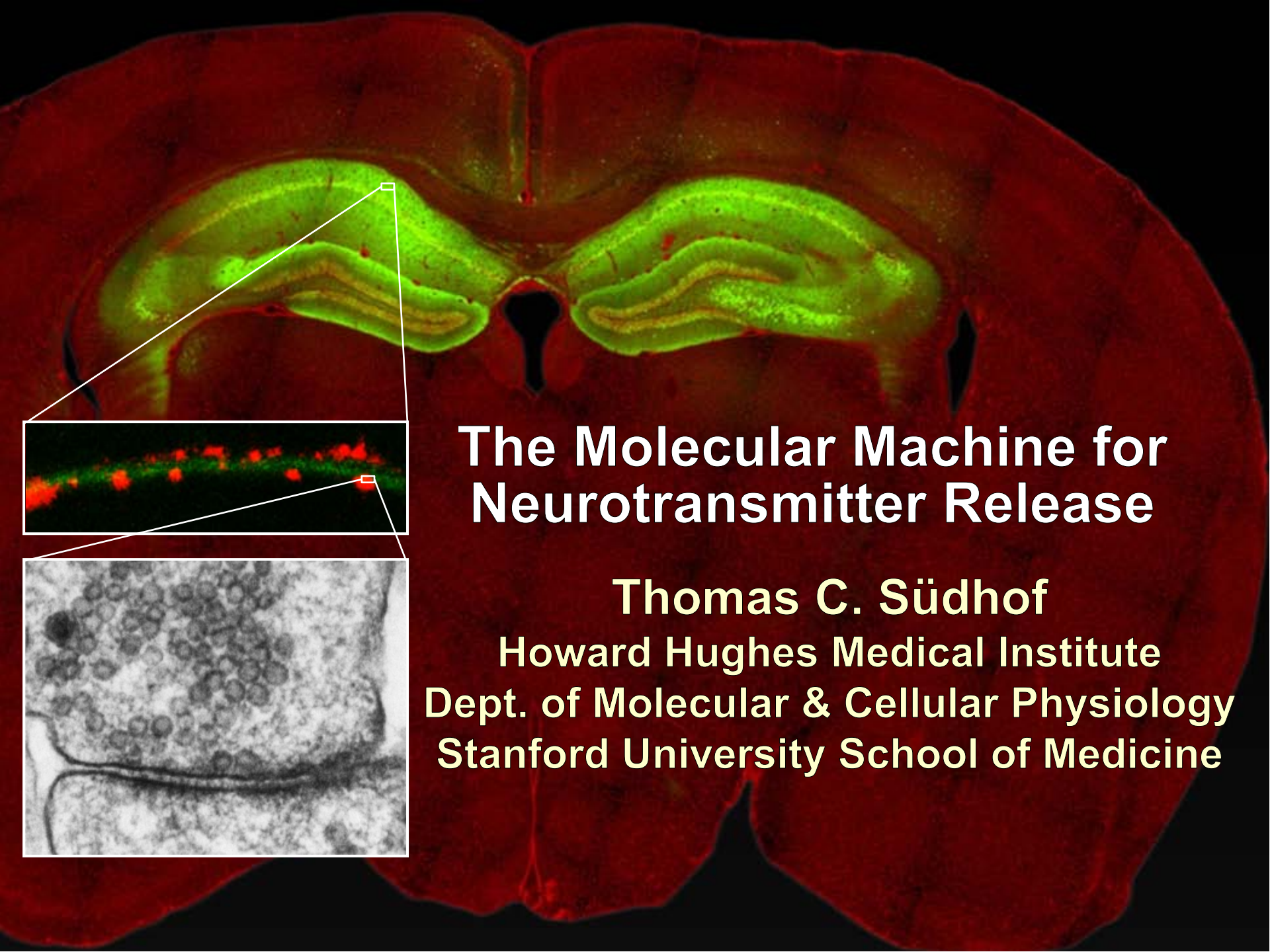


The Molecular Machine for Neurotransmitter Release

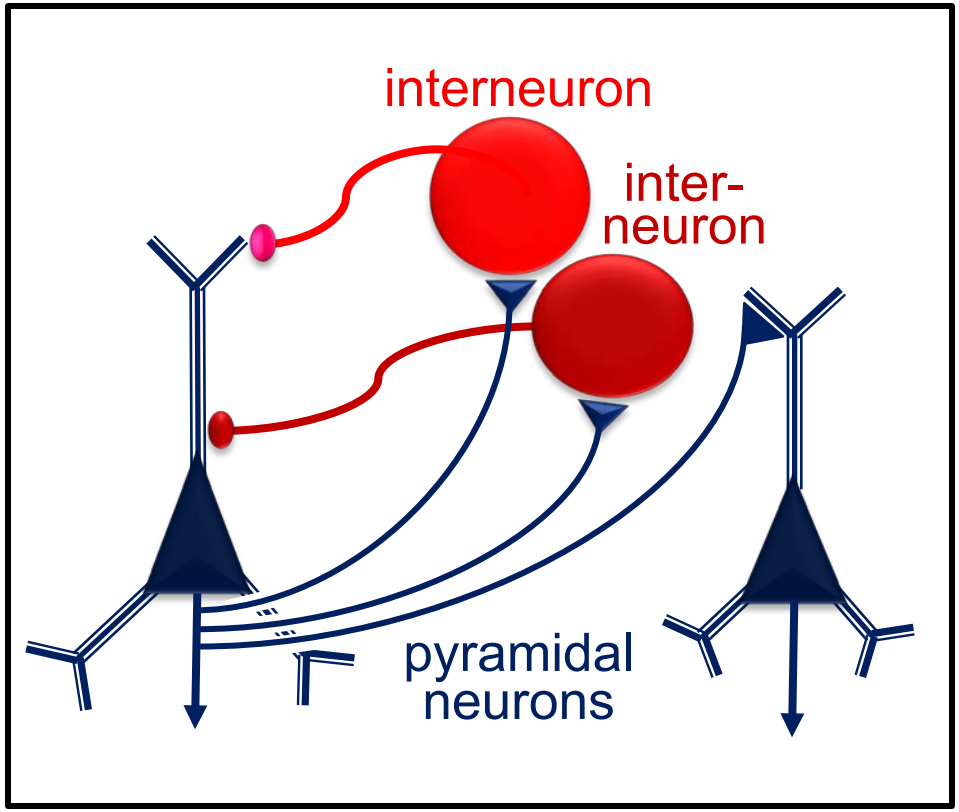
Thomas C. Südhof

Howard Hughes Medical Institute

Dept. of Molecular & Cellular Physiology

Stanford University School of Medicine

Neural Circuits Underlie Brain Function



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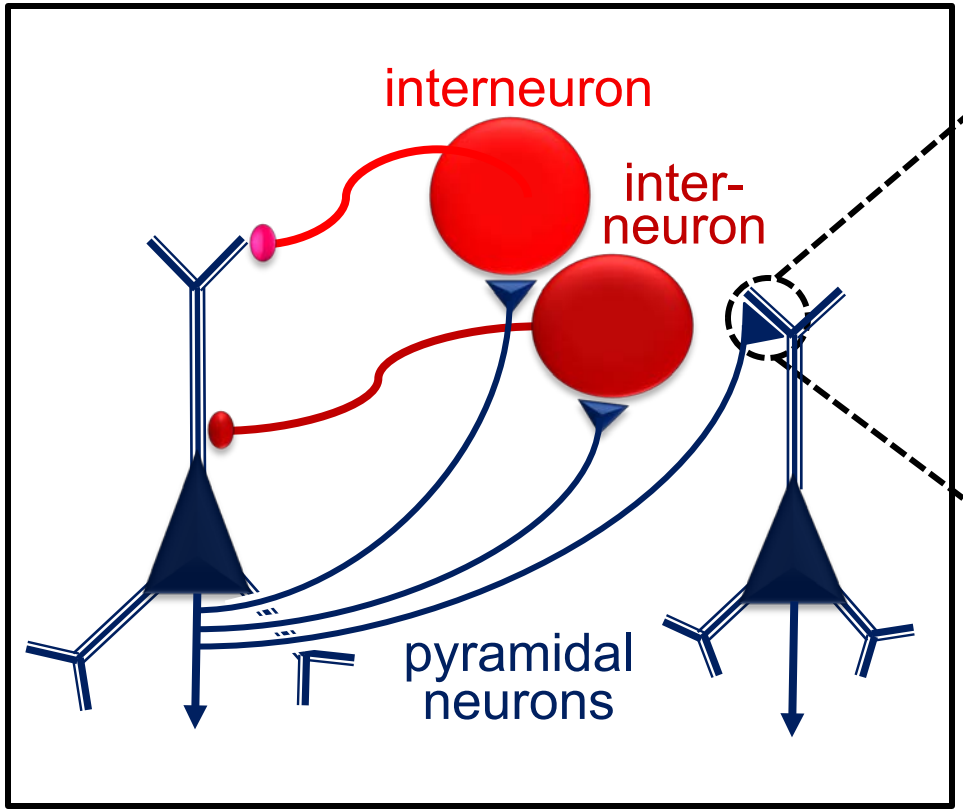

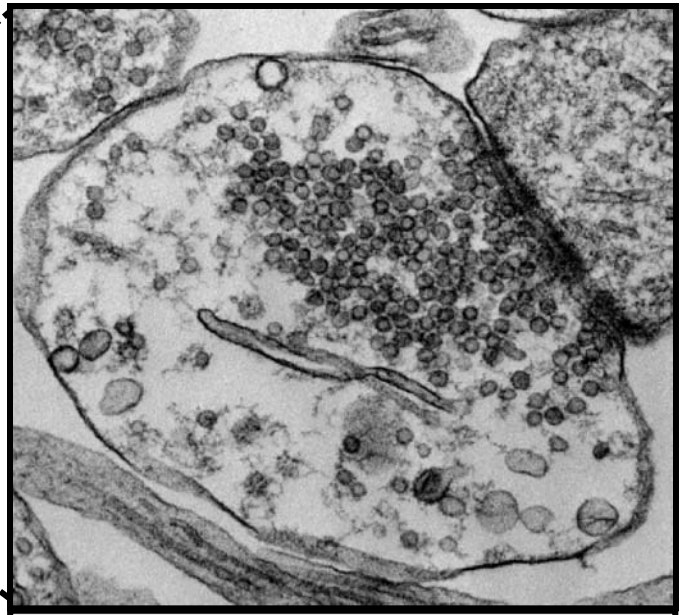

Synapses: the basic computational units of the brain

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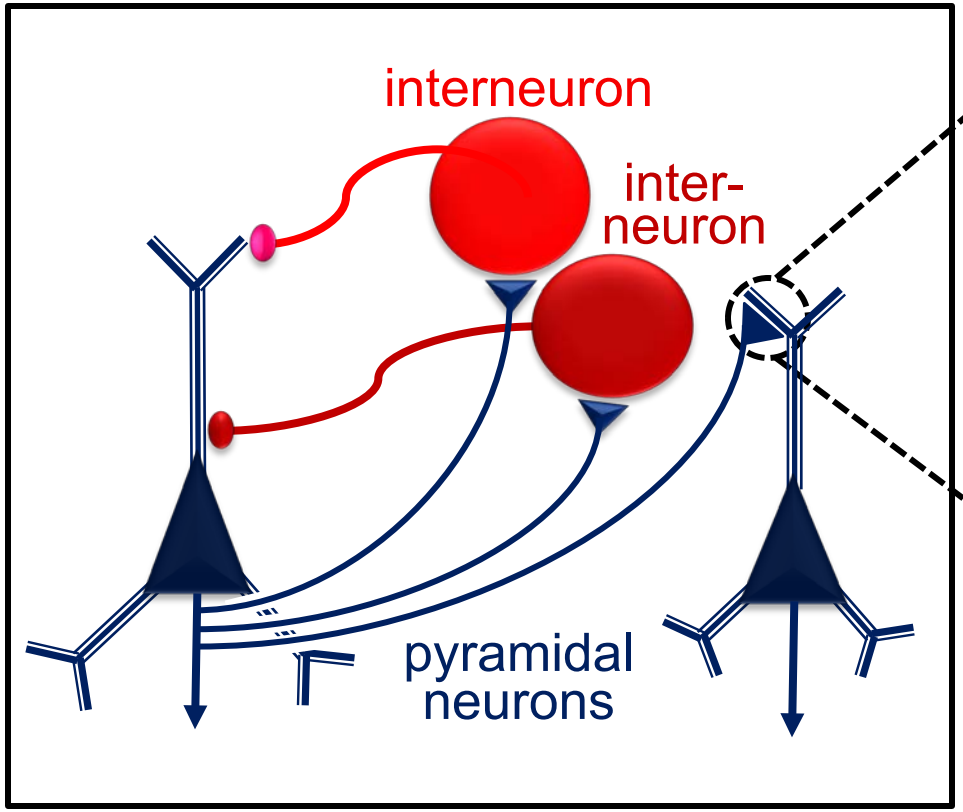

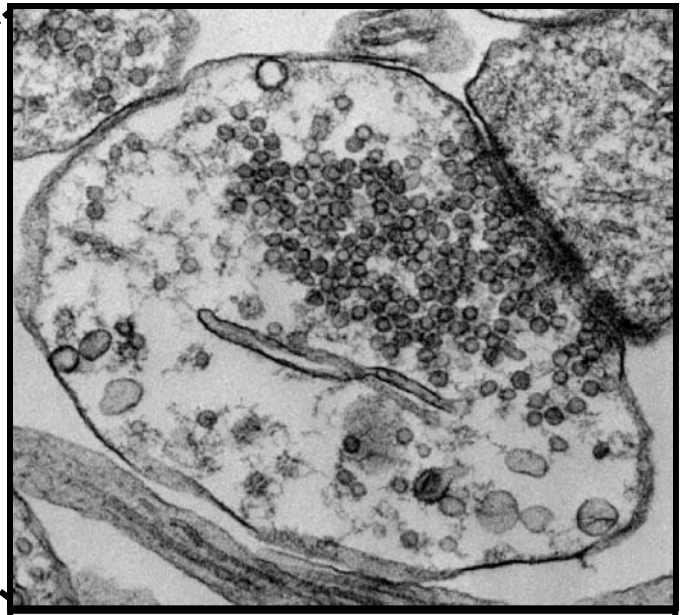

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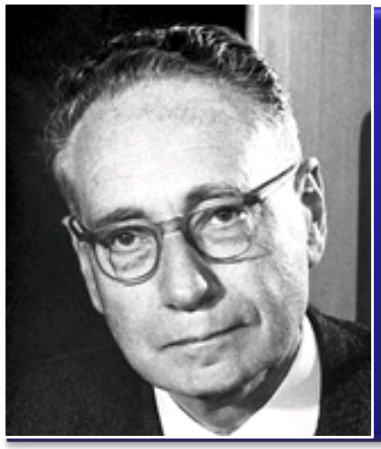
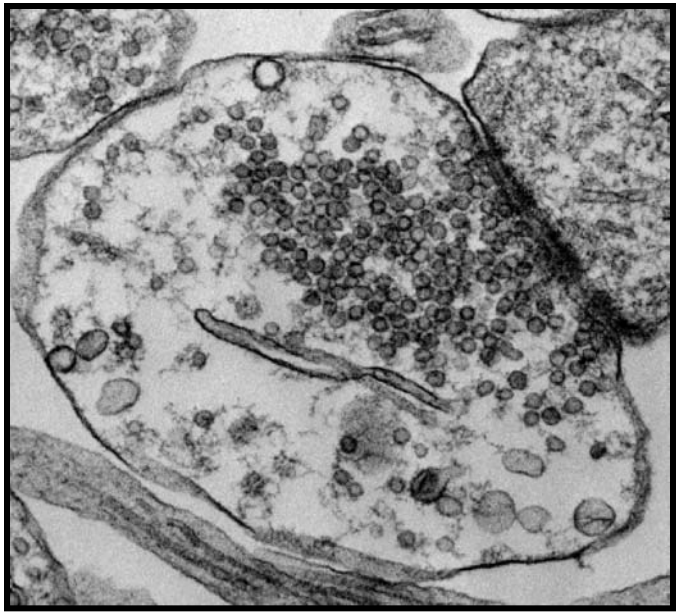


Although synapses differ in properties, all synapses operate by the same principle

Bernard Katz - Nobel Prize, 1970

All Synapses Operate by the Same Principle

An action potential invades the presynaptic nerve terminal



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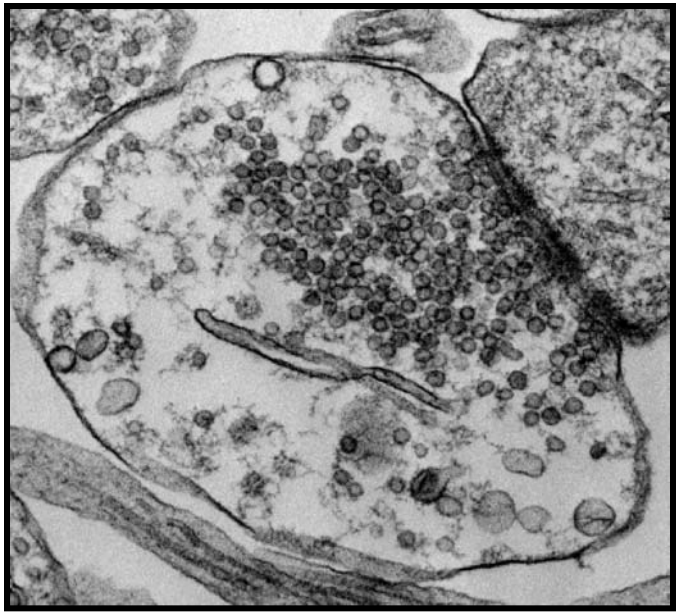
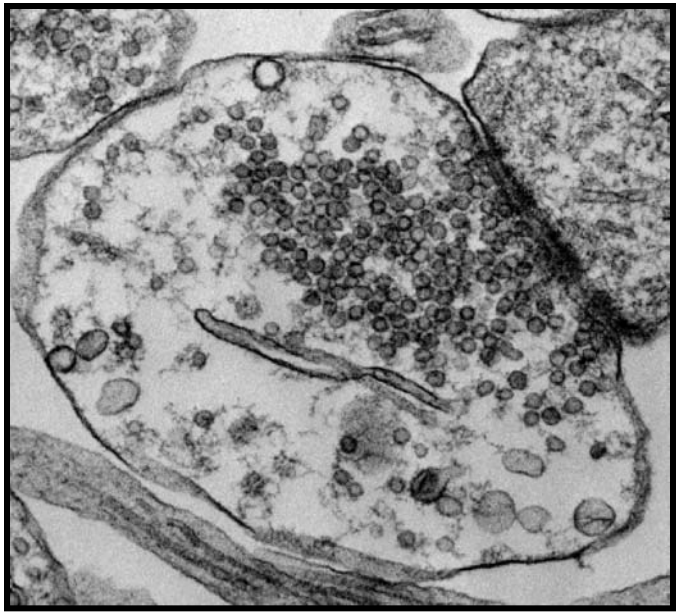
An action potential invades the presynaptic nerve terminal

- **Presynaptic Ca^{2+}-influx triggers neurotransmitter release**



All Synapses Operate by the Same Principle

- An action potential invades the presynaptic nerve terminal
  - Presynaptic Ca^{2+}-influx triggers neurotransmitter release
    - Neurotransmitters bind to postsynaptic receptors & elicit an electrical signal



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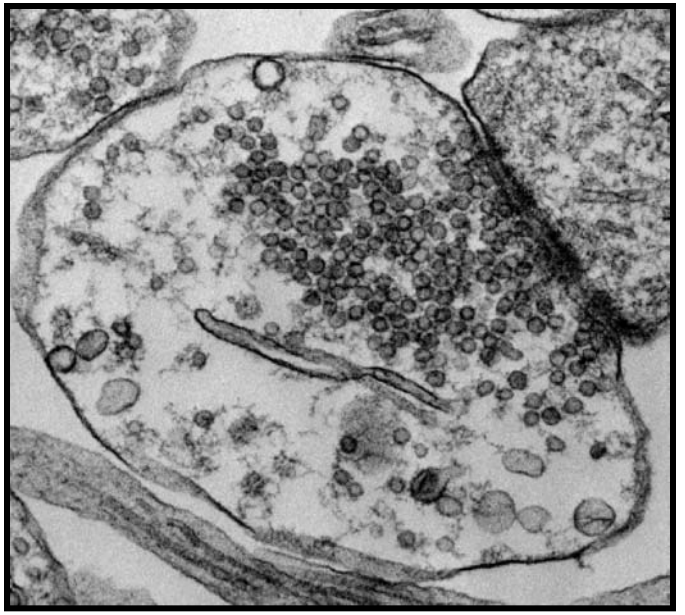
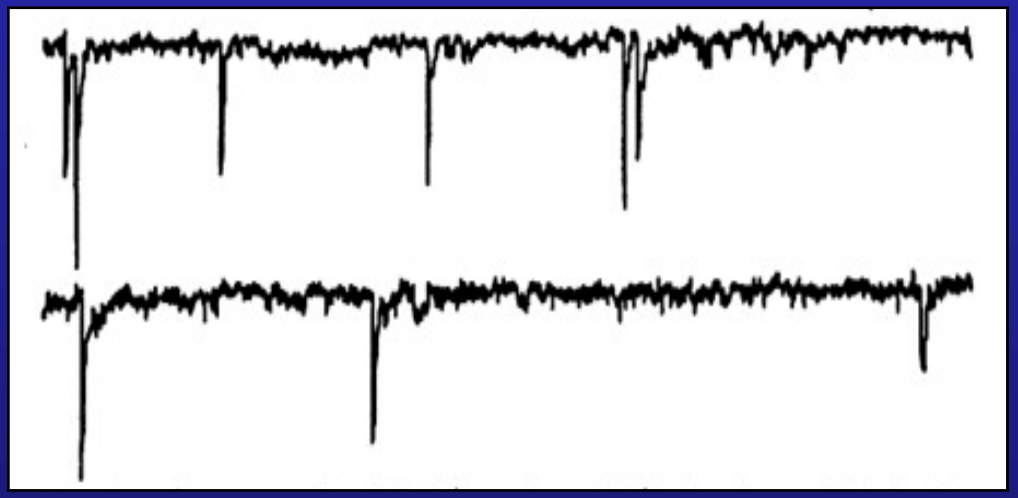


Approach:
Synaptic function is measured **electrophysiologically** via excitatory or inhibitory postsynaptic currents (EPSCs or IPSCs)



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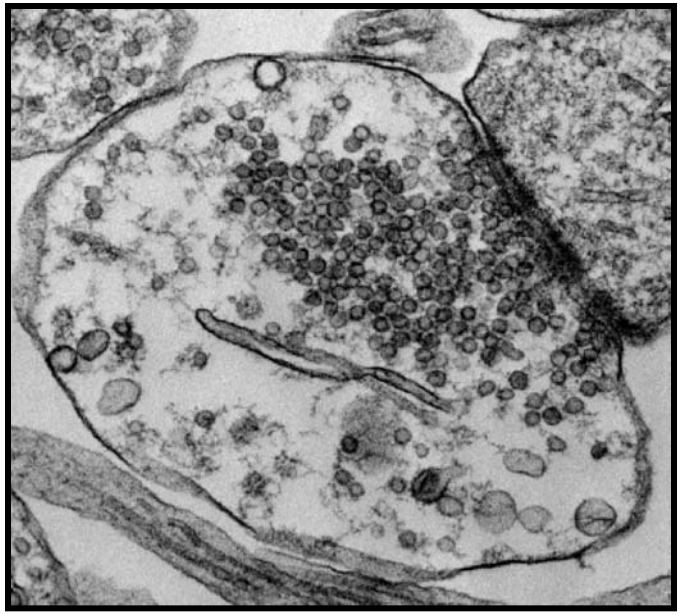

Synaptic transmission is rapid = **1-5 ms**
- key step is neurotransmitter release

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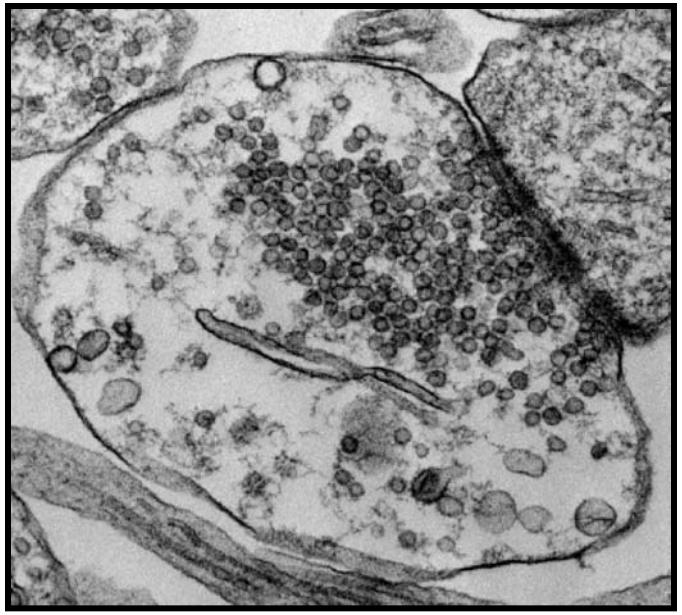

Synaptic transmission is rapid = **1-5 ms**
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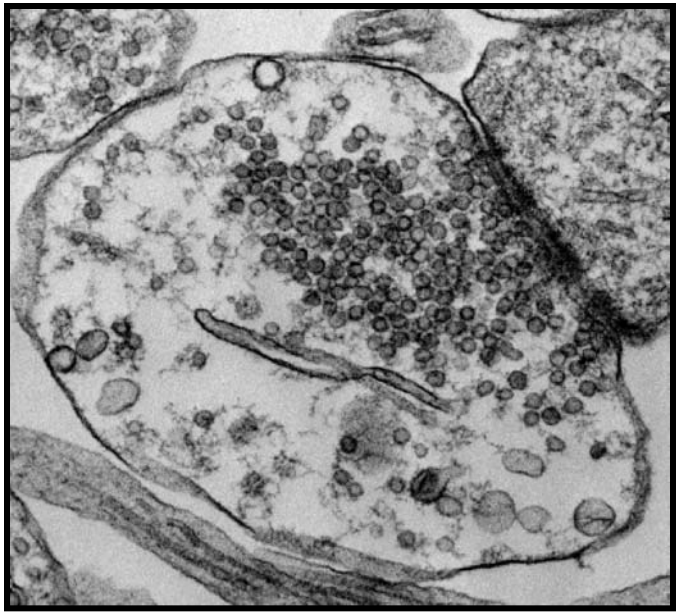
Three basic processes enable rapid release

Three Processes Govern Neurotransmitter Release

1. **Synaptic vesicle fusion**



Three Processes Govern Neurotransmitter Release

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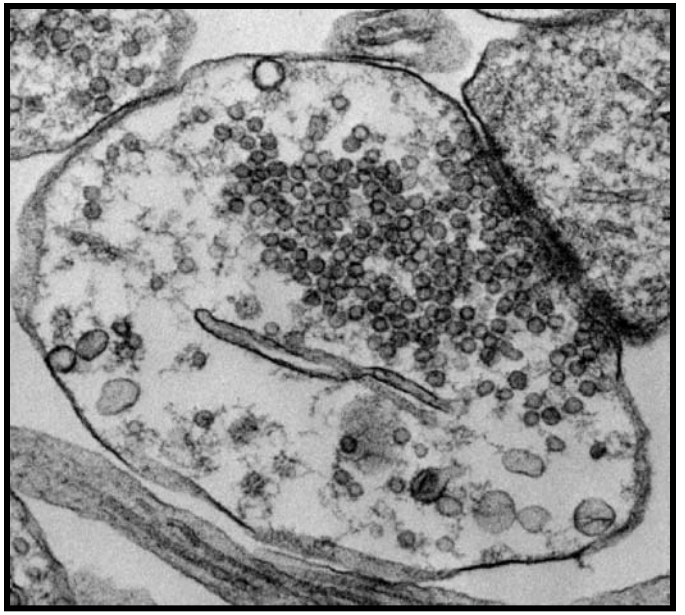
2. Ca^{2+}-triggering of fusion
   - Very fast: ~0.1 msec
   - Cooperative: ~5 Ca^{2+}-ions



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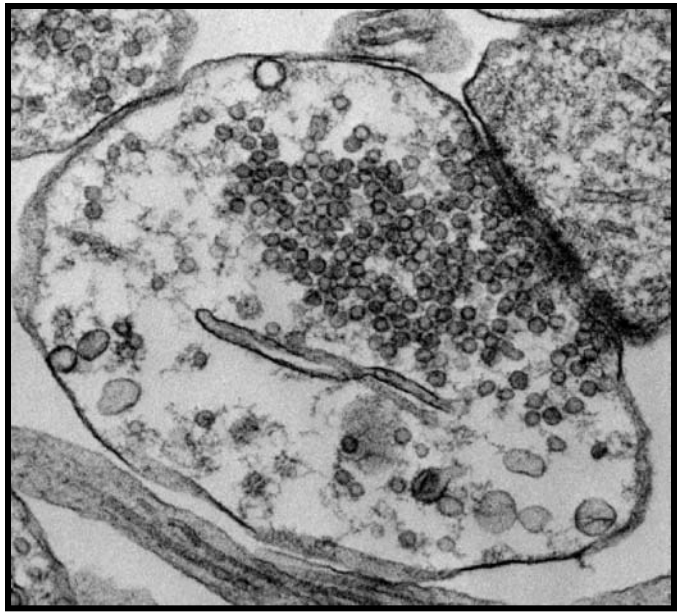

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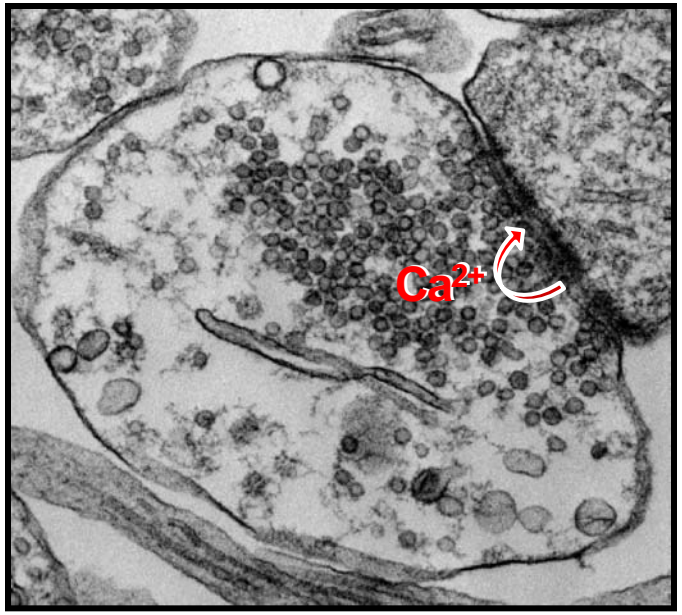

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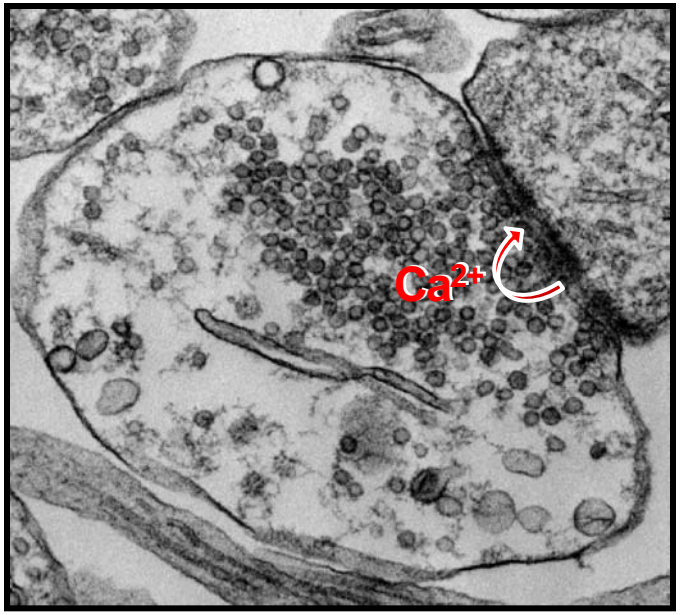
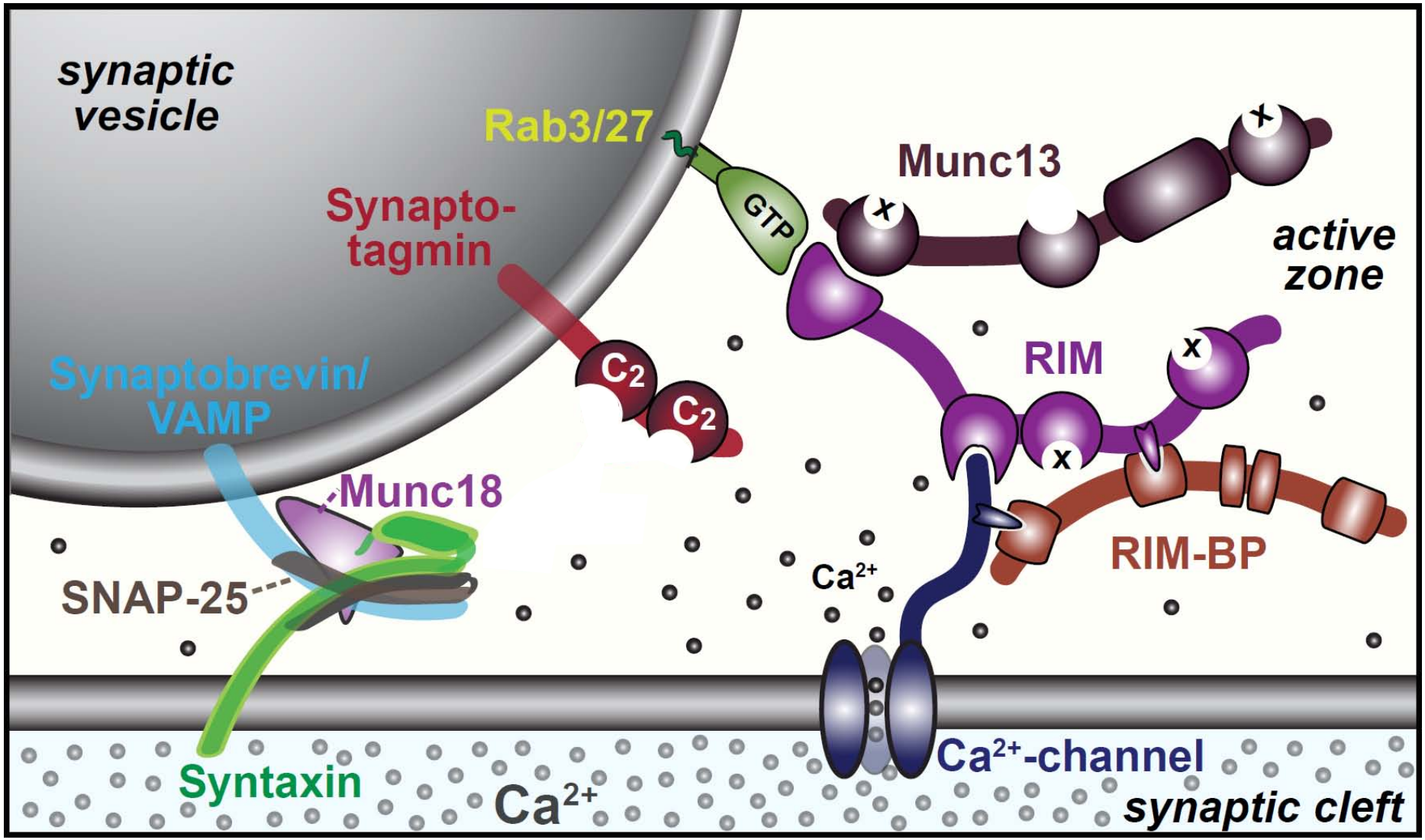


When I started my lab in 1986, neurotransmitter release fascinated me because of its importance, inexplicable speed, and precision – but not a single synapse component had been molecularly characterized

Now – 25 years later – a molecular framework that plausibly explains release in molecular terms has emerged …

A Neurotransmitter Release Machine Mediates Fusion, Ca^{2+}-triggering & Ca^{2+}-Channel Tethering



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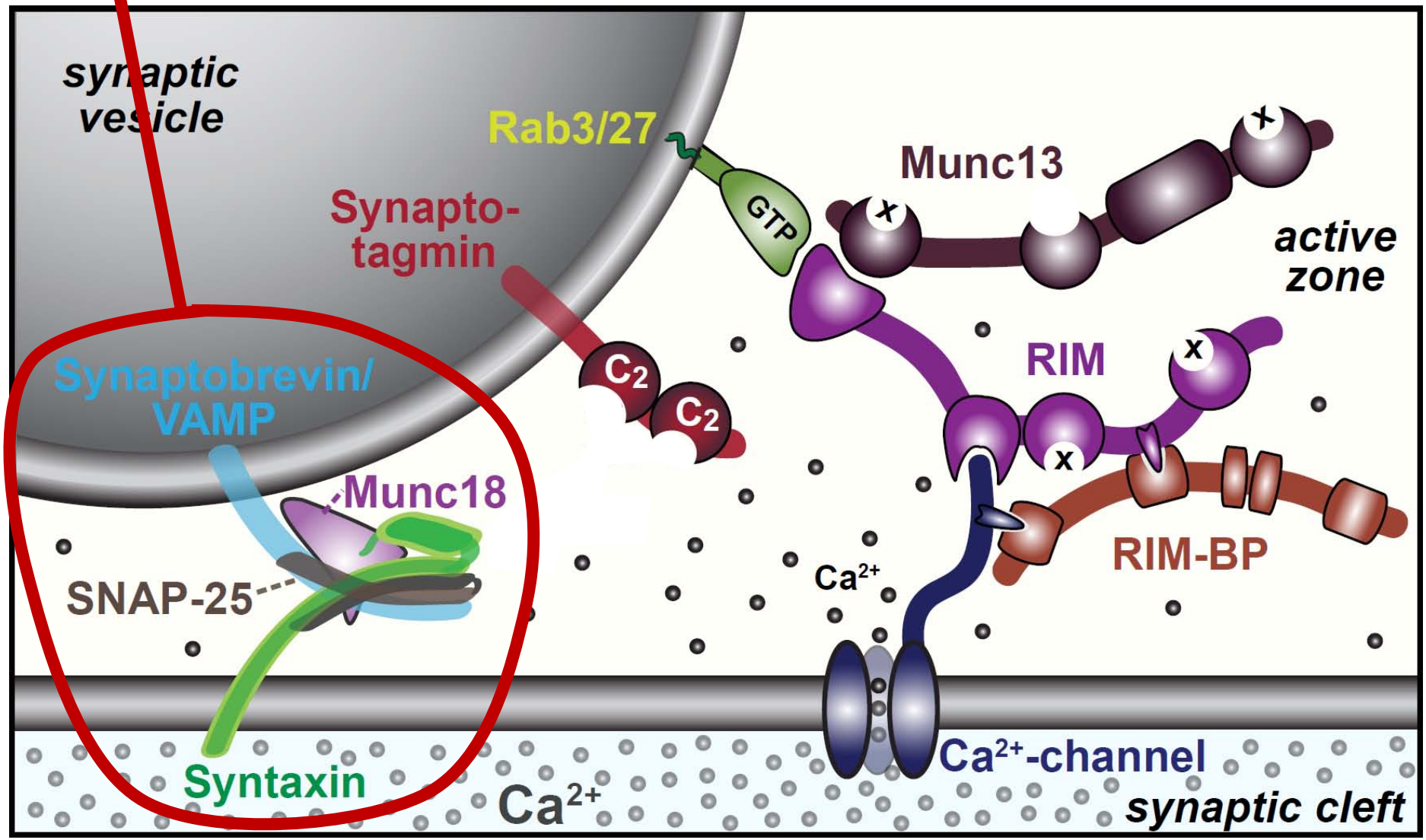

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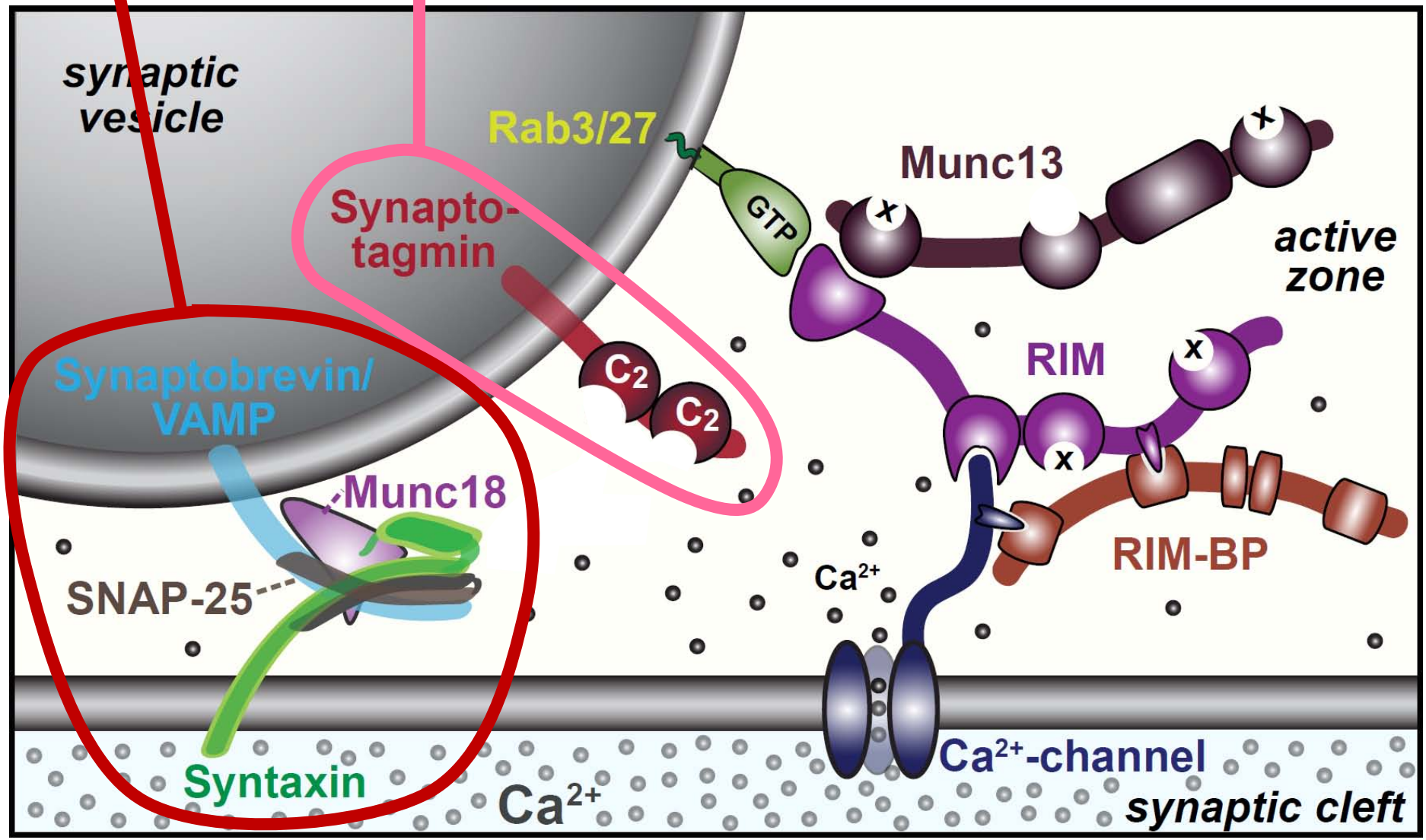

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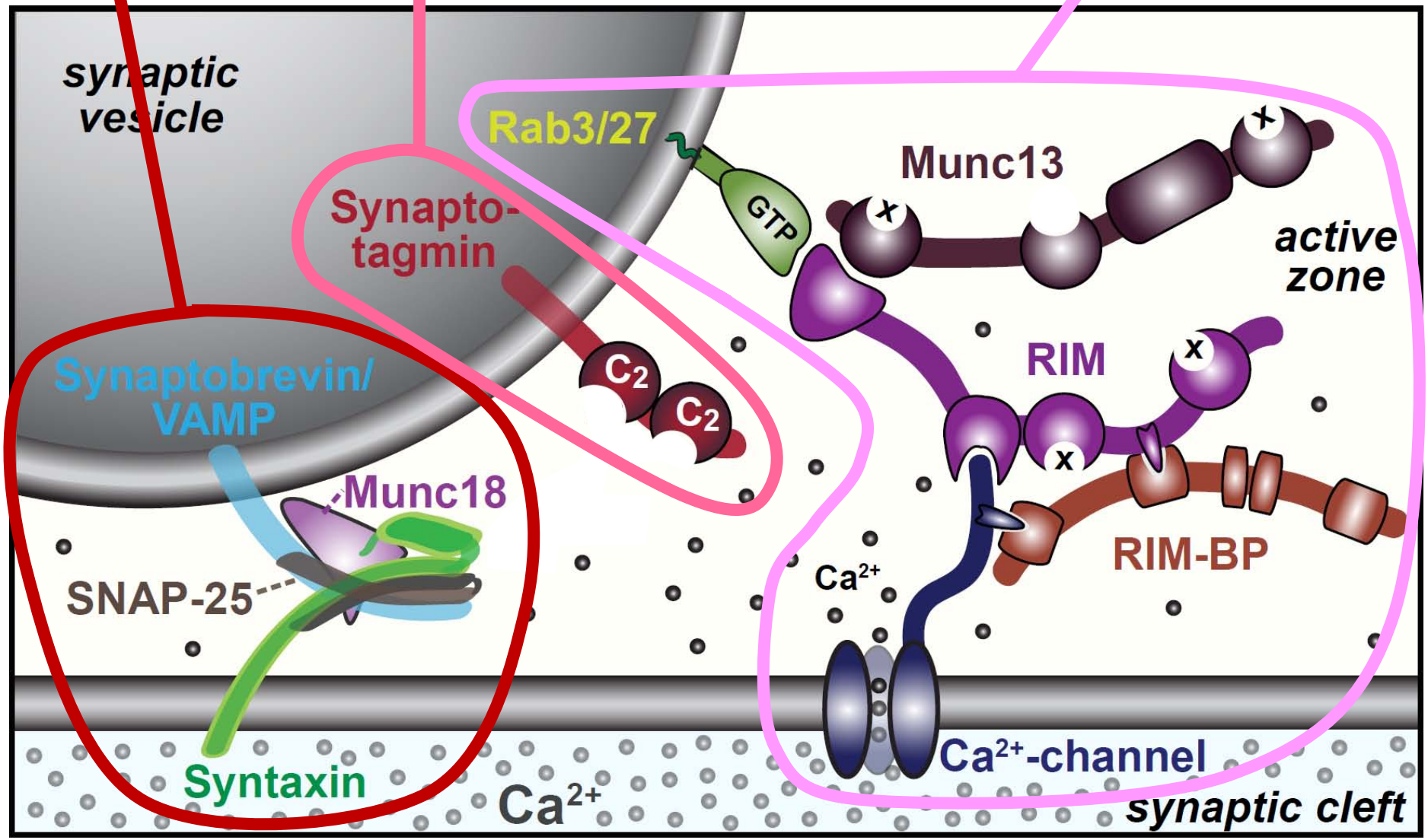

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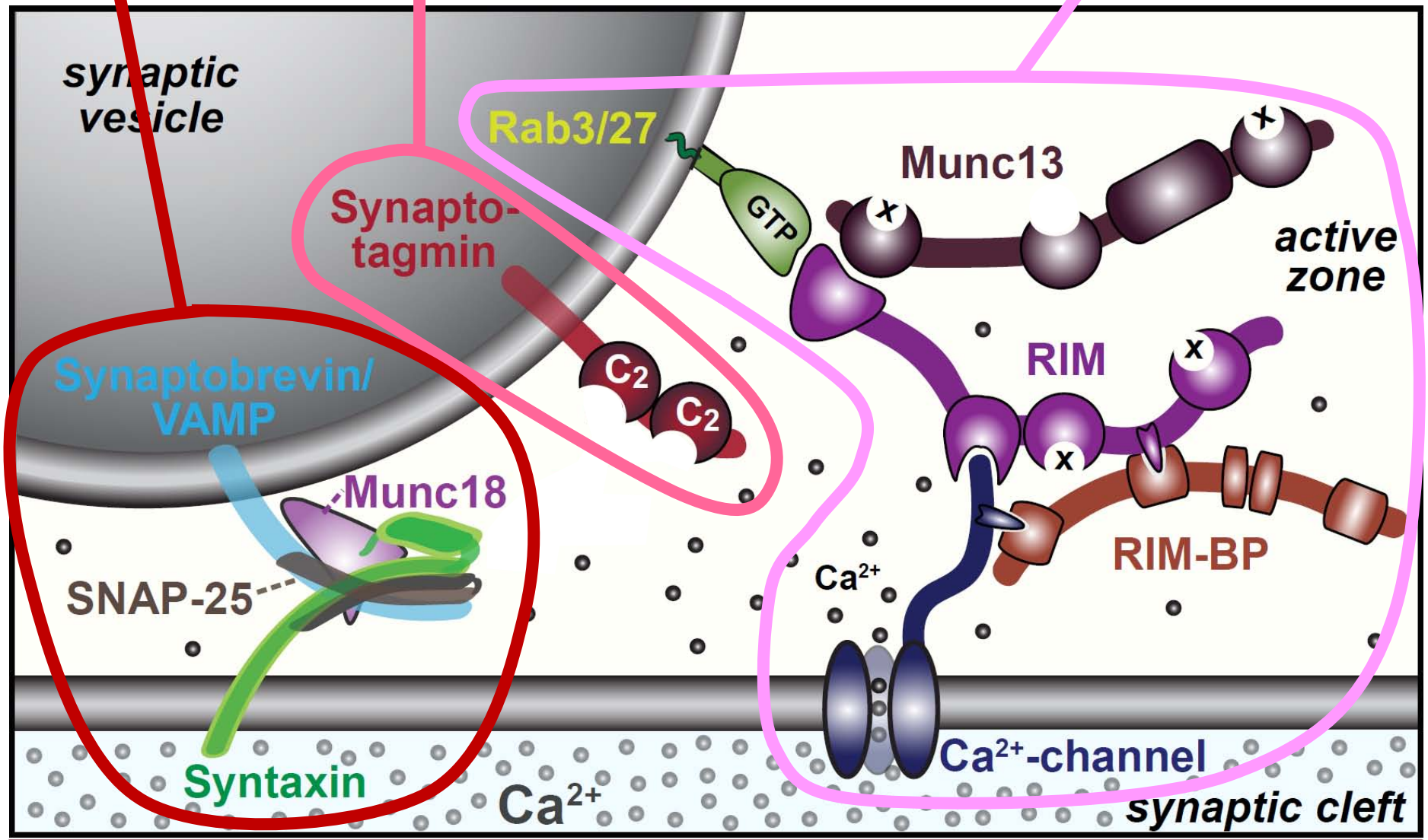

Let's start at the beginning

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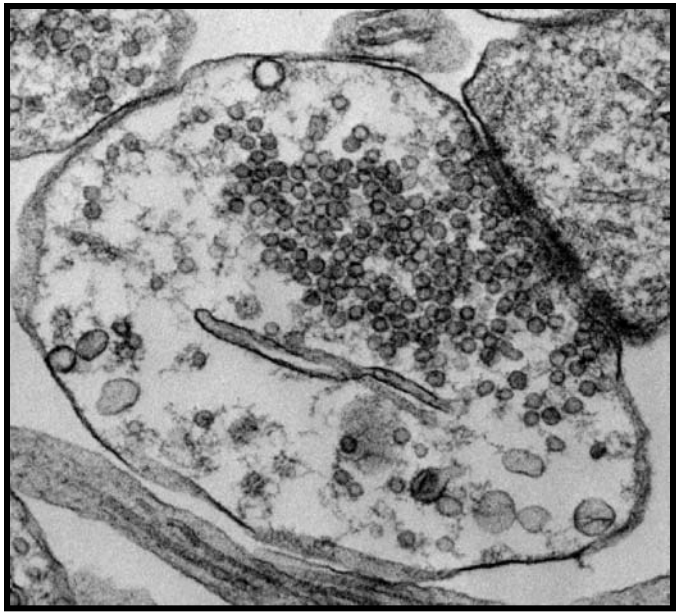
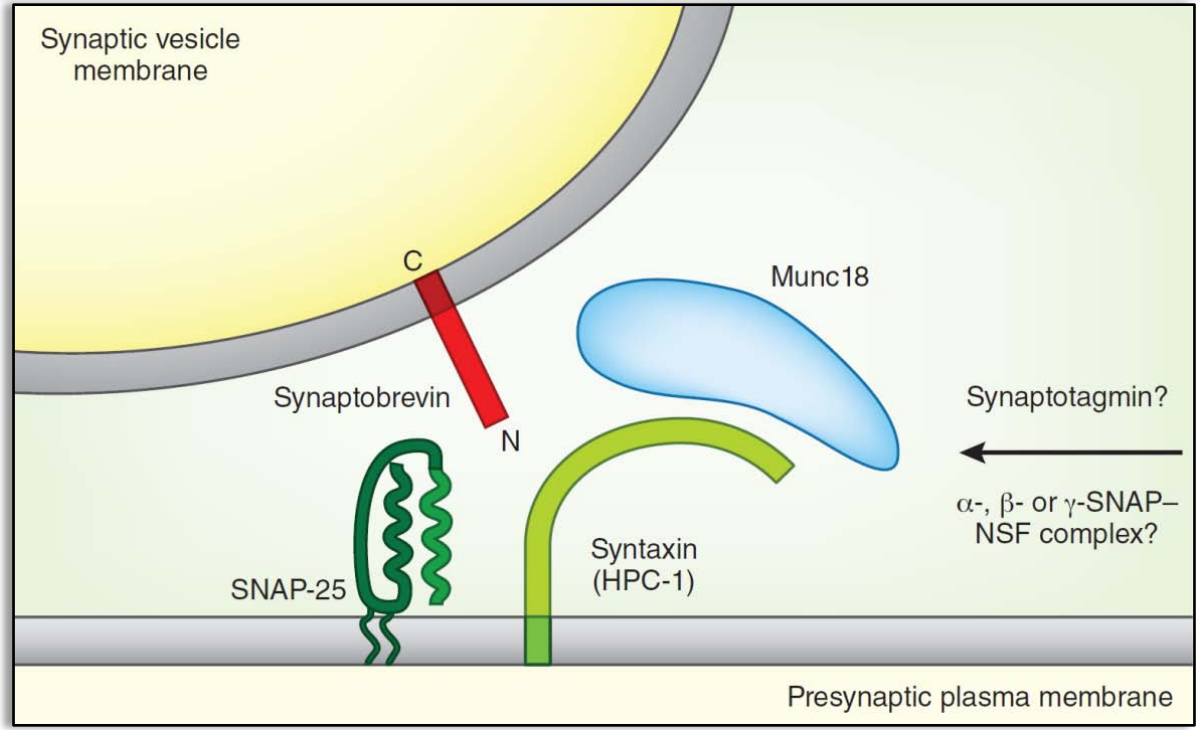


1993 Model of Synaptic Membrane Fusion Machinery



Hata et al., Nature 1993

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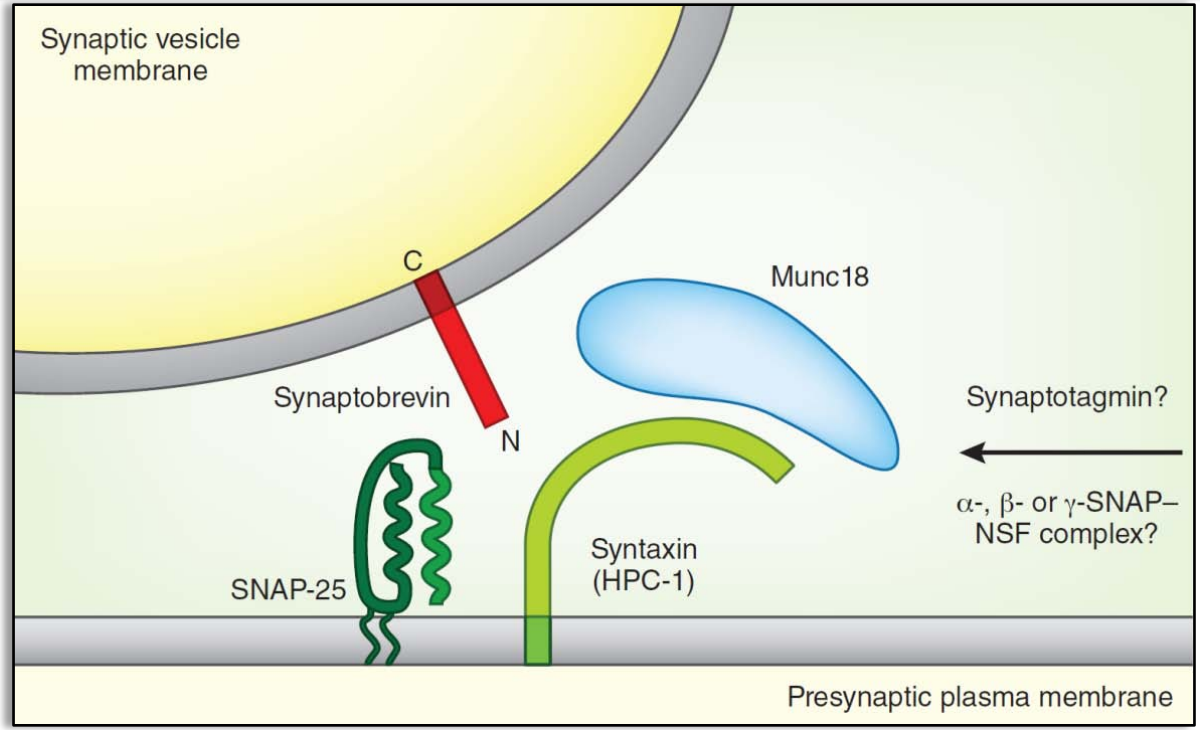

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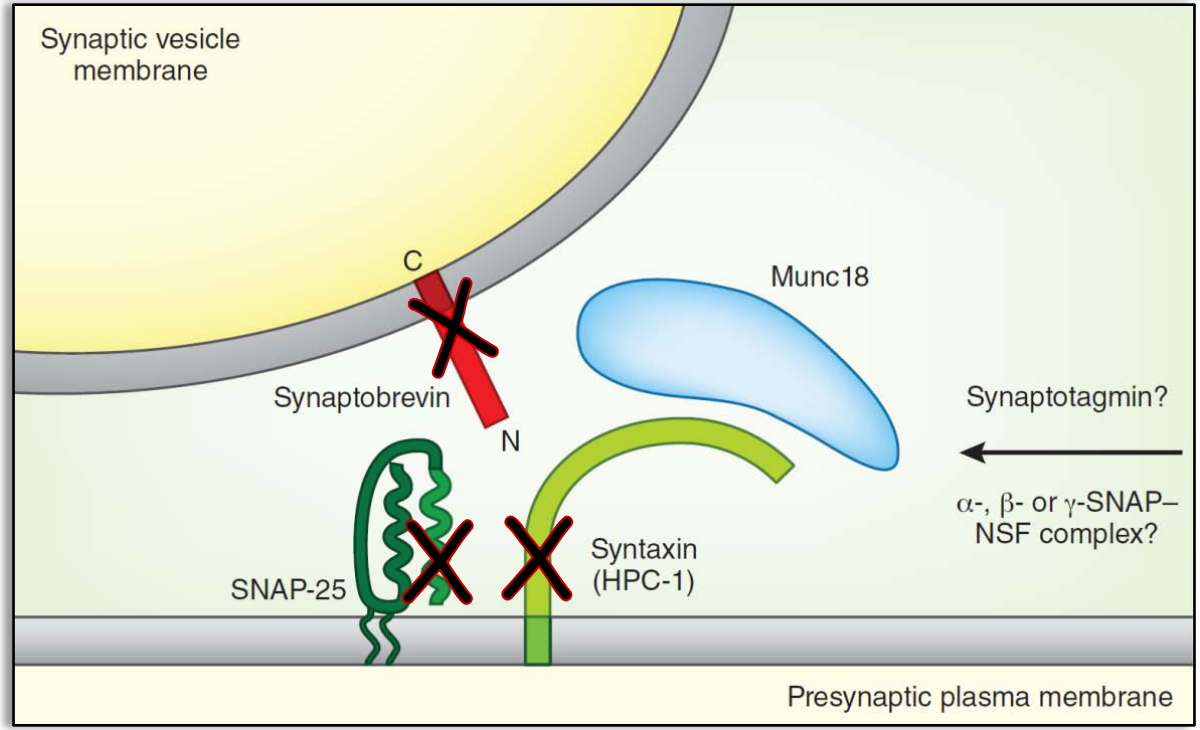

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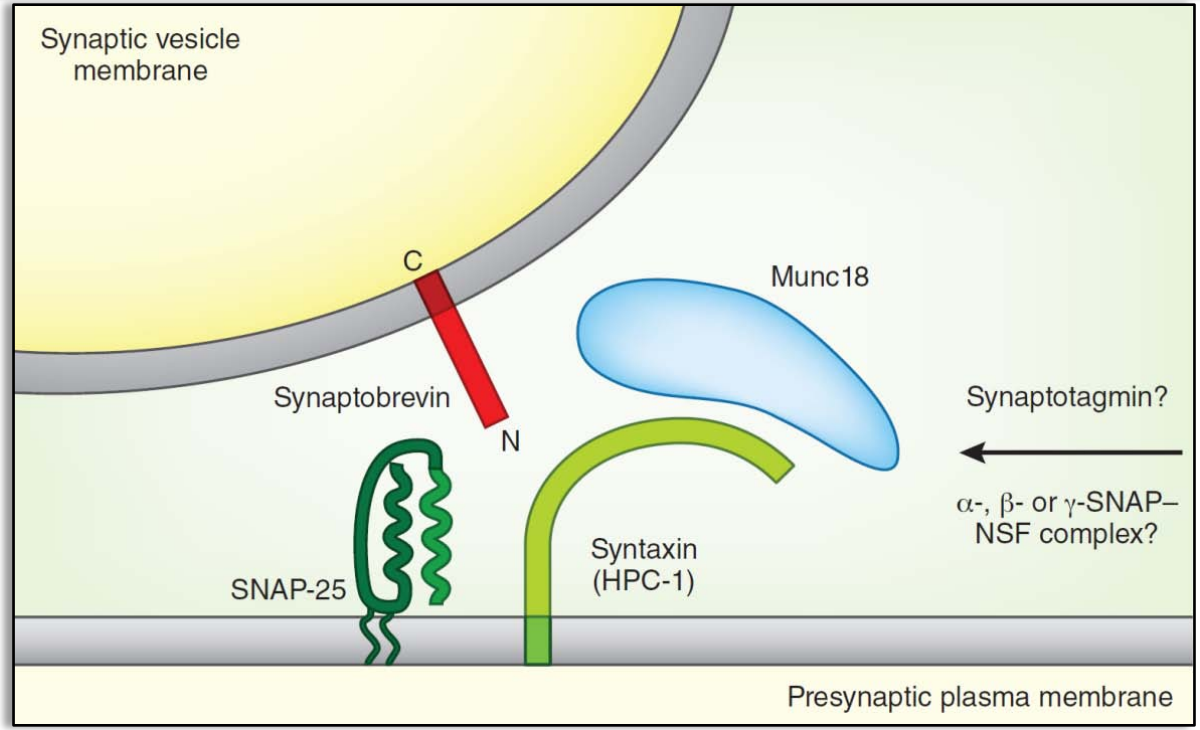

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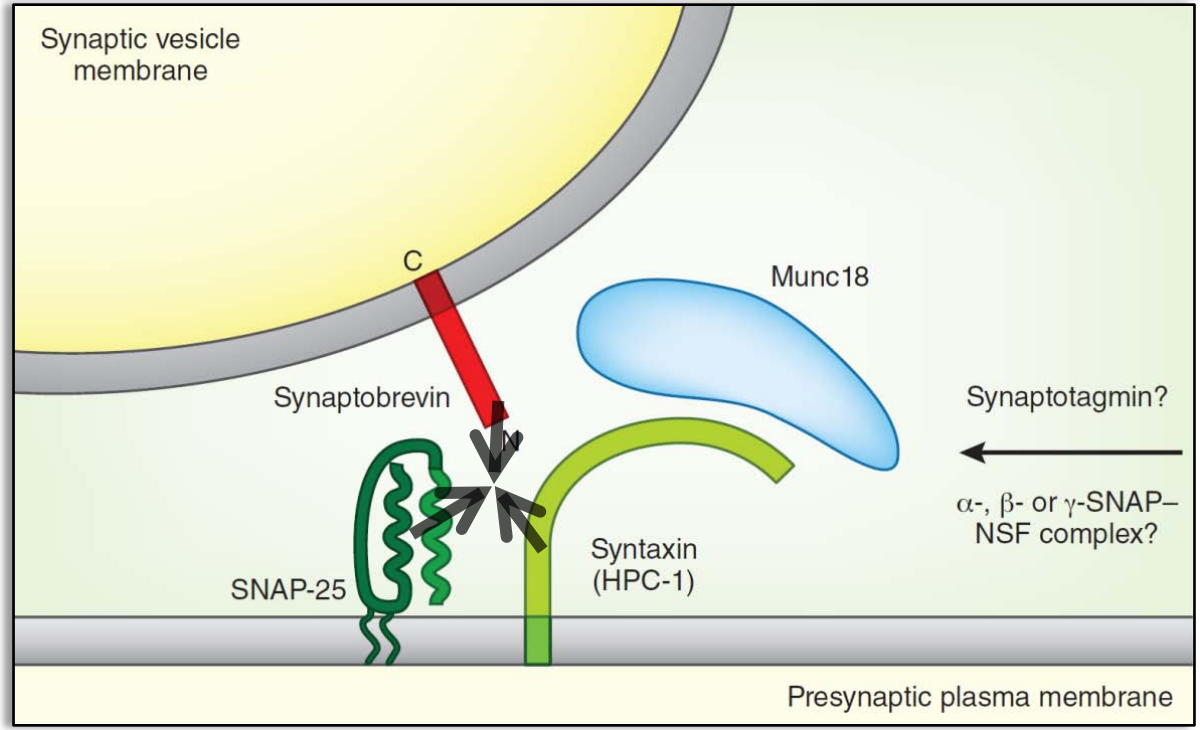

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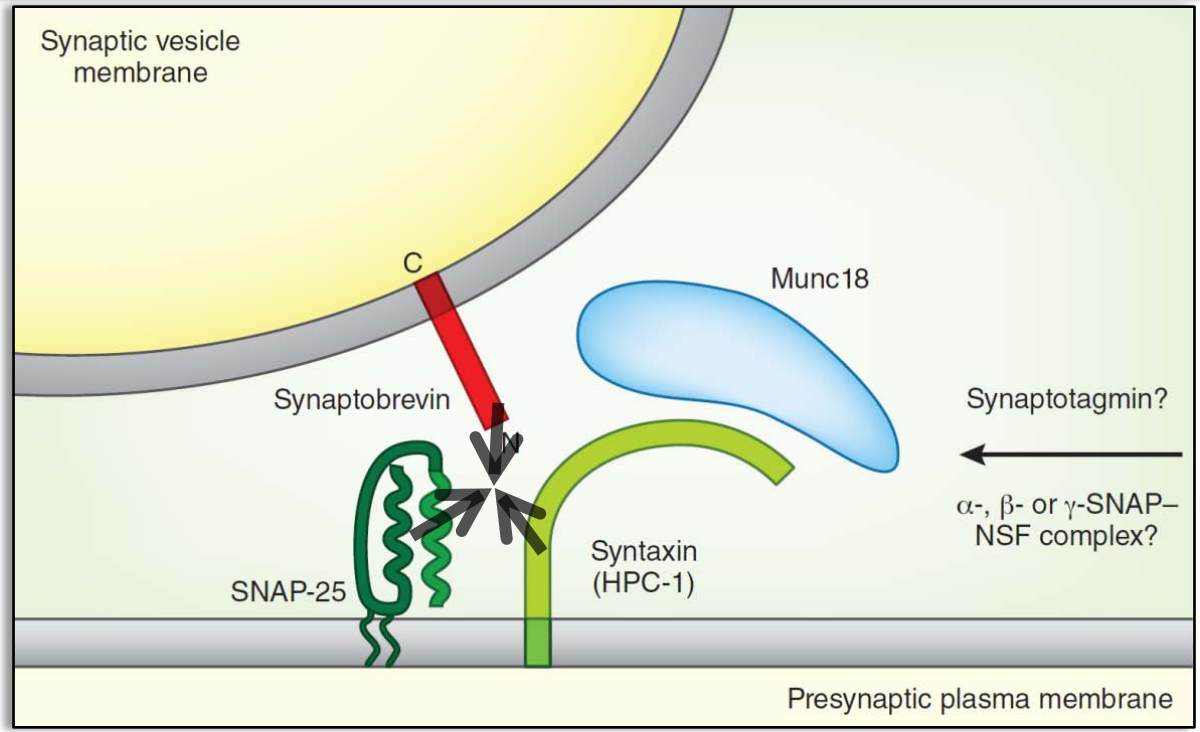

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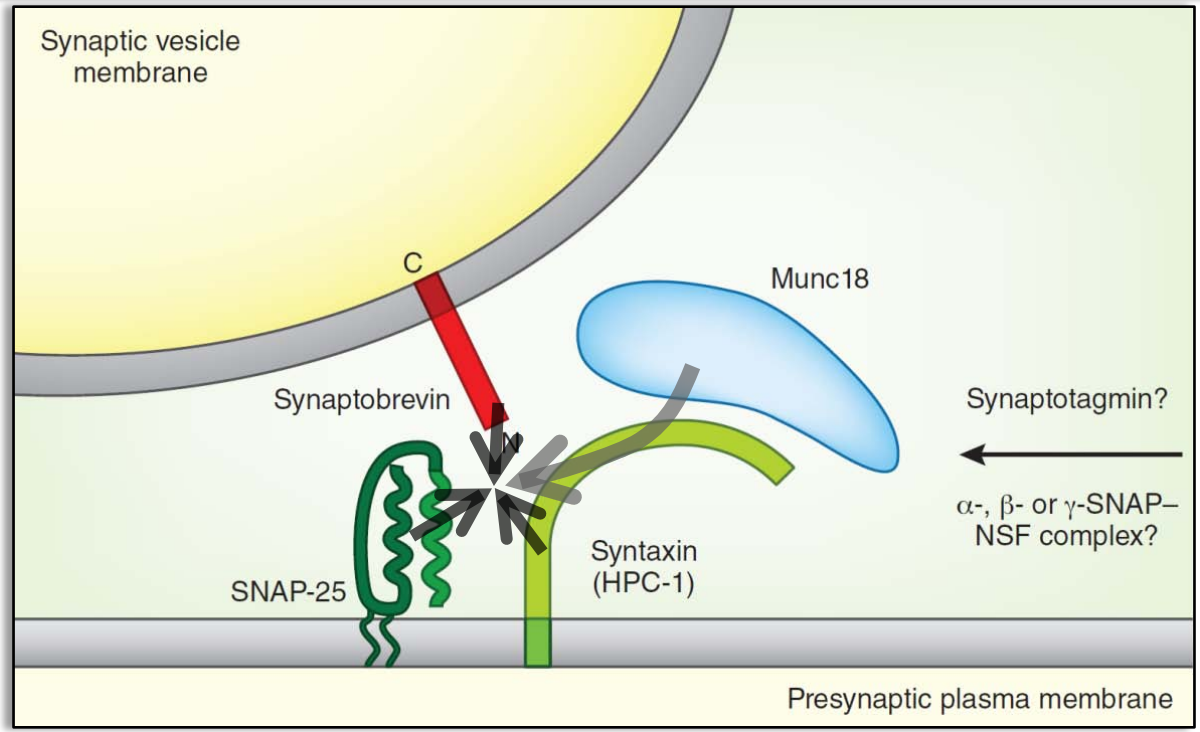

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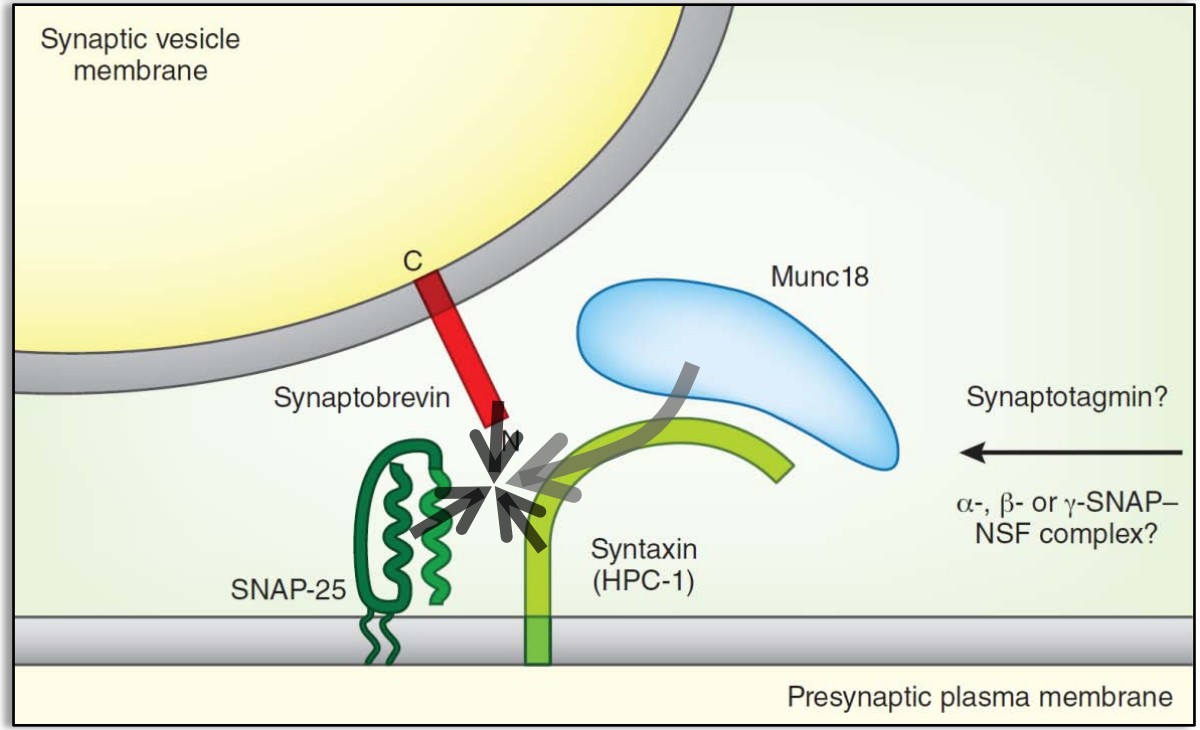

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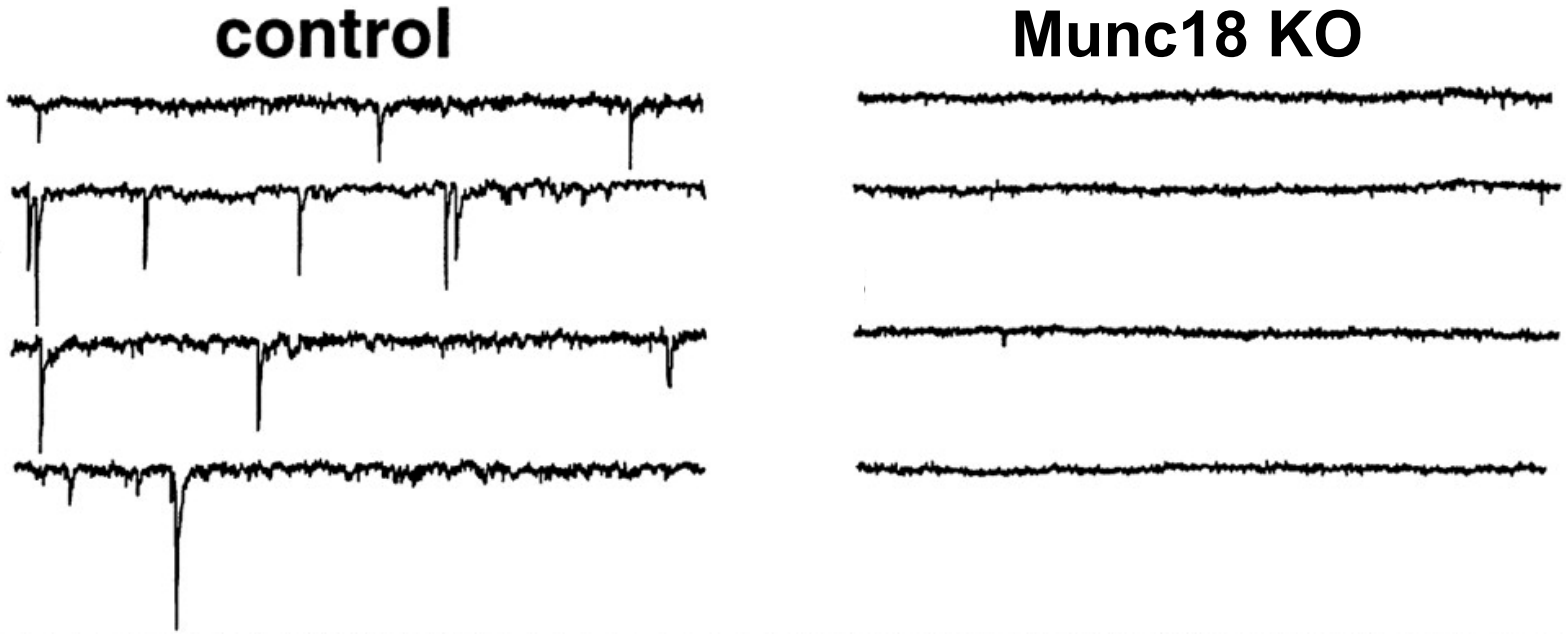
Munc18 is not by-stander but central actor in membrane fusion

Munc18 is <u>absolutely essential</u> for vesicle fusion whereas individual SNAREs are not

Munc18 KO Abolishes Synaptic Membrane Fusion

Spontaneous synaptic activity in the cortex



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Verhage et al., Science 2000

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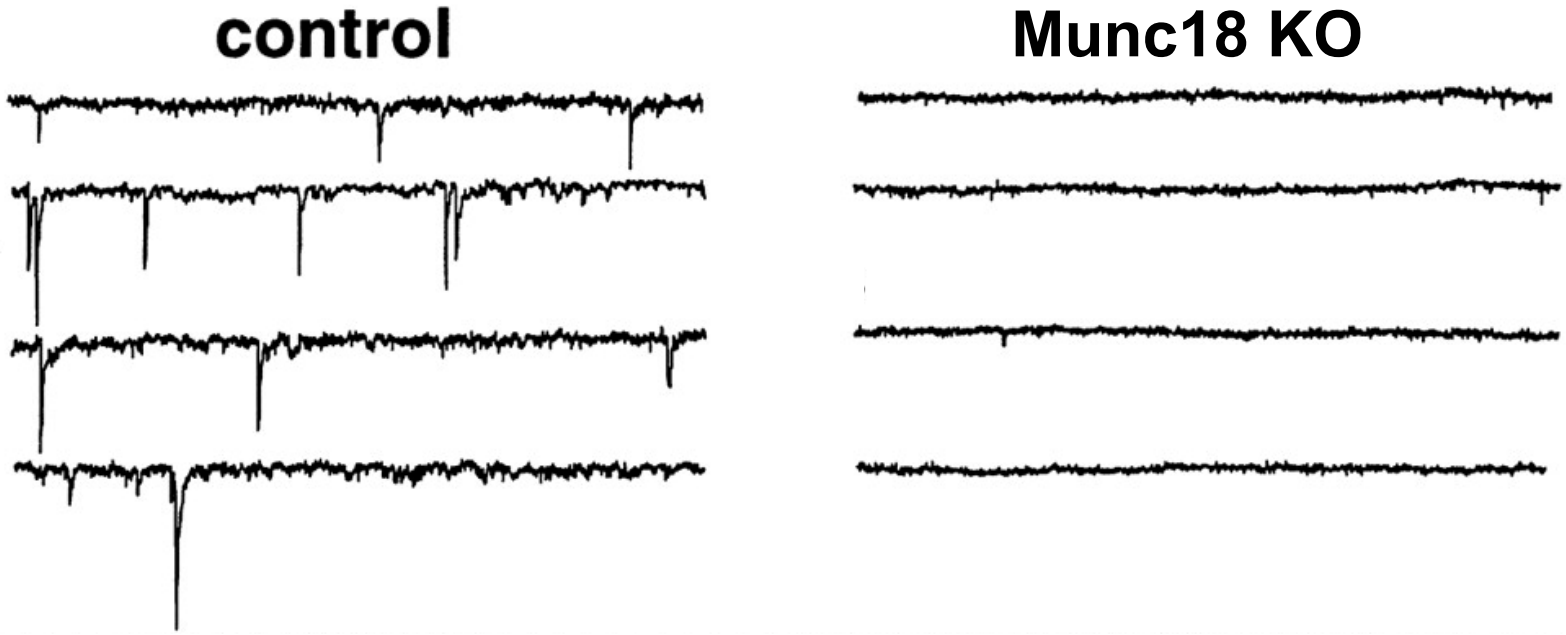

Munc18 KO: Normal synapse formation,
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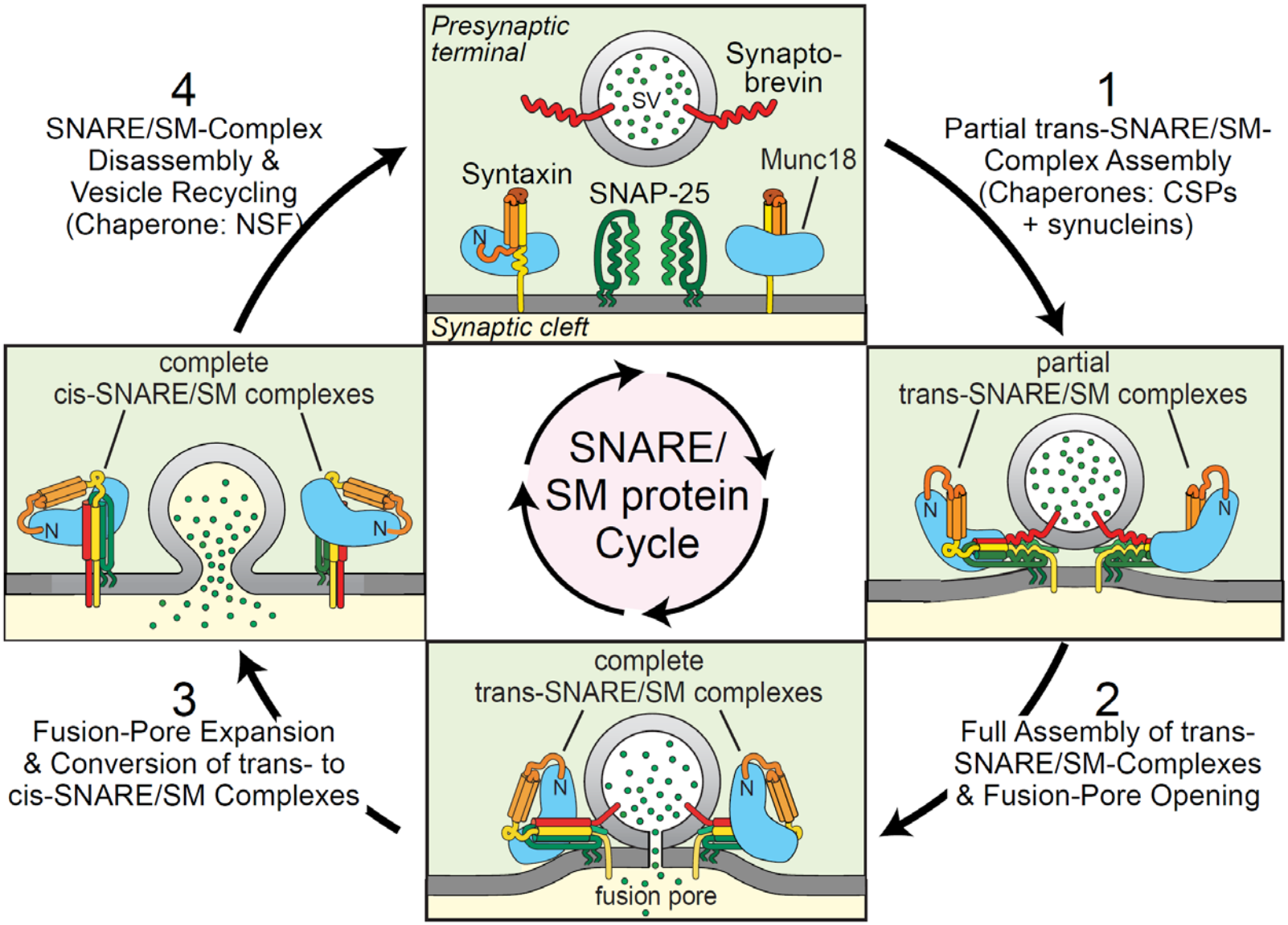
A model for Munc18 function
based on lots of subsequent work …

Verhage et al., Science 2000

SNARE/SM Protein Complex Assembly Drives Fusion



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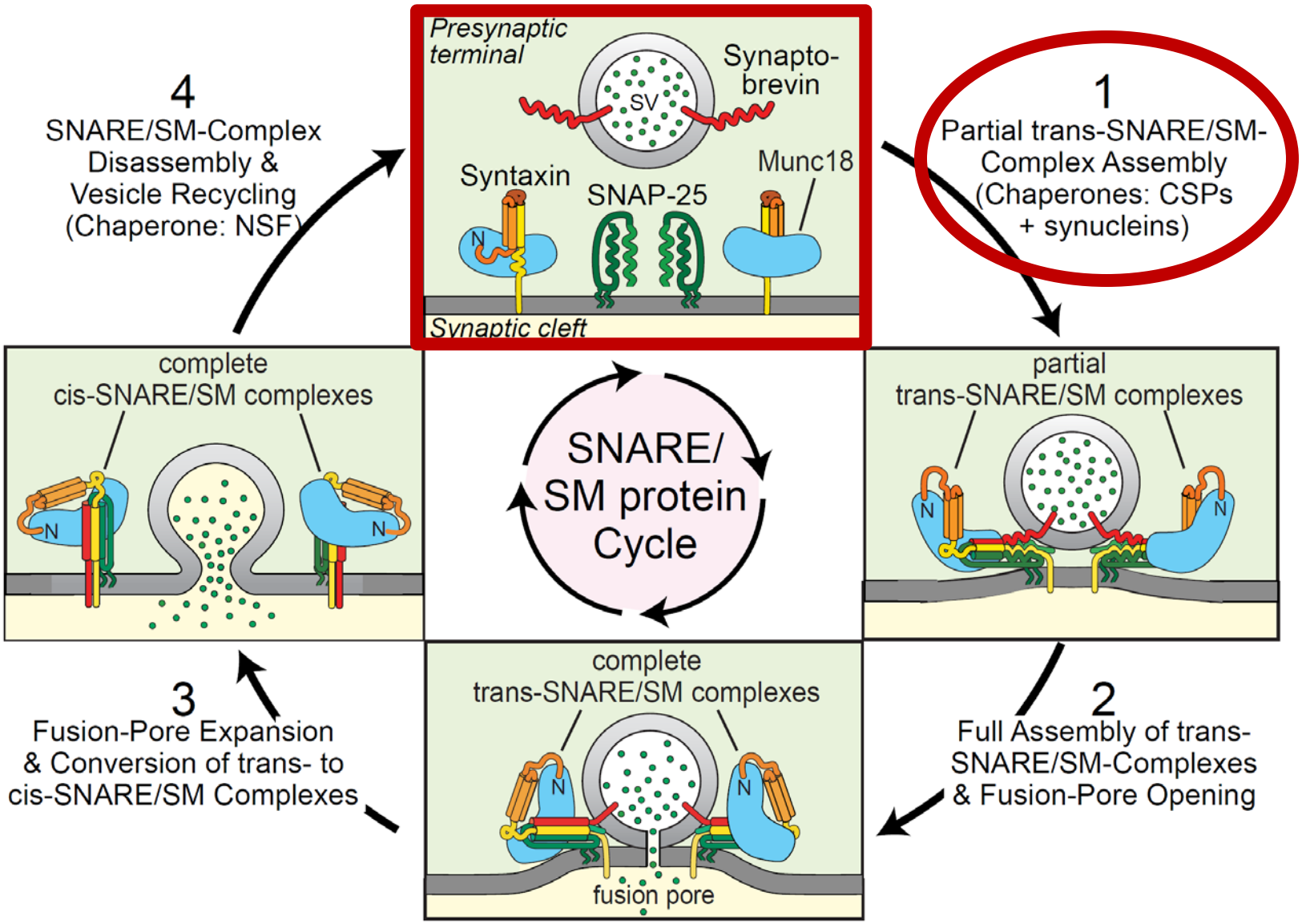

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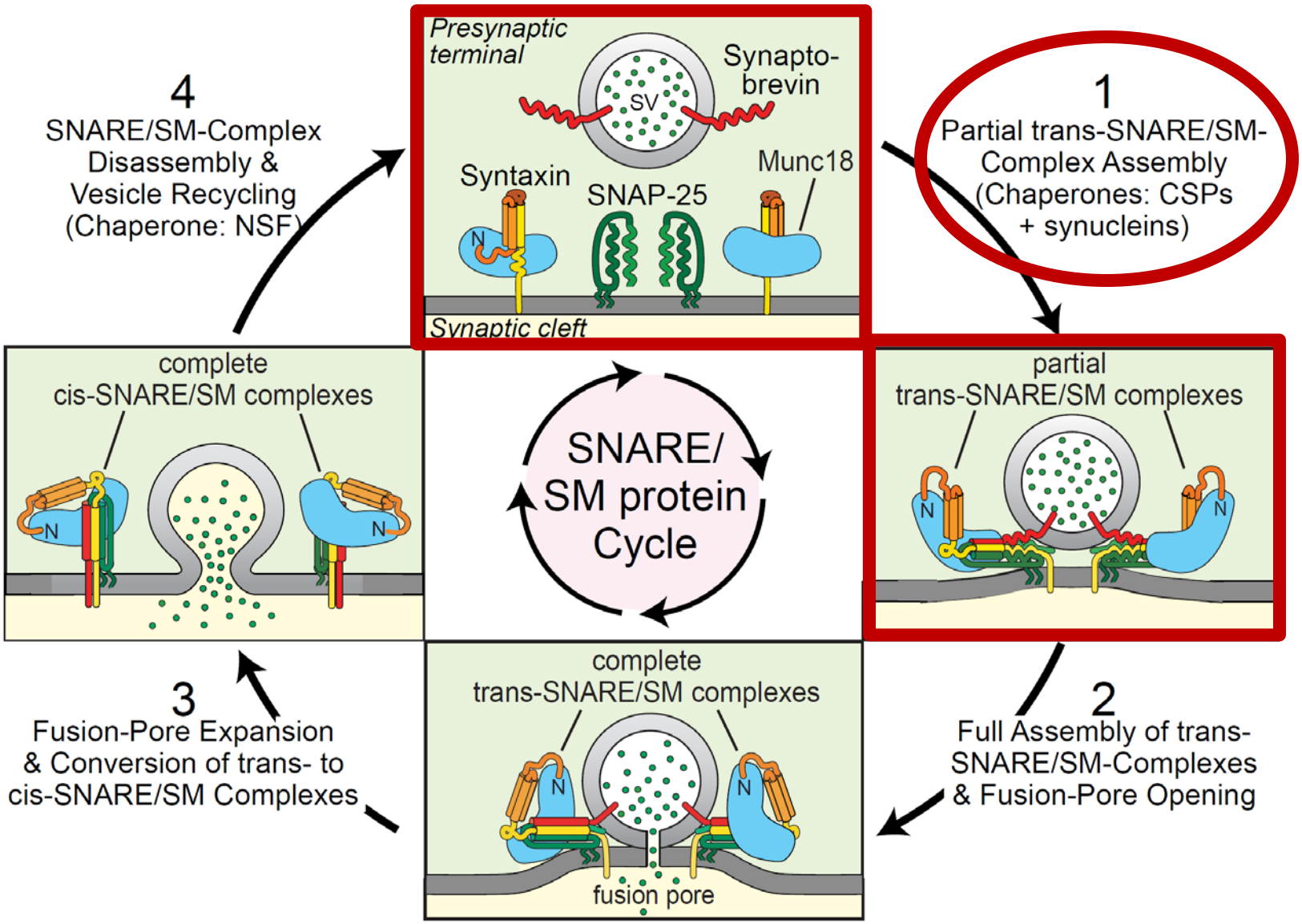

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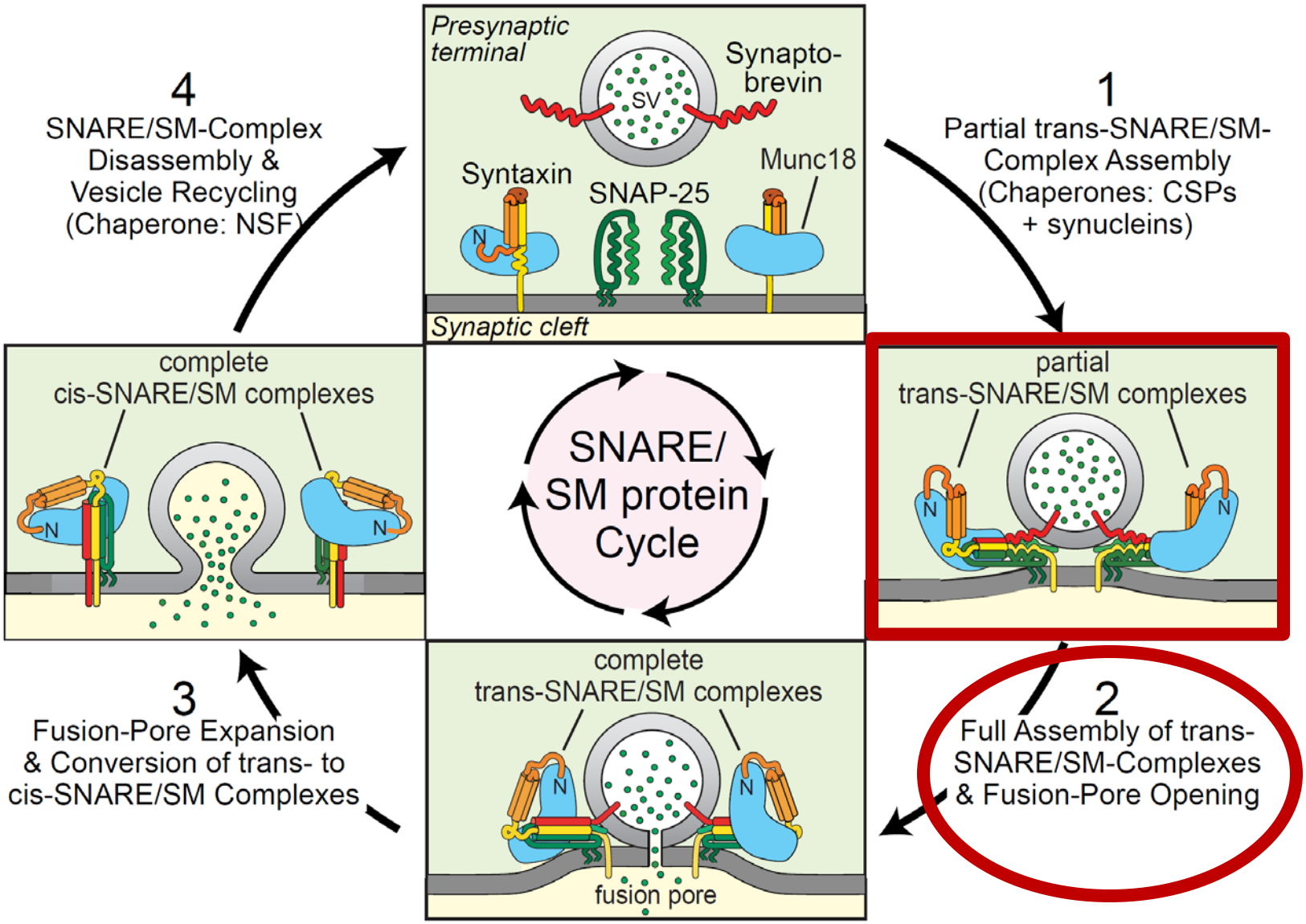

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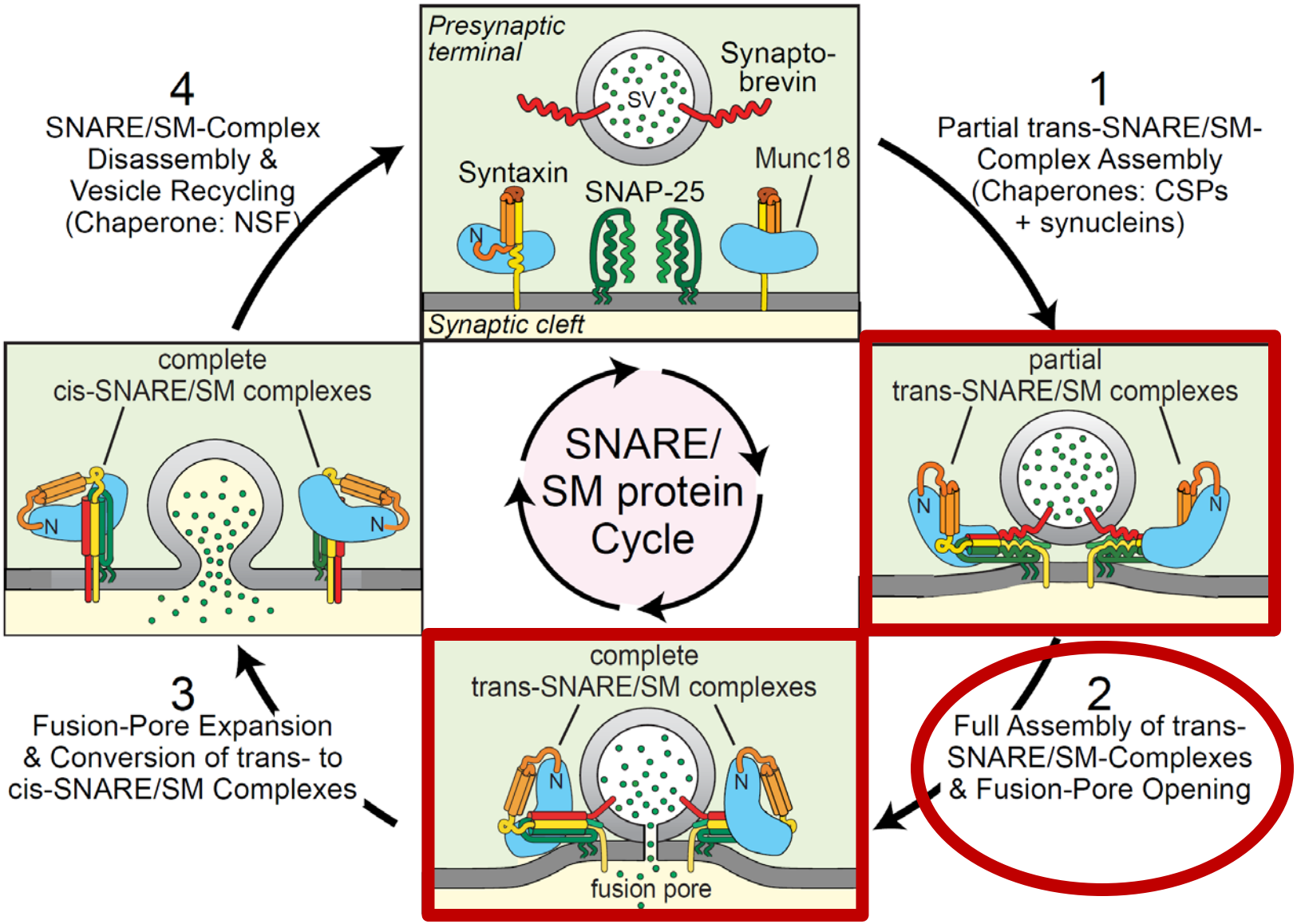

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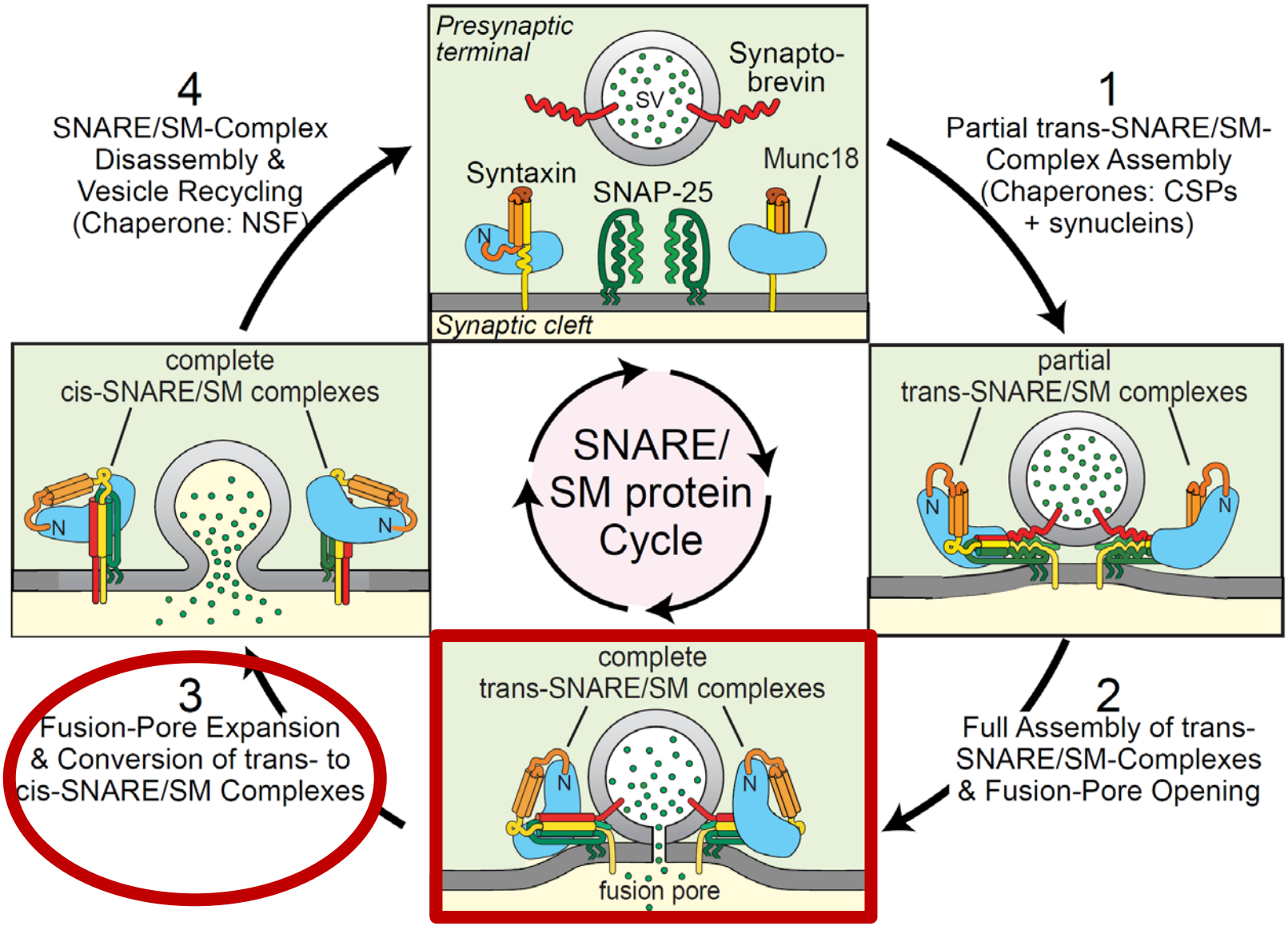

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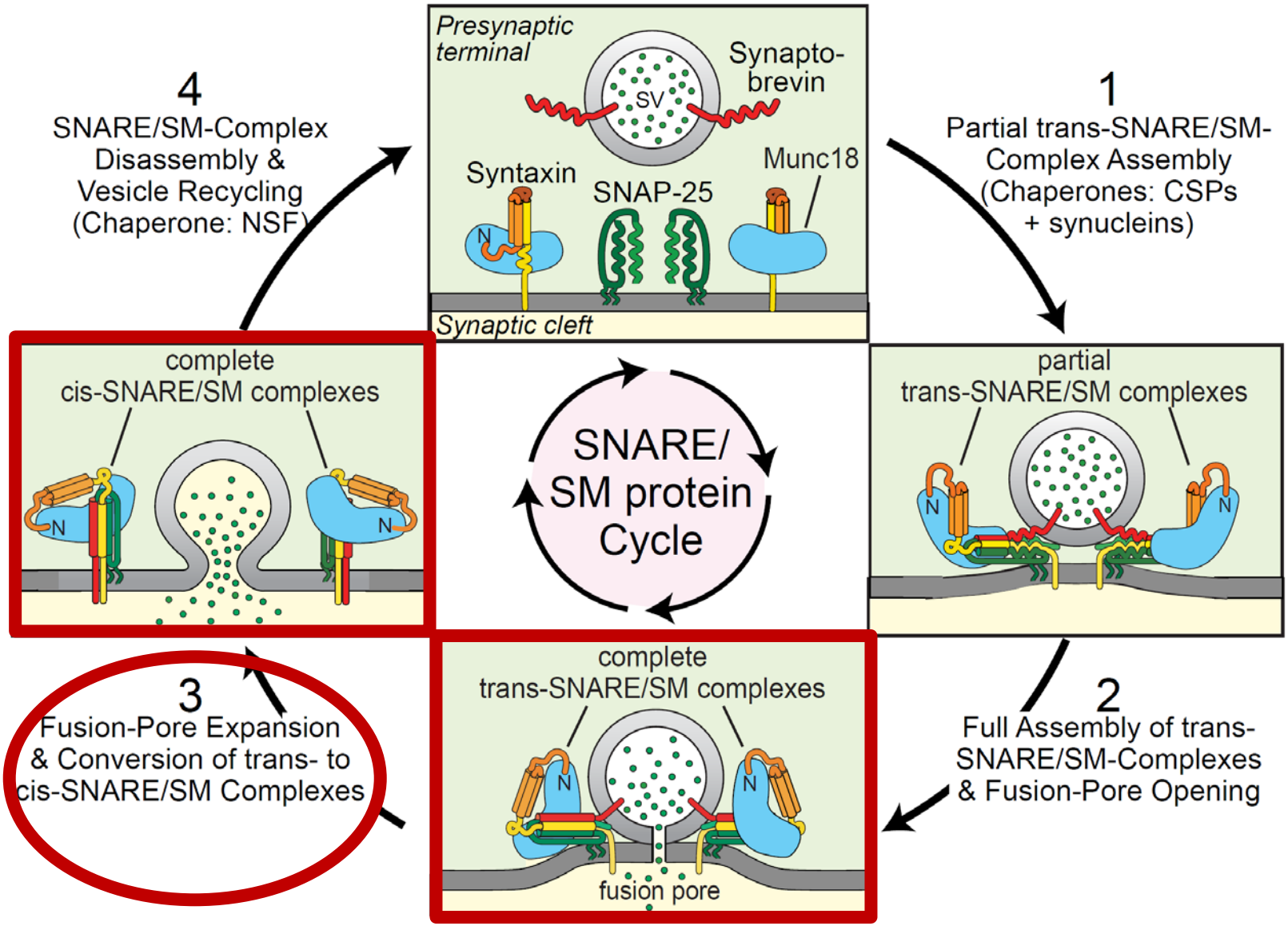

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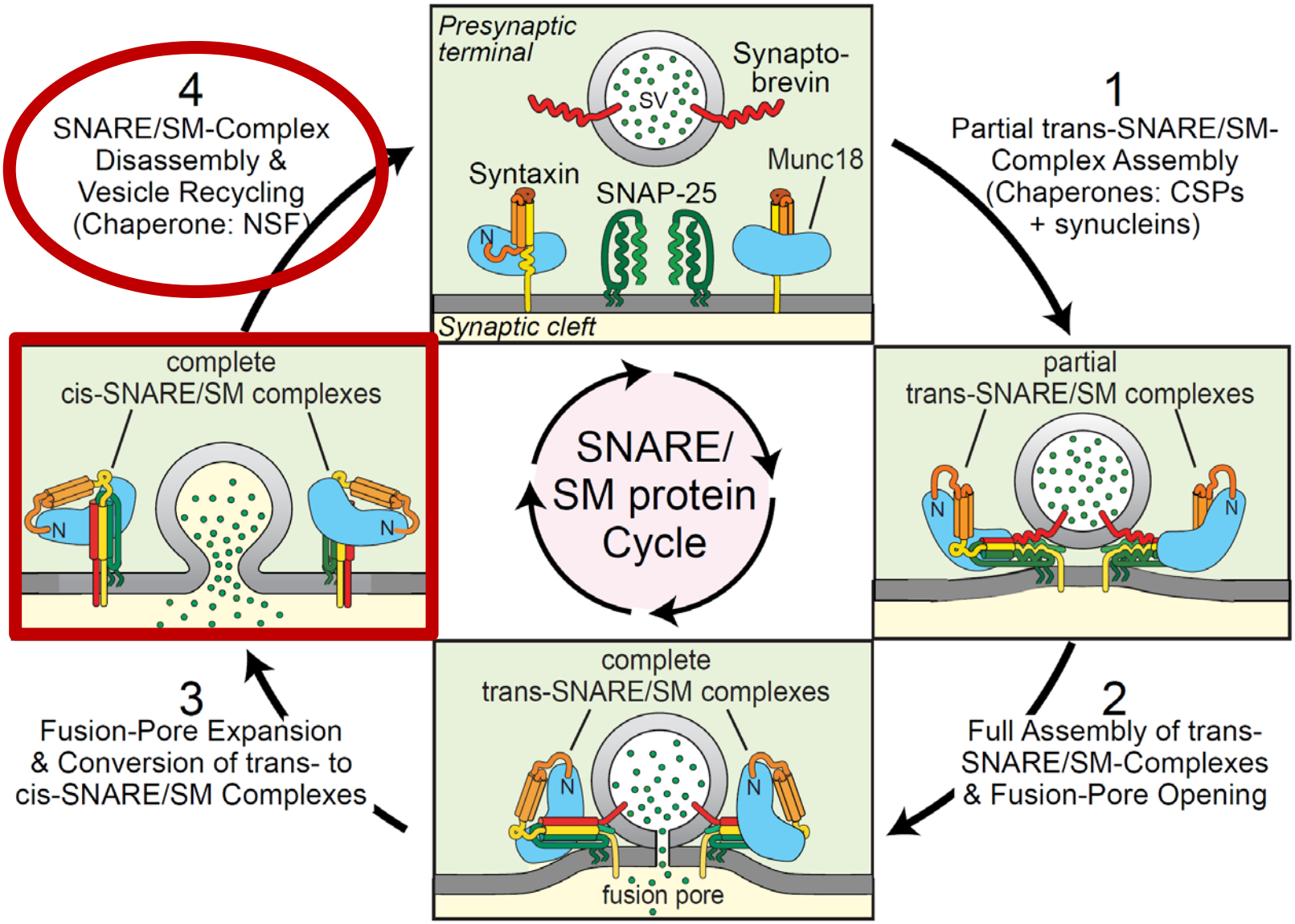

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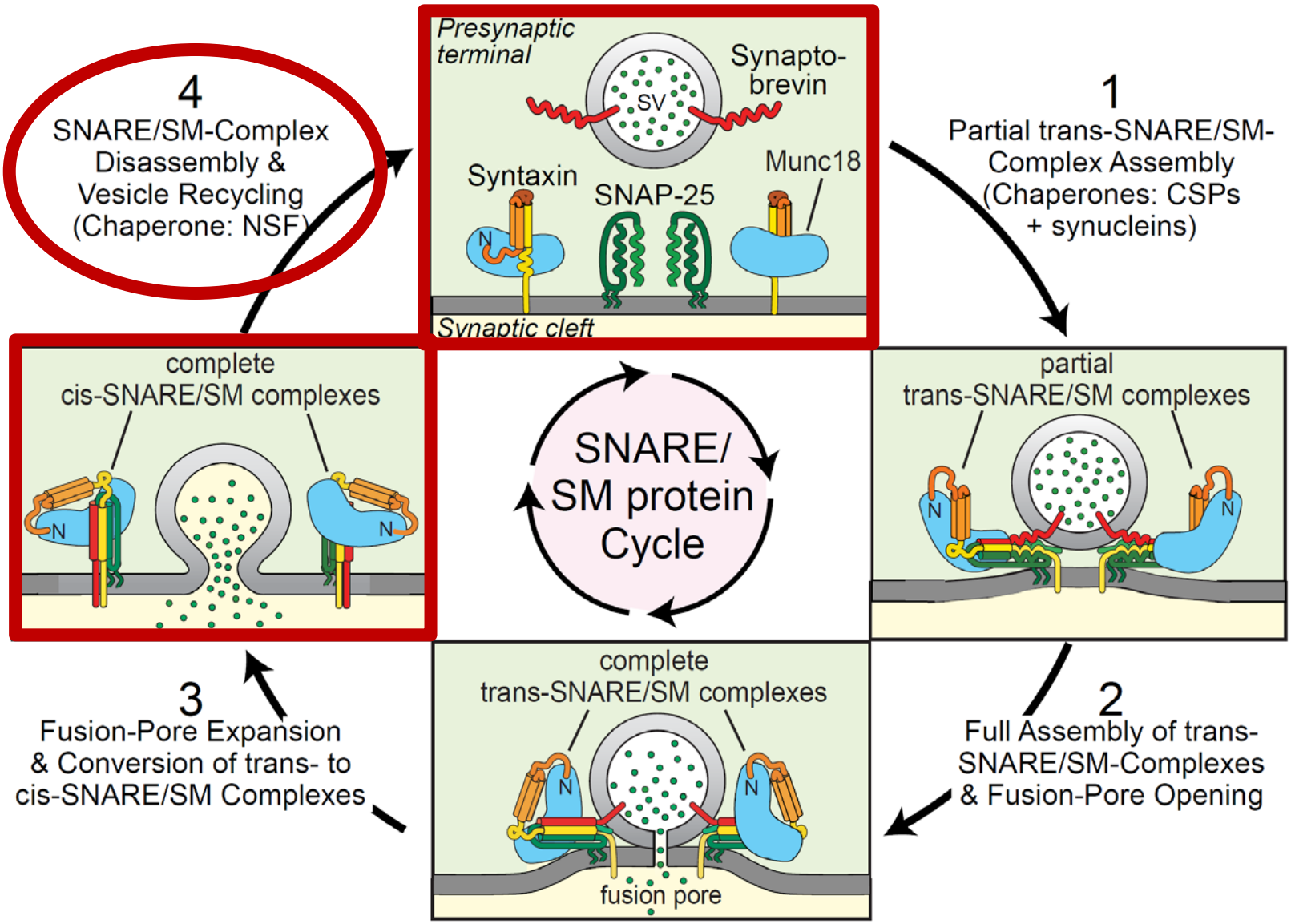

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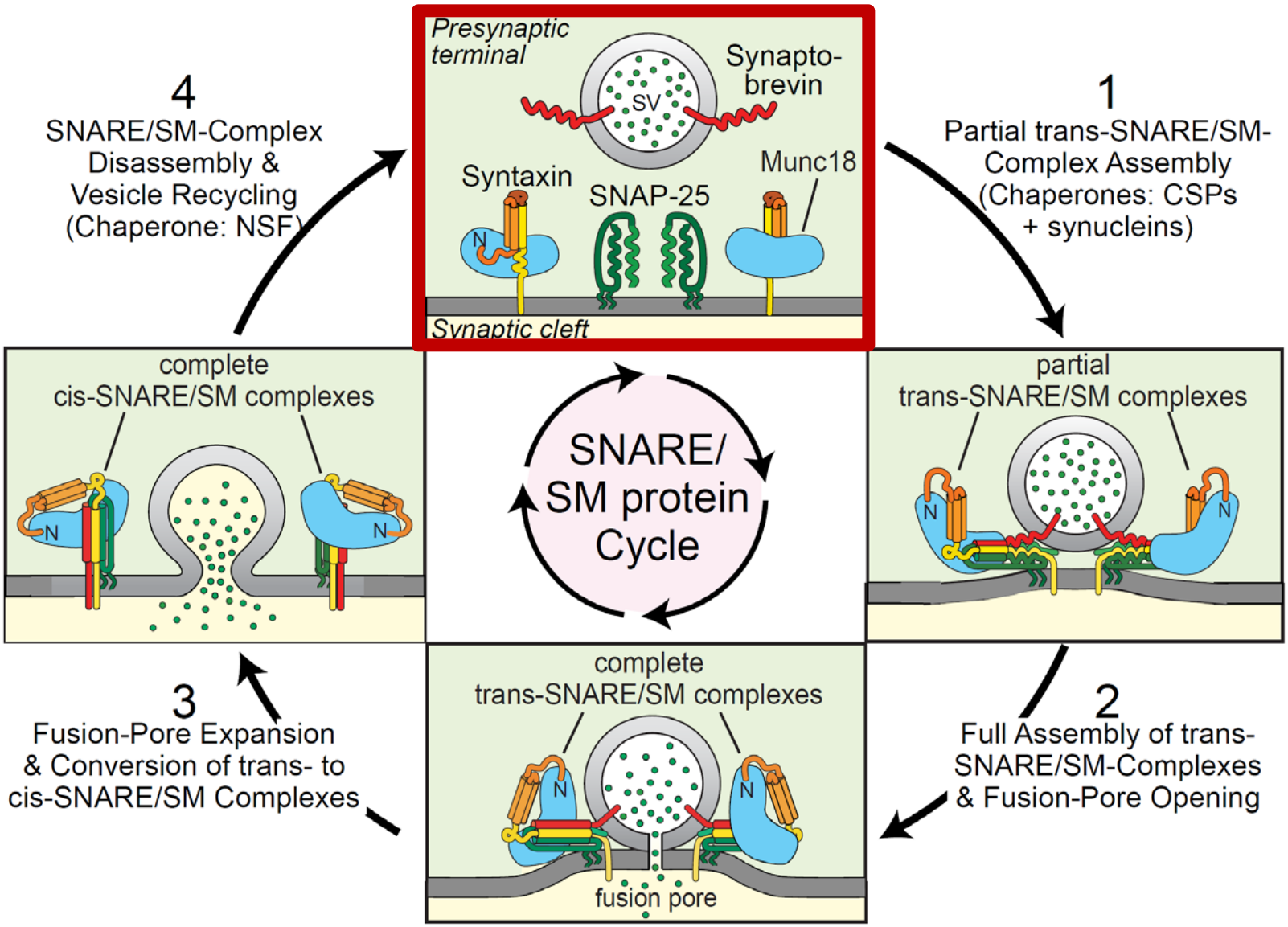

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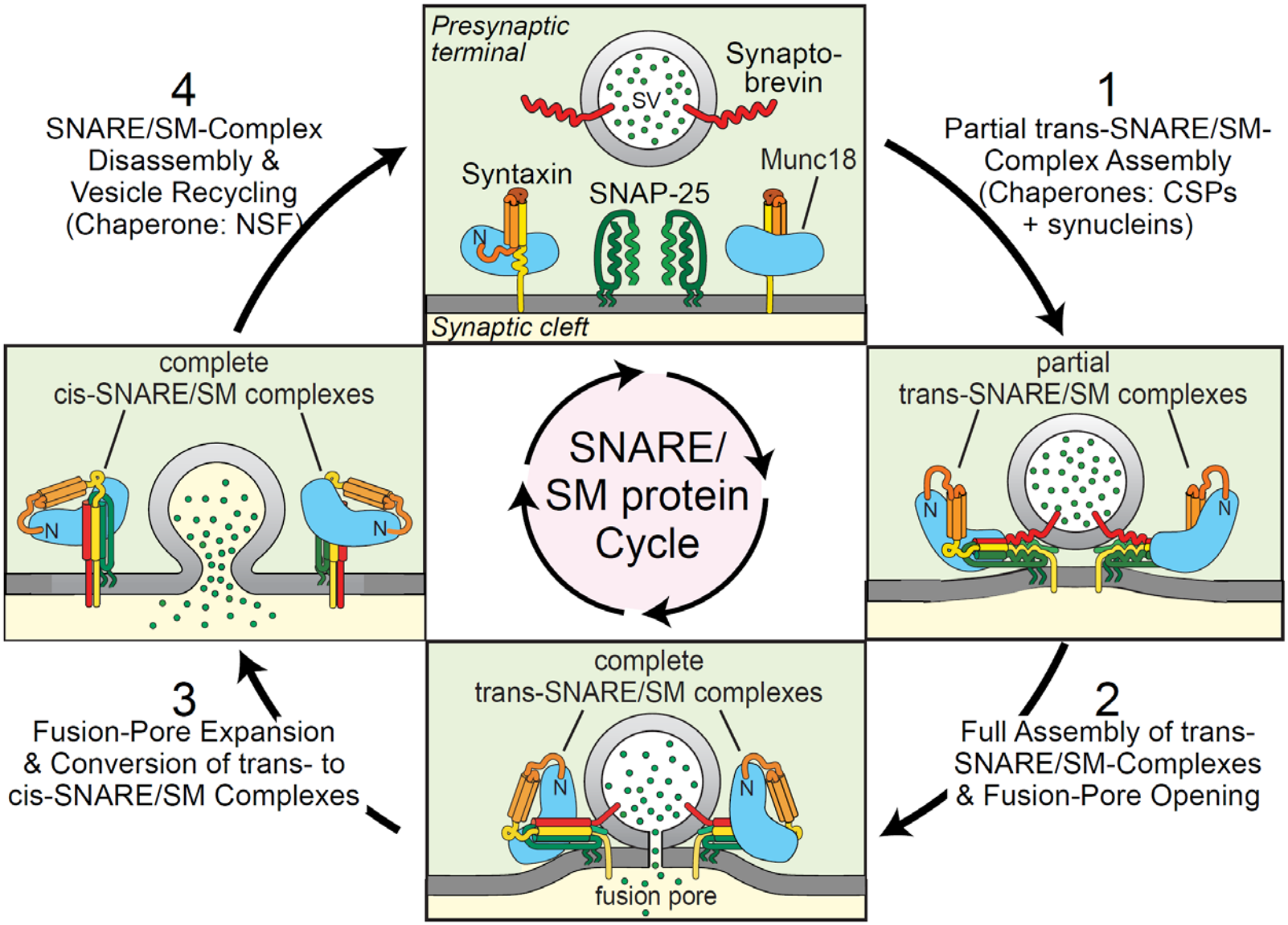
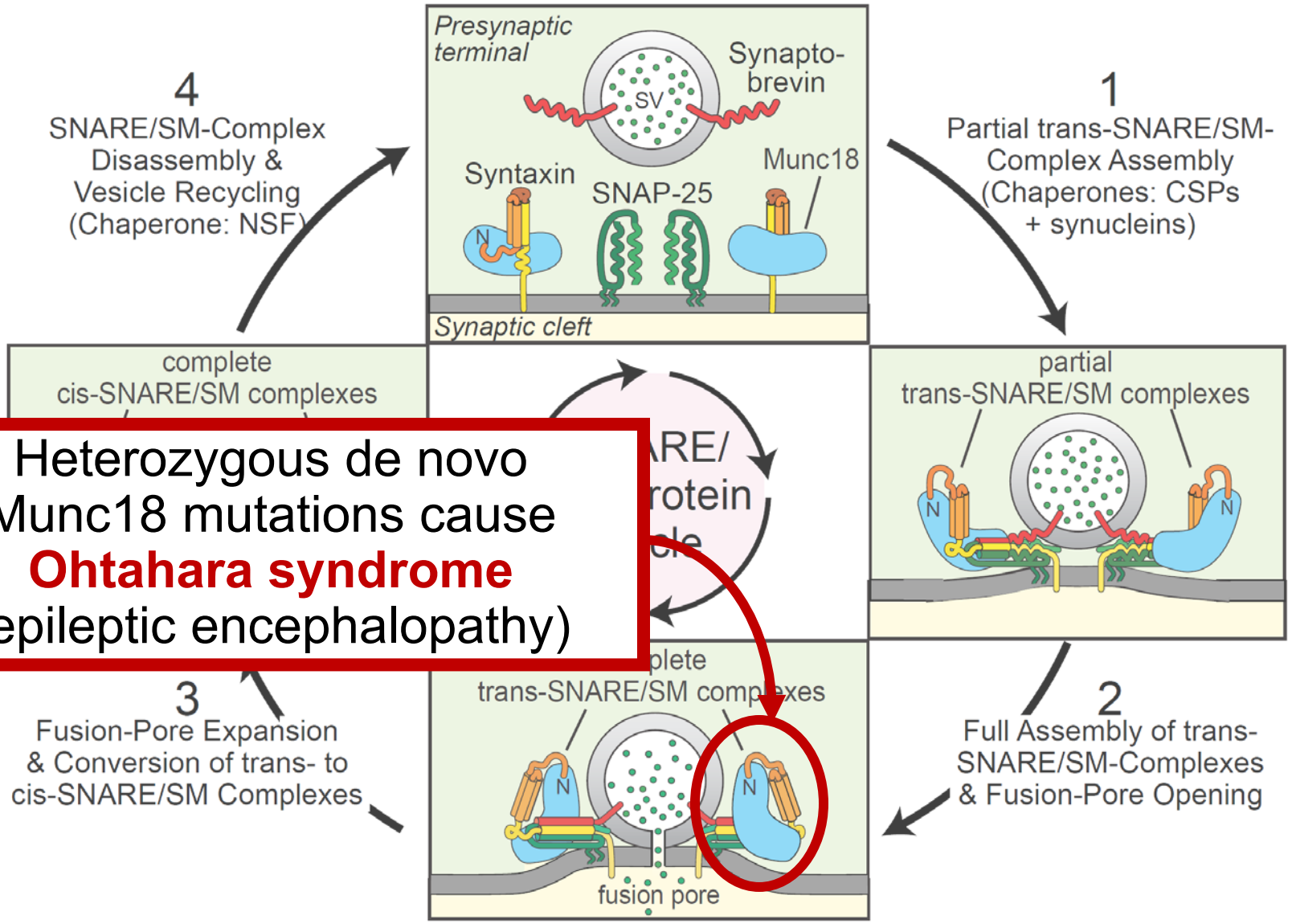


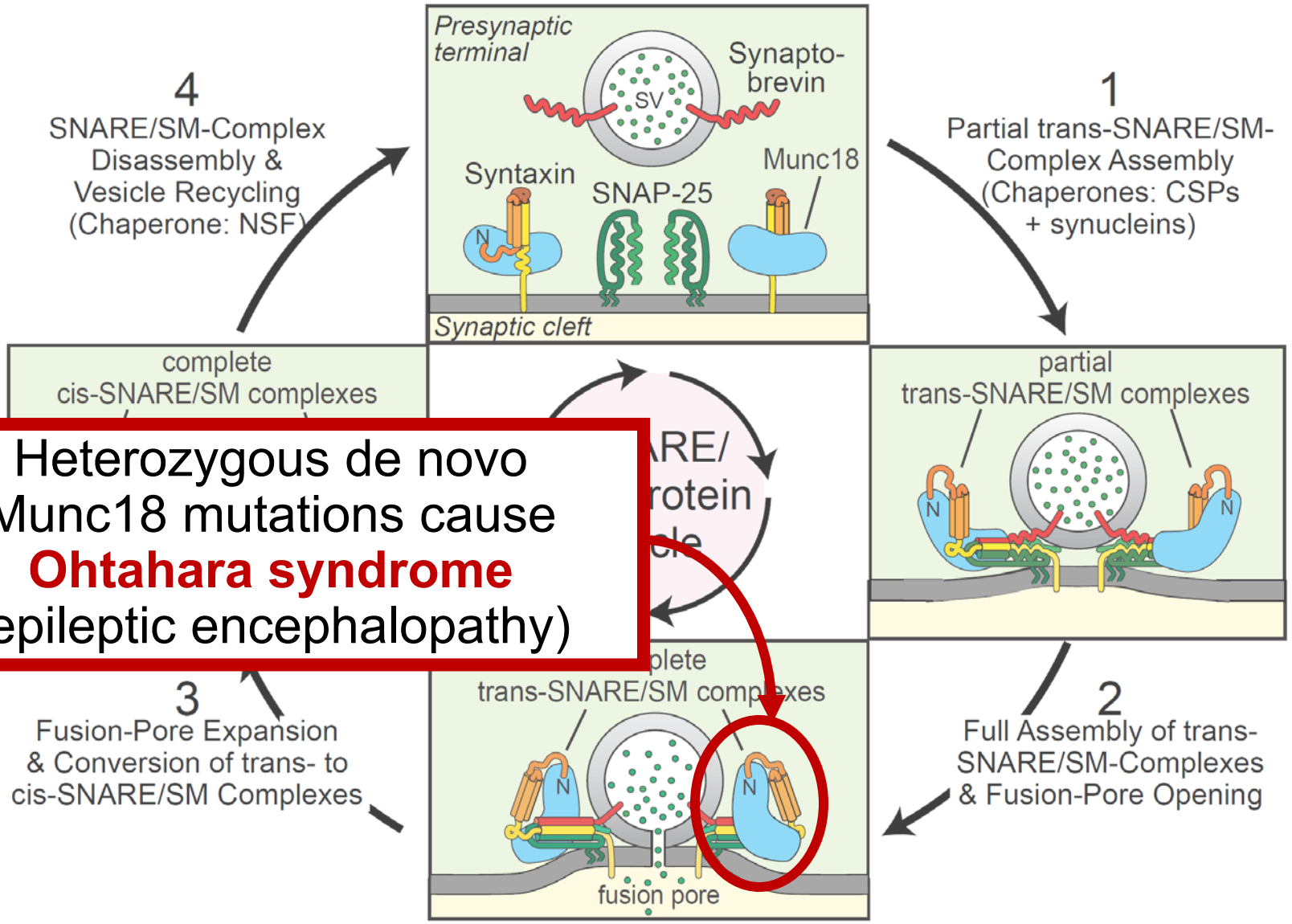
Disease implications

SNARE/SM Protein Complex Assembly Drives Fusion



Heterozygous de novo Munc18 mutations cause **Ohtahara syndrome** (epileptic encephalopathy)

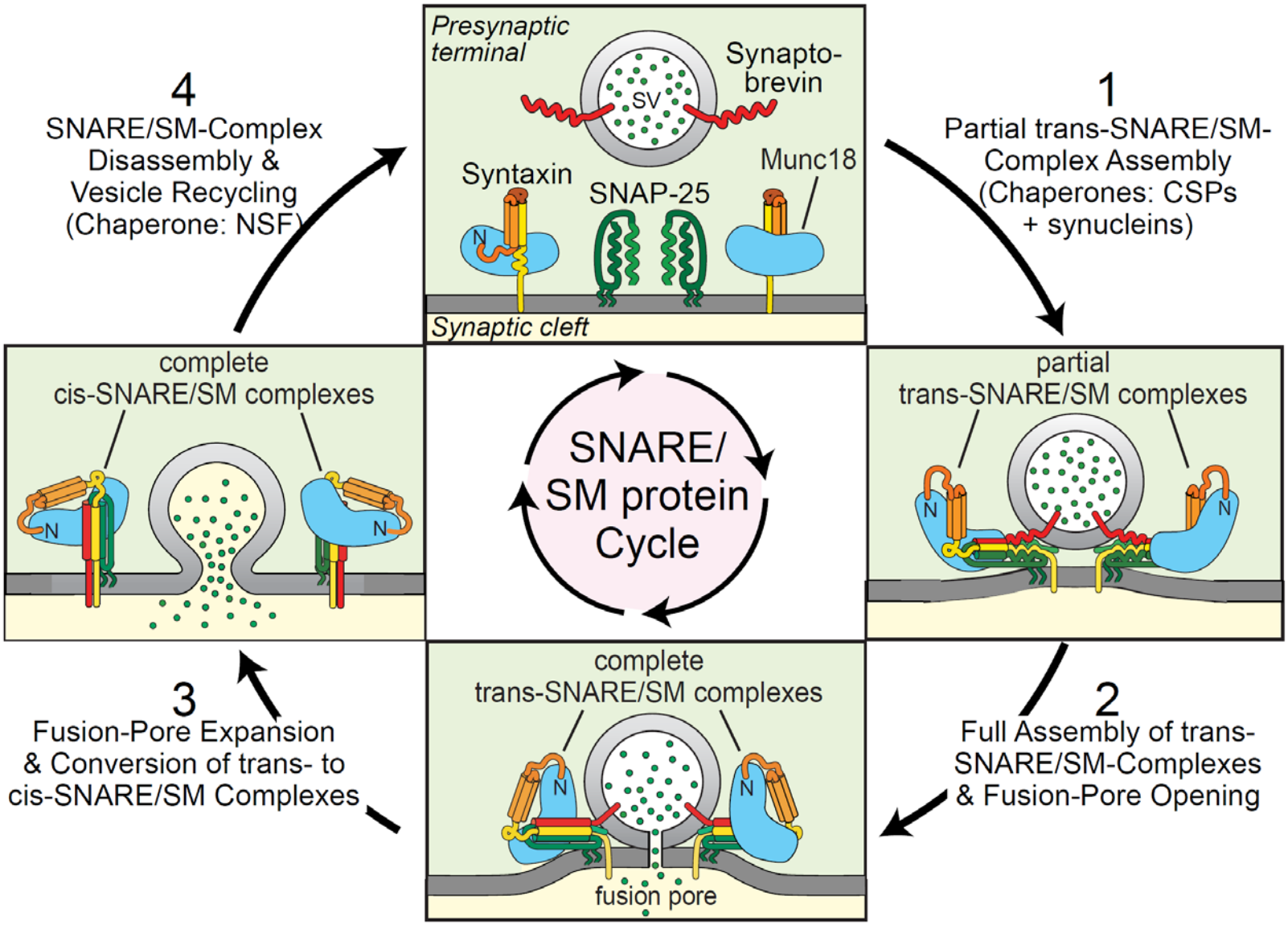
SNARE/SM Protein Complex Assembly Drives Fusion



Heterozygous de novo Munc18 mutations cause **Ohtahara syndrome** (epileptic encephalopathy)

Reinforces the importance of Munc18

SNARE/SM Protein Complex Assembly Drives Fusion



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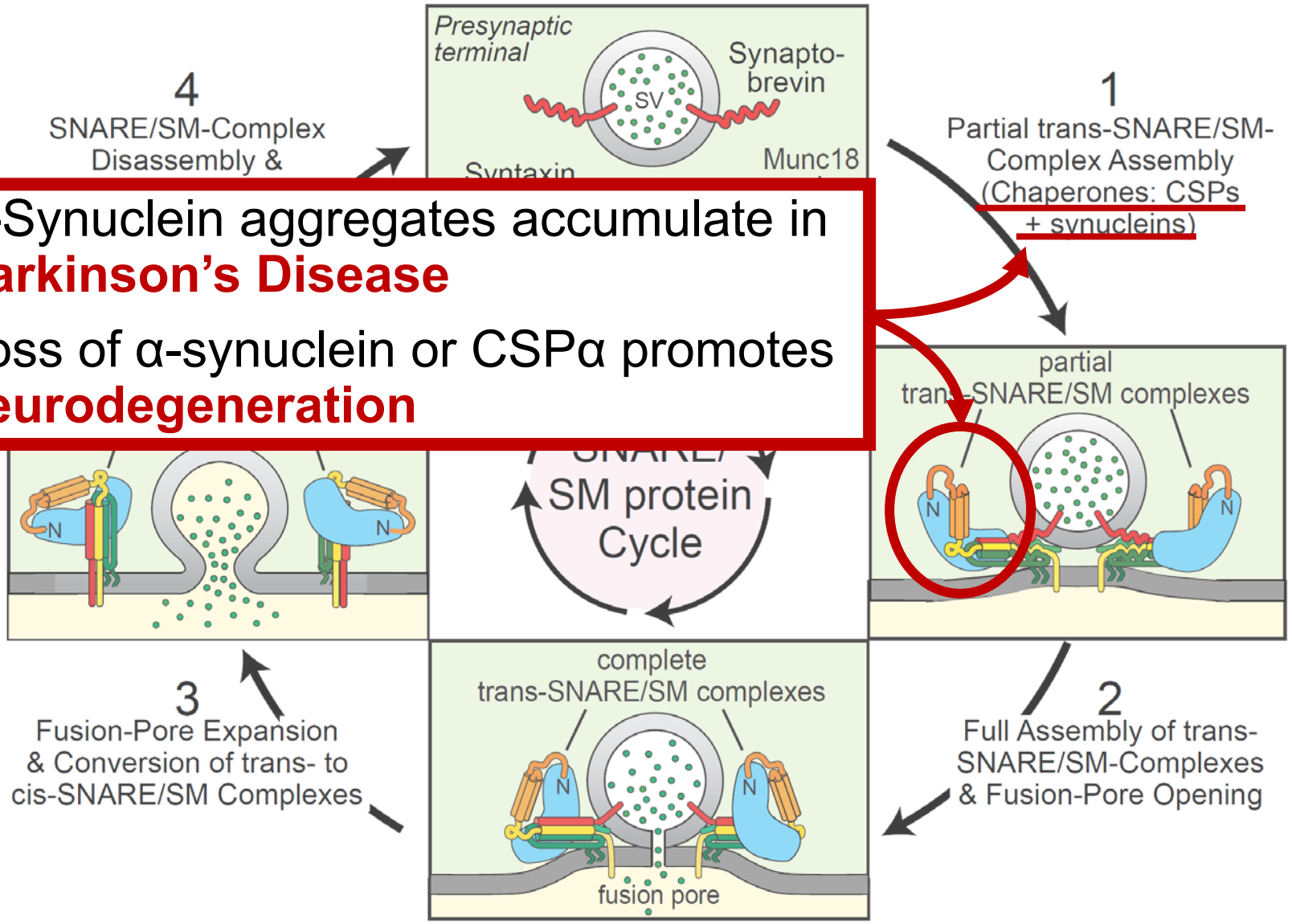

- α-Synuclein aggregates accumulate in **Parkinson's Disease**
- Loss of α-synuclein or CSPα promotes **neurodegeneration**

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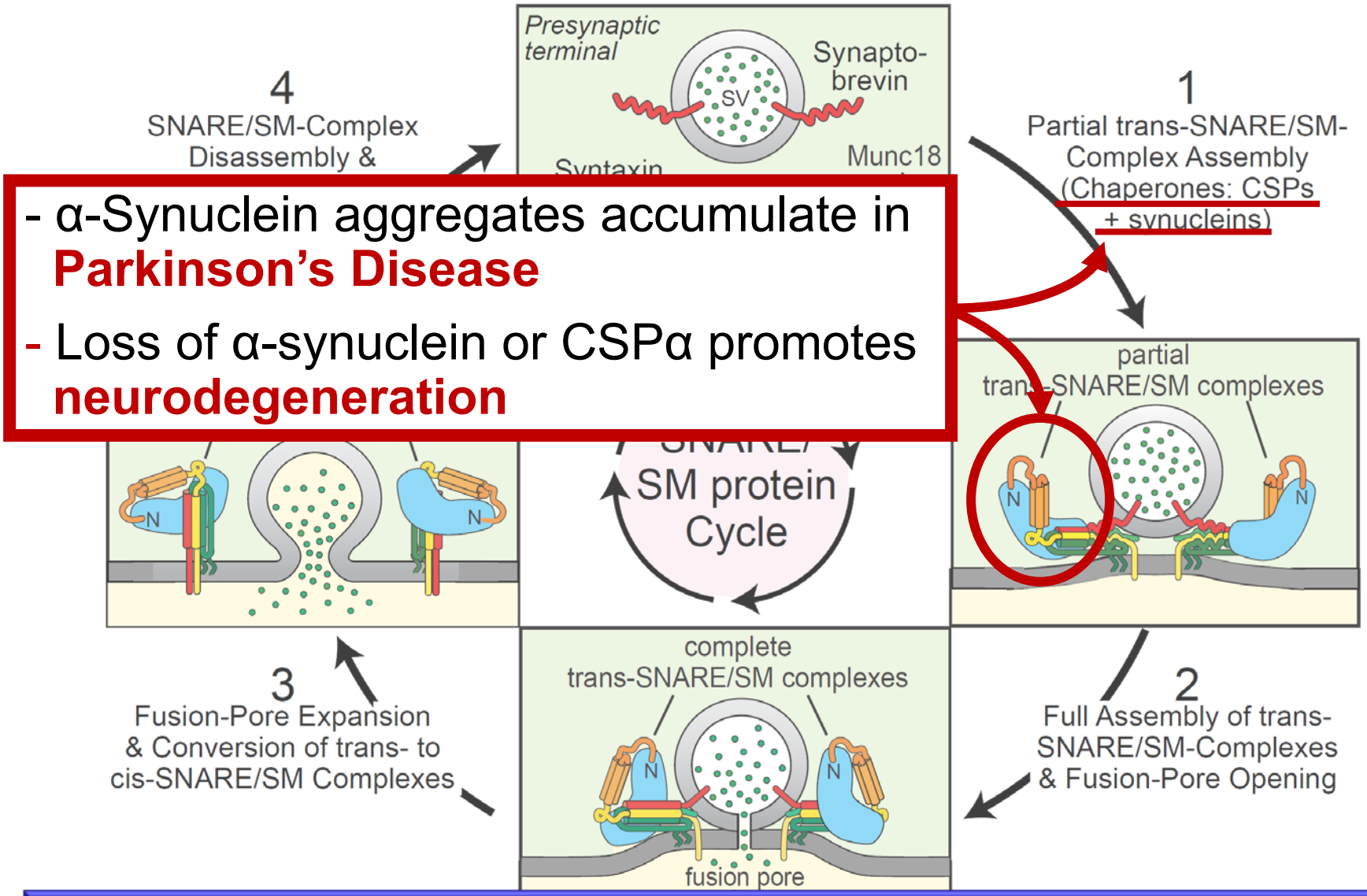
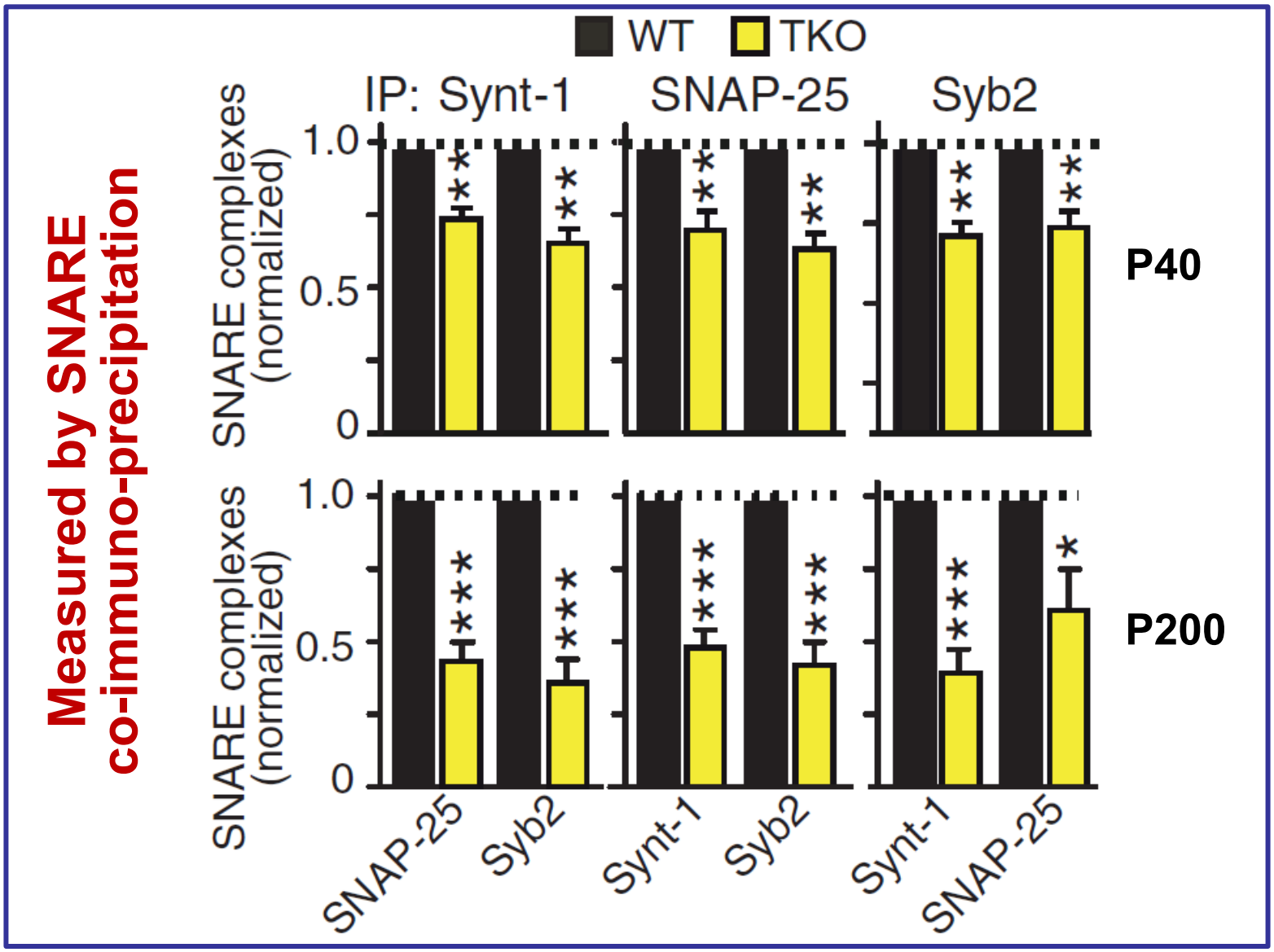


- α-Synuclein aggregates accumulate in **Parkinson's Disease**
- Loss of α-synuclein or CSPα promotes **neurodegeneration**

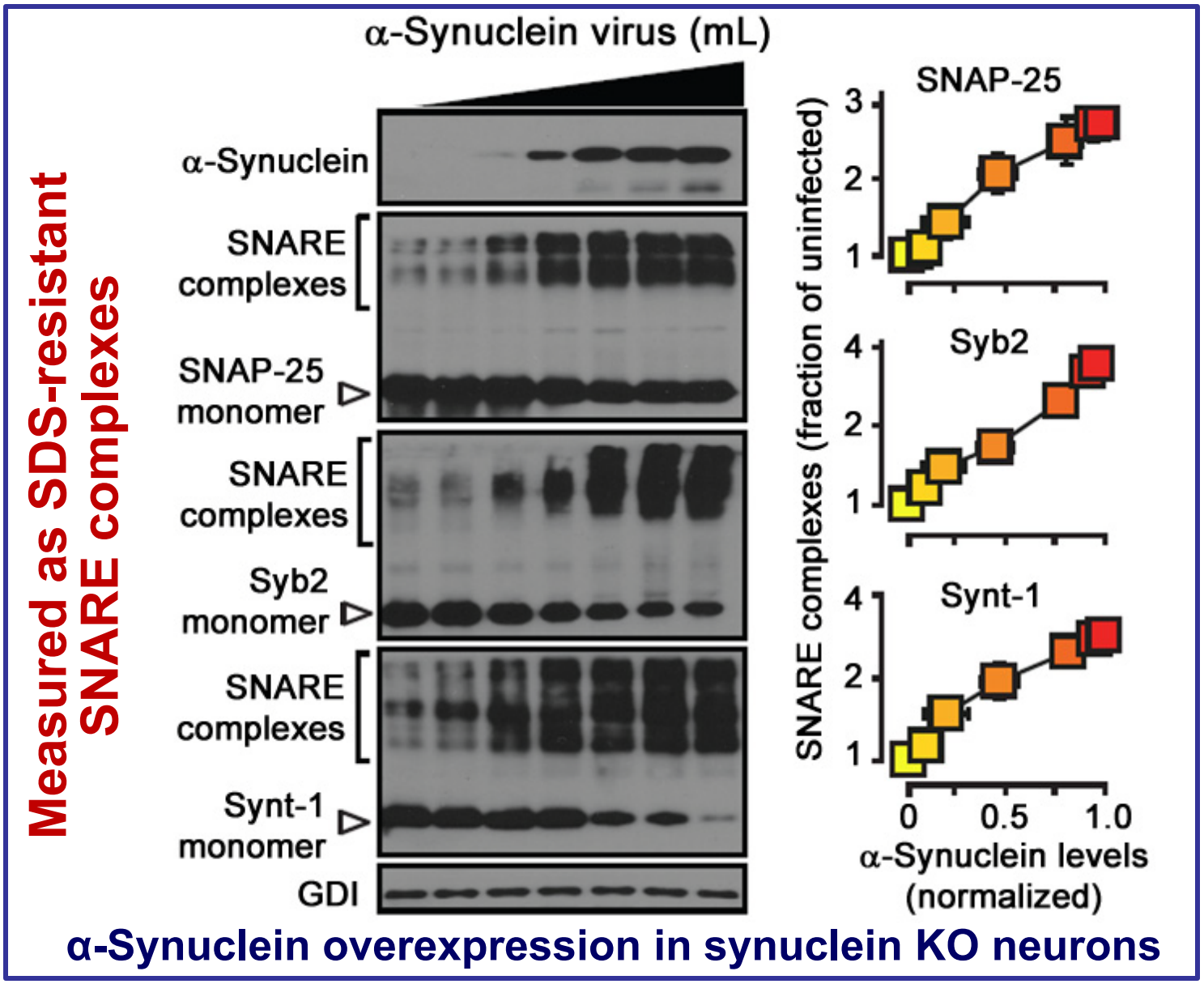
Let me illustrate …

Deletion of Synucleins Causes Age-Dependent Impairment of SNARE-Complex Assembly



Burre et al., Science 2010

α-Synuclein Catalyzes SNARE-Complex Assembly



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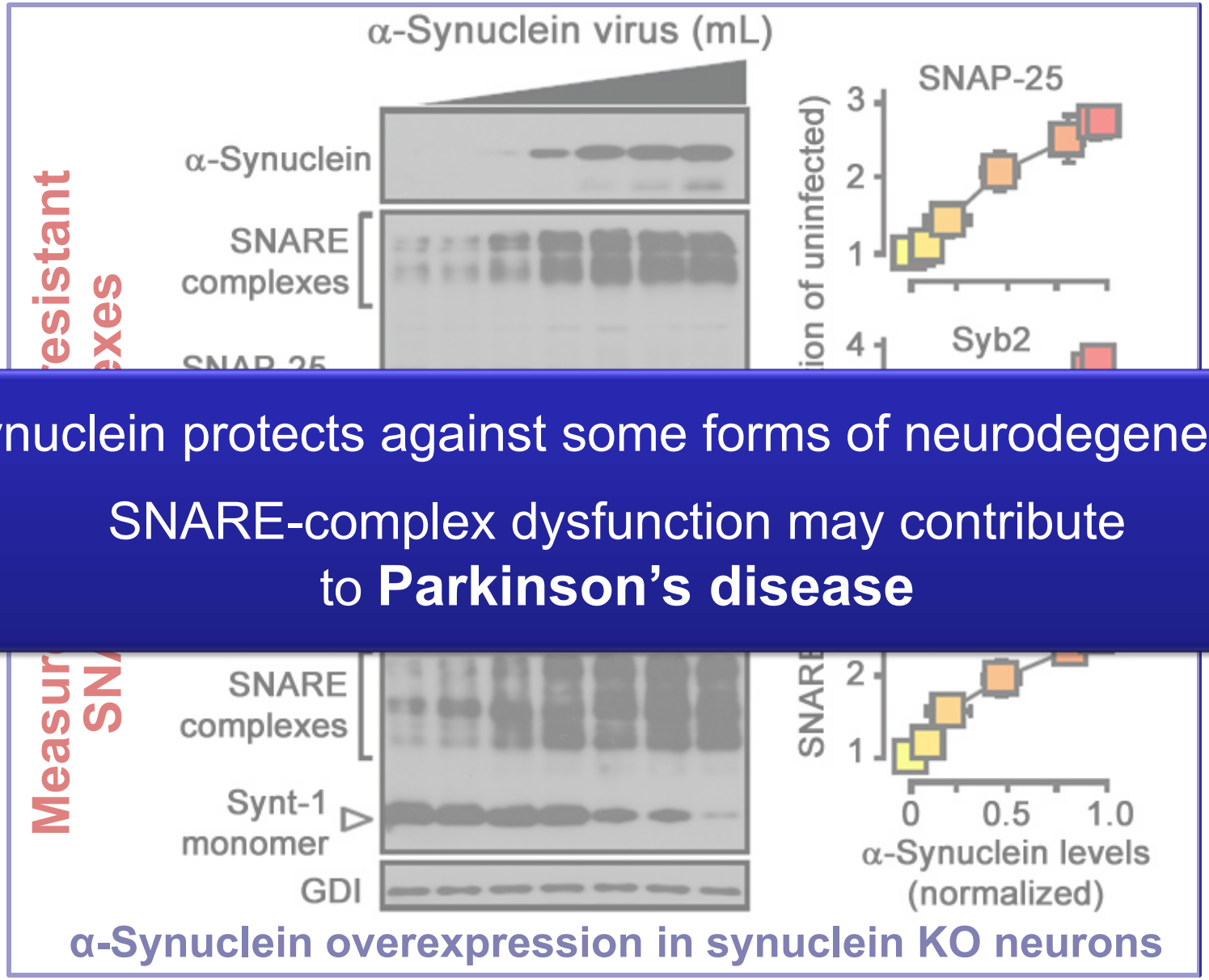


α-Synuclein protects against some forms of neurodegeneration

SNARE-complex dysfunction may contribute to **Parkinson's disease**

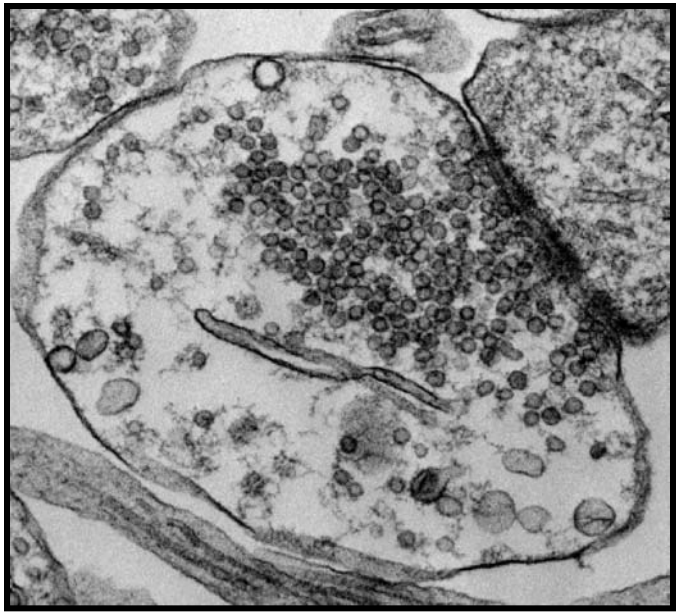
Burre et al., Science 2010

Three Processes Govern Neurotransmitter Release

1. Synaptic vesicle fusion

2. Ca^{2+}-triggering of fusion
   - Very fast: ~0.1 msec
   - Cooperative: ~5 Ca^{2+}-ions

3. Localized Ca^{2+}-influx



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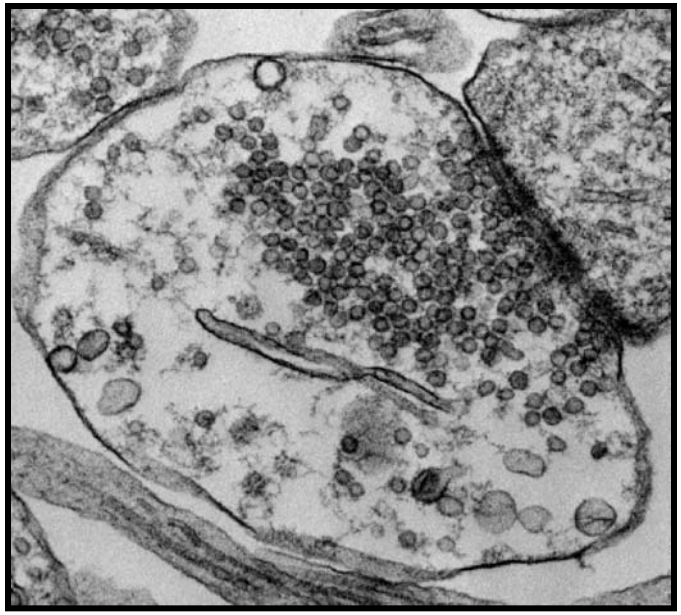
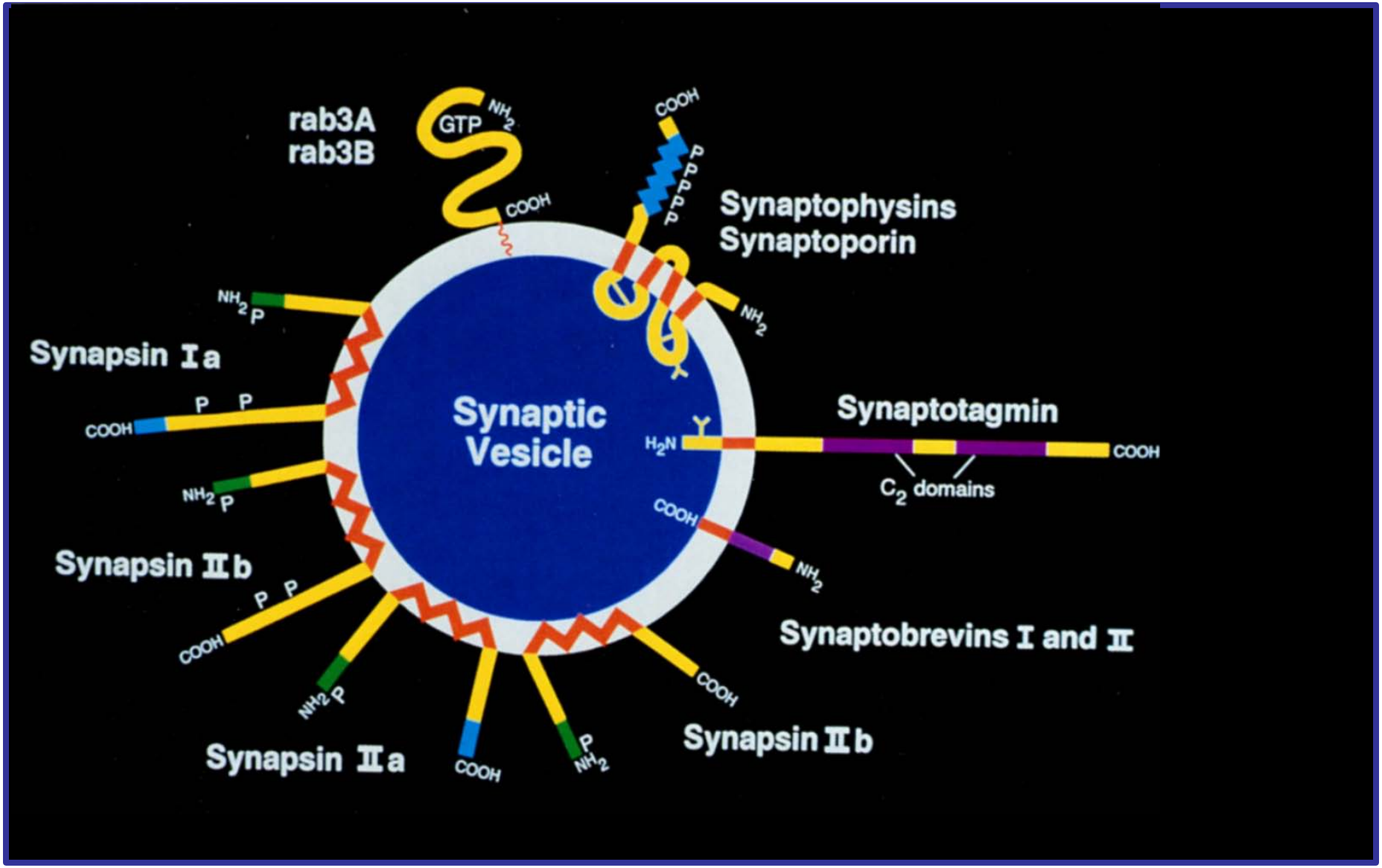


At the same time as we were studying synaptic fusion, we systematically characterized synaptic vesicle proteins

This approach led (among others) to the discovery of synaptotagmin, the Ca^{2+}-sensor for neurotransmitter release

Systematic Analysis of Synaptic Vesicle Proteins Identifies Synaptotagmin-1



Südhof and Jahn, Neuron 1991

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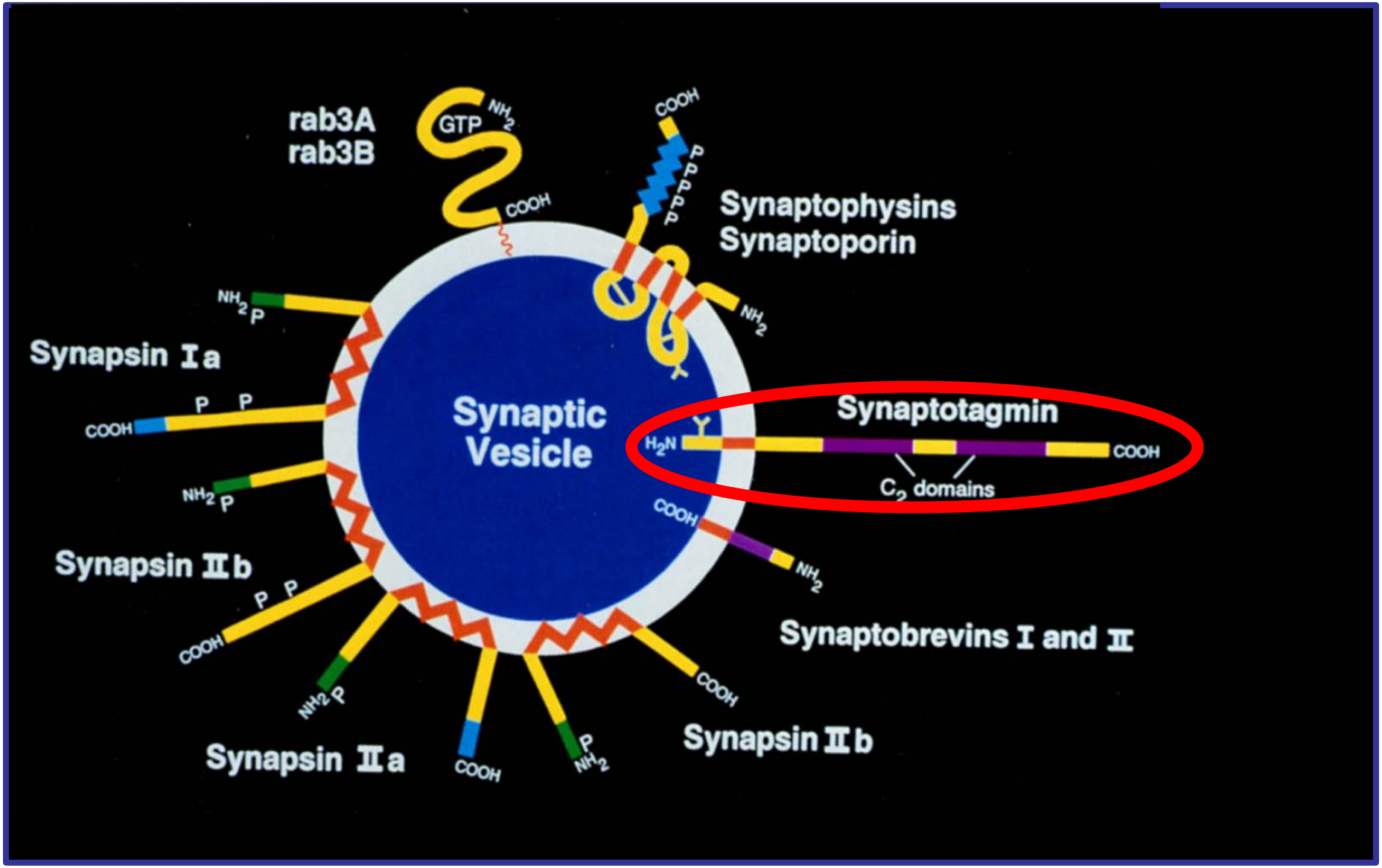

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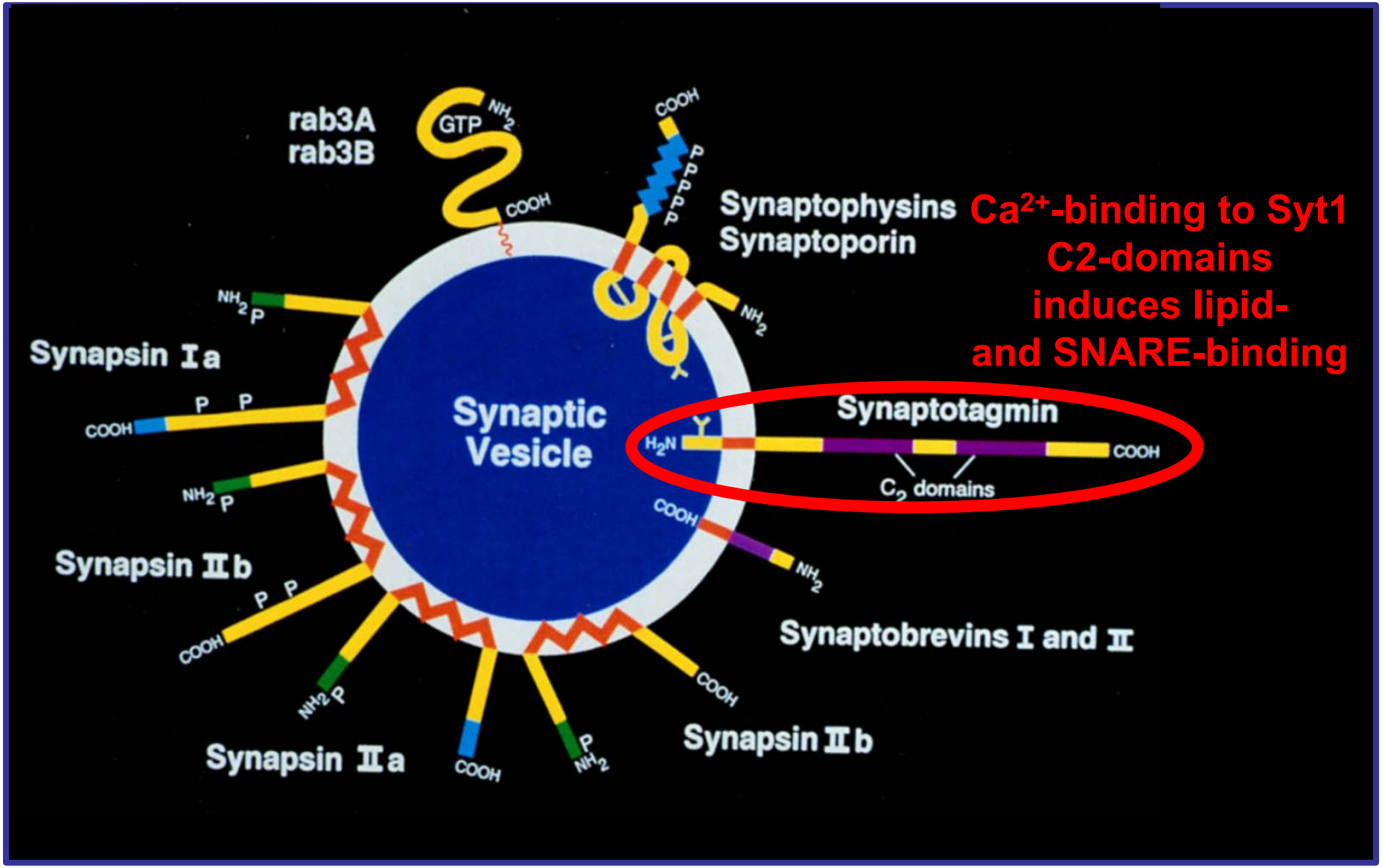

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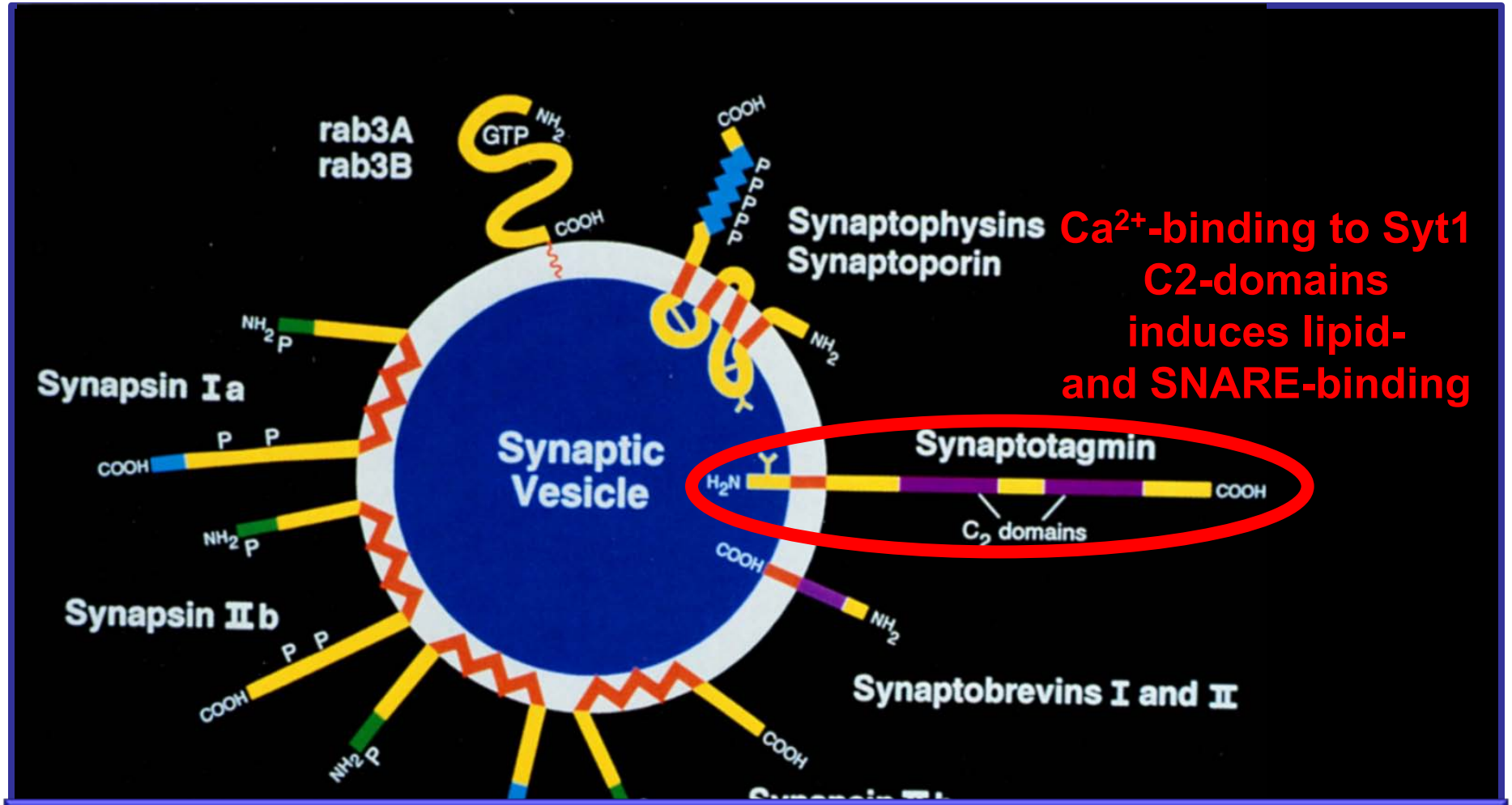
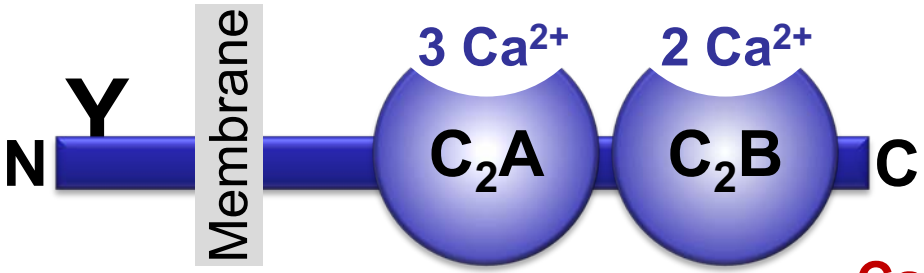
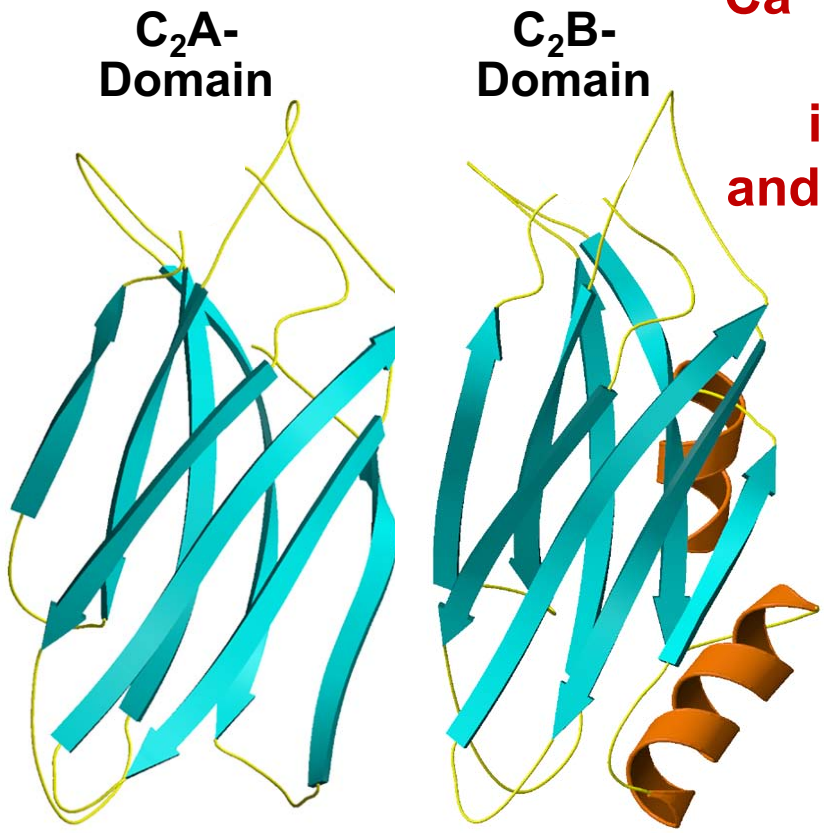


How does synaptotagmin-1 bind Ca^{2+}, and what is its physiological significance?

Synaptotagmin-1 is a Synaptic Vesicle Ca^{2+}-Binding Protein





Ca^{2+}-binding to Syt1 C2-domains induces lipid- and SNARE-binding

Perin et al., Nature 1990; Brose et al., Science 1992; Davletov & Südhof, 1993; Li et al., Nature 1995; Sutton et al., Cell 1995; Chen et al., Neuron 2001

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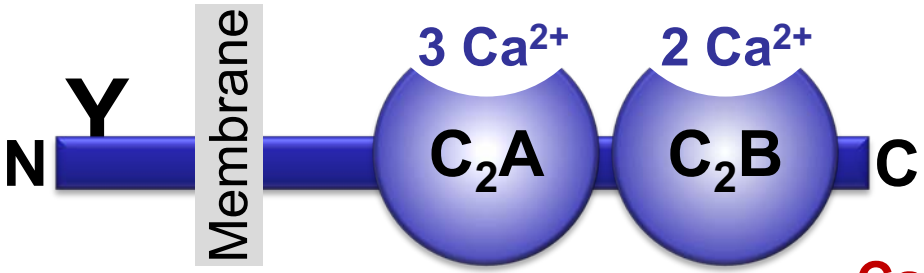

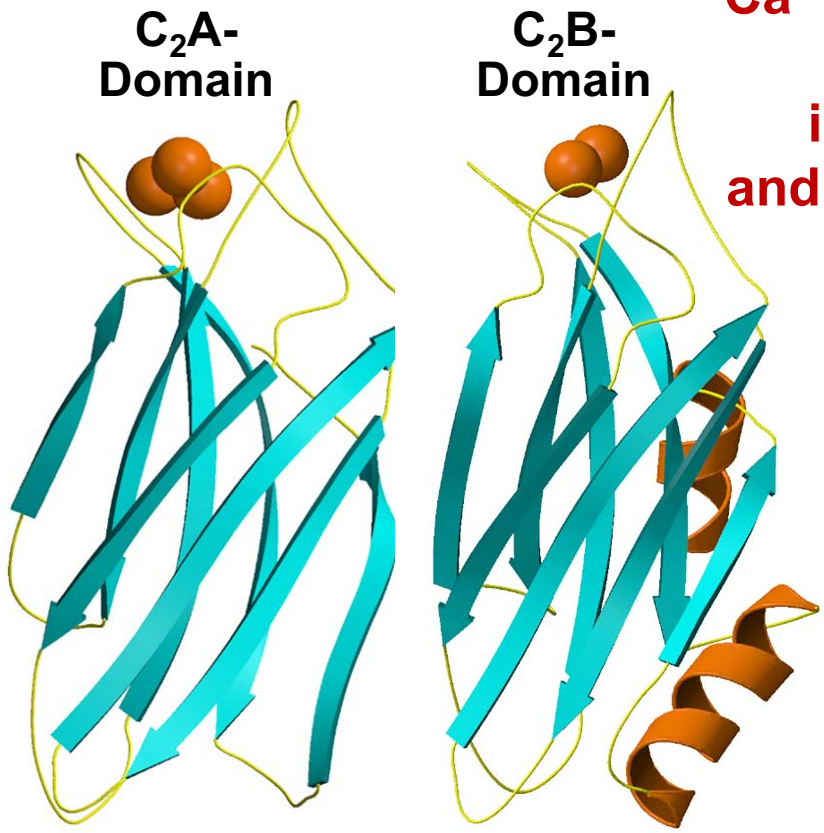

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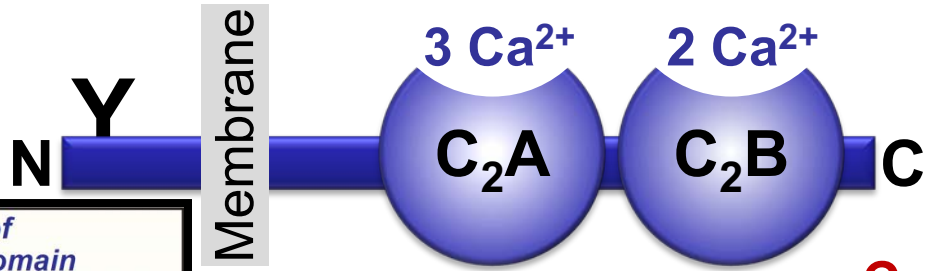
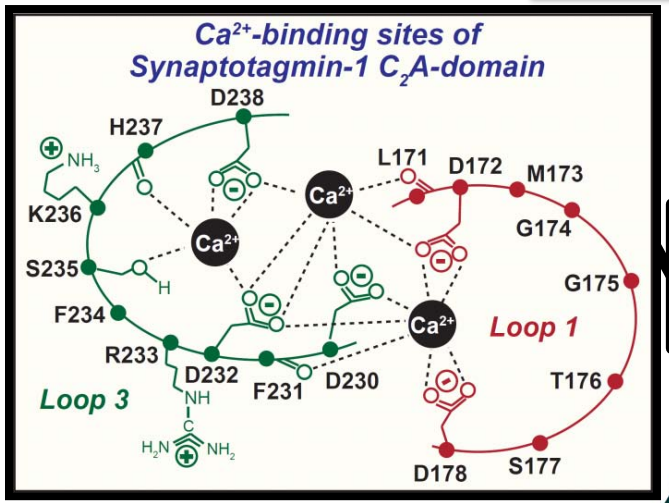
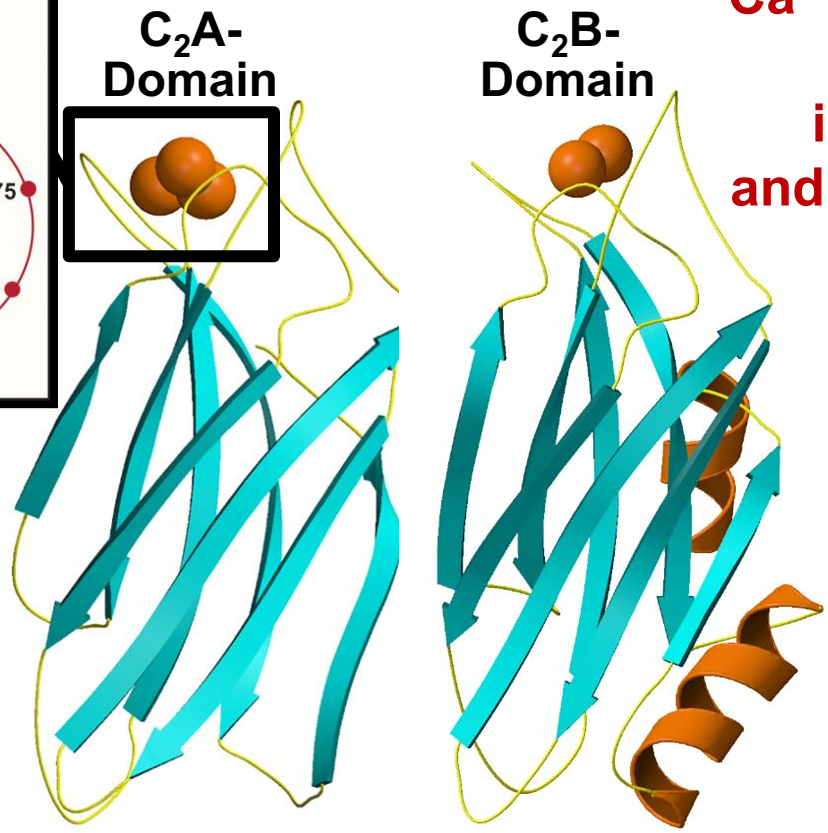
Architecture of Synaptotagmin-1 Ca^{2+}-Binding Sites







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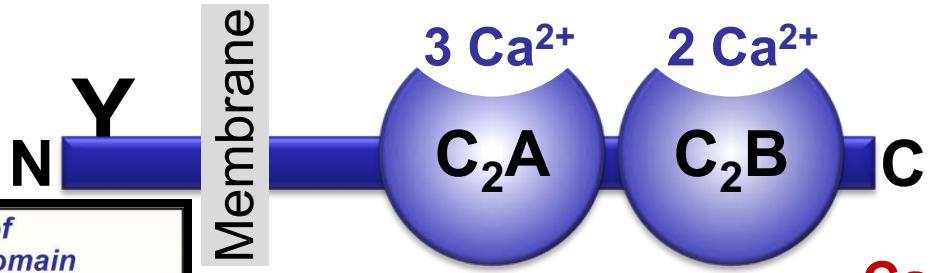
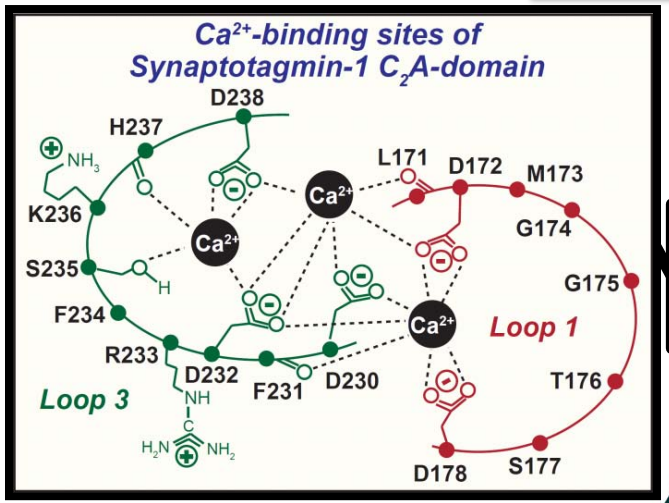
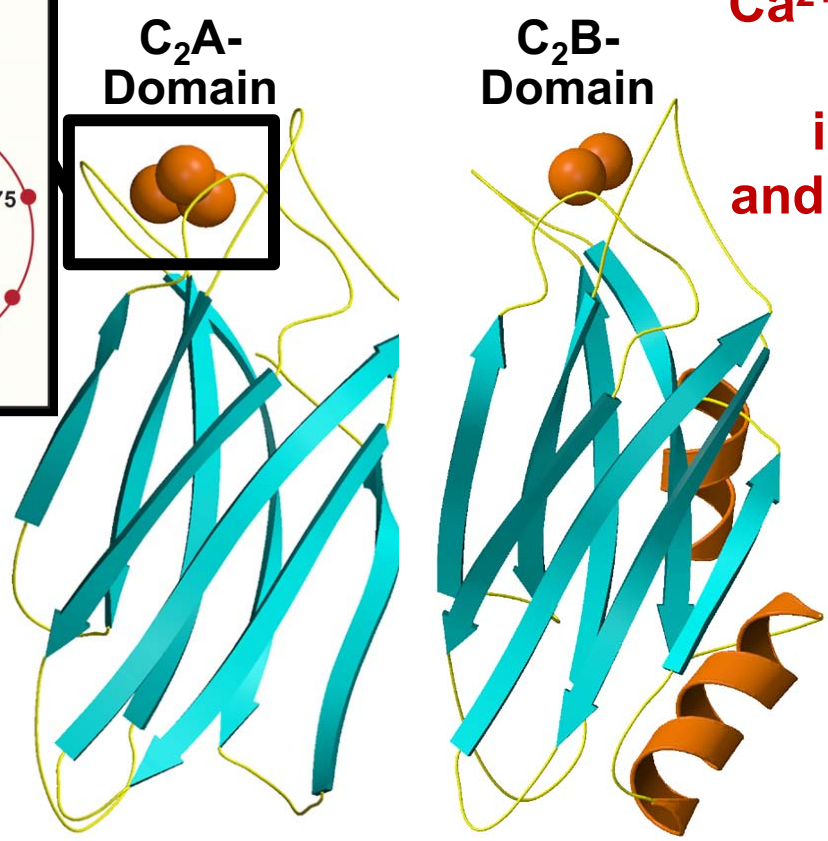


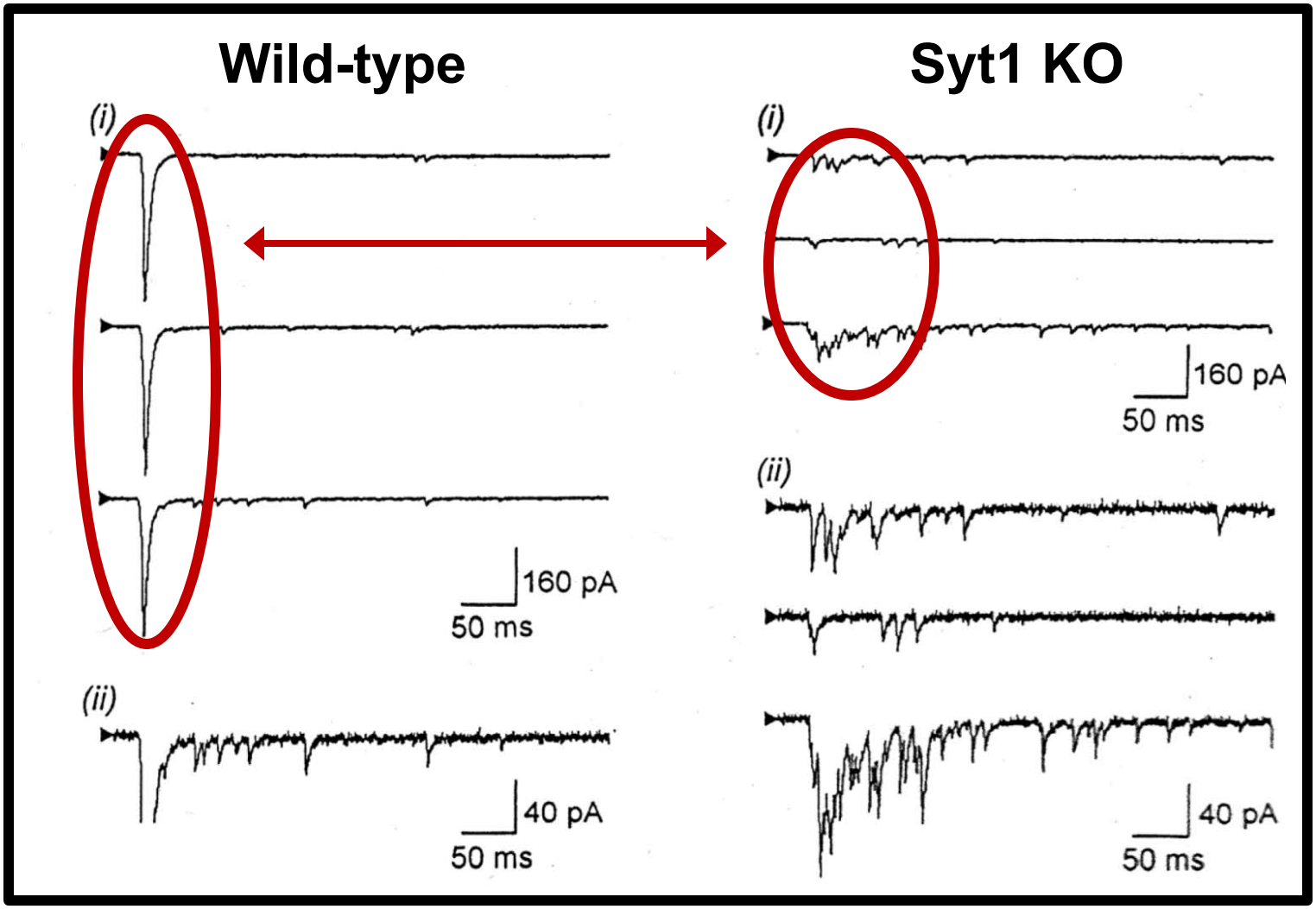




Ca^{2+}-binding to Syt1 C2-domains induces lipid- and SNARE-binding

Does knockout of Syt1 impair Ca^{2+}-triggered release?

Synaptotagmin-1 is Essential for Ca^{2+}-Triggered Neurotransmitter Release

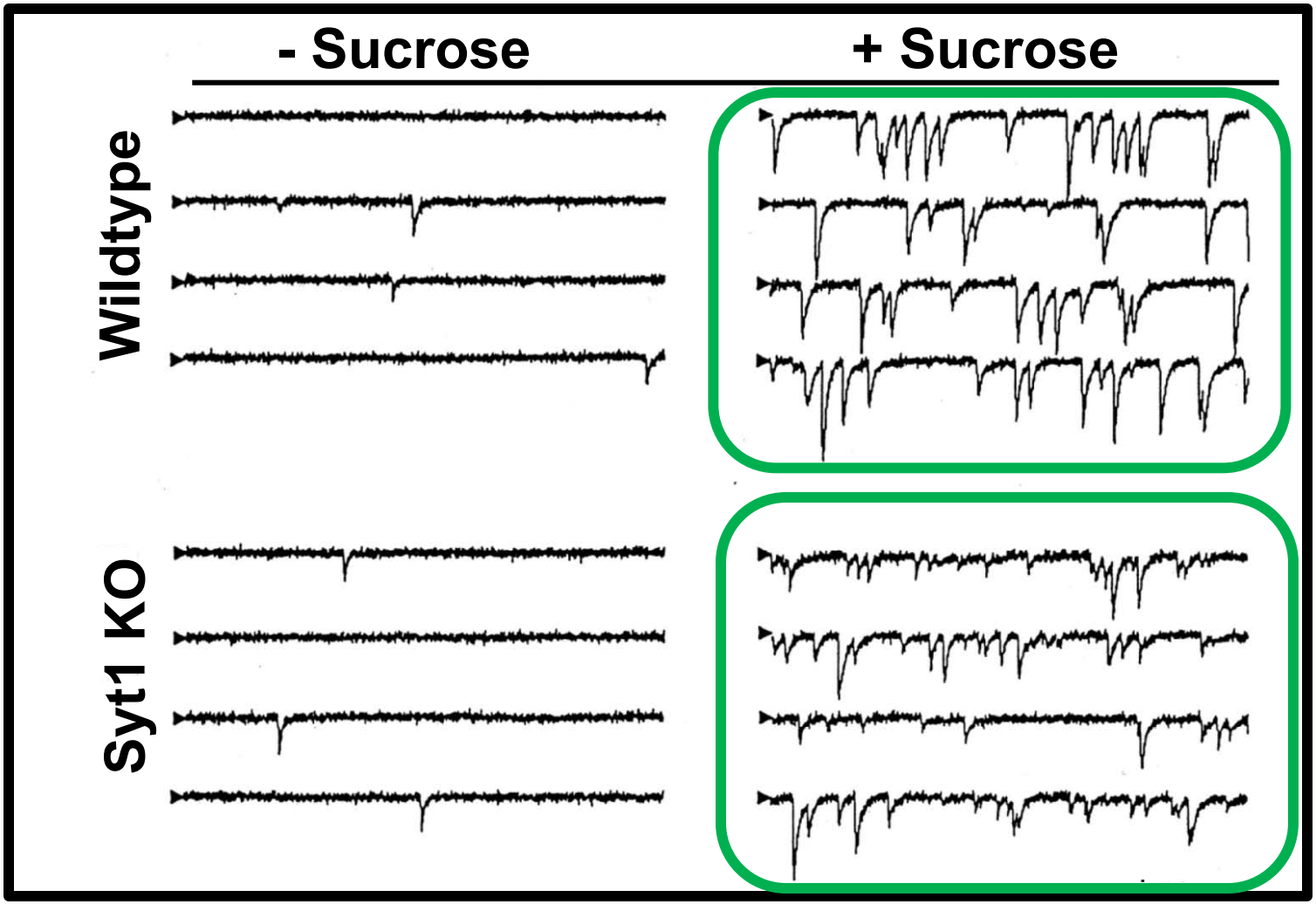


Release stimulated by isolated action potentials

Fast Ca^{2+}-triggered release is gone …

Geppert et al., Cell 1994

Synaptotagmin-1 is Not Essential for Sucrose-Stimulated Neurotransmitter Release

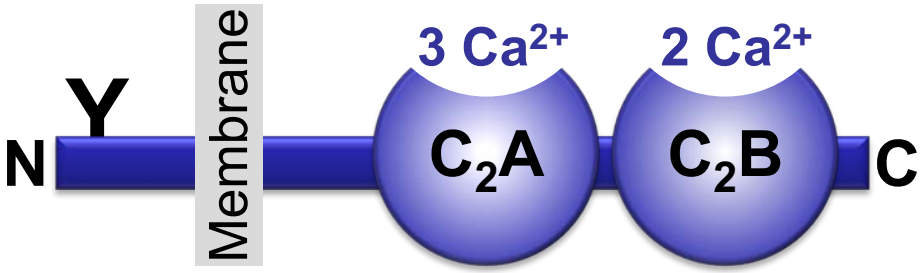


Hypertonic sucrose stimulates release by a Ca^{2+}-independent mechanism

Synaptotagmin is ONLY required for Ca^{2+}-triggered fusion

Geppert et al., Cell 1994

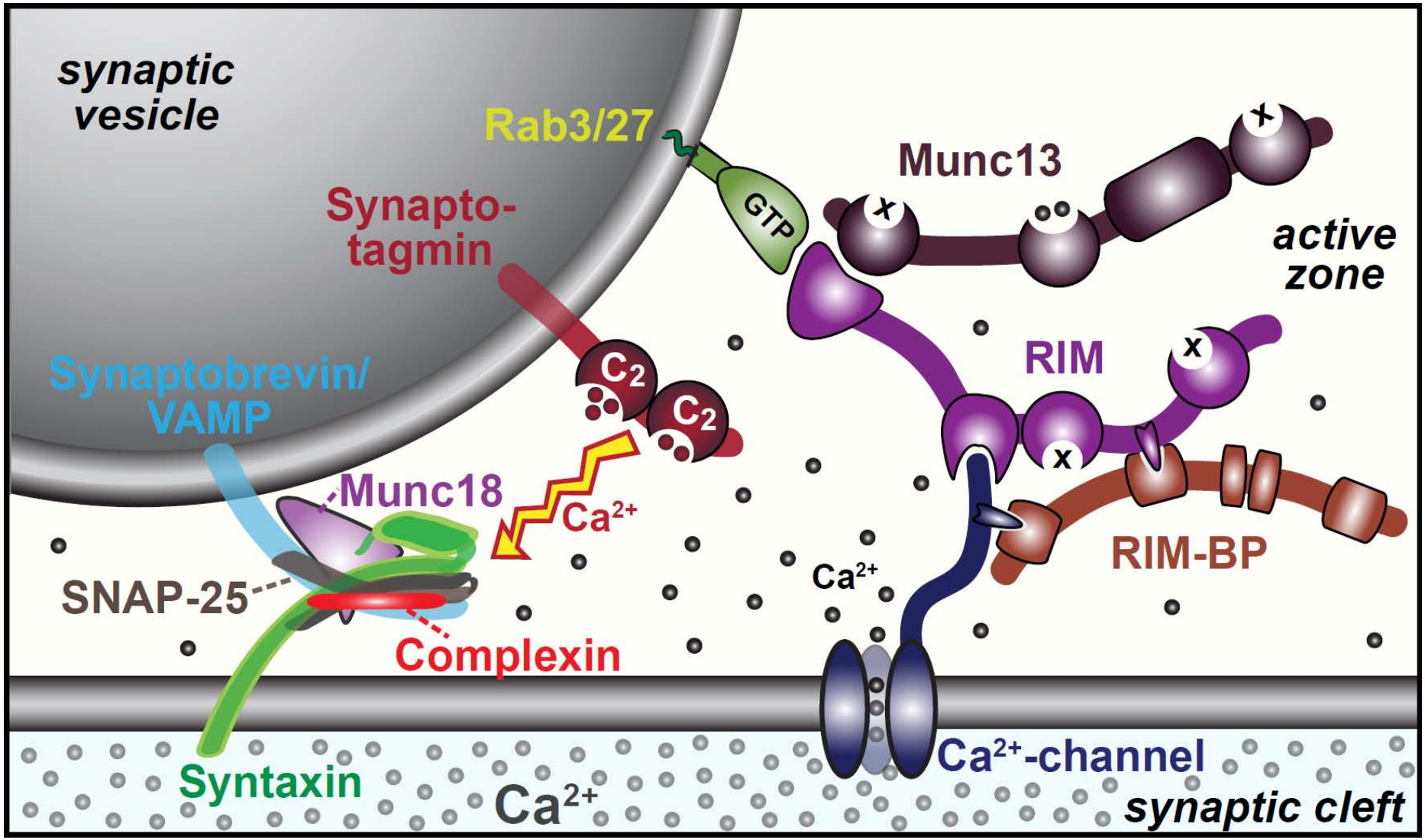
Synaptotagmin-1 is a Synaptic Vesicle Ca^{2+}-Sensor Essential for Ca^{2+}-Triggered Vesicle Fusion



- **Synaptotagmin-1 is a synaptic vesicle Ca^{2+}-binding protein**
- **Synaptotagmin-1 is essential for fast Ca^{2+}-triggered release**

However, synaptotagmin does not act alone - it needs an accomplice = **complexin**

A Neurotransmitter Release Machine Mediates Fusion, Ca^{2+}-triggering & Ca^{2+}-Channel Tethering



McMahon et al., Cell 1995; Chen et al., Neuron 2002

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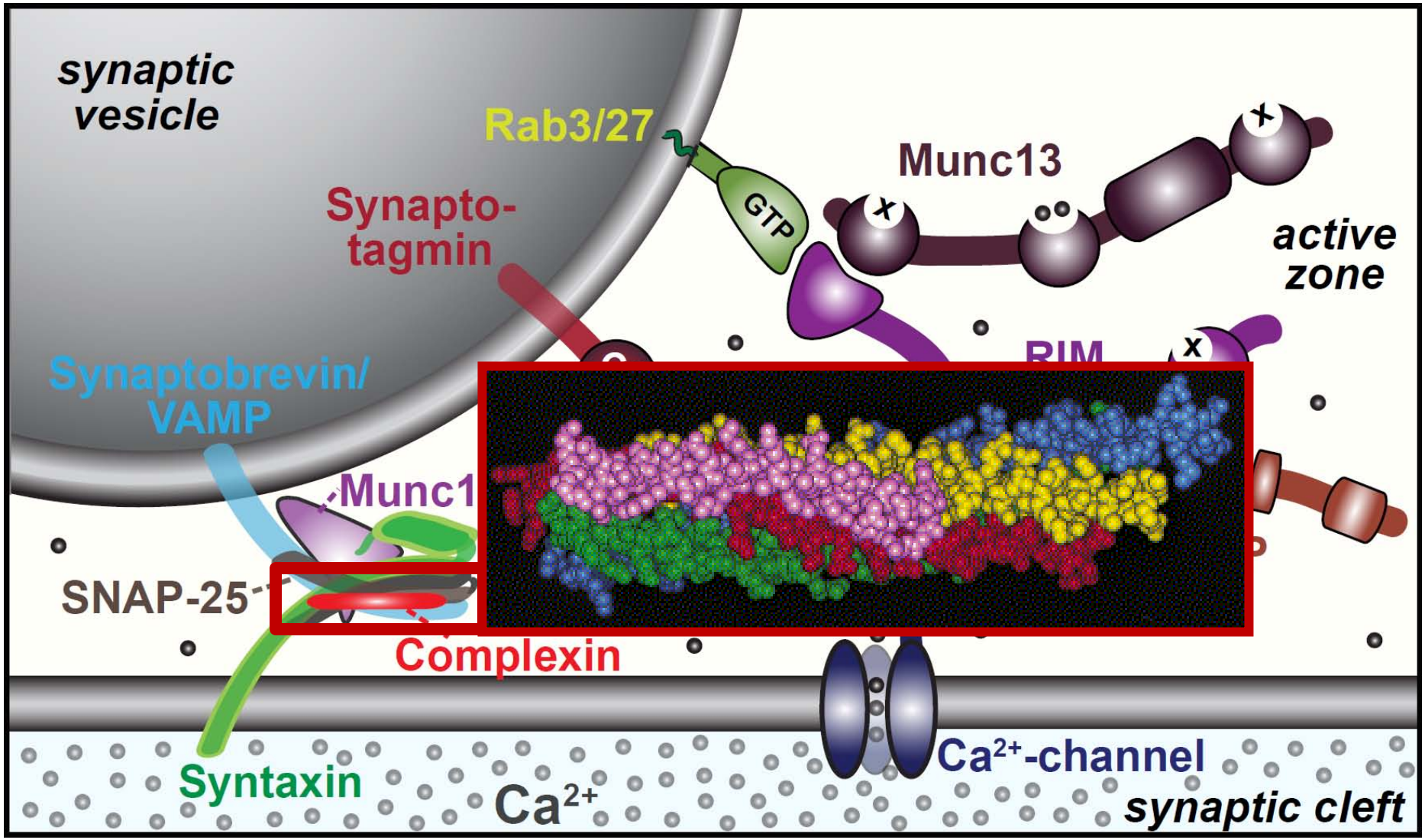

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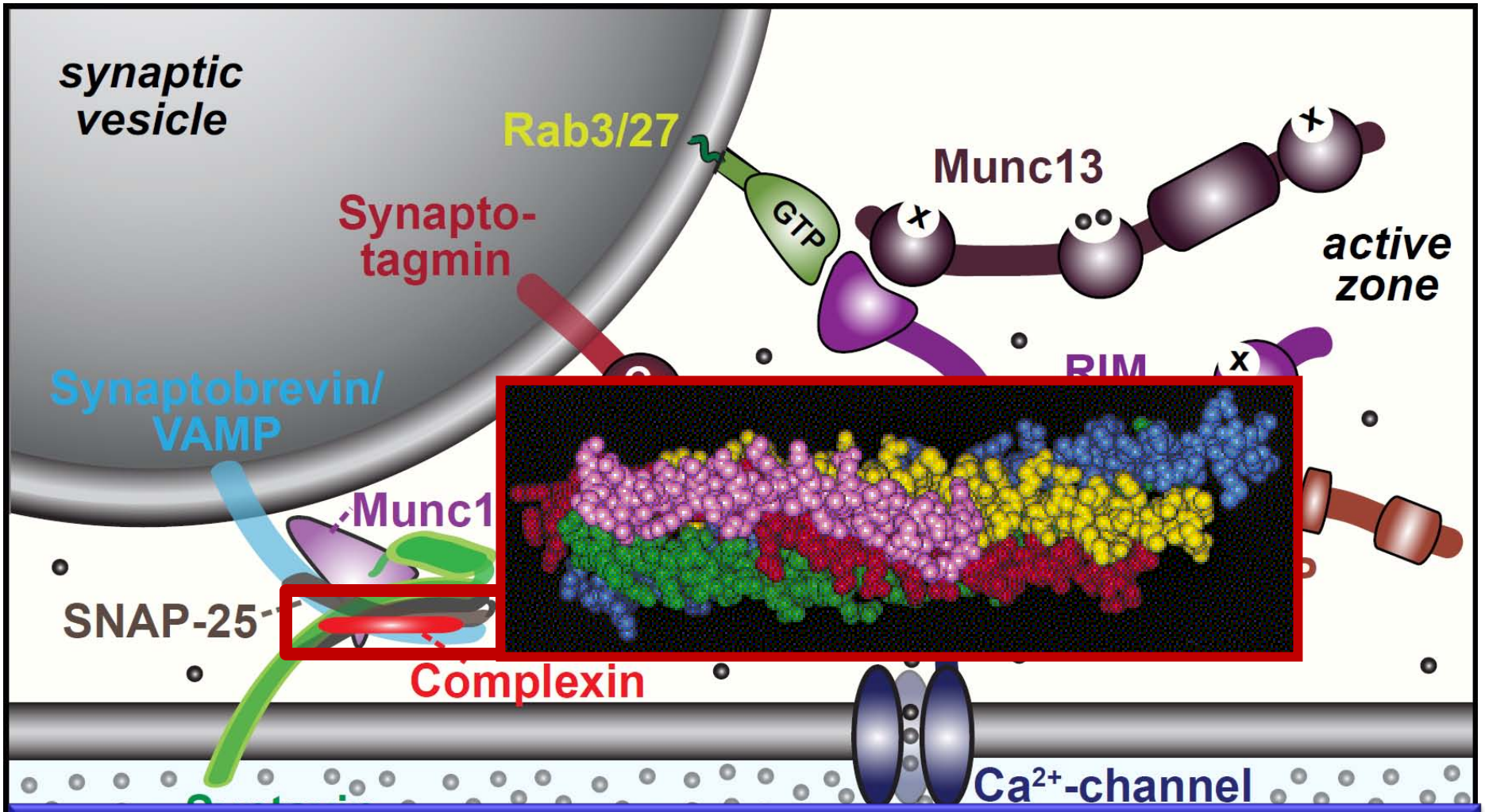

Complexin is an essential activator of synaptotagmin-1 that is evolutionarily conserved – an example

Nomastella Complexin Functions in Mouse Neurons

***Nematostella vectensis* (cnideria)**

Encodes synapto-tagmins & complexins

Yang et al., unpublished

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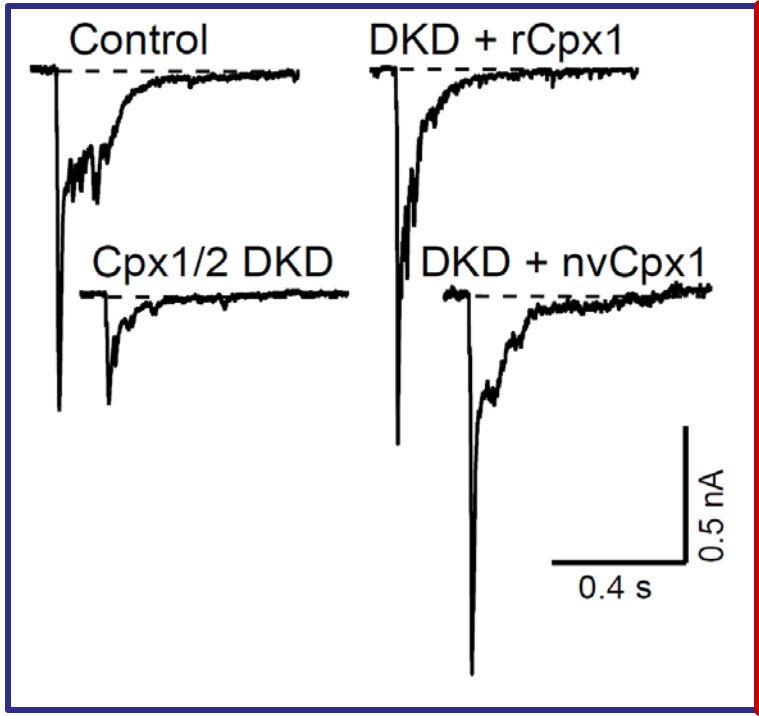
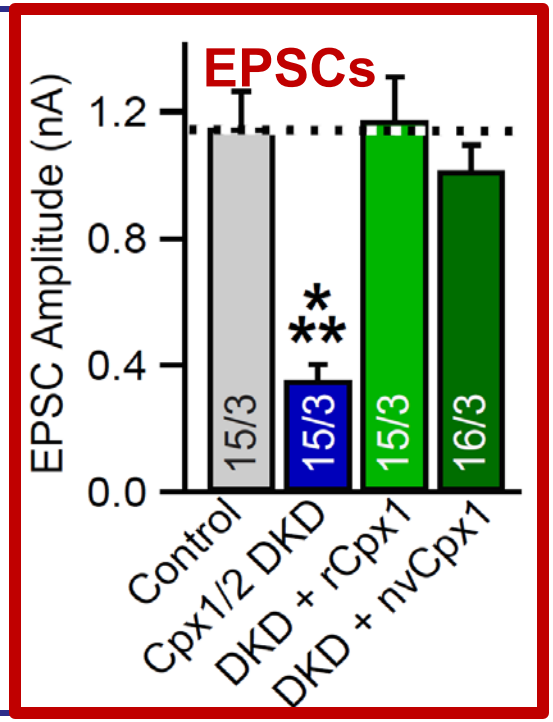
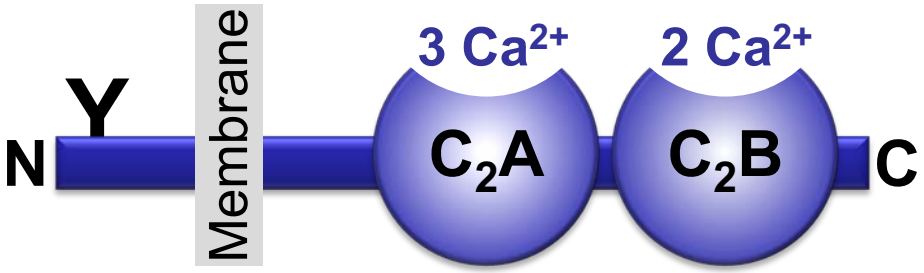




Yang et al., unpublished

Synaptotagmin-1 is a Synaptic Vesicle Ca^{2+}-Sensor Essential for Ca^{2+}-Triggered Vesicle Fusion



- **Synaptotagmin-1 is a synaptic vesicle Ca^{2+}-binding protein**
- **Synaptotagmin-1 is essential for fast Ca^{2+}-triggered release**
- **Synaptotagmin-1 uses complexin as essential co-activator**

This is where we stood in 1995

Südhof Laboratory ~1995

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We had – together with others – identified the major components of the synaptic vesicle membrane fusion machinery and described a candidate Ca^{2+}-sensor for fusion

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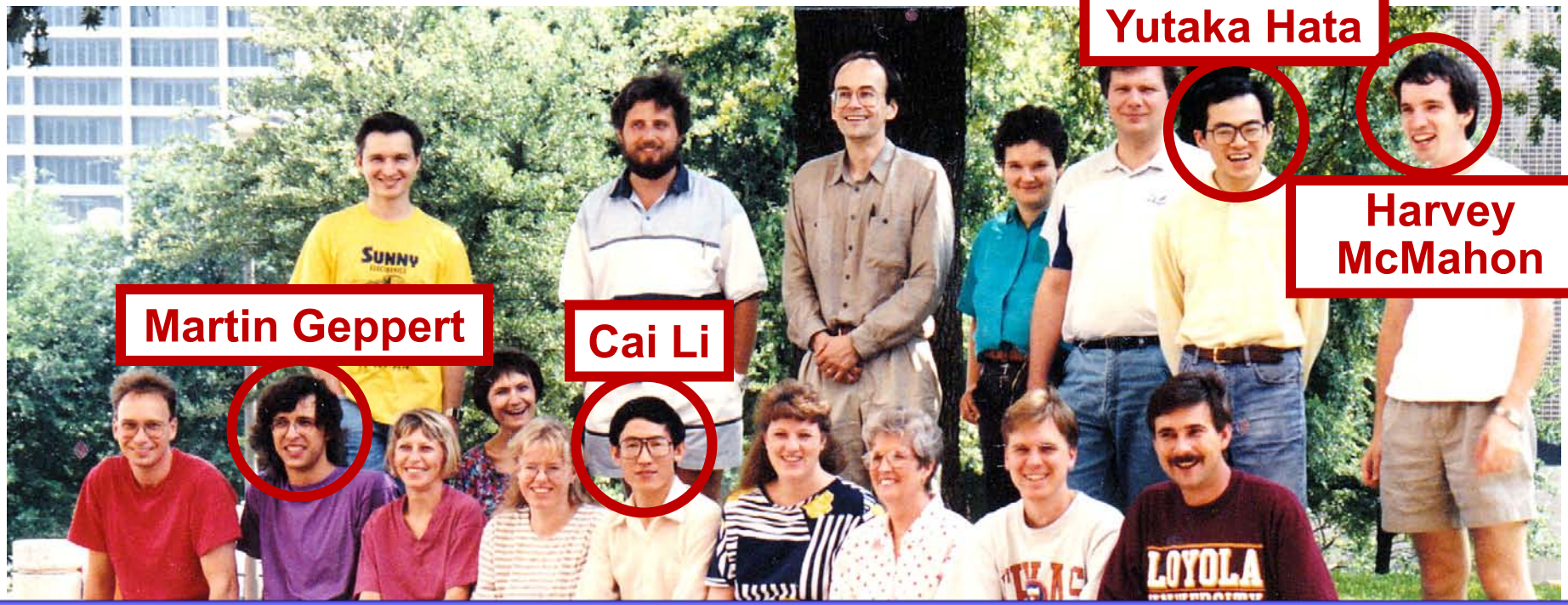

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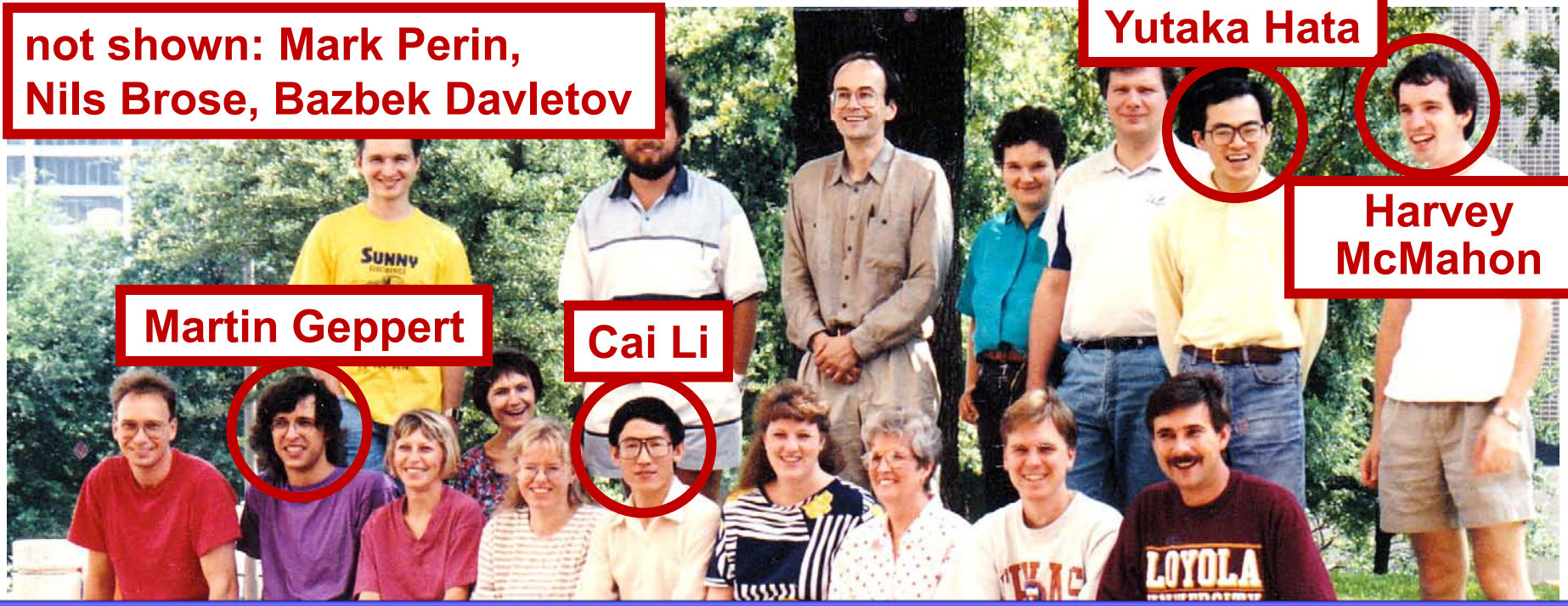


We had – together with others – identified the major components of the synaptic vesicle membrane fusion machinery and described a candidate Ca^{2+}-sensor for fusion

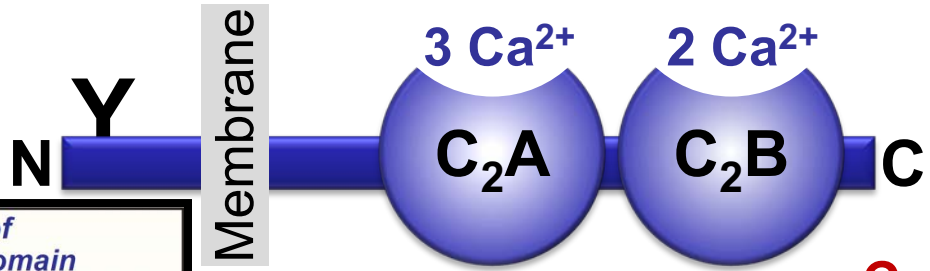
HOWEVER: Many doubted that SNARE & SM proteins 'do' membrane fusion, others suggested that synaptotagmin is a scaffold but NOT a Ca^{2+}-sensor for fusion, and we had no idea how Ca^{2+}-influx is localized to the site of vesicle fusion

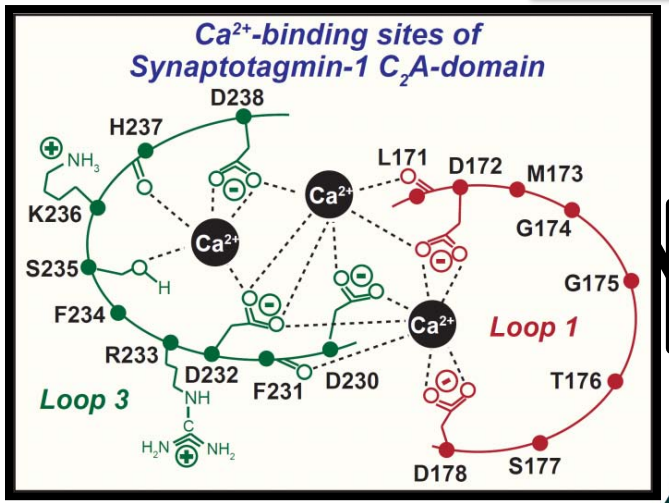
***Remainder of the talk*:**

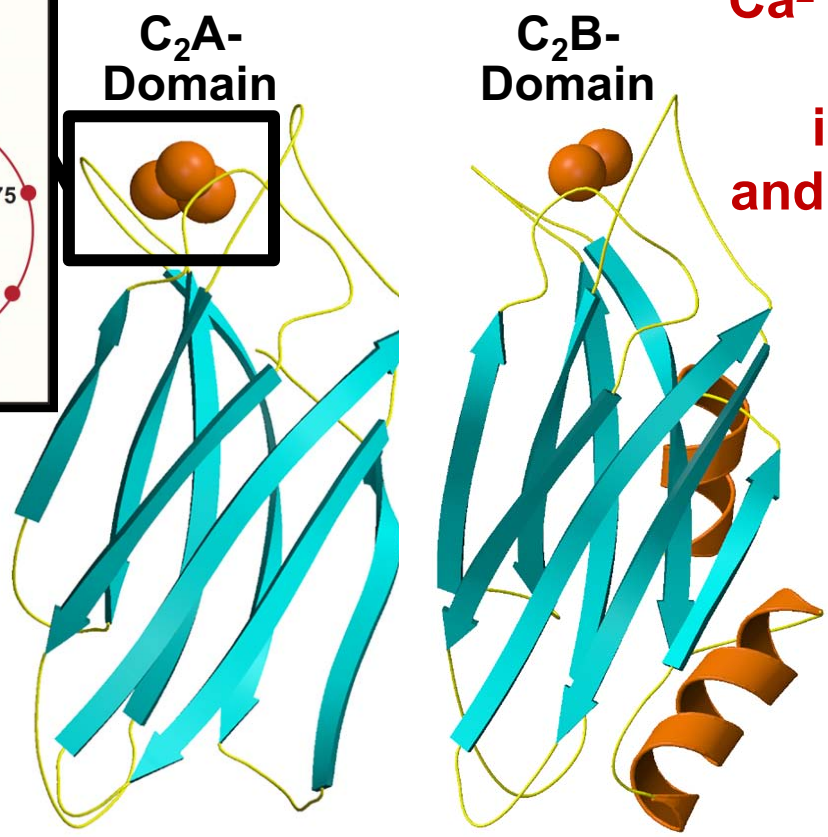
How we addressed the issues of Ca^{2+}-triggering of fusion and of Ca^{2+}-influx

<u>Major question</u>: Does Ca^{2+}-binding to synaptotagmin-1 really trigger fast release?

Architecture of Synaptotagmin-1 Ca^{2+}-Binding Sites







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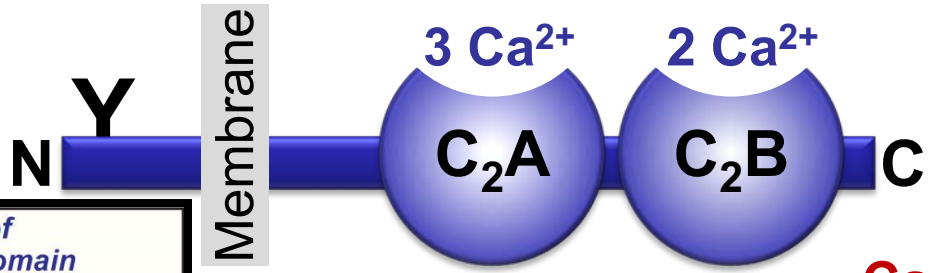
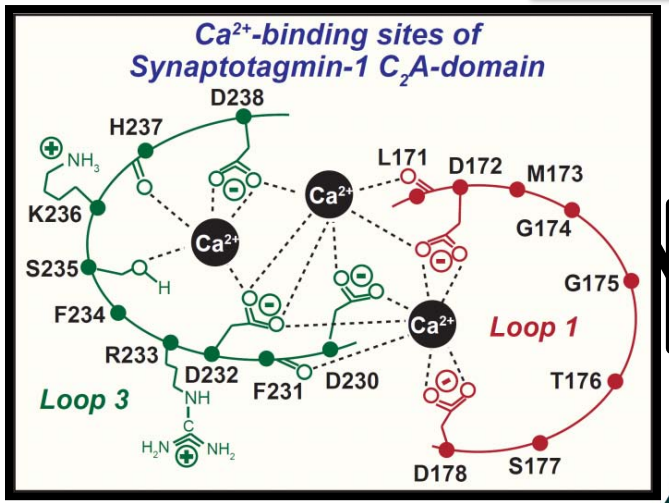
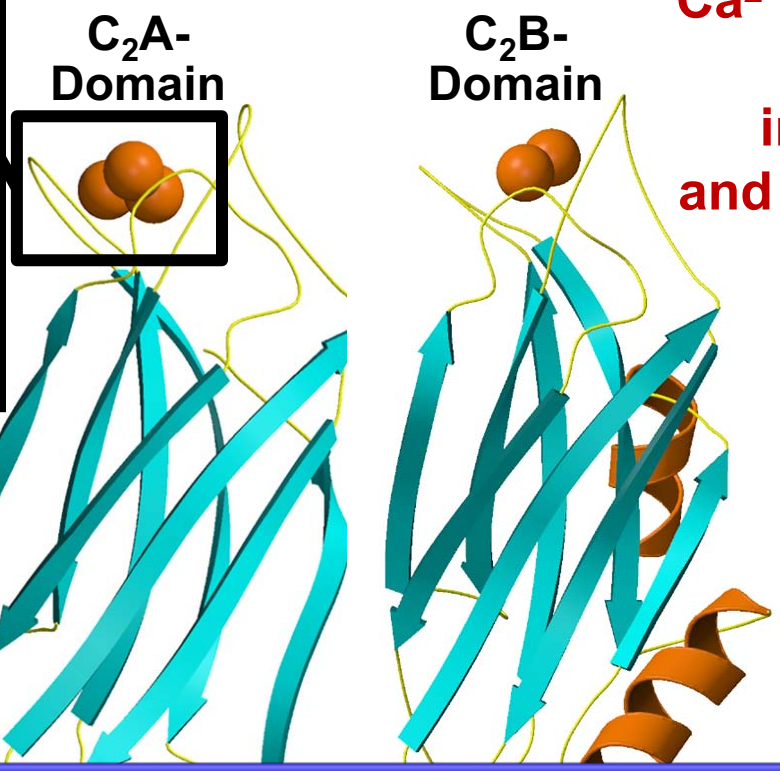


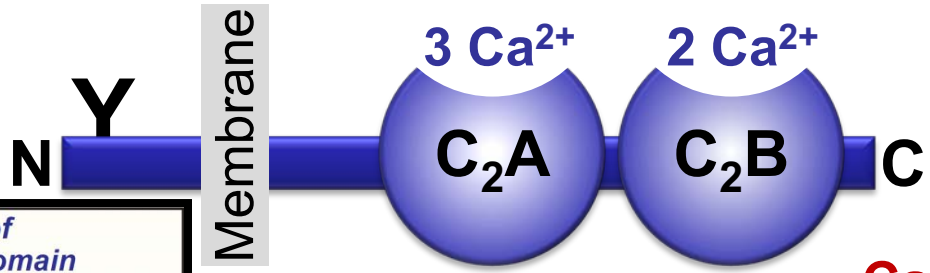


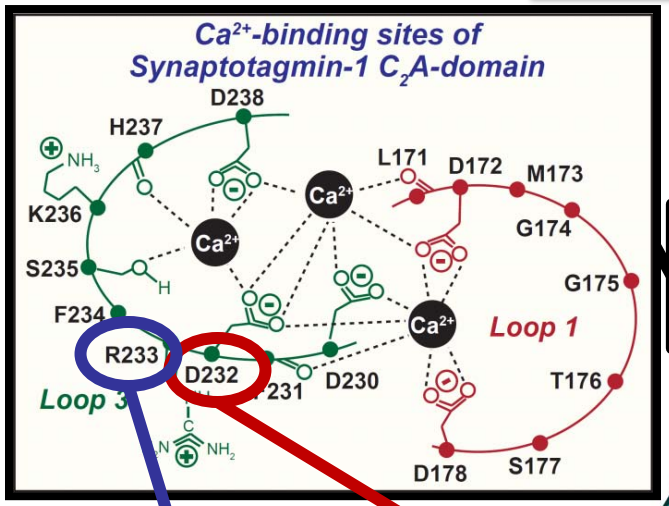


Ca^{2+}-binding to Syt1 C2-domains induces lipid- and SNARE-binding

Design mutations that shift the Ca^{2+}-affinity of synaptotagmin-1 during SNARE- or phospholipid binding

Architecture of Synaptotagmin-1 Ca^{2+}-Binding Sites



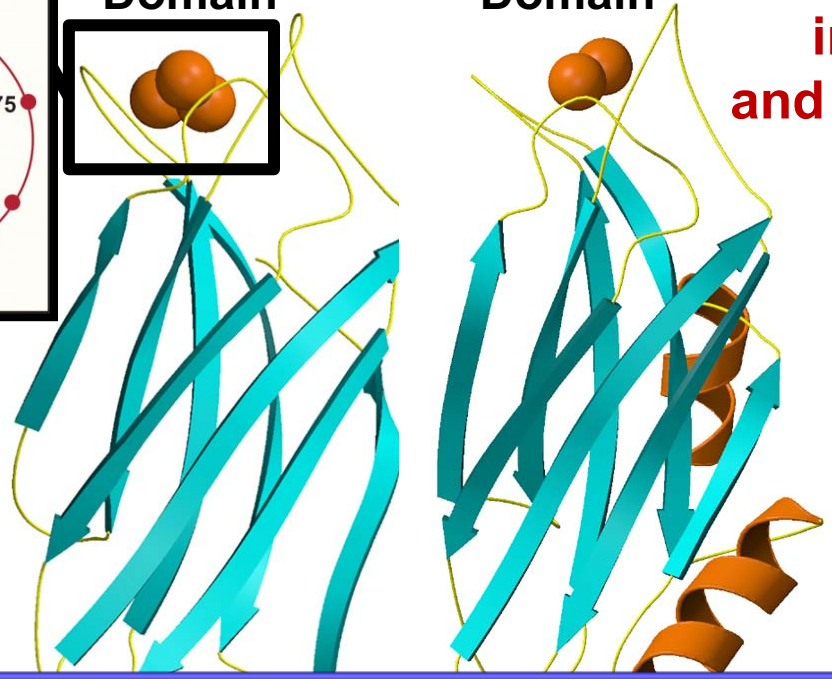


R233Q

D232N

C_2A-Domain

C_2B-Domain

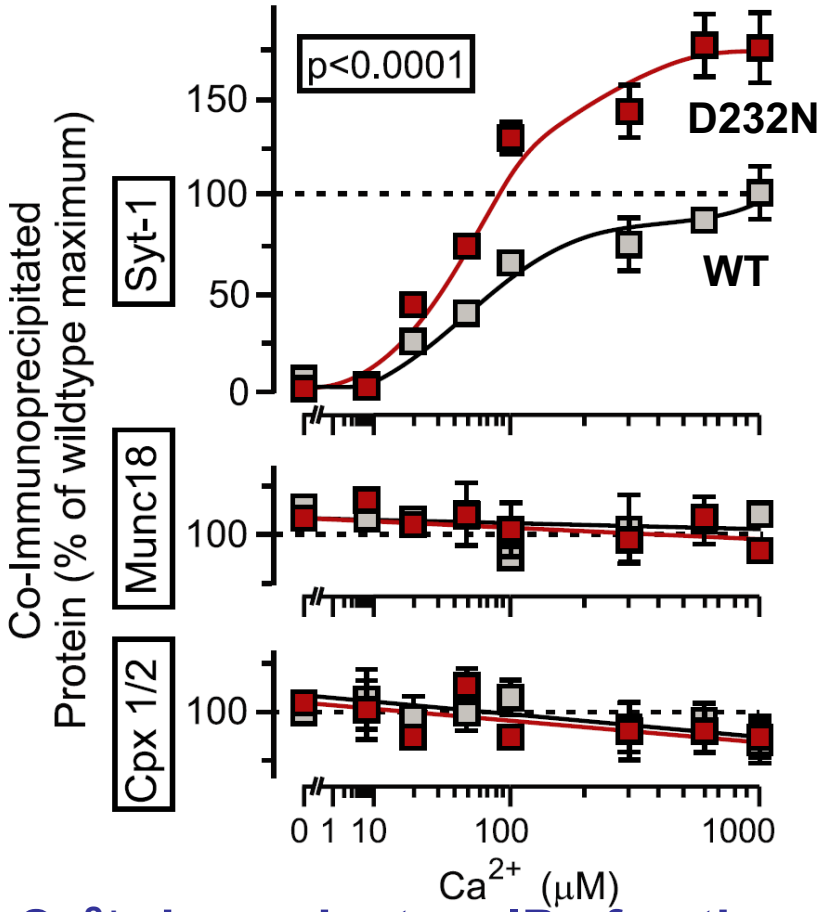


Ca^{2+}-binding to Syt1 C2-domains induces lipid- and SNARE-binding

Design mutations that shift the Ca^{2+}-affinity of synaptotagmin-1 during SNARE- or phospholipid binding

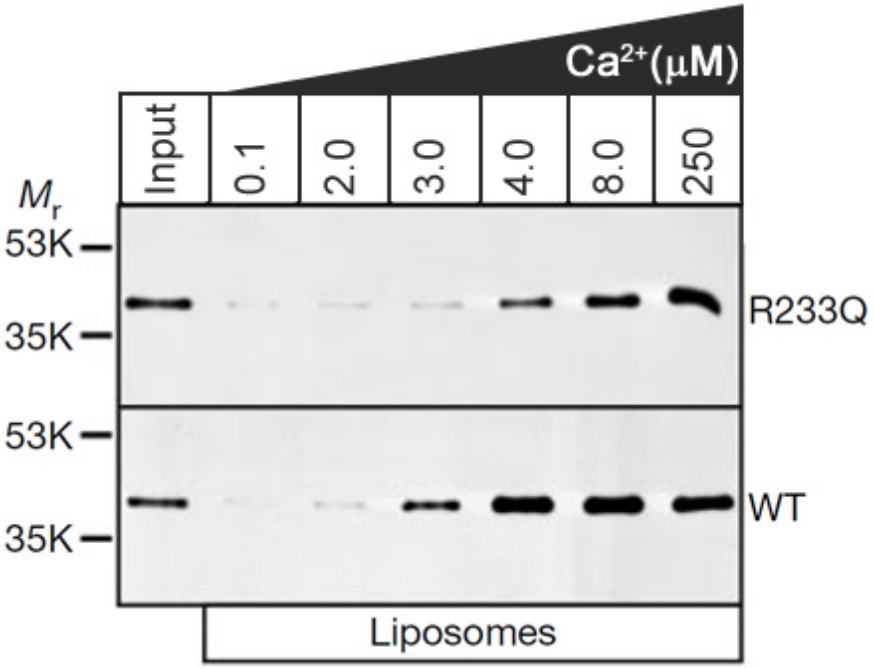
Adjacent C_2A-Domain Mutations (D232N & R233N) Differentially Alter Synaptotagmin-1 Ca^{2+}-Affinity

D232N mutant Syt1



Ca^{2+}-dependent co-IP of native brain Syt1 with SNARE complexes

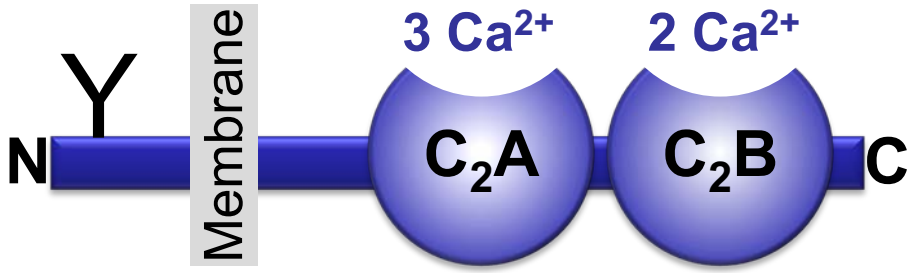
R233Q mutant Syt1



Ca^{2+}-dependent binding of native brain Syt1 to liposomes

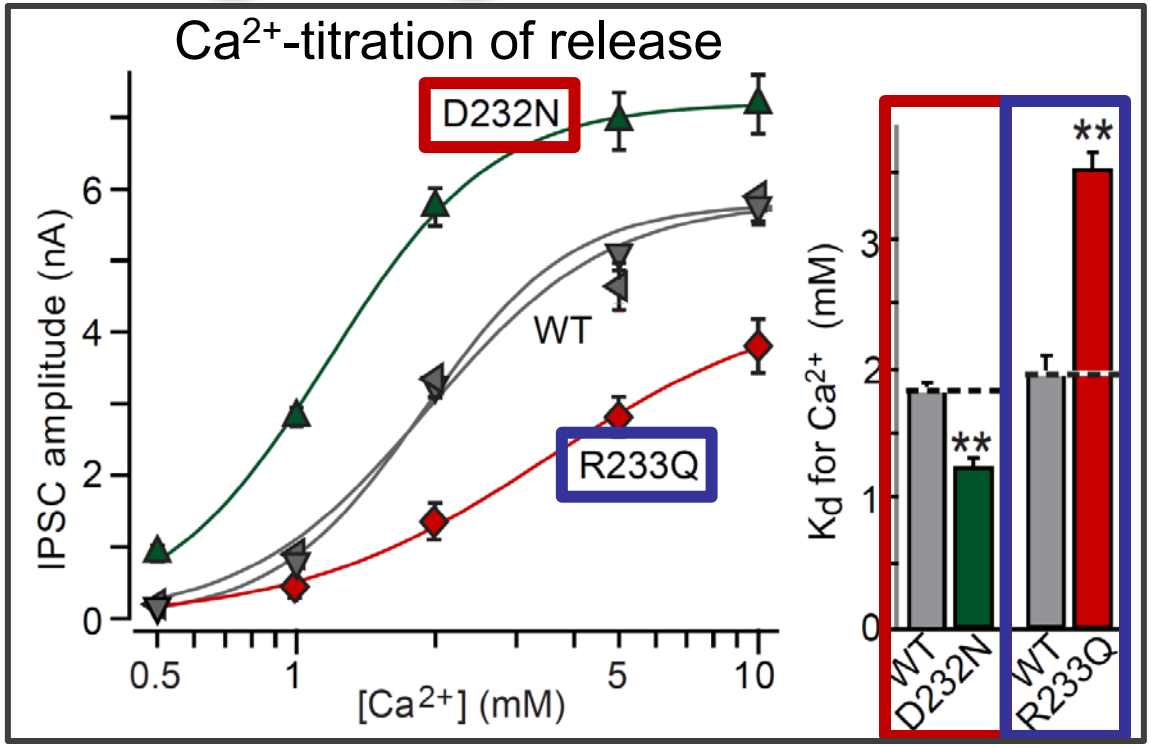
Effect on Ca^{2+}-triggered neurotransmitter release?

Synaptotagmin-1 is a Ca^{2+}-Sensor for Synaptic Vesicle Fusion



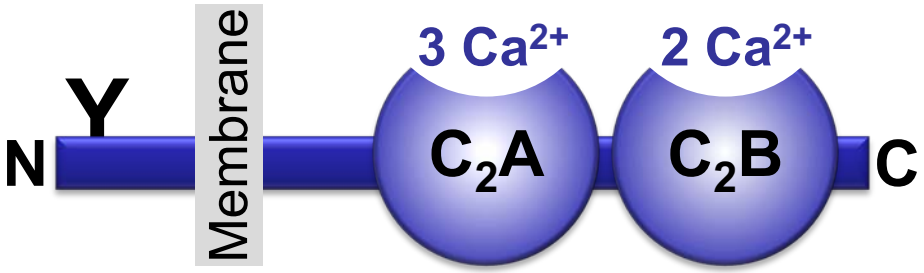
D232N ***increases***
Ca^{2+}-dependent
SNARE binding

R233Q ***decreases***
Ca^{2+}-affinity during
phospholipid binding



Formally proved that Ca^{2+}-binding to synaptotagmin-1 triggers neurotransmitter release

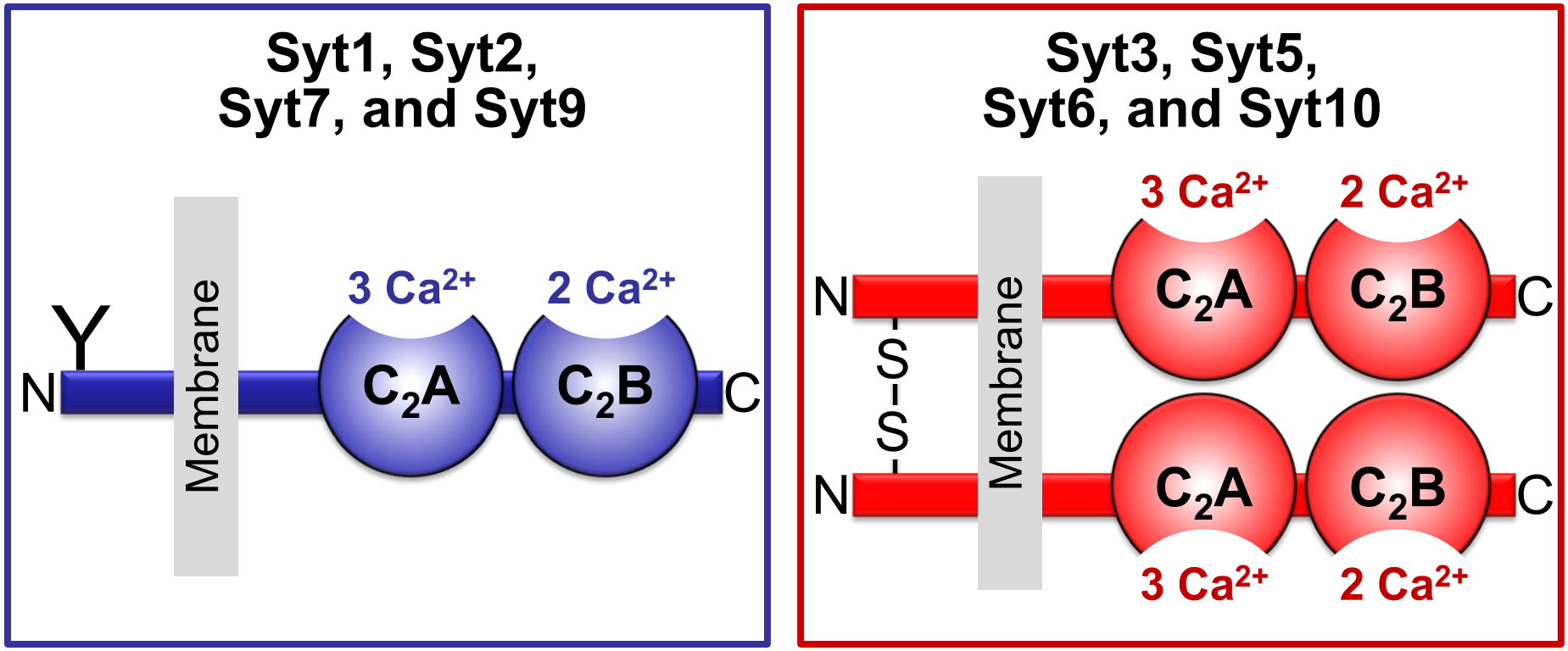
Synaptotagmin-1 is a Ca^{2+}-Sensor for Synaptic Vesicle Fusion



- Synaptotagmin-1 is a synaptic vesicle Ca^{2+}-binding protein
- Synaptotagmin-1 is essential for fast Ca^{2+}-triggered release
- Synaptotagmin-1 uses complexin as essential co-activator
- Ca^{2+}-binding to Synaptotagmin-1 triggers fast release

However, mammals express **16** synaptotagmins!

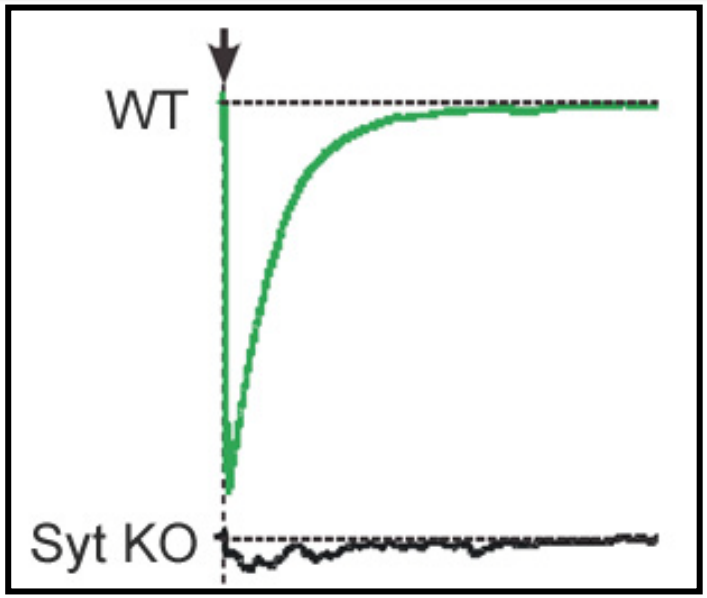
Two Classes of Synaptotagmins Bind Ca^{2+}



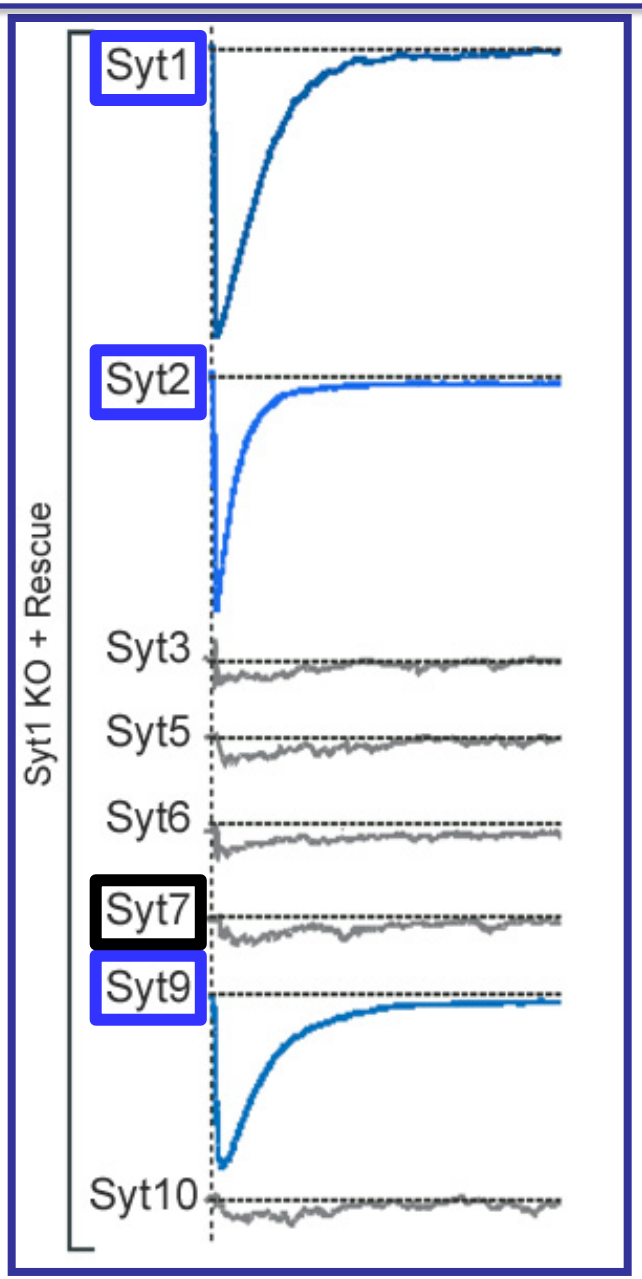
Eight other synaptotagmins do not bind Ca^{2+}

Which synaptotagmins are Ca^{2+}-sensors for fast release?

Syt1, Syt2, and Syt9 Rescue Syt1 KO Phenotype

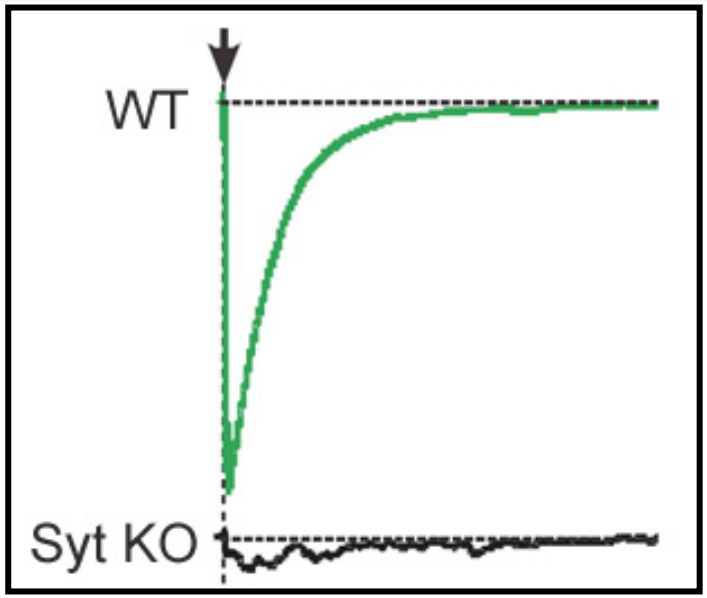


Rescue of Syt1 KO neurons

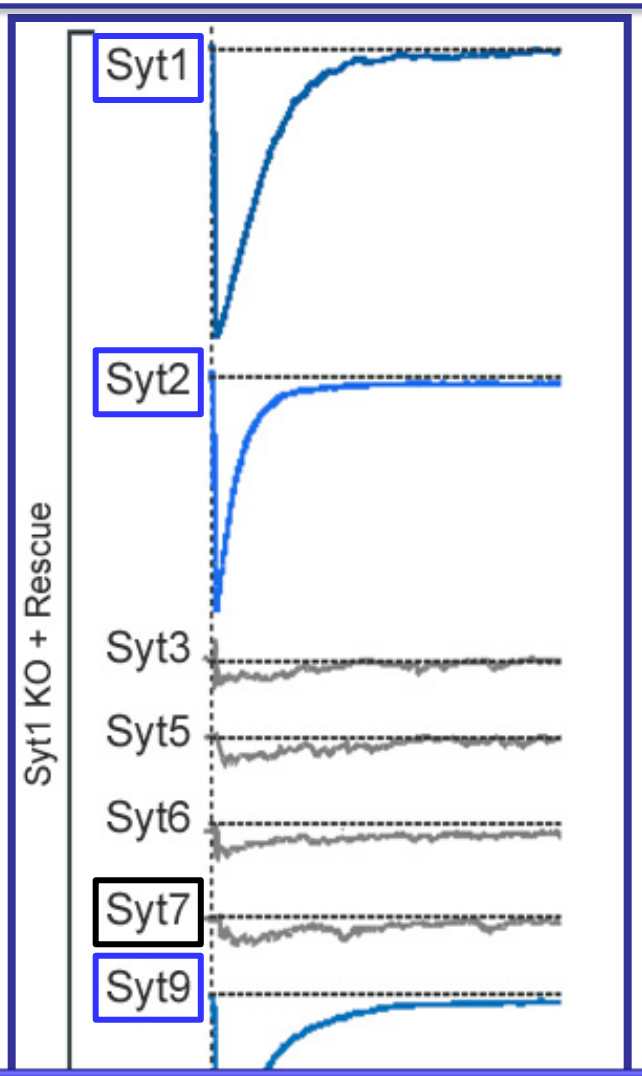


Xu et al., Neuron 2007

Syt1, Syt2, and Syt9 Rescue Syt1 KO Phenotype

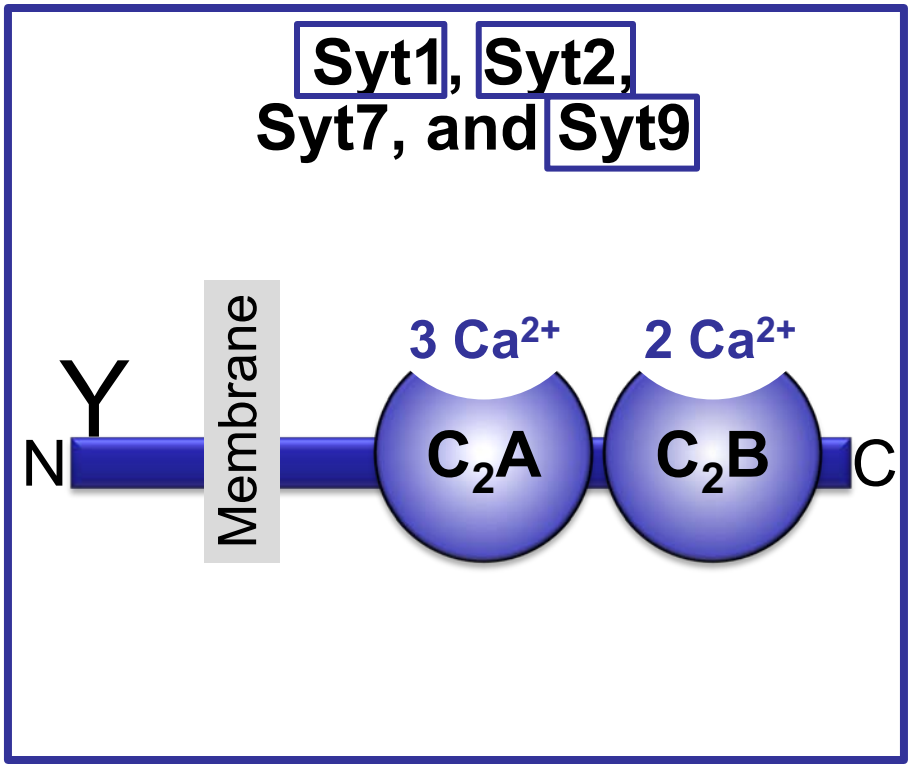


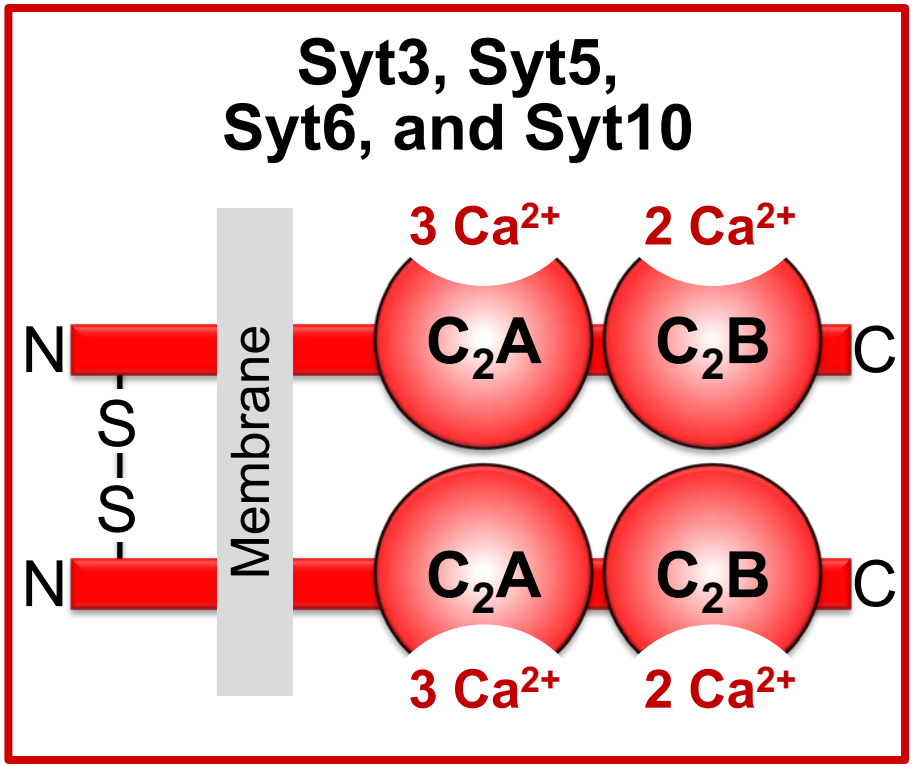
Rescue of Syt1 KO neurons



Syt1, Syt2, and Syt9 selectively rescue fast release in Syt1 KO neurons, but with distinct properties – whereas Syt7 does NOT rescue

Two Classes of Synaptotagmins Bind Ca^{2+}

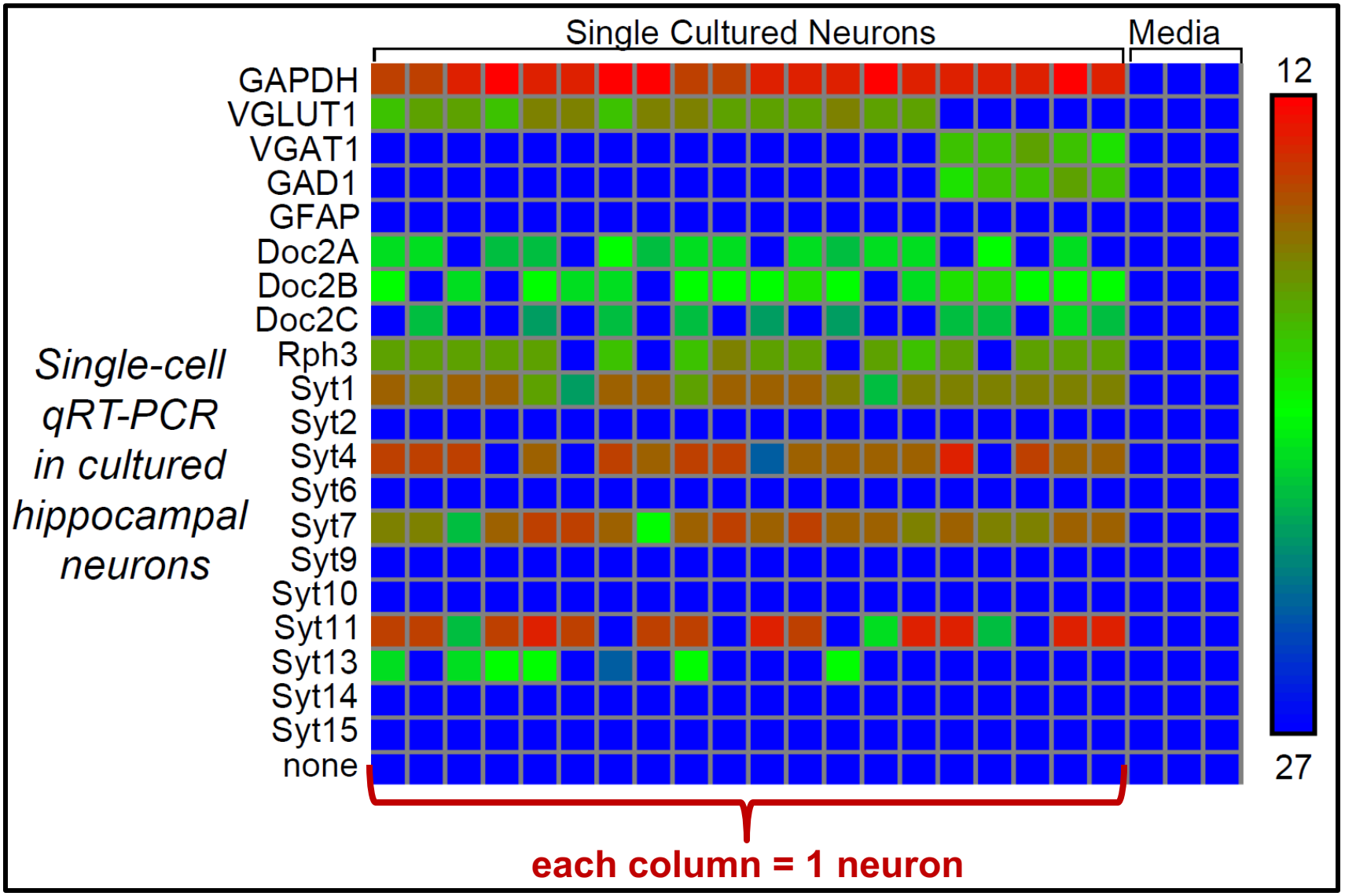




Two new issues:

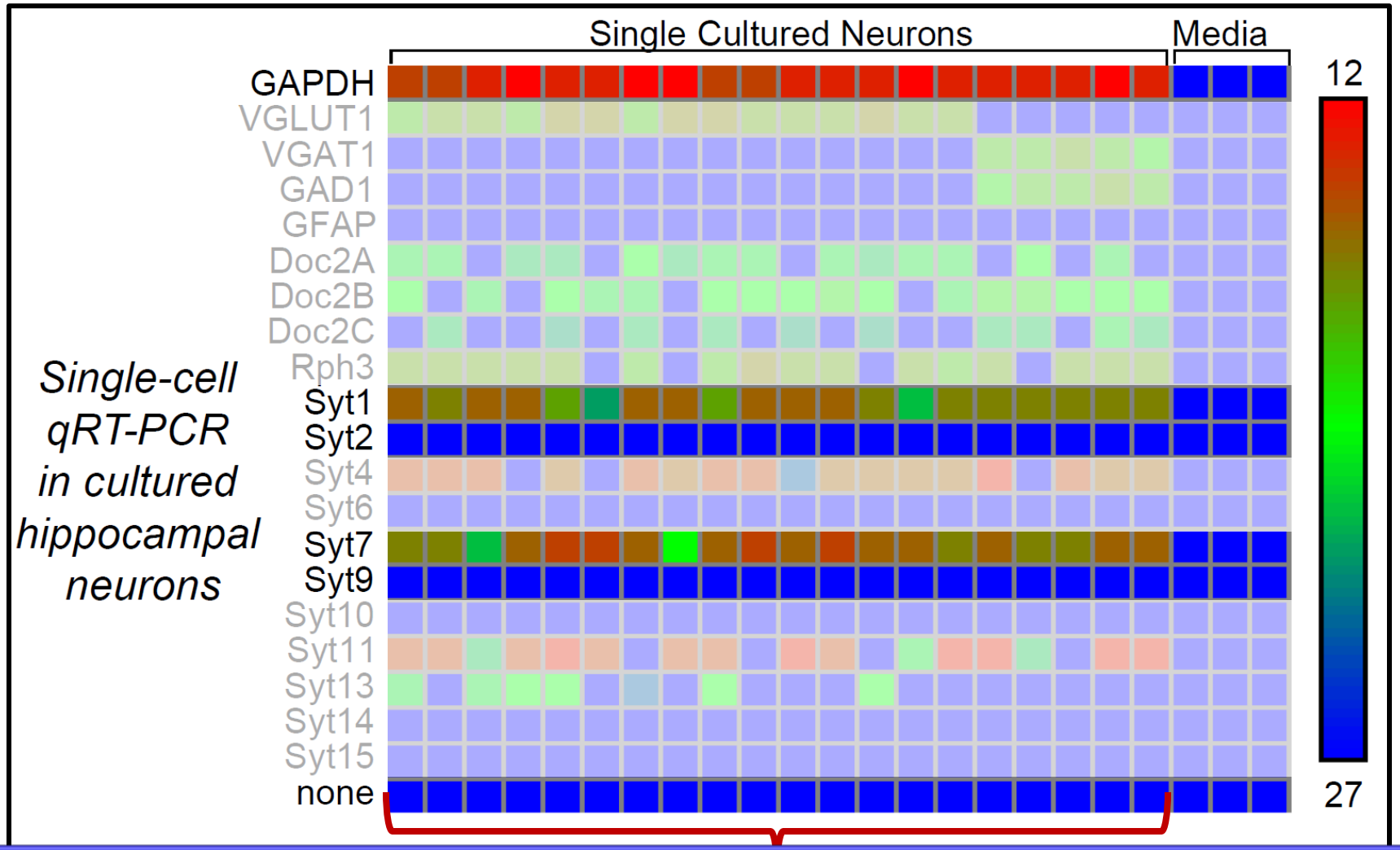
1. Why does the Syt1 KO have a phenotype if Syt2 and Syt9 can compensate?
2. Why doesn't Syt7 function in release if it is so similar to other 'blue' synaptotagmins?

Quantitation of Synaptotagmin mRNA Levels in Single Hippocampal Neurons: Syt2 and Syt9 are Absent



Bacaj et al., Neuron 2013

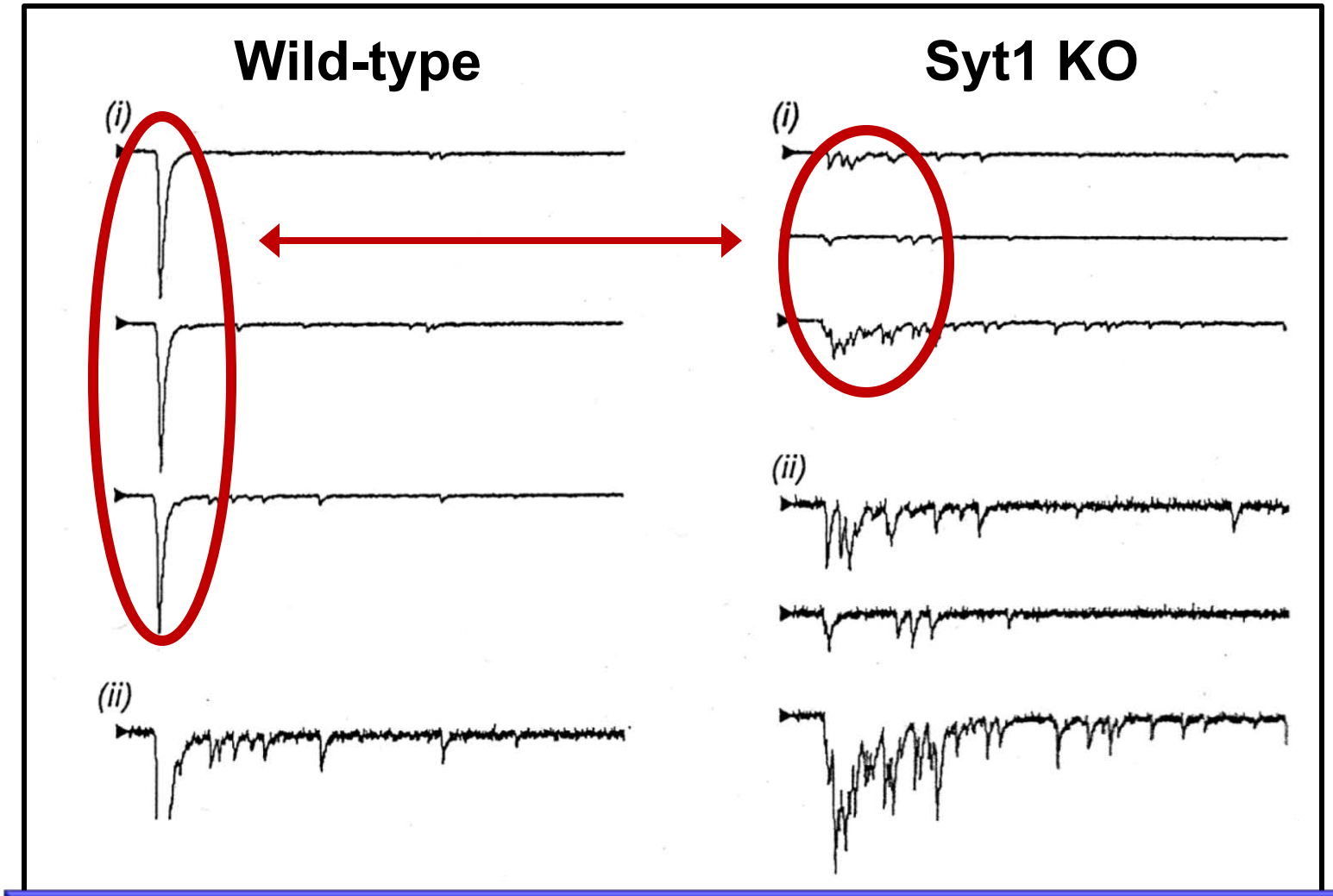
Quantitation of Synaptotagmin mRNA Levels in Single Hippocampal Neurons: Syt2 and Syt9 are Absent



Syt2 & Syt9 are not expressed but Syt7 is highly expressed

What does Syt7 do? Recall the initial KO results …

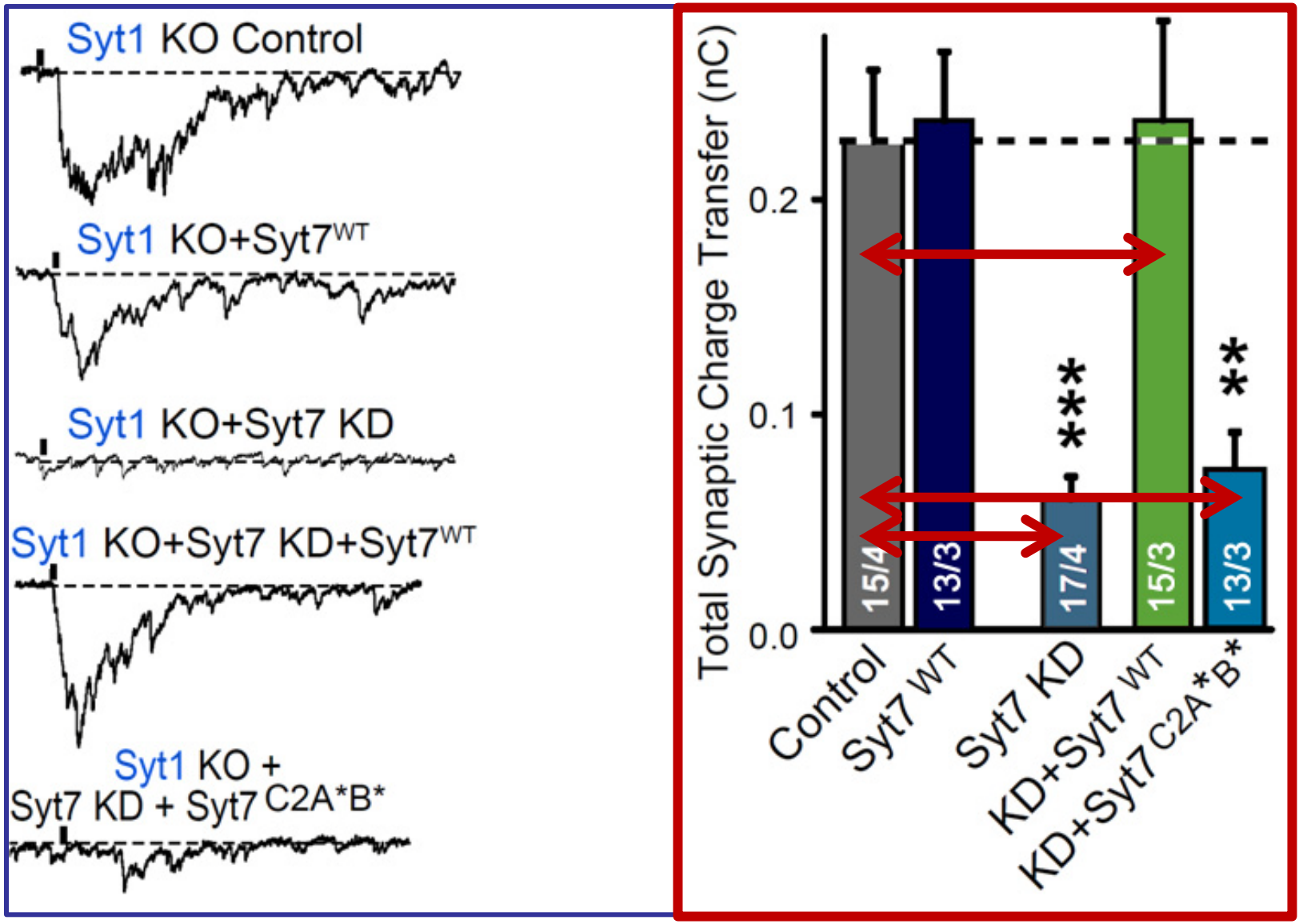
Synaptotagmin-1 is Essential for Ca^{2+}-Triggered Neurotransmitter Release



Release stimulated by isolated action potentials

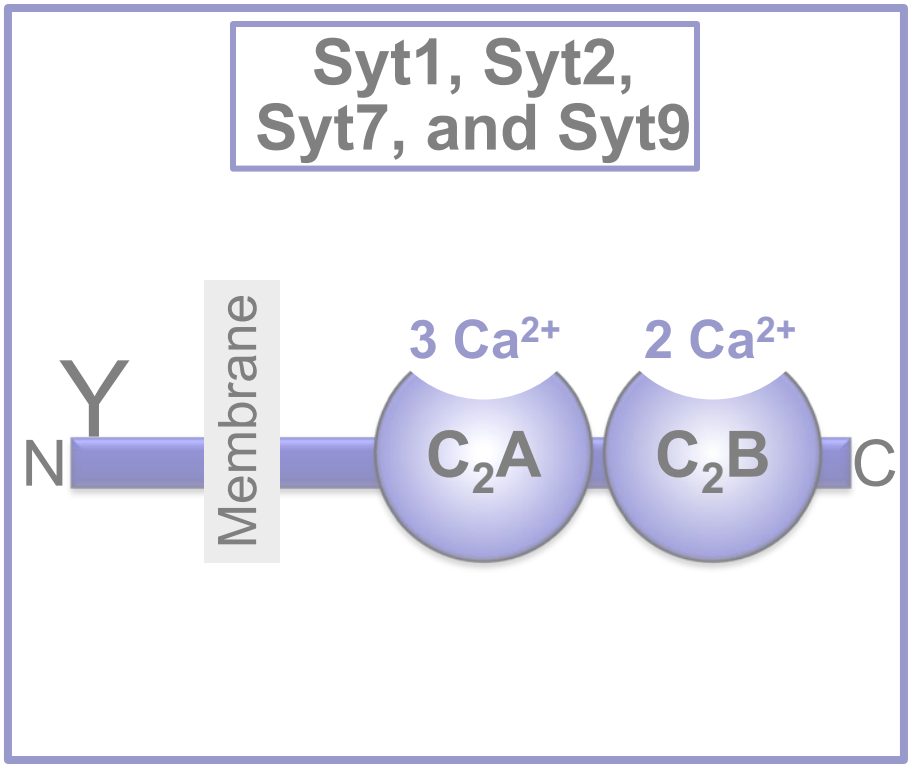
Some residual Ca^{2+}-triggered release remains in synaptotagmin-1 KO neurons

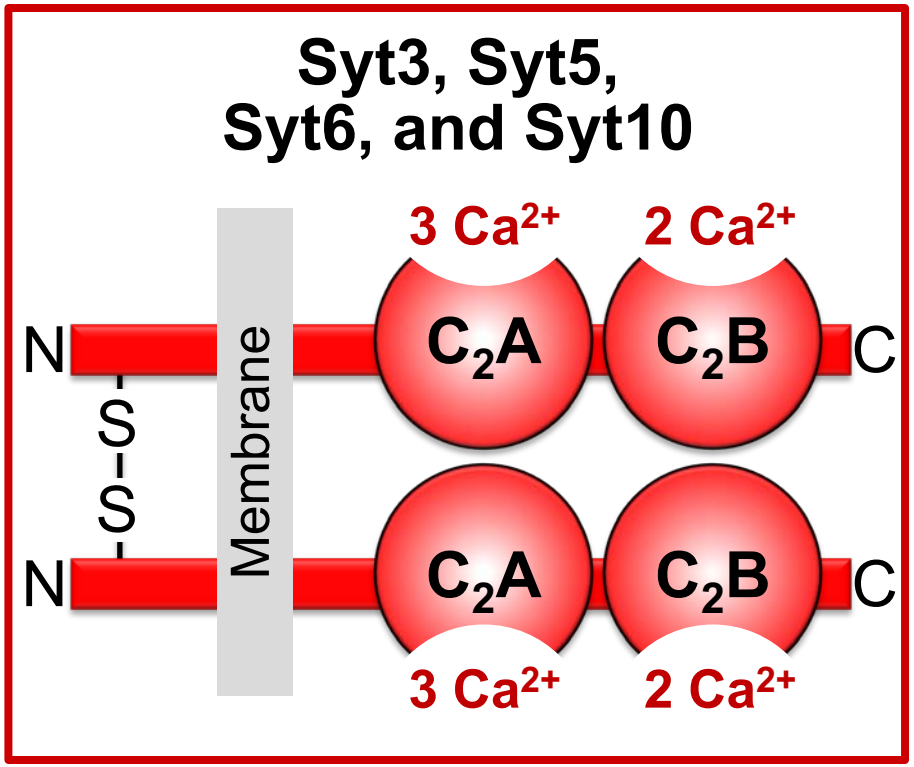
Synaptotagmin-7 Deletion Impairs Remaining Ca^{2+}-Triggered Release in Syt1 KO Neurons



Bacaj et al., Neuron 2013

Two Classes of Synaptotagmins Bind Ca^{2+}

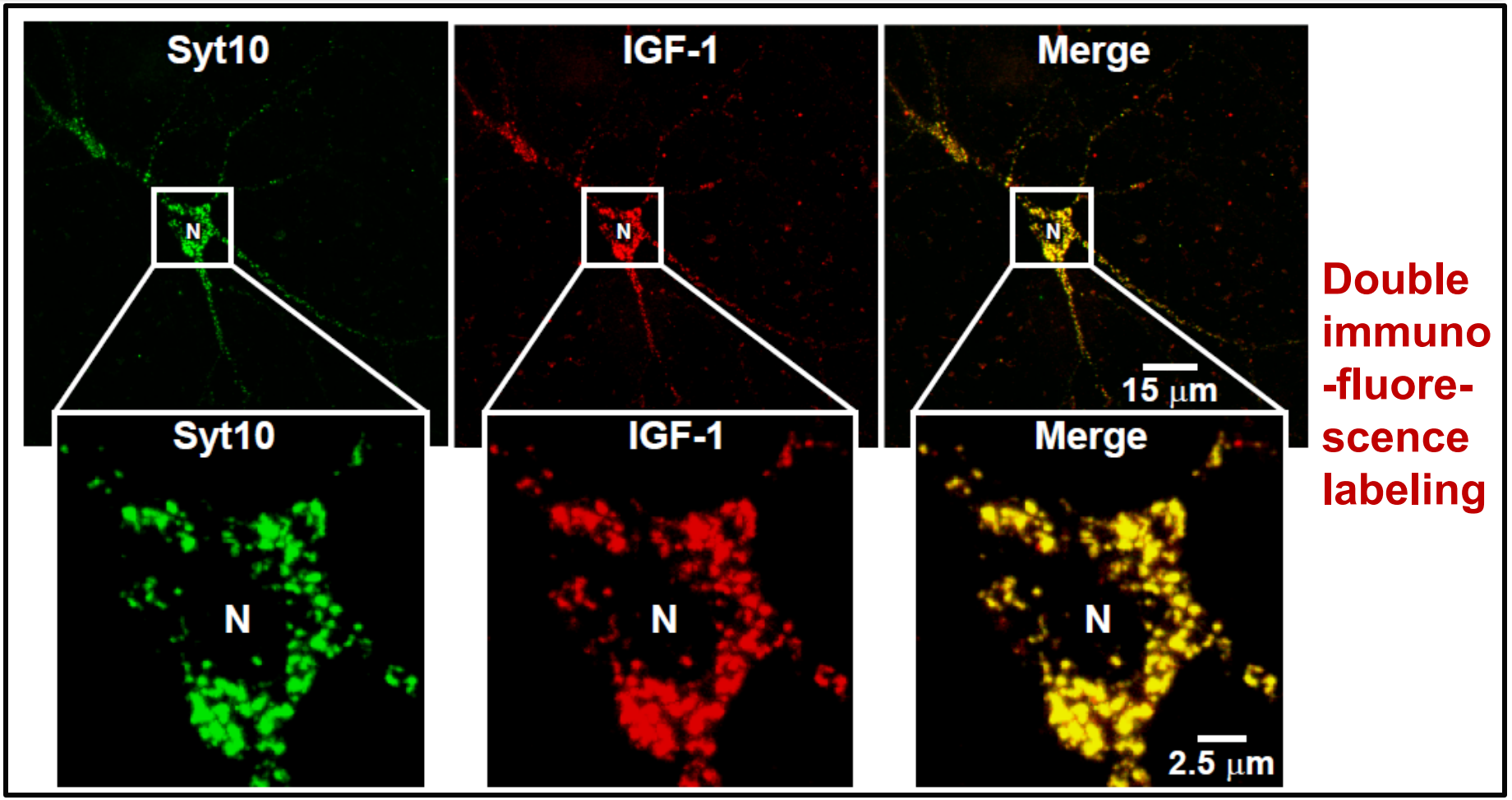




All blue synaptotagmins function in synaptic vesicle fusion but exhibit different Ca^{2+}-triggering kinetics – function also in neuroendecrine/hormone secretion, mast cell degranulation etc.

What about red synaptotagmins? Focus on Syt10 …

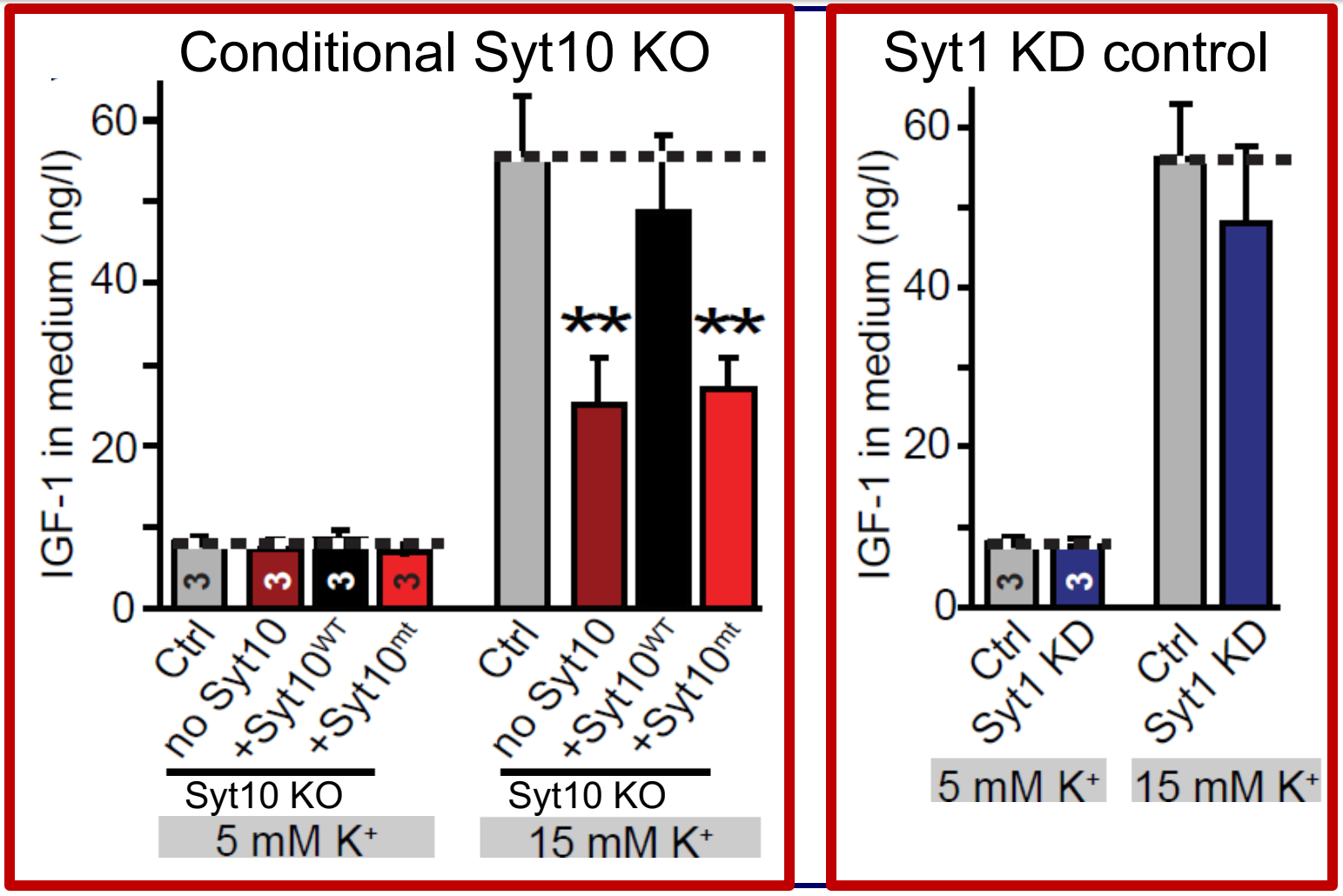
Synaptotagmin-10 Co-Localizes with IGF-1 in Olfactory Bulb Neurons



Double immuno-fluore-scence labeling

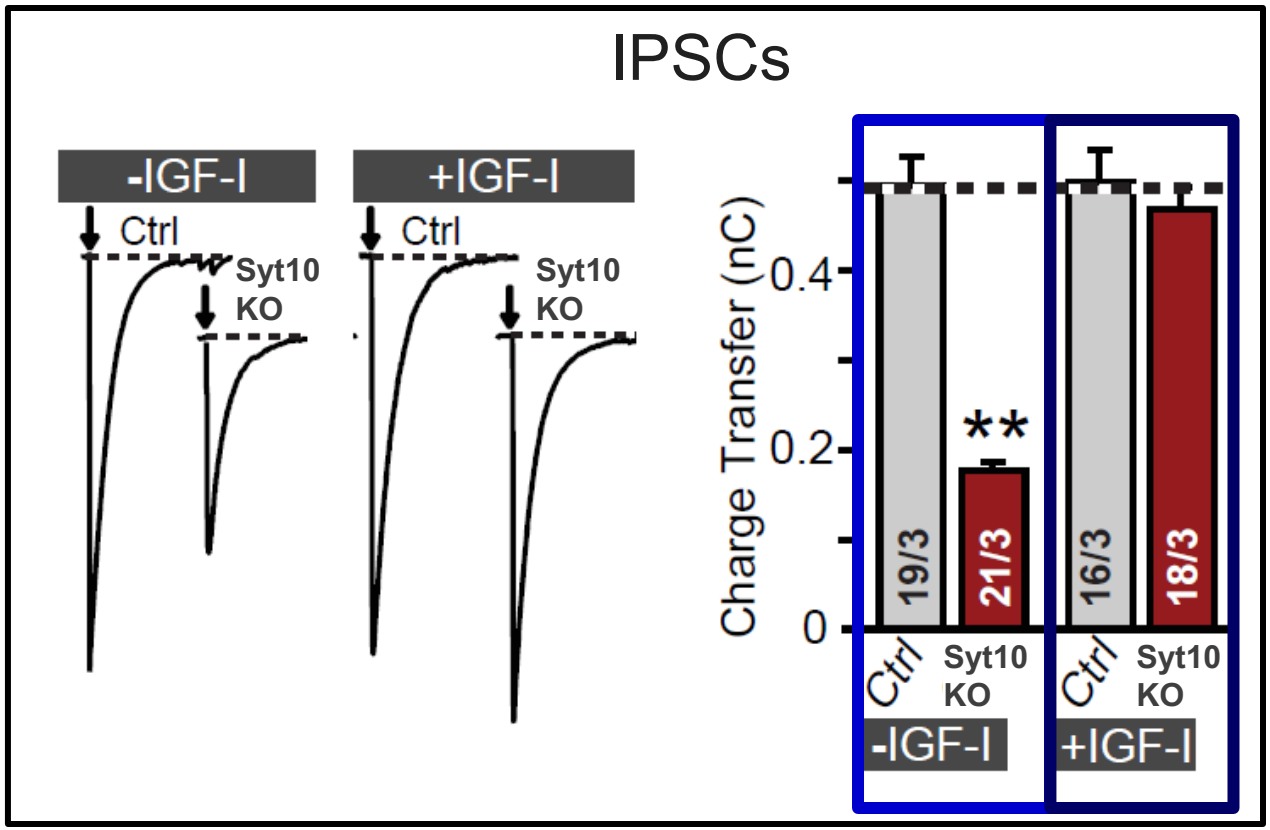
Cao et al., Cell 2011

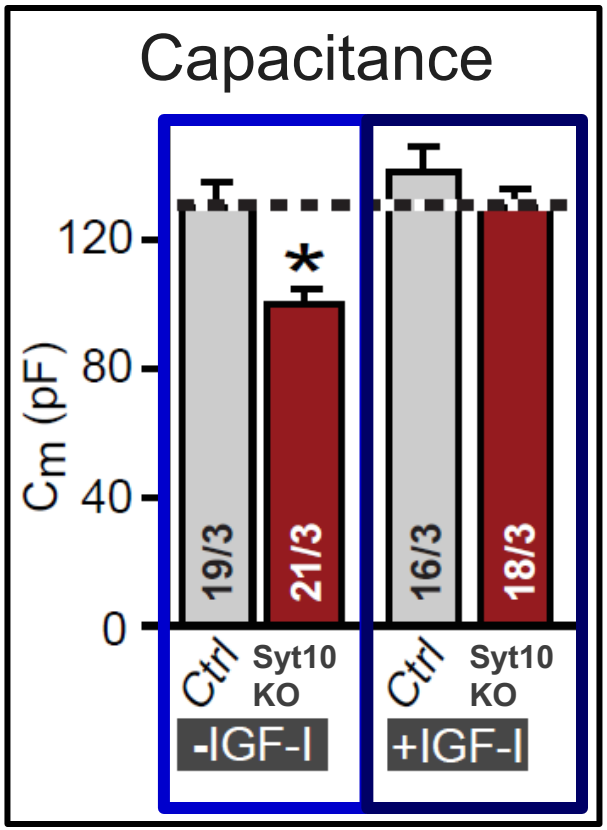
Synaptotagmin-10 Knockout Impairs Depolarization-Induced IGF-1 Secretion



Loss of IGF-1 secretion decreases neuron size and synapse numbers – rescue with IGF-1

Syt10 KO Decreases Total Synaptic Responses & Capacitance of Neurons - Rescue with IGF-1

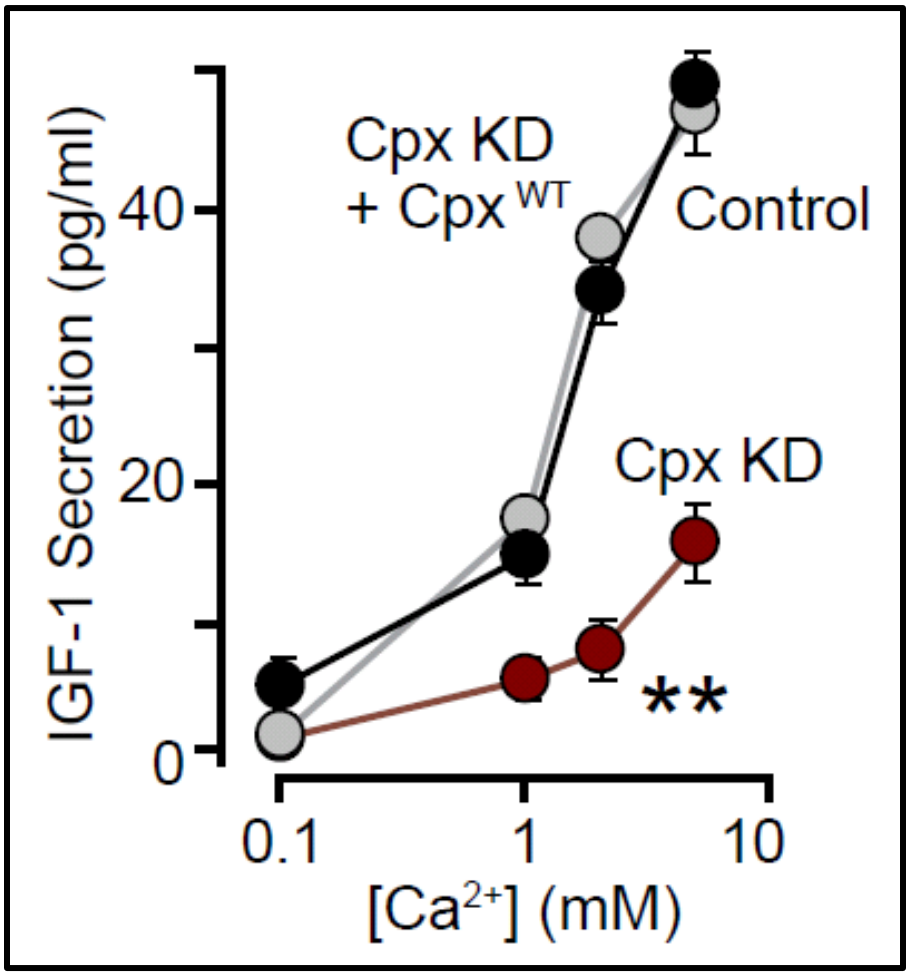




Syt10 is a Ca^{2+}-sensor for IGF-1 exocytosis – does Syt10 use complexin as a co-factor?

Cao et al., Cell 2011

Complexin Depletion Impairs Synaptotagmin-10 Dependent IGF-1 Secretion

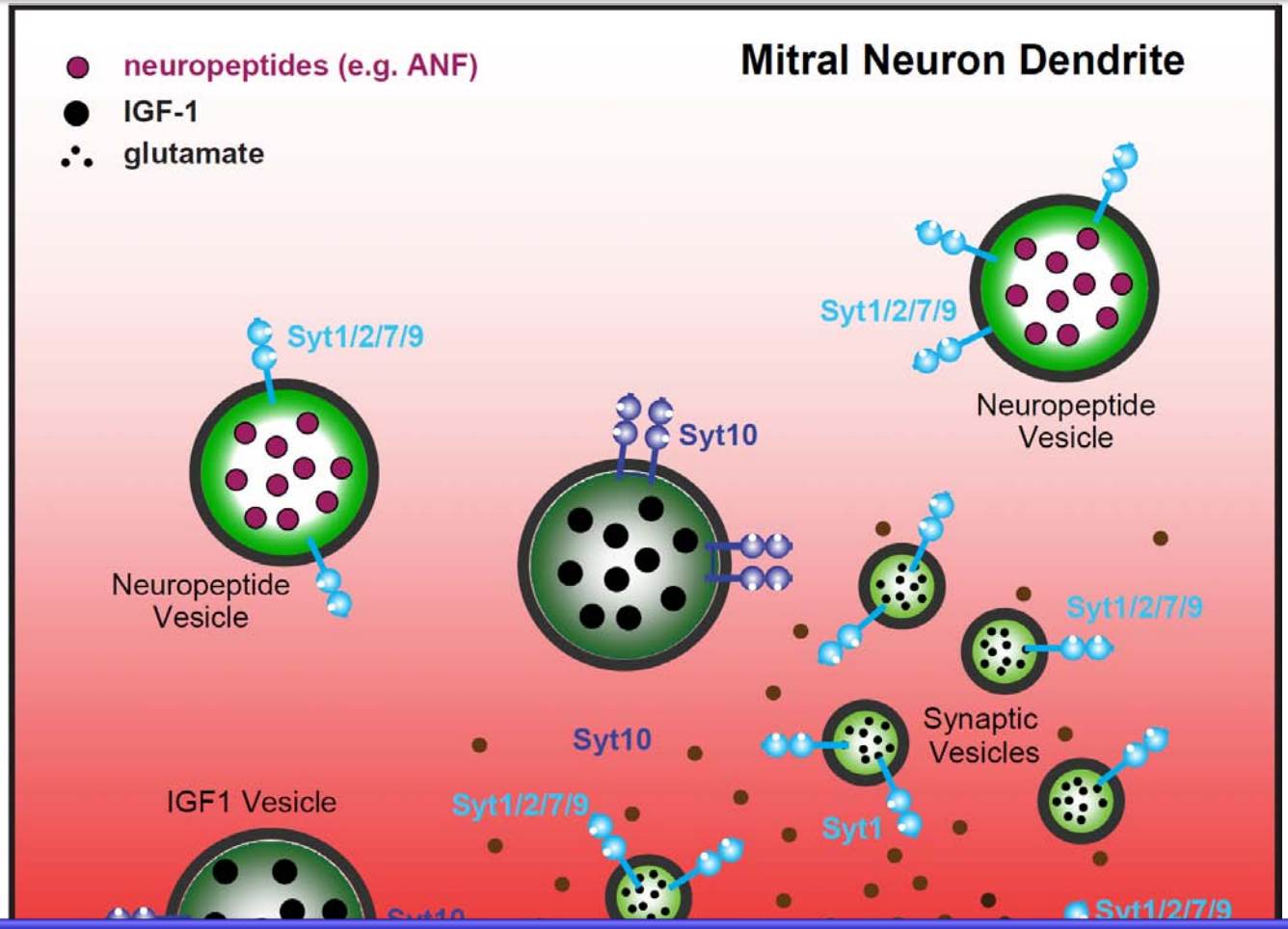


IGF-1 secretion measured at different extracellular Ca^{2+}-concentrations

Implications for synaptotagmin function

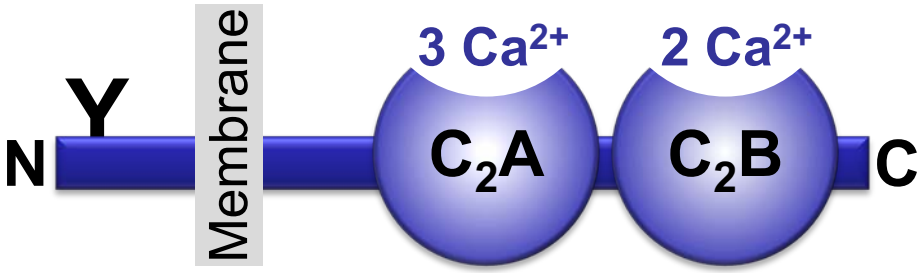
Cao et al., J. Neurosci. 2012

Multiple Pathways of Ca^{2+}-Triggered Exocytosis Controlled by Different Synaptotagmins



Diverse non-redundant synaptotagmins use the same complexin-dependent mechanism for different Ca^{2+}-dependent membrane fusion reactions

Synaptotagmins Are Universal Ca^{2+}-Sensors for Ca^{2+}-Triggered Vesicle Fusion



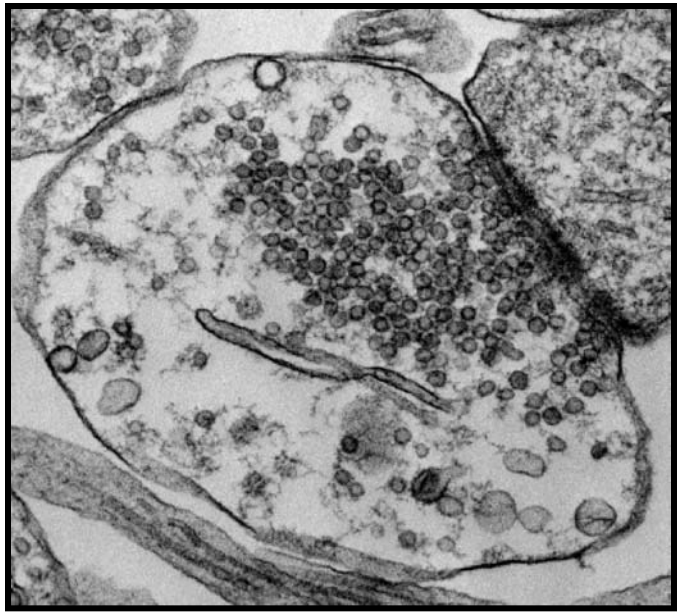
- Synaptotagmin-1 is a synaptic vesicle Ca^{2+}-binding protein
- Synaptotagmin-1 is essential for fast Ca^{2+}-triggered release
- Synaptotagmin-1 uses complexin as essential co-activator
- Ca^{2+}-binding to Synaptotagmin-1 triggers fast release
- Other synaptotagmins perform analogous functions in Ca^{2+}-triggered release with complexin as co-factor

Three Processes Govern Neurotransmitter Release

1. Synaptic vesicle fusion

2. Ca^{2+}-triggering of fusion
   - Very fast: ~0.1 msec
   - Cooperative: ~5 Ca^{2+}-ions

3. Localized Ca^{2+}-influx



These studies thus established synaptotagmins as Ca^{2+}-sensors for membrane fusion and generalized their functions in most if not all Ca^{2+}-dependent fusion reactions

What about Ca^{2+}-influx?

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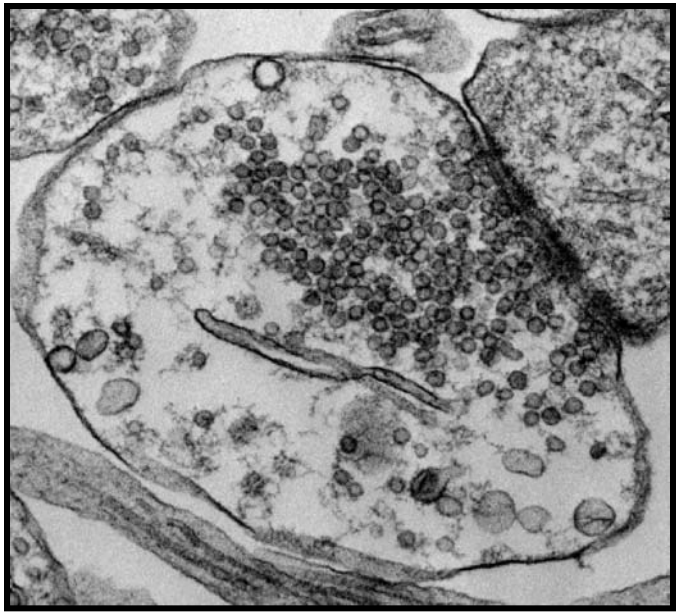

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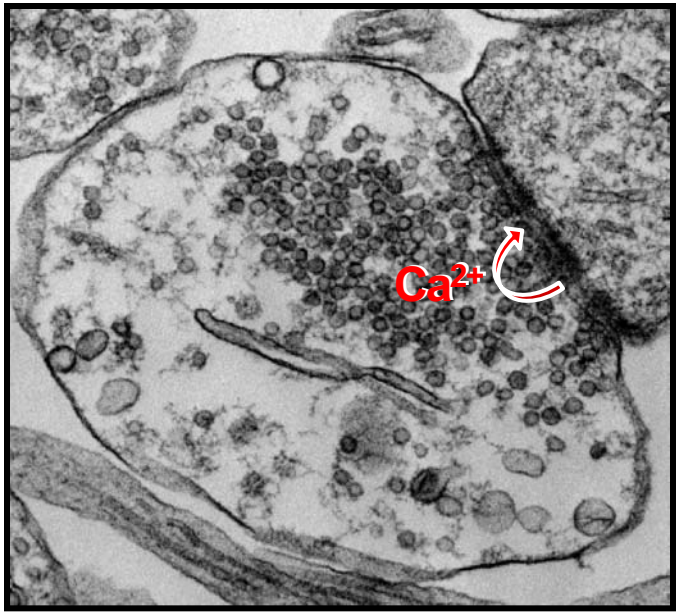

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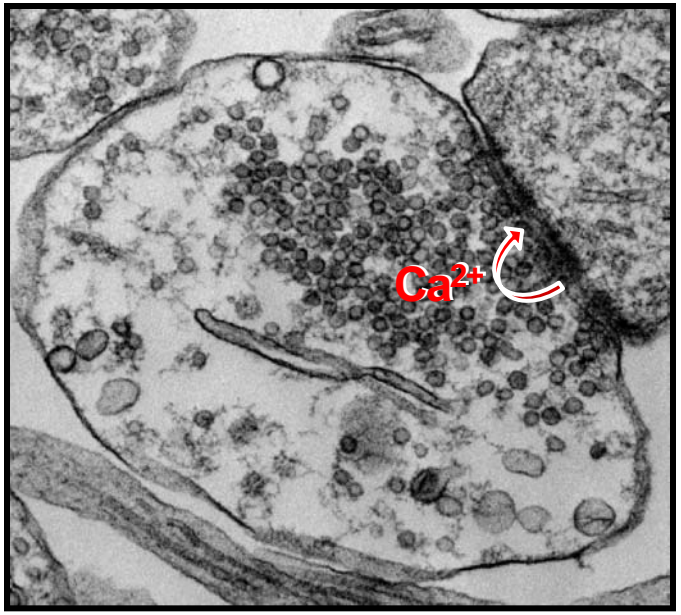
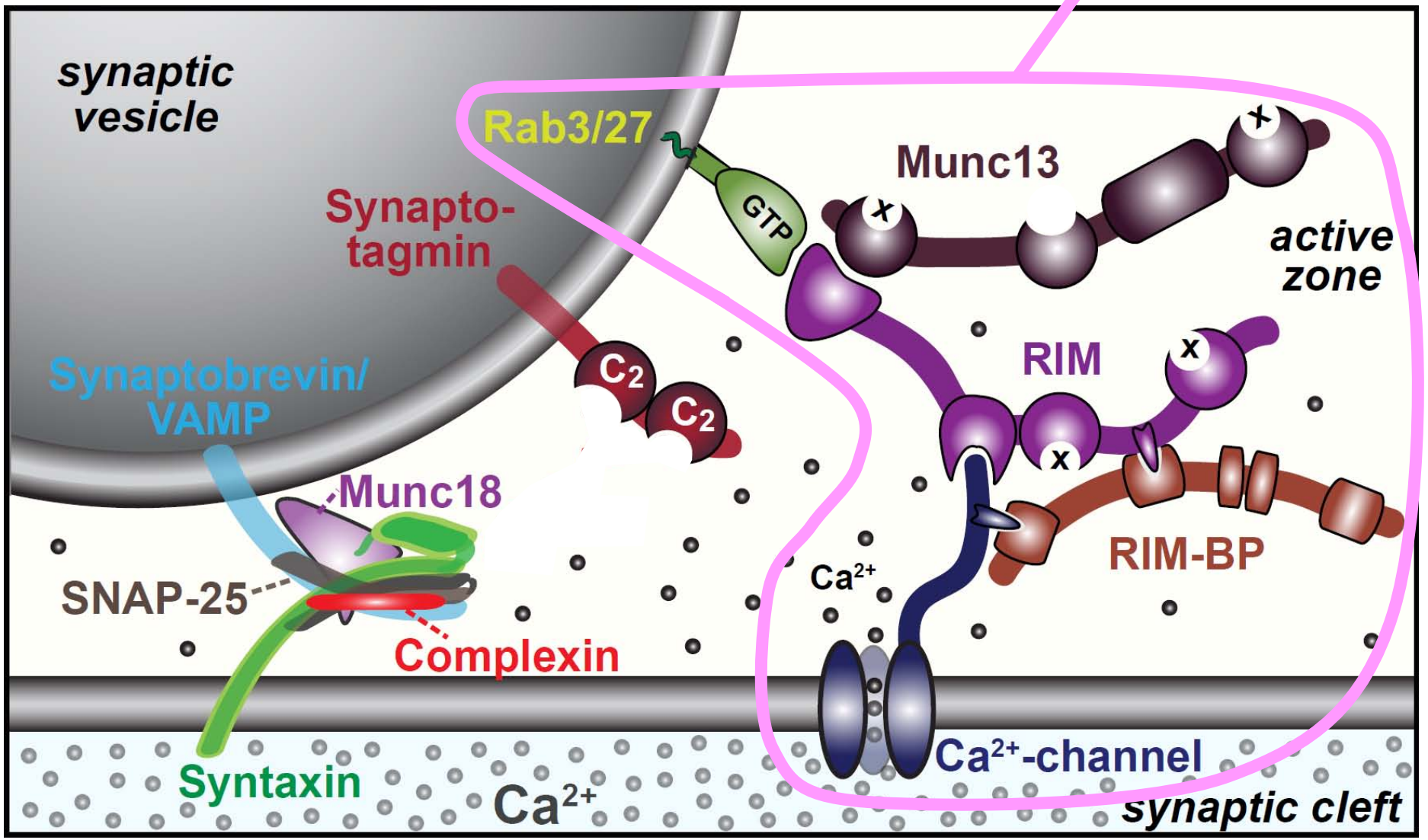


Without localized Ca^{2+}-influx at the active zone, action potentials and release become uncoupled, and release is desynchronized and decelerated

The importance of localized Ca^{2+}-influx cannot be overestimated – like in real estate, location is everything!

A Neurotransmitter Release Machine Mediates Fusion, Ca^{2+}-triggering & Ca^{2+}-Channel Tethering



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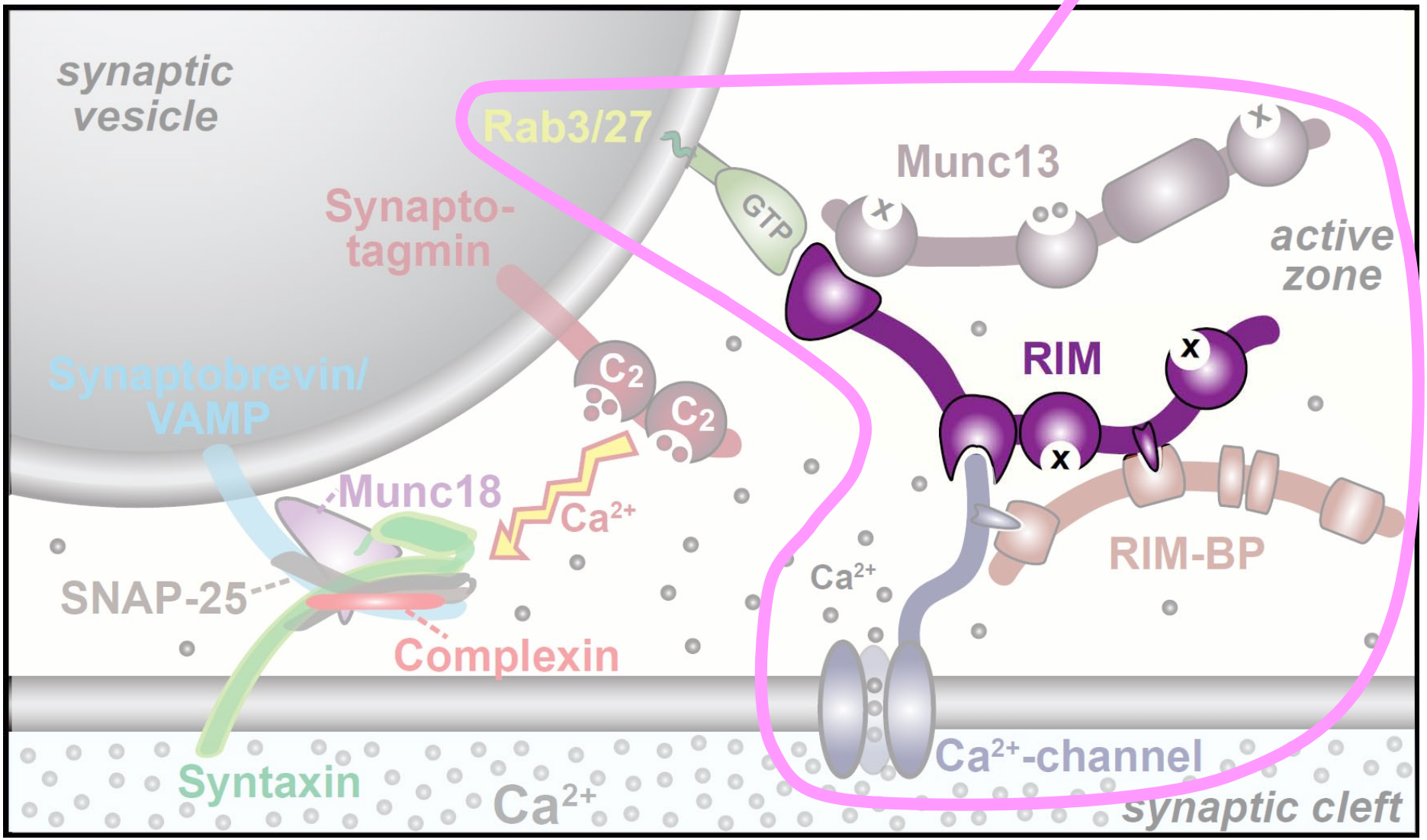
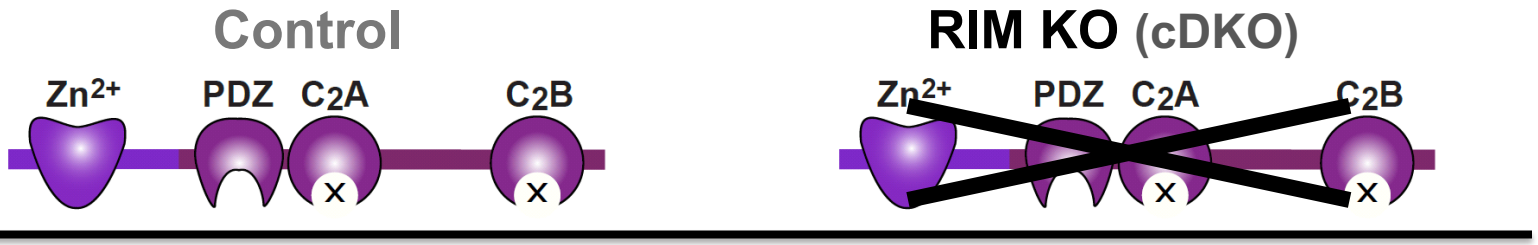
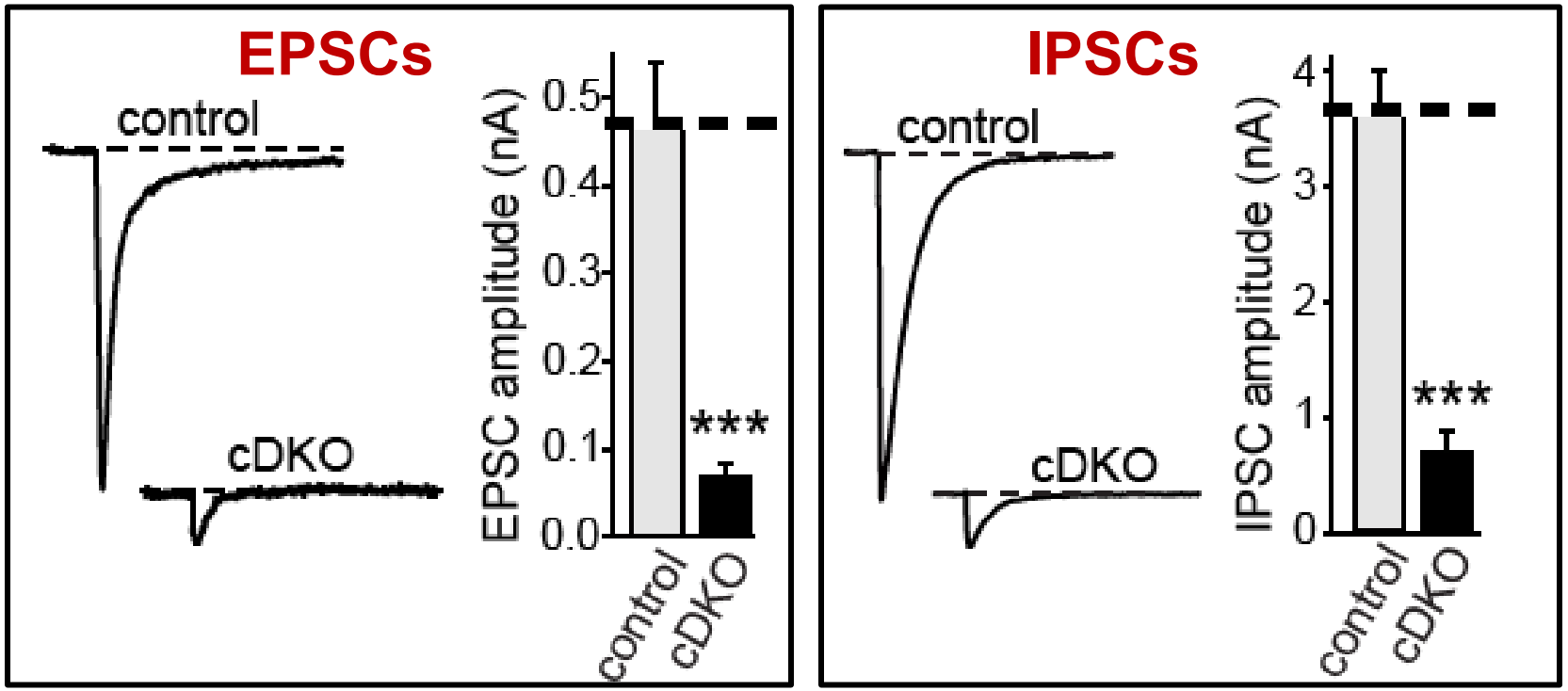


RIM is the central component of the active zone

Deletion of RIM Severely Impairs Release

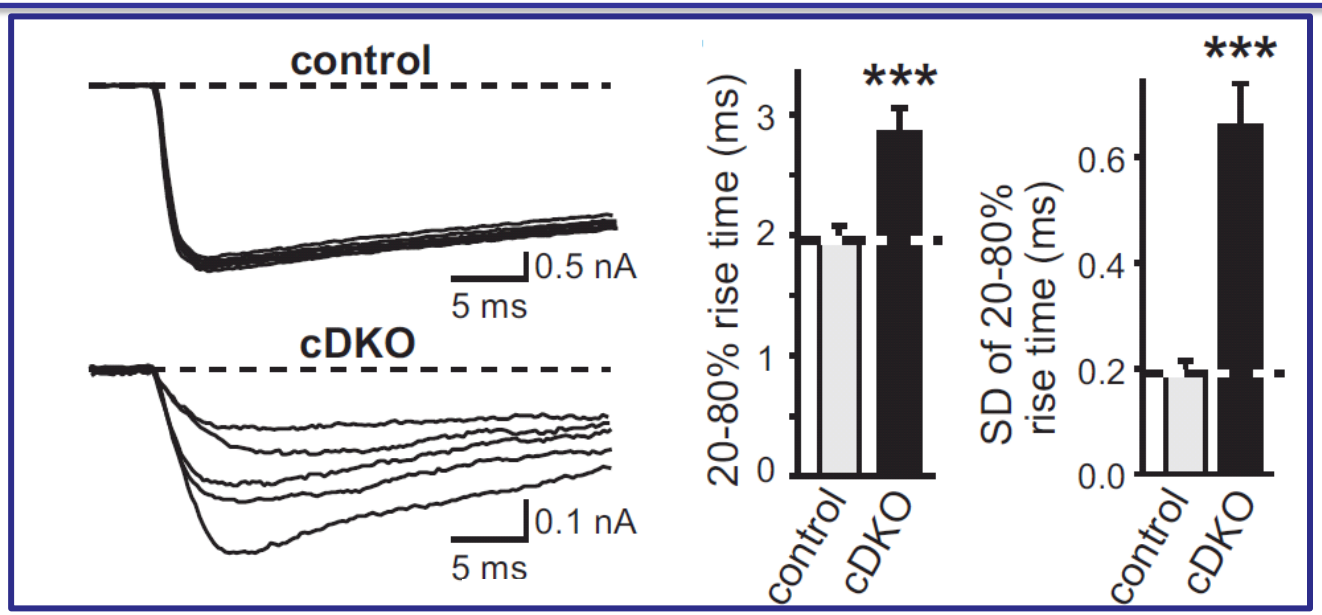




Is release impaired because of a defect in Ca^{2+}-influx?

Kaeser et al., Cell 2011; Deng et al., Neuron 2011; Han et al., Neuron 2011

RIM Deletion Decelerates & Desynchronizes Release: Renders Release Sensitive to Slow Ca^{2+}-Buffers



Speed & synchronicity of IPSCs

Kaeser et al., Cell 2011; Deng et al., Neuron 2011; Han et al., Neuron 2011

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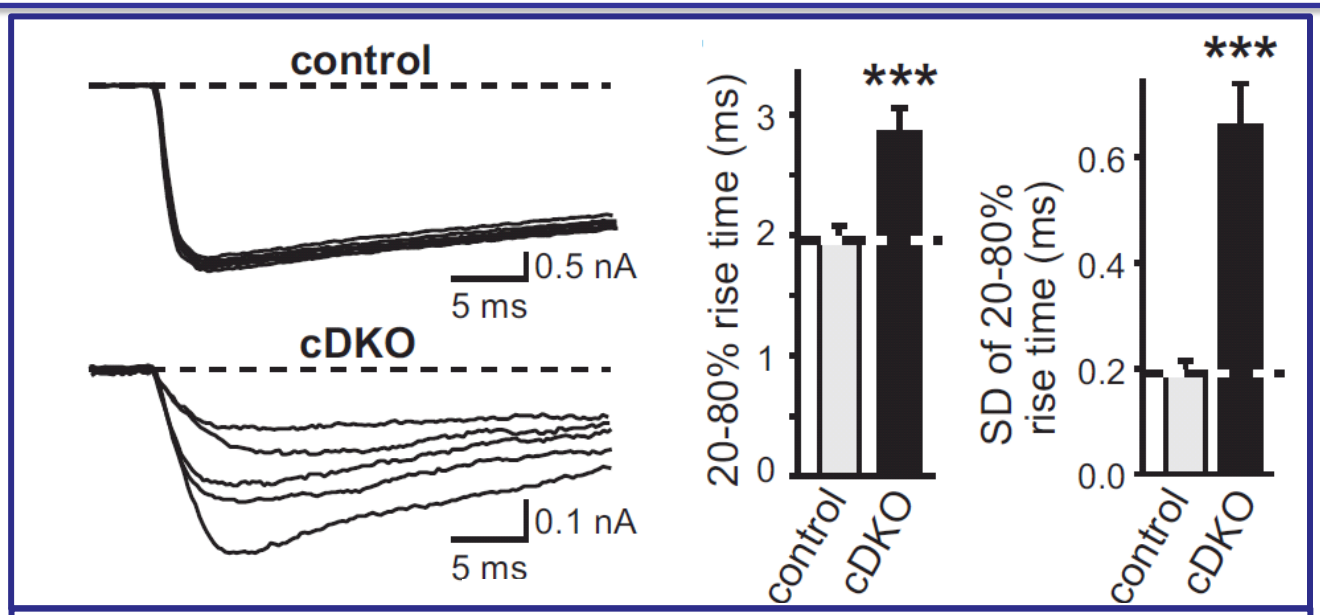
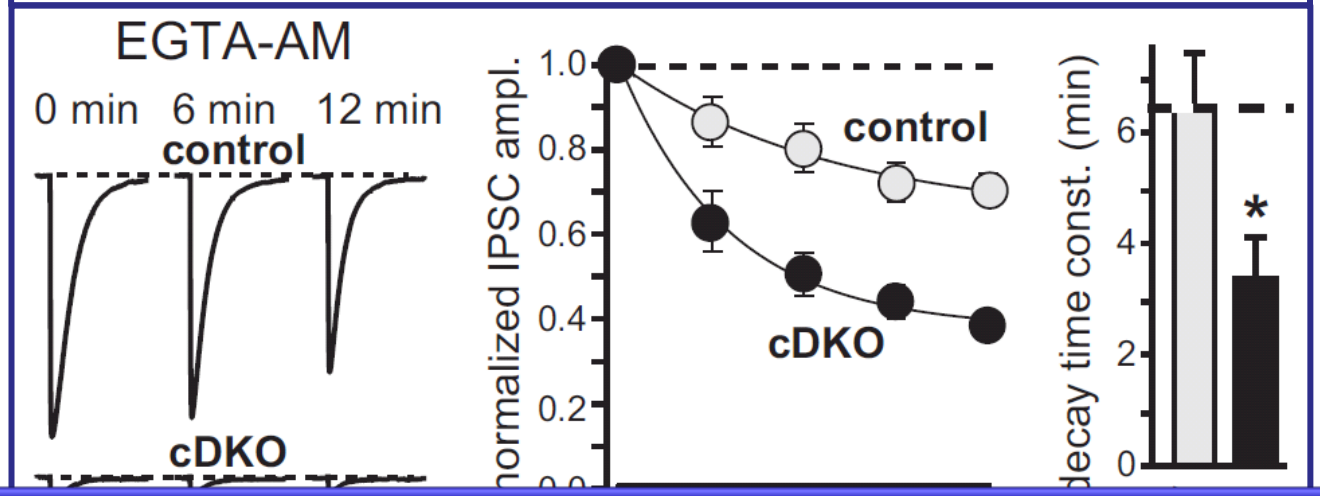


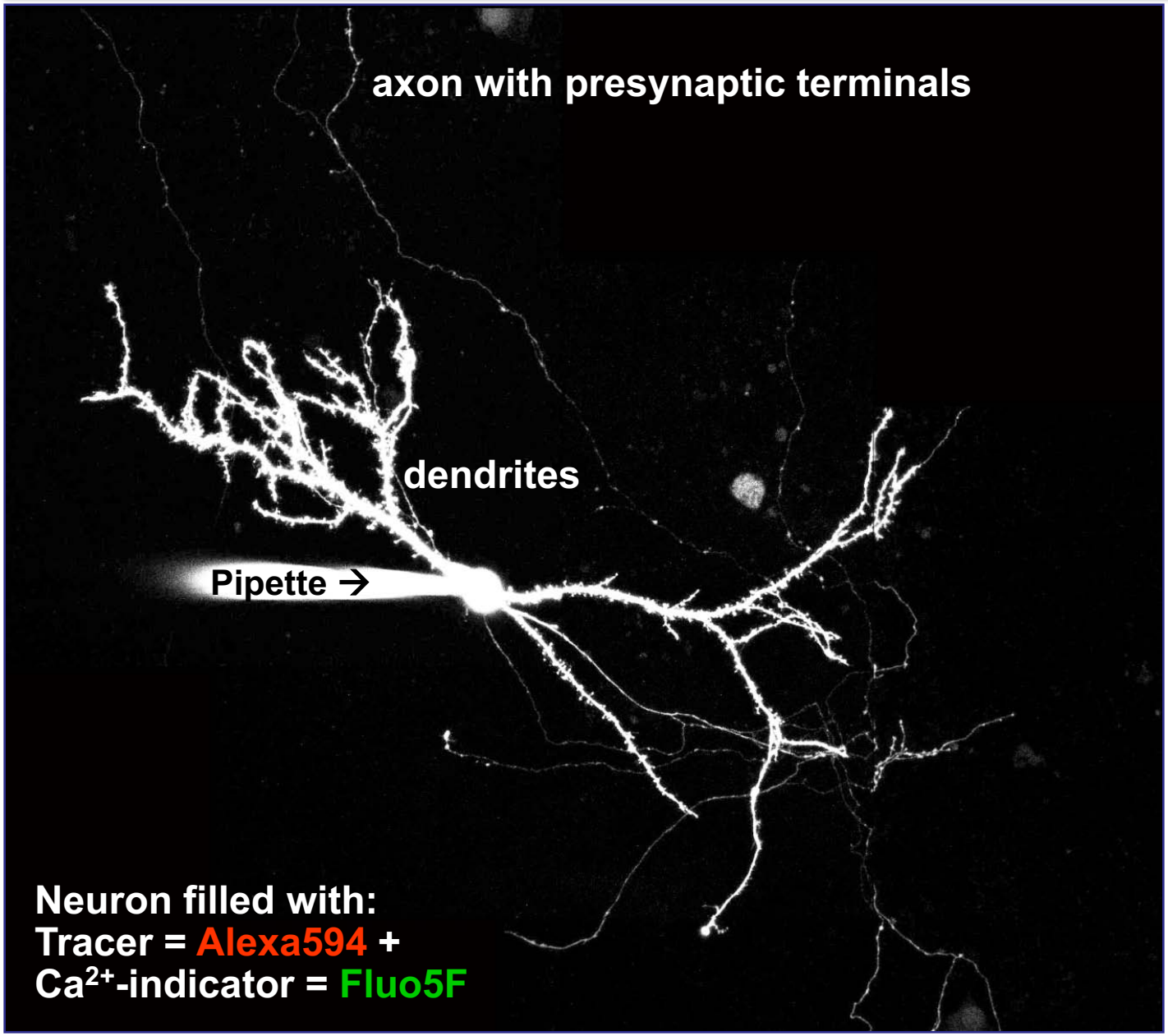
Speed & synchronicity of IPSCs



Effect of slow Ca^{2+}-buffers

Consistent with impaired Ca^{2+}-influx → measure the role of RIM in Ca^{2+}-influx directly

Measurement of Ca^{2+}-Transients in Hippocampal Neurons



Kaeser et al., Cell 2011

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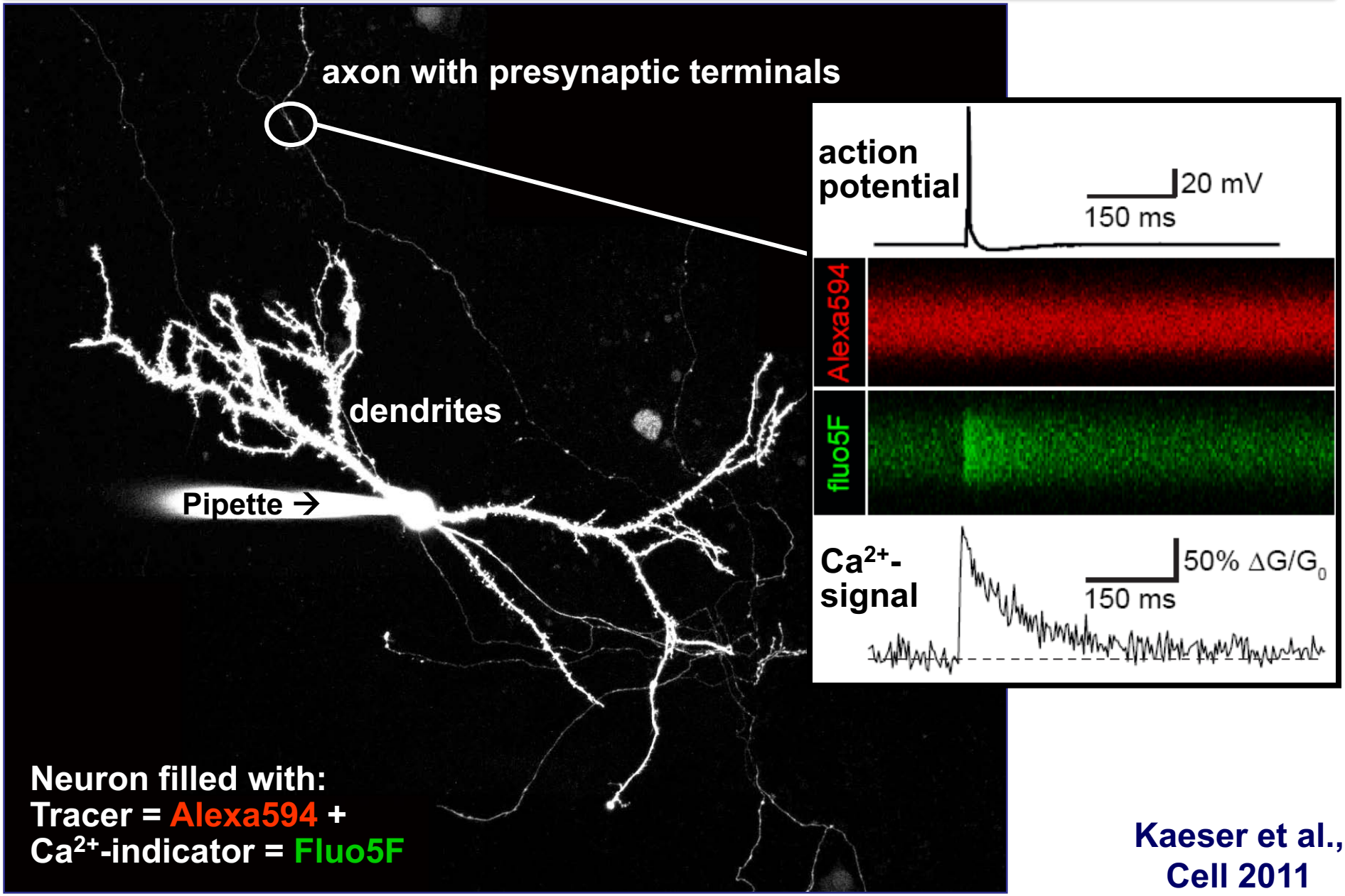
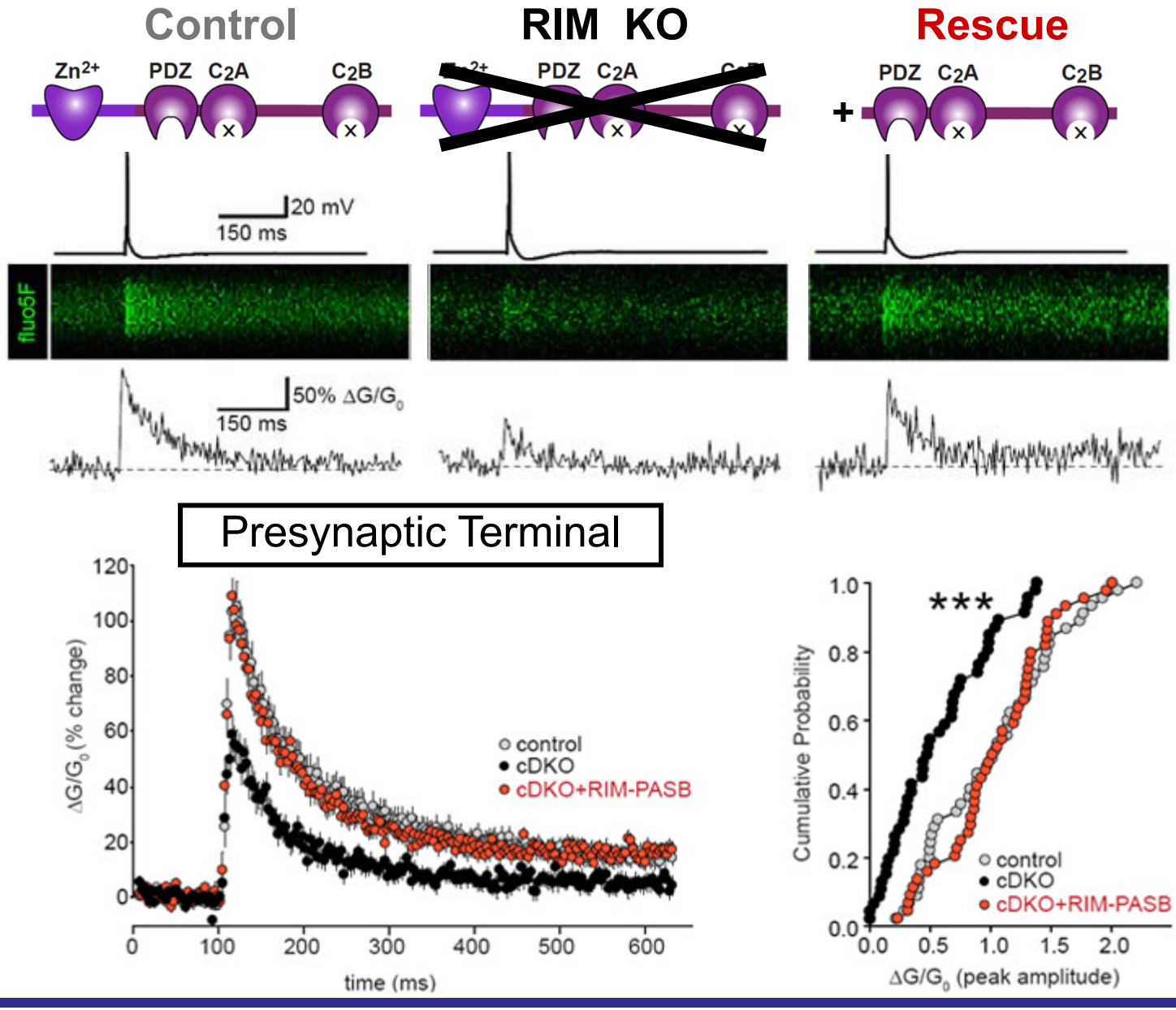


Kaeser et al., Cell 2011

RIM Deletion Impairs Presynaptic Ca^{2+}-Influx



Kaeser et al., Cell 2011

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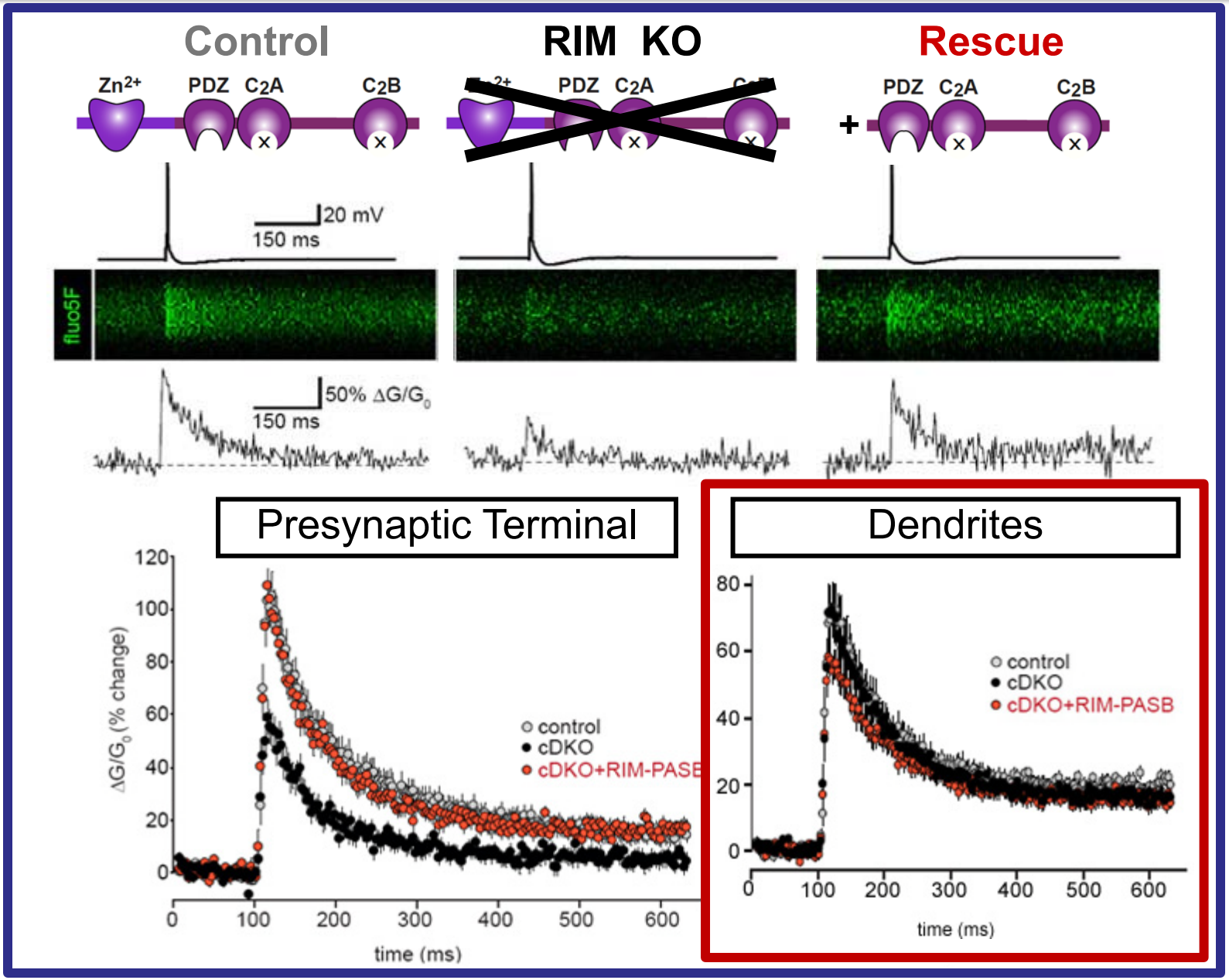
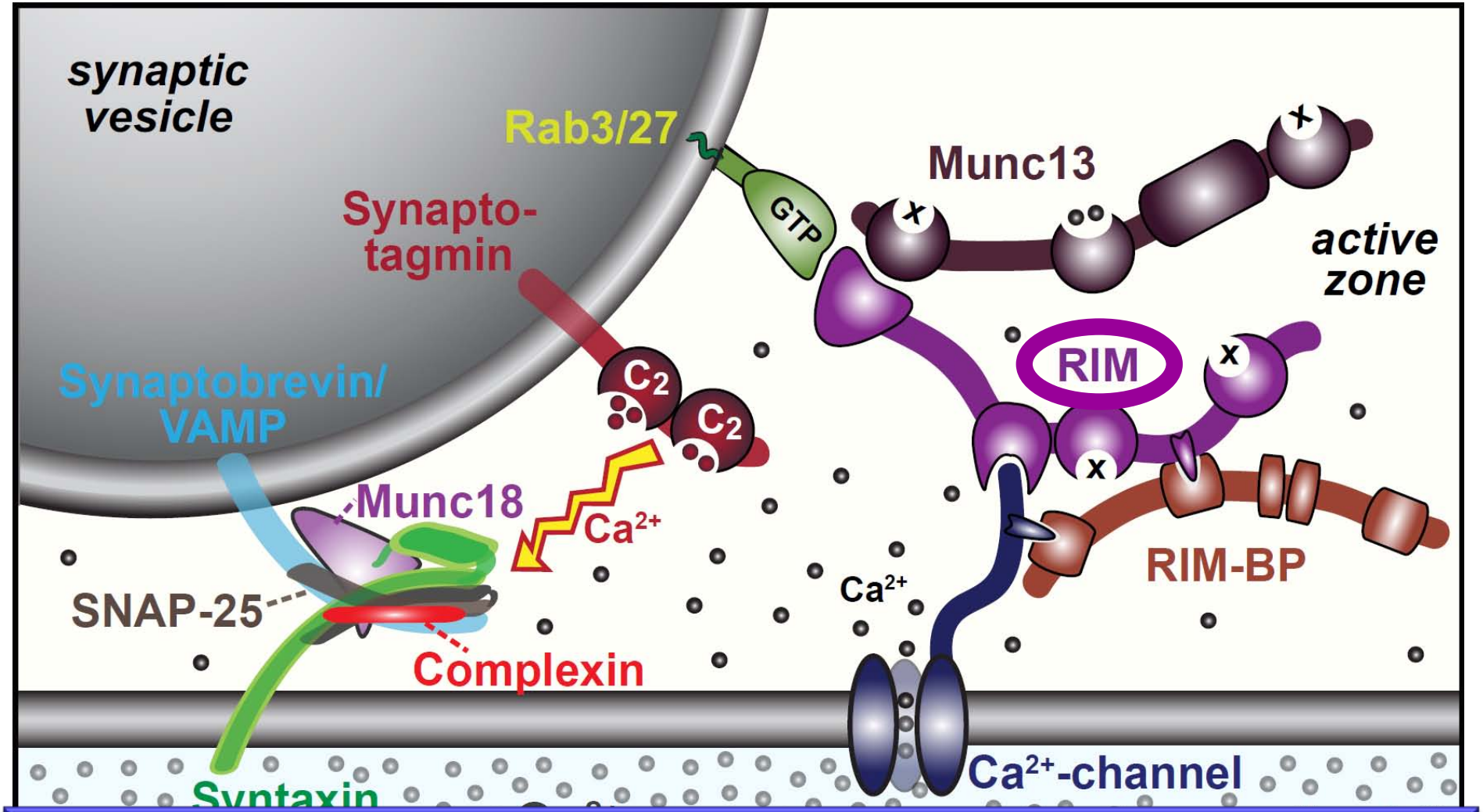


Kaeser et al., Cell 2011

A Neurotransmitter Release Machine Mediates Fusion, Ca^{2+}-triggering & Ca^{2+}-Channel Tethering



RIM also mediates synaptic vesicle docking, enable synaptic plasticity, and activates Munc13 for vesicle priming

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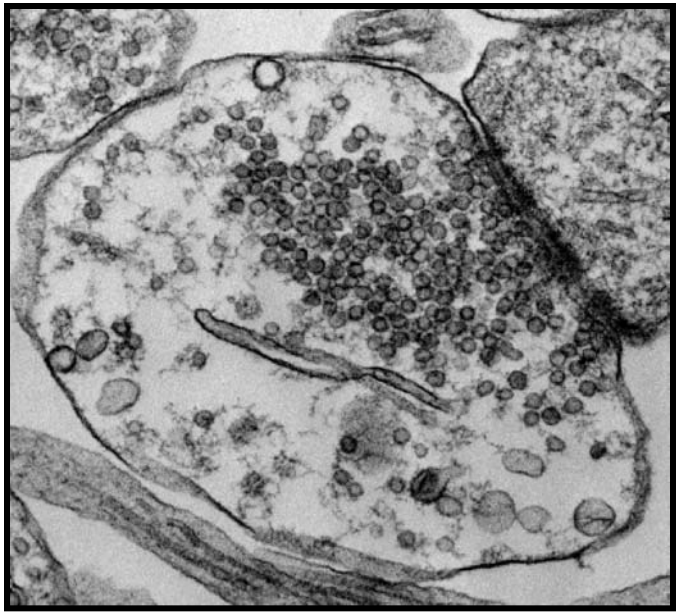

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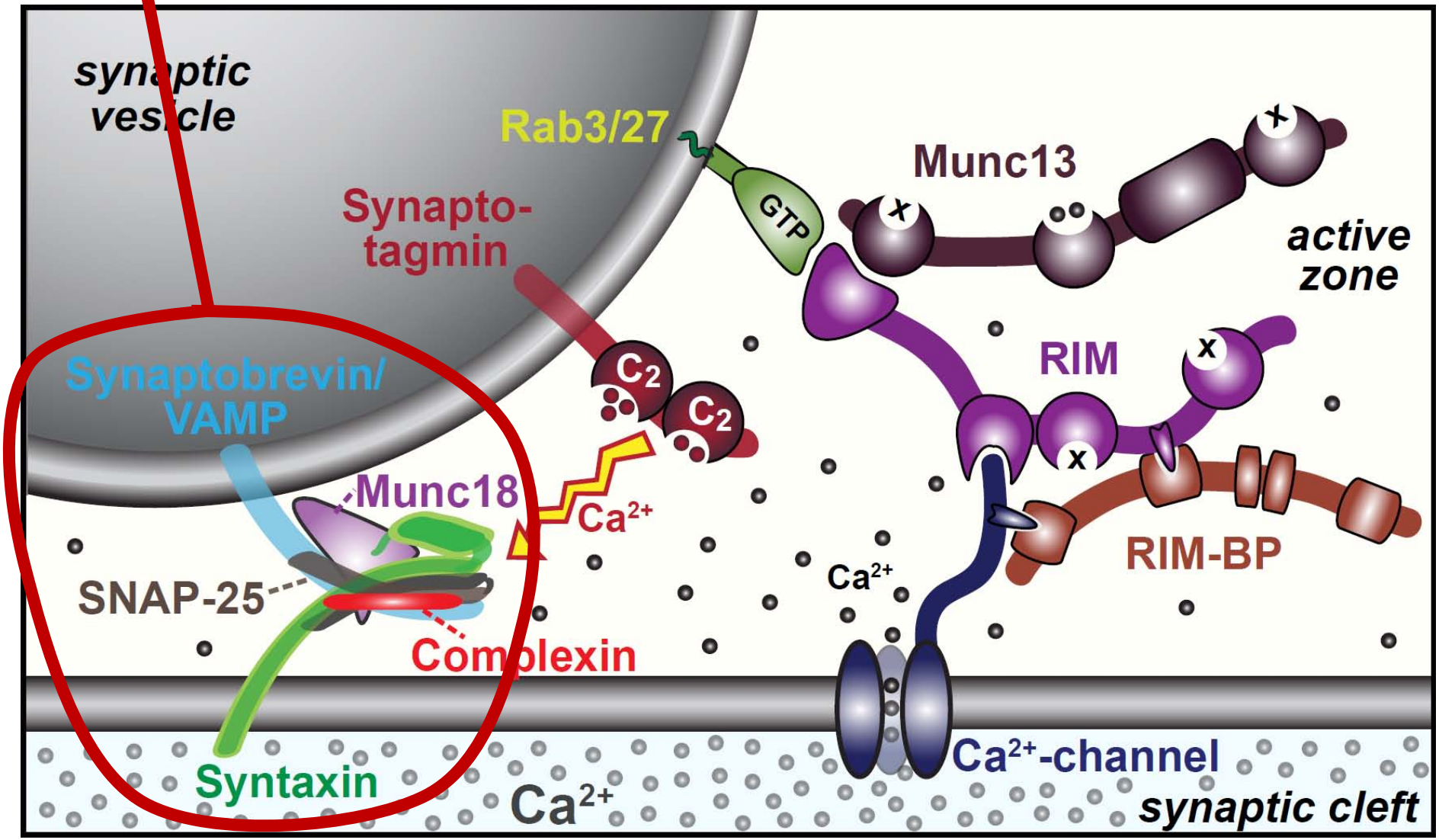

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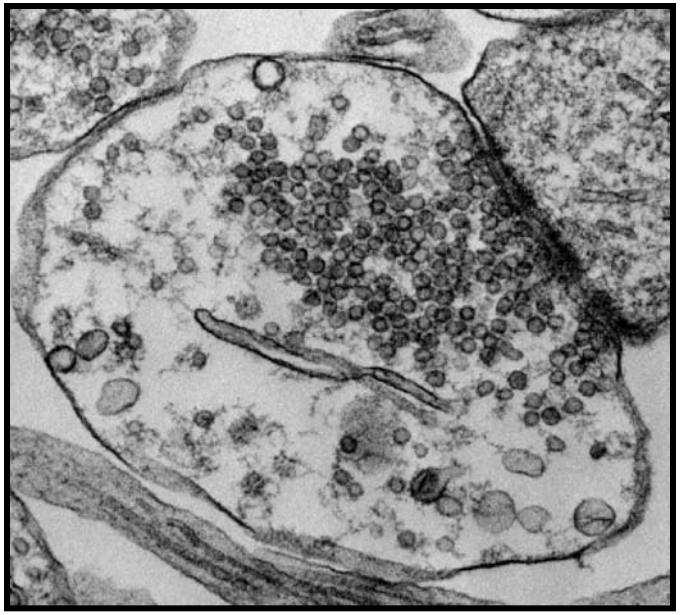
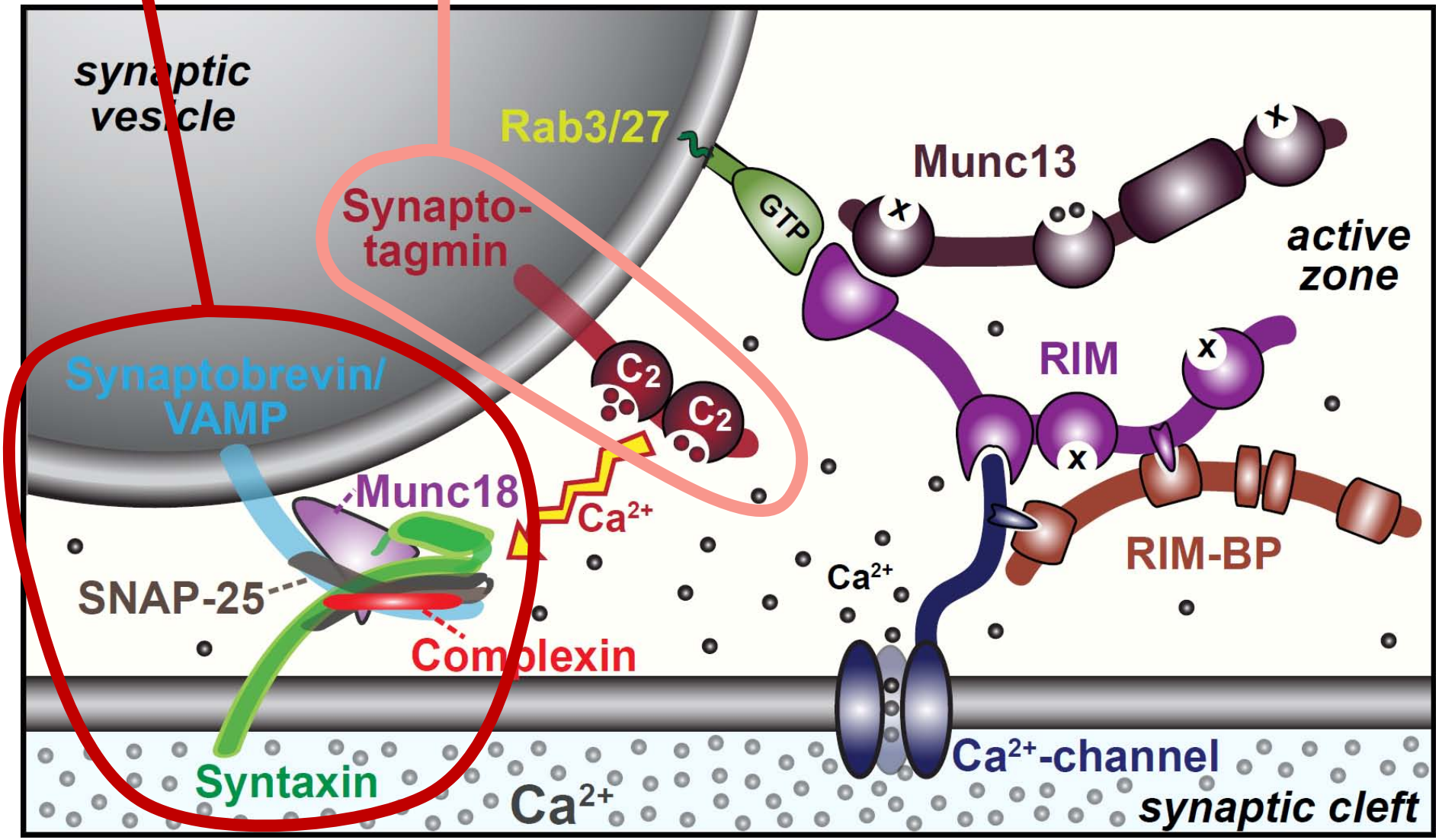
   - Cooperative: ~5 Ca^{2+}-ions

3. Localized Ca^{2+}-influx



A Neurotransmitter Release Machine Mediates Fusion Ca^{2+}-triggering & Ca^{2+}-Channel Tethering

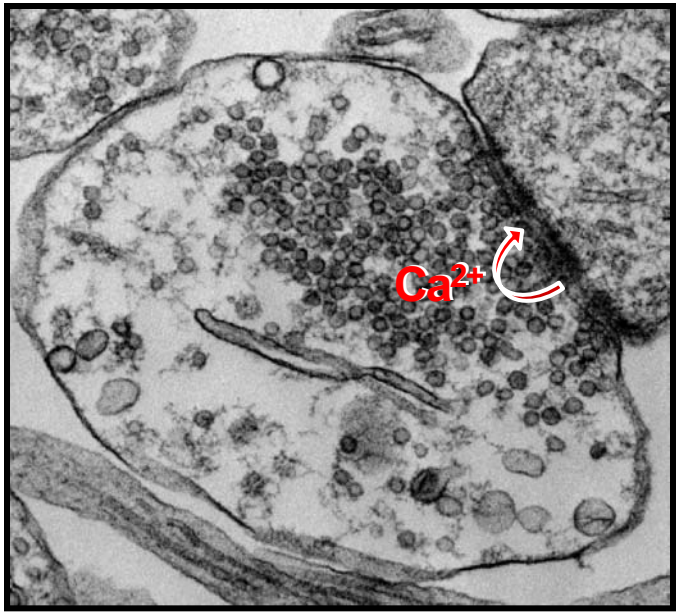


Three Processes Govern Neurotransmitter Release

1. Synaptic vesicle fusion

2. Ca^{2+}-triggering of fusion
   - Very fast: ~0.1 msec
   - Cooperative: ~5 Ca^{2+}-ions

3. Localized Ca^{2+}-influx



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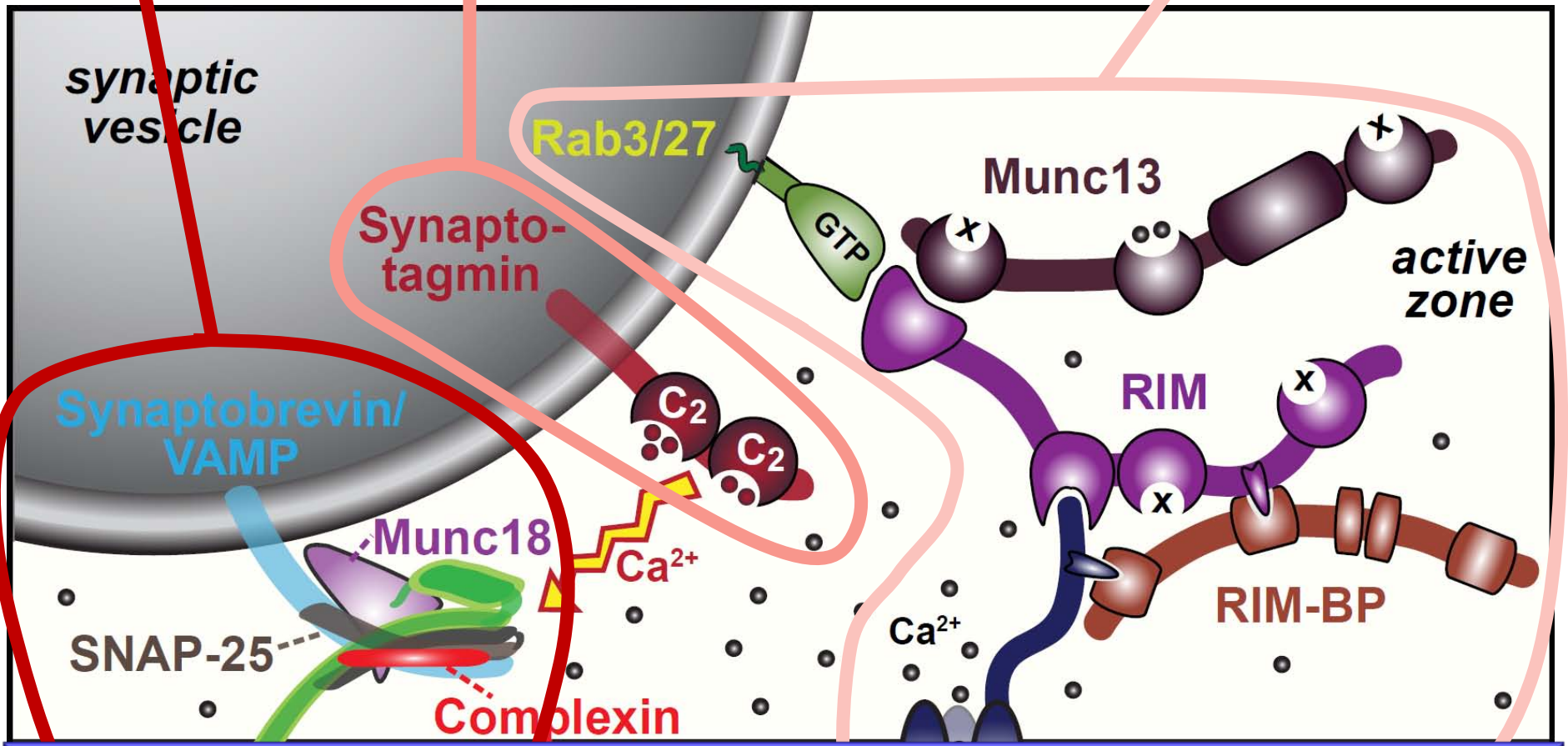

Functionally, the fusion, Ca^{2+}-triggering, and active zone complexes form a single interacting nanomachine mediating fast transmitter release

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