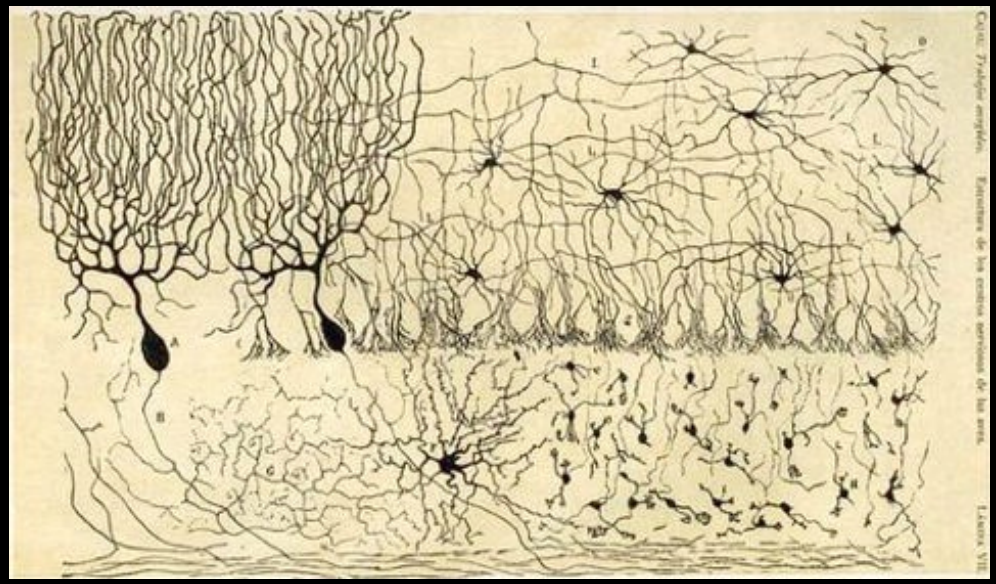
OUR AMAZING BRAIN

http://research.vtc.vt.edu/news/2013/feb/13/brain-awareness-week-designed-highlight-advances-b/

Neurons form amazing networks

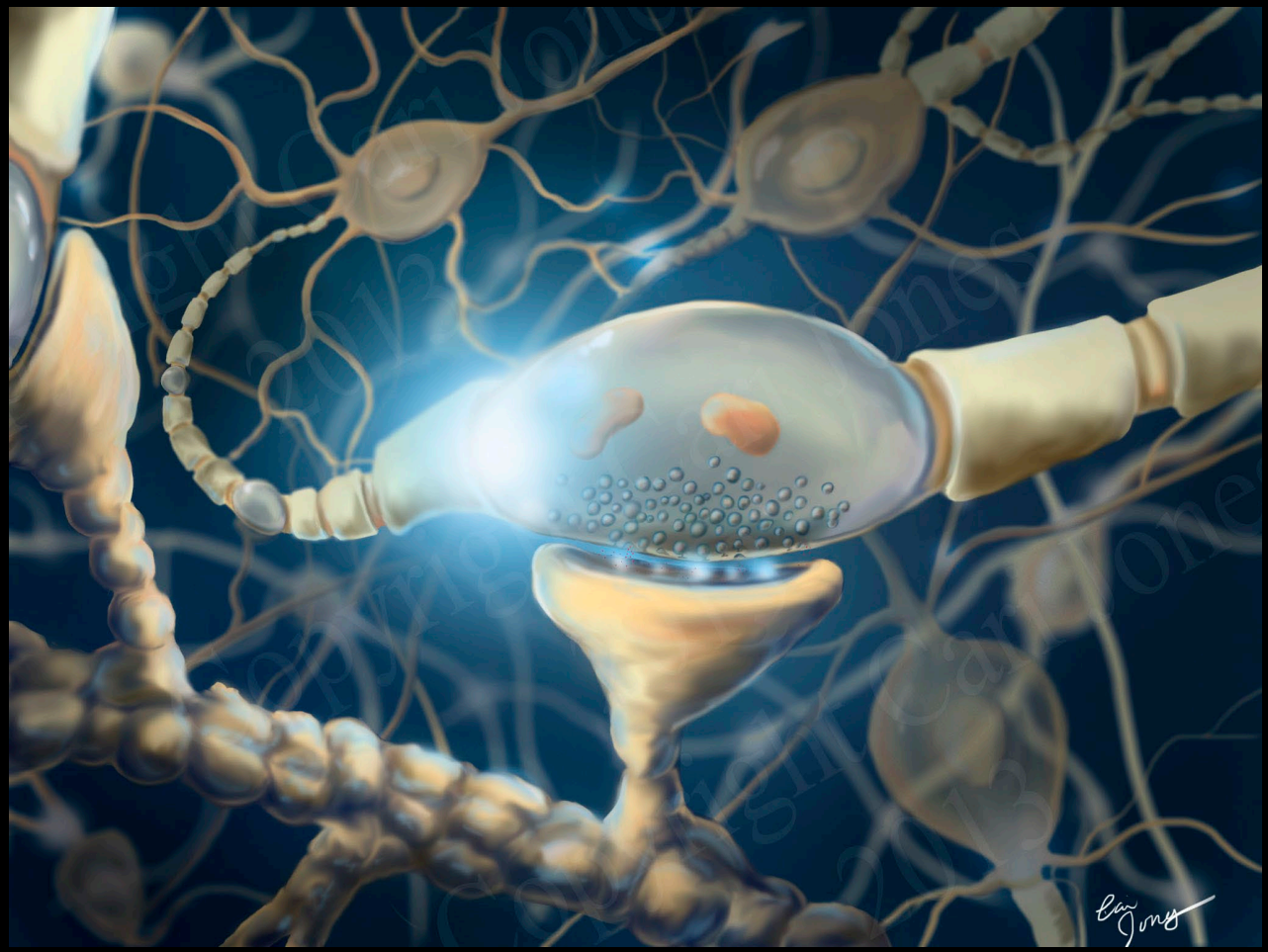
www.the-scientist.com



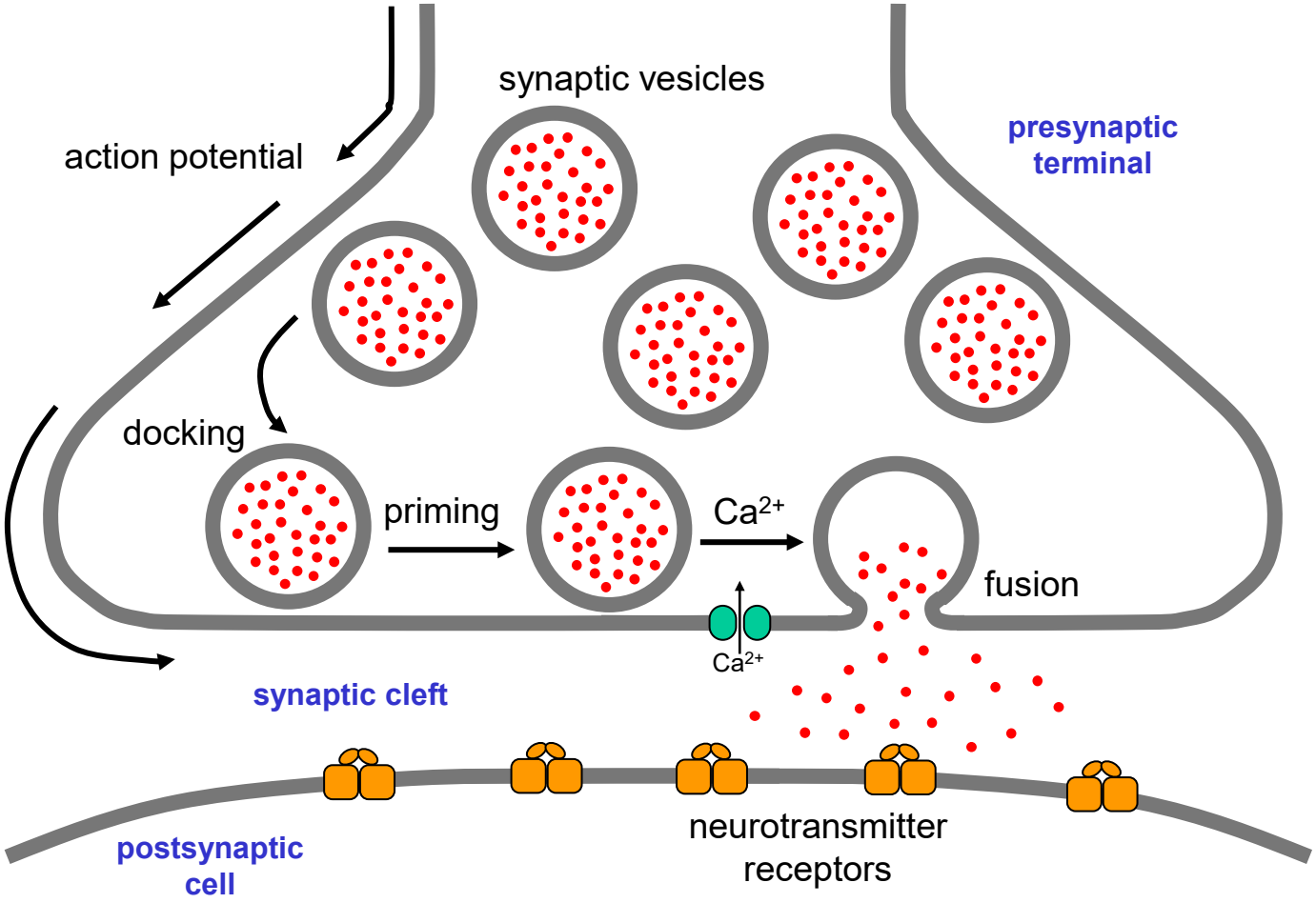
neuronico.net

(drawings by Santiago Ramon y Cajal)

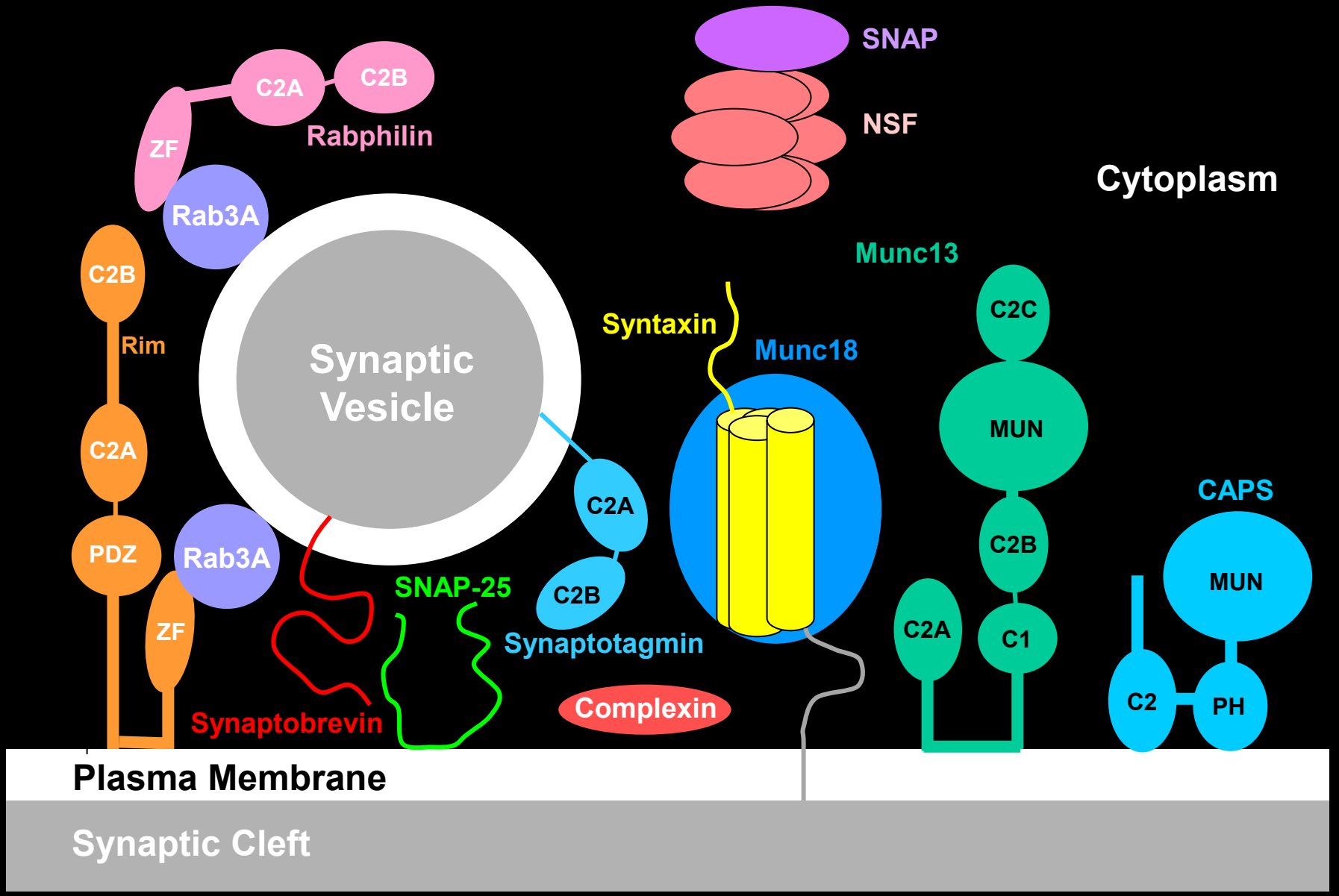
Synaptic transmission occurs at synapses



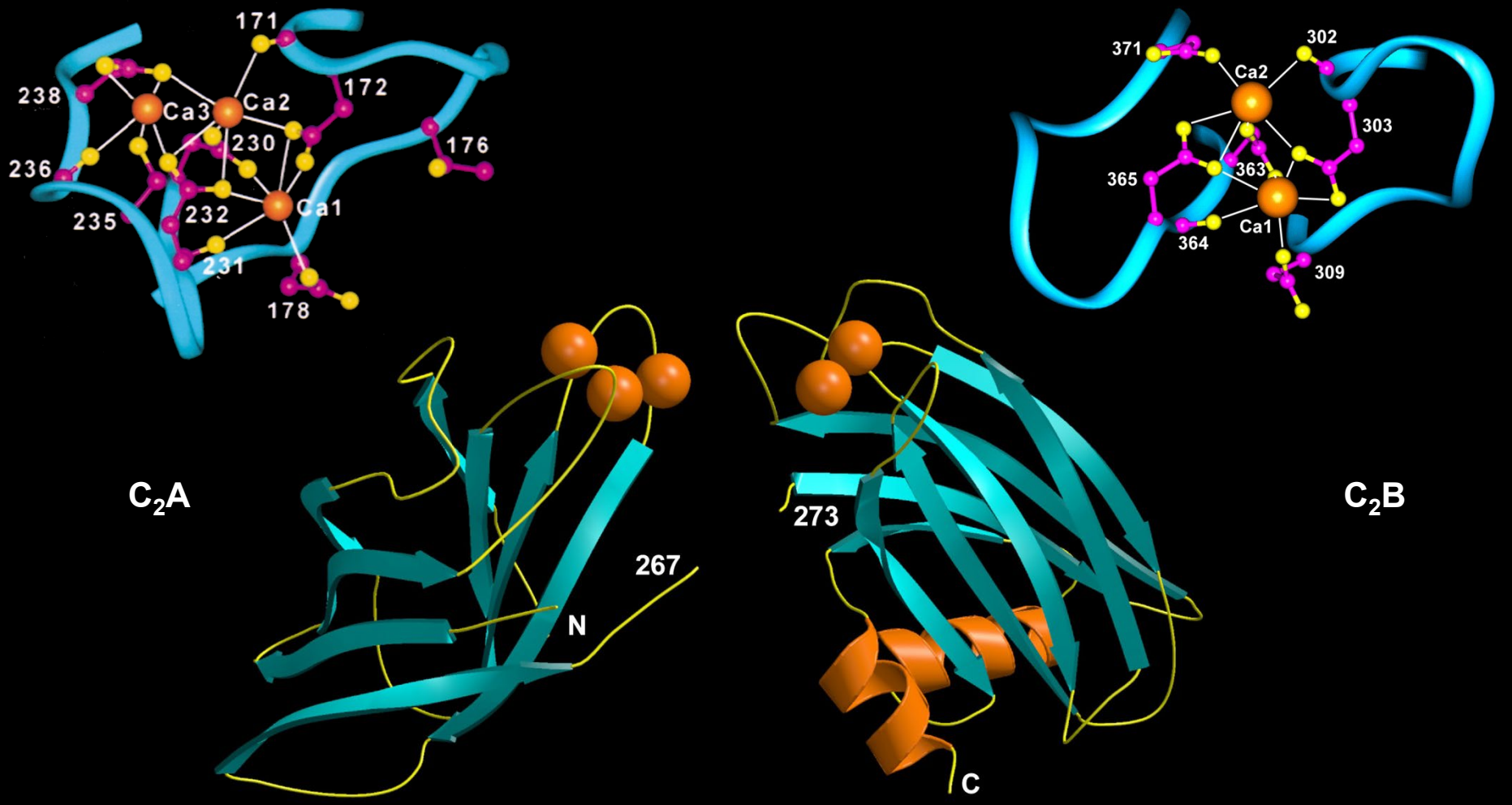
Synaptic vesicle fusion is key for interneuronal communication



TWO fundamental jobs for the release machinery:
Stimulate AND control membrane fusion



Structures and Ca^{2+} binding modes of the synaptotagmin-1 C_2 domains

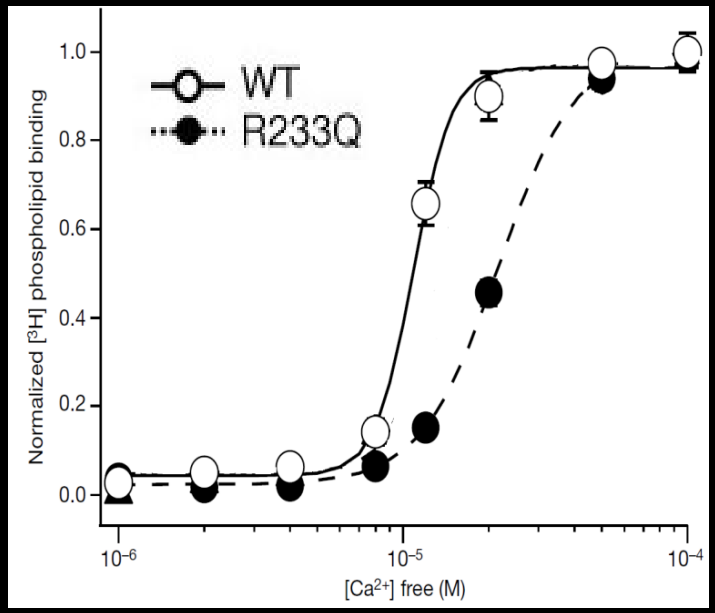


Shao et al. Science 273, 248 (1996)
Shao et al. Biochemistry 37, 16106 (1998)

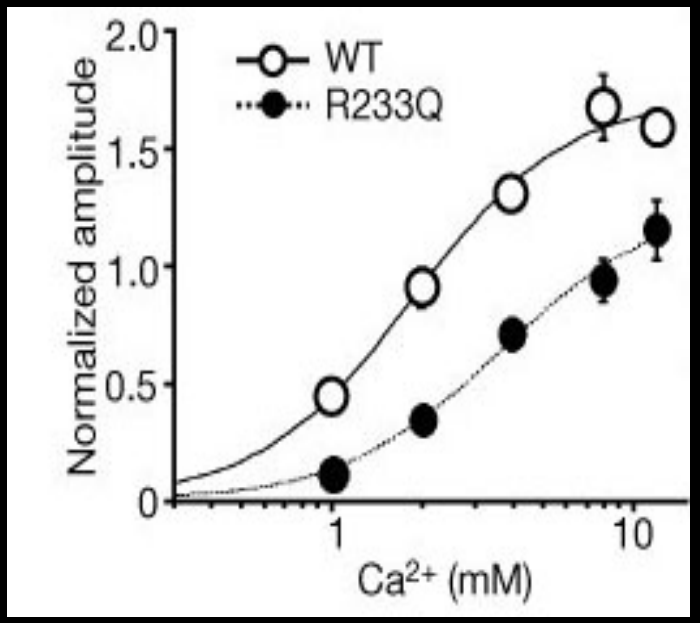
Ubach et al. EMBO J. 17, 3921 (1998)
Fernandez et al. Neuron 32, 1057 (2001)

Synaptotagmin I acts as a Ca2+ sensor in neurotransmitter release

In vitro Ca2+-dependent phospholipid binding



In vivo Ca2+-dependence of neurotransmitter release



Fernandez-Chacon et al. Nature 410, 41 (2001)

The Nobel Prize in Physiology or Medicine 2013
James E. Rothman, Randy W. Schekman, Thomas C. Südhof

The Nobel Prize in Physiology or Medicine 2013

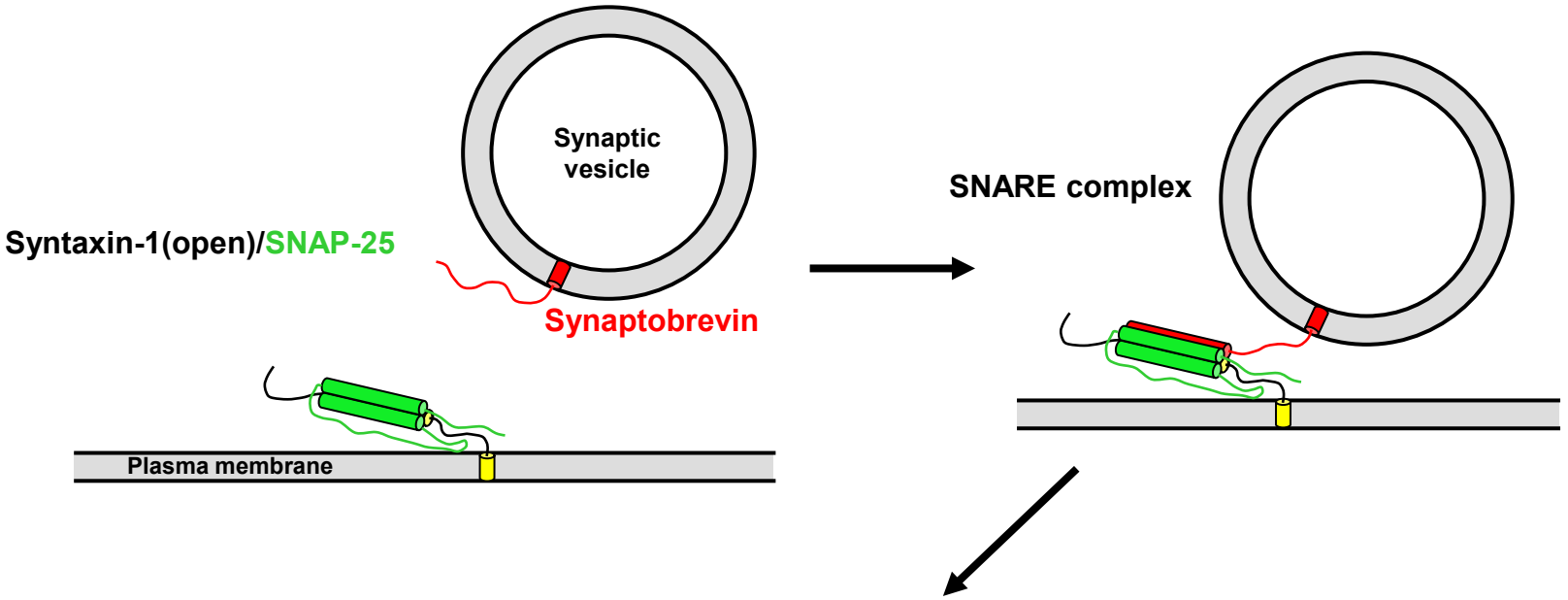
Photo: © Yale University
James E. Rothman

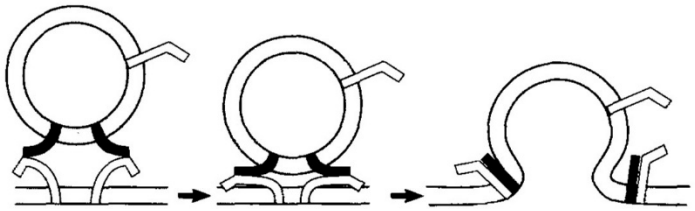
Photo: H. Goren. © HHMI
Randy W. Schekman

Photo: © S. Fisch
Thomas C. Südhof

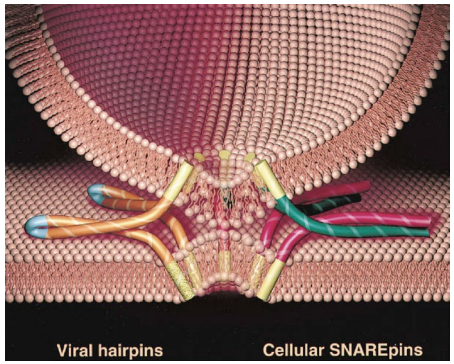
The Nobel Prize in Physiology or Medicine 2013 was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof ***"for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells"***.

Textbook model of SNARE function in synaptic vesicle fusion

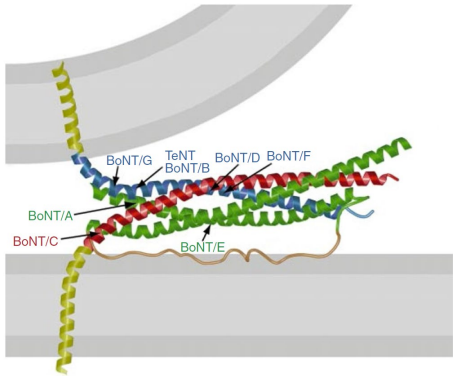




Hanson et al. (1997) Cell 90, 523
Hanson et al. (1997) Curr. Opin. Neurobiol 7, 310

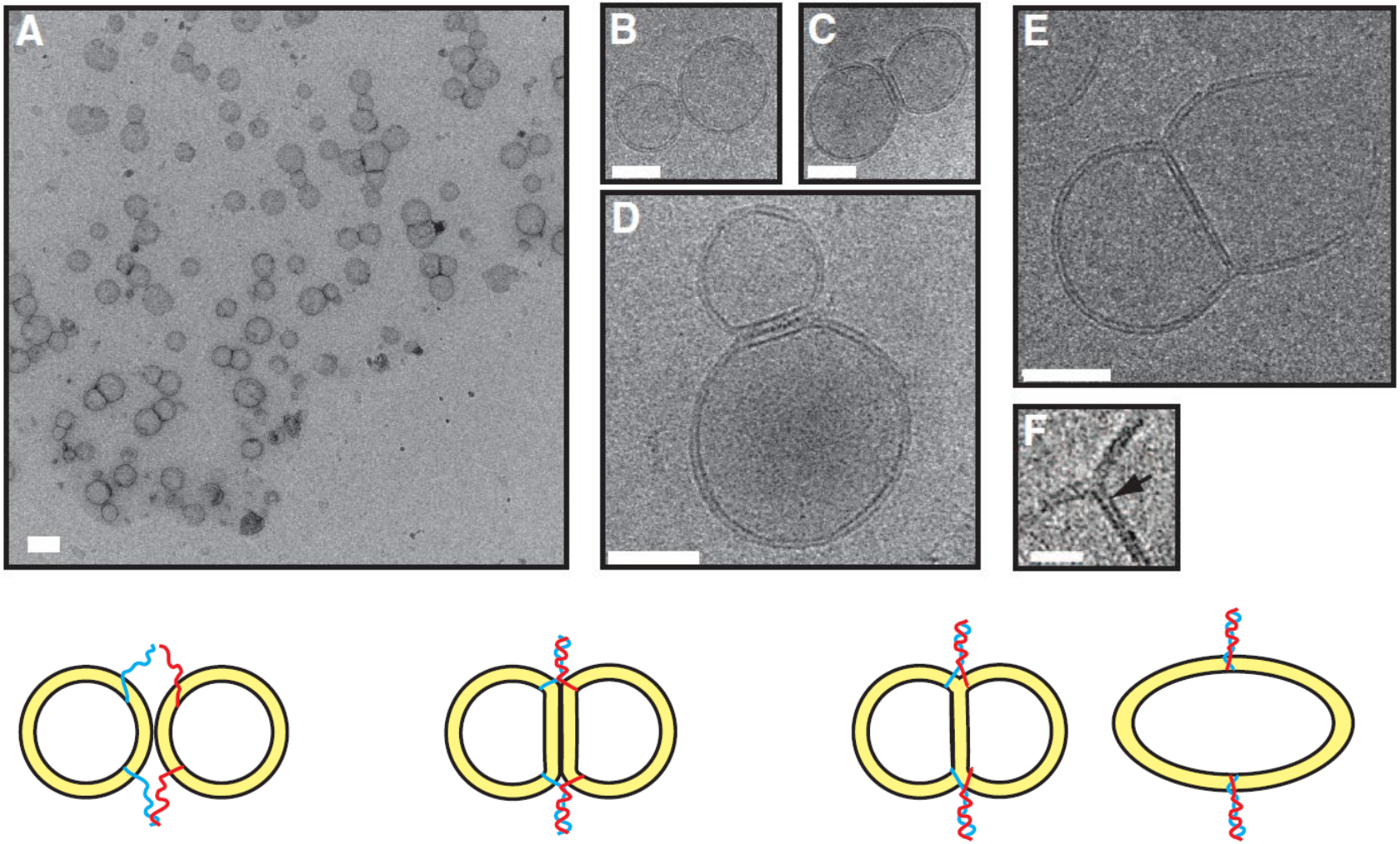


Weber et al. (1998) Cell 92, 759



Sutton et al. (1998) Nature 395, 347

Can the neuronal SNAREs alone induce membrane fusion in less than < 60 μs?



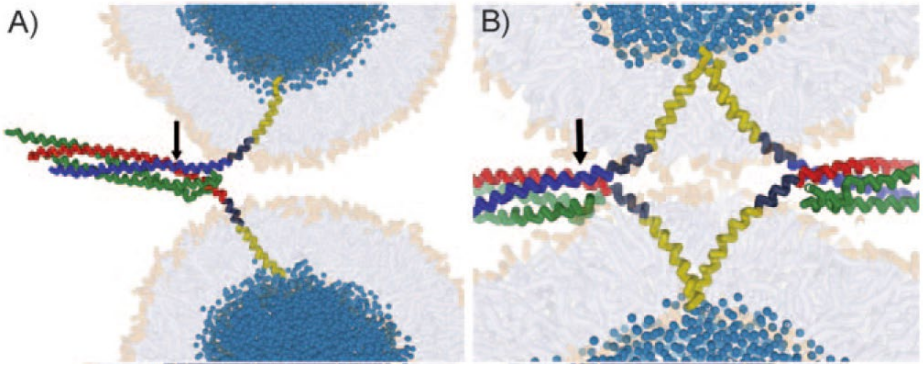
Hernandez et al. (2012) Science 336, 1581

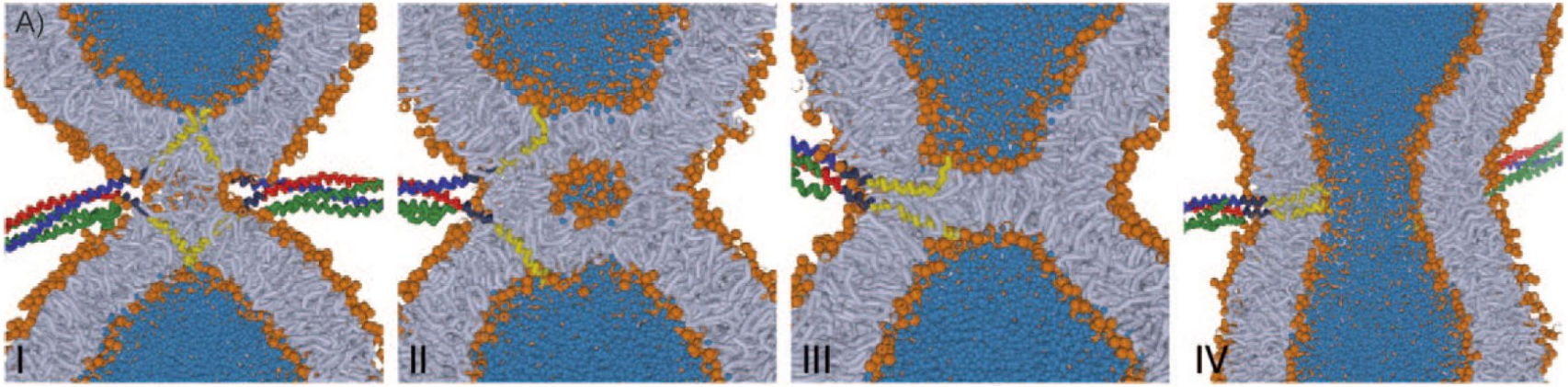
Coarse-grained MD simulations of SNARE-mediated membrane fusion assumed continuous SNARE helices

Caught in the Act: Visualization of SNARE-Mediated Fusion Events in Molecular Detail

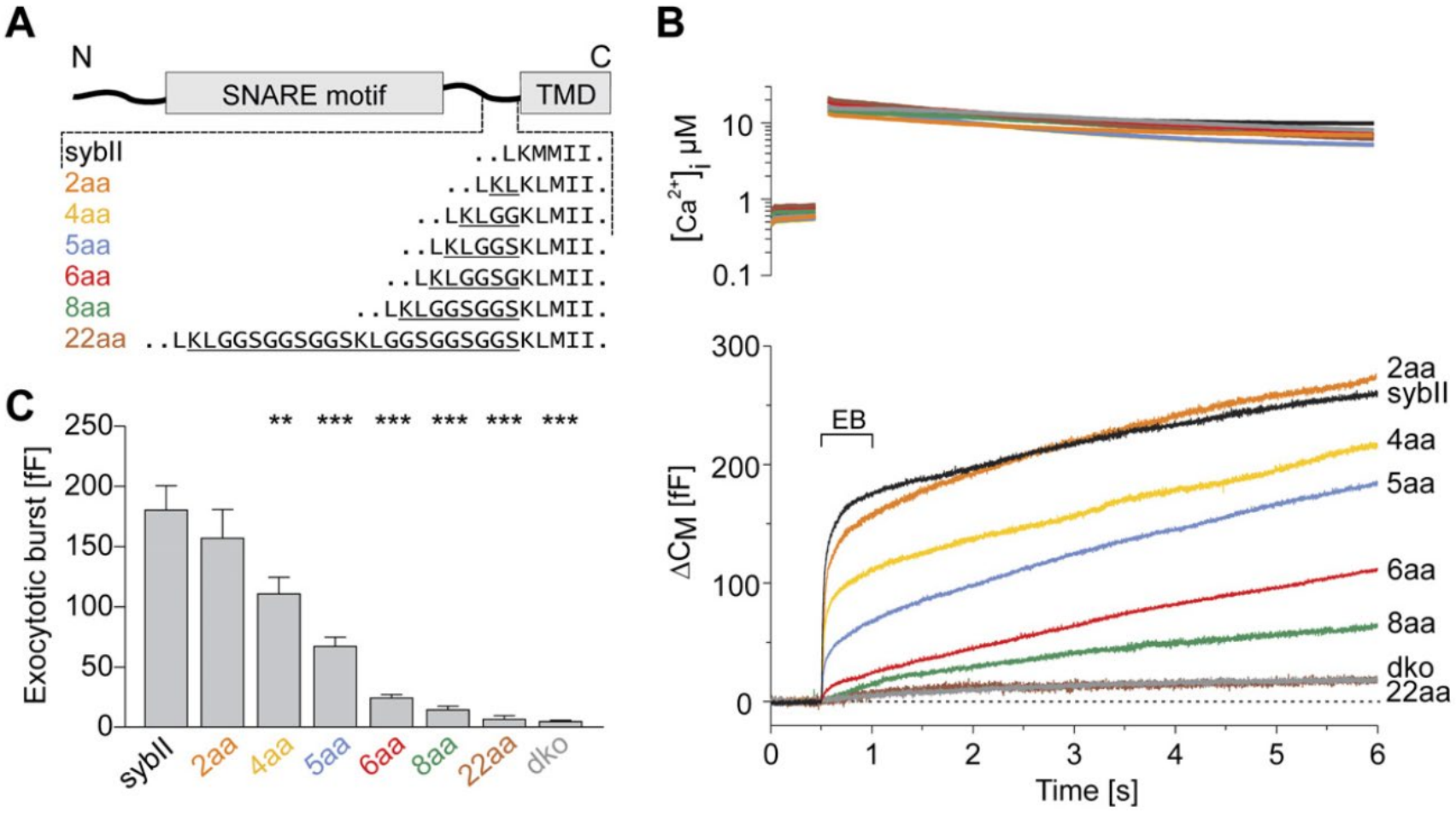
Herre Jelger Risselada, Carsten Kutzner, and Helmut Grubmüller*[a]

ChemBioChem **2011**, *12*, 1049 – 1055 WILEY ONLINE LIBRARY



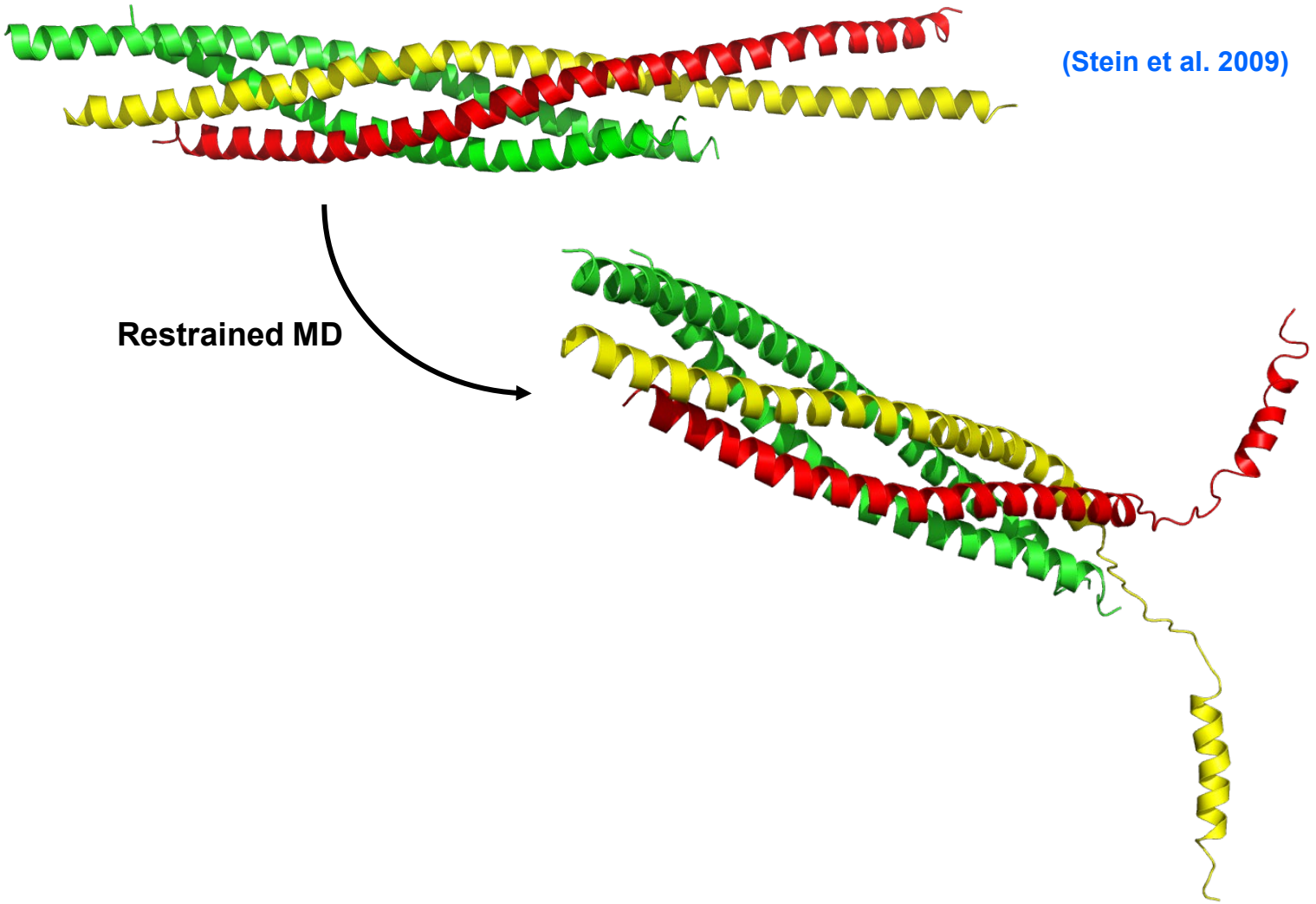


Short linker insertions in synaptobrevin allow robust exocytotic burst



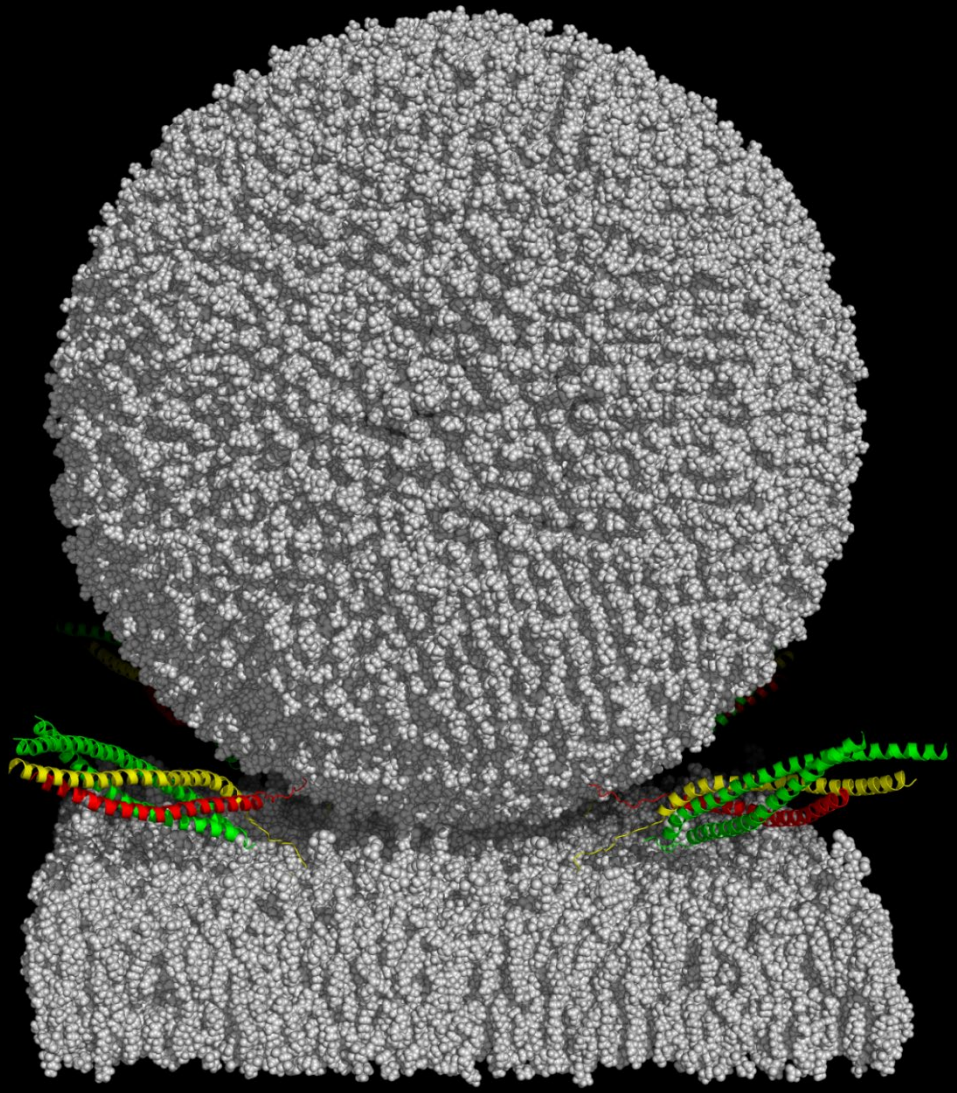
Kesavan et al. (2007) Cell 131, 351

All-atom molecular dynamics simulations with flexible linkers



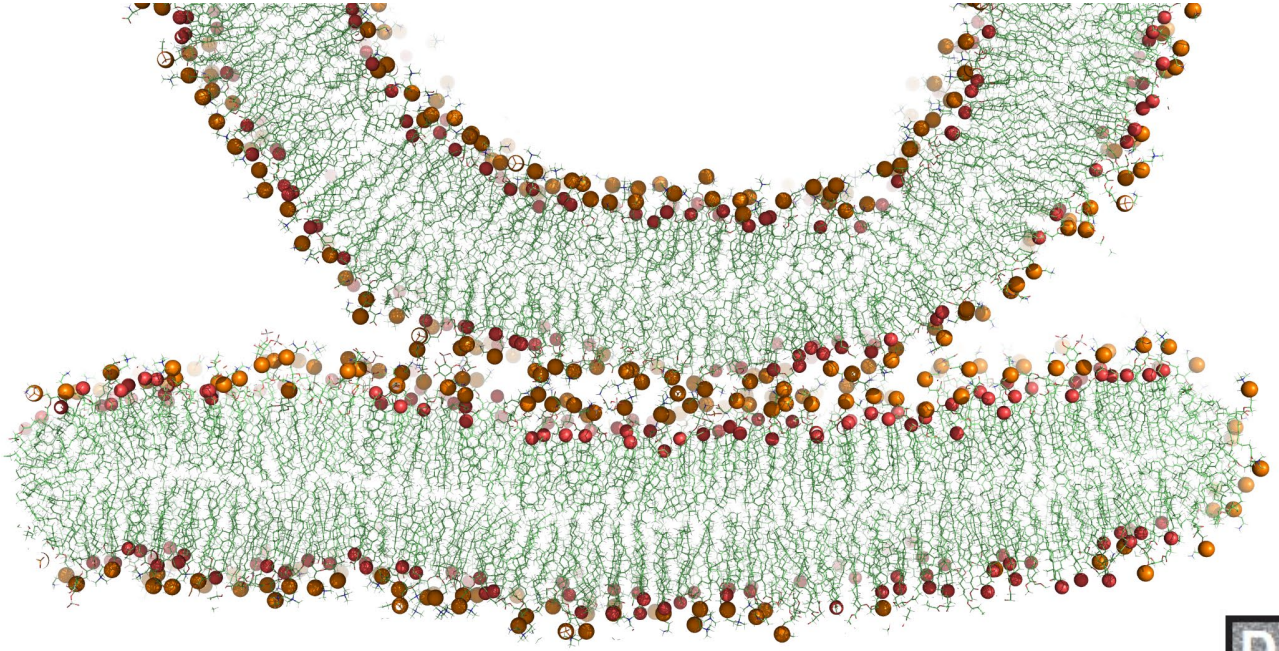
Rizo et al. (2022) *eLife 11*, e76356

Four trans-SNARE complexes with unstructured linkers bridging a vesicle and a planar bilayer

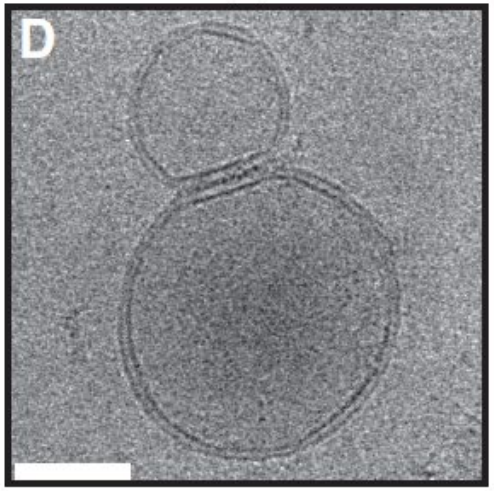


Rizo et al. (2022) *eLife 11*, e76356

Extended vesicle-flat bilayer interface formed after 280 ns resembles liposome-liposome interfaces observed by cryo-EM

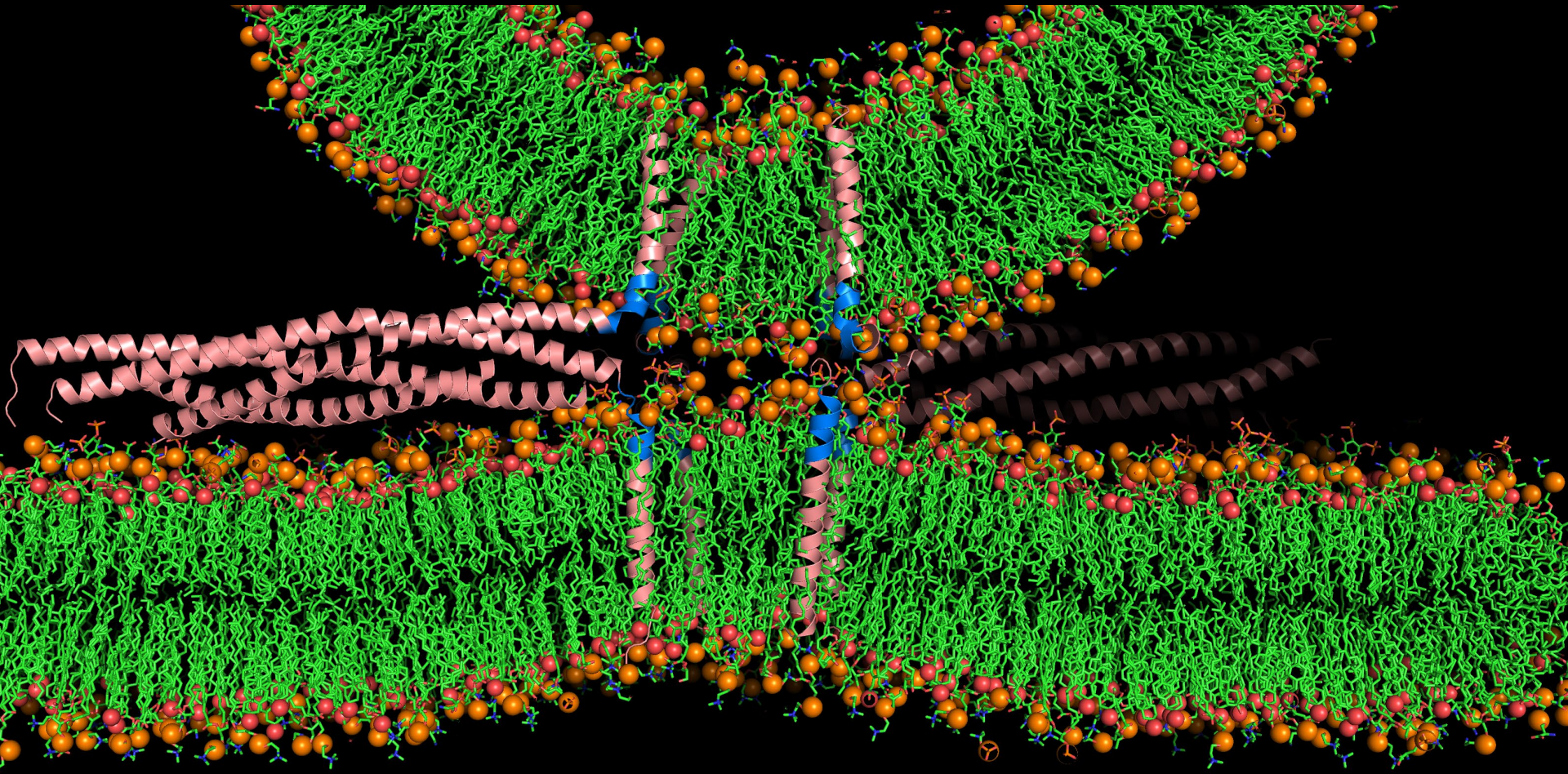


Rizo et al. (2022) *eLife 11*, e76356

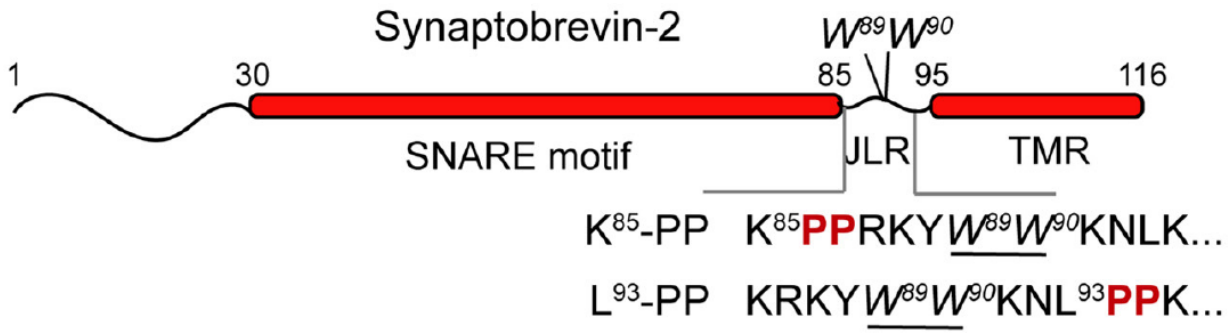


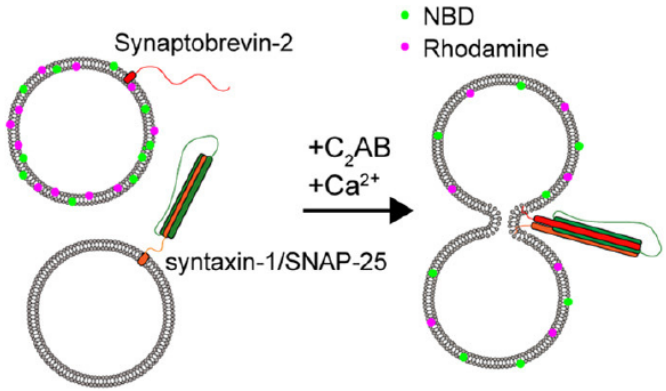
Hernandez et al. (2012) Science 336, 1581

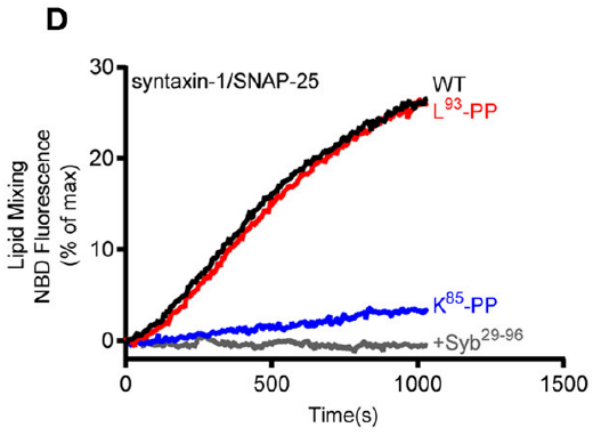
Simulation with kinked helices at linkers: bilayer-bilayer contact but no initiation of lipid mixing in 2 μs

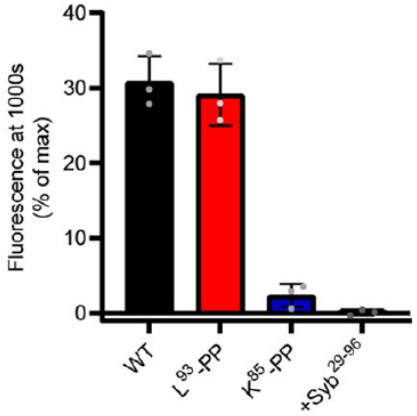


PP insertion between SNARE motif and juxtmembrane linker of synaptobrevin strongly disrupts liposome fusion



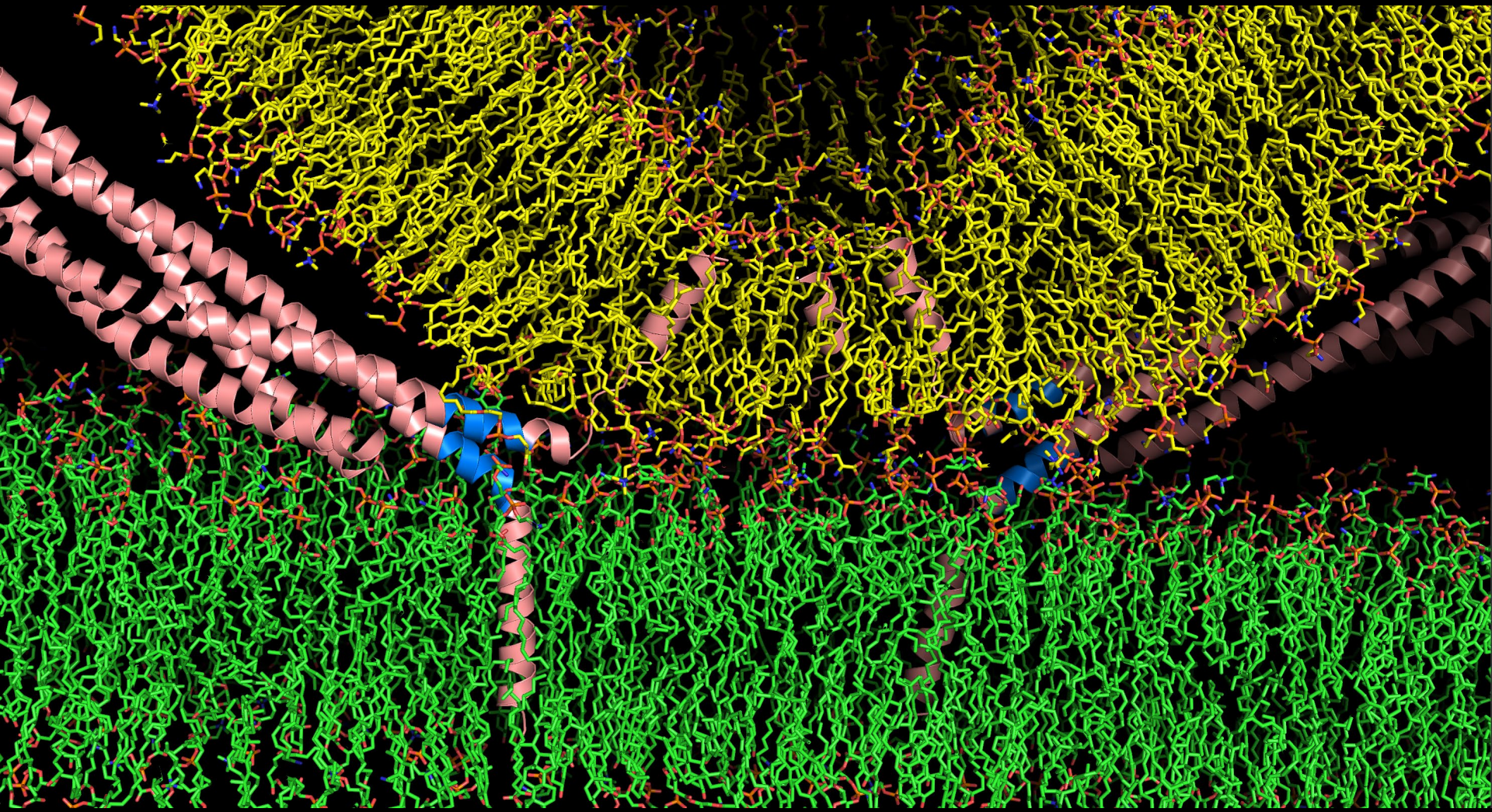






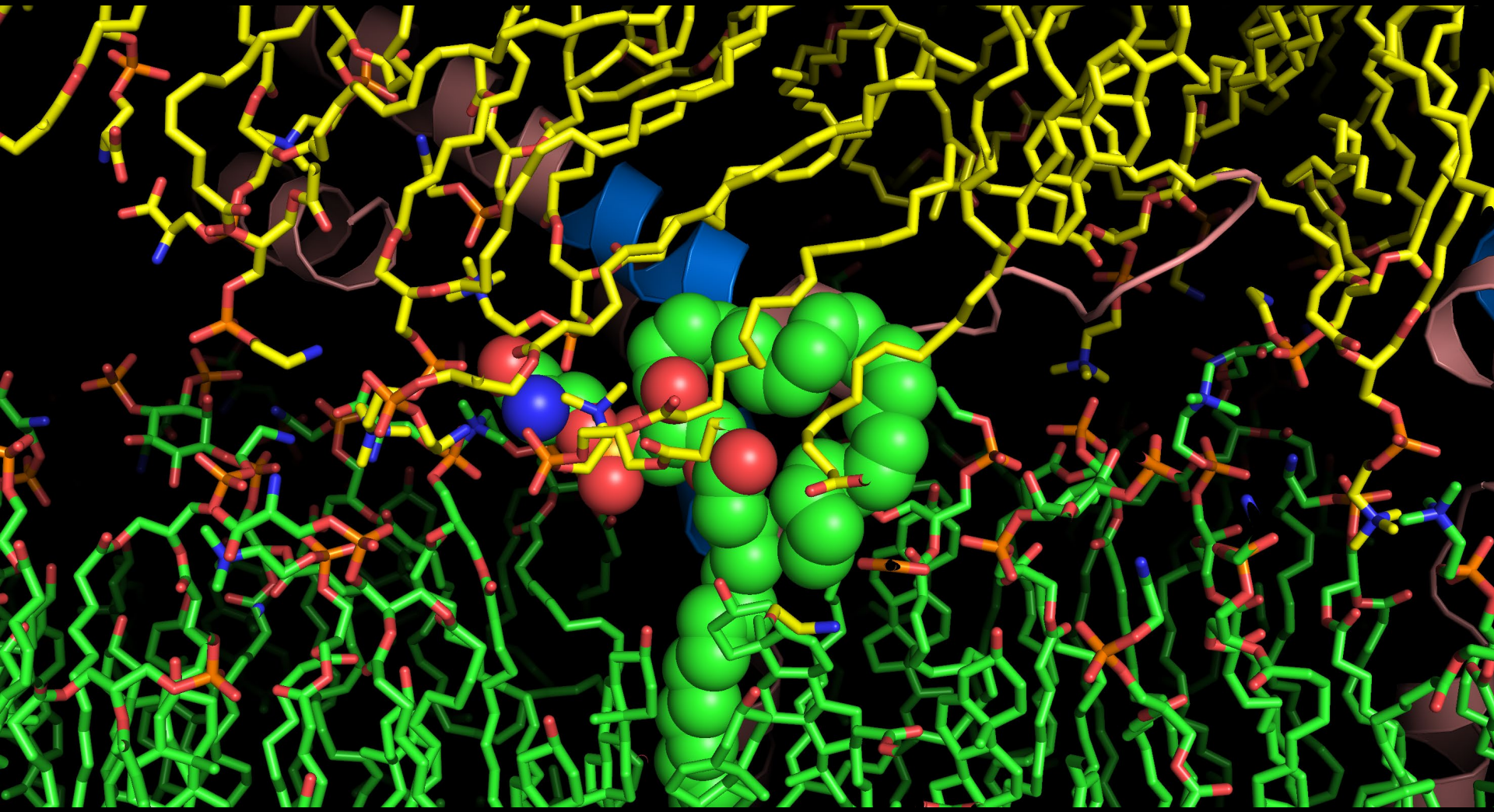
Hu et al. (2020) Front Cell Dev Biol, 8, 609708

Simulation with zippered linkers



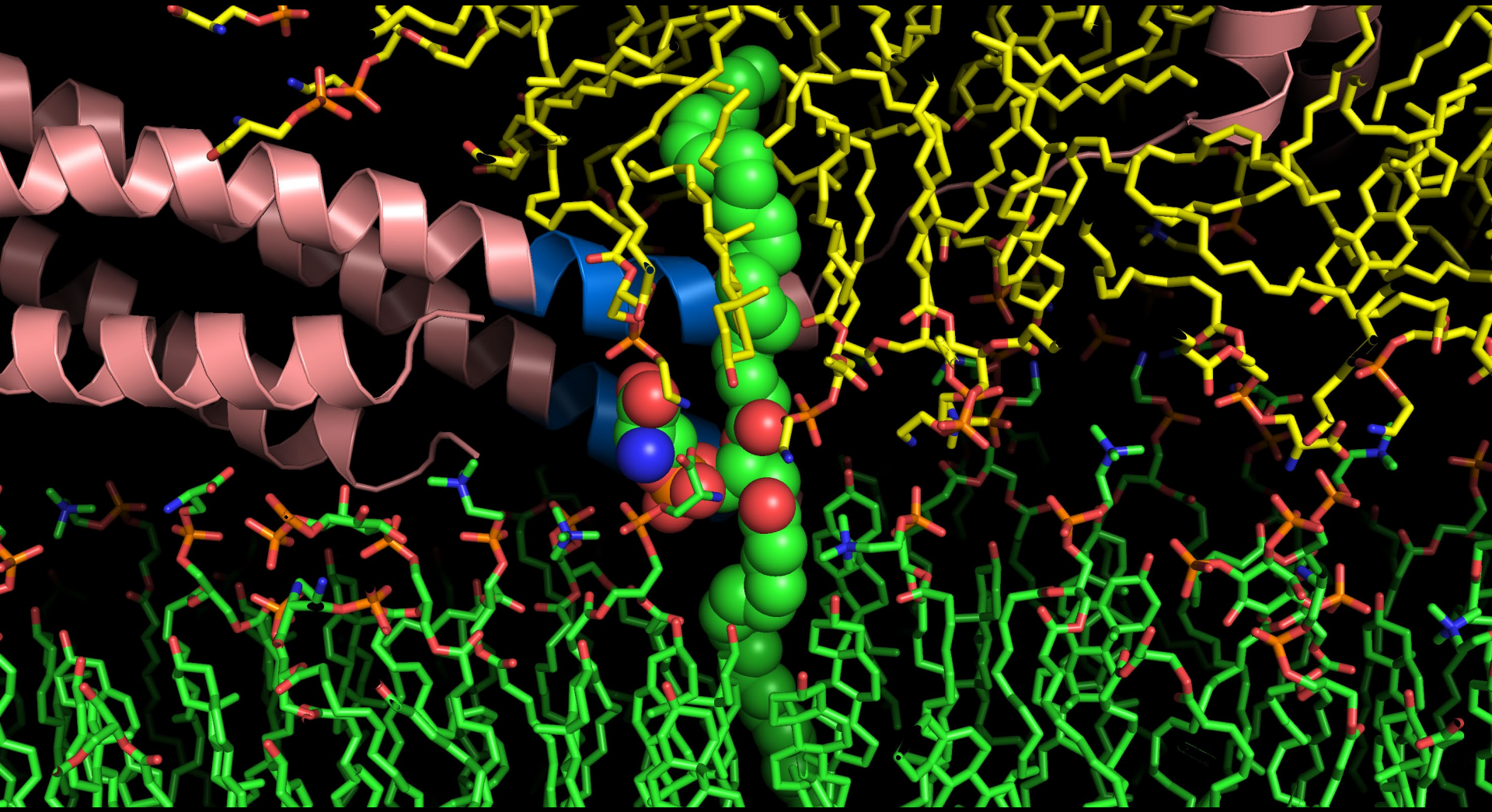
100 ns

A 22:6 acyl chain starts splaying
next to a zippered linker



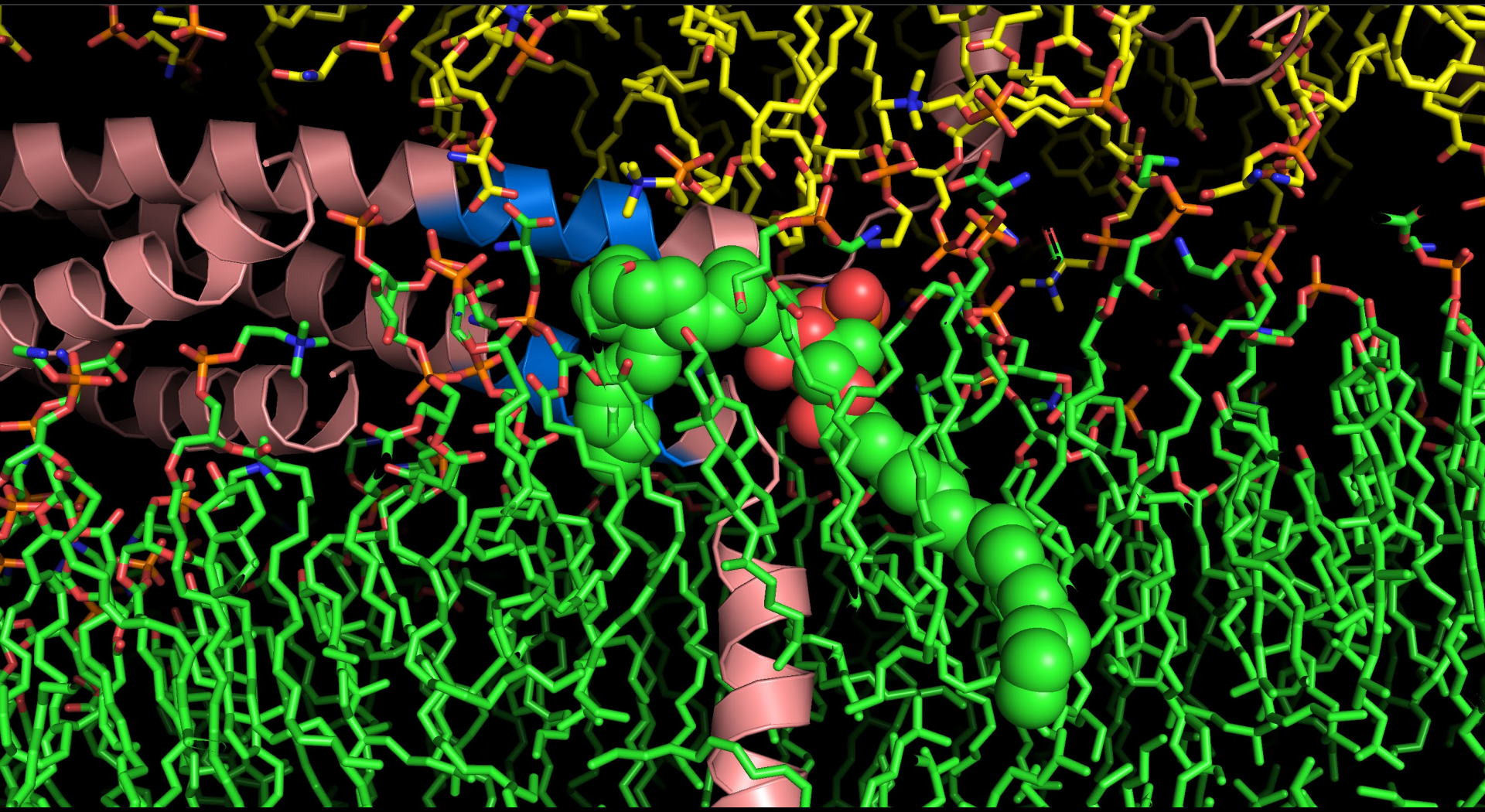
150 ns

The 22:6 acyl chain is splayed
but nothing remarkable happens there for hundreds of ns



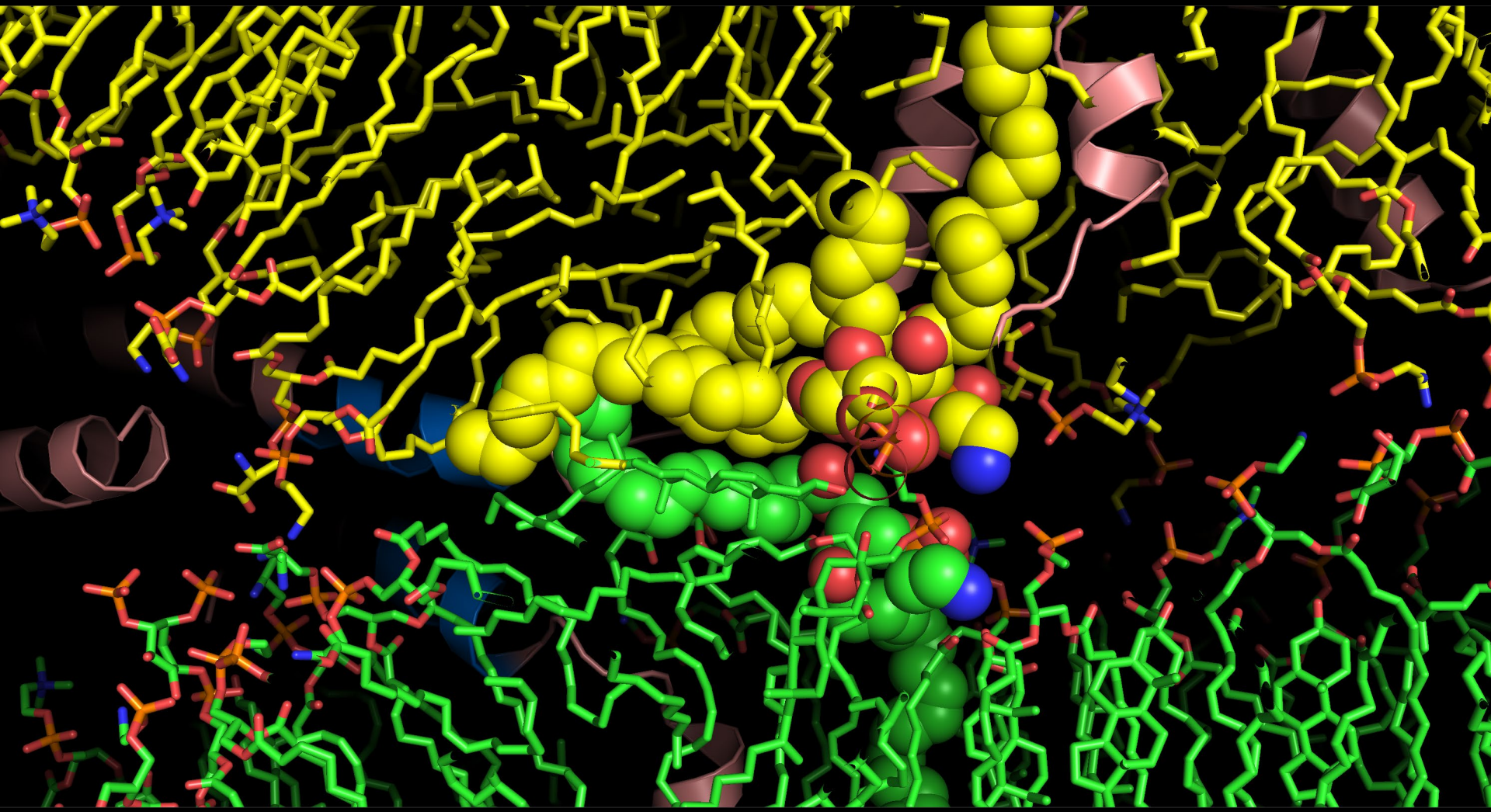
350 ns

Another 22:6 acyl chain starts splaying
next to another zippered linker



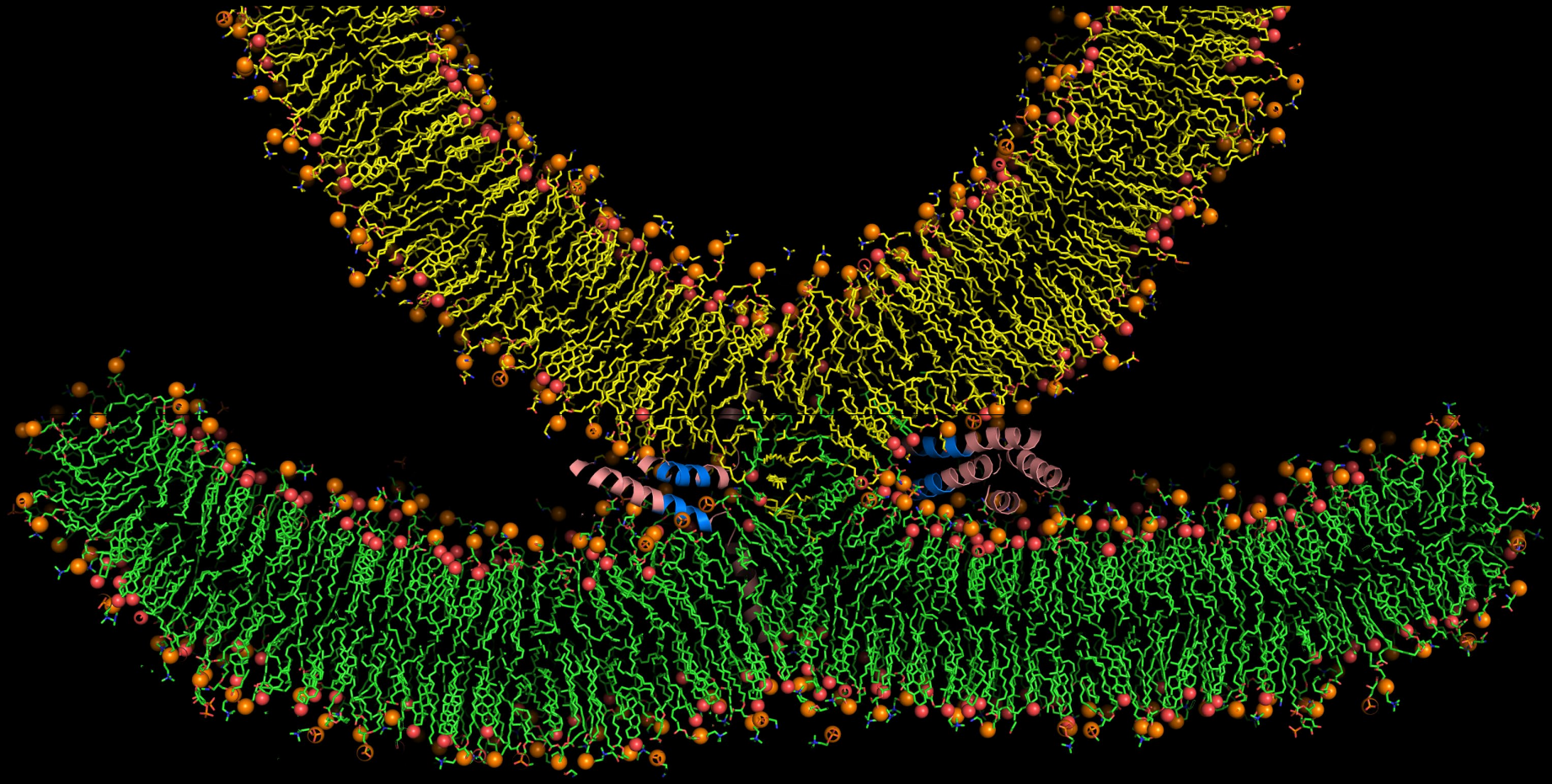
450 ns

The splayed 22:6 lipid contacts two 20:4 lipids
next to a zippered linker, forming a hydrophobic seed

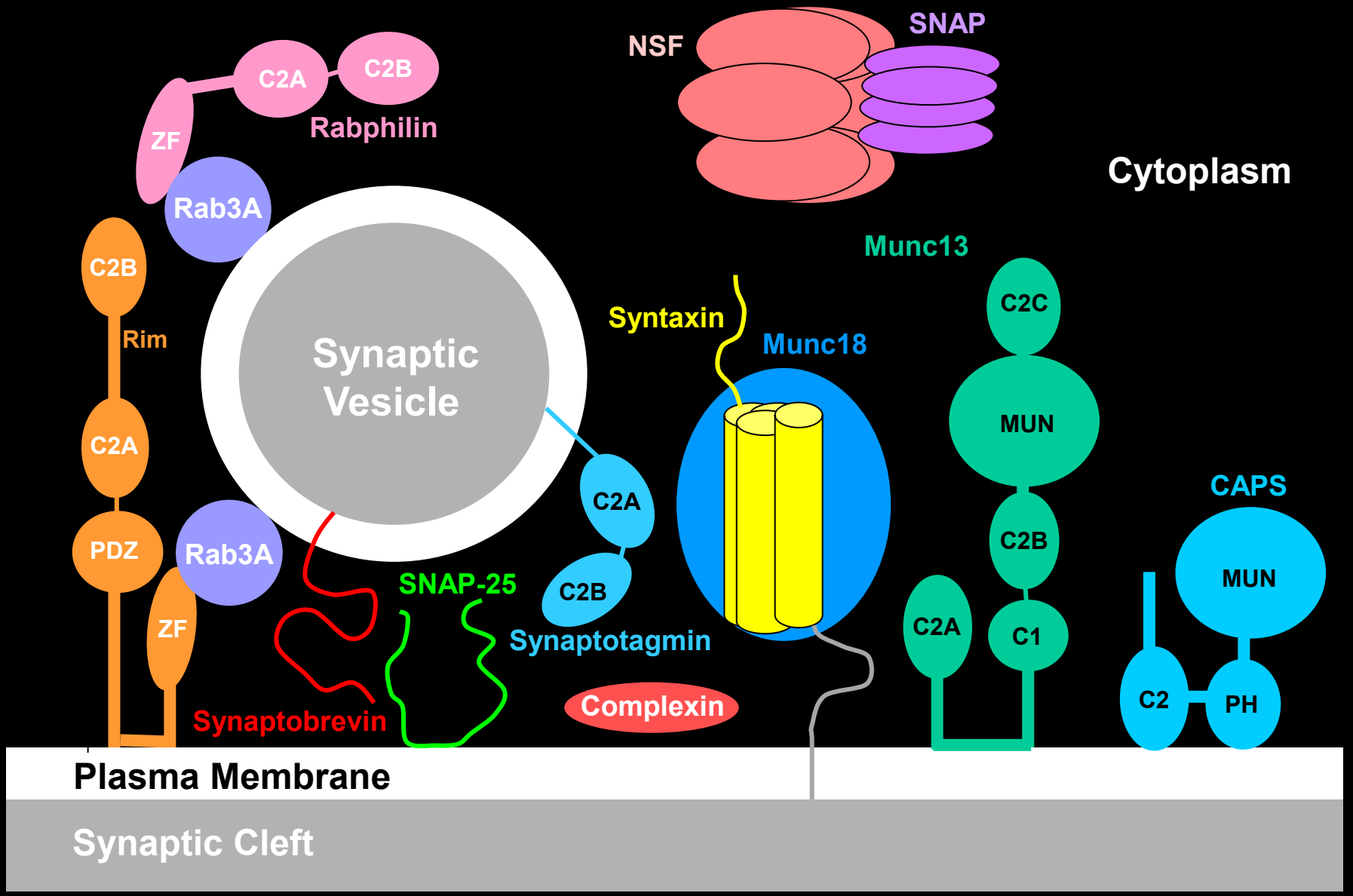


650 ns

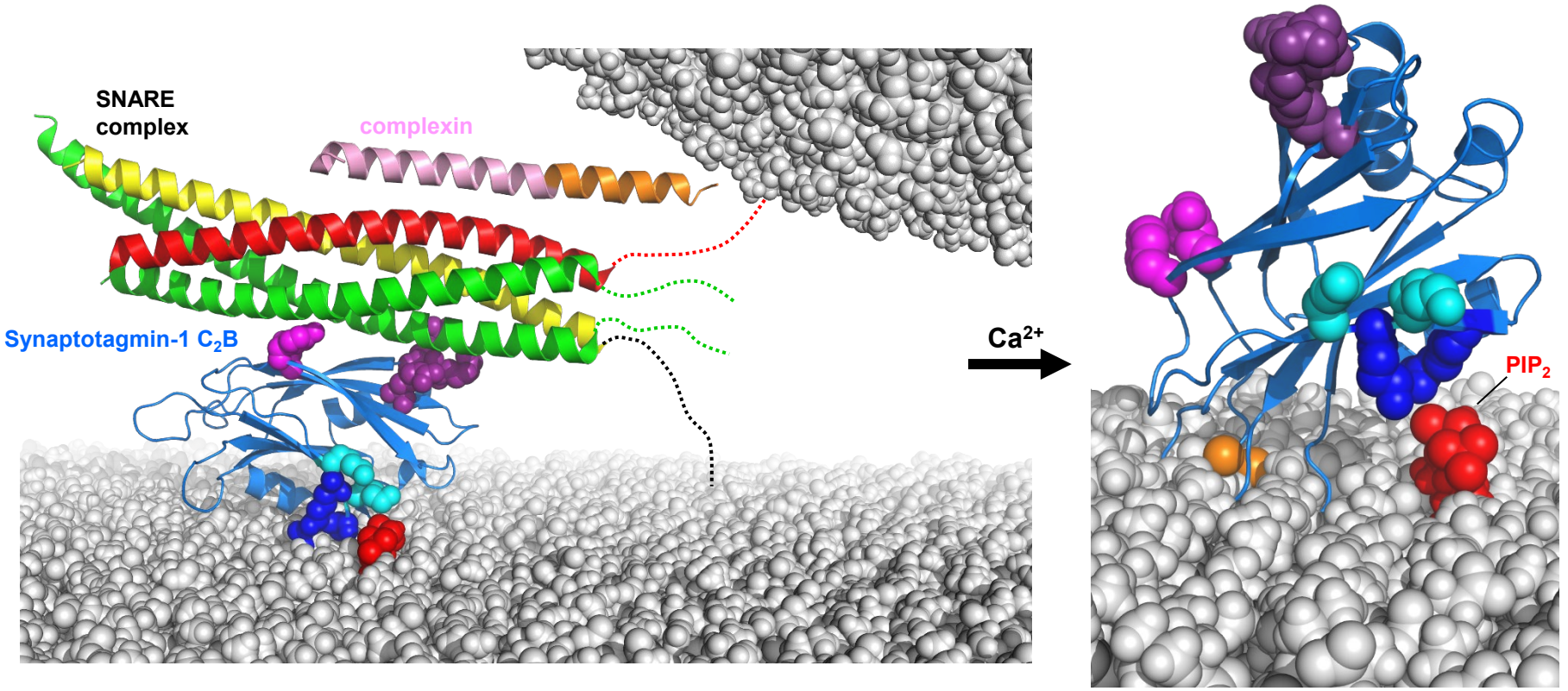
A stalk is formed within 200 ns



TWO fundamental jobs for the release machinery:
Stimulate AND control membrane fusion



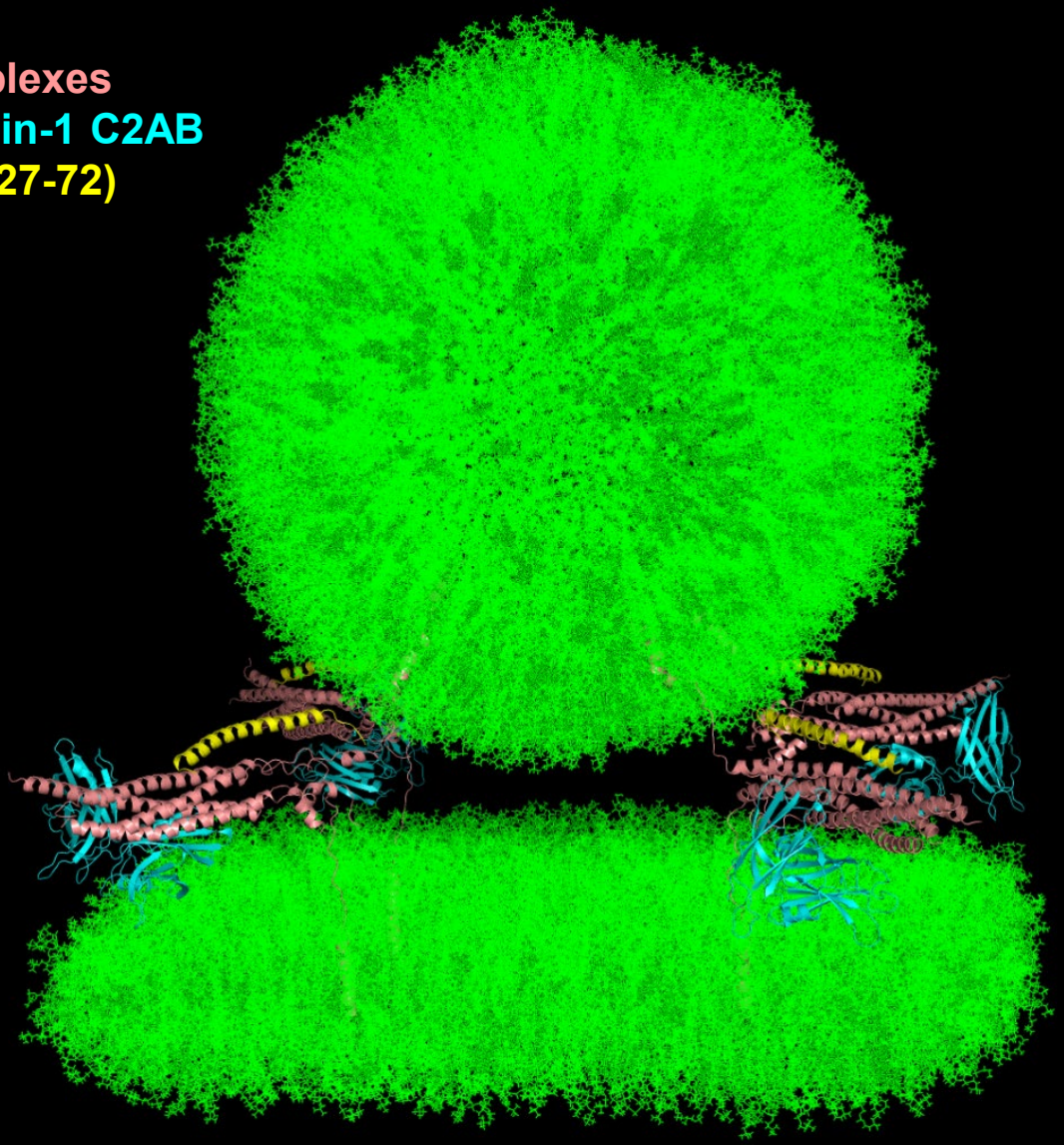
Ca^{2+}- and PIP_2-dependent dissociation of Synaptotagmin-1 from the SNARE complex may be crucial to trigger neurotransmitter release



Voleti et al. (2020) eLife 9, e57154

Simulation of primed synaptotagmin-1-SNARE-complexin complexes

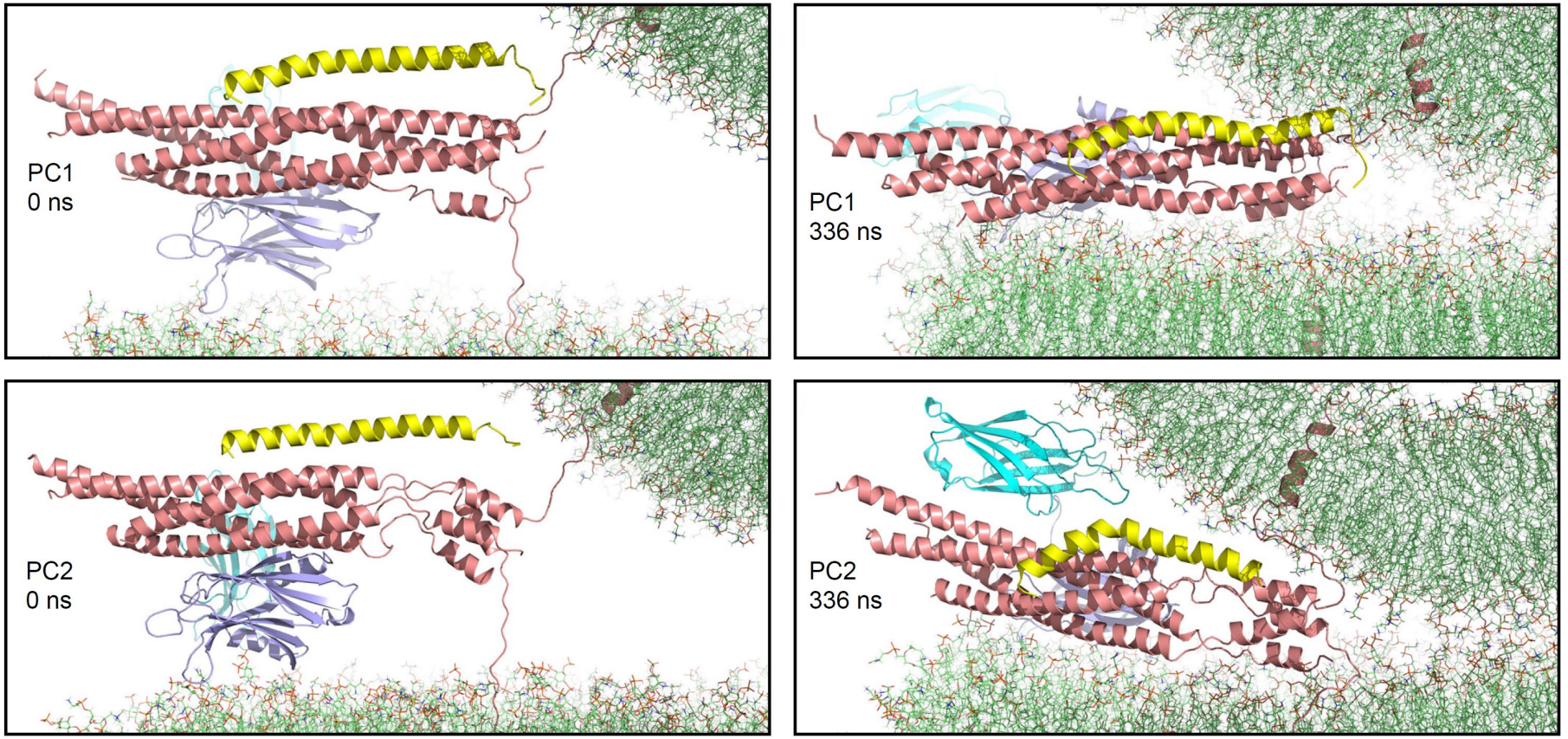
SNARE complexes
Synaptotagmin-1 C2AB
complexin-1(27-72)
no Ca^{2+}



5 million atoms
Including solvent

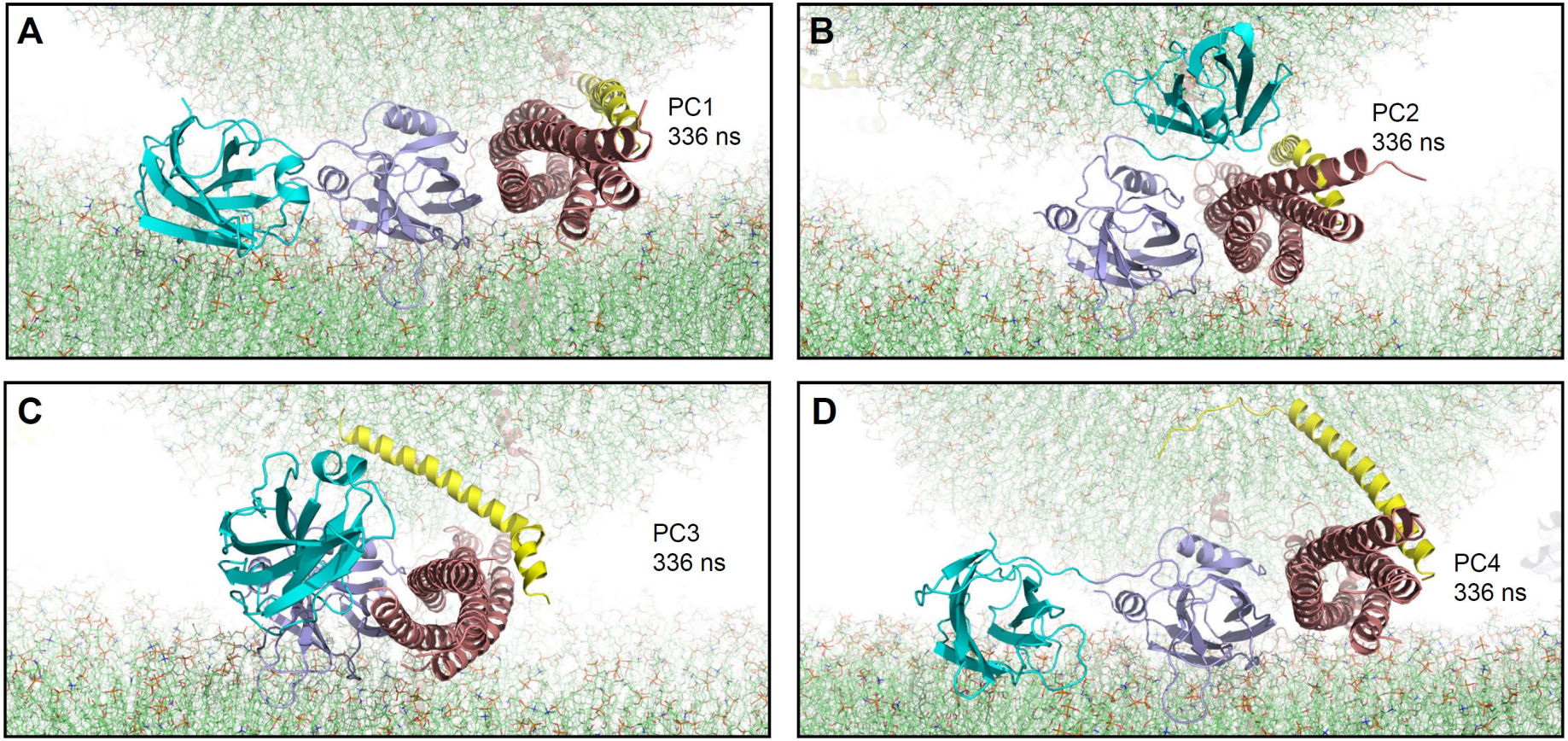
Rizo et al. (2022) *eLife 11*, e76356

Complexin-1 bumps with the vesicle
C-terminal zippering of the SNARE complex is slow



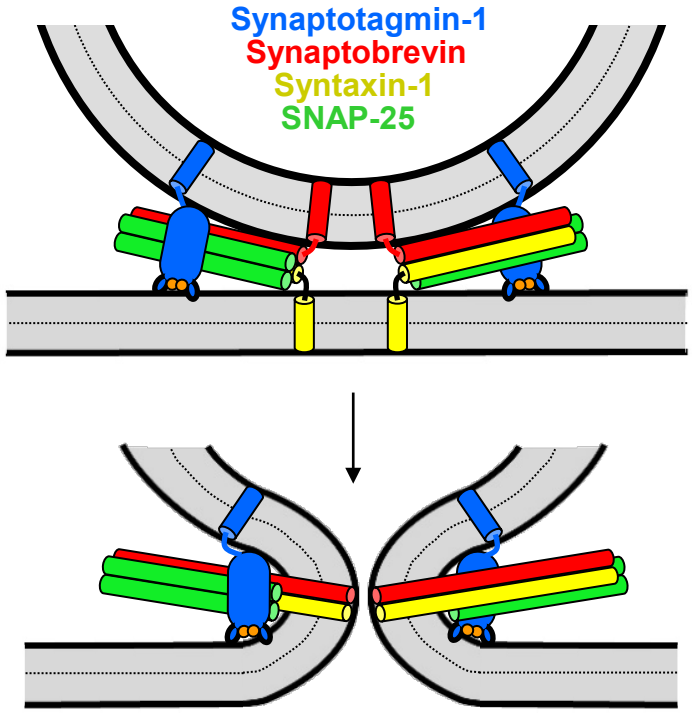
Rizo et al. (2022) *eLife 11*, e76356

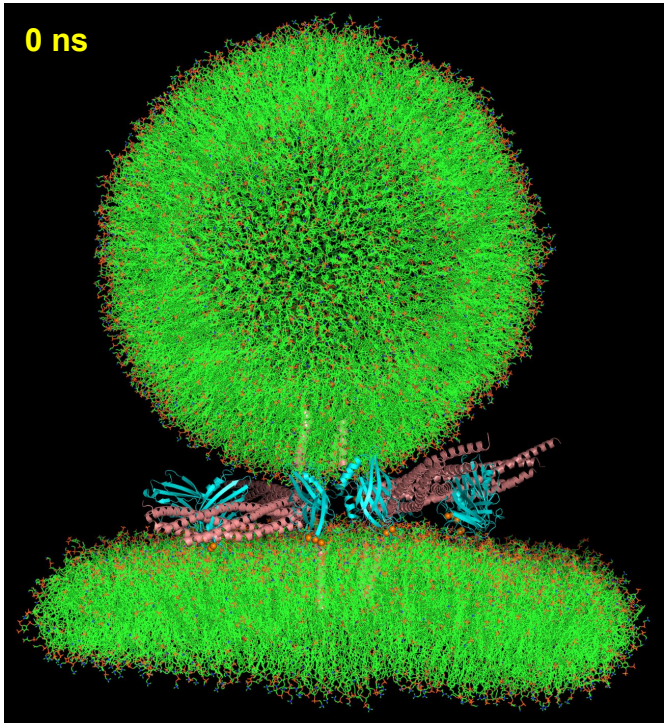
Both synaptotagmin-1 C_2B and the SNARE complex interact extensively with the flat bilayer

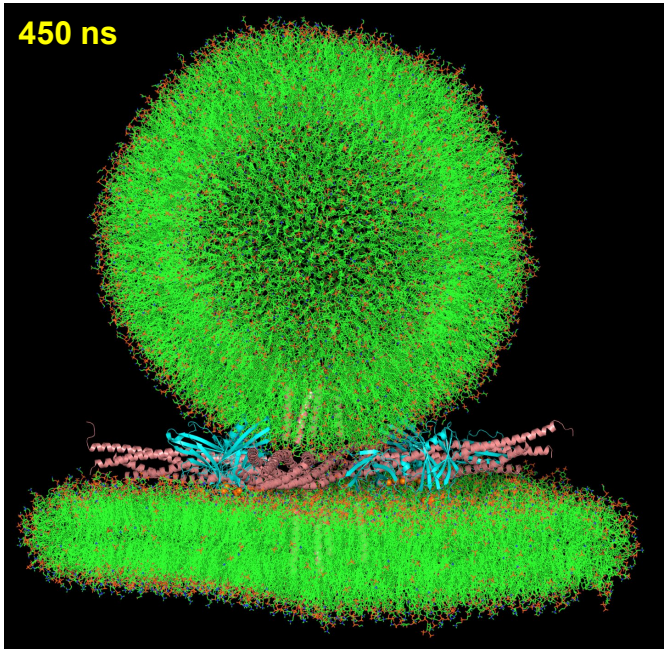


Rizo et al. (2022) *eLife 11*, e76356

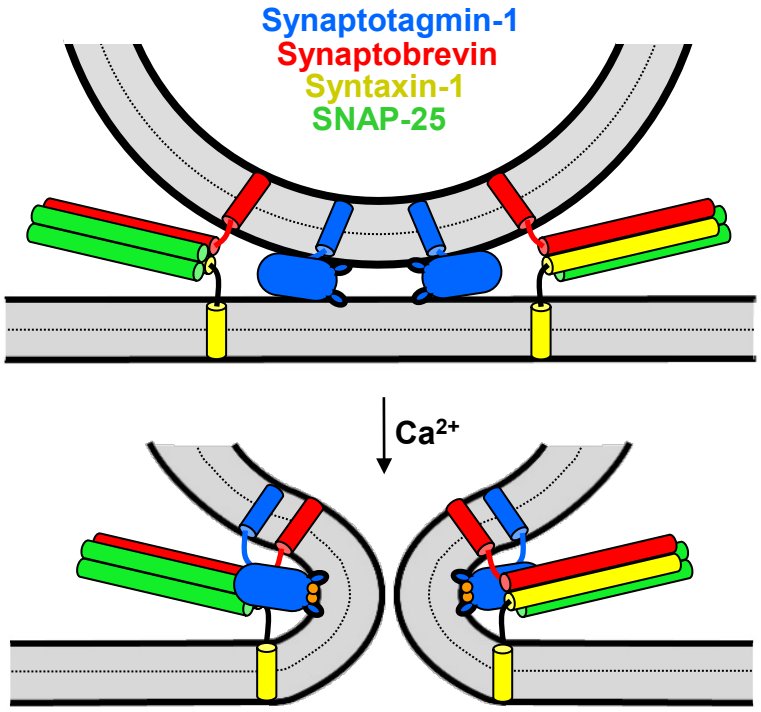
Synaptotagmin-1 C_2 domains bridging two membranes hinder the action of the SNAREs in drawing them together

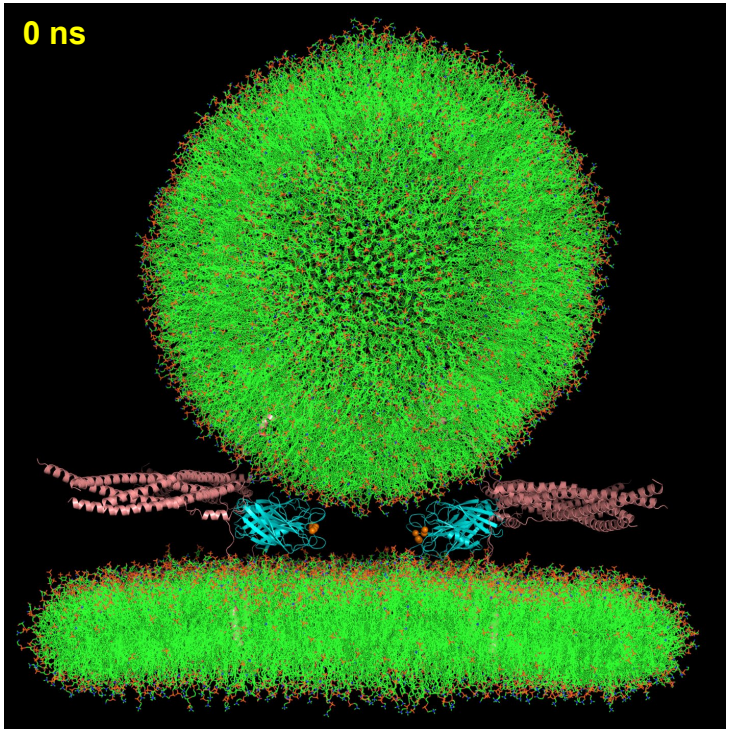


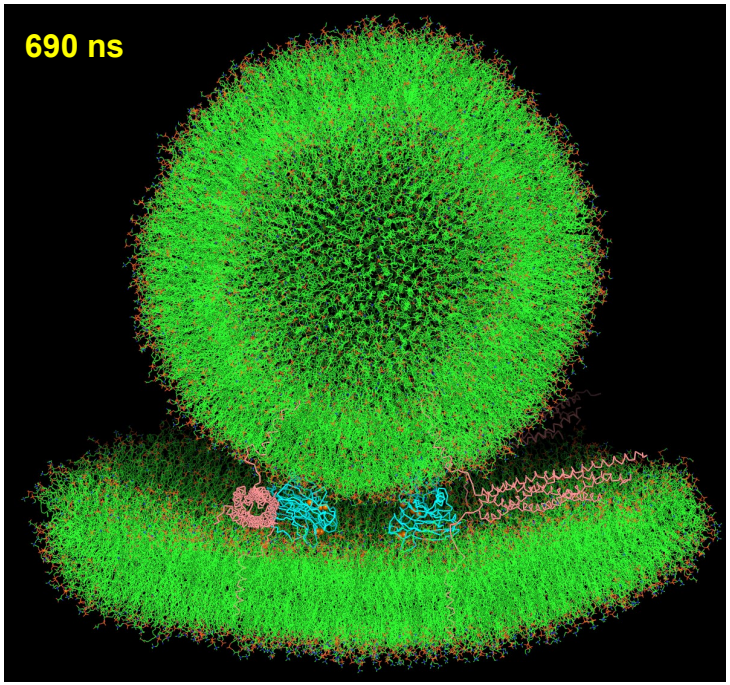




Synaptotagmin-1 C_2 domain Ca^{2+}-binding loops binding two membranes hinder the action of the SNAREs in drawing them together

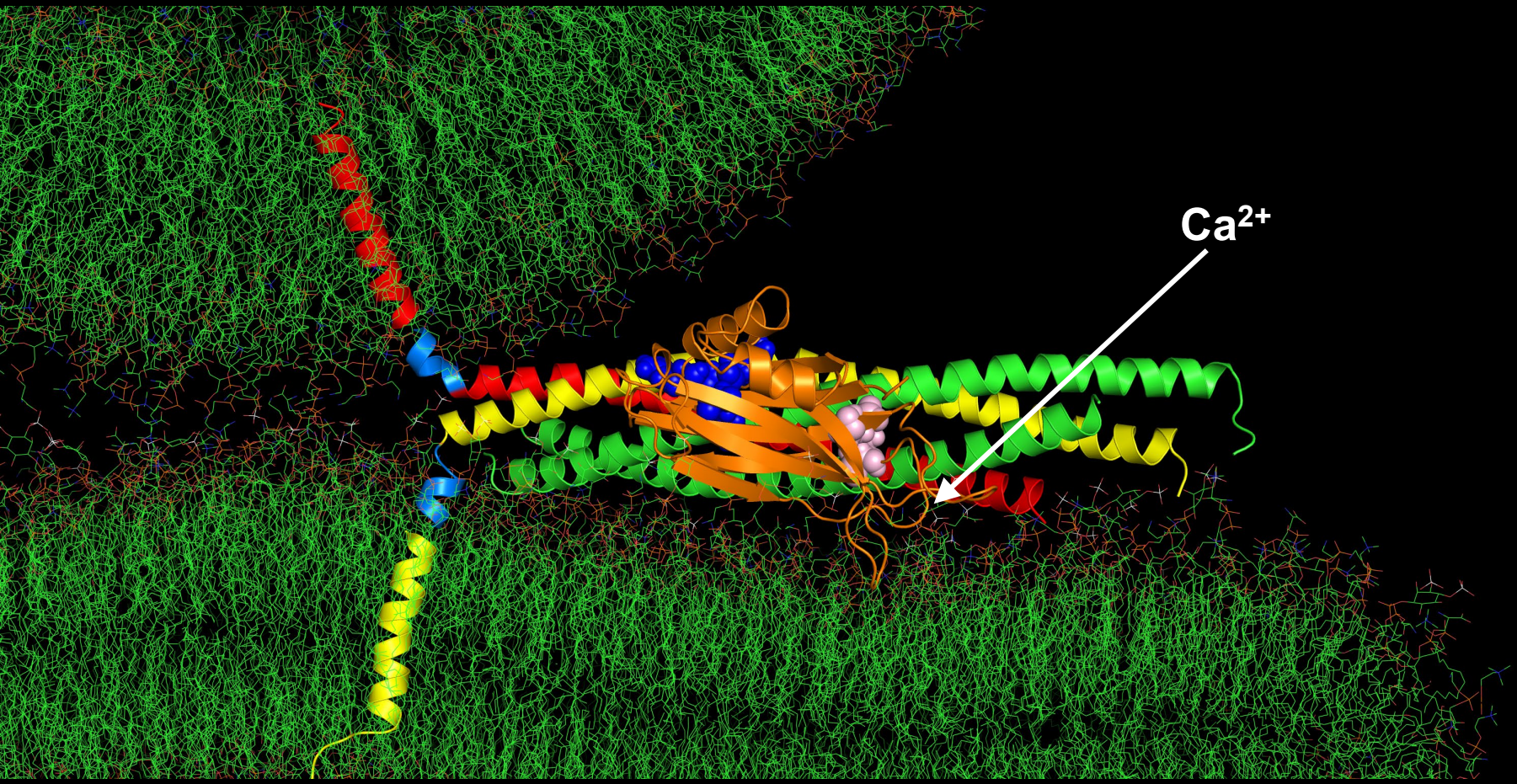




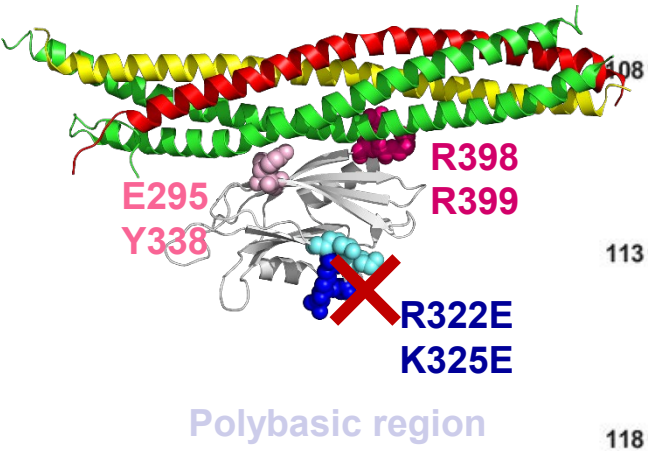


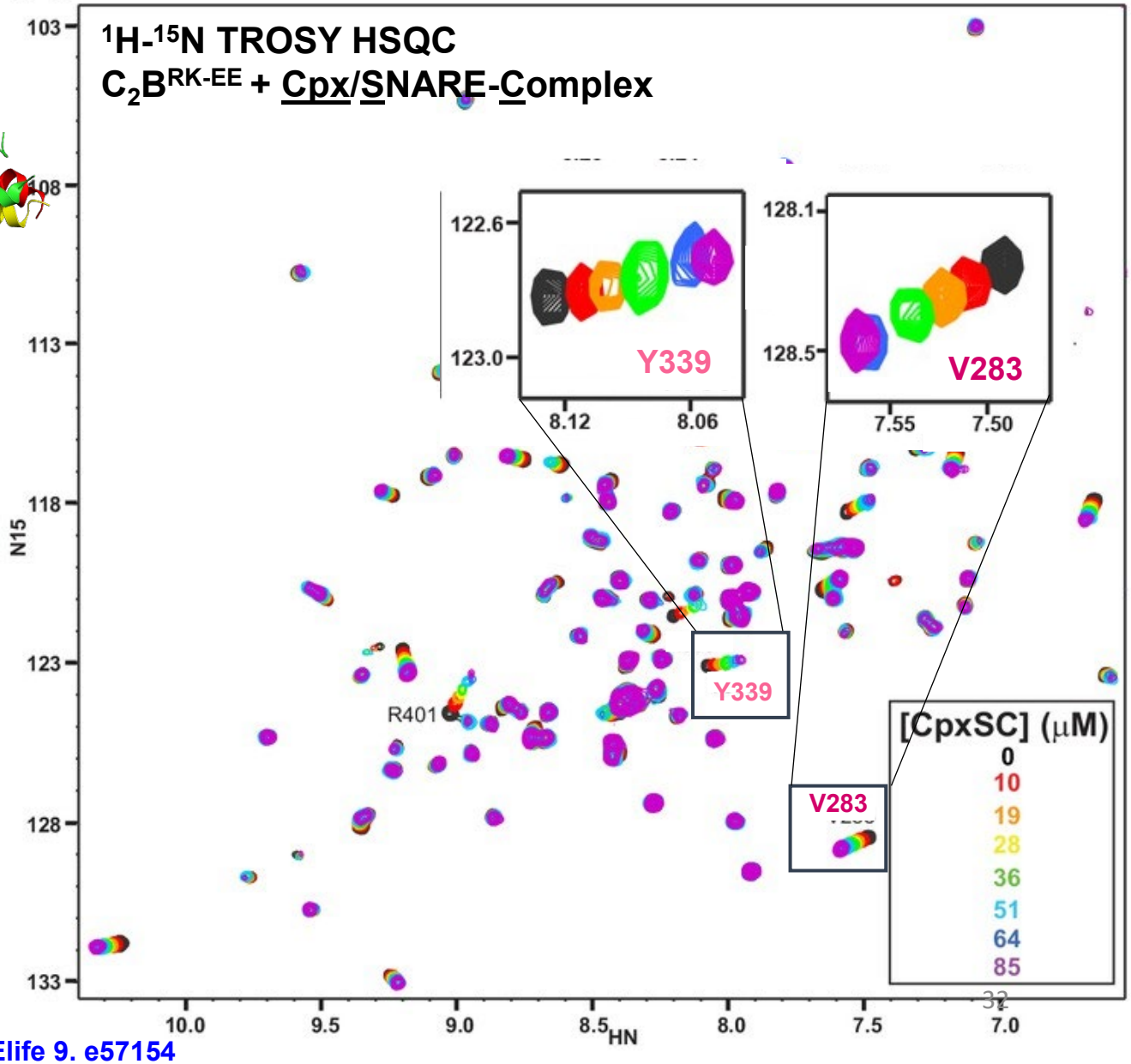
Induction of membrane curvature by synaptotagmin-1 C_2 domains also seems highly unlikely

Primed synaptotagmin-1-SNARE complex



Diagnostic HSQC cross-peaks reflect binding of synaptotagmin-1 to the SNARE complex through two regions of the primary interface

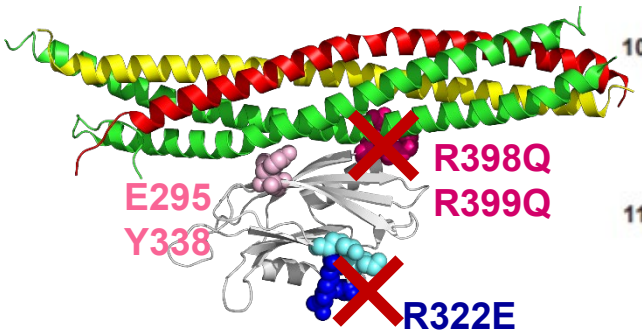




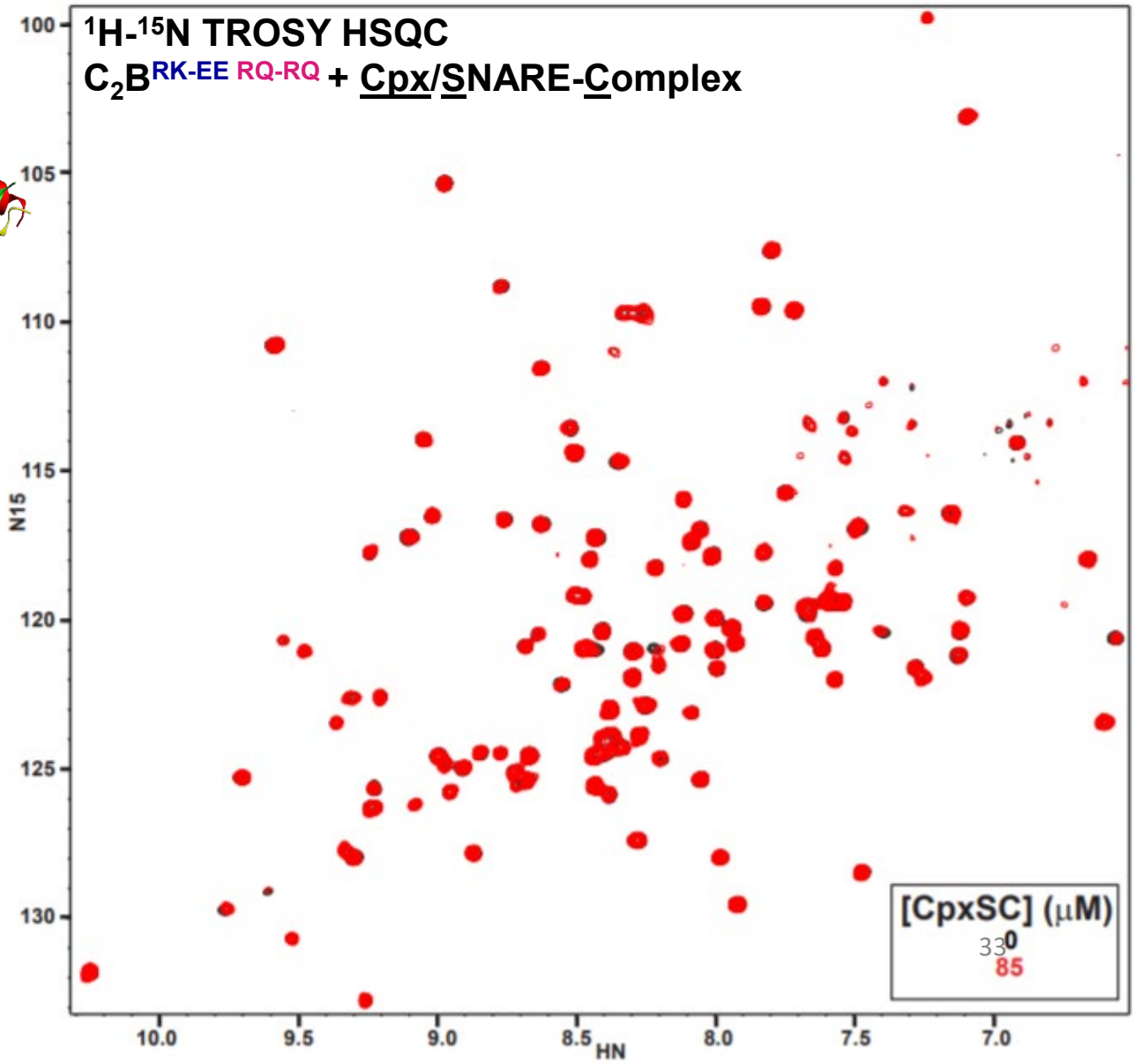
Voleti*, Jaczynska* and Rizo (2020) Elife 9. e57154

R322E/K325E/R398Q/R399Q mutation abolishes synaptotagmin-1-SNARE complex binding

Primary interface



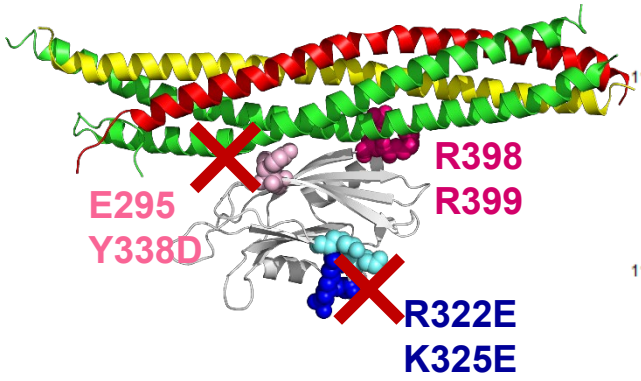
Polybasic region



Voleti*, Jaczynska* and Rizo (2020) Elife 9. e57154

Y338D mutation abolishes binding to region I
But binding to R398/R399 remains!!!!!

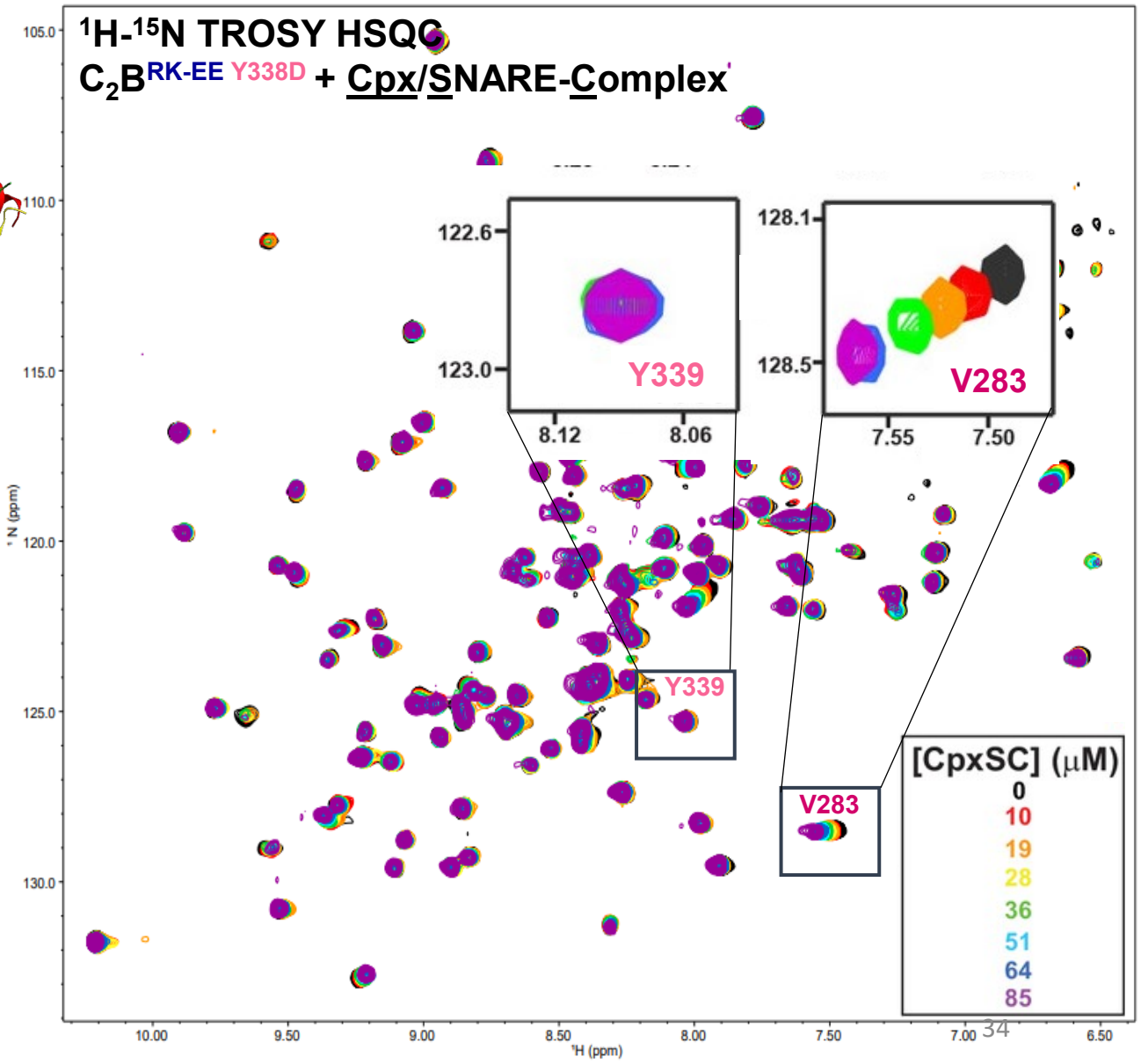
Primary interface
only RR-region binds



Polybasic region

^{1}H-^{15}N TROSY HSQC

$C_2B^{RK\text{-}EE\ Y338D}$ + Cpx/SNARE-Complex



Voleti*, Jaczynska* and Rizo (2020) Elife 9. e57154

Model of fast (μs) Ca^{2+}-evoked neurotransmitter release

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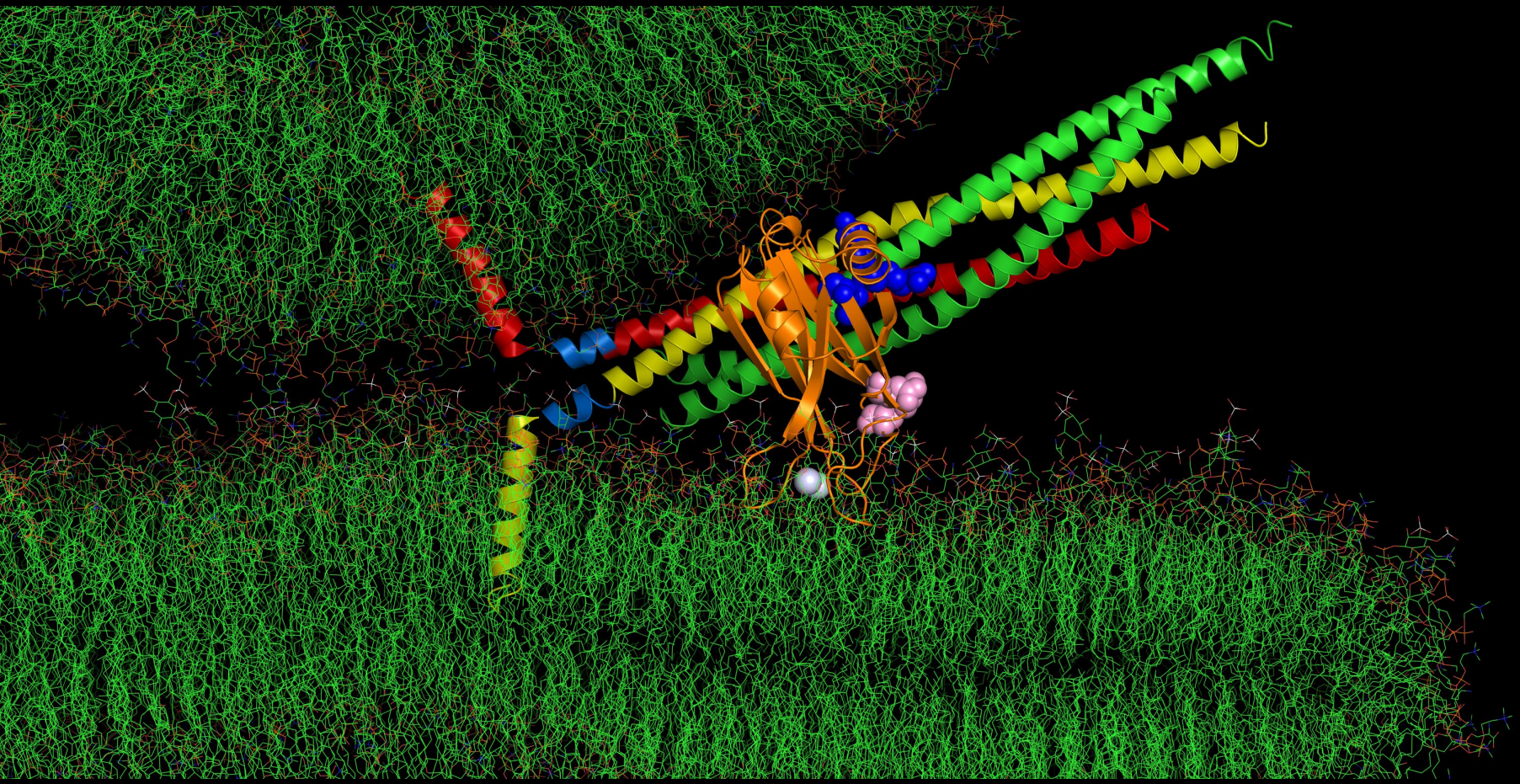


KLAUDIA JACZYNSKA

MILO LIN
LEVENT SARI
WONPIL IM
YIFEI QI

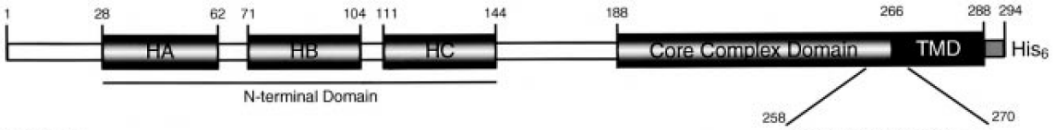
THOMAS SUDHOF
CHRISTIAN ROSENMUND

Pathways and LARC allocations Frontera (TACC) - XSEDE
NIH
Welch Foundation
Virginia Lazenby O'Hara Chair

Reminder of primed state: image of beginning of the movie

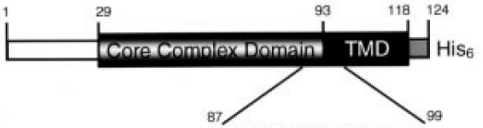
Flexible linker insertions impair but do not abolish liposome lipid mixing

Syntaxin 1A

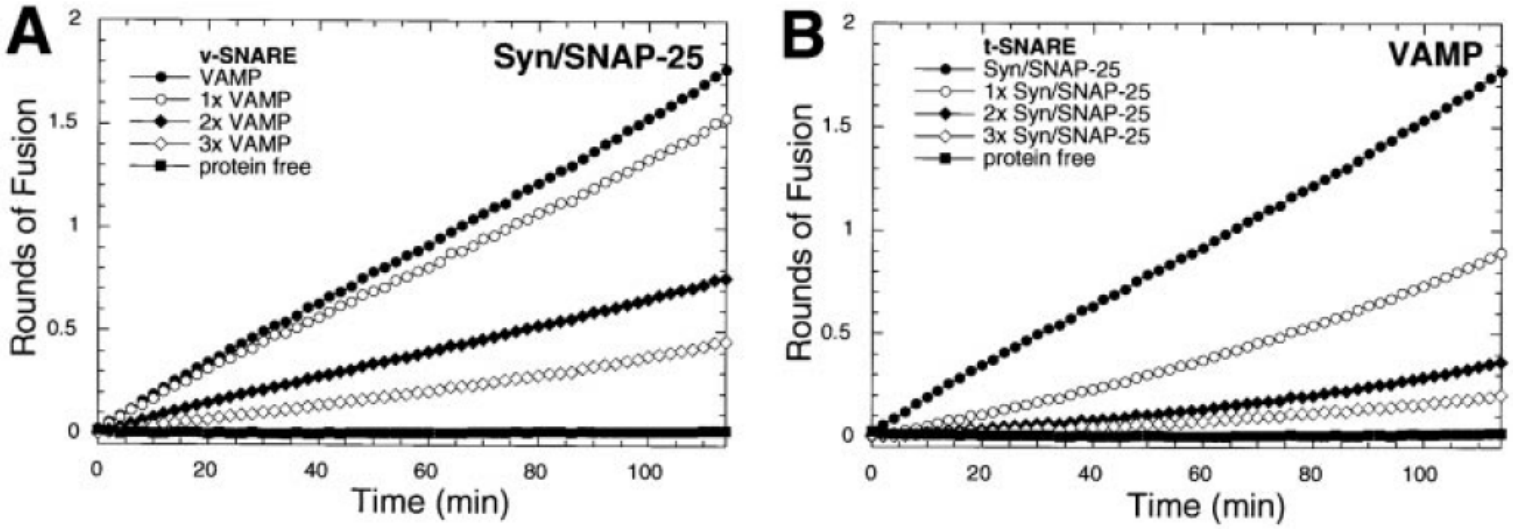


	Syntaxin	...QSKARRKKIMIII...
Syn	Syntaxin	...QSKARRK**KL**MIII...
Syn-PP	Syntaxin-PP	...QSKARRK**KLGPPKL**MIII...
1x Syn	1x linker Syntaxin	...QSKARRK**KLGGSGGSGGSKL**MIII...
2x Syn	2x linker Syntaxin	...QSKARRK**KLGGSGGSGGSKLGGSGGSGGSKL**MIII...
3x Syn	3x linker Syntaxin	...QSKARRK**KLGGSGGSGGSKLGGSGGSGGSKLGGSGGSGGSKL**MIII...

VAMP2 (synaptobrevin)

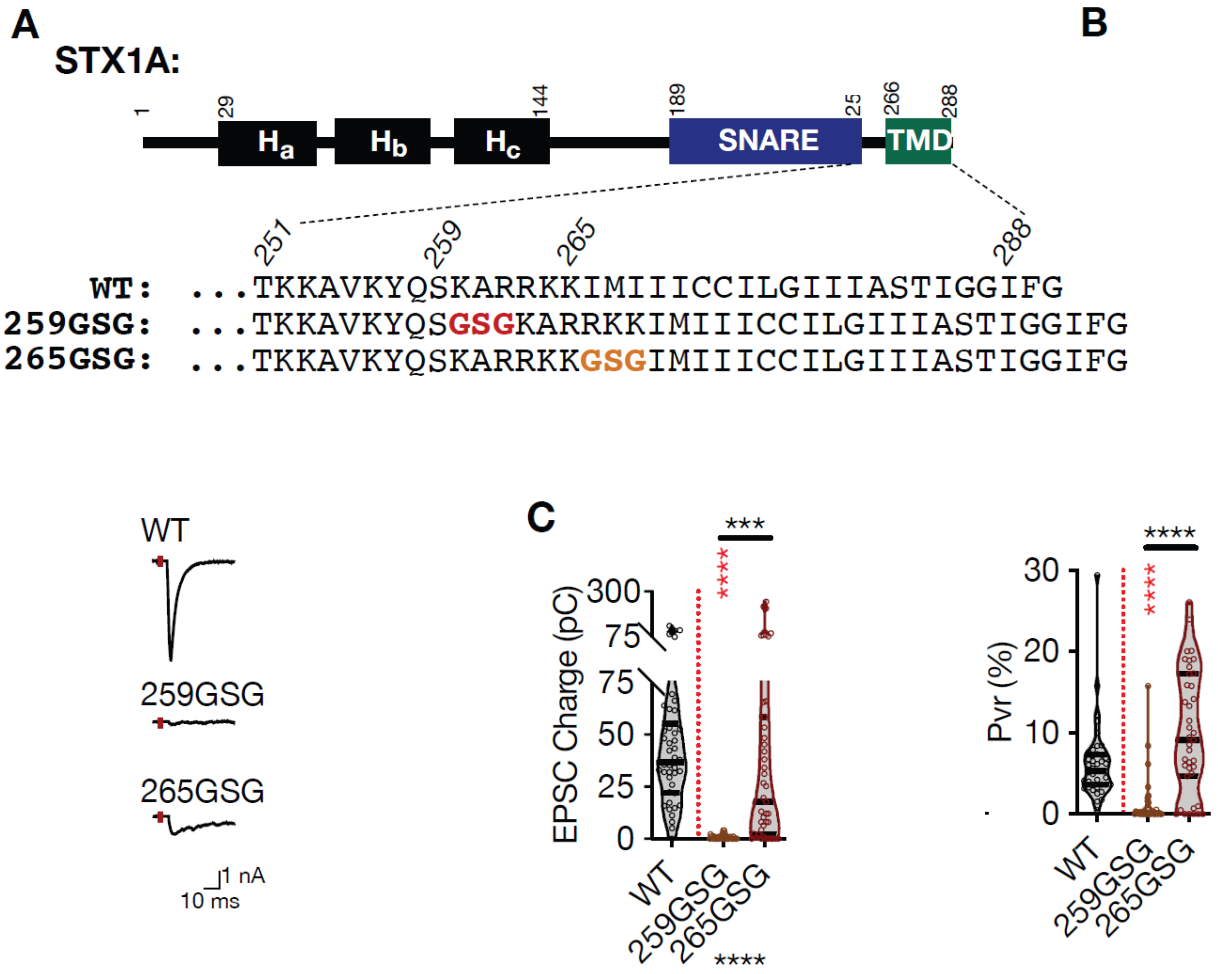


	VAMP	...KYWWKNLKMMIIL...
V	VAMP	...KYWWKNL**KL**MIIL...
V-PP	VAMP-PP	...KYWWKNL**KLGPPKL**MIIL...
1x V	1x linker VAMP	...KYWWKNL**KLGGSGGSGGSKL**MIIL...
2x V	2x linker VAMP	...KYWWKNL**KLGGSGGSGGSKLGGSGGSGGSKL**MIIL...
3x V	3x linker VAMP	...KYWWKNL**KLGGSGGSGGSKLGGSGGSGGSKLGGSGGSGGSKL**MIIL...



McNew et al. (1999) *Mol. Cell 4*, 415

Spontaneous release is enhanced by insertion between SNARE motif and Jx linker and strongly enhanced by insertion between Jx linker and TM



Vardar et al. (2022) eLife 11, e78182