Prima Riunione Gruppo SIN
Rete Italiana Tossina Botulinica

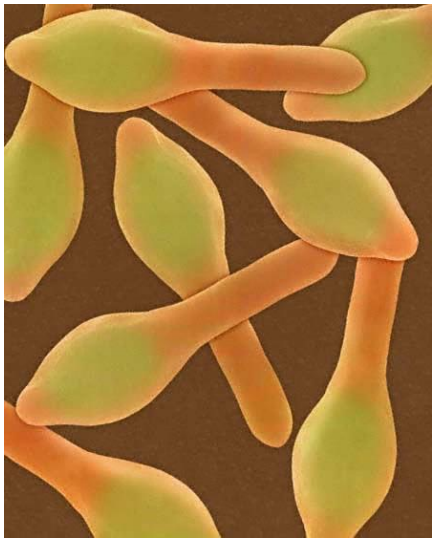
Roma 17 marzo 2017

Upgrade sui meccanismi d'azione delle Neurotossine Botuliniche

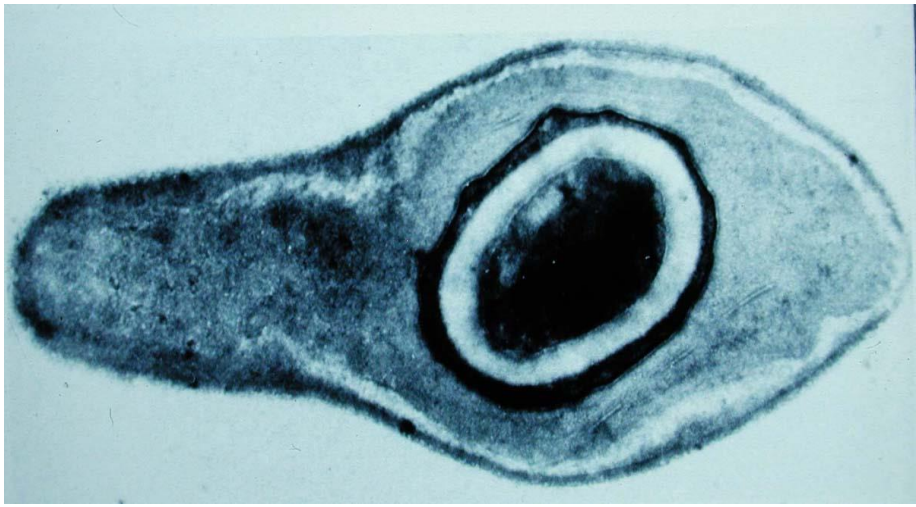
Cesare Montecucco
Università di Padova

Clostridium spp.

- Gram-positive
- Naturally found in soil
- Obligate anaerobic
- Form subterminal endospores (≈ 1 µm)

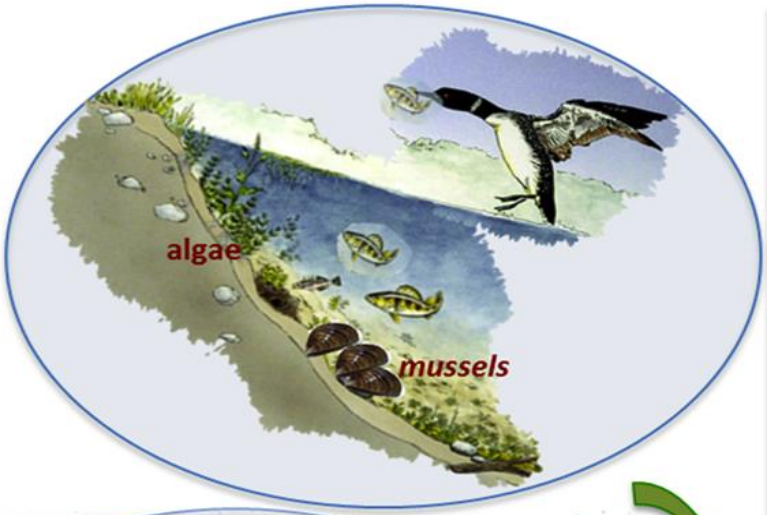


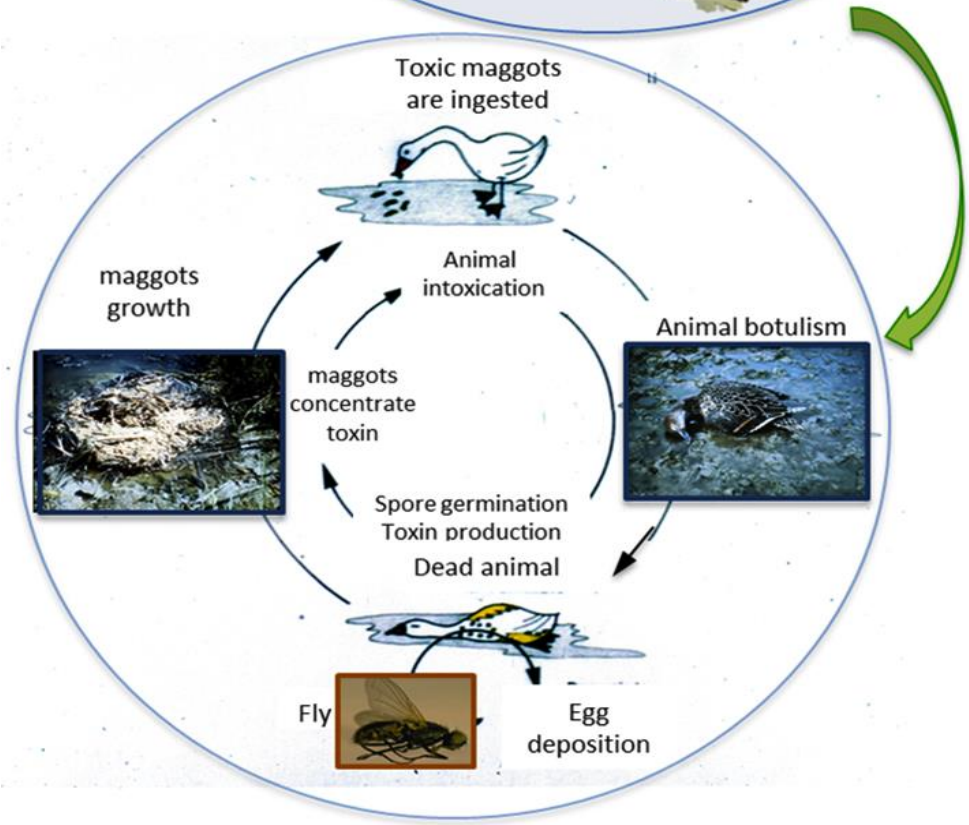
http://www.textbookofbacteriology.net/clostridia_4.html



//www.ciriscience.org/ph_137-Clostridium_botulinum_Copyright_Dennis_Kunkel_Microscopy

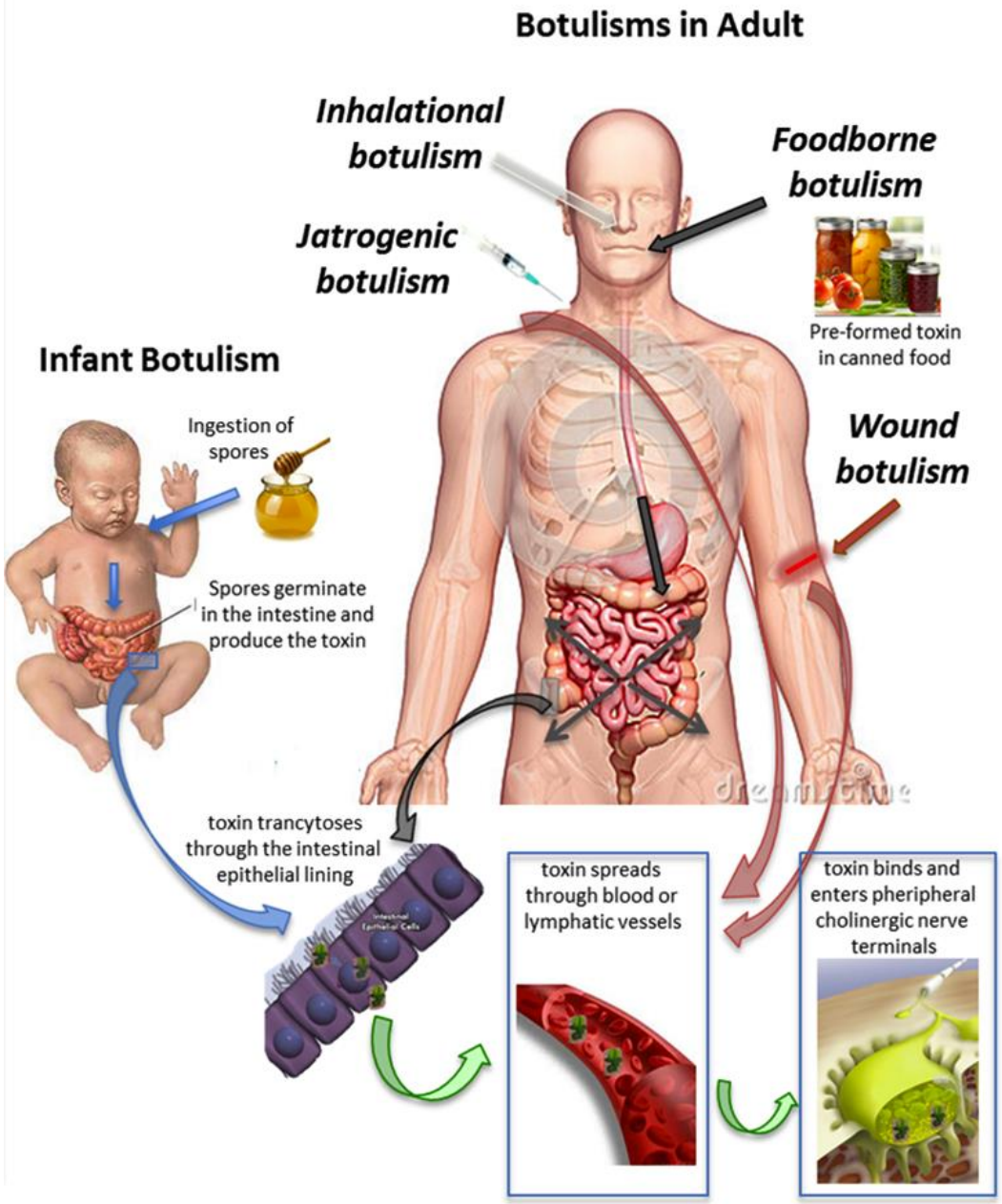
Botulism in wildlife





Rossetto et al.,
Nature Rev. Microbiol. 2014

Human Botulism



Rossetto et al.,
Nature Rev. Microbiol. 2014

THERE ARE **MANY** BOTULINUM NEUROTOXINS

THEIR NUMBER IS RAPIDLY GROWING !!!

> 70 BoNT

Table 1: Serotypes and subtypes of *Clostridium botulinum*

	Proteolytic *C. botulinum* Group I	Non-proteolytic *C. botulinum* Group II	*C. botulinum* Group III	*C. argentinense* (or group IV)	*C. butyricum*	*C. baratii*
Type	A, B, F, H	B, E, F	C, D	G	E	F
Subtype	A1, A2, A3, A4, A5, A6, A7, A8 B1, B2, B3, B5 (Ba), B6, B7, A(B), Ab, Af, Af84, Bf, F1, F2, F3, F4, F5	B4, E1, E2, E3, E6, E7, E8, E9, E10, E11, F6	C, D, C/D, D/C		E4, E5	F7

.

Clostridium tetani produces only one Tetanus Neurotoxin

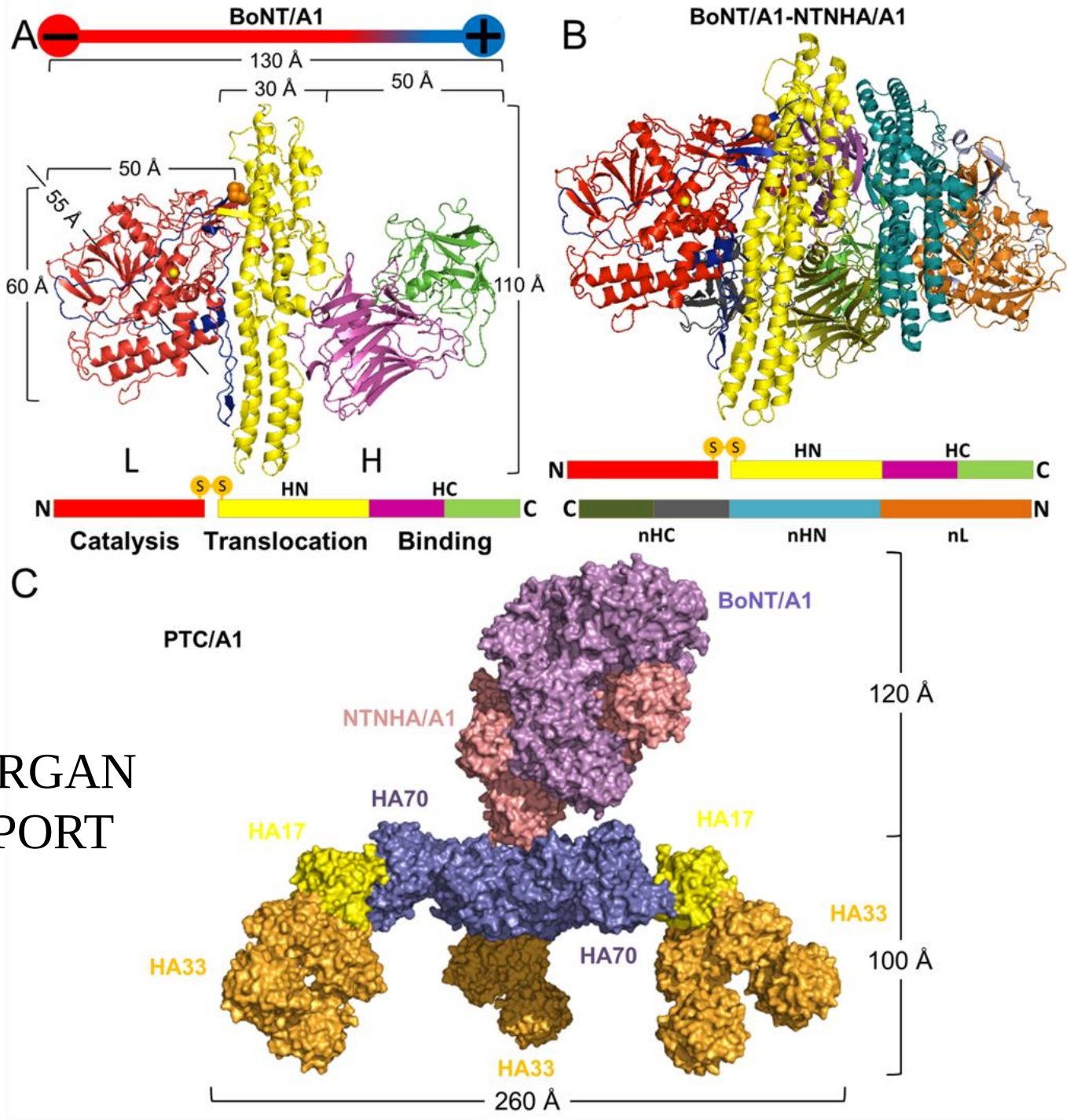
Rossetto et al., Nat. Rev. Microbiol 2014

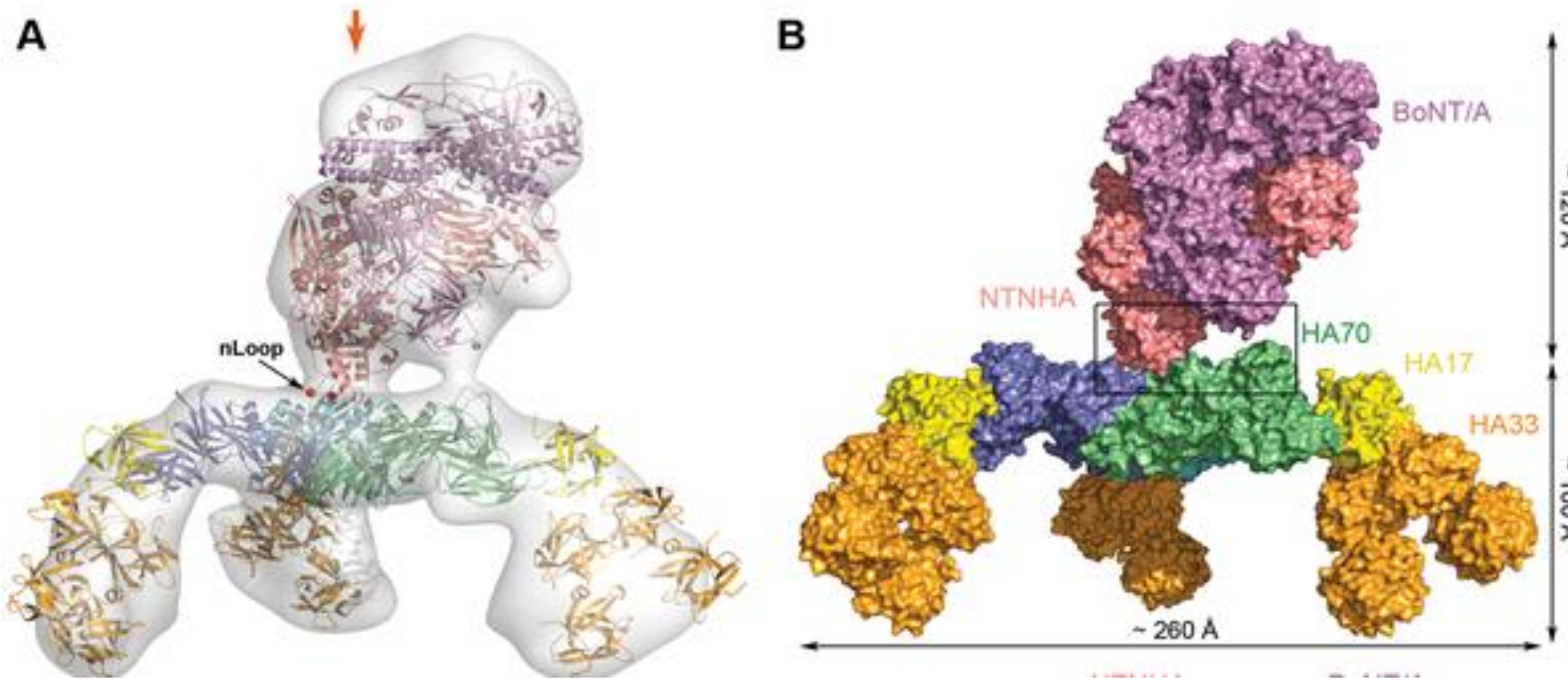
SEVEN BOTULINUM NEUROTOXIN SEROTYPES: A, B, C, D, E, F, G

		(mouse LD50 ng/Kg)
BoNT/A, B, E, (F):	**Human and Animal Botulism**	**(0.1 -1.2)**
BoNT/C	**Botulism of Birds**	
BoNT/D	**Animal botulism**	
BoNT/E	**Botulism in Aquatic Animals**	
BoNT/G:	***?***	

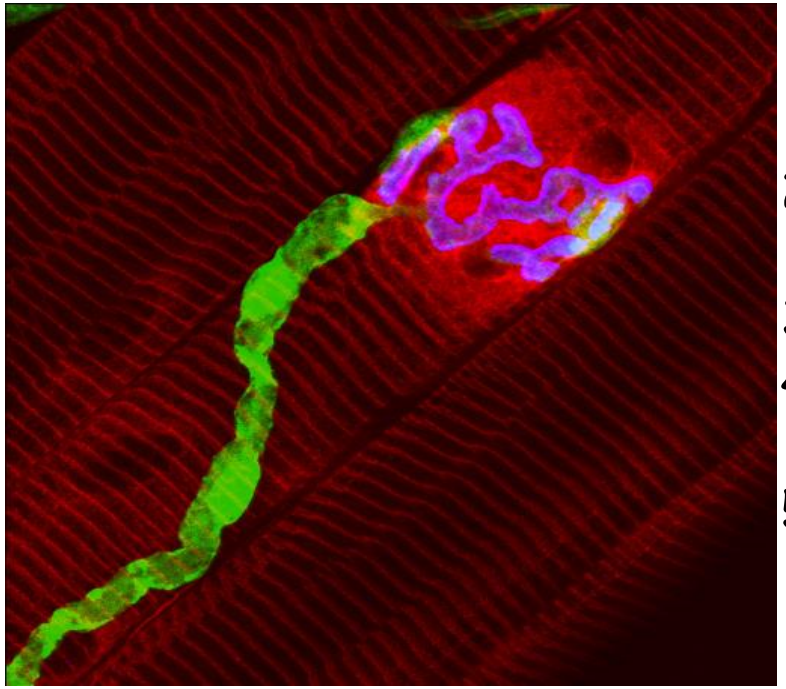
MERZ

ALLERGAN
DYSPORT



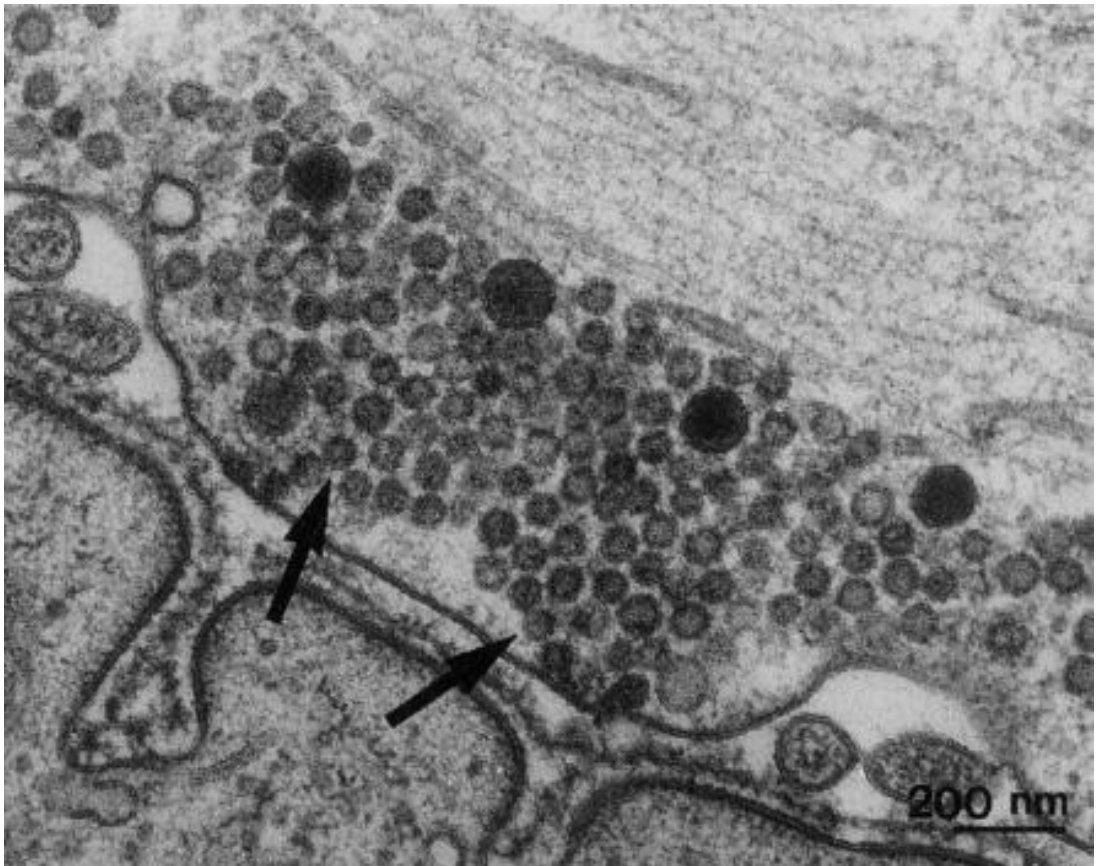


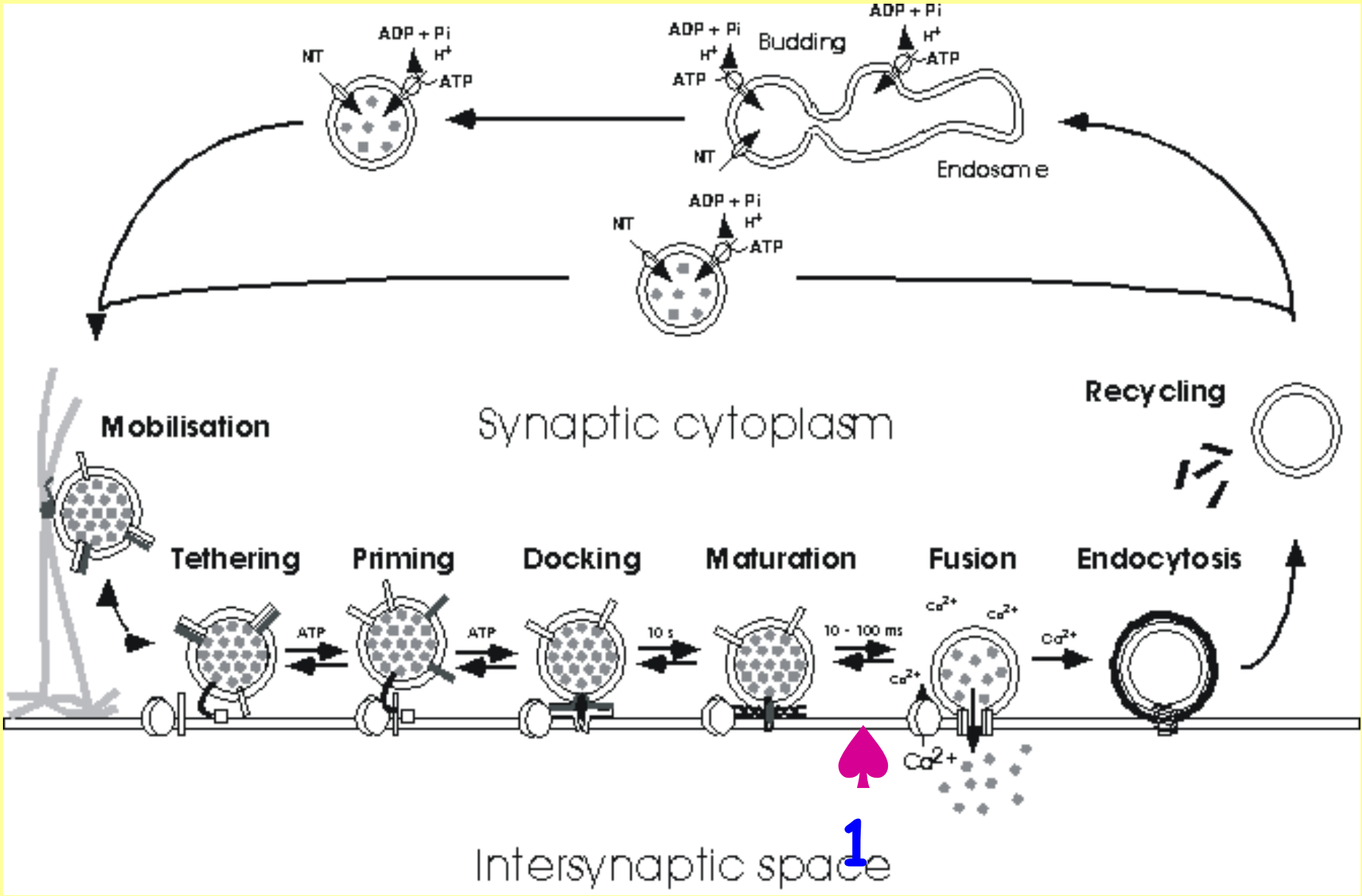
The complex of BoNT/A with accessory proteins (NTNHA e HA70, HA17, HA33) rapidly dissociates at the neutral pH of tissues freeing BoNT/A which can bind to cholinergic terminals.



BoNT/A:

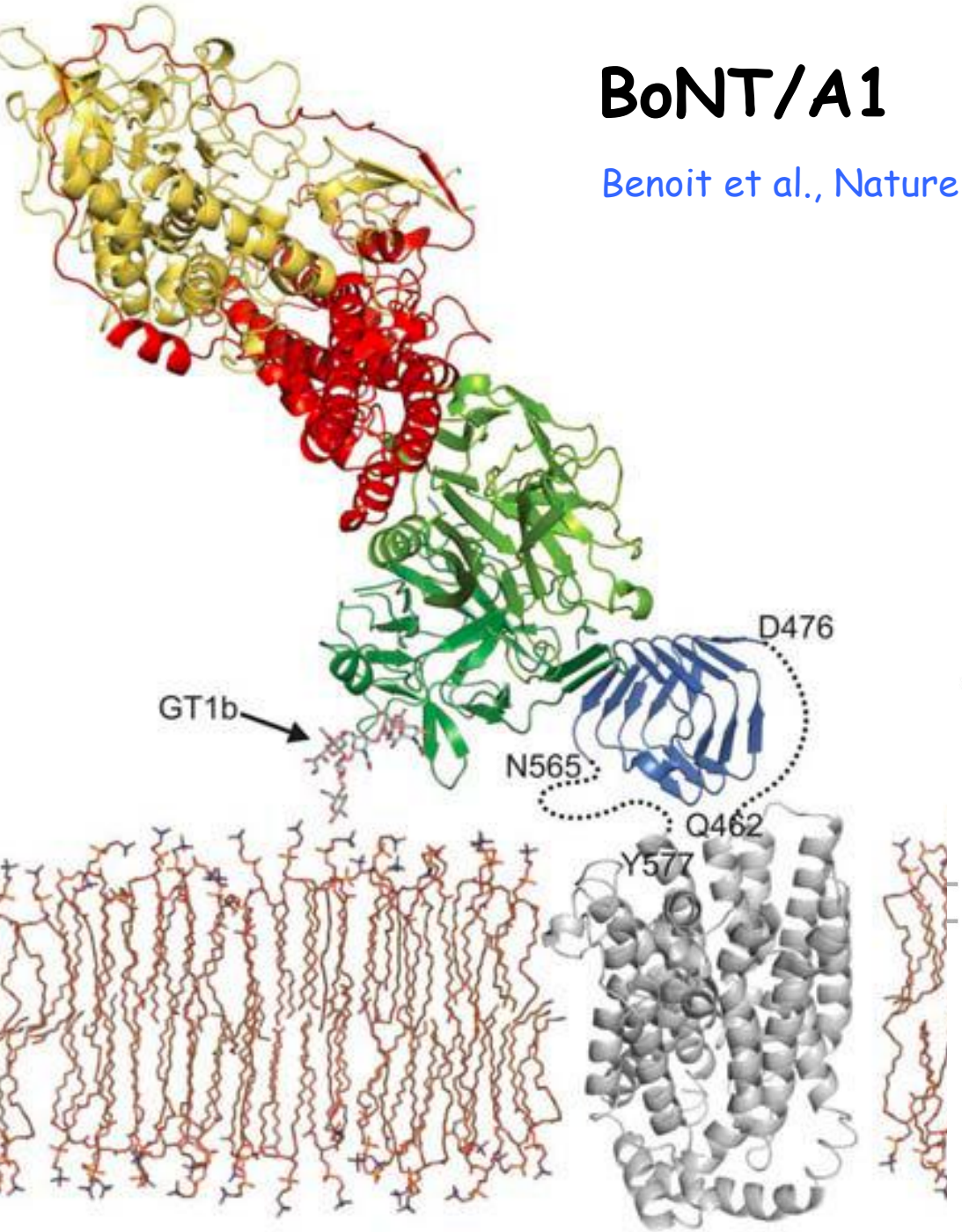
1) is highly neurospecific
2) it causes a localized and long lasting neuroparalysis
3) This neuroparalysis is totally reversible
4) It does not diffuse significantly from the site of injection
5) It is poorly immunogenic

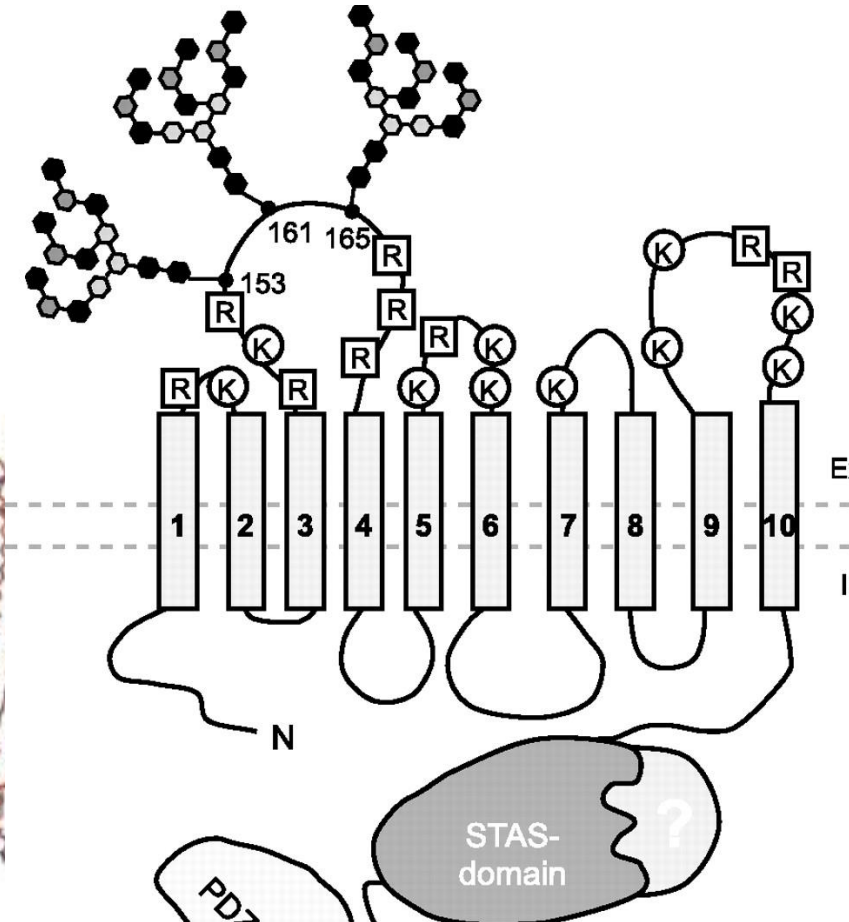


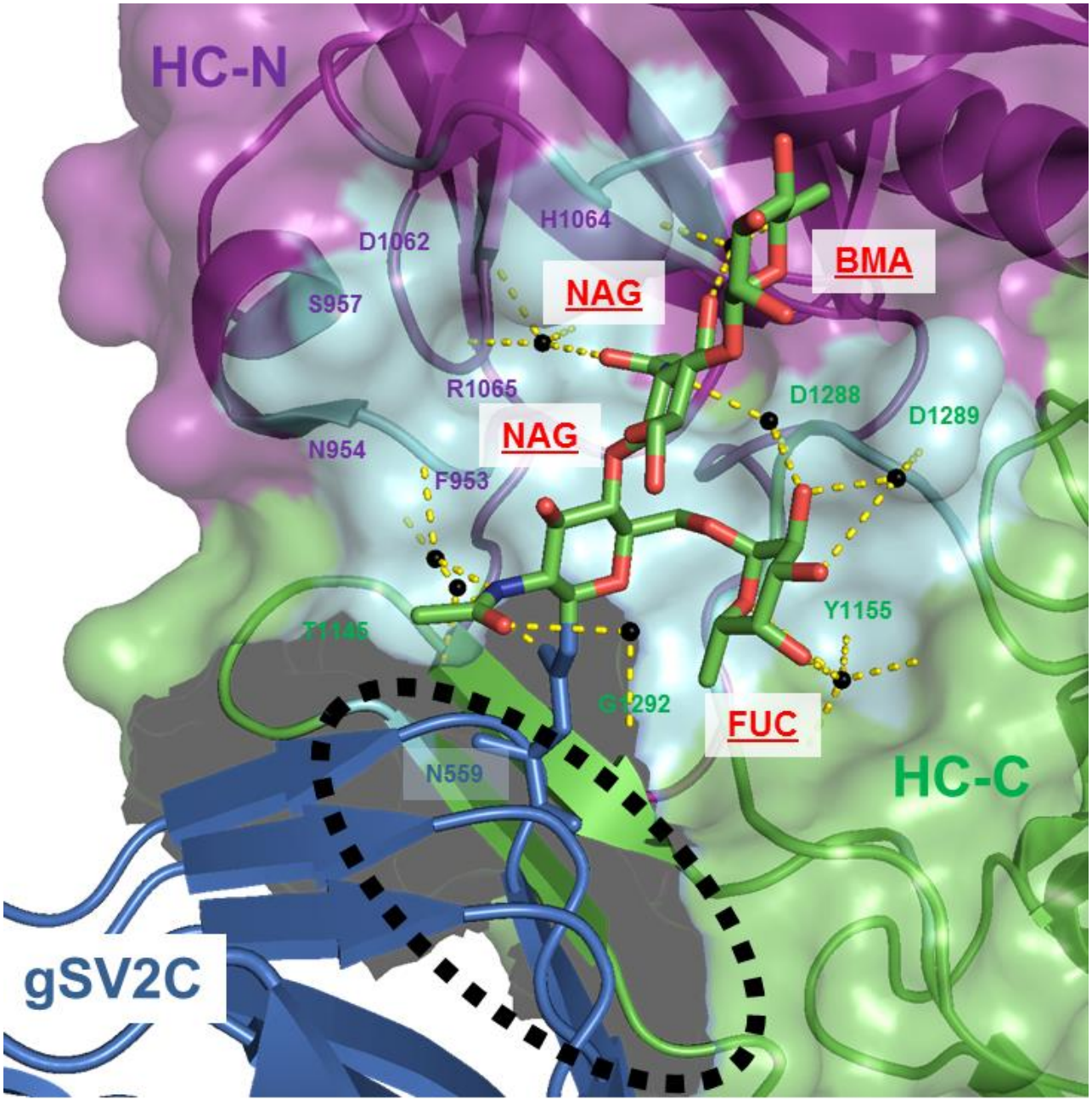

ADP + Pi
H+
ATP
NT
Budding
Endosome
Mobilisation
Synaptic cytoplasm
Recycling
Tethering
Priming
Docking
Maturation
Fusion
Endocytosis
ATP
10 s
10 - 100 ms
Ca2+
Intersynaptic space
1

BoNT/A1

Benoit et al., Nature 2013

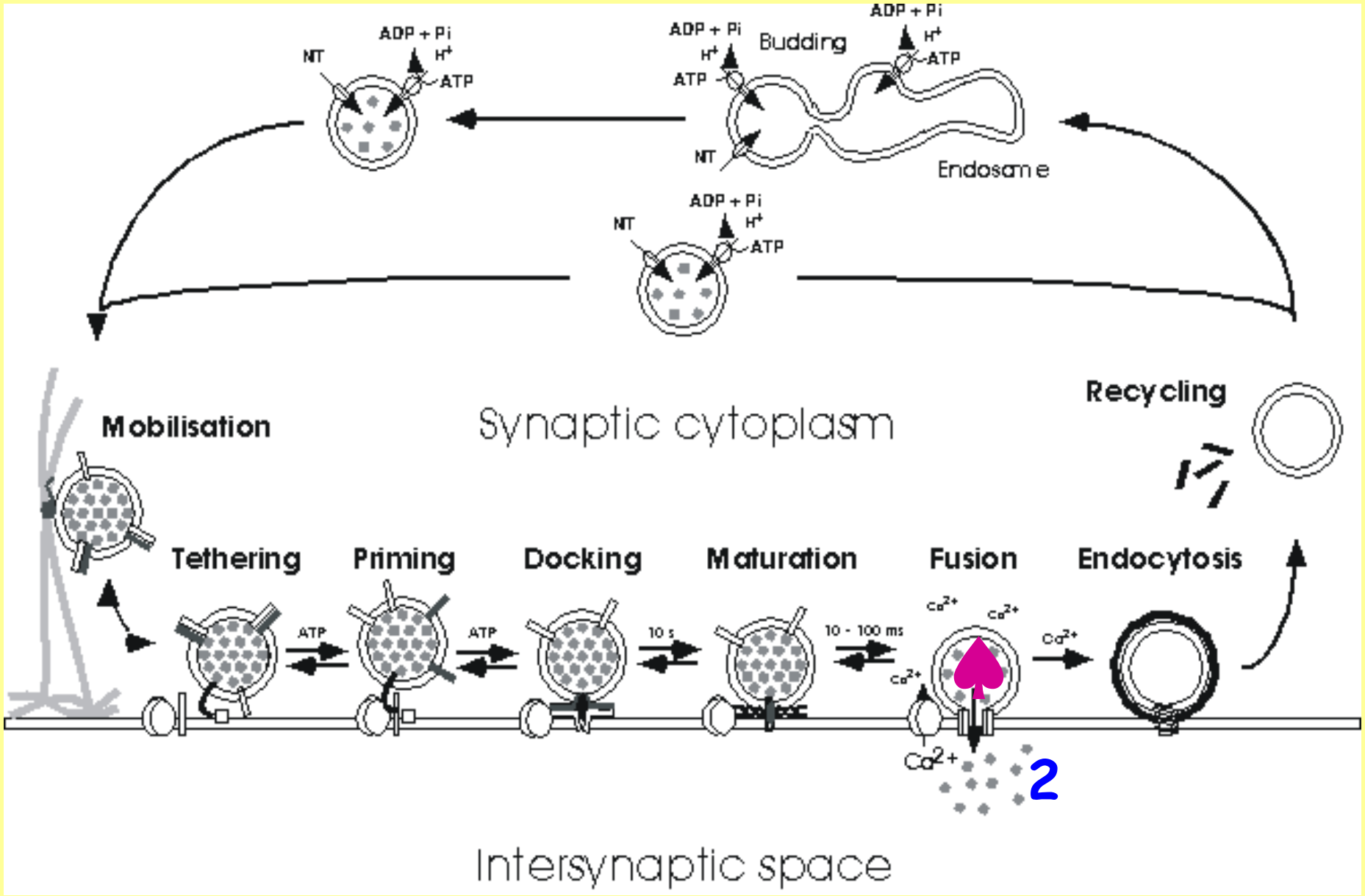


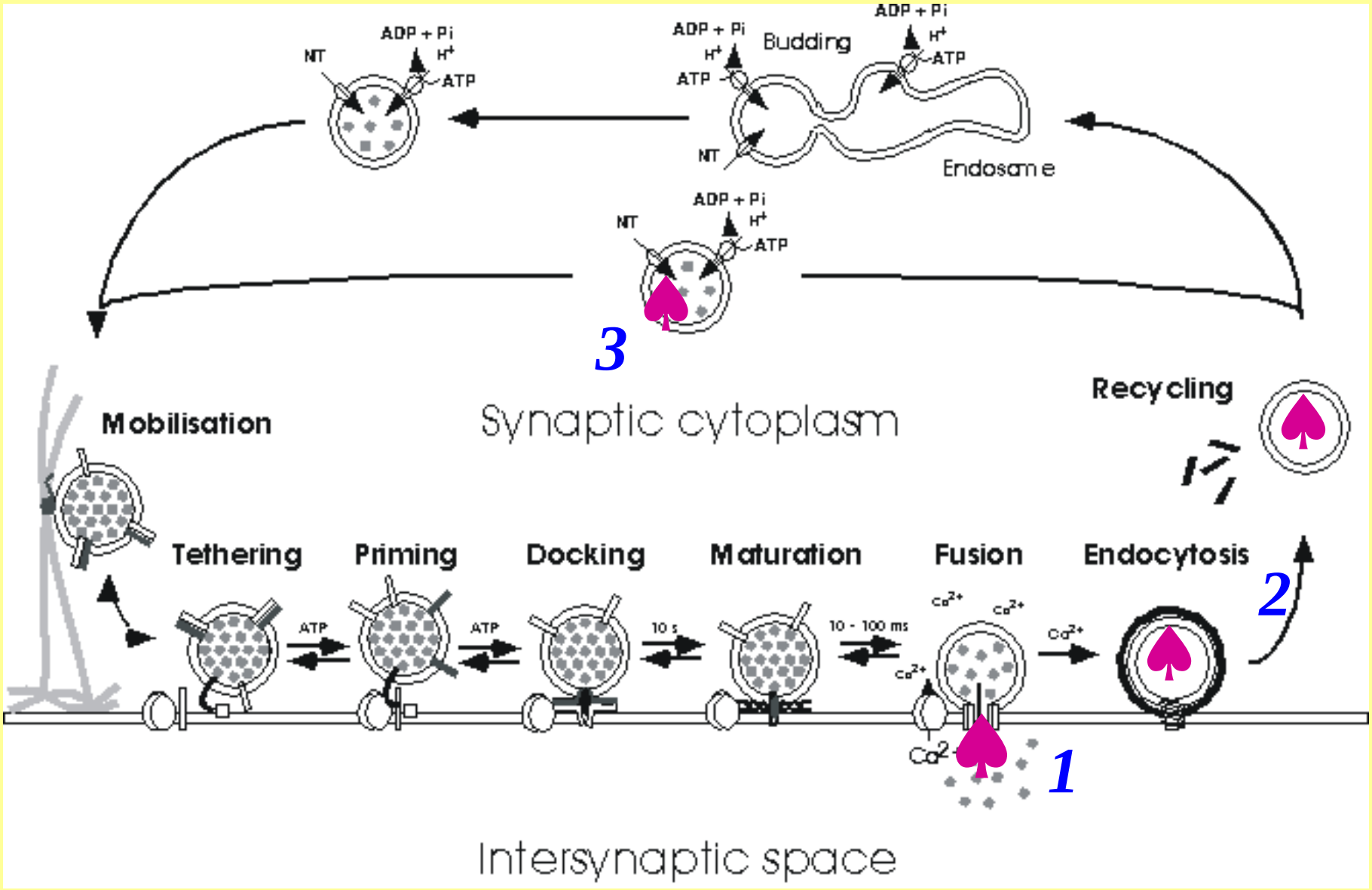


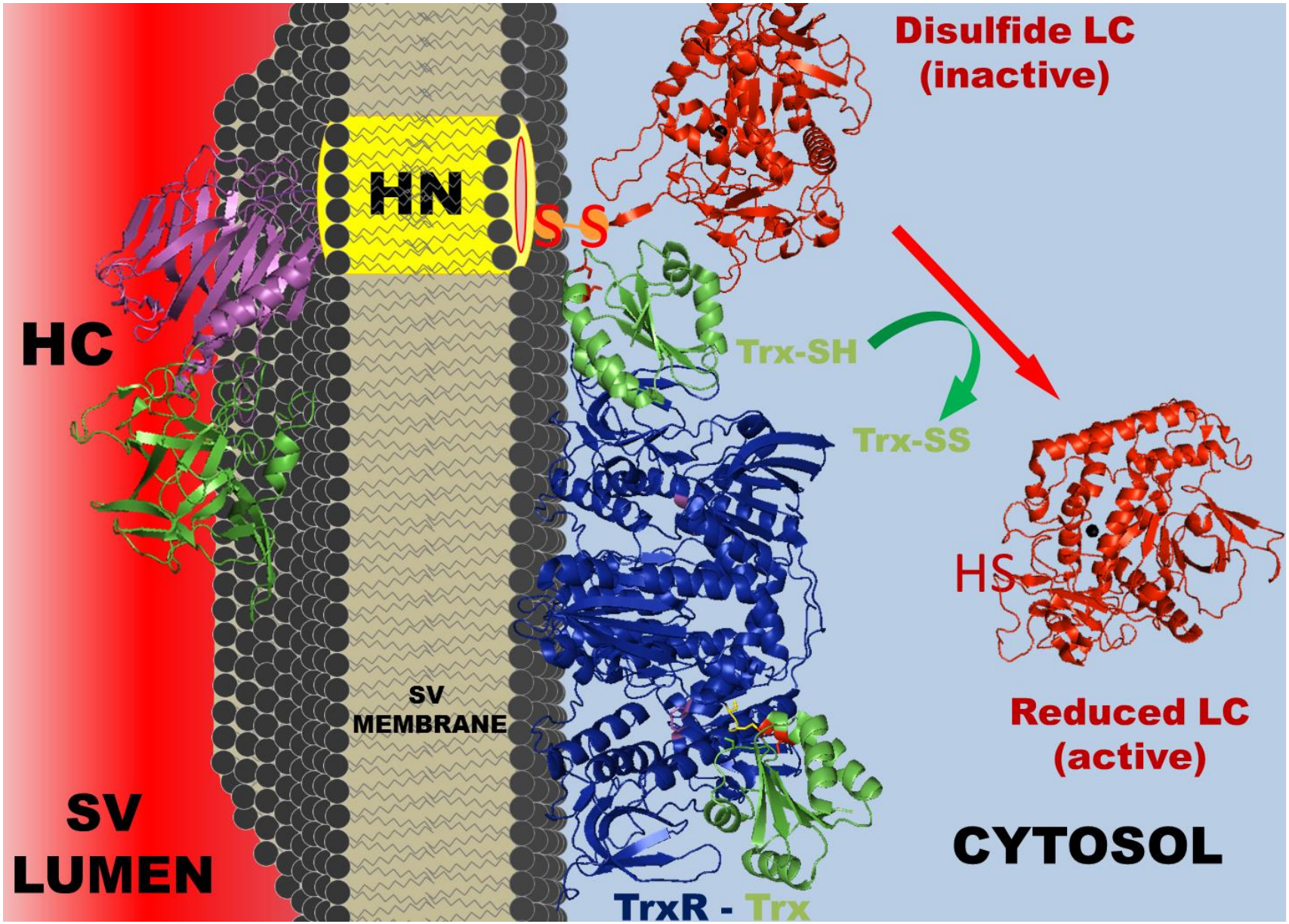


N-glycans vary from individual to individual and could contribute to explain the different response To BoNT/A1 among individual patients

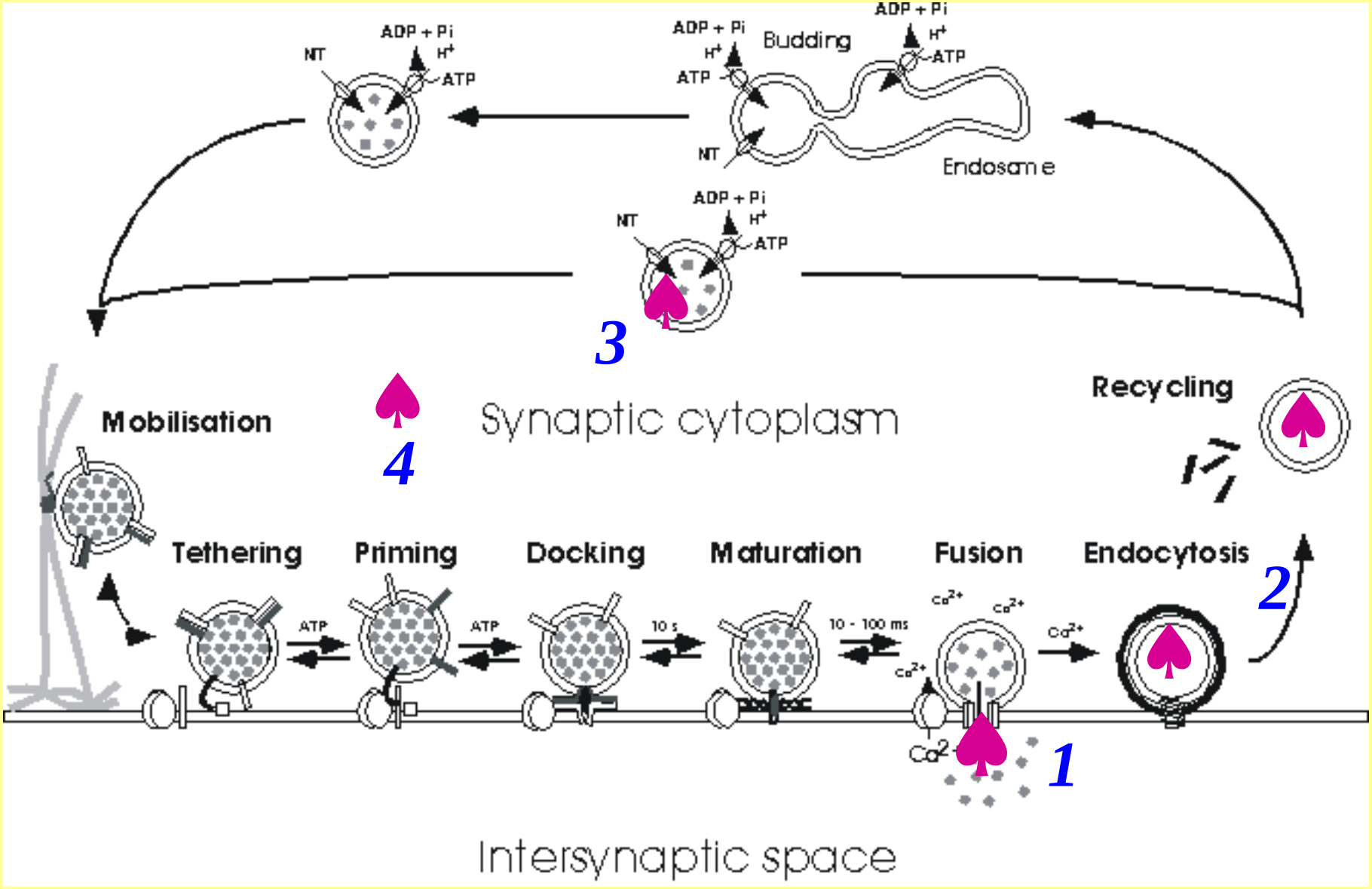
Yao et al., Nat Struct Mol Biol. 2016


ADP + Pi
H+
ATP
NT
Budding
Endosome
Mobilisation
Synaptic cytoplasm
Recycling
Tethering
Priming
Docking
Maturation
Fusion
Endocytosis
ATP
10 s
10 - 100 ms
Ca2+
2
Intersynaptic space


ADP + Pi
H+
ATP
NT
Budding
Endosome
3
Synaptic cytoplasm
Recycling
Mobilisation
Tethering
Priming
Docking
Maturation
Fusion
Endocytosis
ATP
10 s
10 - 100 ms
Ca2+
2
1
Intersynaptic space



Pirazzini et al, Cell Rep. 2014


ADP + Pi
H+
ATP
NT
Budding
Endosome
3
Synaptic cytoplasm
Mobilisation
4
Recycling
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Priming
Docking
Maturation
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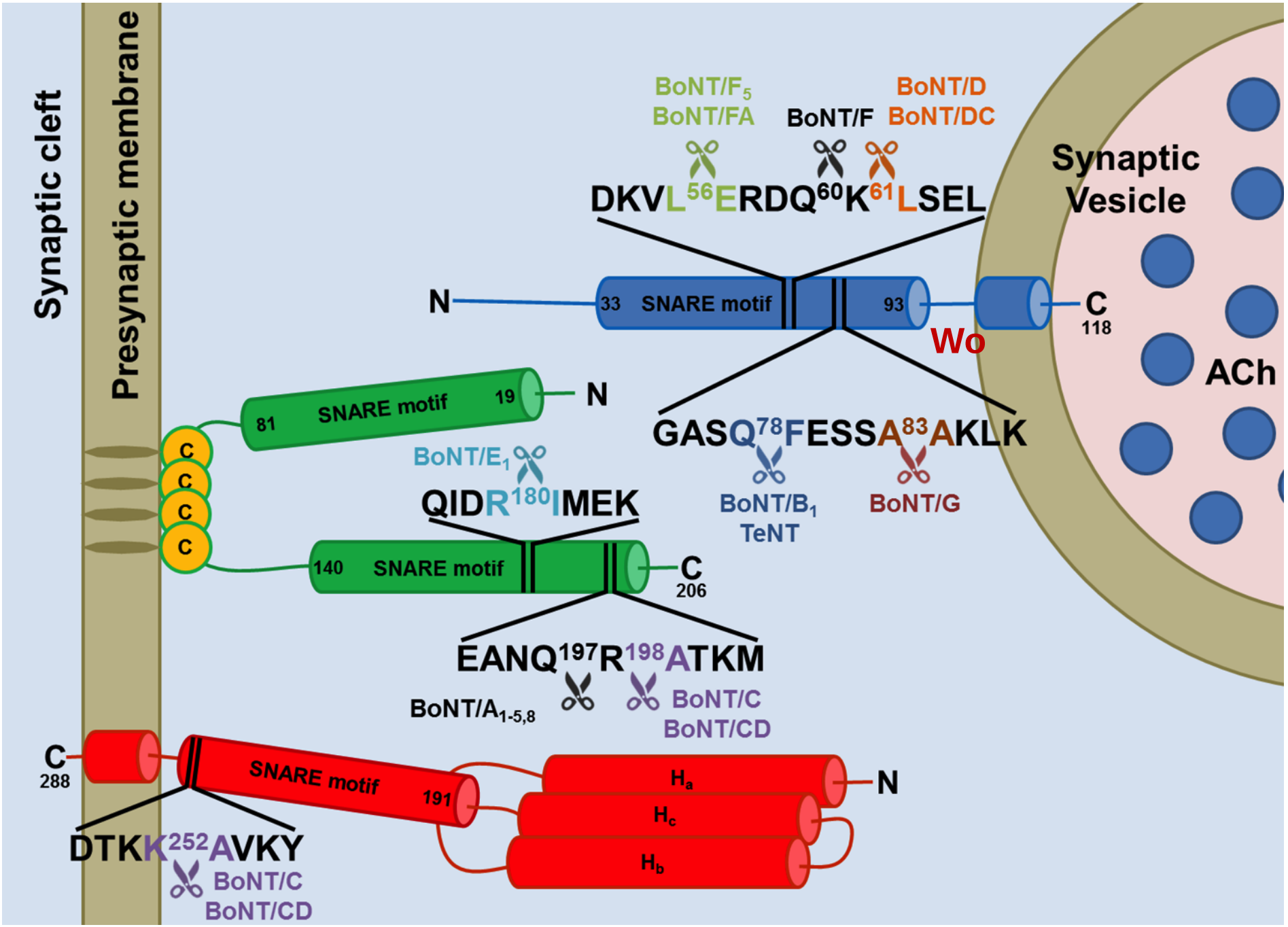
ATP
10 s
10 - 100 ms
Ca2+
1
Intersynaptic space



Pirazzini et al, *Pharmacol Reviews, 2017*

Weissella oryzae, probiotico capace di fermentare il riso

BoNT intoxication is fully reversible

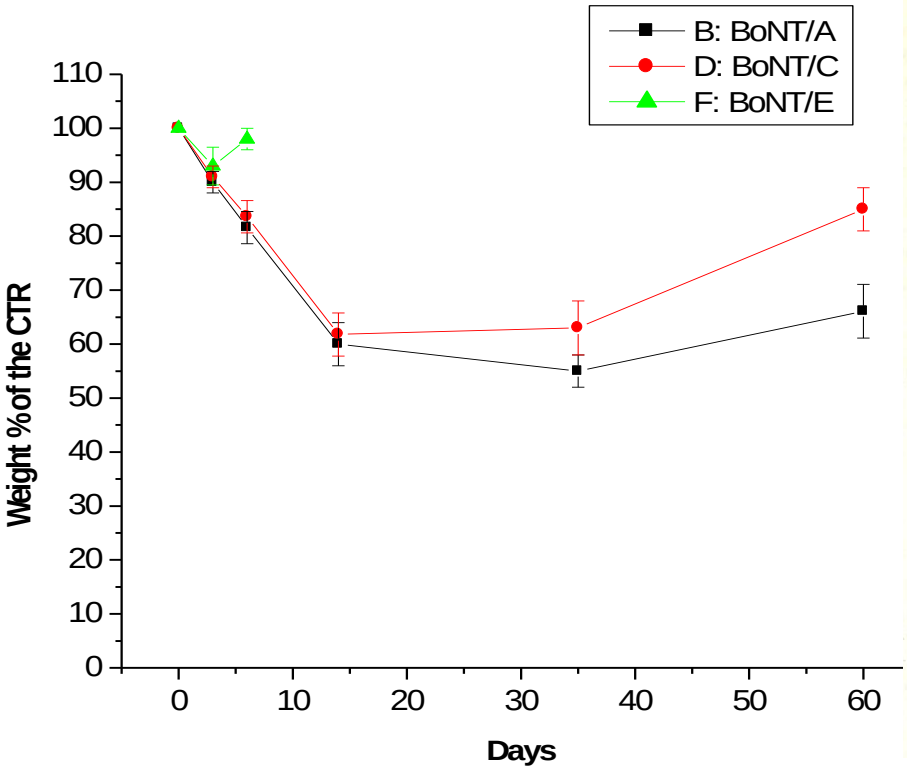
The time course of the recovery of the function of a cholinergic nerve terminal poisoned by BoNT depends on:

1. **The animal species**

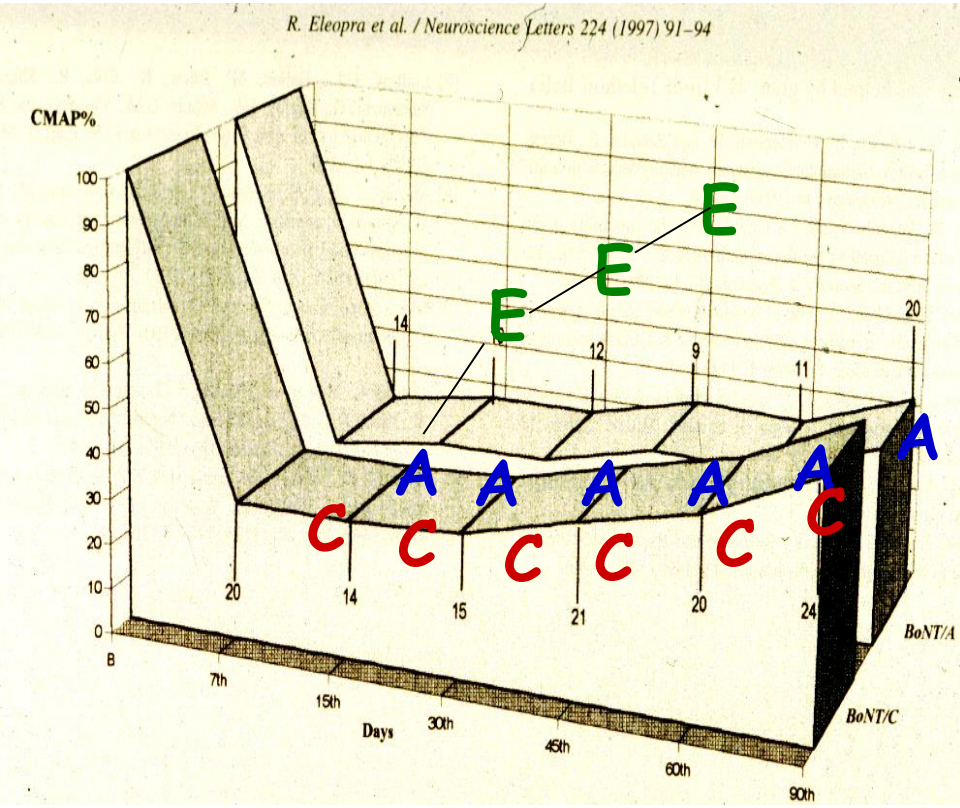
1. **the BoNT serotype and the DOSE**

3. the type of terminal
(skeletal *"rapid"*, autonomic *"slow"*)

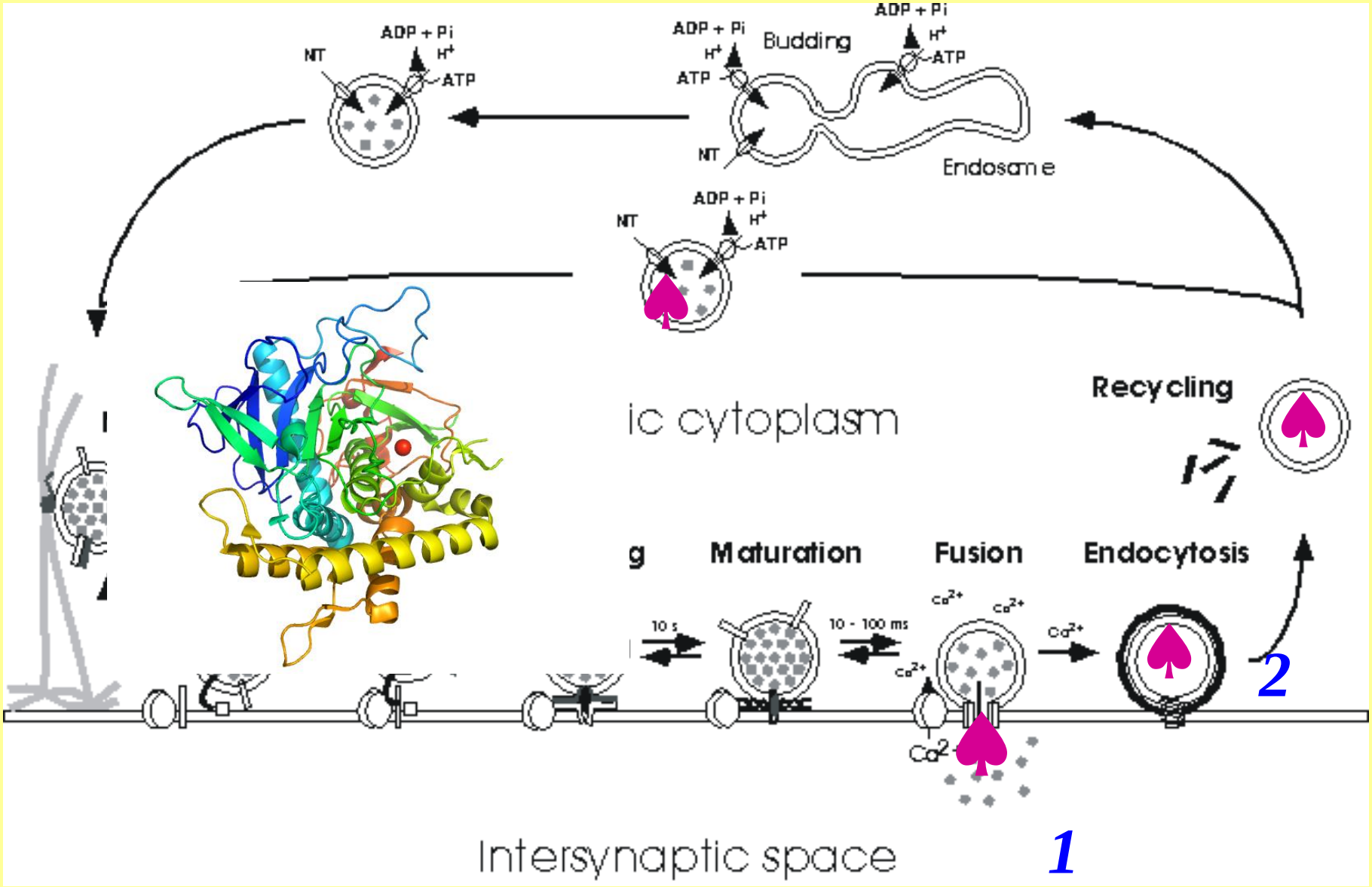
Duration of the muscle paralysis induced by BoNT/A, /C and /E in mice and humans



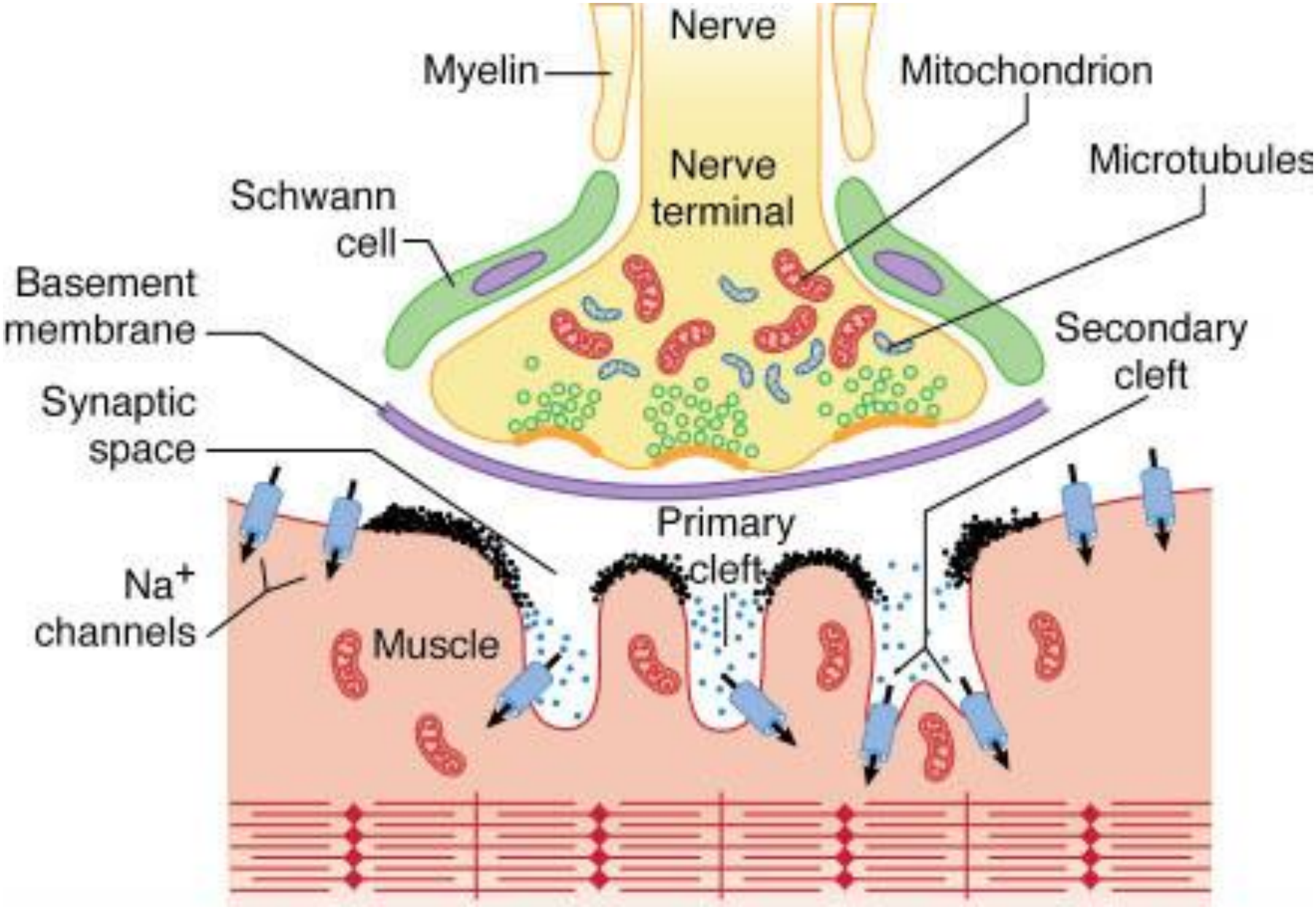
Morbiato et al. 2007

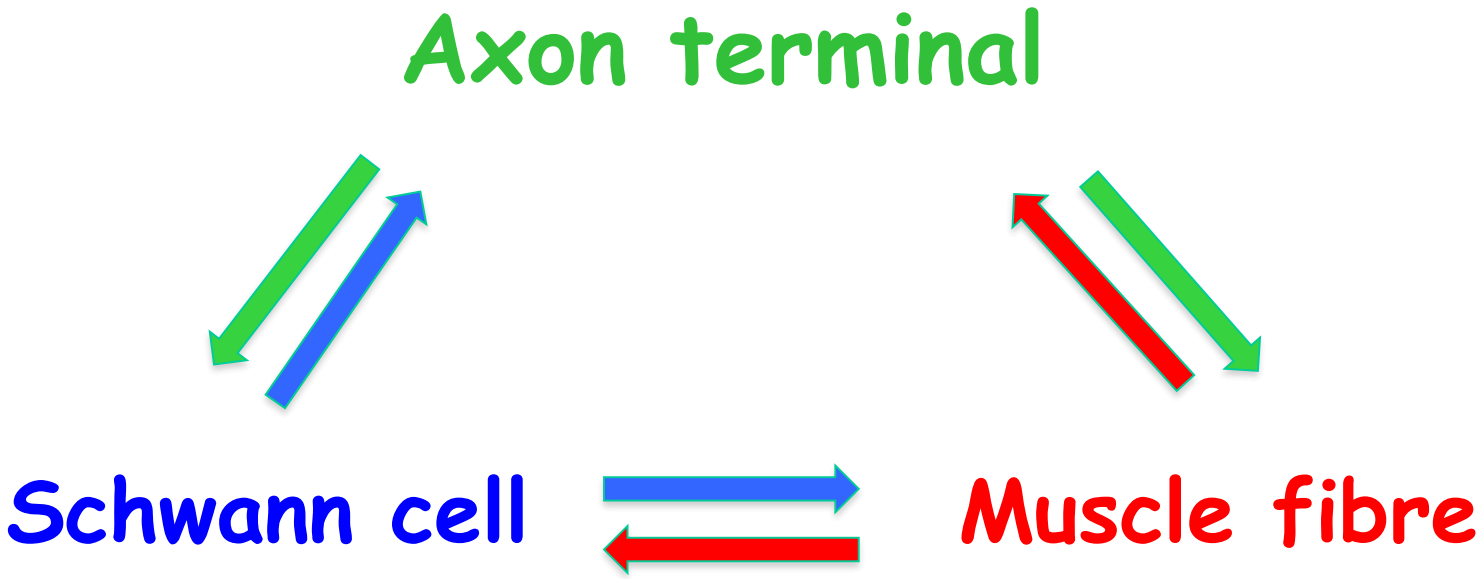


Eleopra et al. 1997



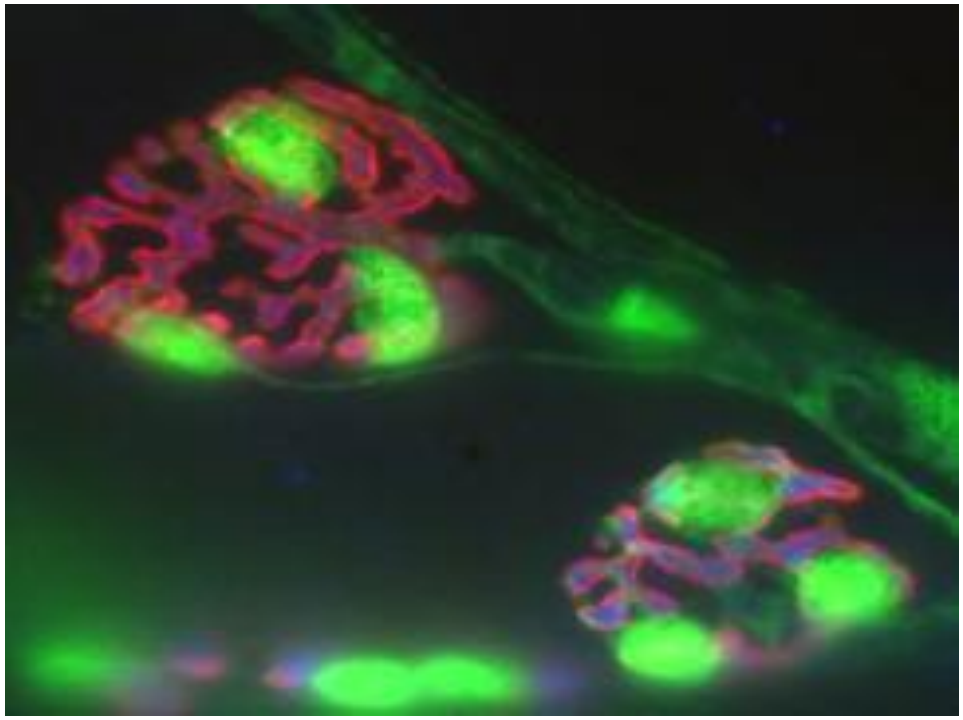
Common explanation: **different lifetimes of the different toxins**


Nerve
Myelin
Mitochondrion
Nerve terminal
Microtubules
Schwann cell
Basement membrane
Secondary cleft
Synaptic space
Primary cleft
Na+ channels
Muscle



BoNT/A intoxicated nerve terminals send anterograde signals which activate perisynaptic Schwann cells and muscle fibers which, in turn, release retrograde signals which stimulate the motor axon terminal to resume their electrophysiological signalling activity

The duration of paralysis of the NMJ induced by BoNT is also determined by the response of the peripheral Schwann Cells and Muscle fibre to the blockade of neuroexocytosis.



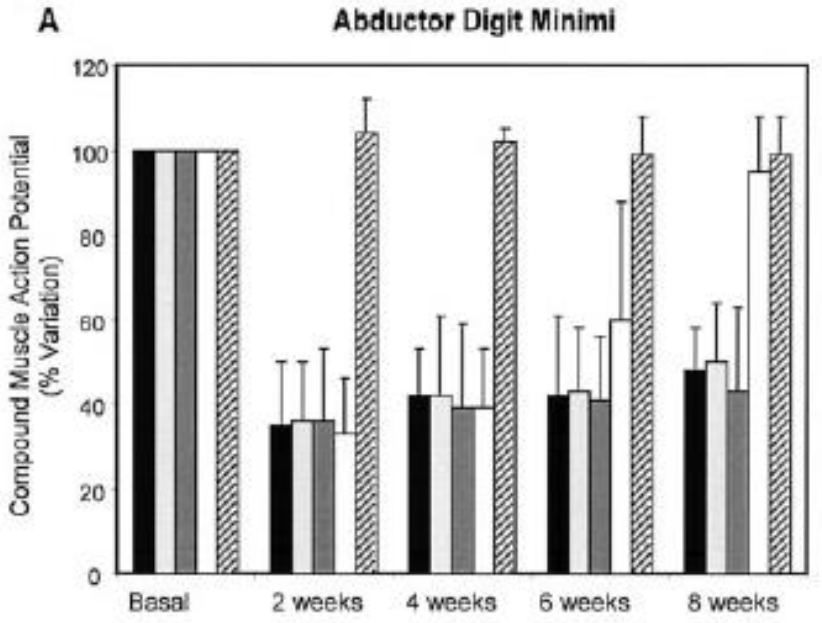
This provides and explanation for the long duration of action of BoNT/A1 and BoNT/B1 at autonomic nerve terminals and in neurons in culture with respect to the shorter duration of action at skeletal nerve terminals.

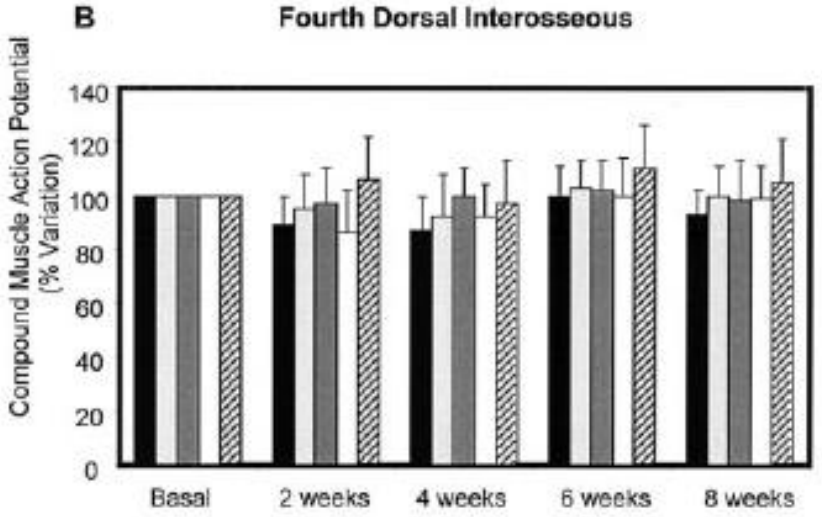
1. **The problem of botulinum neurotoxin diffusion from the site of injection**

2. The problem of immunization with production of toxin neutralizing antibodies

There is little if any spreading into adjacent muscles

Eleopra et al. 2004





Black bars, BoNT/A; light gray bars, BoNT/B; dark gray bars, BoNT/C; white bars, BoNT/F; striped bars, placebo.

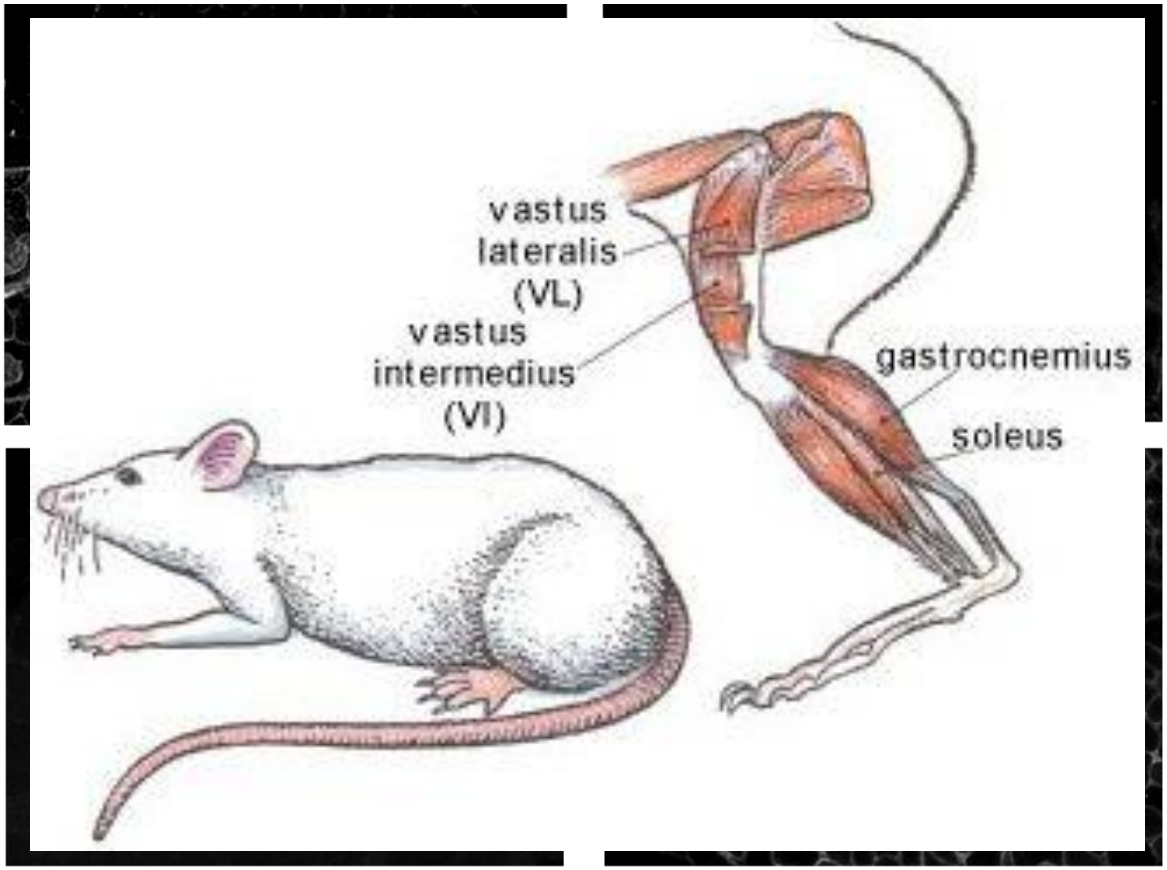
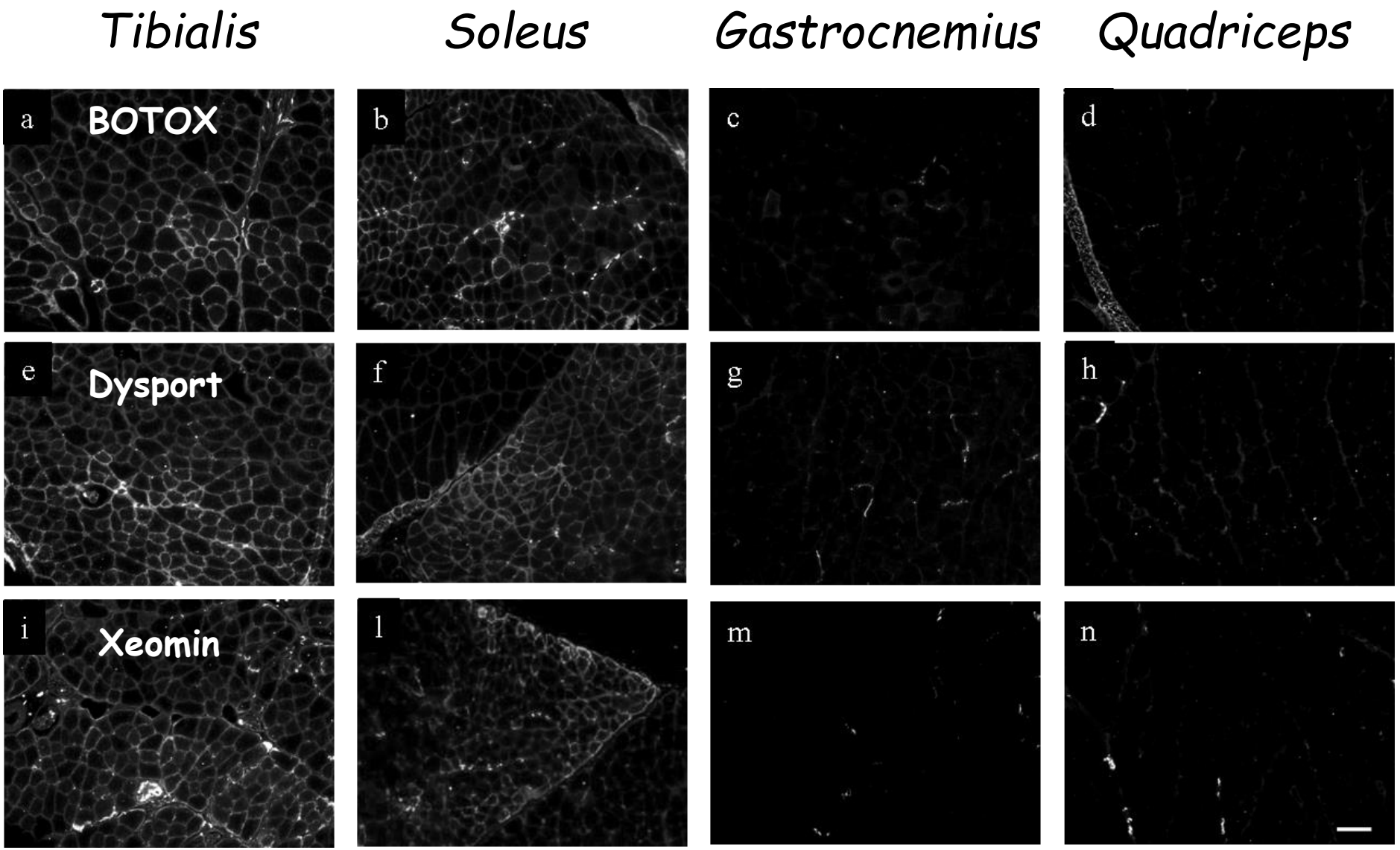


Fig. X: *Tibialis anterior* transverse cryosections labelled with anti-N-CAM antibody.
(a) 7 days denervated muscle; (b) Control muscle; (c) saline injected; (d) Botox injected.

Carli et al., Muscle & Nerve 2009



Carli et al., Muscle & Nerve 2009

BoNT diffusion della BoNT around the site if injection depends on the injected VOLUME.

Smaller volumes ≈ lower diffusion

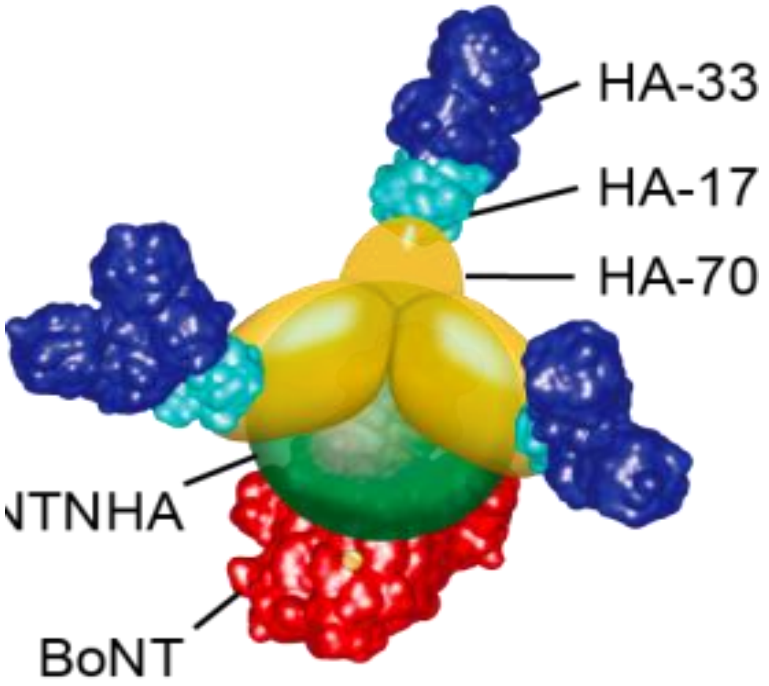
Larger injected volumes ≈ larger diffusion

2. The problem of immunization with production of toxin neutralizing antibodies depends on:

a) The nature of the antigen (i.e. tetanus toxin is a strong antigen, botulinum neurotoxins are not.

b) Quality of the antigen (purity of the toxin, if the toxin preparation contains other bacterial proteins,they can act as carriers or adjuvants)

Protein content of BoNT/A preparations



able 4: Comparison of Botulinum Neurotoxins products marketed in Europe and North merica

Brand name	Botox®/Vistabel®	Dysport®/Azzalure®	Xeomin®/Bocouture®	Neurobloc®/Myobloc®
Generic name	OnabotulinumtoxinA	AbobotulinumtoxinA	IncobotulinumtoxinA	RimabotulinumtoxinB
Manufacturer	Allergan (USA)	Ipsen Pharmaceuticals (France)	Merz Pharmaceuticals (Germany)	US WorldMeds (USA)
. botulinum strain	Hall A-hyper	Hall A	Hall A (ATCC 3502)	Bean
Toxin type	A1	A1	A1	B1
MW (PTCs[a])	900 kDa complex (Yes)	MW not reported (Yes)	150 kDa None	MW not reported (Yes)
Pharmaceutical form	Vacuum-dried powder for reconstitution	Freeze-dried powder for reconstitution	Freeze-dried powder for reconstitution	Ready-to-use solution
Shelf life	2-8 °C 36 months	2-8 °C 24 months	Room temperature 36 months	2-8 °C 24 months
H (reconstituted)	7.4	7.4	7.4	5.6
Excipients	In 100 IU vial: HSA[b] 500 µg NaCl (900 µg/vial)	In 500 IU vial: HSA[b] 125 µg Lactose (2.5 mg/vial)	In 100 IU vial: HSA[b] 1000 µg Sucrose (4.7 mg/vial)	HSA[b] 500 µg/ml Succinate 10 mM NaCl 100mM
Unit*/vial	100 IU or 200 IU Botox® 50 IU Vistabel®	300 IU or 500 IU Dysport® 125 IU Azzalure®	100 IU or 200 IU Xeomin® 50 IU Bocouture®	2500 IU/0.5ml 5000 IU/1ml 10000 IU/2ml
Protein load/vial	5 ng/100U	4.35 ng/500U	0.44 ng/100U[c]	55 ng/2500U
Clinical activity in relation to Botox®	1	1:2 - 1:3	1	1:40-50

nits are manufacturer specific and are not interchangeable. [a]Progenitor Toxin Complex. [b]Human Serum Albumin eurotoxin concentration measured by ELISA (Frevert, 2010).

Pirazzini et al. *Pharmacol. Rev*, 2017

- La tossina, anche se pura dal punto di vista chimico può essere in parte denaturata (fisicamente aggregata, non solubile). L'aggregato potrebbe essere immunogenico

- Quantità di tossina iniettata
- Luogo d'iniezione: maggiore pericolo d'immunizzazione se in zona ricca in linfonodi regionali (collo).
- Quantità di tossina iniettata: maggiore è la quantità maggiore è il pericolo.
- Frequenza di trattamento: tanto più frequente è il numero d'iniezioni tanto maggiore è il rischio d'immunizzazione.