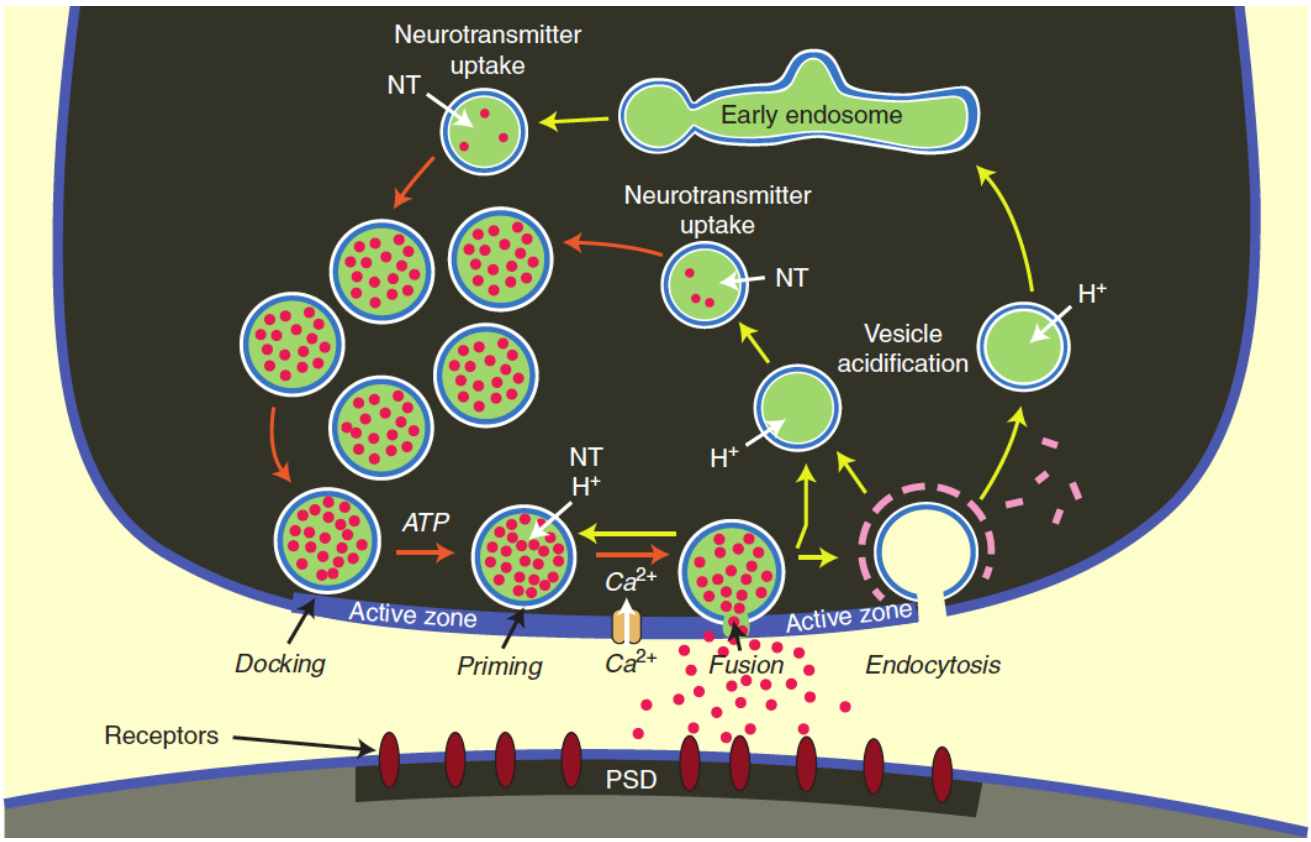
Synaptic Vesicle Cycle

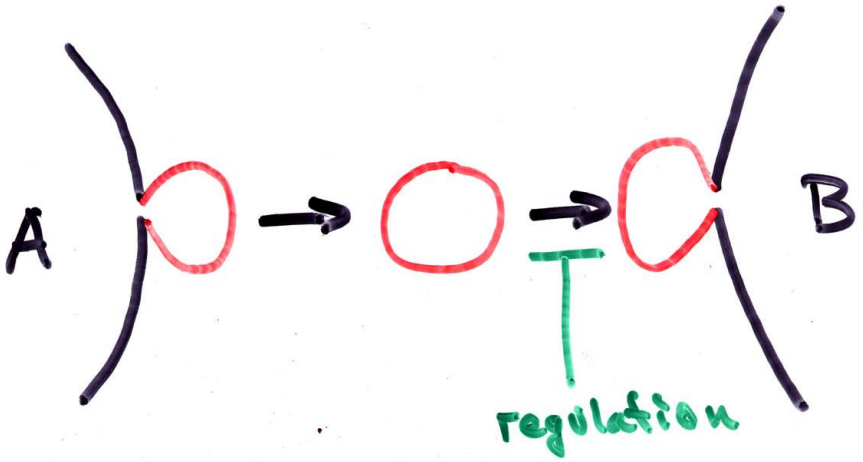
quantal release has many advantages
but imposes several requirements

high potential for regulation
huge variation in release probability
multiple modes of release
process information



vesicular transport: a general mechanism

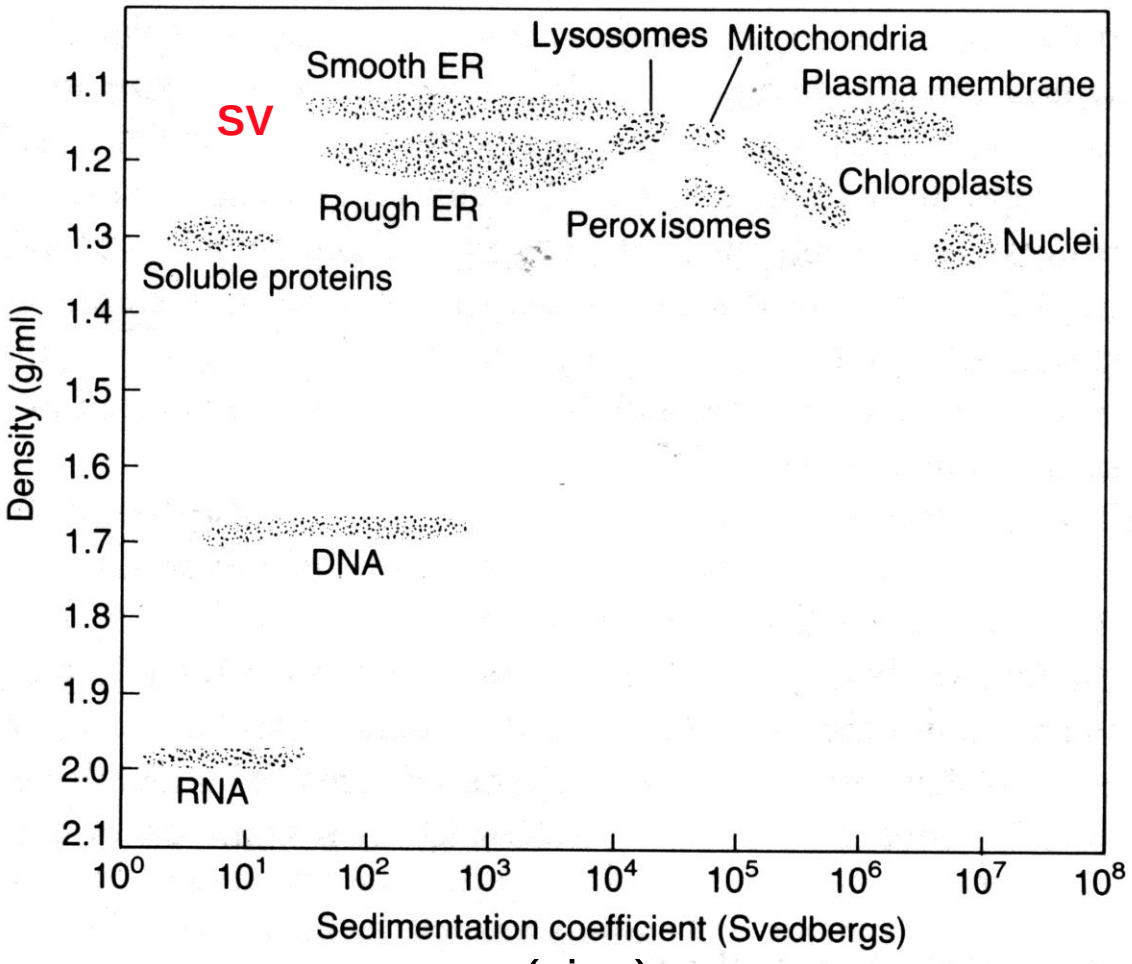
transport vesicles form at compartment A
fuse with compartment B
what are alternative possibilities?



usually constitutive
vesicles do not accumulate
cannot isolate key intermediate
--unless process regulated

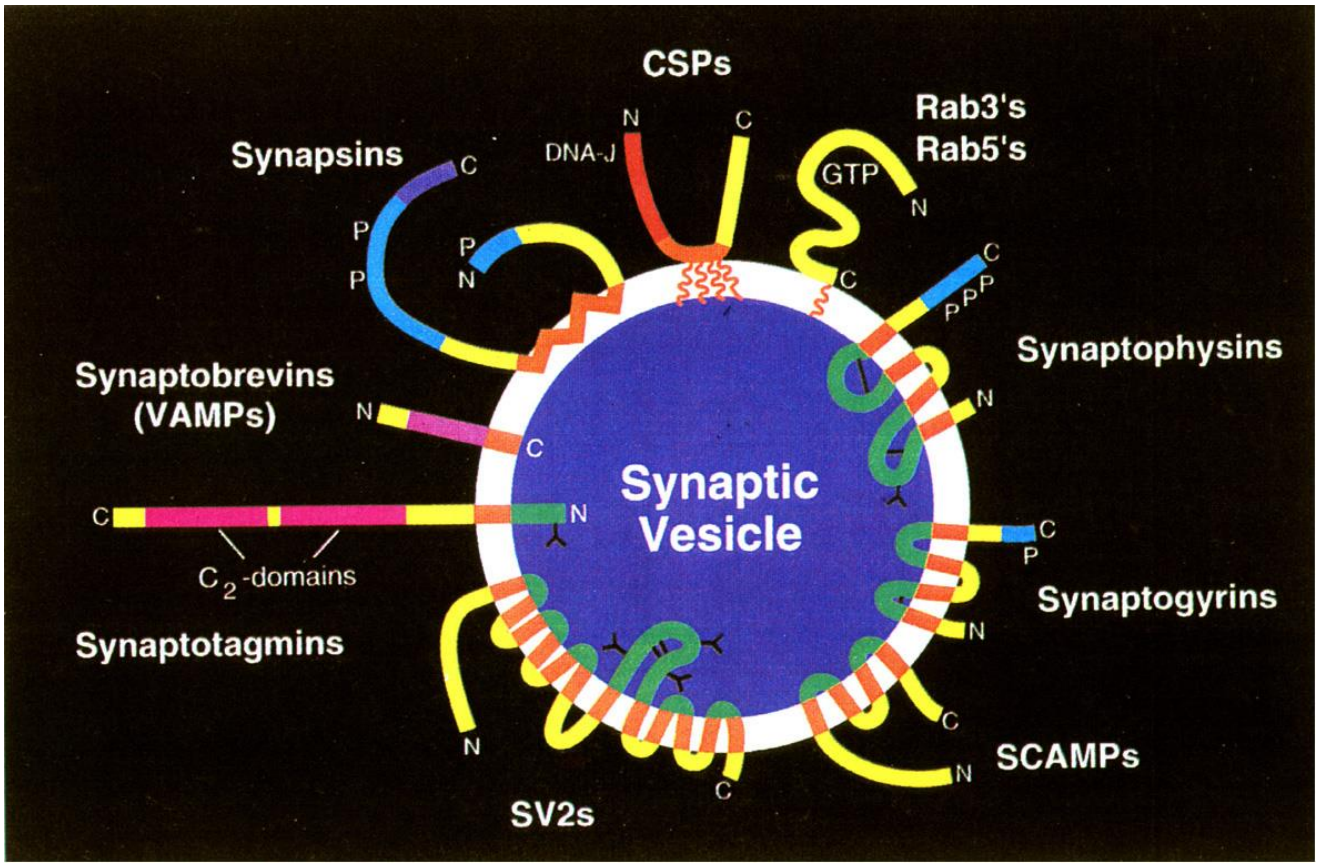
SV Purification

synaptic vesicles the smallest biological membranes
homogeneous in size, shape
--separate by density (equilibrium sedimentation) and size (velocity sedimentation, size ex-exclusion chromatography)



(size)

proteins excised from gel, sequenced:



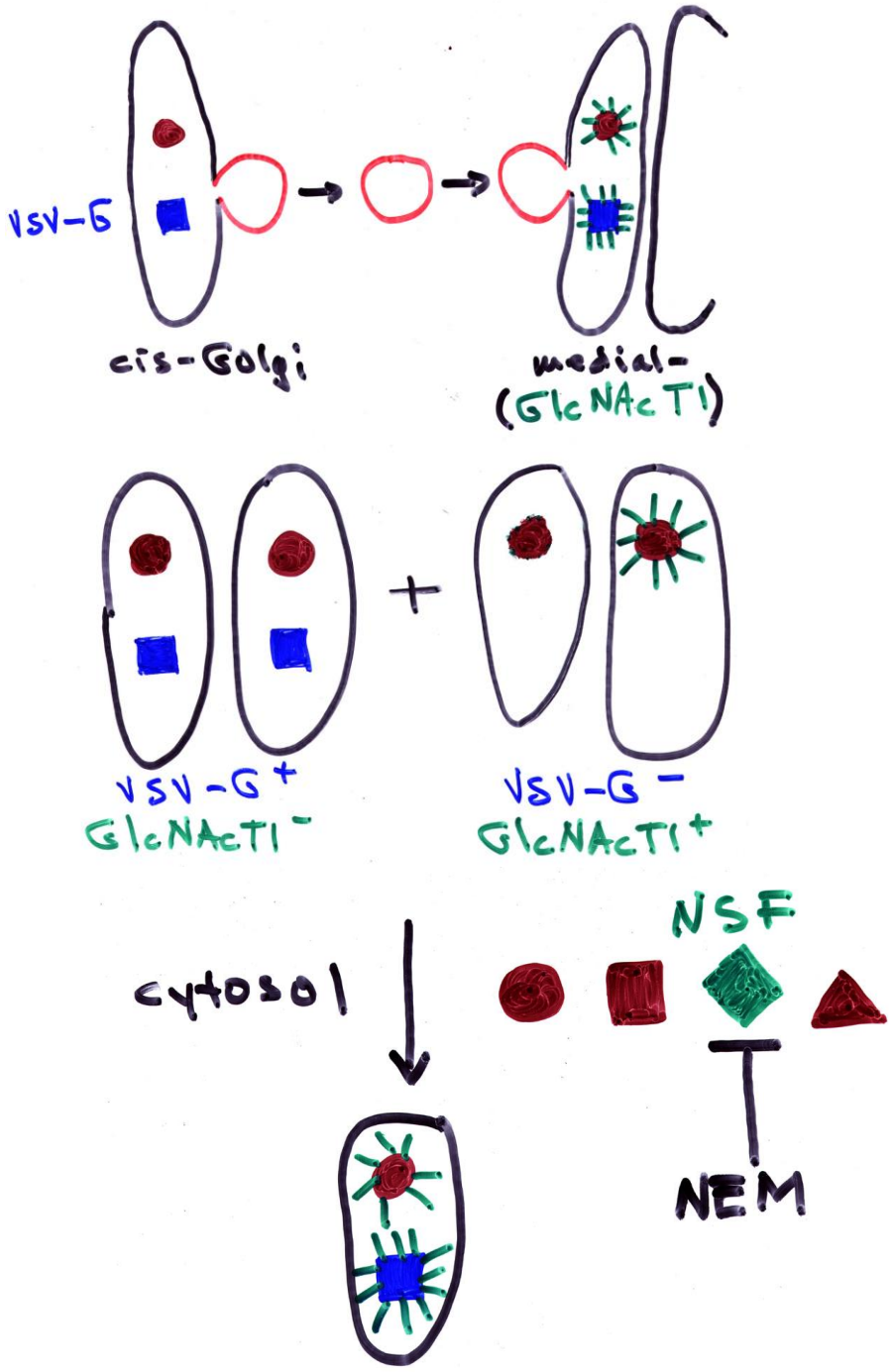
mechanisms of membrane association
single TMD (type 2 synaptobrevin, 1 synaptotagmin)
polytopic (synaptophysin, SV2)
peripheral membrane proteins (synapsin, synuclein)
lipid-anchored (rab3, cysteine string protein)

proteomics has quantified components

the function of most remain unknown

trafficking assay (Rothman)

for vesicular transport within Golgi complex



assay for glycosylation of VSV-G protein

requires transport between stacks of different cells

activity requires many proteins

identified by functional complementation

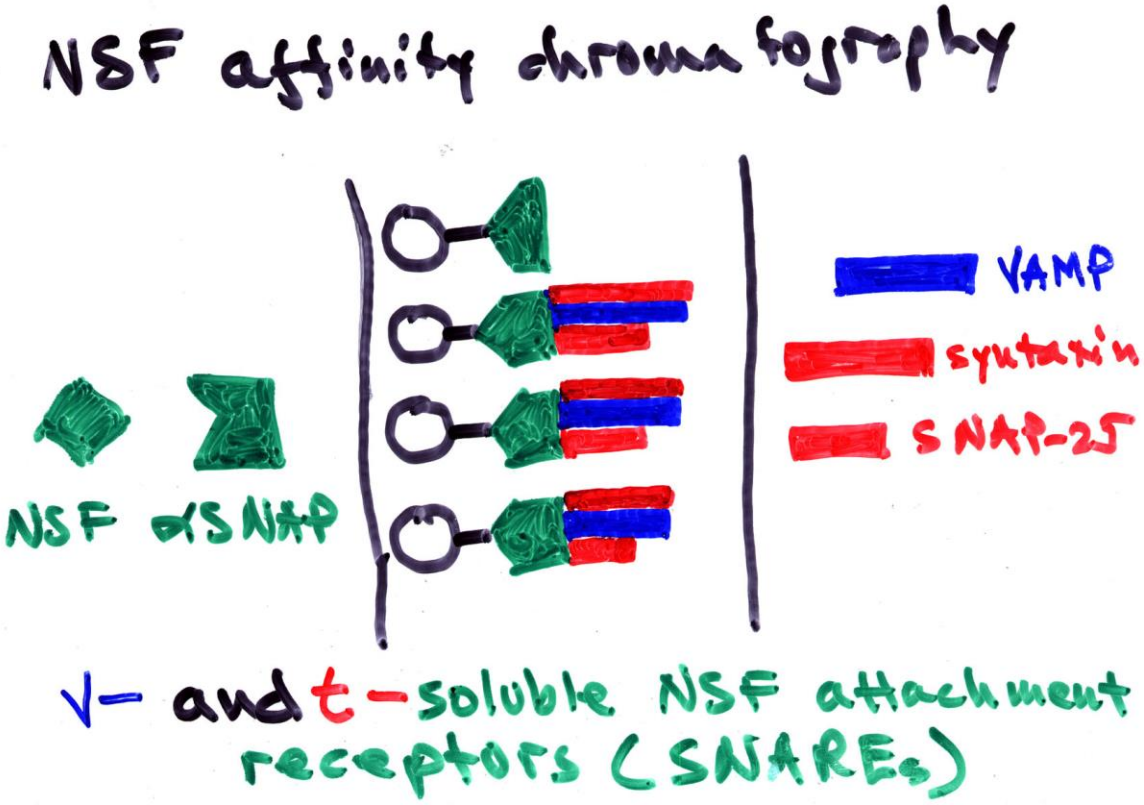
inactivate extract with NEM (-SH reagent)

rescue with untreated extract

purify rescuing component:

--NEM-sensitive factor (NSF)

NSF is an ATPase--used to find associated proteins:



binding in non-hydrolyzable ATP

elute with ATP--releases only those bound

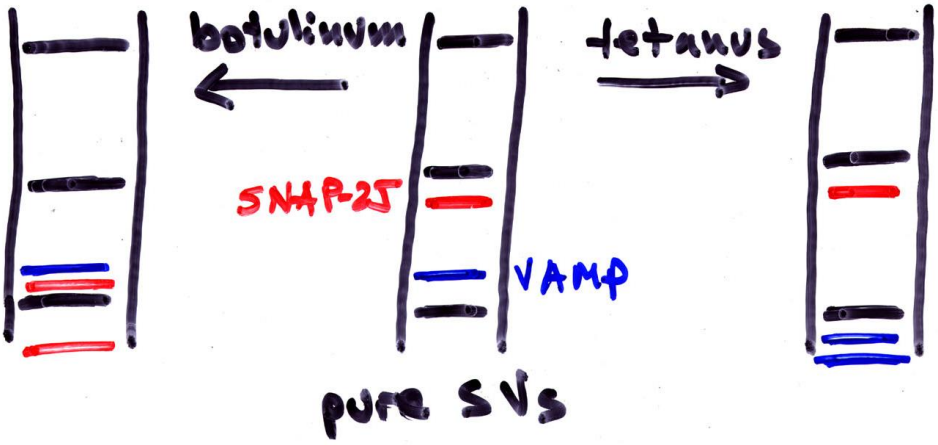
dependent on ATP

--soluble NSF attachment receptors (SNAREs)

function

clostridial toxins block NT release

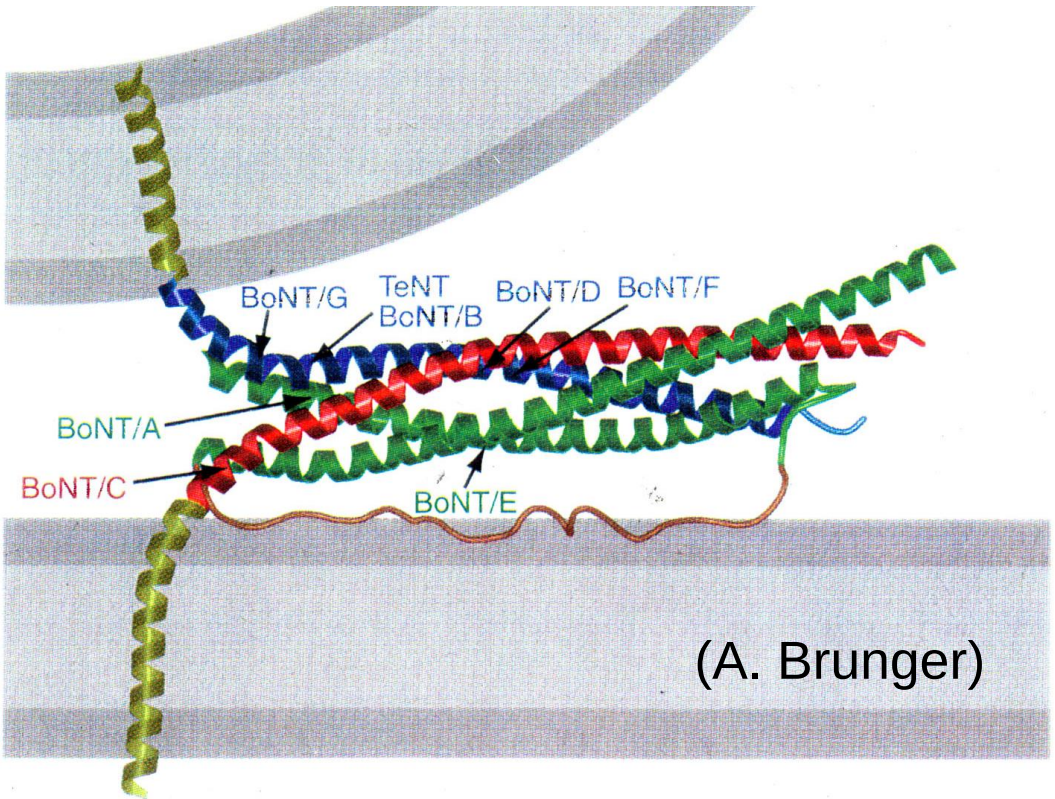
--Zn-dependent proteases



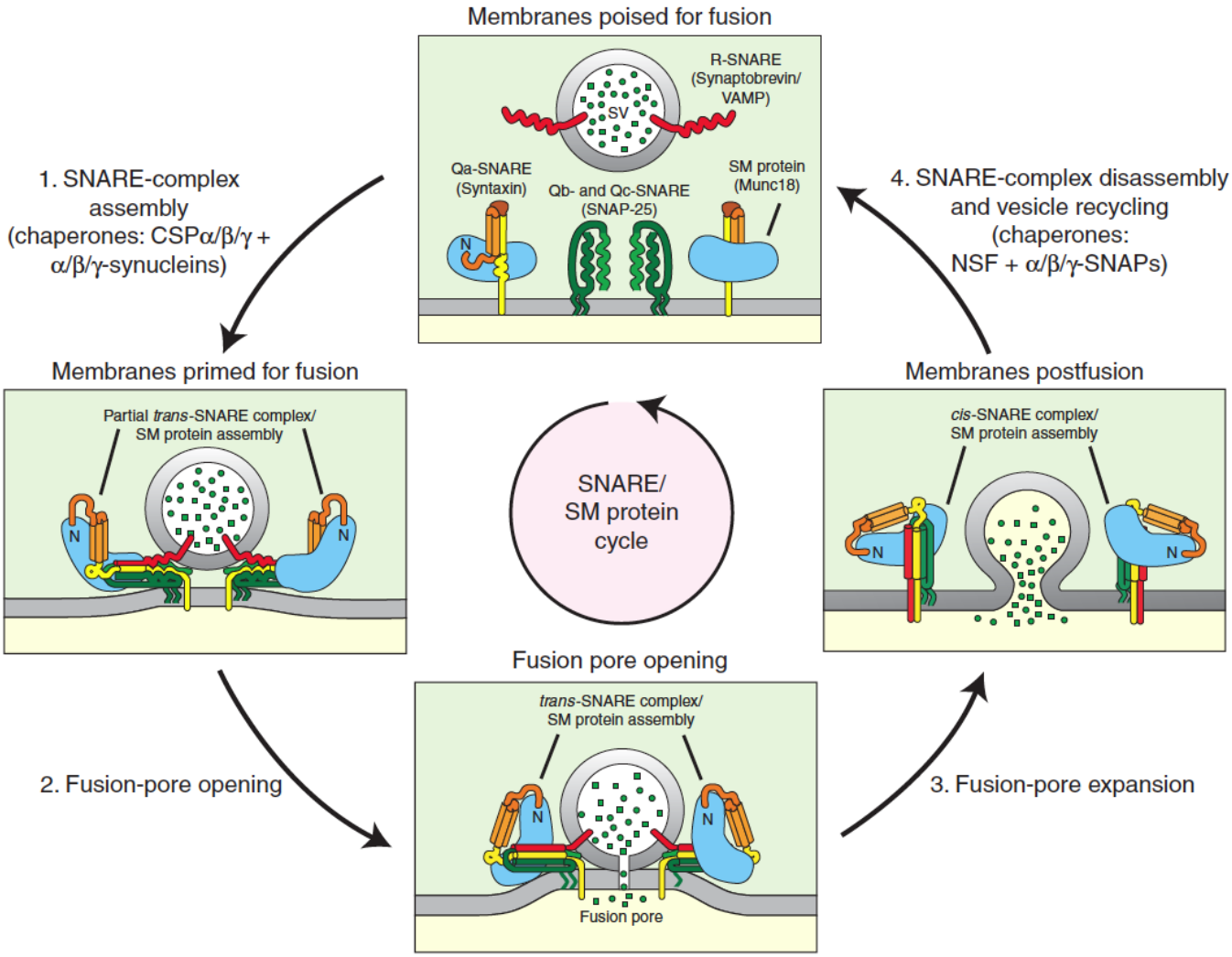
specifically cleave SNAREs

--less effect on spontaneous than evoked release

structure



revised model

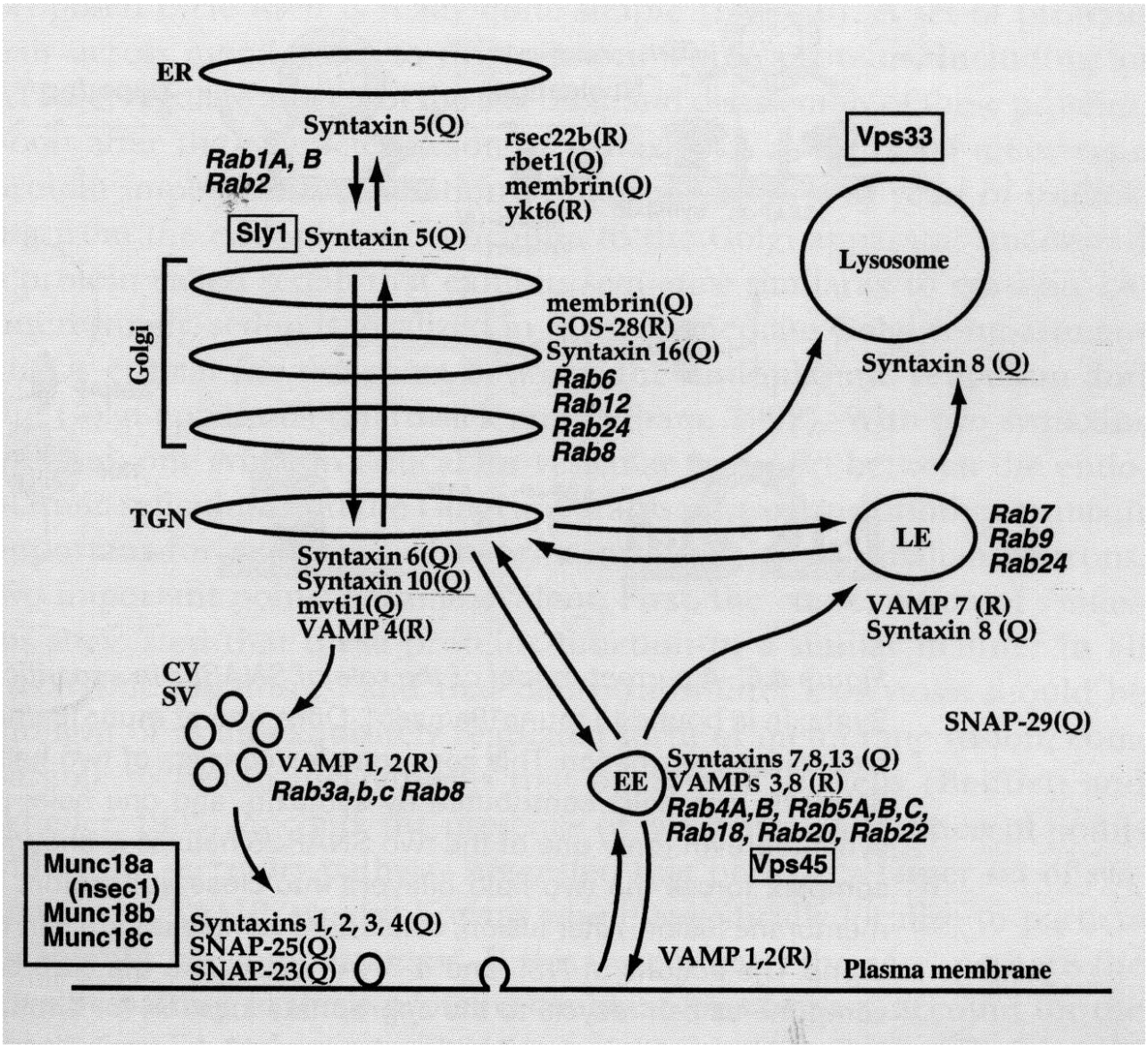


zippering mechanism provides energy
N-termini of v- and t-SNAREs interact first
energy for fusion provided by binding
    SNARE complex very stable
    --dissociates only by boiling in SDS

OR addition of ATP to NSF
    to dissociate SNAREs before endocytosis,
    leaving t-SNAREs on plasma membrane

SNARE distribution

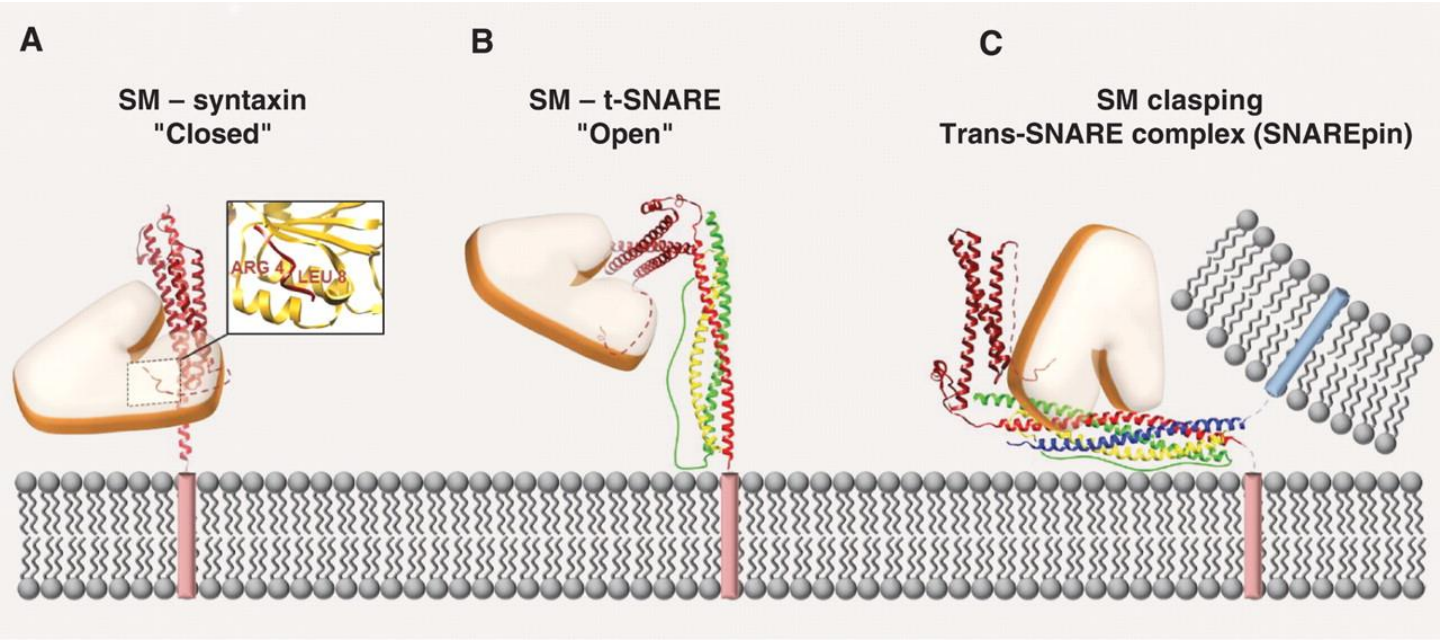
suggests specificity:



BUT SNARE complex formation is promiscuous
AND only some complexes produce fusion
?role for transmembrane domains?
what regulates SNARE complex formation?

docking and priming: SM proteins

regulation at t-SNARE itself:
syntaxin has an **auto-inhibitory** domain
--must be removed to form SNARE complex



(Sudhof and Rothman, 2009)

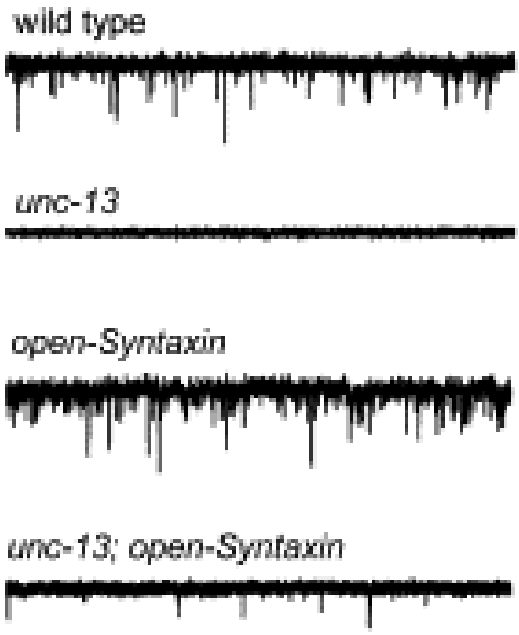
SM proteins *essential* for fusion in cells (not *in vitro*)
munc18 (n-**s**ec1) stabilizes closed state of syntaxin
--site of regulation: open point mutant
but loss blocks fusion--catalytic

munc18 also binds to assembled SNARE complex
--positive regulator as well as negative

another SNARE regulator: unc-13

required for glutamate release (not IPSCs)

EPSCs

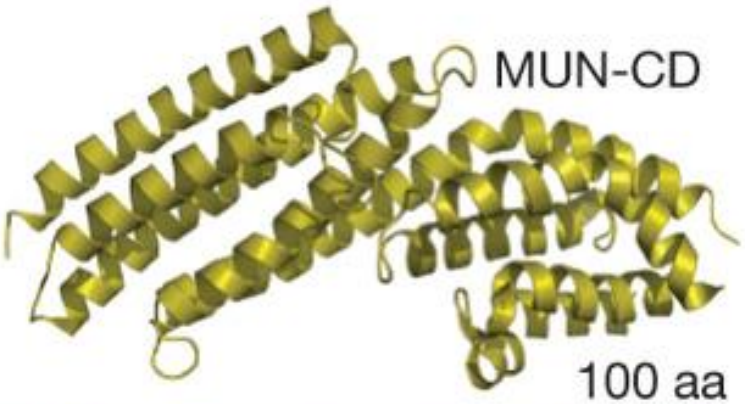


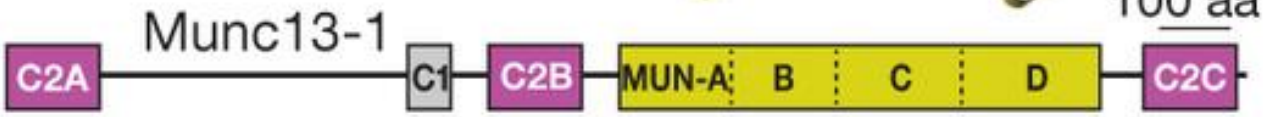
(McEwen et al, 2006)

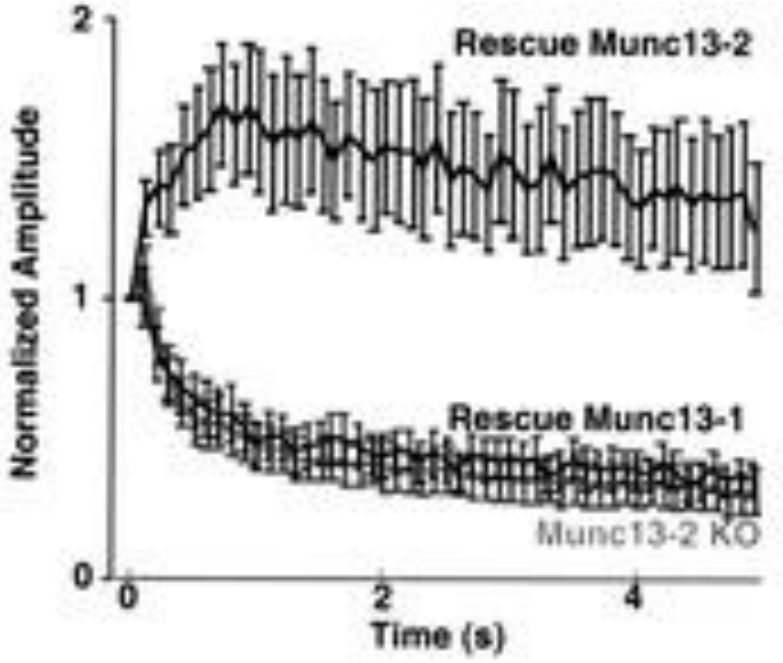
open syntaxin lacking autoinhibitory domain rescues unc13 null

--role in priming?

munc13







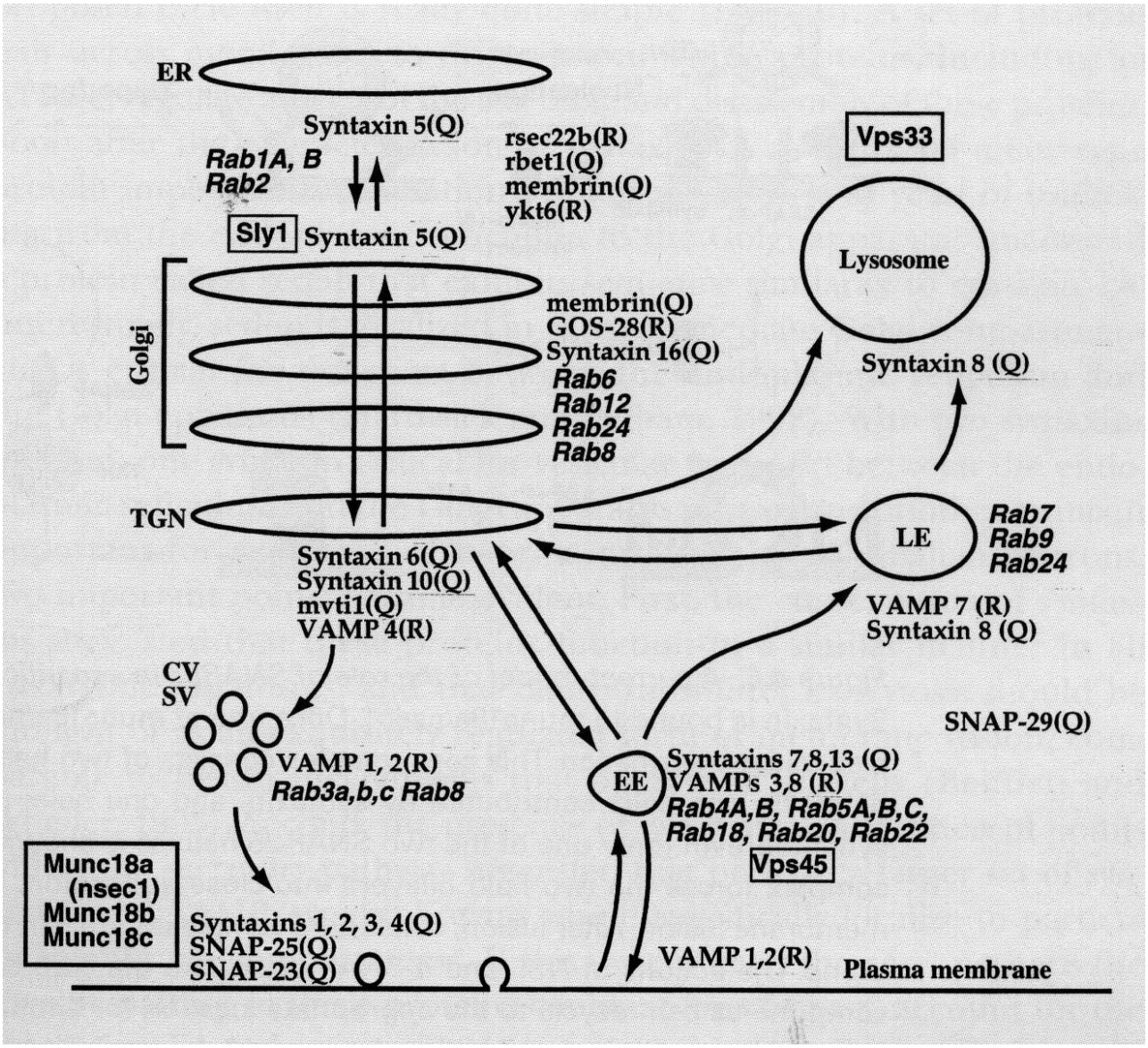
(Rosenmund et al, 2002)

double KO = no release
rescue with different isoforms
alone confers different forms
of short-term plasticity
(due to changes in Pr)

what confers specificity to SNARE assembly?

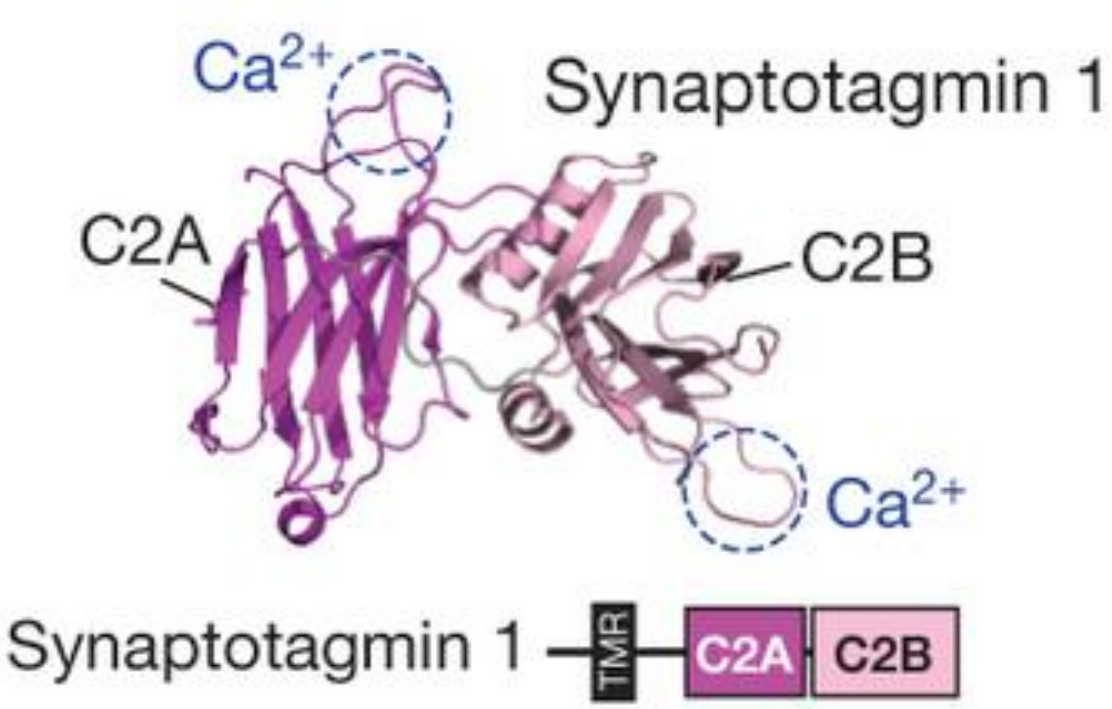
not SNAREs

only a few SM proteins (operate at multiple sites)



?rabs

Ca^{++}-dependent triggering



type I SV protein (N-term lumenal)

C2s mediate Ca^{++}-dependent phospholipid binding
- could mediate Ca^{++}-evoked release
- interact with SNARE proteins

dimer contains 4 C2 domains:
- does this confer the sensitivity to $[Ca^{++}]^4$?
- --delete one of the two C2 domains:

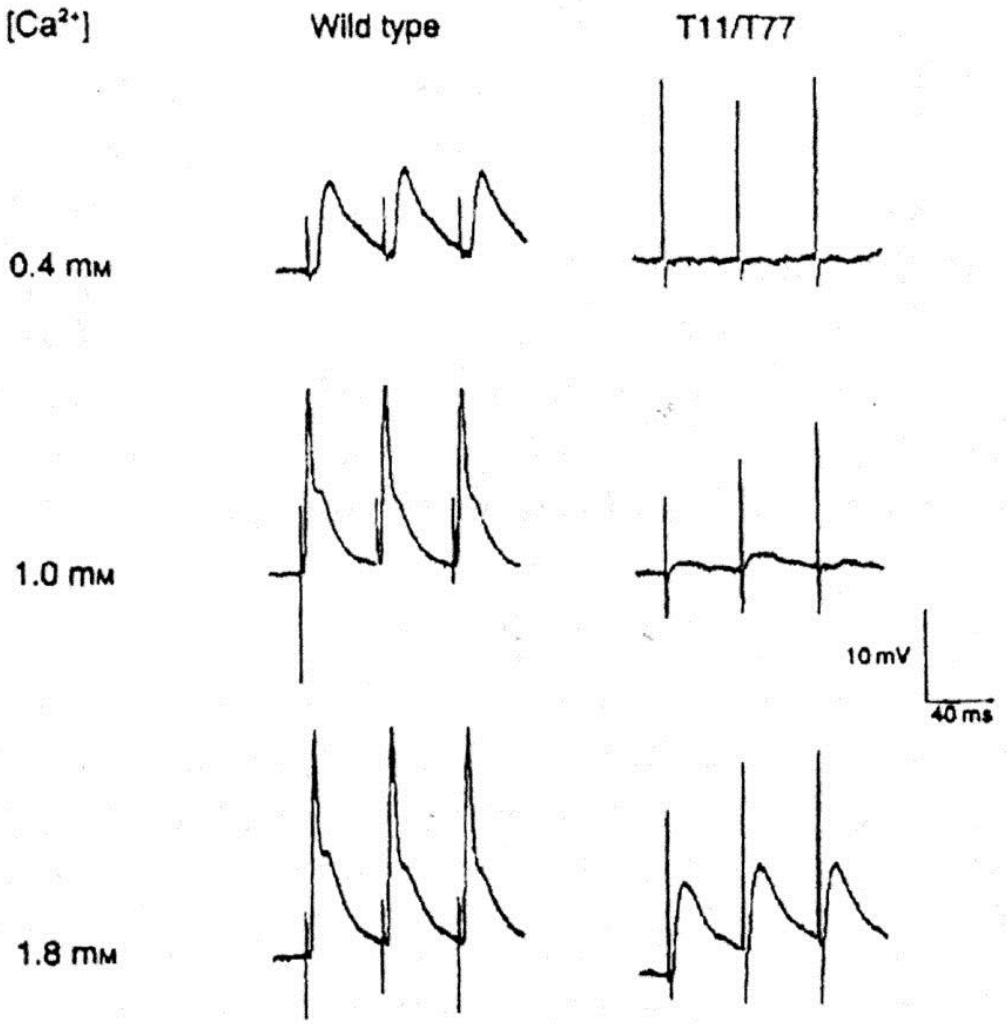
Drosophila

loss of syt eliminates evoked release

increases spontaneous release

delete one C2 domain:

single C2 domain



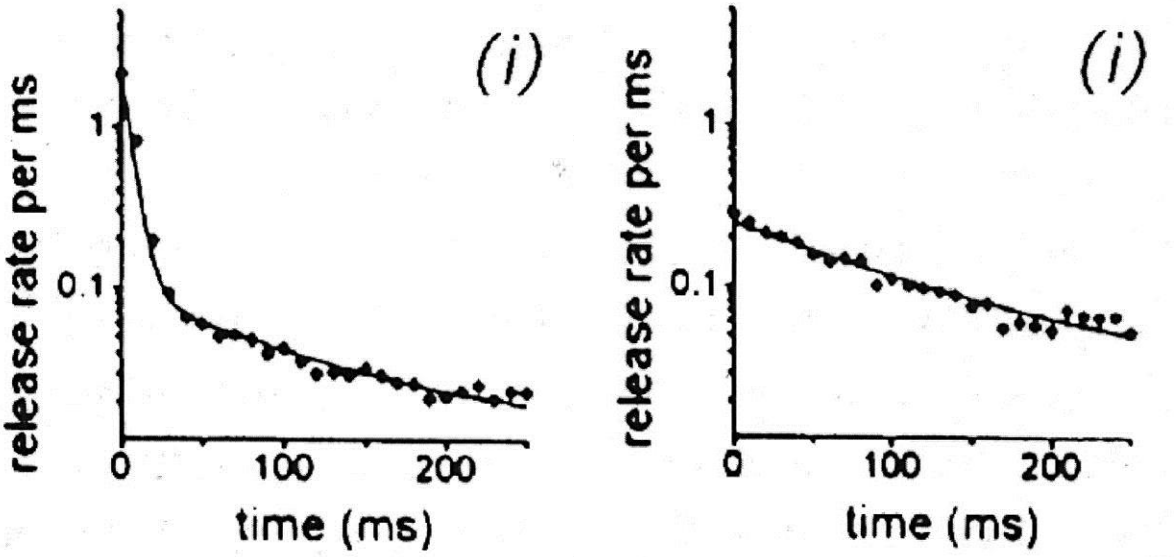
(Littleton and Bellen, 1994)

Hill coefficient reduced from ~4 to ~2

WT interpreted as dimer (4 C2 domains)

syt 1 KO (mouse)

wt KO

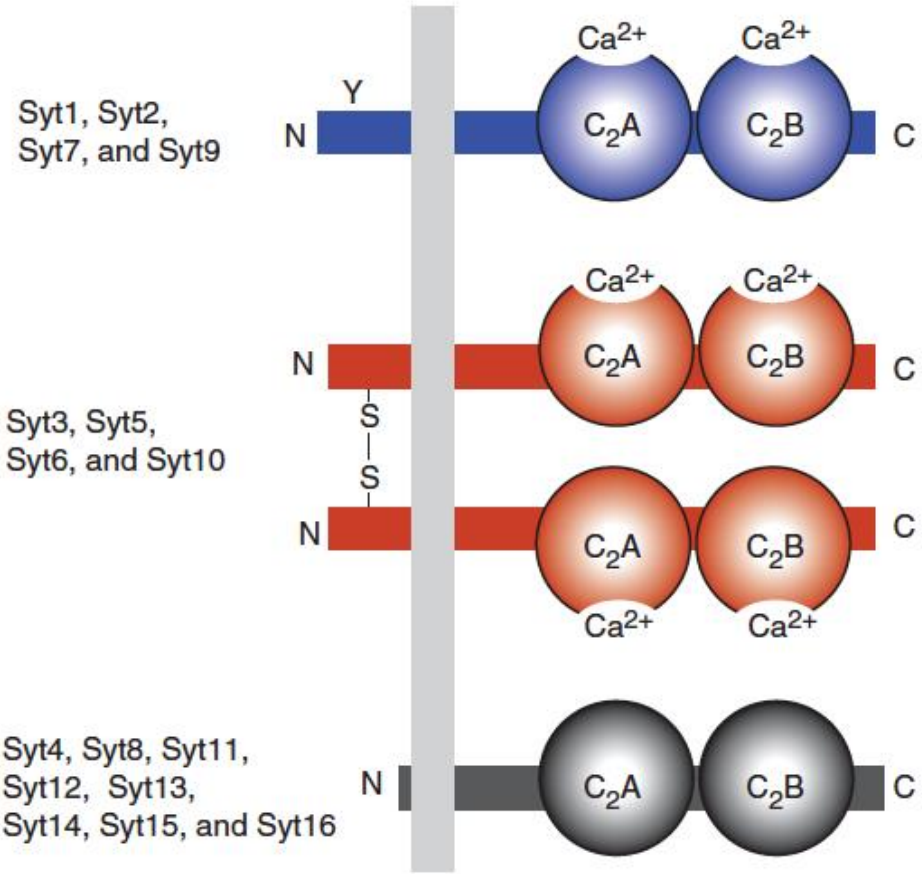


(Geppert et al, 1994)

KO reduces synchronous release
- no effect on asynchronous

increased spontaneous release
- still Ca^{++}-sensitive
- unmasking a distinct Ca^{++} sensor
- higher affinity

multiple synaptotagmins



differ in Ca^{++} affinity

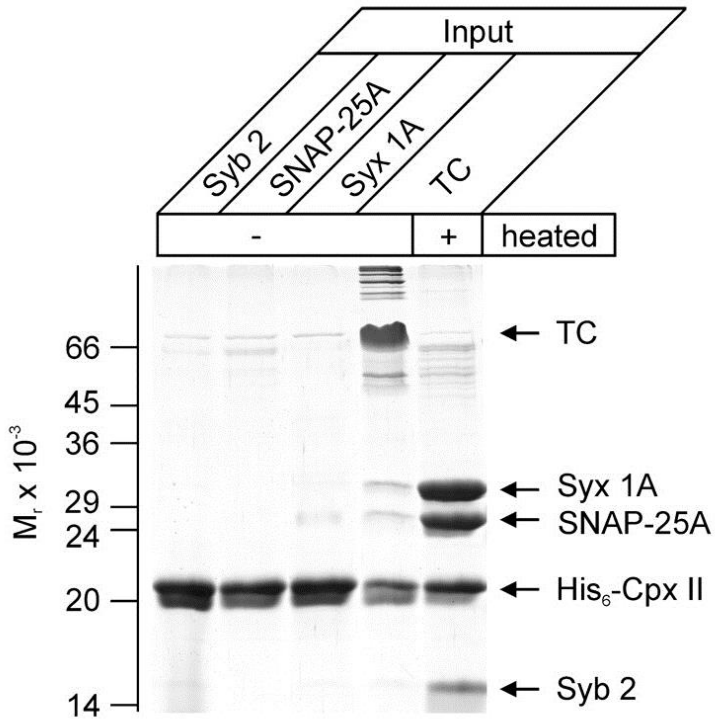
Syt7 on LDCVs and lysosomes

required for facilitation: how?

contributes to regulated secretion

function of others unknown

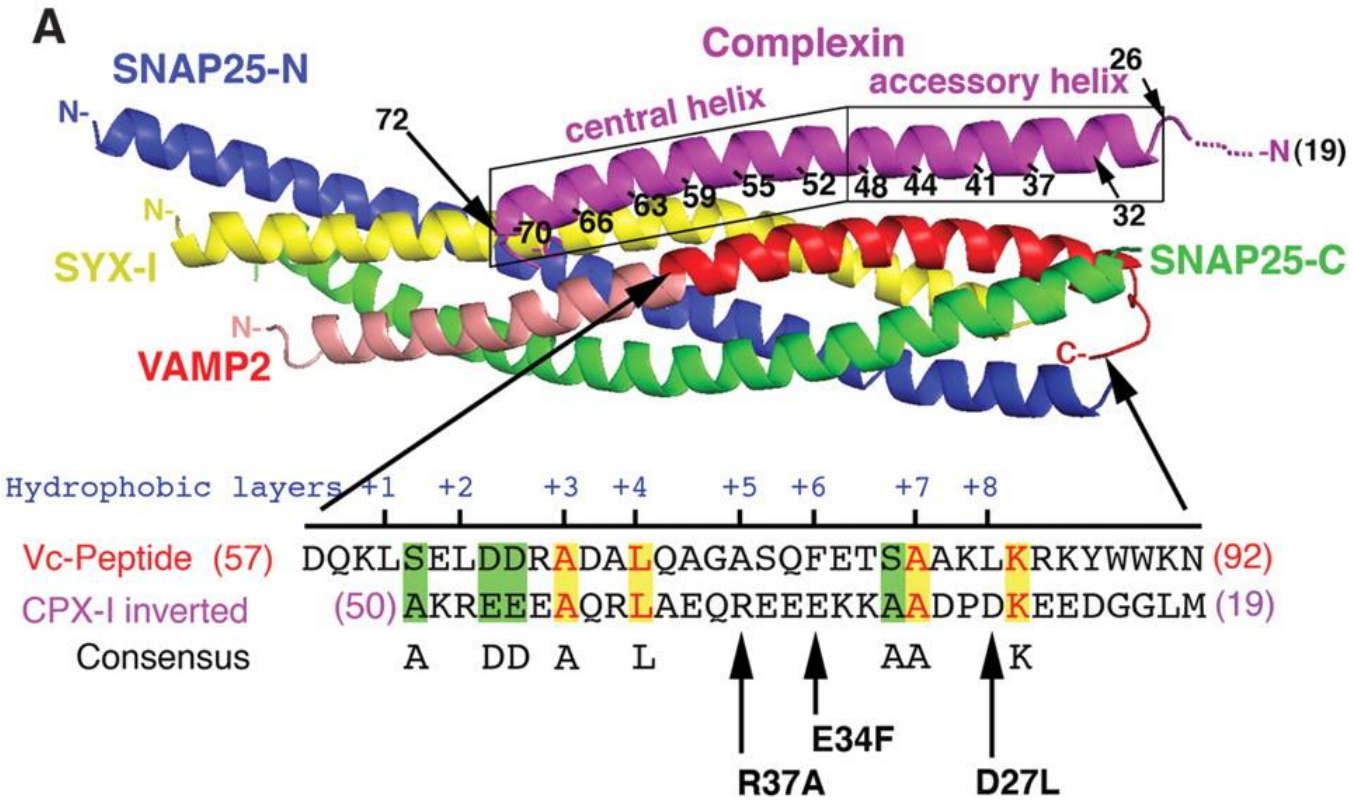
complexin binds to SNARE complex



(Pabst et al, 2000)

--not to individual SNAREs

over-expression **inhibits** release
complexin KO ~synaptotagmin KO



(Giraudo et al., 2009)

experimental issue with studying NT release

presynaptic manipulation problematic in slices

- cannot make KI mice for each mutation
- --mass cultures vs. autapses

loss of complexins

- reduces evoked release
- increases spontaneous release

central helix binds to SNARE complex

- orientation opposite to SNARE proteins
- accessory helix clamps, blocking fusion
- N-terminus activates fusion

model: synaptotagmin displaces to trigger fusion?

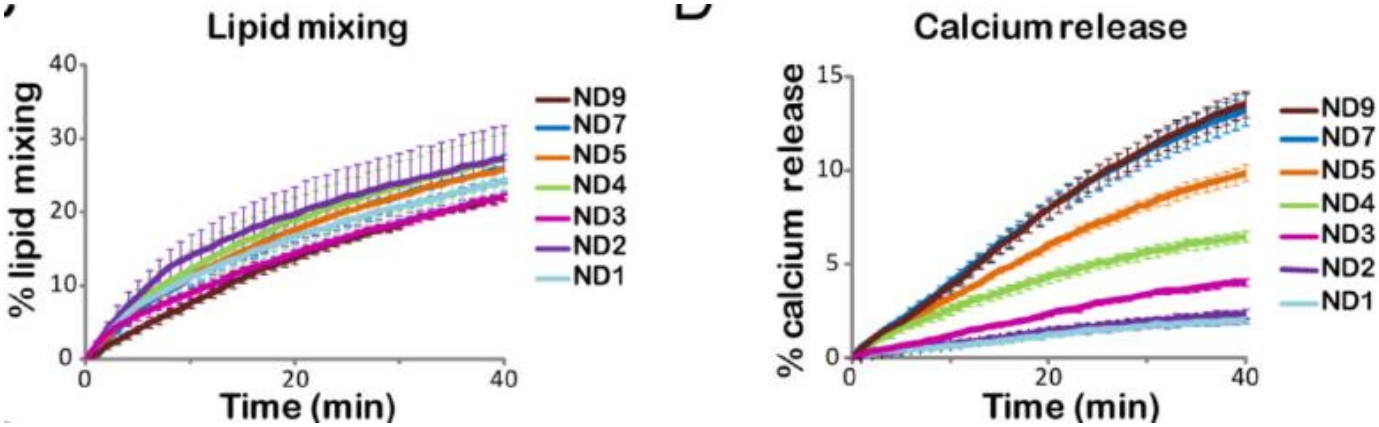
number of SNAREs

one for fusion in chromaffin cells

one for hemifusion, 3 for full fusion:

reconstituted nanodiscs

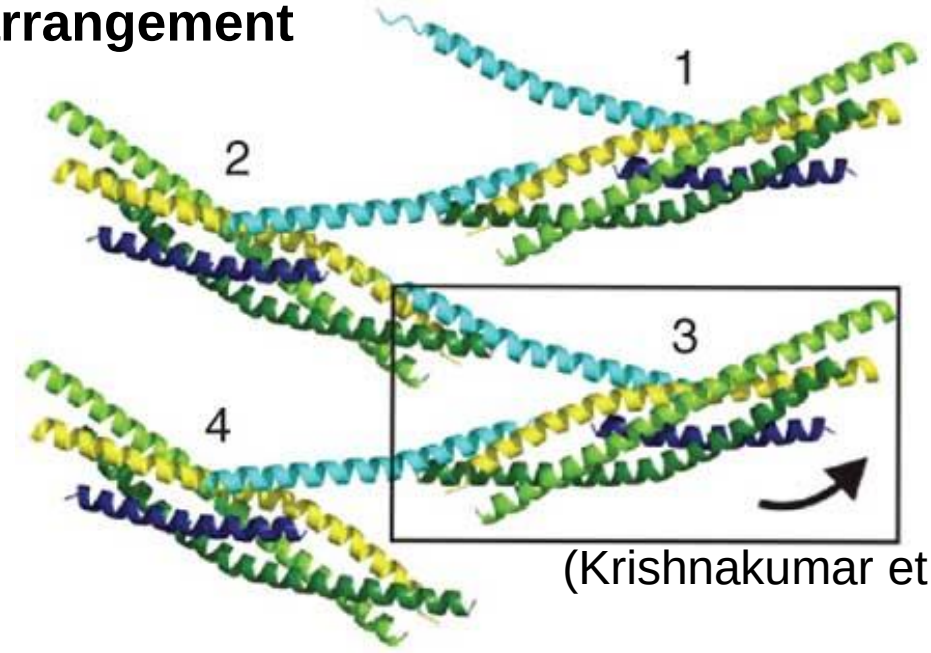
(# v-SNAREs)



(Shi et al., 2012)

arrangement

zigzag



(Krishnakumar et al., 2011)

complexin accessory helix clamps

adjacent SNARE complex

active zone

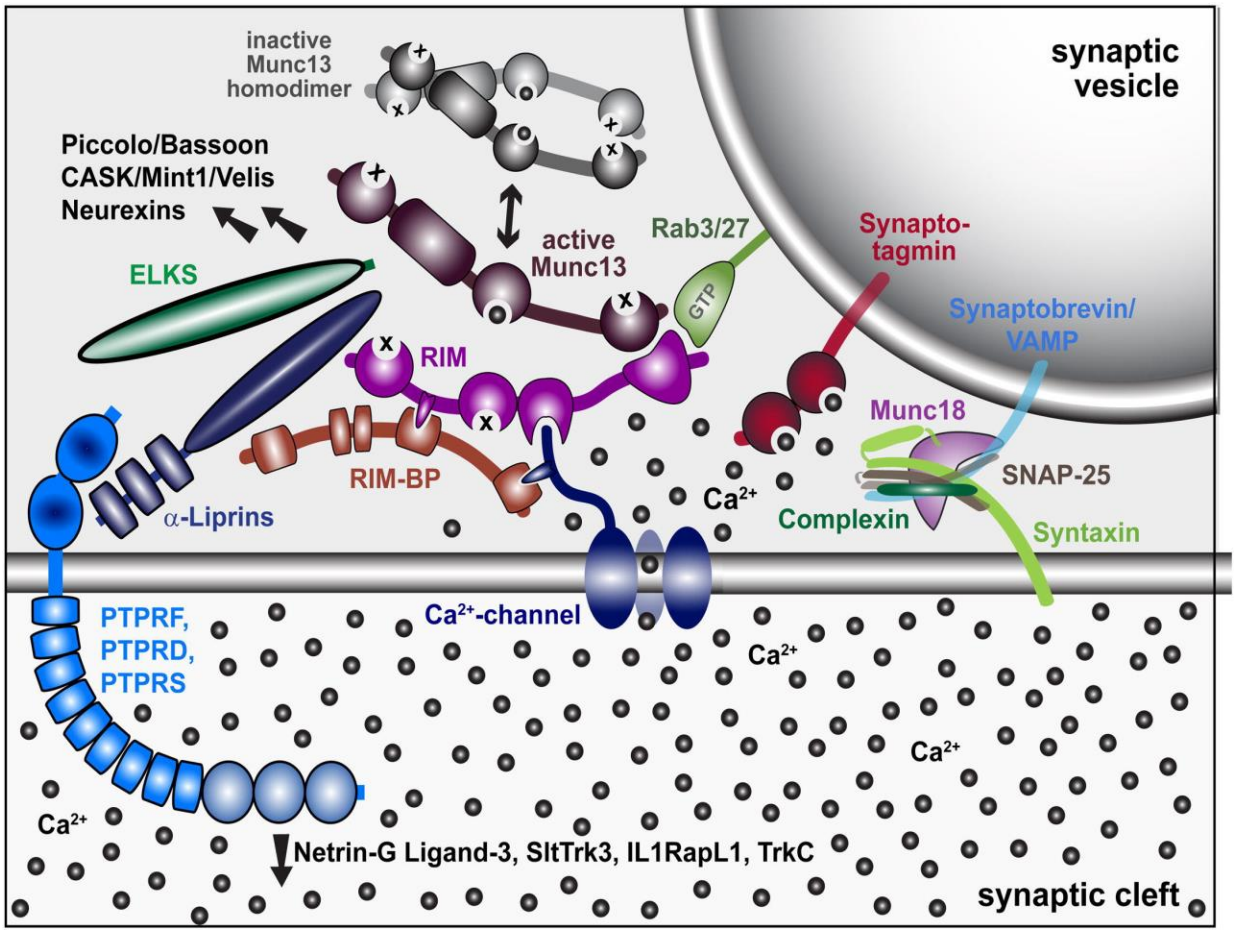
sites of preferred exocytosis

t-SNAREs axonal (not just active zone)

specified by munc-13, RIM, RIM-binding protein (RBP), α-liprin

cytomatrix proteins: bassoon, piccolo, ELKS/CAST

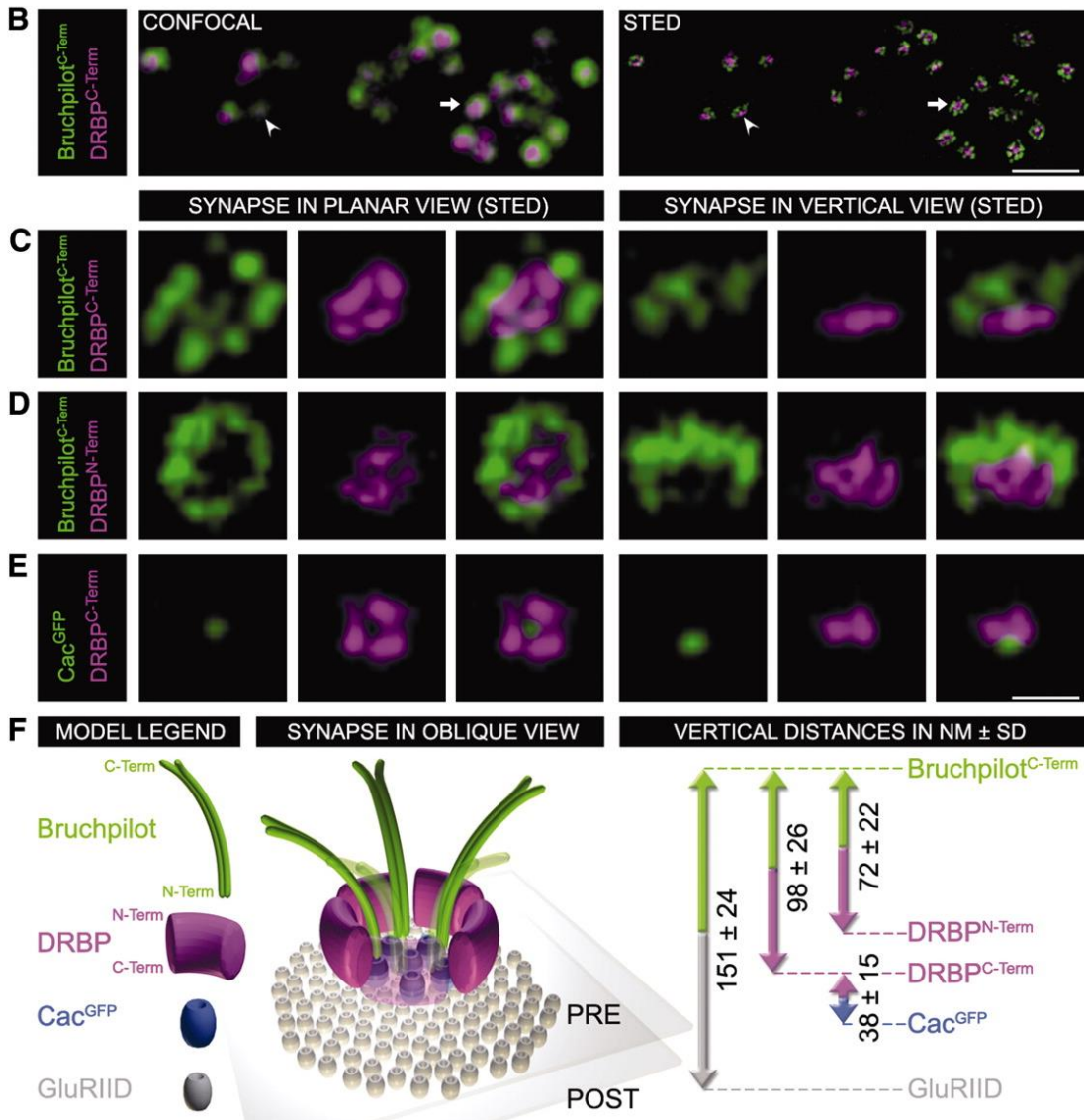
calcium channels



role for active zone proteins in priming

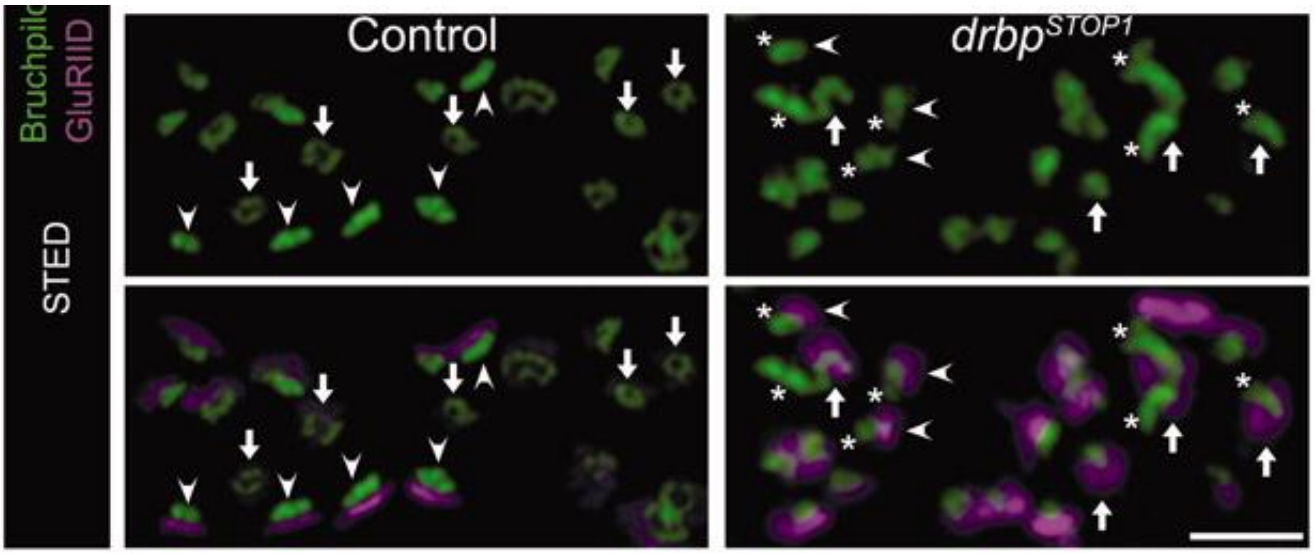
RIM mutant: reduced release
- evoked and spontaneous
- normal docking
- rescued by open syntaxin
- rescued by dimerization-deficient munc13

RIM-binding protein

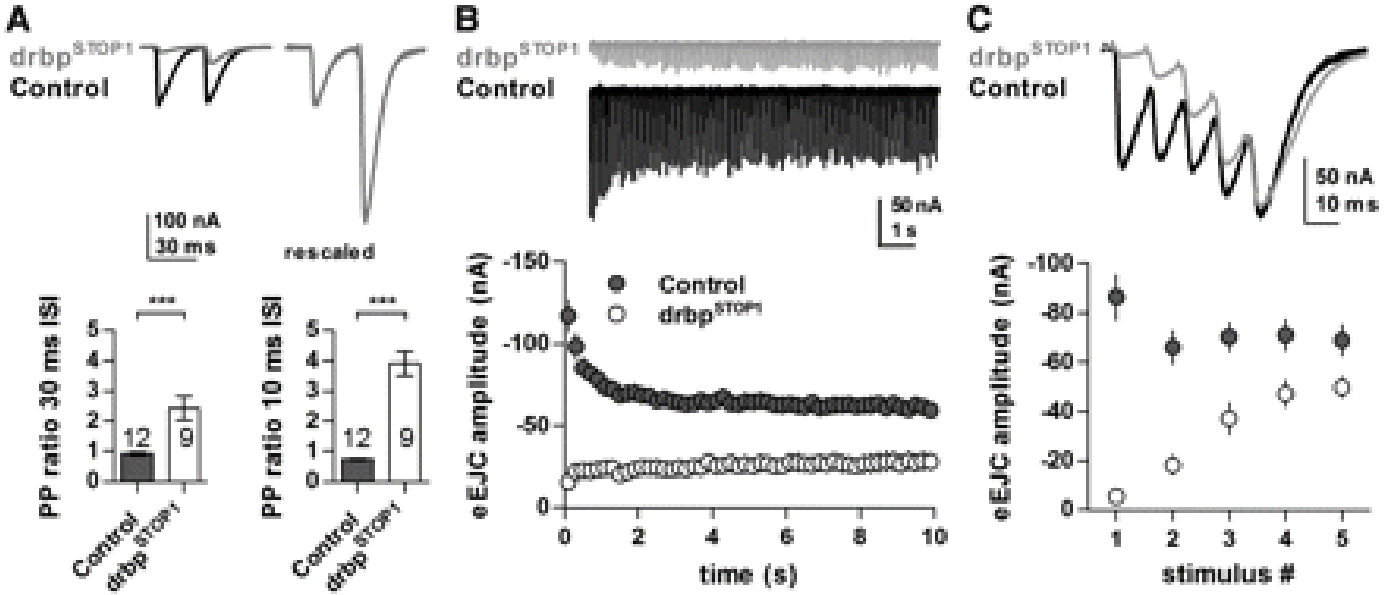


(Liu et al., 2011)

(Liu et al., 2011)

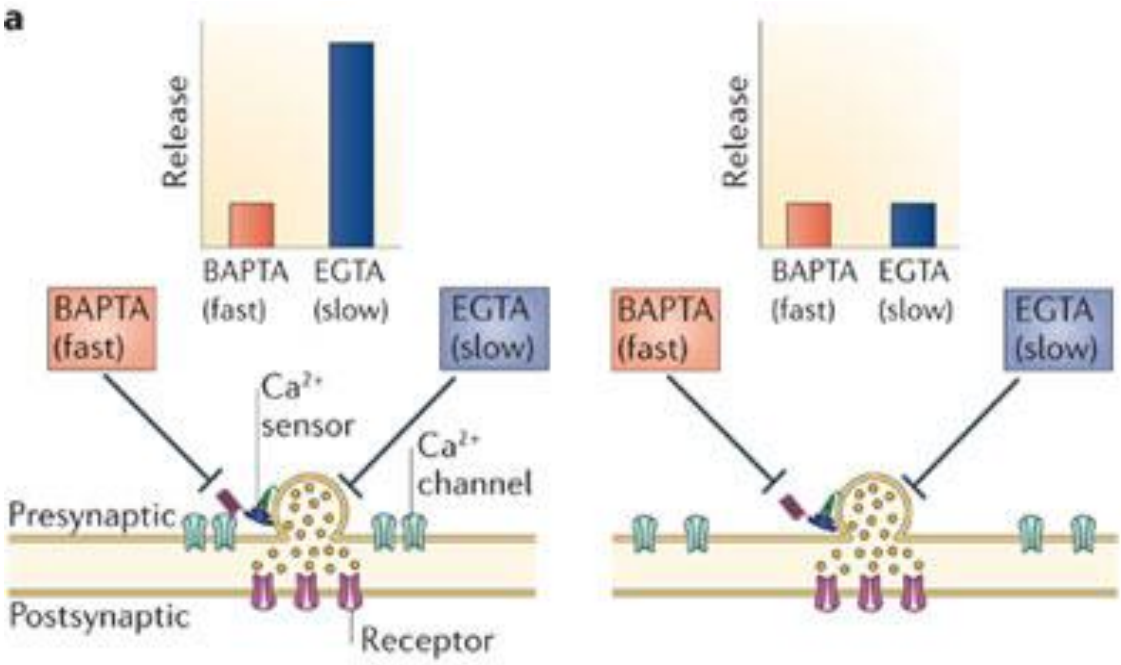


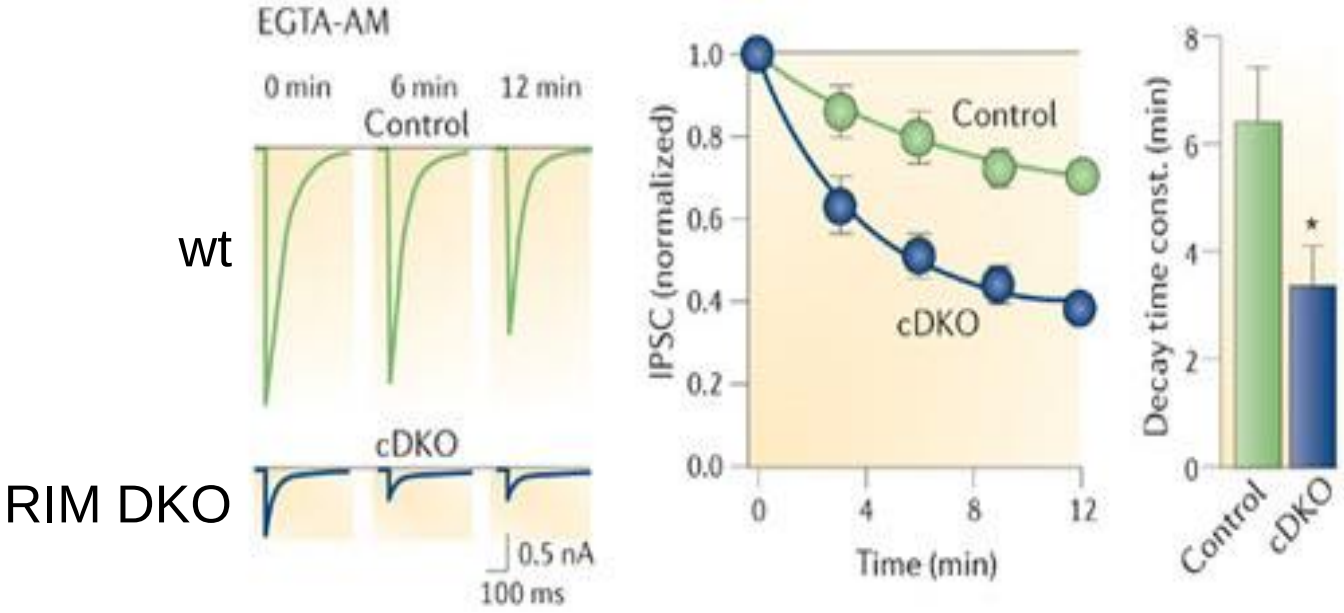
loss of RBP disrupts Brp (ELKS)



RBP mutant recovers with stimulus train

coupling to calcium entry





(Eggermann et al., 2011)

direct visualization of NT release

biochemical reconstitution
- dissects steps (difficult with other approaches)
- amenable to manipulation of components

FM dyes
- fluoresce only in membrane
- loaded by stimulation
- wash to remove free dye
- stimulate to unload dye

pHluorin reporters
- quenched at low pH of SVs
- unquenched by exocytosis
- membrane proteins quenched again after endocytosis due to reacidification
- soluble proteins released (slowly)

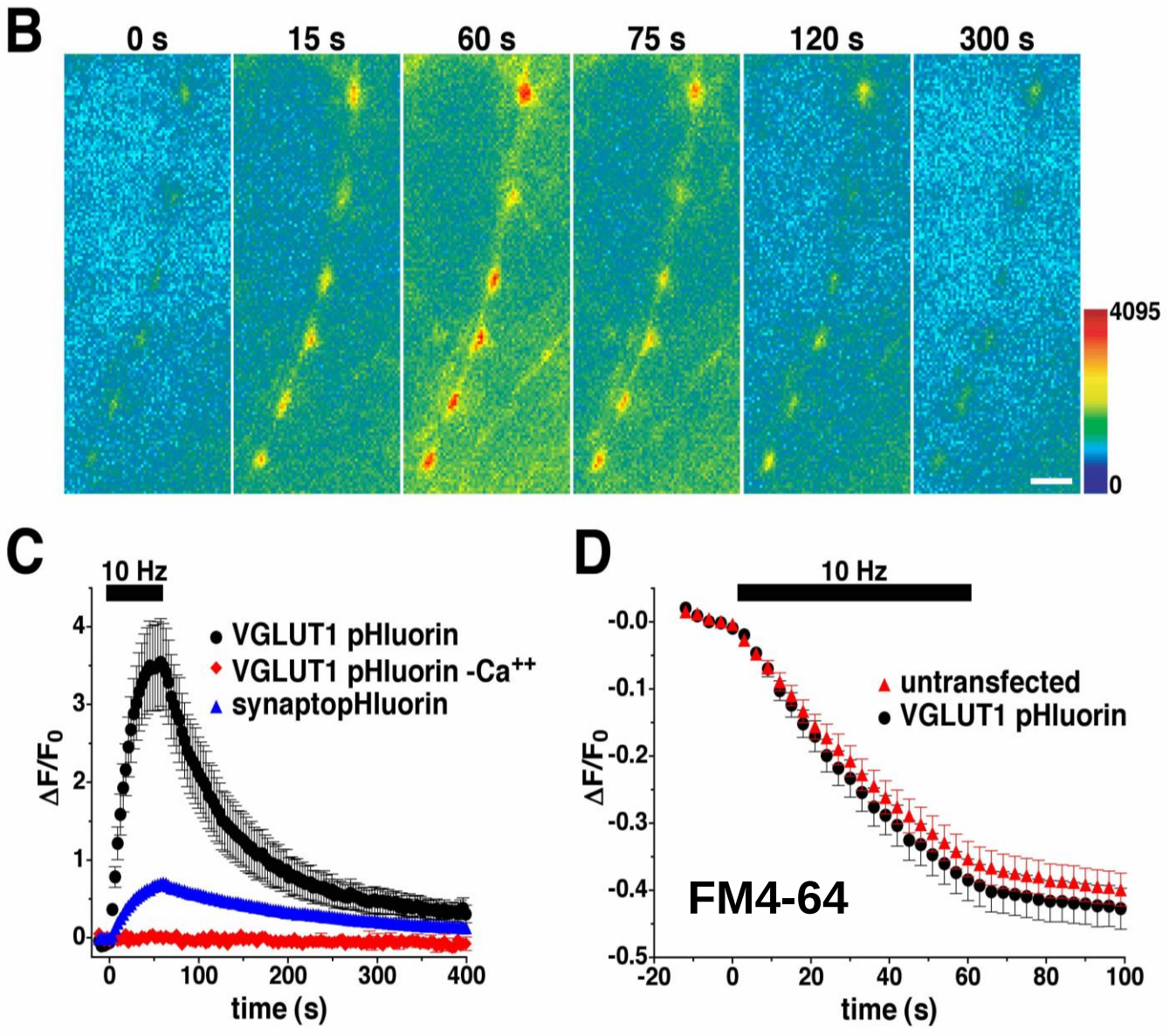
amperometry
- direct measurement of monoamines
- can detect single SV fusion

capacitance
- detects cumulative fusion
- except in cell-attached patch mode

total internal reflection fluorescence microscopy
- detects individual exocytic events
- brightens on entering TIRF plane

false fluorescent neurotransmitters
- loaded by vesicular transporters (many molecules)
- monitor fast phase of release
- can detect pH-insensitive FFNs before fusion

VGLUT1-pHluorin



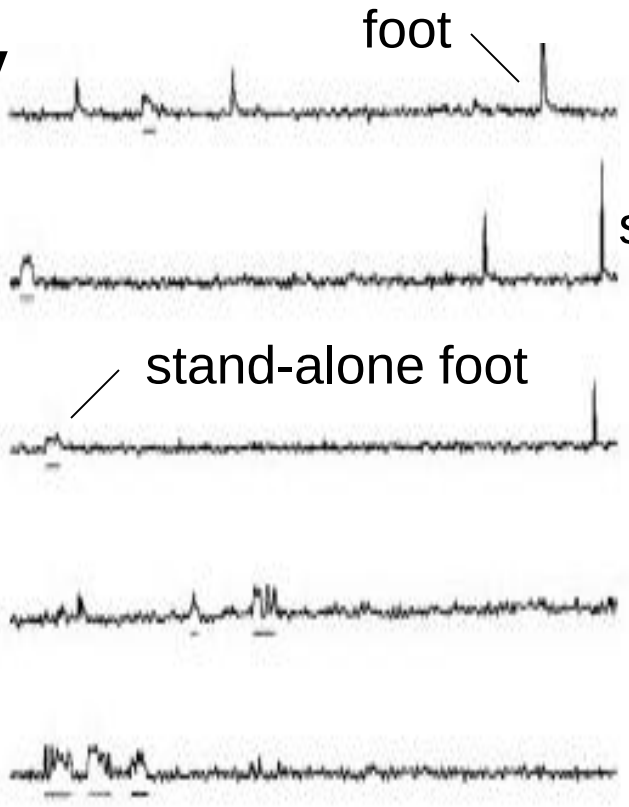
--difficult to detect individual SVs

these methods cannot detect kinetics of single events

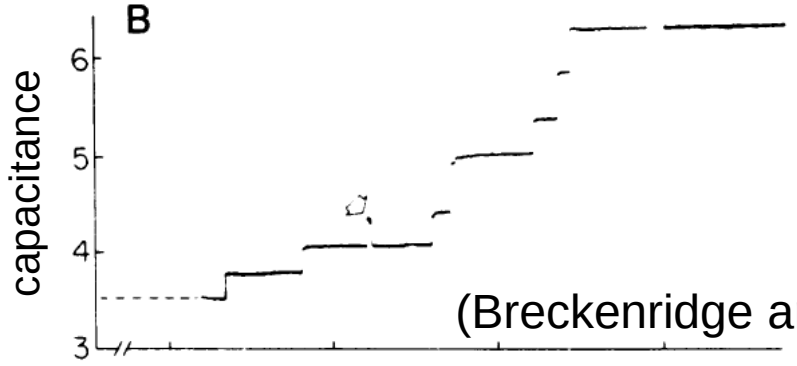
physiological mechanism unknown

large dense core vesicles (LDCVs): release kinetics

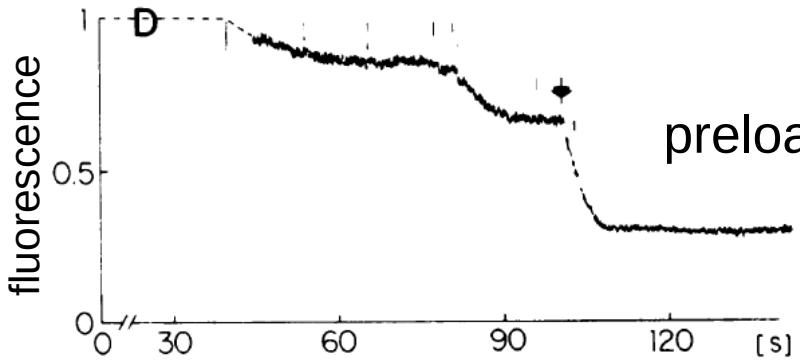
amperometry



capacitance: oscillating voltage
individual vesicles in mast cells--

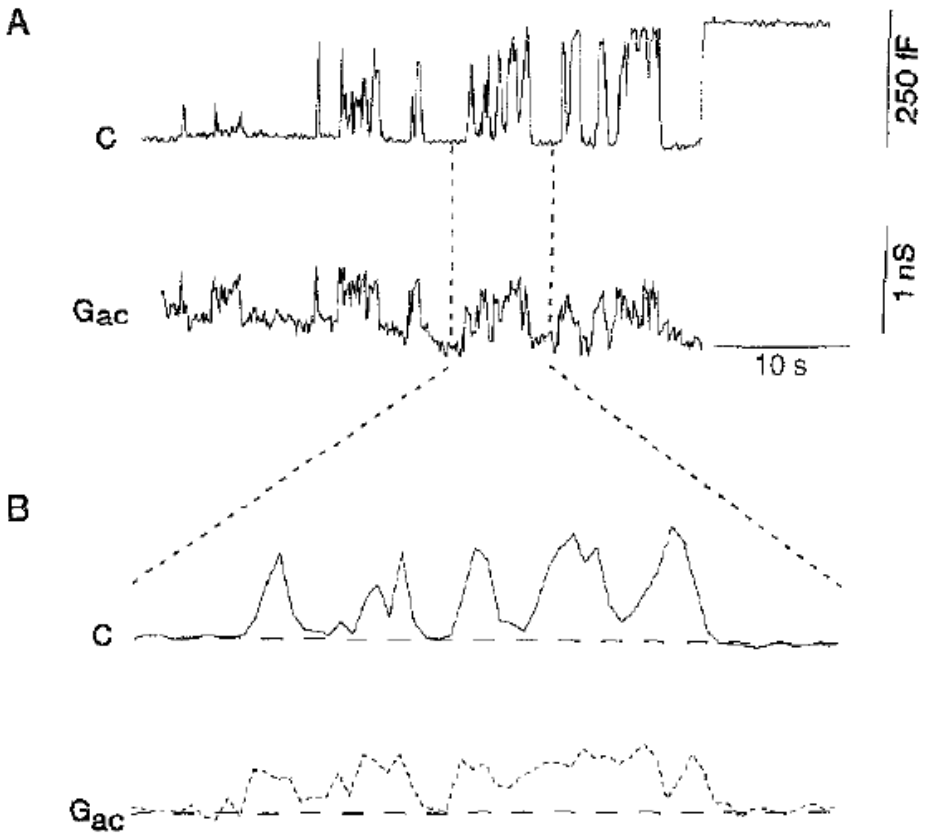


(Breckenridge and Almers, 1987)



preloaded quinacrine

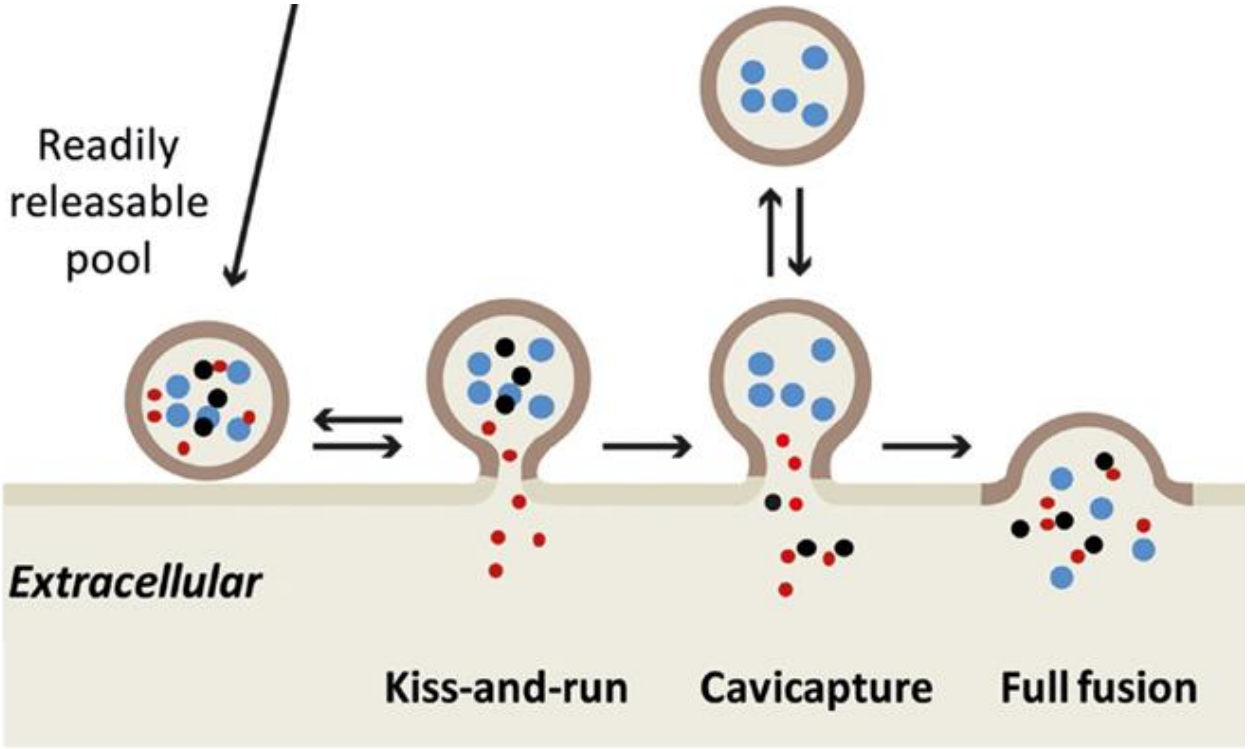
capacitance flicker



(Spruce et al., 1990)

kiss-and-run:
reversible opening of fusion pore

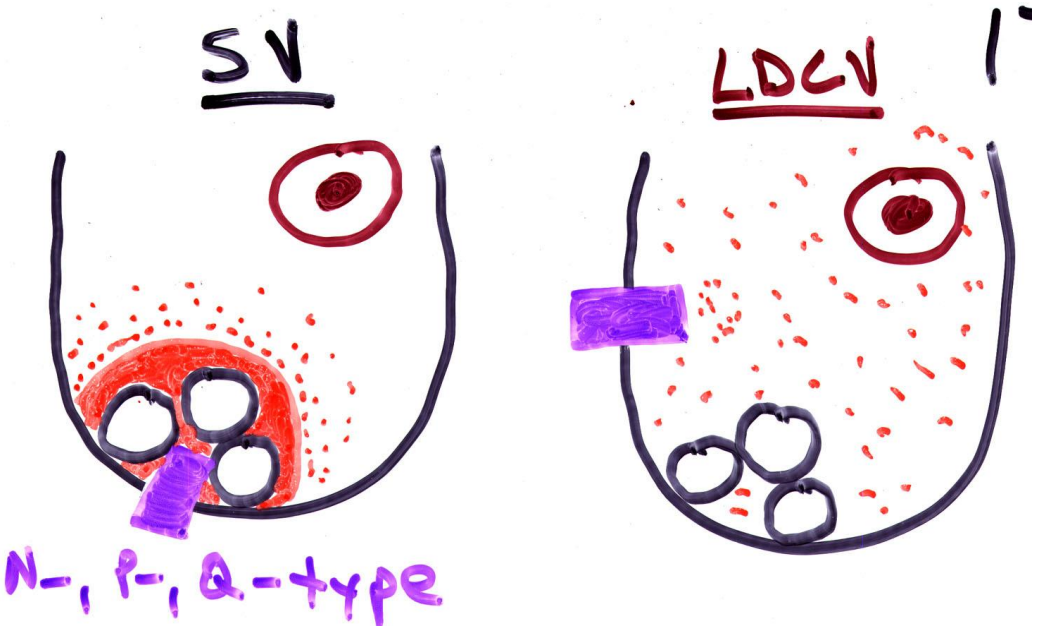
fusion pore



fusion pore dilation regulates
- which peptides released
- --characteristic order of release
- less affect on classical transmitters

how is the fusion pore regulated?
- does this affect release from SVs?

peptidergic vesicles
(large dense core vesicles)



different calcium requirements

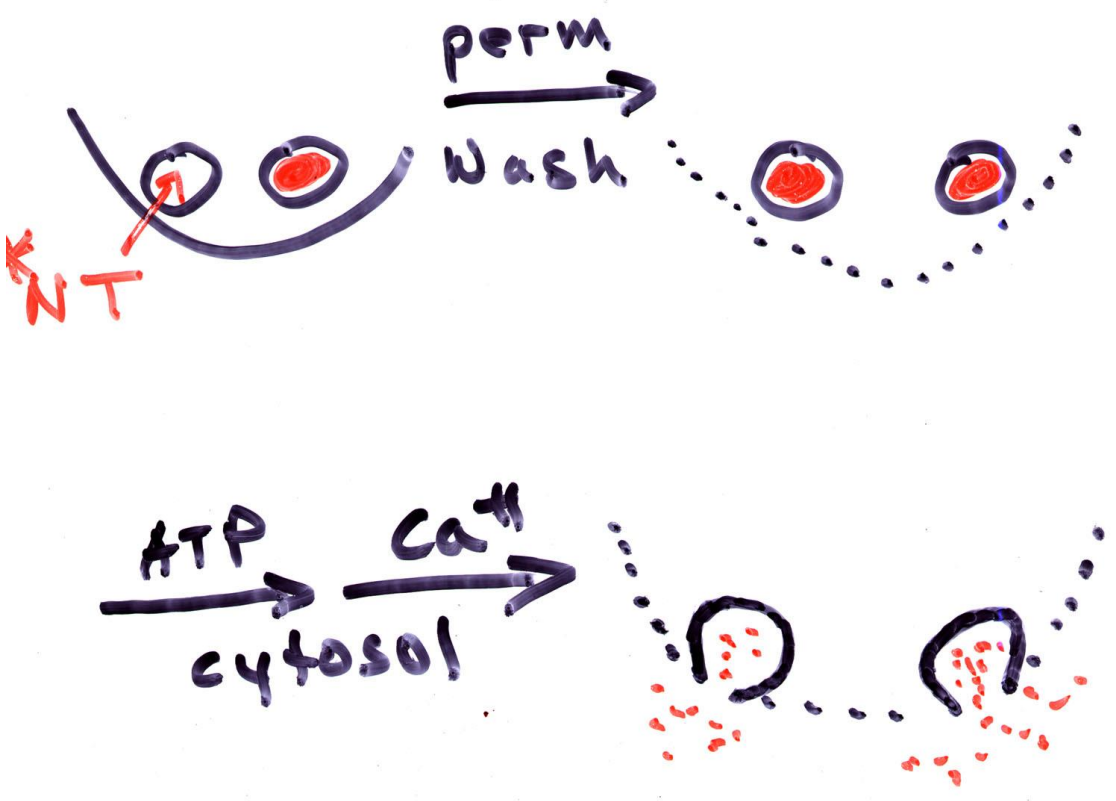
LDCVs require more stimulation

BUT have higher intrinsic Ca^{++} sensitivity

--further from Ca^{++} entry sites

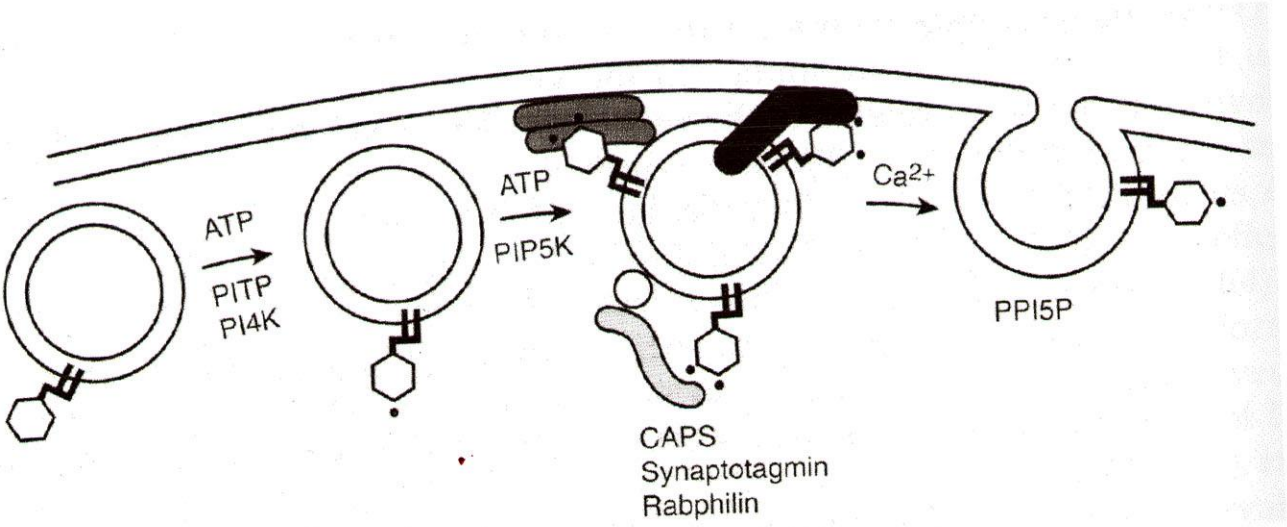
what about the membranes?

PC12 permeabilized cell assay (Martin)



ATP-dependent priming
Ca^{++}-dependent triggering

sequential lipid modification



1) PI phosphorylation (3 proteins required):
   PI transfer protein
   PI4K
   PIP5K

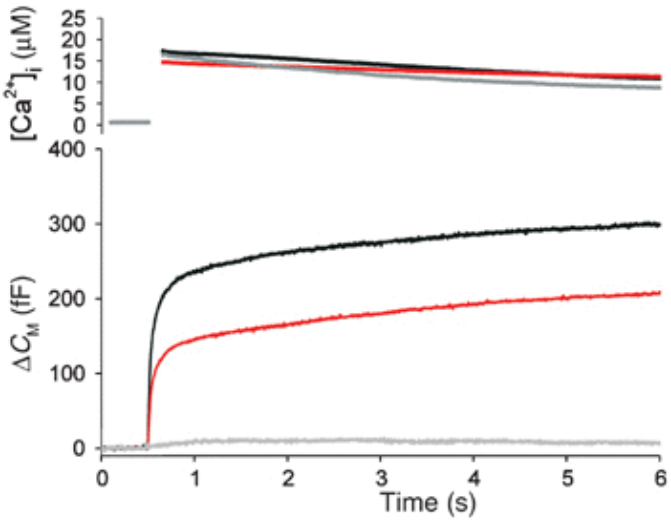
2) CAPS (Ca^{++}-activated protein for secretion)

not unique to LDCVs:
   CAPS ~munc 13
      promotes SNARE assembly

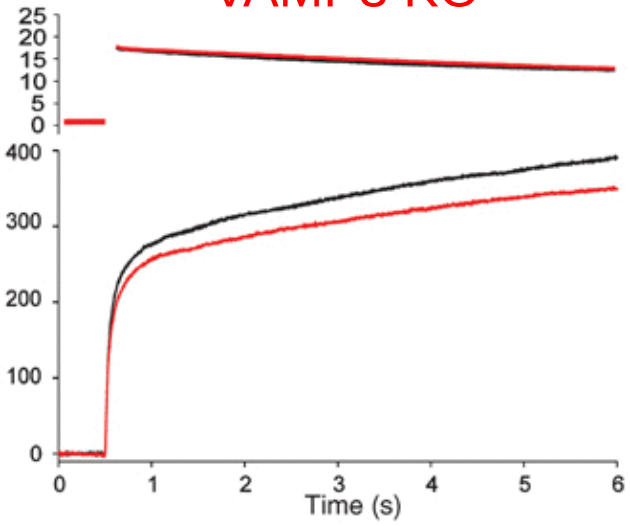
adrenal chromaffin cells

Ca^{++} uncaging

VAMP2 KO



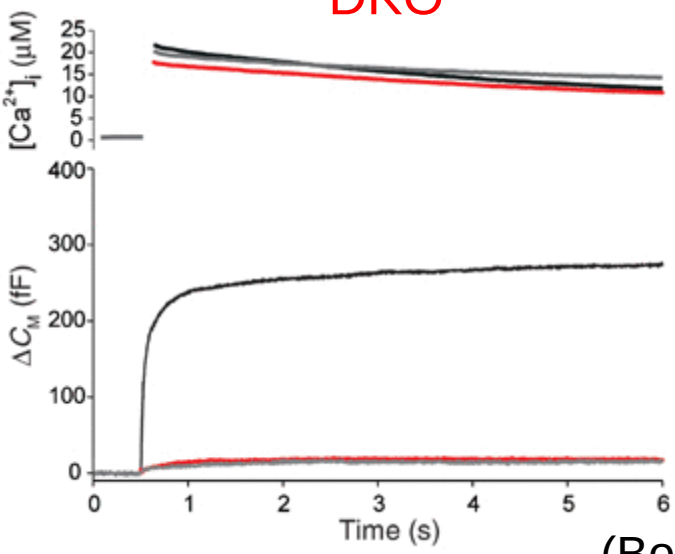
VAMP3 KO



affects fast phase

affects slow phase

DKO



DKO: release gone

(Borisovska, 2005)

cannot detect individual events (amperometry)

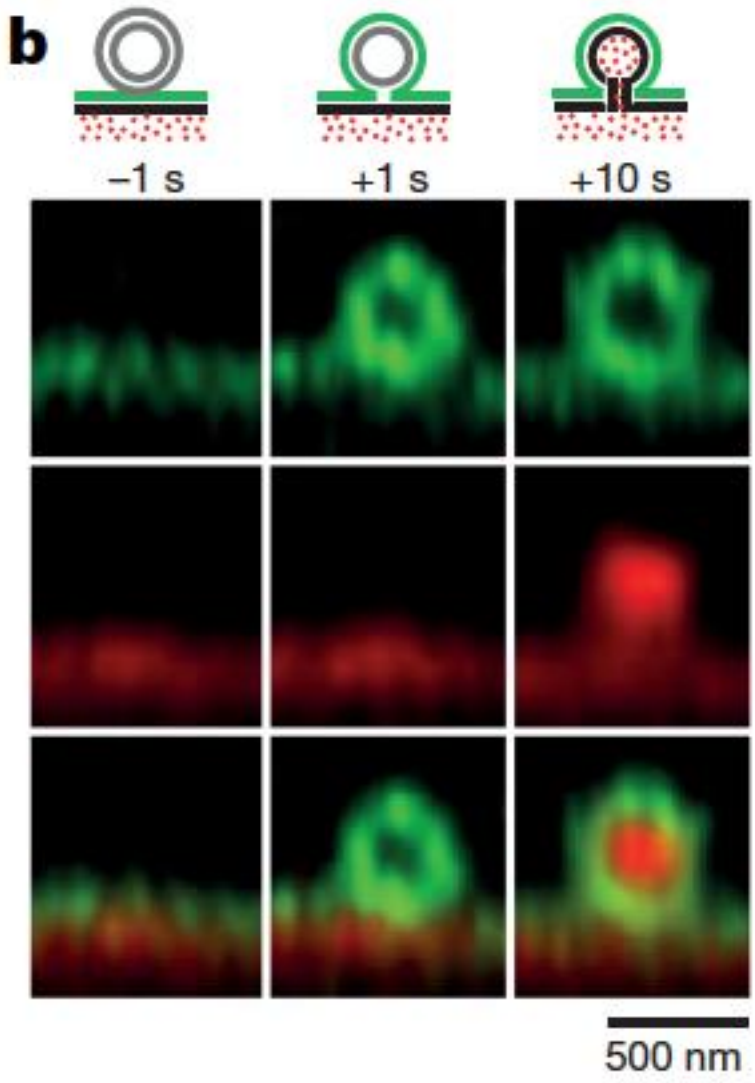
why do VAMP1 and 2 KOs differ?

hemifusion precedes fusion

PH-GFP labels plasma membrane

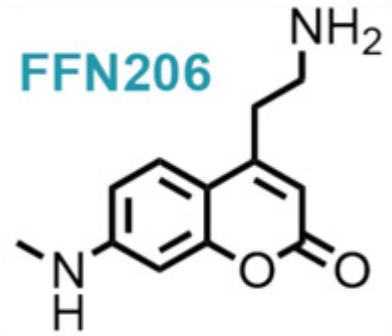
Alexa dye in medium

--enters with full fusion



(Zhao et al., 2016)

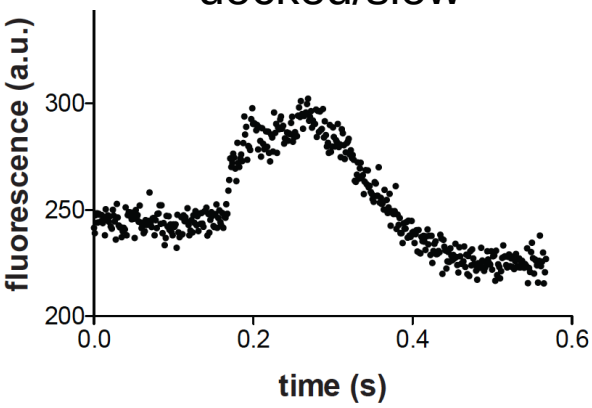
delay means hemifusion can be stable



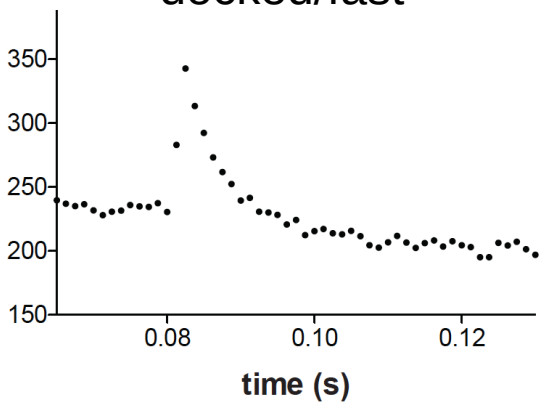
VMAT2 substrate
load 1 h before imaging
FFN206 is pH-insensitive (detect before fusion)
many molecules per vesicle
    works in adrenal chromaffin cells
    ?neurons?

imaging 800 Hz

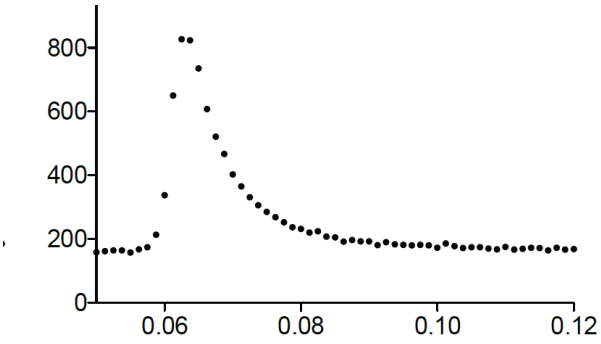
docked/slow



docked/fast



new arrivals



--3 event types
what is responsible?
are there multiple pathways
    to exocytosis?

Reading: The Synapse, edited by Sheng, Sabatini and Sudhof, pp. 49-78

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