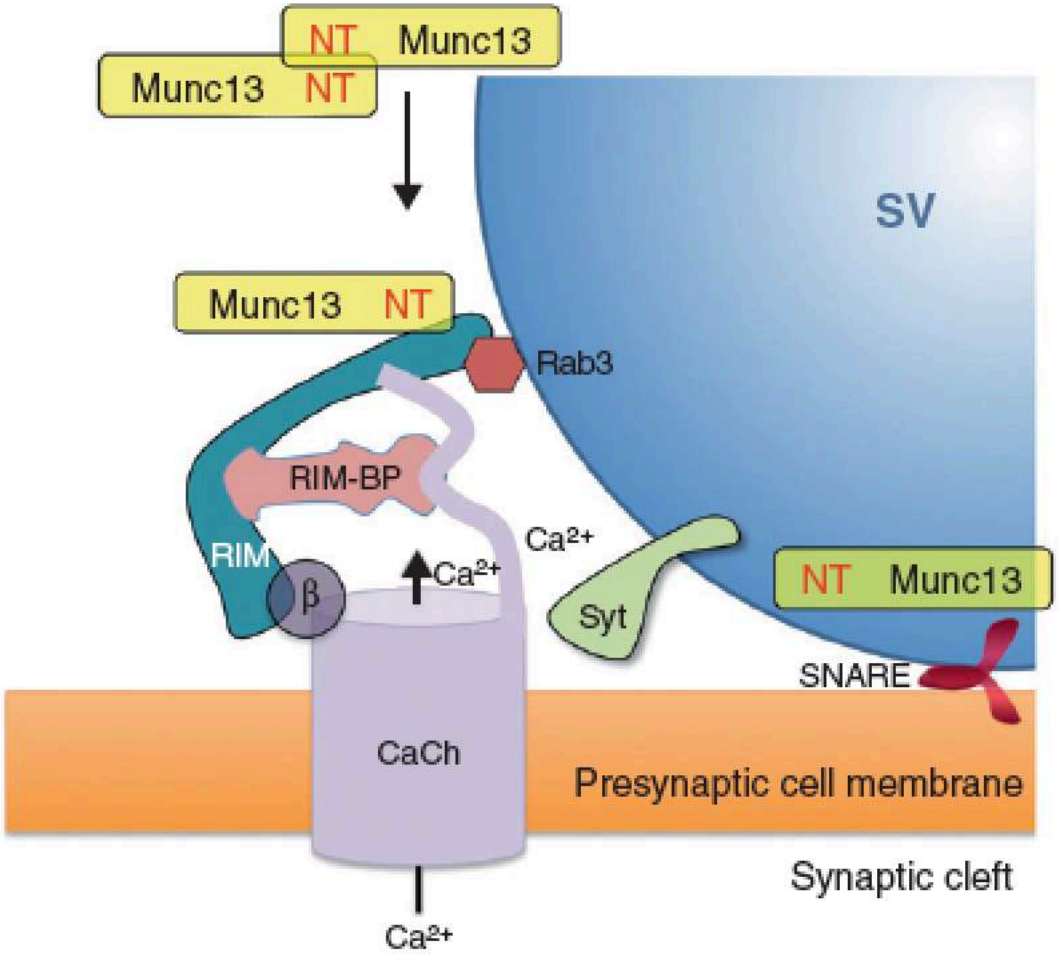
Transmitter Molecule	Derived From	Site of Synthesis
Acetylcholine	Choline	CNS, parasympathetic nerves
Serotonin 5-Hydroxytryptamine (5-HT)	Tryptophan	CNS, chromaffin cells of the gut, enteric cells
GABA	Glutamate	CNS
Glutamate		CNS
Aspartate		CNS
Glycine		spinal cord
Histamine	Histidine	hypothalamus
Epinephrine	Tyrosine	adrenal medulla, some CNS cells
Norpinephrine	Tyrosine	CNS, sympathetic nerves
Dopamine	Tyrosine	CNS
Adenosine	ATP	CNS, periperal nerves

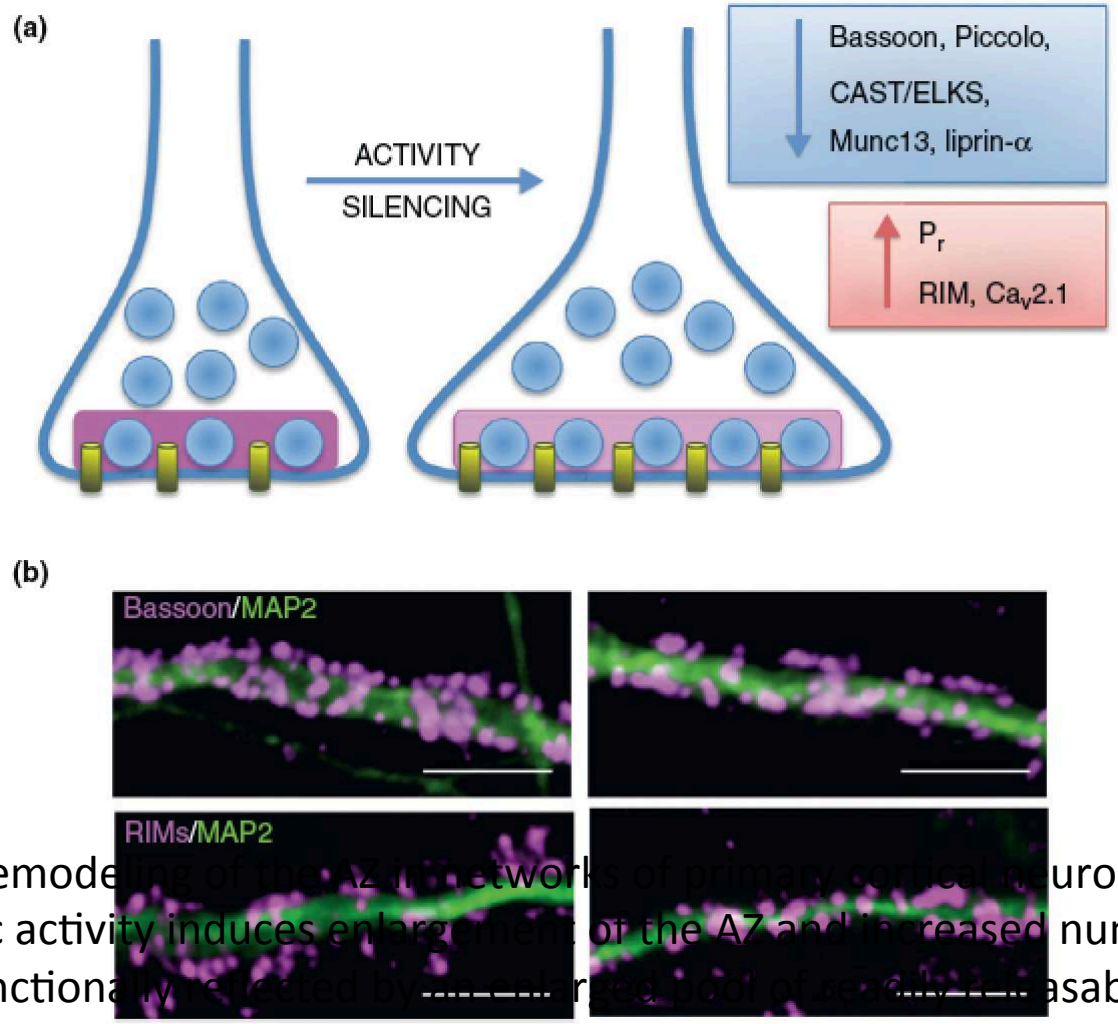
Neurotrans.	Types of receptors	Mode of action	Result in postsynaptic cell	Target
Acetylcholine	Nicotinic Muscarinic	Opens ion channels	EPSP	CNS neurons; skeletal muscle
Serotonin	Two main classes; multiple subclasses	G-protein coupled receptors; both AC and IP3/DAG	Depends on receptor type	Platelet aggregation, smooth muscle contraction, satiety, vomiting
GABA	GABA-A GABA-B	Receptor Cl- channel G-linked K+ channel	IPSP in all cases	Throughout CNS and in retina
Norepinephrine	α Receptor β receptor	G-protein linked to cAMP G-protein linked to cAMP	IPSP EPSP	Relaxes smooth muscles of gut, bronchial tree, and vessels to skel. muscle Increases rate and strength of cardiac contraction; excites smooth muscle in vessels
Dopamine	D1, D2, D3, D4, and D5	G-protein linked to cAMP, direct channel opening, cAMP to K + channel opening	EPSP and IPSP	D1-3 are located in the striatum of the CNS, and the basal ganglia D3-5 play a role in mood, psychosis and neuroprotection


VAMP
(Rab)
Nucleation
t-Sec1
t-Sec1
t-Sec1
SNAP-25
Syntaxin
Ca2+
Zipping
Membrane
fusion
α-SNAP
NSF
NSF
α-SNAP
ADP+Pi
ATP
t-Sec1
NSF
α-SNAP



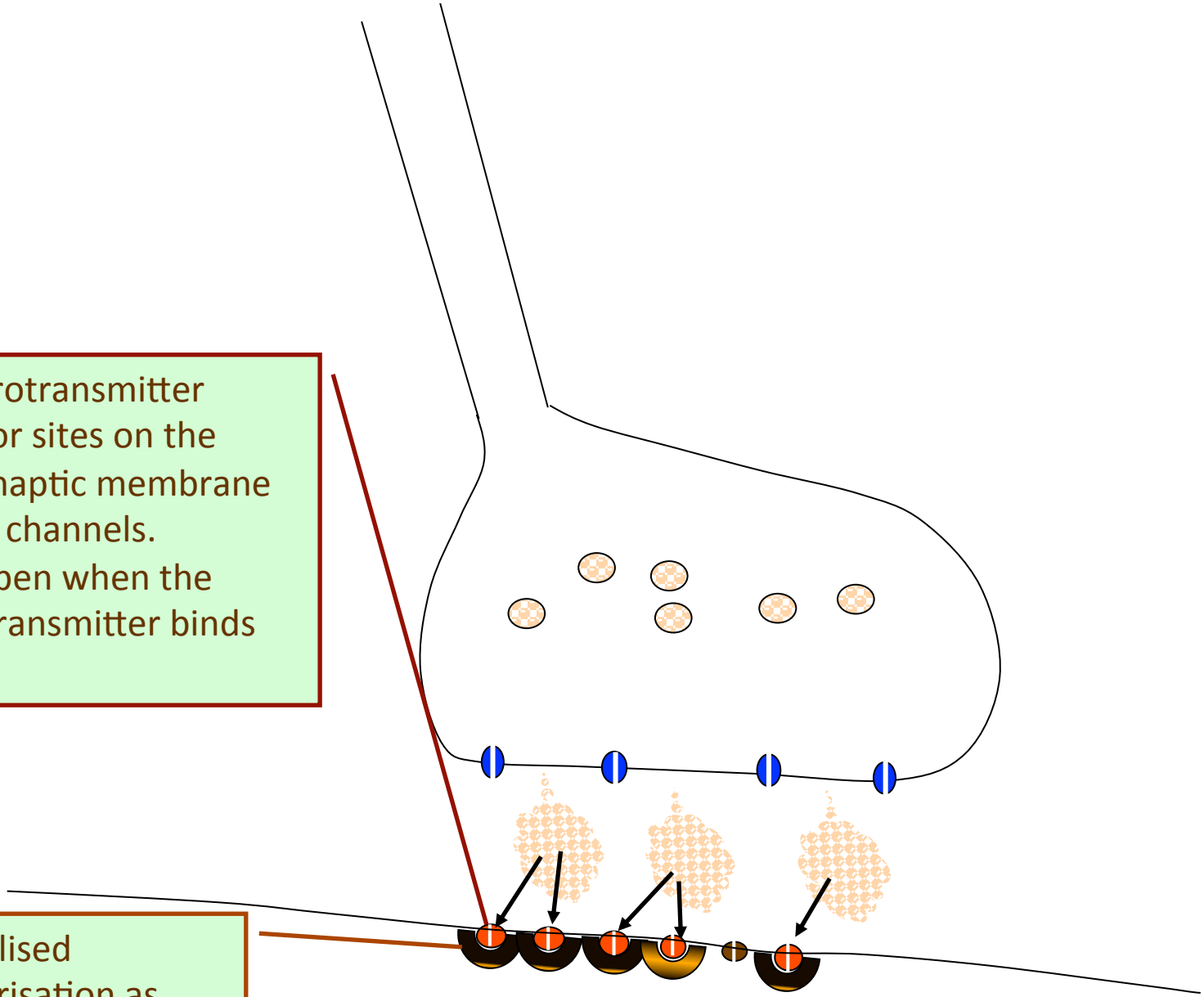
Scheme of the ultra-structural organization and the localization of some proteins at the active zone of conventional brain synapses. (i) Immunogold localization of epitopes against Piccolo/Aczonin (Pclo) N-terminal portion (six epitopes), (ii) C-terminal portion of Pclo (five epitopes), (iii) Bassoon (Bsn) C-terminal portion, (iv) Munc13 N-terminus, (v) RIM N-terminus, (vi) P/Q-type calcium channel CaV2.1, (vii) Bsn N-terminal portion and (viii) CAST/ ELKS2. Note: epitopes (vi)–(viii) and some of (i) and (ii) have only been mapped for their distance from the presynaptic cell membrane and not relative to the dense projections. Epitopes (i)–(vi) were taken from Ref. [17__] and (vii), (viii) from Ref. [21]. Epitope mapping with superresolution light microscopy favorably supports the data for RIM, Bsn and Pclo [20_].


NT Munc13
Munc13 NT
SV
Munc13 NT
Rab3
RIM-BP
RIM
β
Ca2+
Ca2+
Syt
NT Munc13
SNARE
CaCh
Presynaptic cell membrane
Synaptic cleft
Ca2+



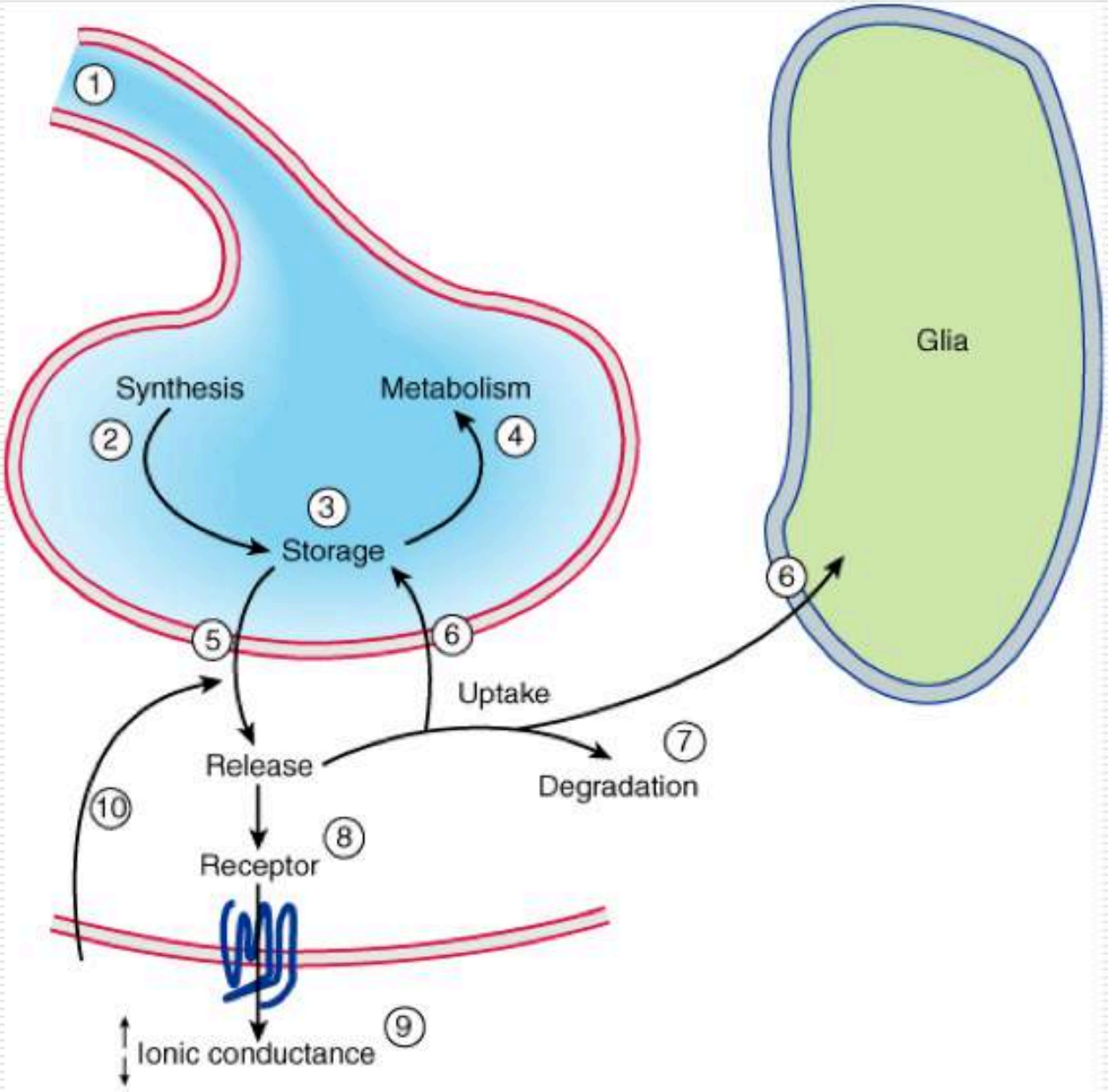
Activity silencing-induced remodeling of the AZ in networks of primary cortical neurons. (a) Chronic silencing of synaptic activity induces enlargement of the AZ and increased number of docked vesicles, which is functionally reflected by an enlarged pool of readily releasable vesicles and higher synaptic release probability (Pr) [58]. This structural and functional remodeling is associated with the reduction of most CAZ proteins in synapses, the redistribution of RIM to the most active synapses and an allover increase of synaptic P/Q-type CaV2.1 channels. (b) Examples of immunostaining showing depletion of Bassoon and redistribution of RIM at synapses of proximal dendrites of rat cortical neurons (visualized by anti-MAP2 staining) upon 48-h pharmacological inhibition of glutamate receptors using D-AP5 and CNQX (for details see [60_]). Scale bar, 5 mm.

5. Neurotransmitter receptor sites on the postsynaptic membrane are ion channels.
They open when the neurotransmitter binds

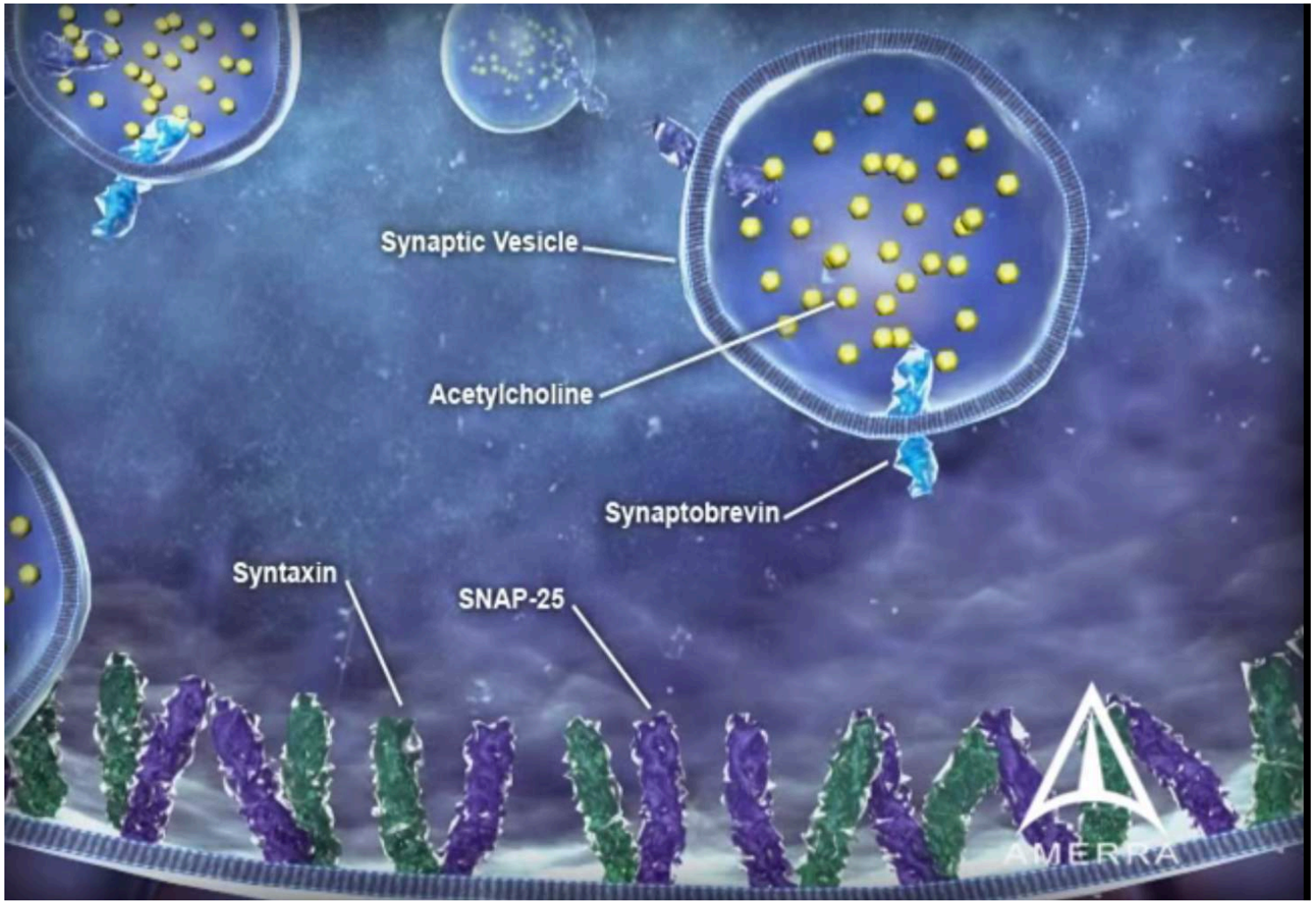


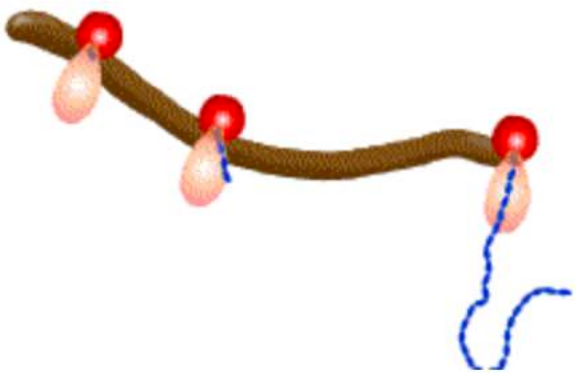
6. Localised depolarisation as ions leak in or out of membrane.

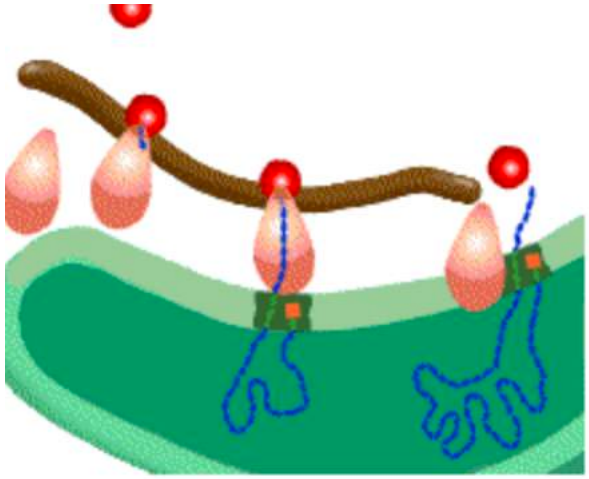
Site of drug action

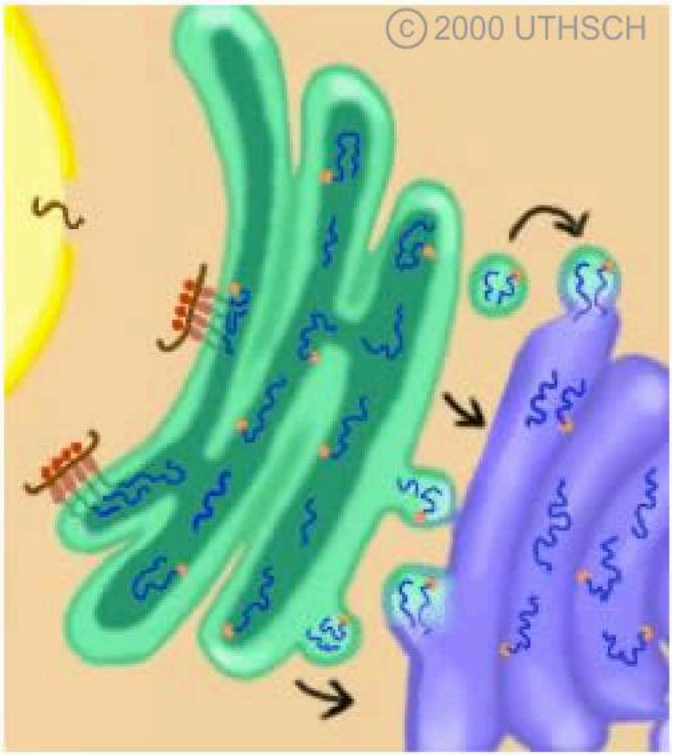


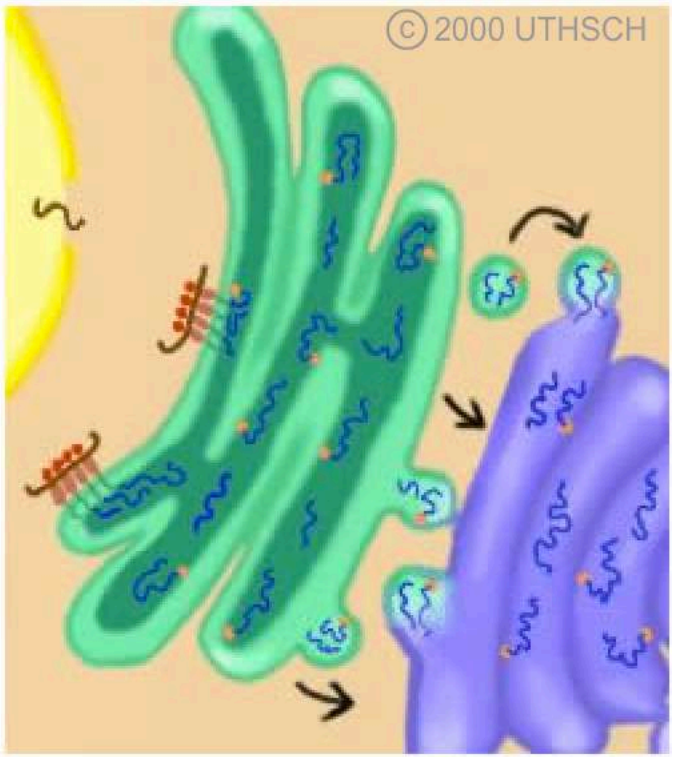
Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com


Synaptic Vesicle
Acetylcholine
Synaptobrevin
Syntaxin
SNAP-25
AMERRA

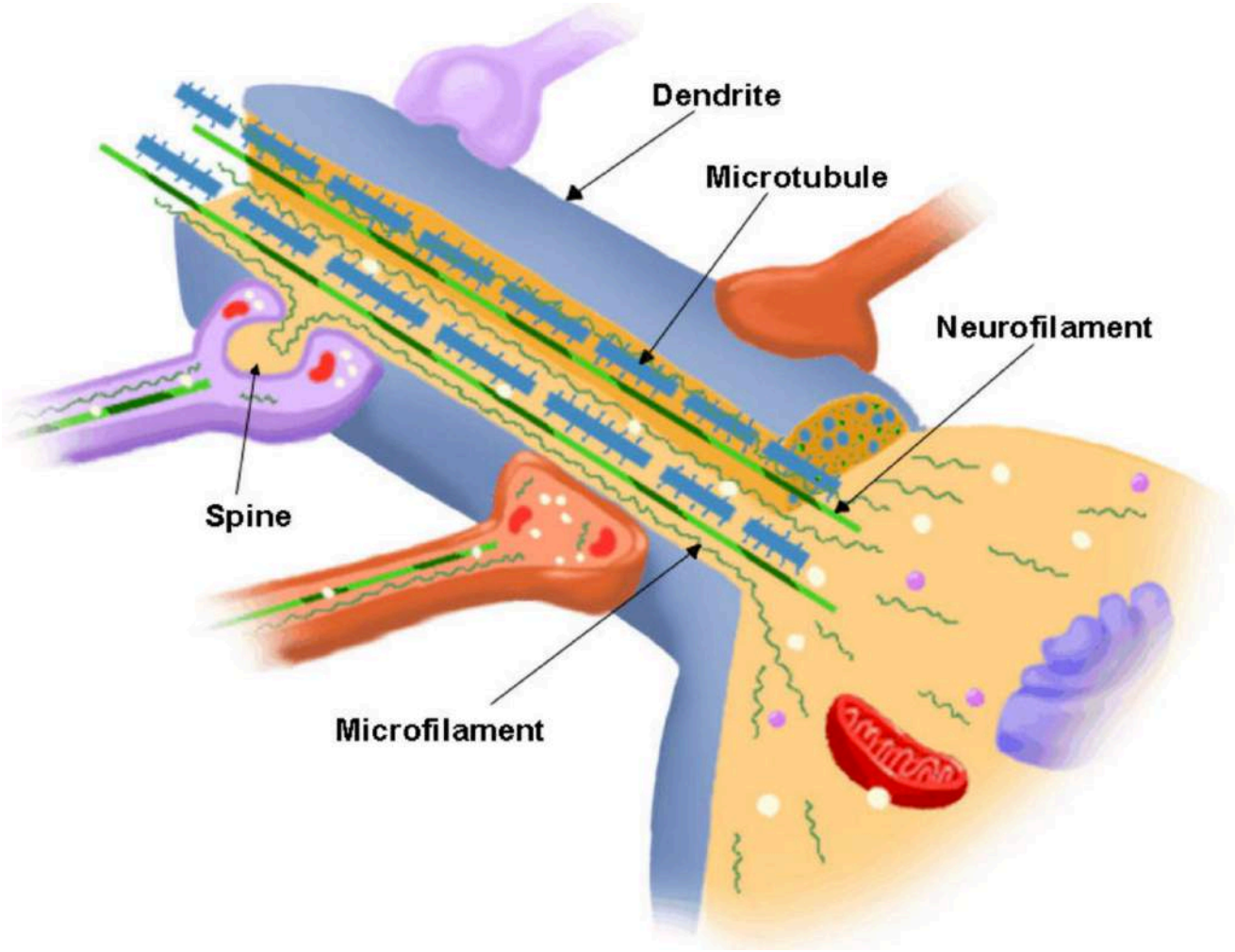


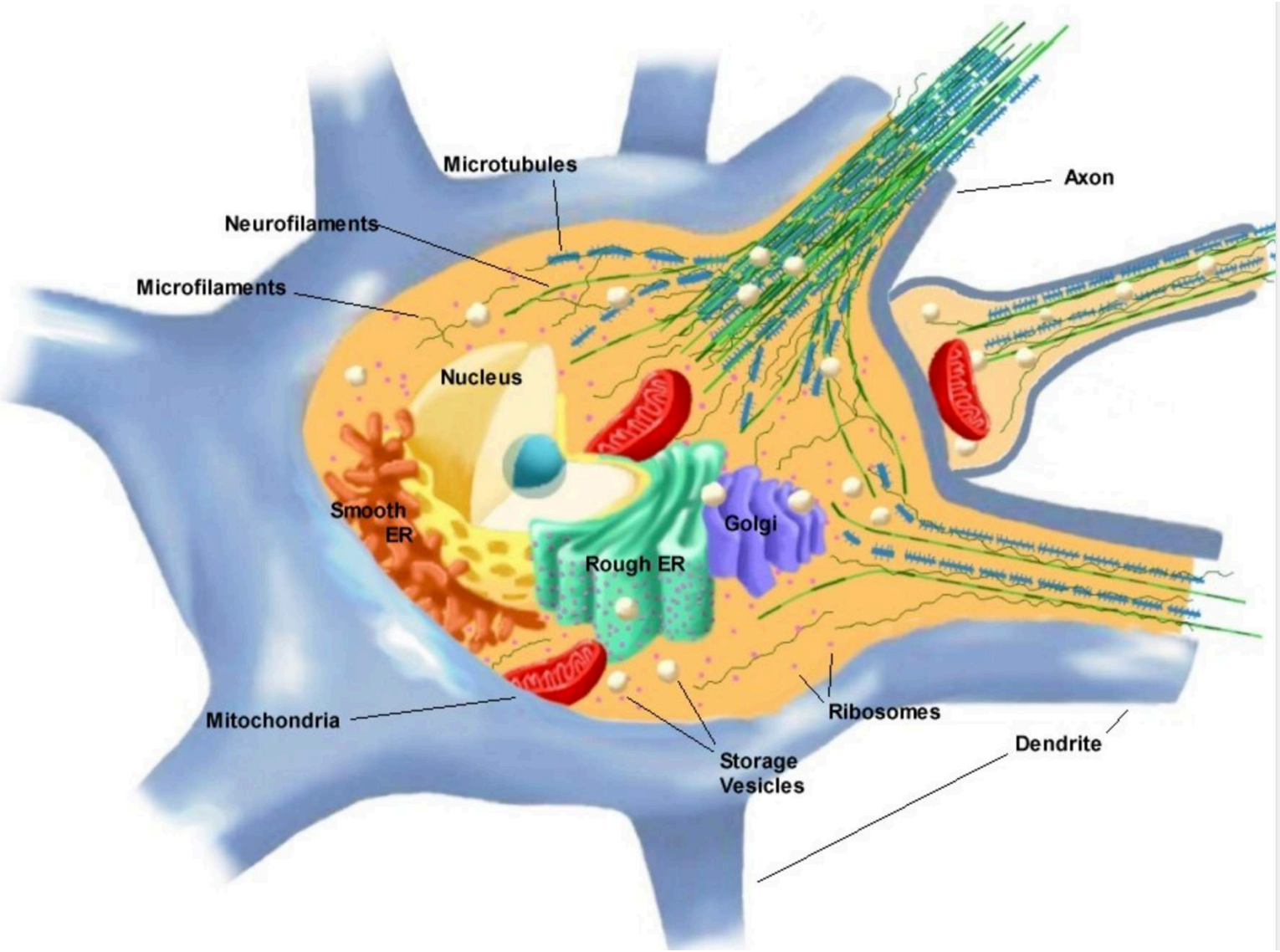



© 2000 UTHSCH



the movement of the secretory vesicles through the rough and the smooth ER. The smooth ER extends from the RER and serves as a site for lipid biosynthesis for the production of endosomes, lysosomes and plasma membrane as well as for the neurotransmitter vesicles. New membrane protein that begins its synthesis in the RER continues in the SER where pieces of the SER bud off to form transport vesicles that shuttle to the Golgi apparatus with their contents.


Dendrite
Microtubule
Neurofilament
Spine
Microfilament


Microtubules
Axon
Neurofilaments
Microfilaments
Nucleus
Smooth ER
Golgi
Rough ER
Mitochondria
Ribosomes
Storage Vesicles
Dendrite

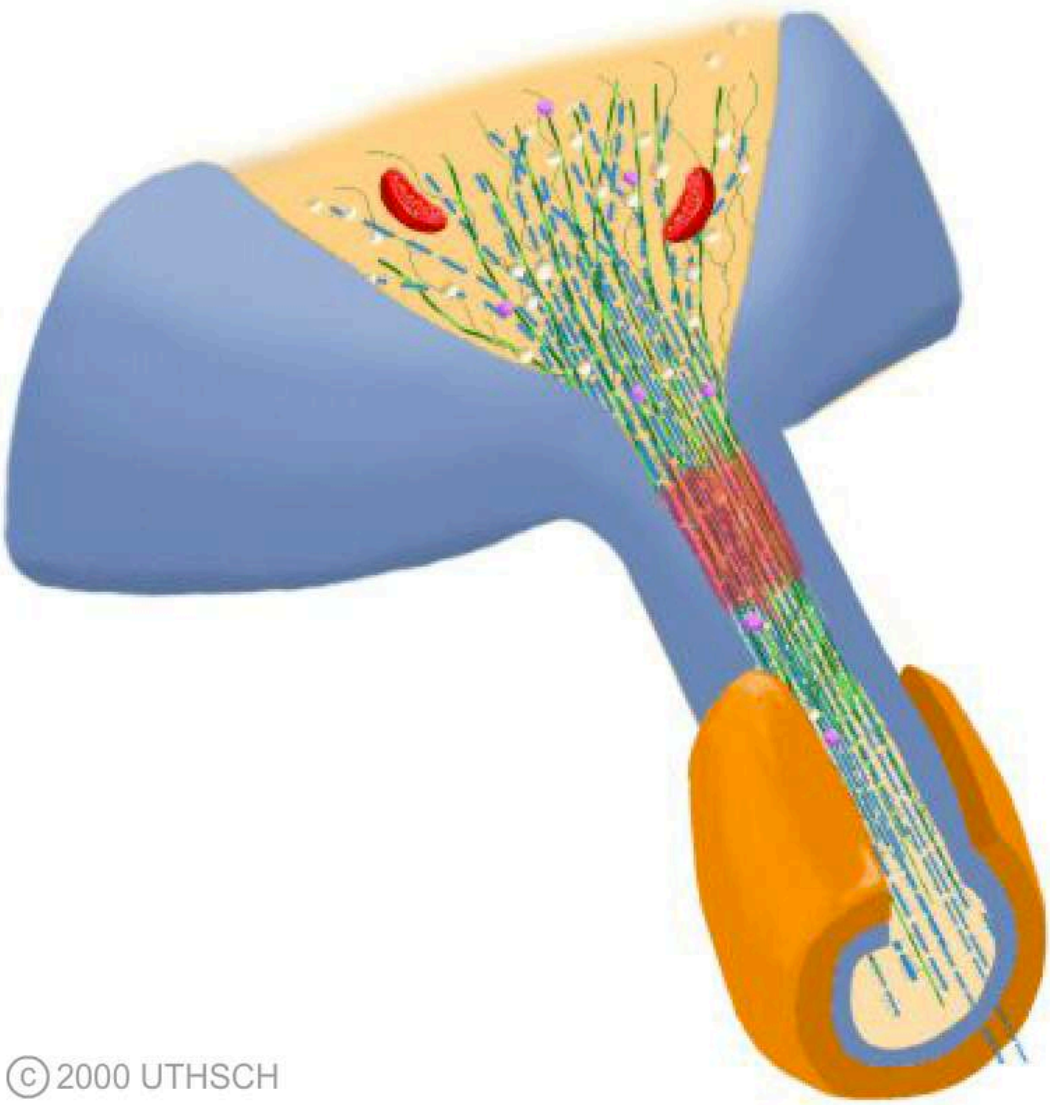
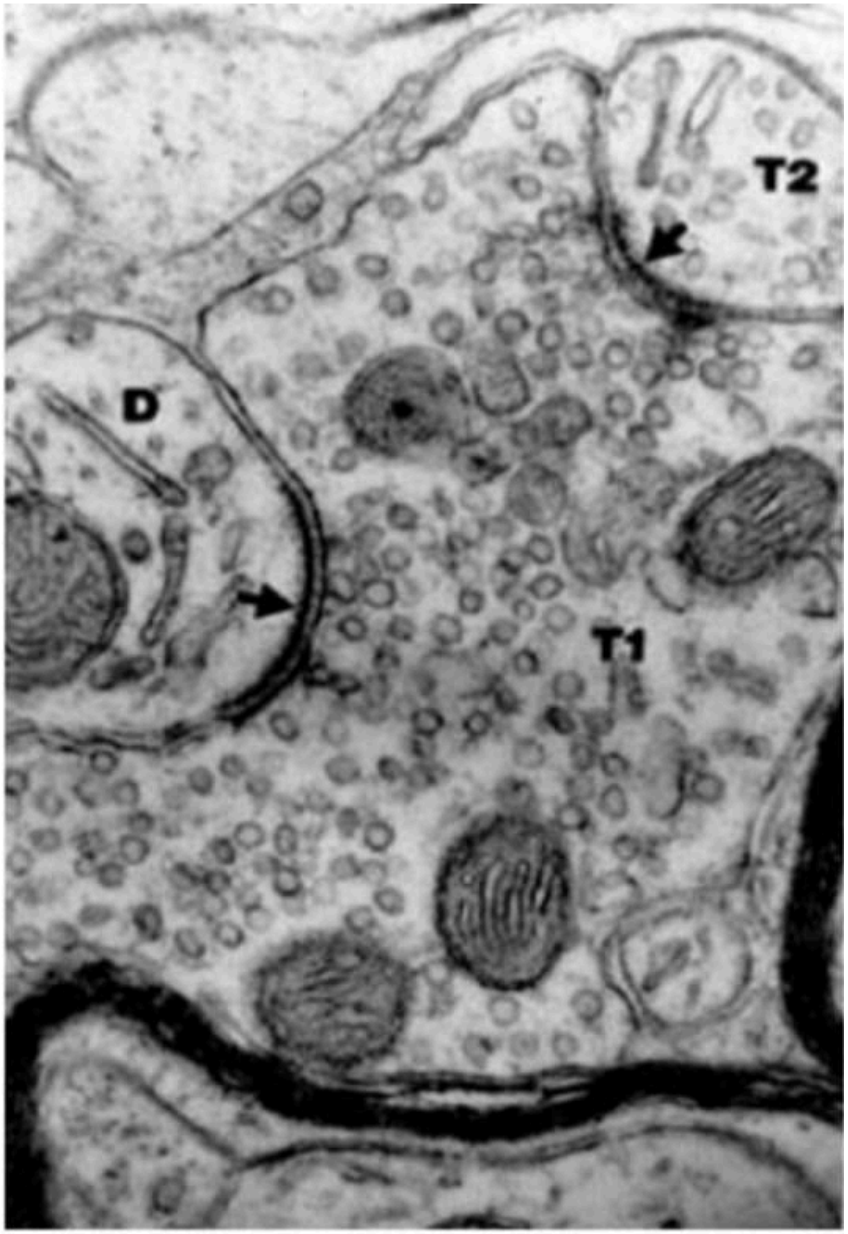
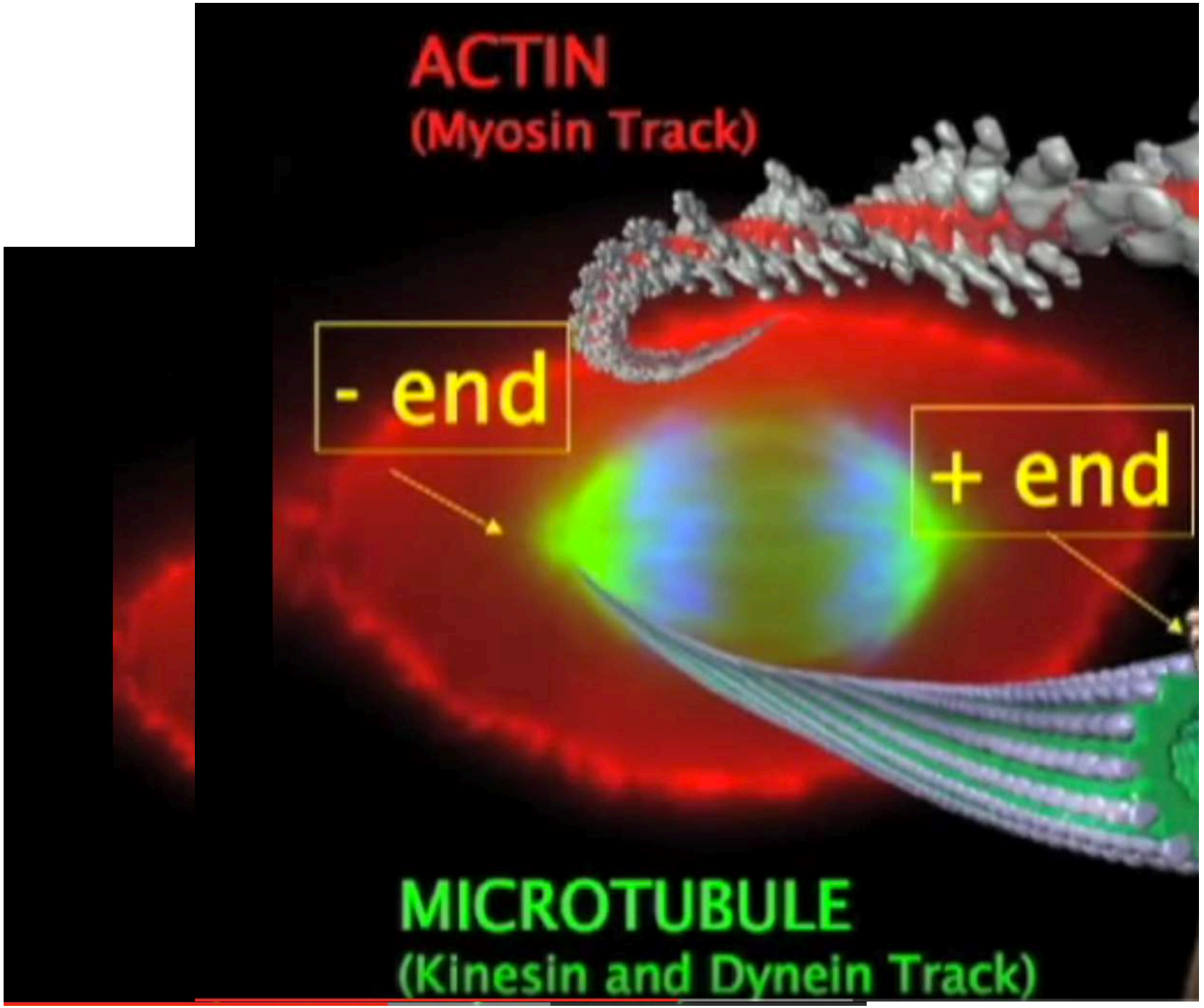
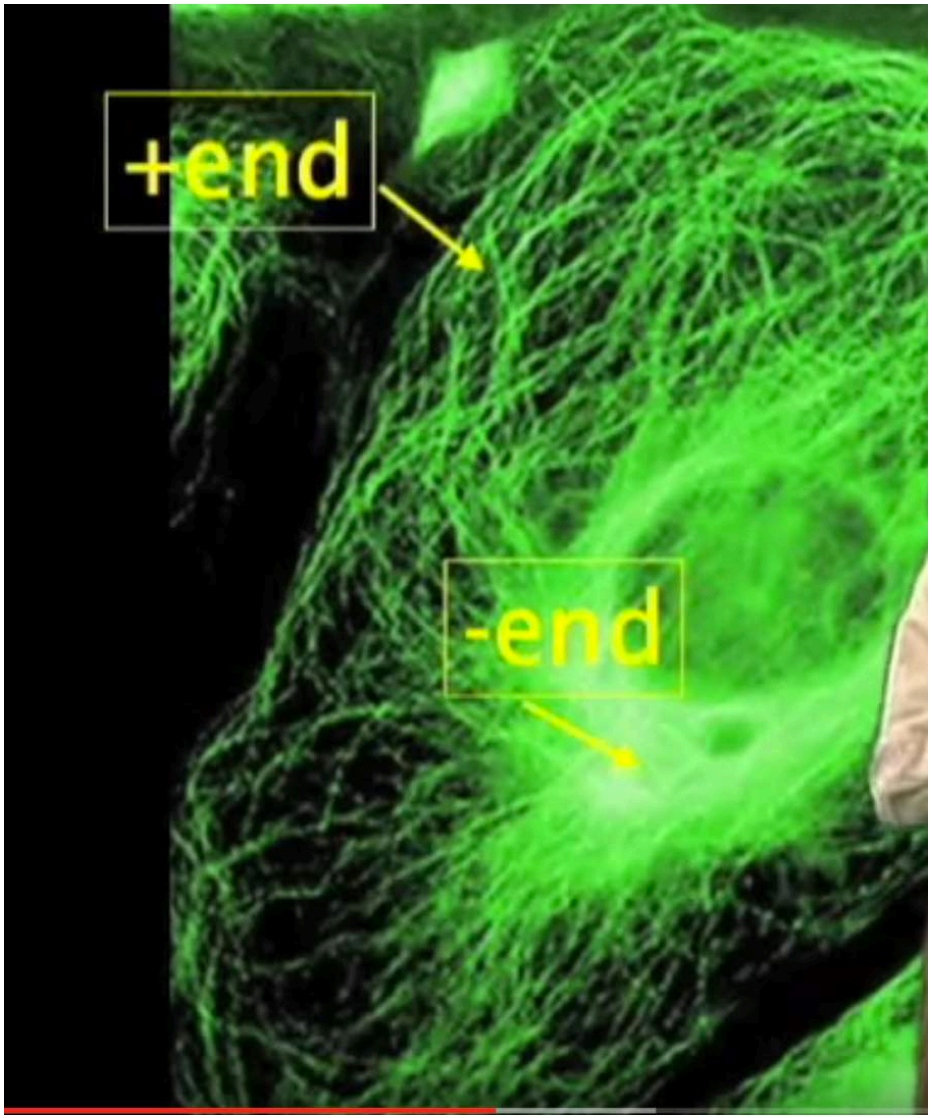


Figure 8.4 (See enlarged view)
Diagrammatic representation of the initial segment of a neuron, emphasizing the areas in which the action potential is initiated.

Синапс – электронная фотография

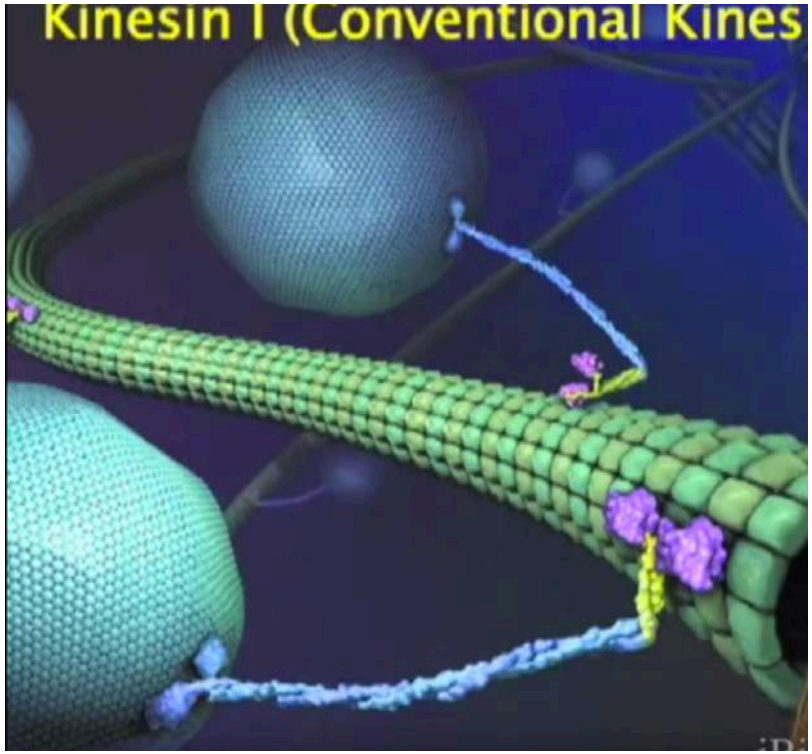


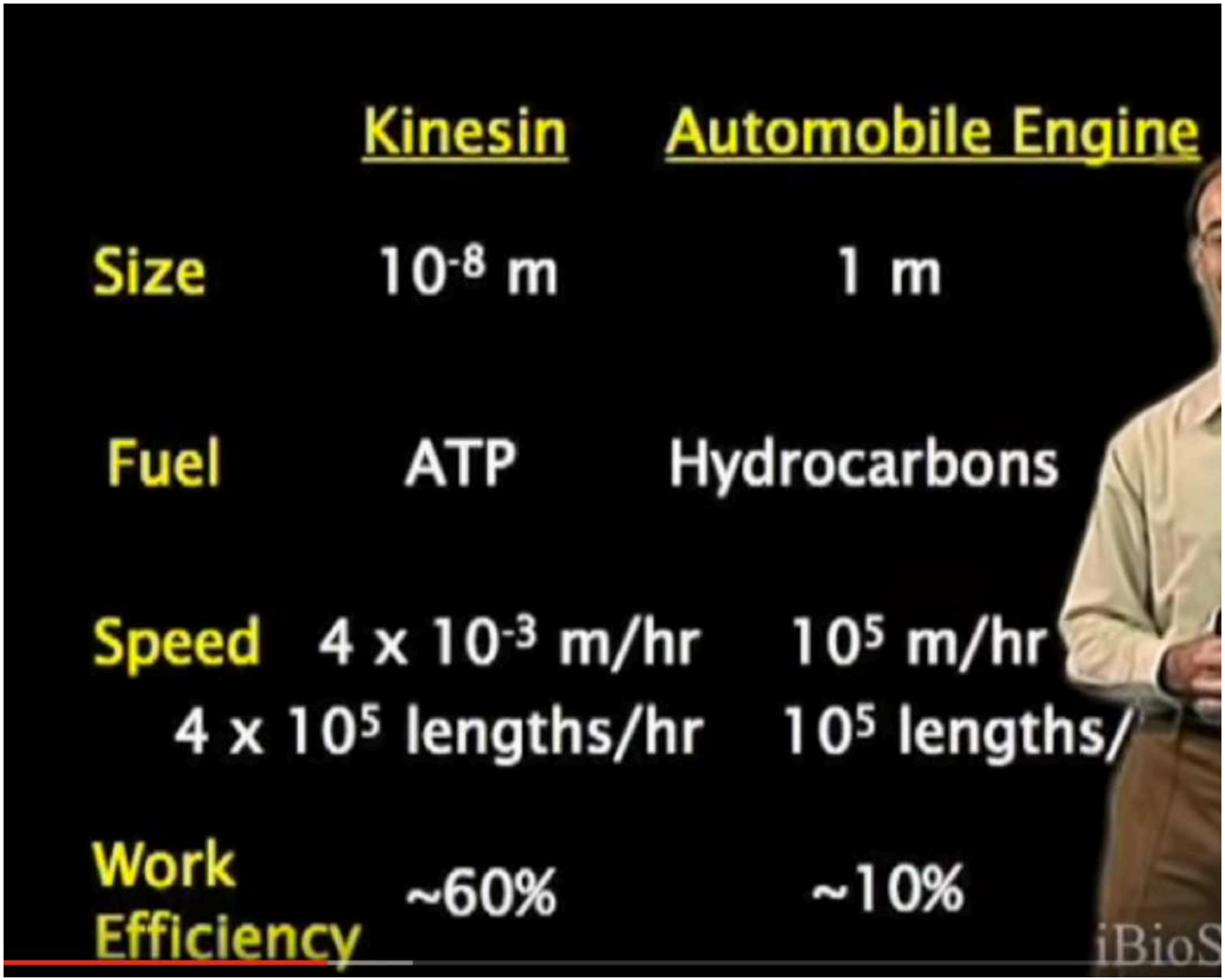

ACTIN
(Myosin Track)
- end
+ end
MICROTUBULE
(Kinesin and Dynein Track)



Моторные белки различают полярность и знают в каком направлении двигаться

Это – фантастически эффективная, сложная и надежно организованная система внутриклеточного транспорта


Kinesin I (Conventional Kines

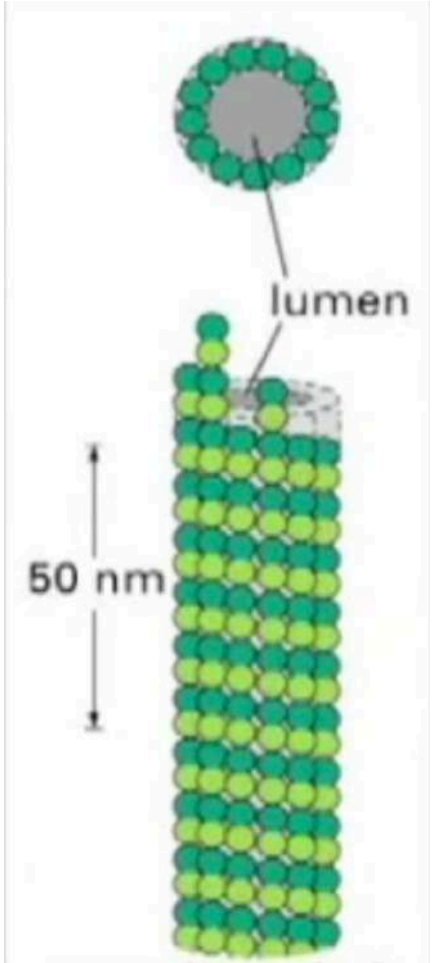

Kinesin
Automobile Engine
Size
10-8 m
1 m
Fuel
ATP
Hydrocarbons
Speed
4 x 10-3 m/hr
10⁵ m/hr
4 x 10⁵ lengths/hr
10⁵ lengths/
Work Efficiency
~60%
~10%

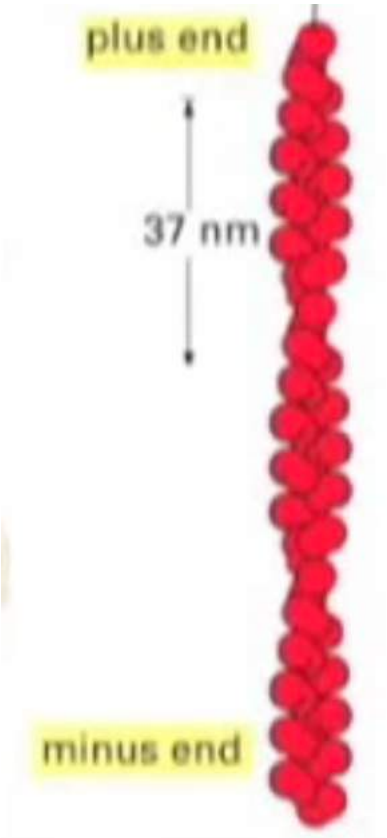
Relevance to medicine:

Transport defects can cause disease.

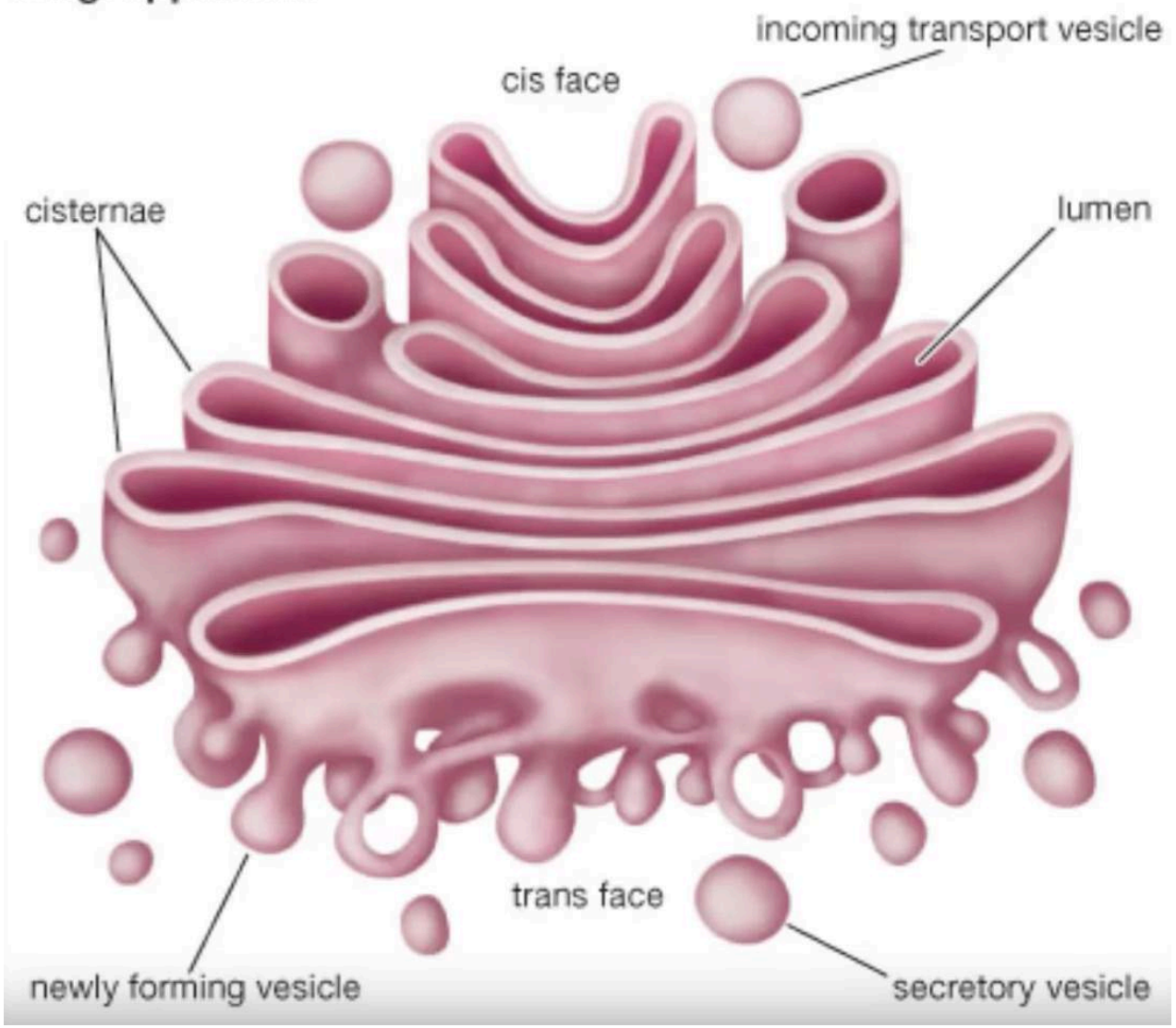
Inhibition or enhancement of motor protein activity may have therapeutic benefit.

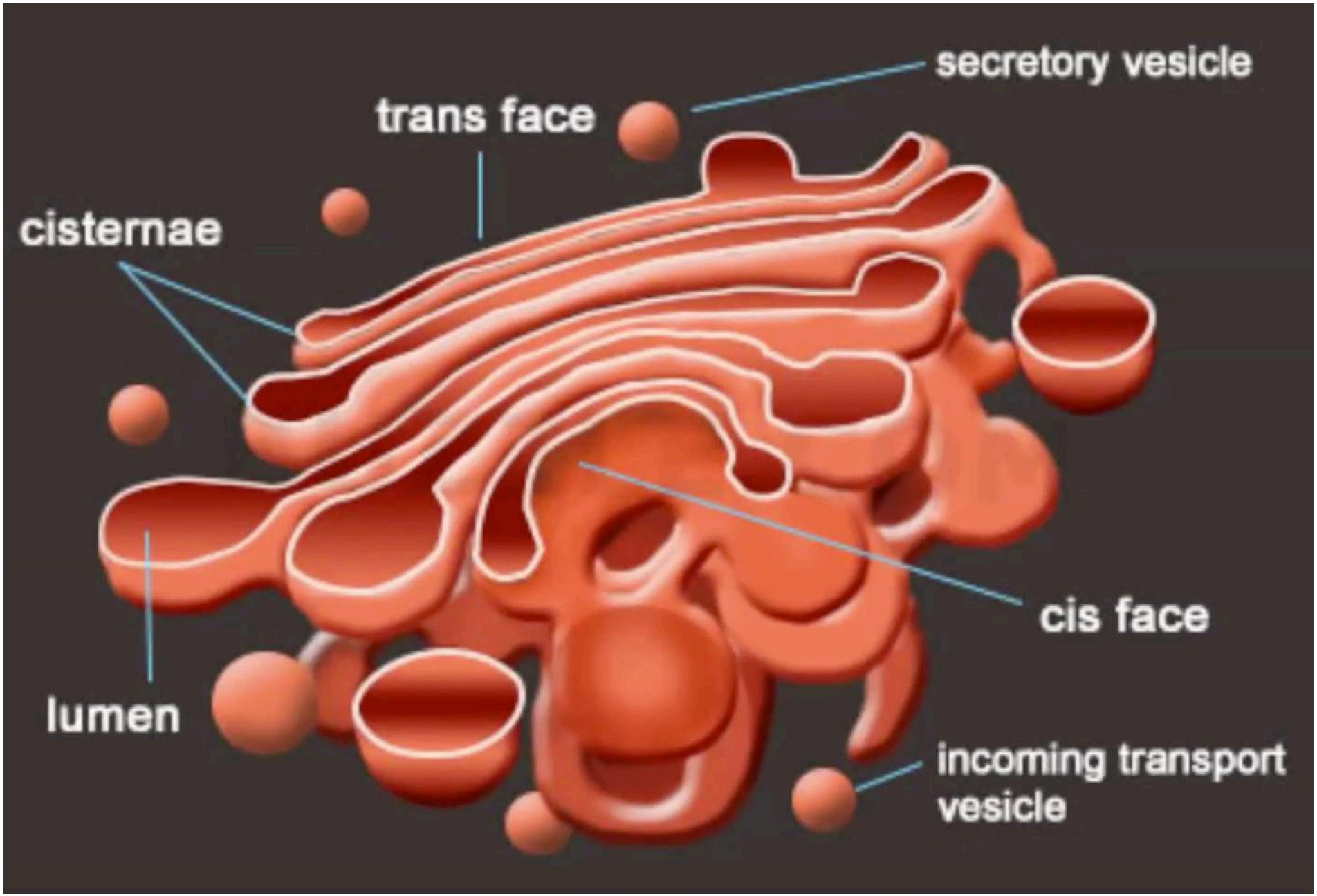
Ключевые компоненты цитоплазматического транспорта

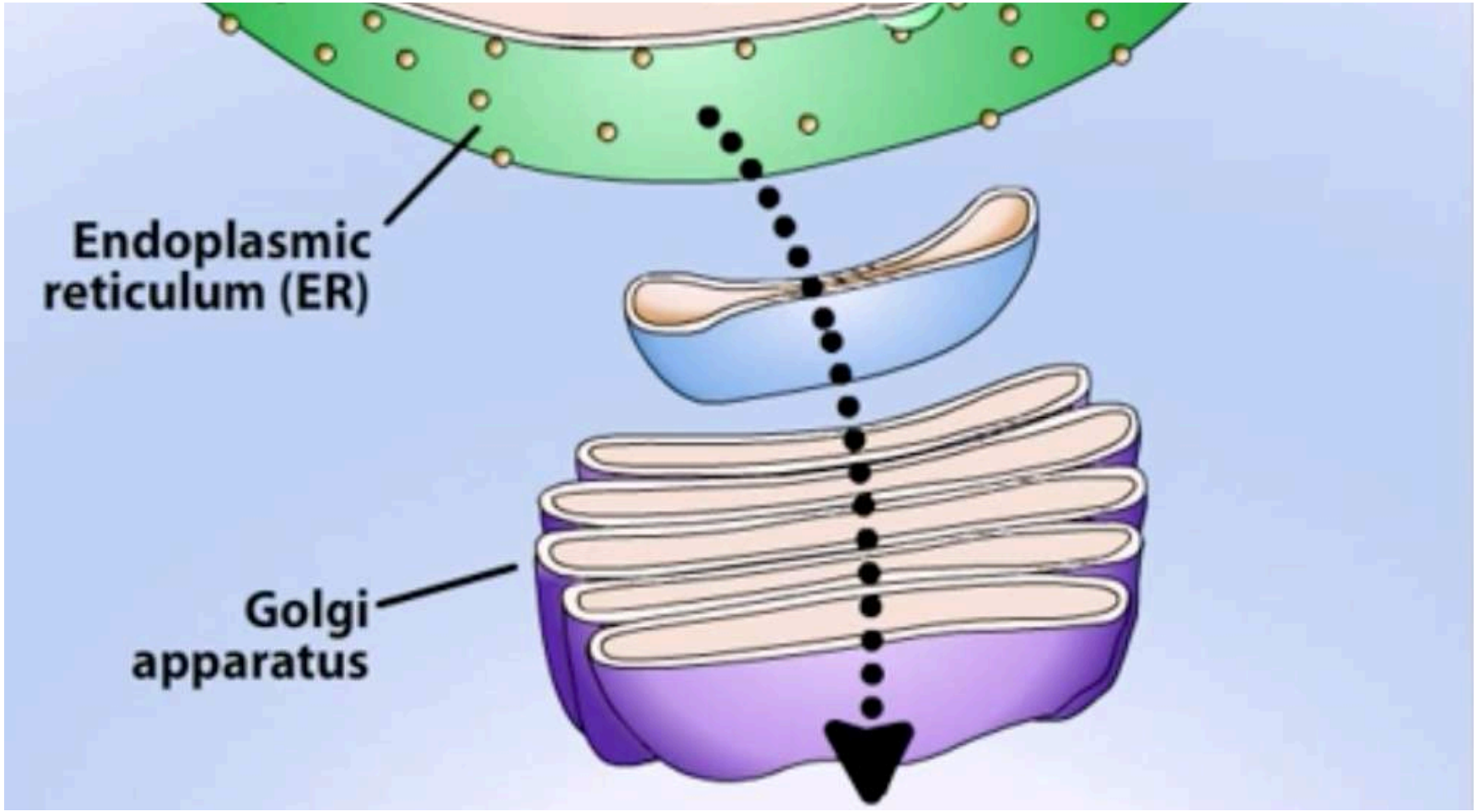


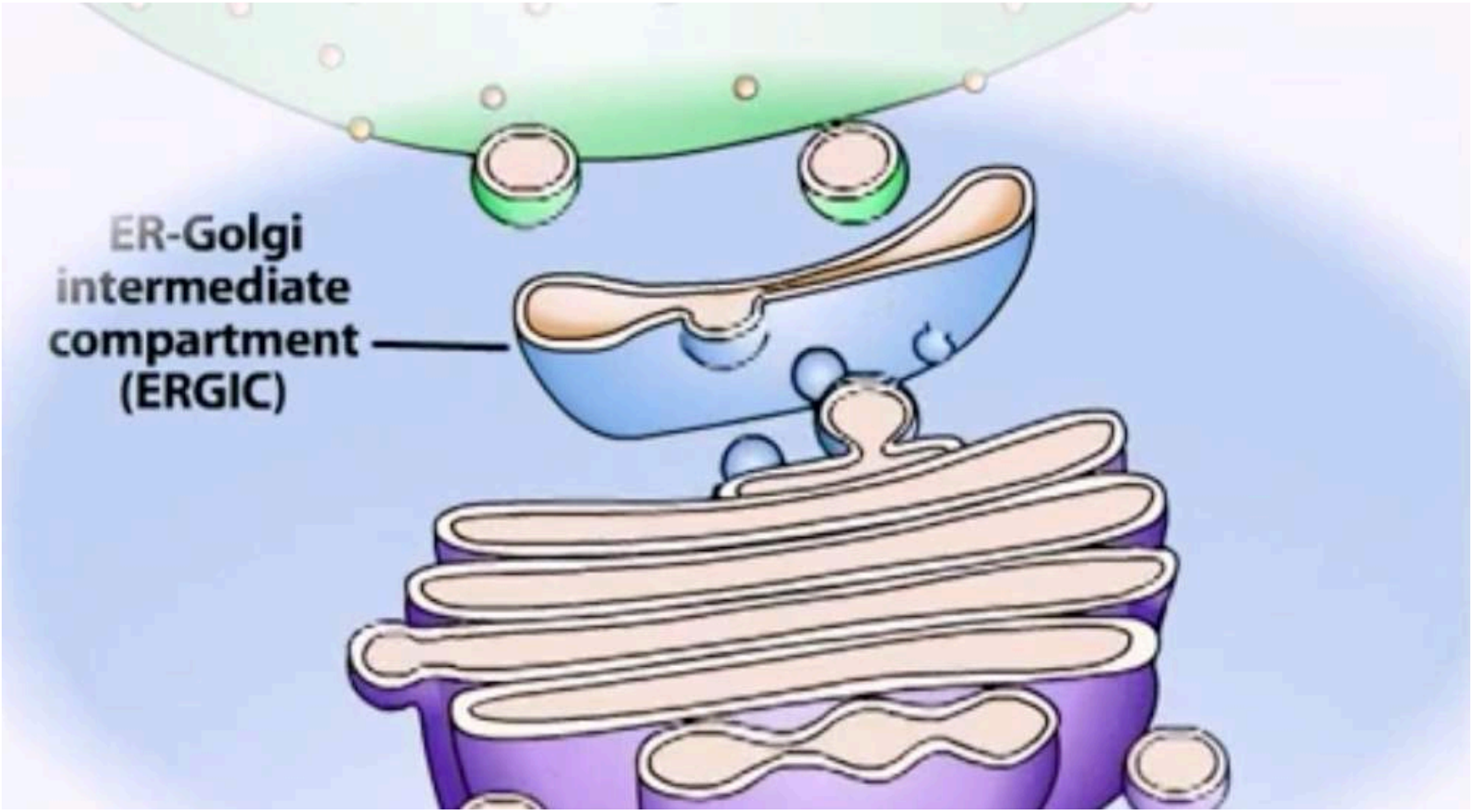


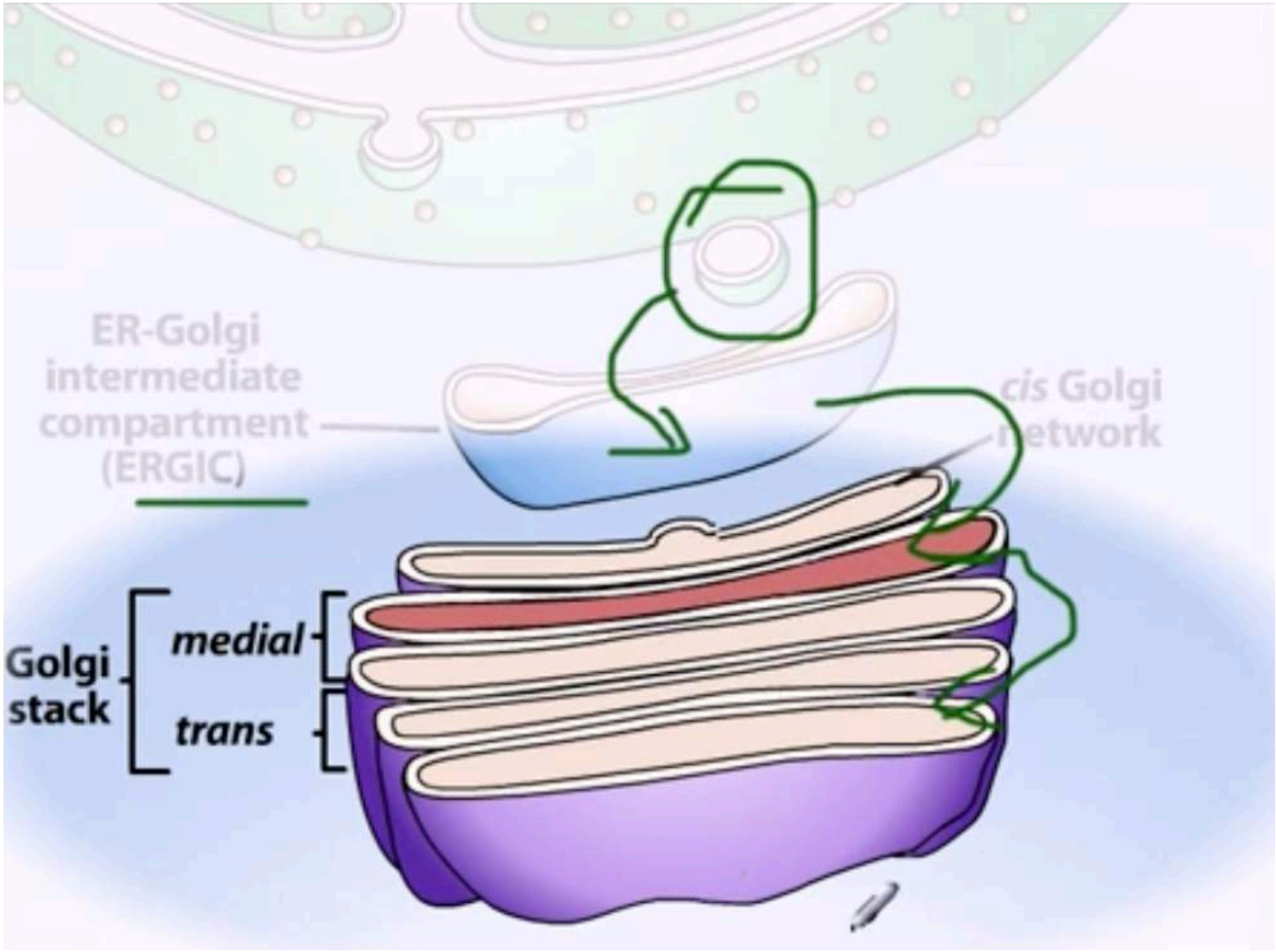
Golgi apparatus



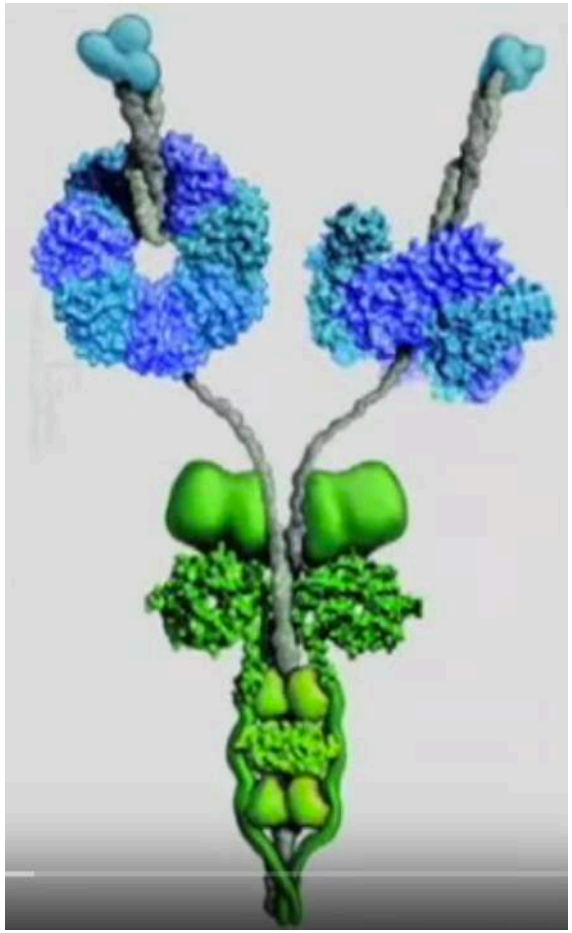

secretory vesicle
trans face
cisternae
cis face
lumen
incoming transport vesicle

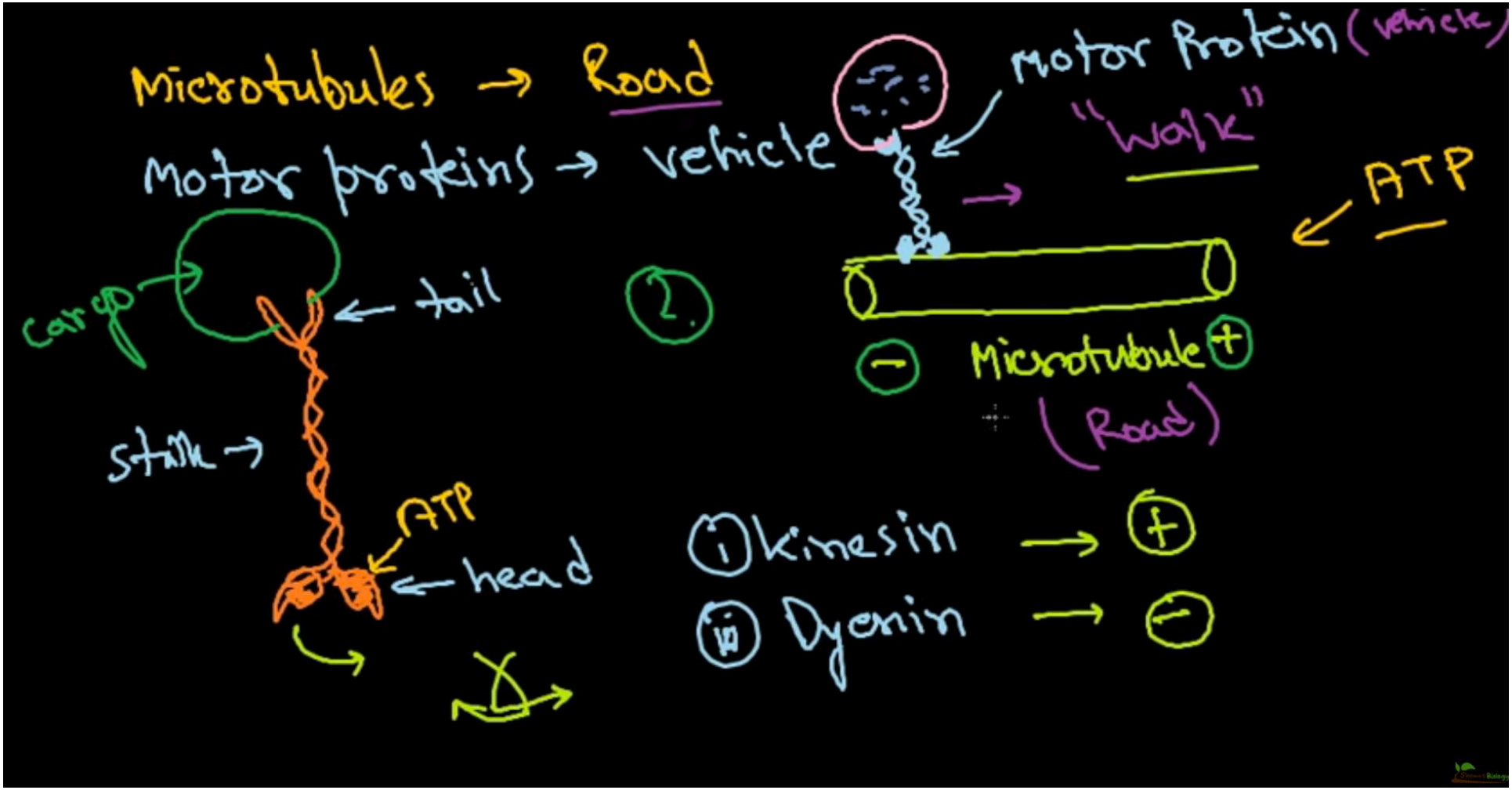

Endoplasmic
reticulum (ER)
Golgi
apparatus


ER-Golgi
intermediate
compartment
(ERGIC)


ER-Golgi
intermediate
compartment
(ERGIC)
cis Golgi
network
Golgi
stack
medial
trans

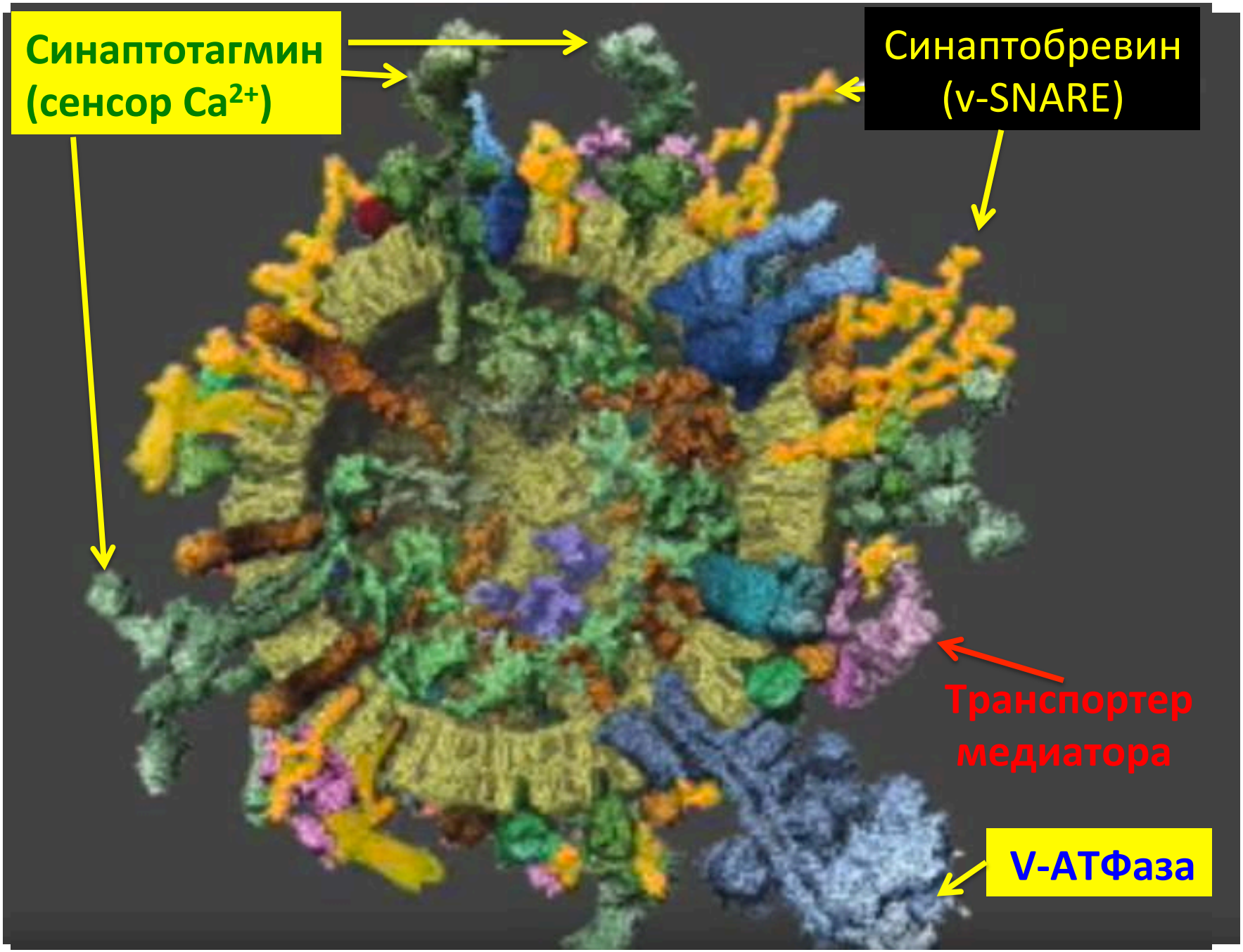
Динеин – моторный белок ретроградного транспорта

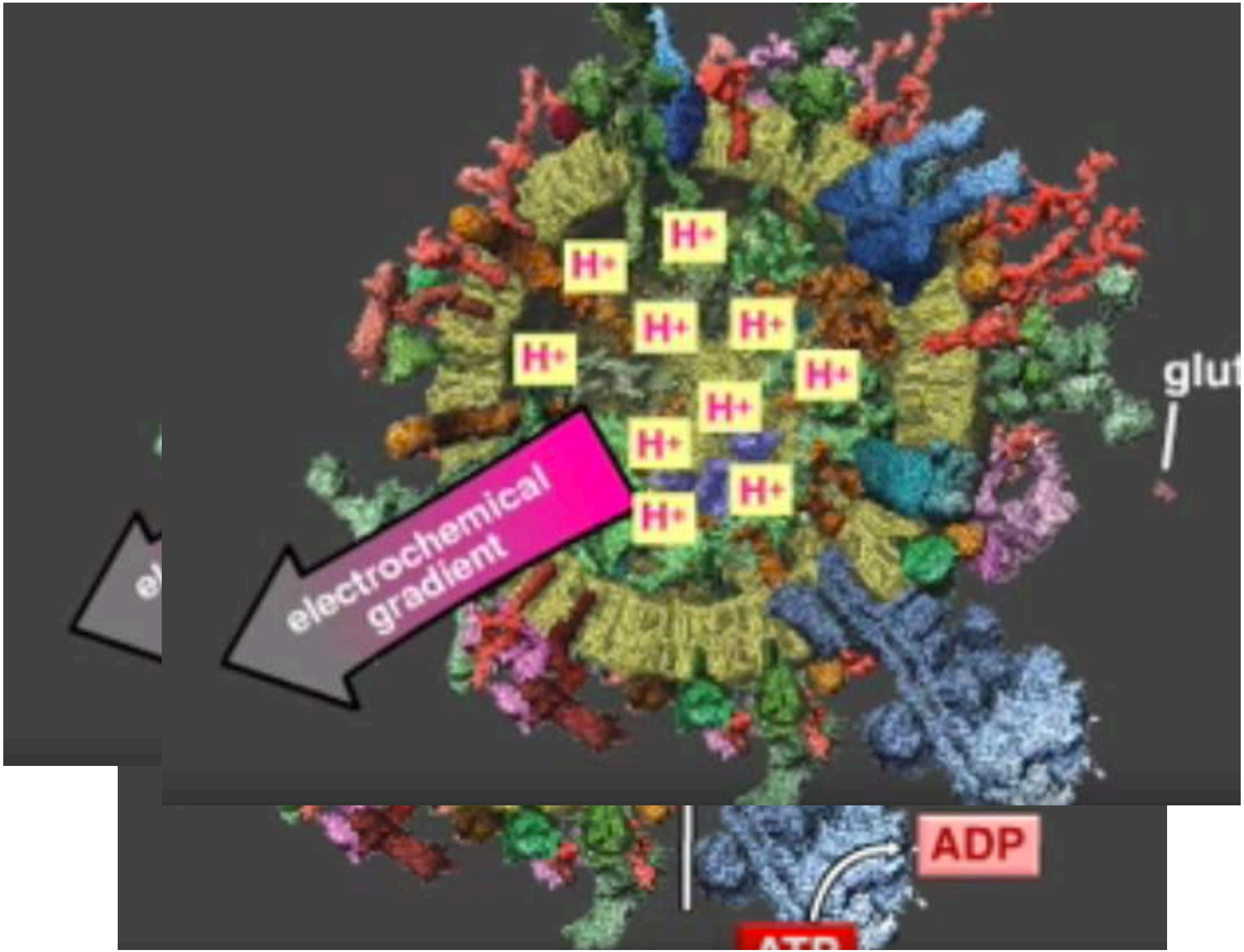


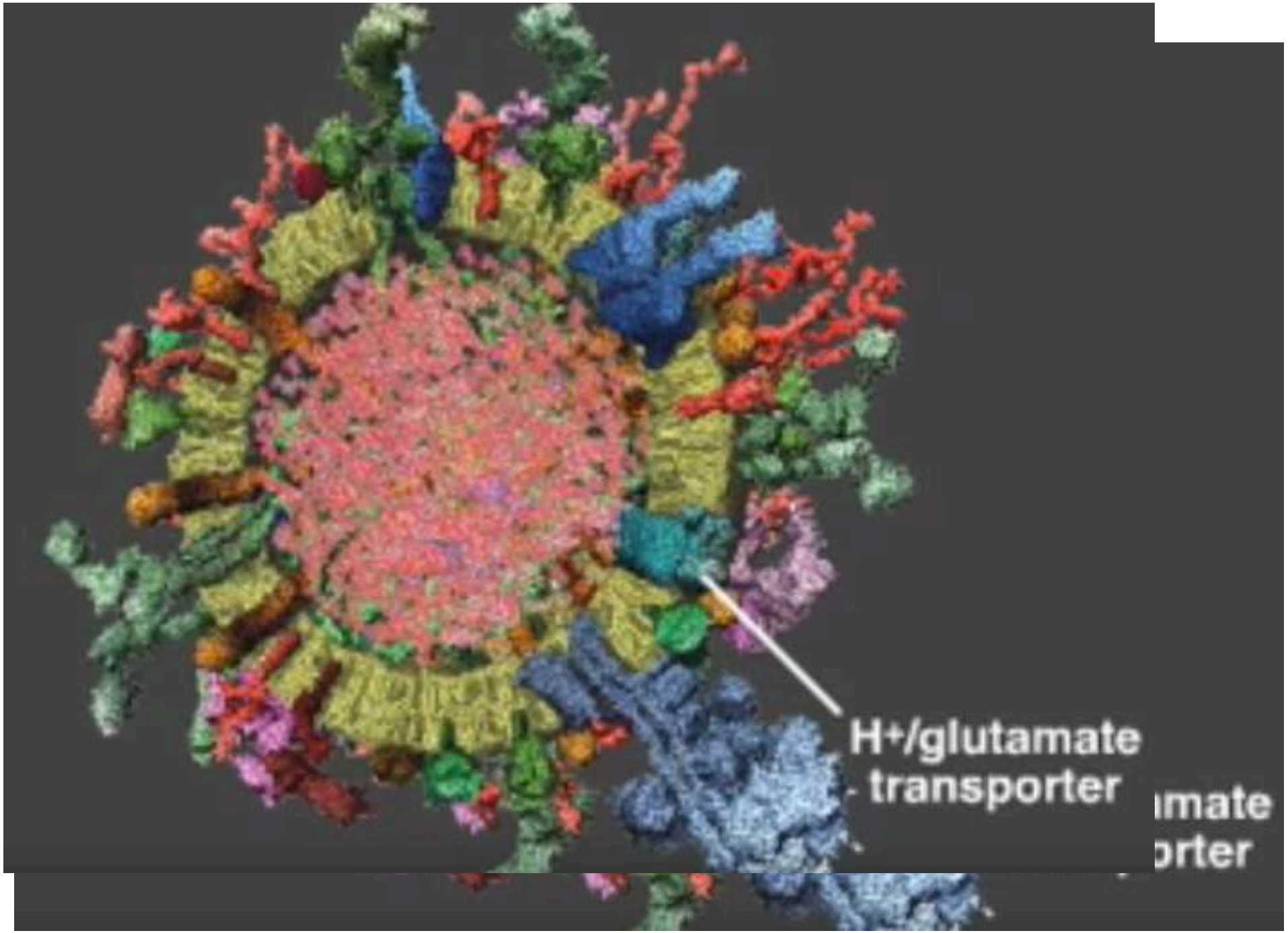

Microtubules → Road
Motor proteins → vehicle
motor Protein (vehicle)
"walk"
ATP
cargo
tail
stalk →
ATP
head
Microtubule
(Road)
(i) kinesin → +
(ii) Dynein → −

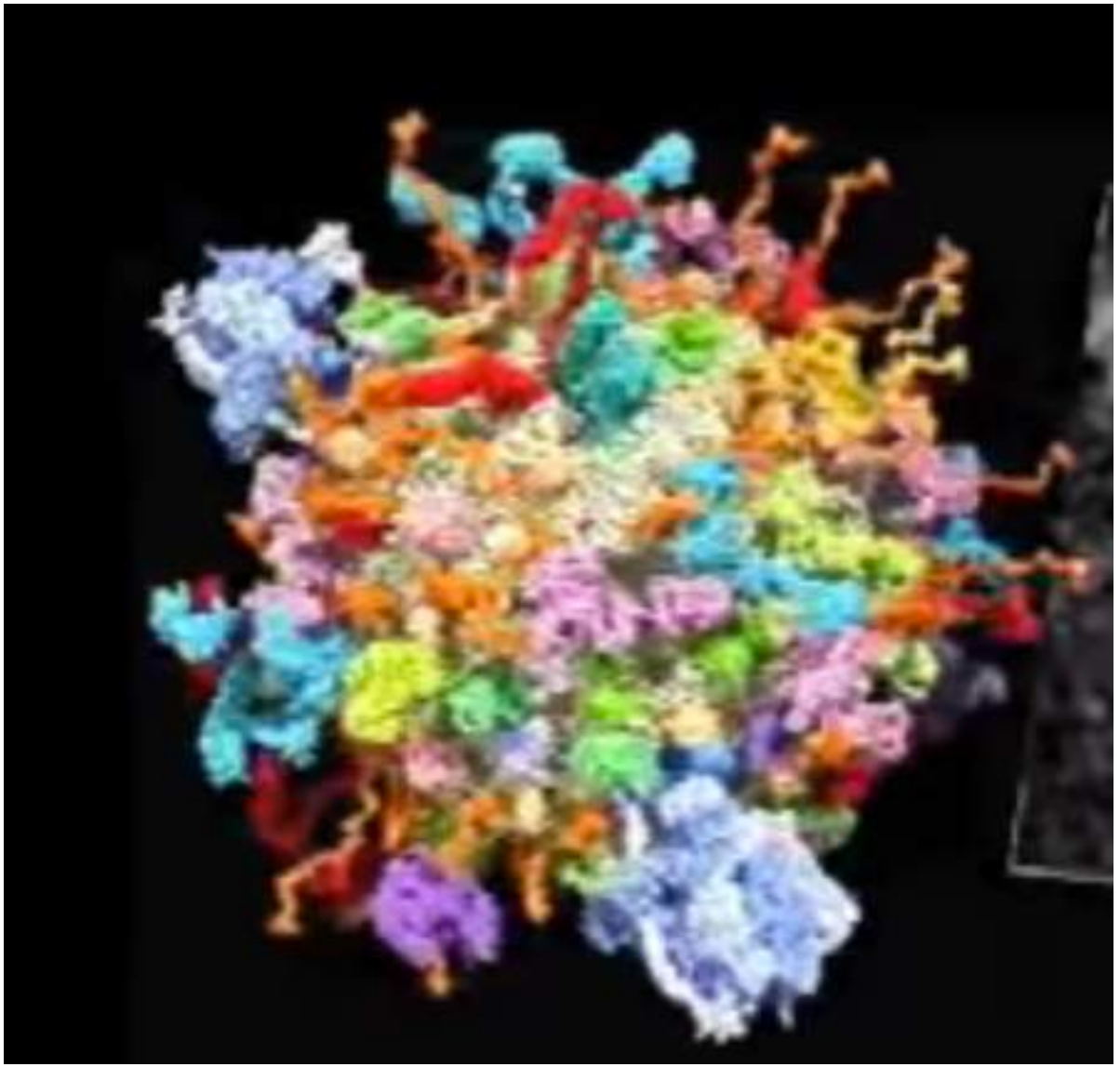
Функции кинезинов

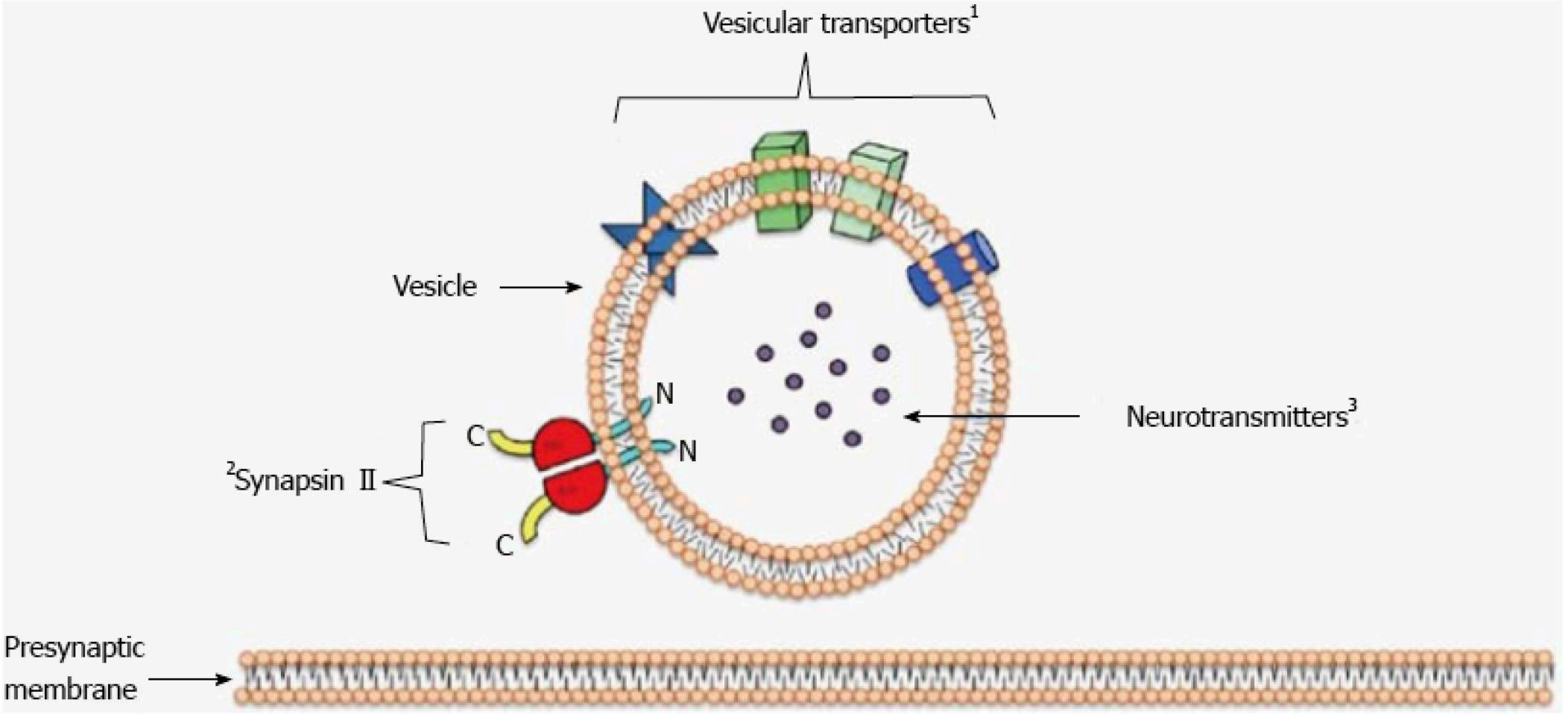
- Транспорт органелл
- Транспорт РНК и белков
- Сборка жгутиков и ресничек
- Формирование веретен деления при митозе и движение хромосом

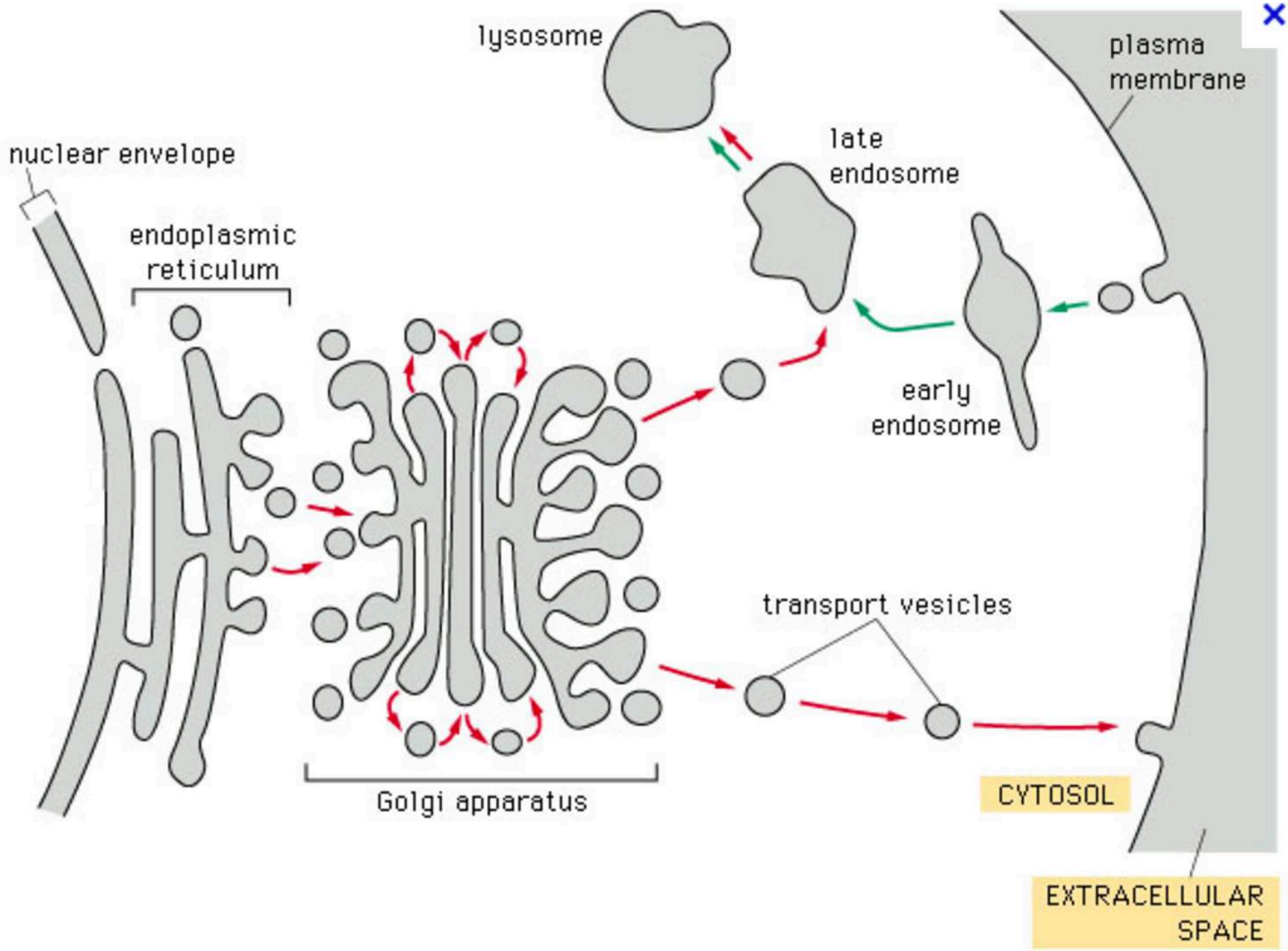

Синаптотагмин
(сенсор Ca^{2+})
Синаптобревин
(v-SNARE)
Транспортер
медиатора
V-АТФаза


H+
H+
H+
H+
H+
H+
H+
H+
H+
H+
electrochemical gradient
glut
ADP
ATP

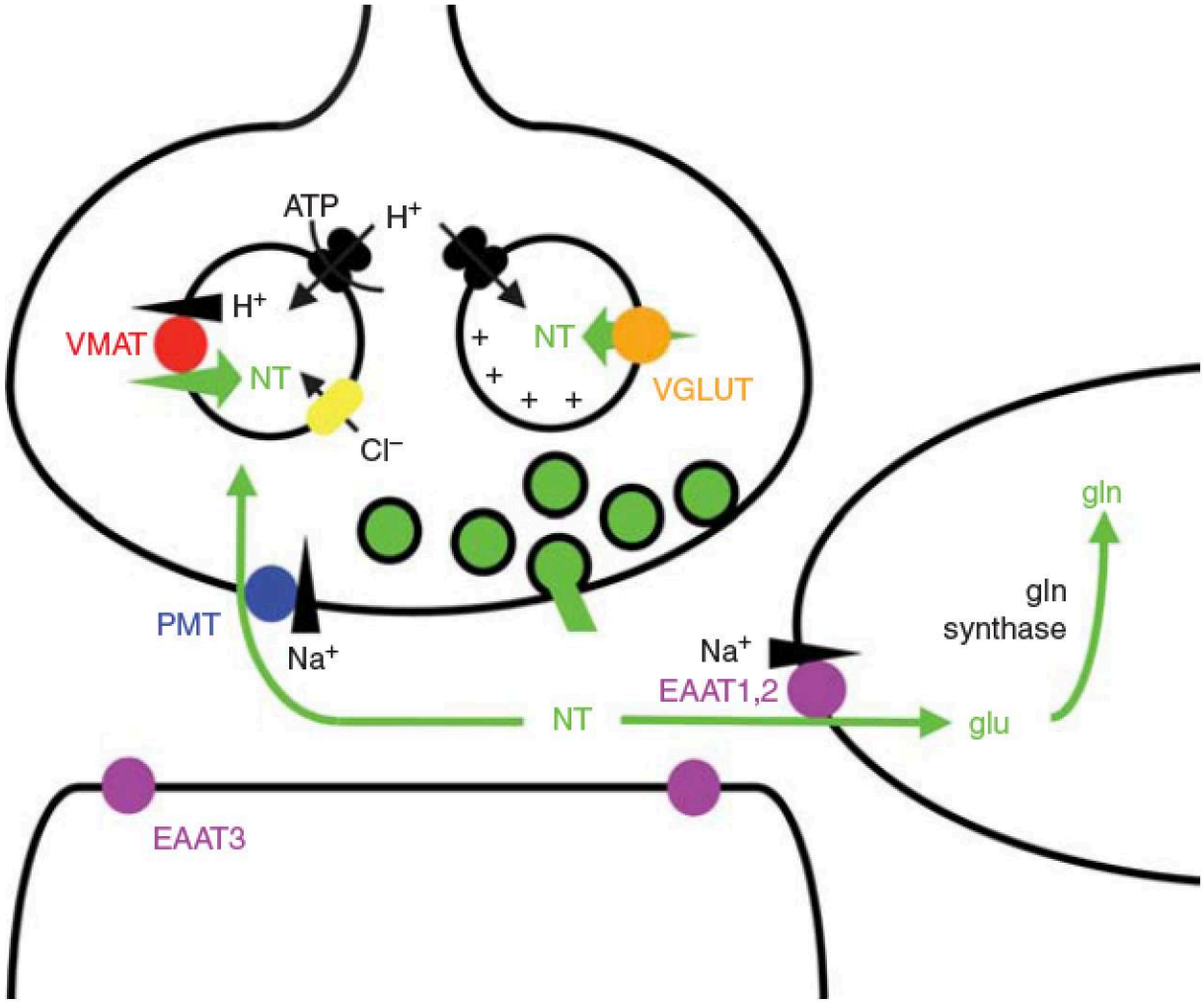

H+/glutamate
transporter



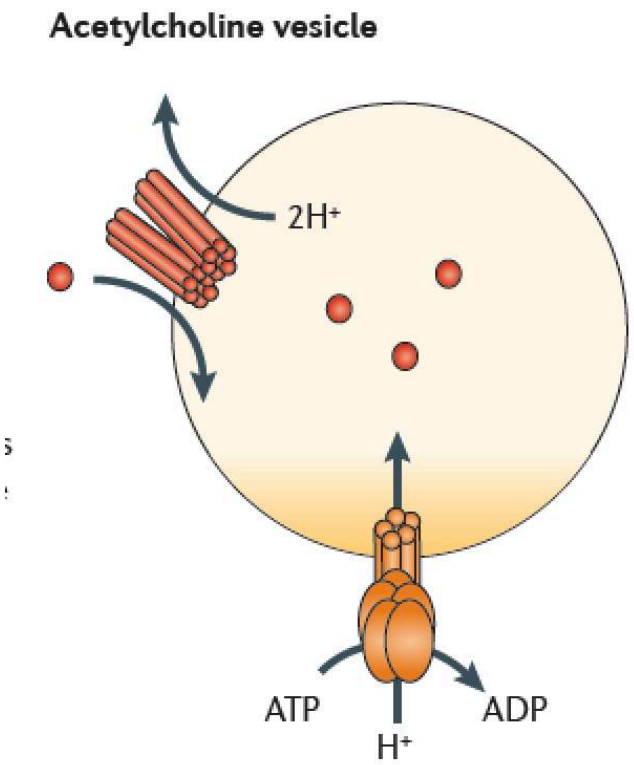

Vesicular transporters[1]
Vesicle
N
N
C
C
[2]Synapsin II
Neurotransmitters[3]
Presynaptic membrane

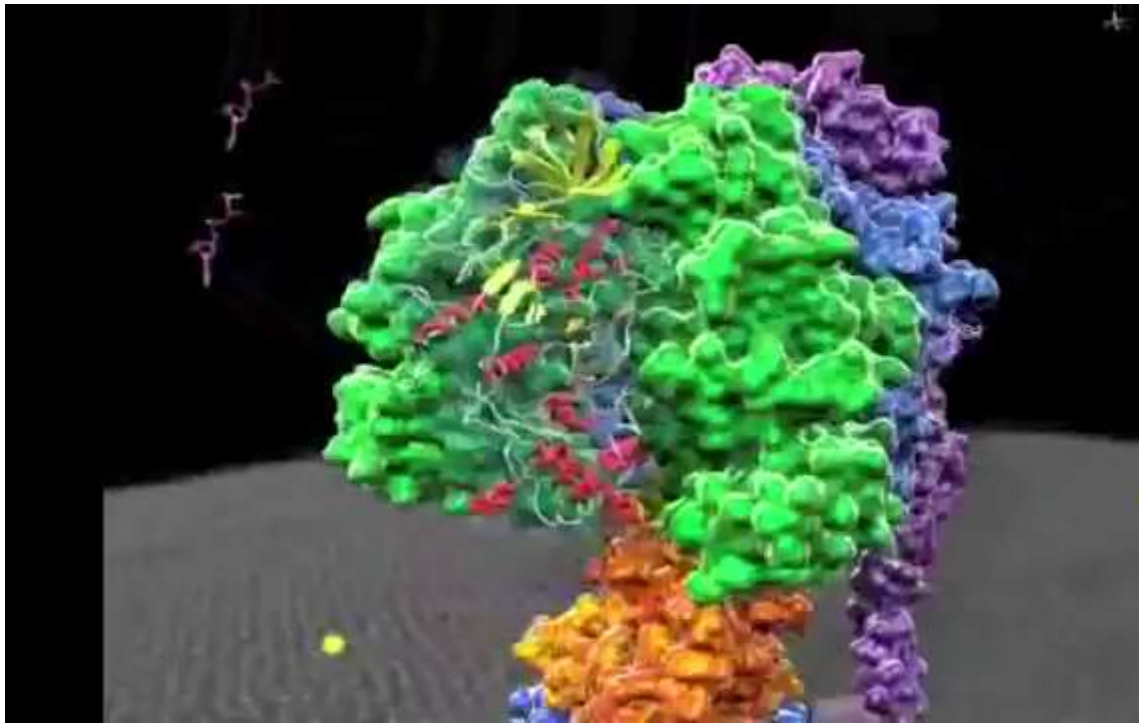

lysosome
plasma membrane
late endosome
nuclear envelope
endoplasmic reticulum
early endosome
transport vesicles
Golgi apparatus
CYTOSOL
EXTRACELLULAR SPACE

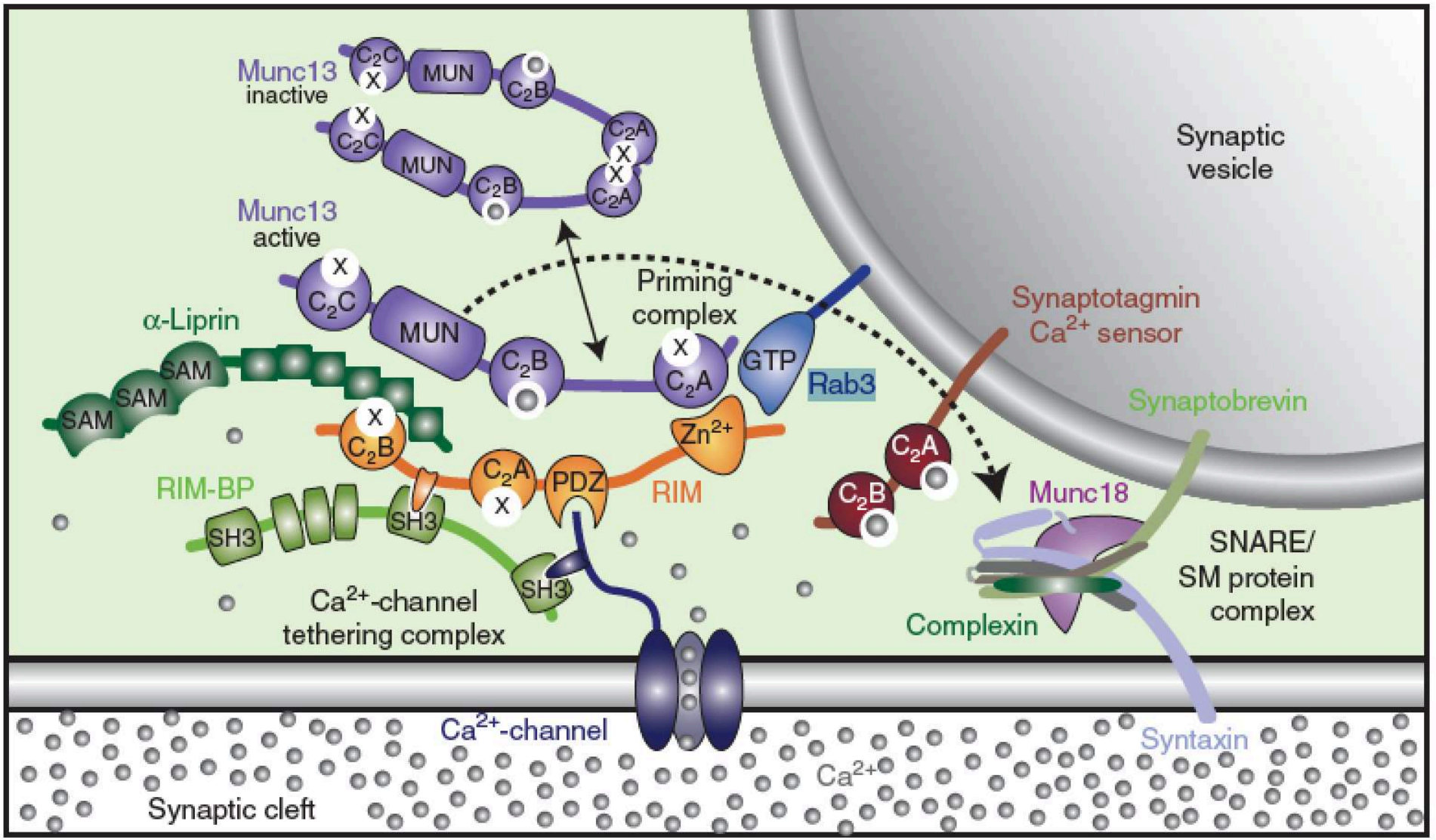
Транспортеры нейромедиаторов

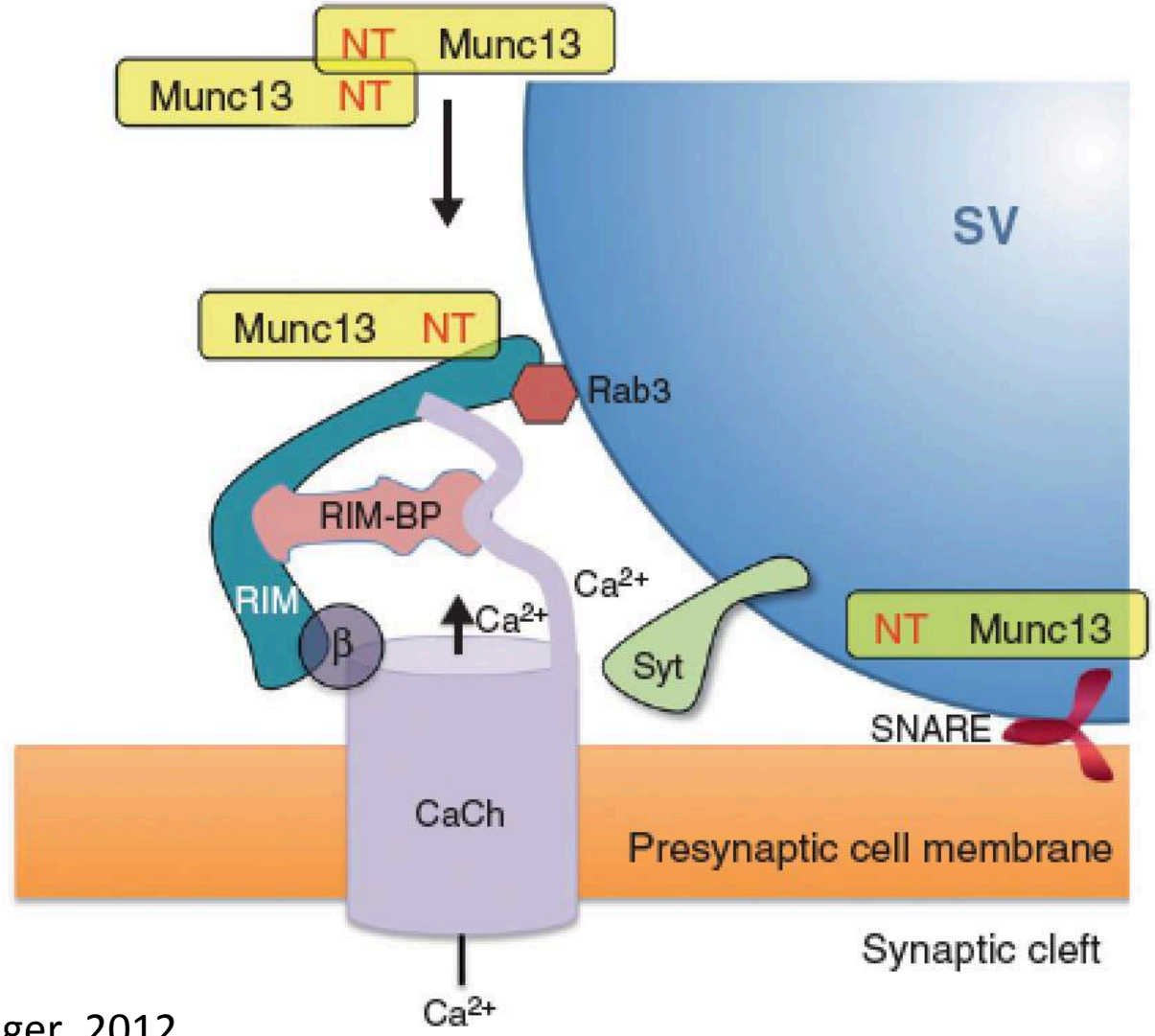


Транспортеры нейромедиаторов





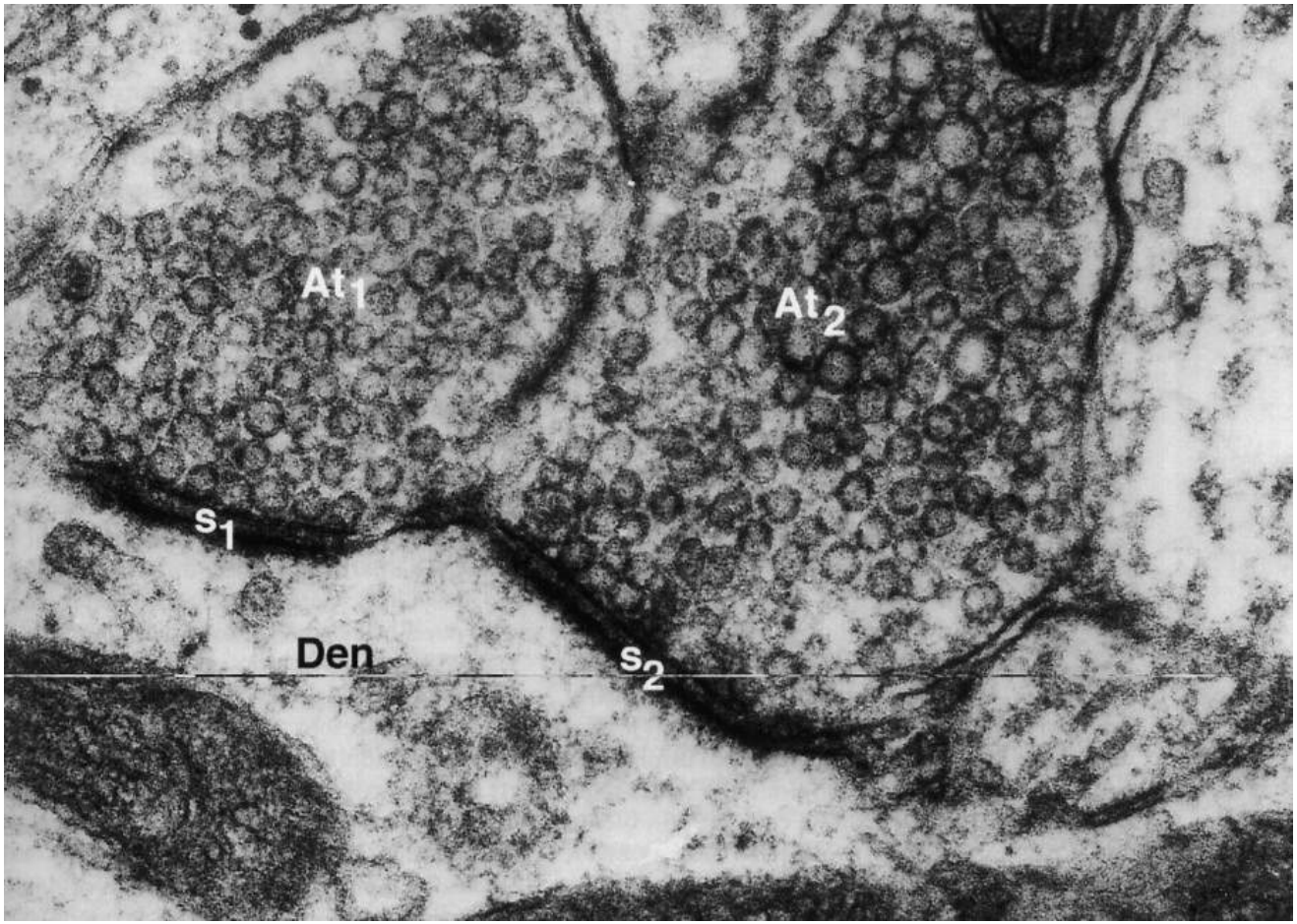

Munc13
inactive
C2C
MUN
C2B
C2A
Munc13
active
Priming
complex
α-Liprin
SAM
SAM
SAM
GTP
Rab3
Synaptic
vesicle
Synaptotagmin
Ca2+ sensor
Synaptobrevin
Zn2+
PDZ
RIM
RIM-BP
SH3
SH3
SH3
Munc18
SNARE/
SM protein
complex
Complexin
Ca2+-channel
tethering complex
Ca2+-channel
Ca2+
Syntaxin
Synaptic cleft



Gundelfinger, 2012

Designer signals

- Преимущество использования Природой нейропередатчиков в том, что обеспечивается высокая **специфичность** работы синапсов
- Нейромедиаторы могут контролировать **возбуждение** и **торможение**
- Много лекарственных препаратов нервный расстройств действуют на синаптические белки



Dept of Biology, Saint Louis University