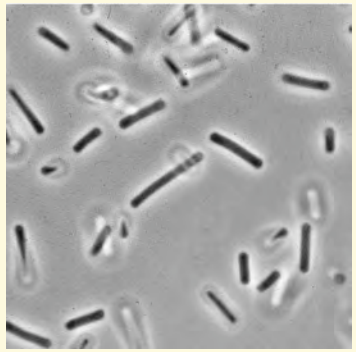
Basic mode of action of clostridial toxins

Clostridium

- Rod-shaped
- Gram positive
- Anaerobic
- Sporulated



C. perfringens

C. difficile

- fermentative metabolism
- no dissimilatorly sulfate reduction

- Main habitat : environment

DNA low % G+C

Toxigenic *Clostridia*

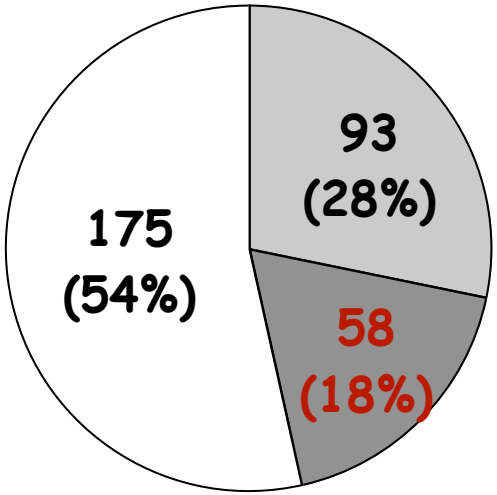
15 toxigenic *Clostridium* species among about 200 species

Genus *Clostridium* produces
the largest number of toxins
and the most potent toxins

Clostridium	Toxins (Total 58)	16S rDNa cluster
C. argentinense	1	I
C. baratii	2	I
C. bifermentans	3	XI
C. botulinum	3	I
C. butyricum	1	I
C. chauvoei	4	I
C. difficile	3	XI
C. haemolyticum	3	I
C. histolyticum	5	II
C. novyi	8	I
C. perfringens	14	I
C. septicum	4	I
C. sordellii	4	XI
C. spiroforme	1	XVIII
C. tetani	2	I

Toxins from non clostridial Gram-positive bacteria

Toxins from Gram-negative bacteria



Toxins from Gram-positive bacteria
152
(46%)

Clostridial Toxins

Total number of known bacterial toxins : 327

	dose létale souris µg/kg souris
toxine botulique *A*	0,0003
toxine tétanique	0,001
C. perfringens epsilon toxin	0,1
C. sordellii lethal toxin	0,1
toxine diphtérique	0,16 (cobaye)
C. perfringens beta toxin	0,4
C. difficile toxin*A*	0,5
Shiga toxin	1,3
C. perfringens alpha toxin	5
Pseudomonas exotoxin *A*	3
perfringolysin	16
C. perfringens enterotoxin	80
E. coli heat labile enterotoxin	250 i.v.
Cholera toxin	250

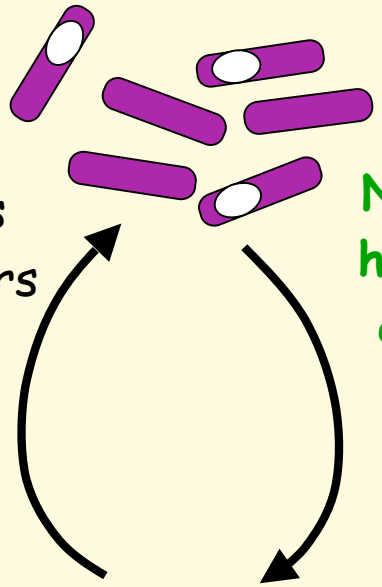
Deficient in many genes for
- aa biosynthesis
- tricarboxylic acid cycle
- respiratory chain

Clostridium

Entry into organisms

Non invasive
No specific adherence

- oral route
- wound



Numerous transporters

Numerous hydrolytic enzymes

local colonization

Toxins

Toxins

Disease

toxins have evolved from certain secreted enzymes?

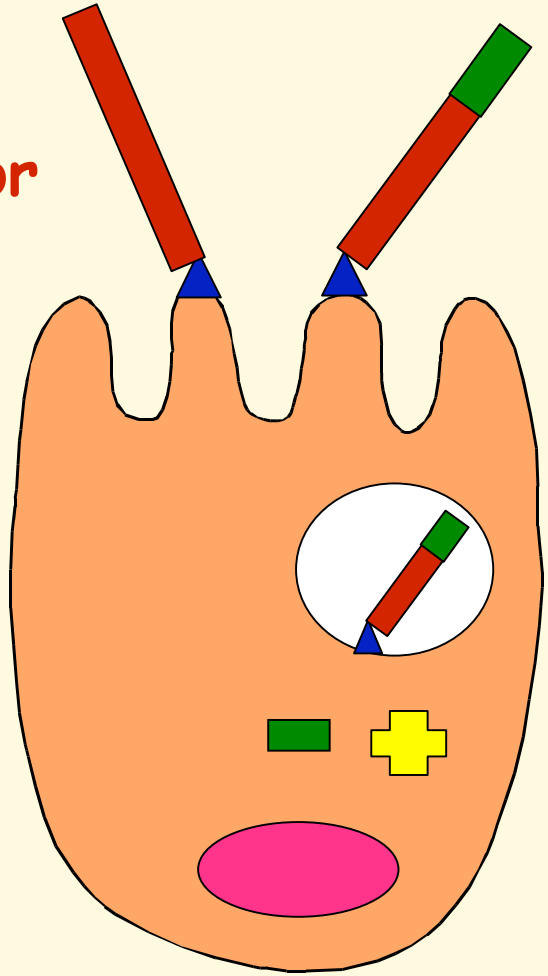
Degradation of organic matter (cadavers, plants ...)

Environment

- intestinal diseases
- food borne disease
- gangrene

Toxin-cell interaction

recognition of a
cell surface receptor



Toxins active at the cell surface

- pore formation
- enzymatic activity

Toxins active intracellularly

- endocytosis
- translocation of the enzymatic domain
- recognition and enzymatic modification of an intracellular target
- cellular effect

Clostridial toxins

Toxins active on extracellular matrix

C. histolyticum collagenases	gangrene

Toxins active on the cell surface (membrane damaging toxins)

Pore-forming toxins

Cholesterol-dependent cytotoxins

Perfringolysin related toxins in *C. tetani*, *C. botulinum*, *C. septicum*, *C. histolyticum*, *C. novyi*, *C. chauvoei*, *C. bifermentans*, *C. sordellii*	gangrene
C. perfringens enterotoxin Beta, Beta2, NetB, Delta	enteritis

Aerolysin family

C. perfringens epsilon toxin	general disease
C. septicum alpha toxin	gangrene

Enzymatic activity on cell surface (Phospholipases)

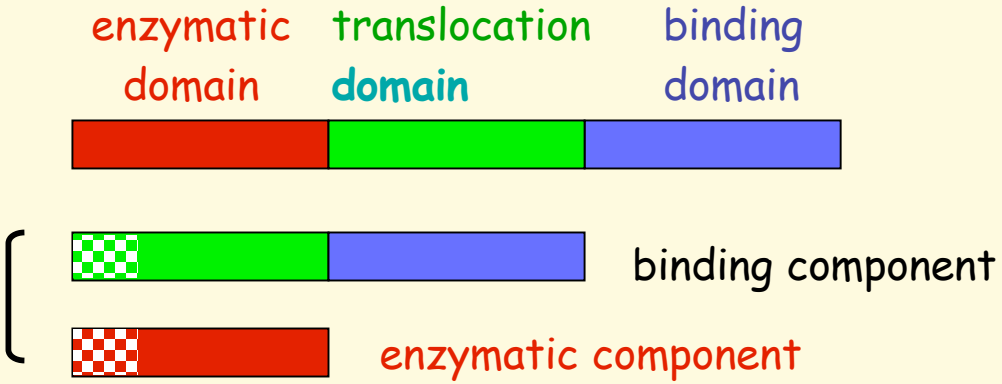
C. perfringens alpha toxin related toxins in *C. novyi*, *C. sordellii*, *C. bifermentans*	gangrene

Clostridial toxins

Intracellularly active Toxins

Single chain proteins

Binary toxins



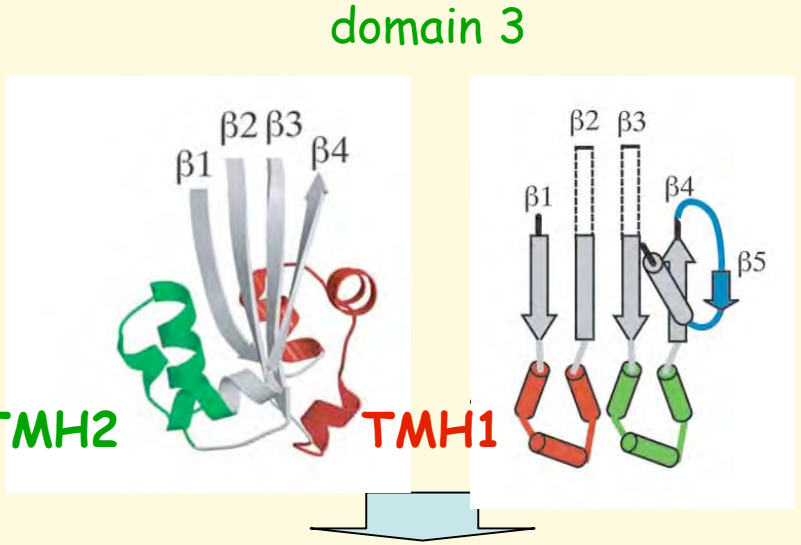
Modification of the actin cytoskeleton

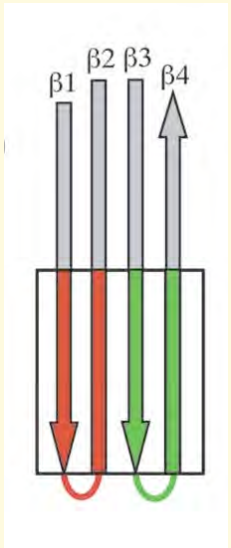
	Enzymatic activity	Target
Large clostridial toxins	glucosylation	Rho/Ras-GTPases
C3 enzyme	ADP-ribosylation	Rho
Clostridial binary toxins	ADP-ribosylation	actin

Blockade of the exocytosis machinery

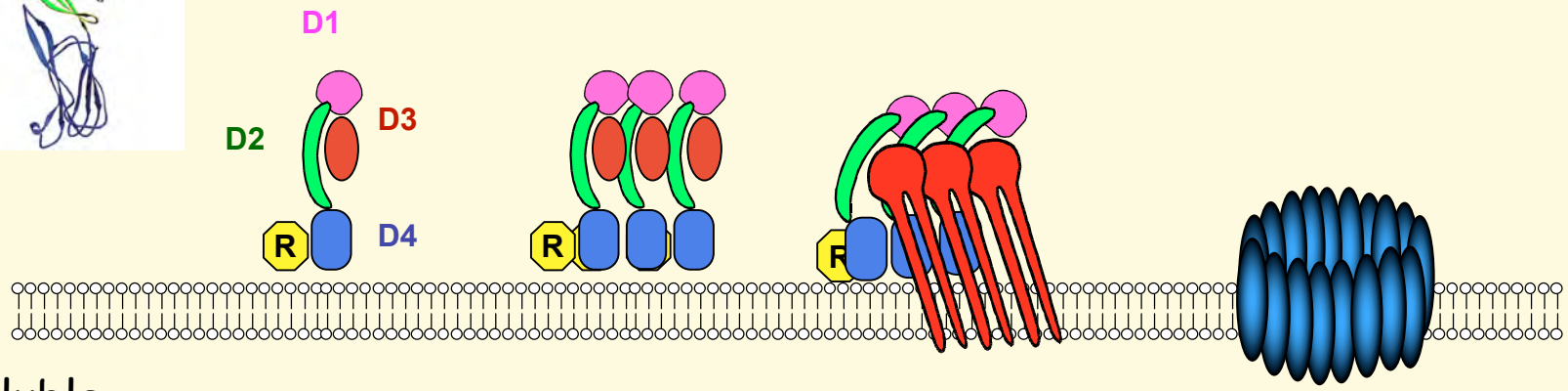
Botulinum neurotoxins Tetanus toxin	Protease	SNARE proteins

Perfringolysin, the paradigm of cholesterol-dependent cytolysin





Perfringolysin : Pore formation



soluble monomer

soluble monomer bound to receptor

oligomerization prepore formation

unfloding of a-helices of D3 in 2 β-hairpins, insertion of the resulting β-barrel into the lipid bilayer

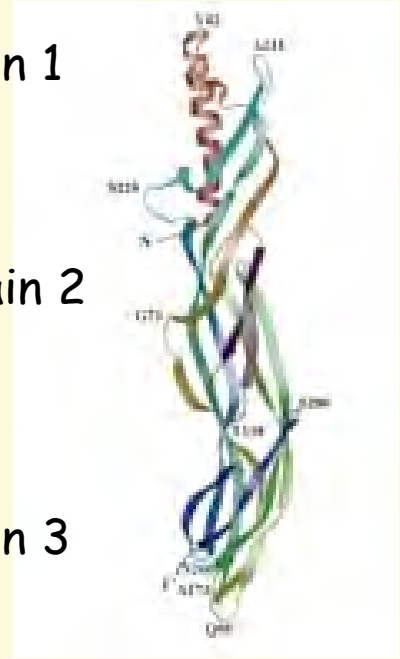
large pores 35-50 monomers 250-300 Å

Epsilon toxin

domain 1

domain 2

domain 3



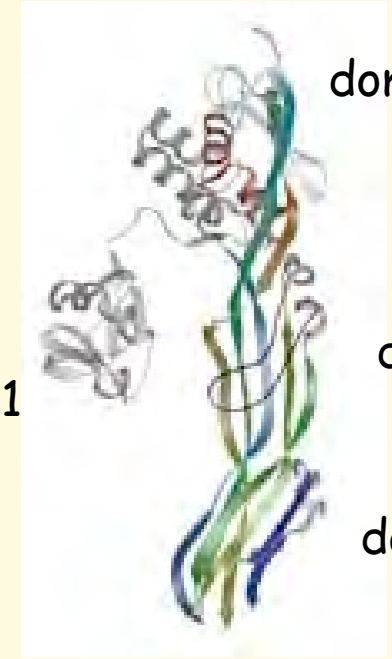
Aerolysin

domain 2

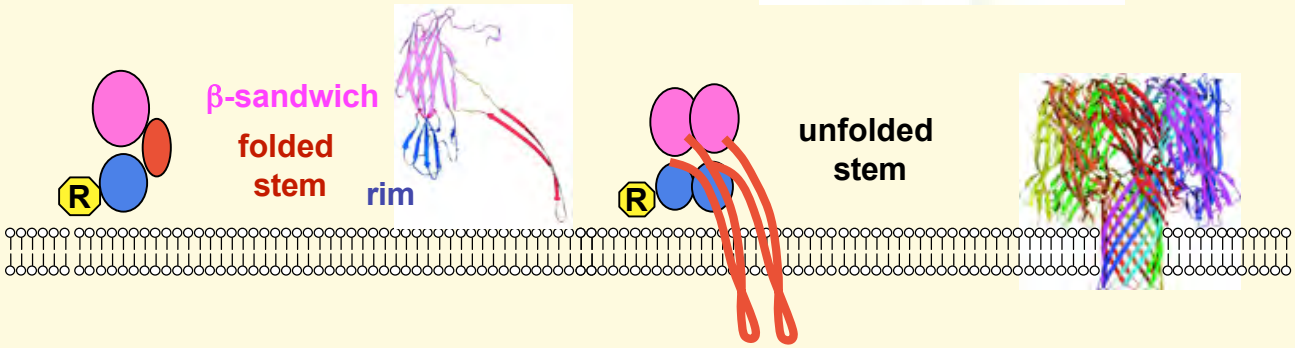
domain 1

domain 3

domain 4



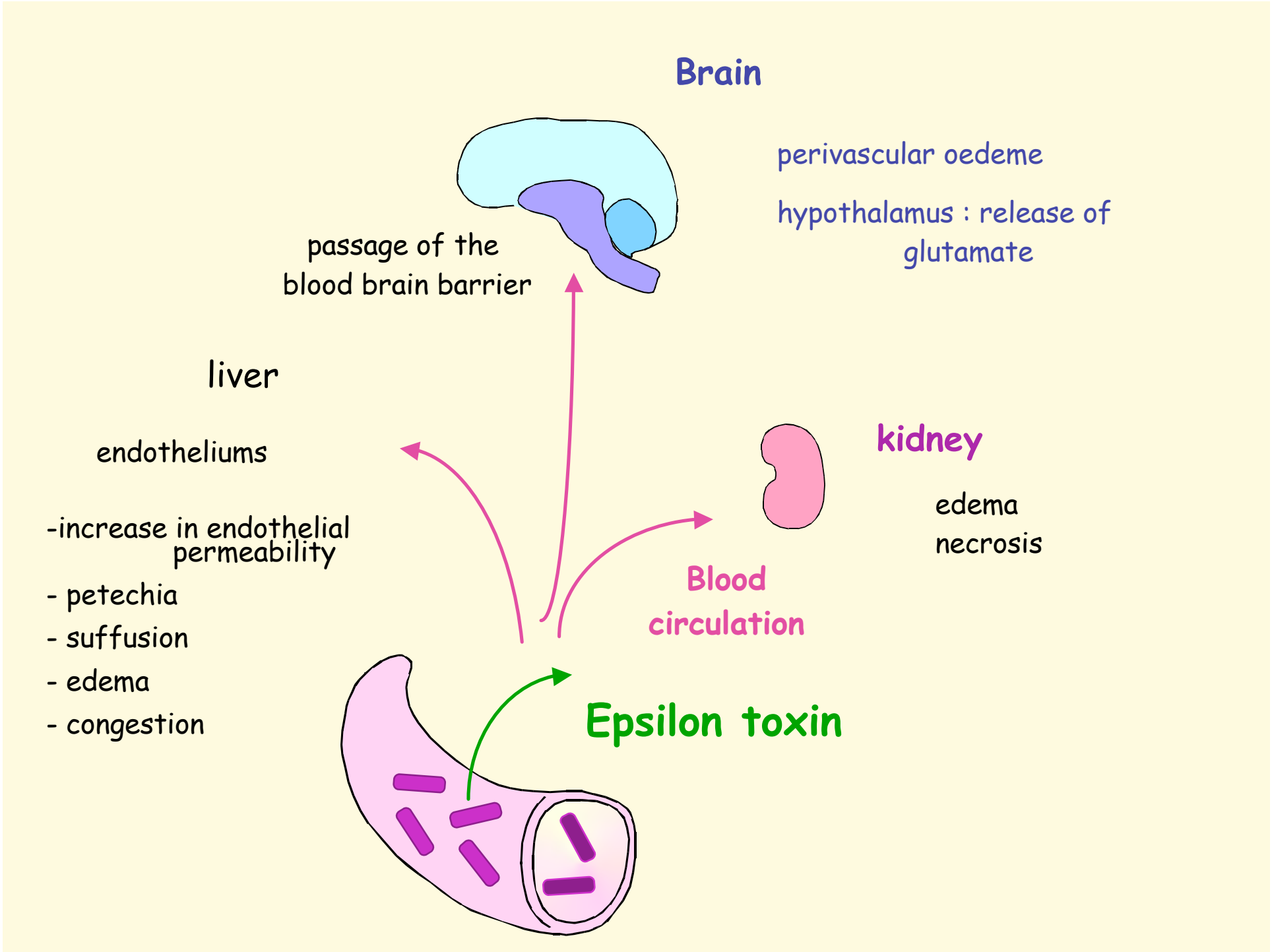
Staphylococcus α-toxin



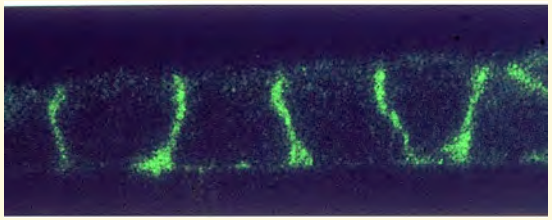
soluble monomer
bound to receptor

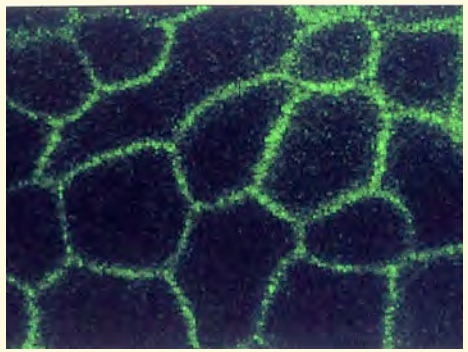
heptamerization and
unfolding of the stem domain,
association of **one β-hairpin**
from each monomer in b-barrel

insertion of the prepore
into the lipid bilayer
small pore 15-30Å


Brain
perivascular oedeme
hypothalamus : release of glutamate
passage of the blood brain barrier
liver
endotheliums
-increase in endothelial permeability
- petechia
- suffusion
- edema
- congestion
kidney
edema
necrosis
Blood circulation
Epsilon toxin

Epsilon toxin



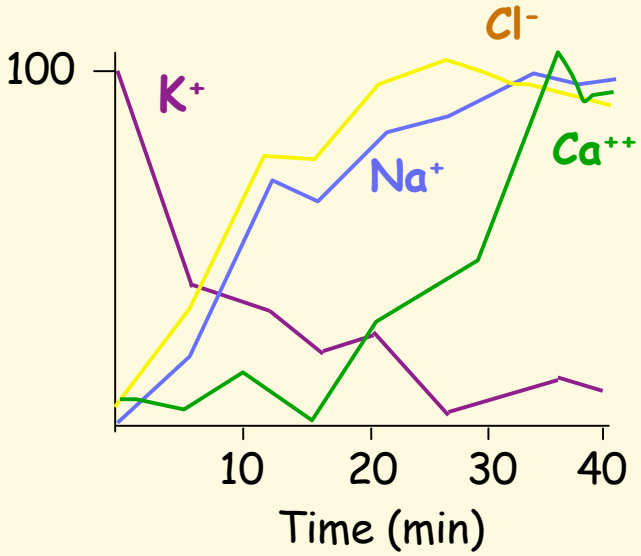


binding to cell surface
no entry into the cytosol

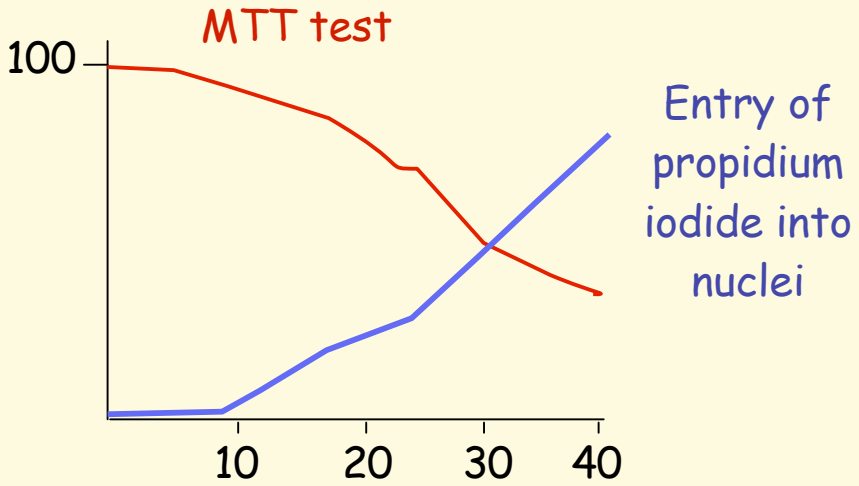
- swelling
- blebing

MDCK cells

Epsilon toxin forms pore through the cell membrane



Epsilon toxin rapidly kills the cells



Epsilon toxin

Binding to a specific cell surface receptor

↓

oligomerization, pore formation

↓

K^+ leakage, Na^+ entry

↓

ATP depletion ⇄ ? mitochondria alteration

↓ ?

Bax, AMPK activation

? ↗

↓

cytochrome *c* release

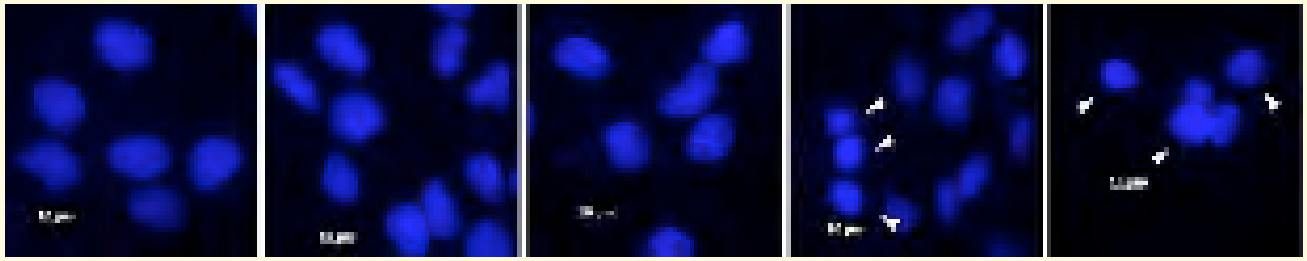
↓

AIF translocation to nuclei

↓

cell necrosis

epsilon 30 min



0 10^{-10} 10^{-9} 10^{-8} 10^{-7} M

DAPI

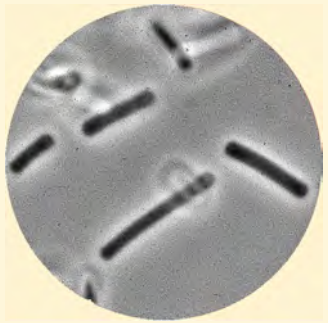
C. perfringens enterotoxin

food
contamination

preparation in large
volume and in advance

Environment

C. perfringens
multiplication
15< <50°C

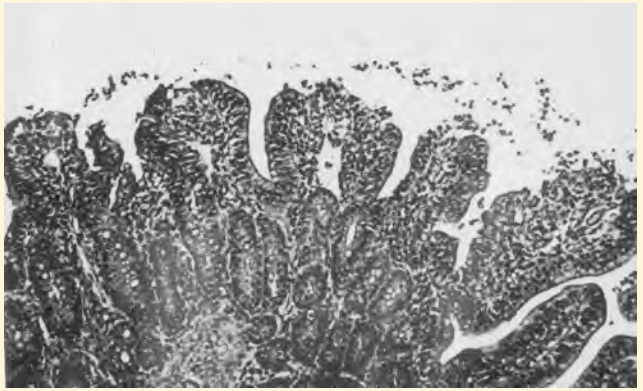


Ingestion of a large

number of *C. perfringens*

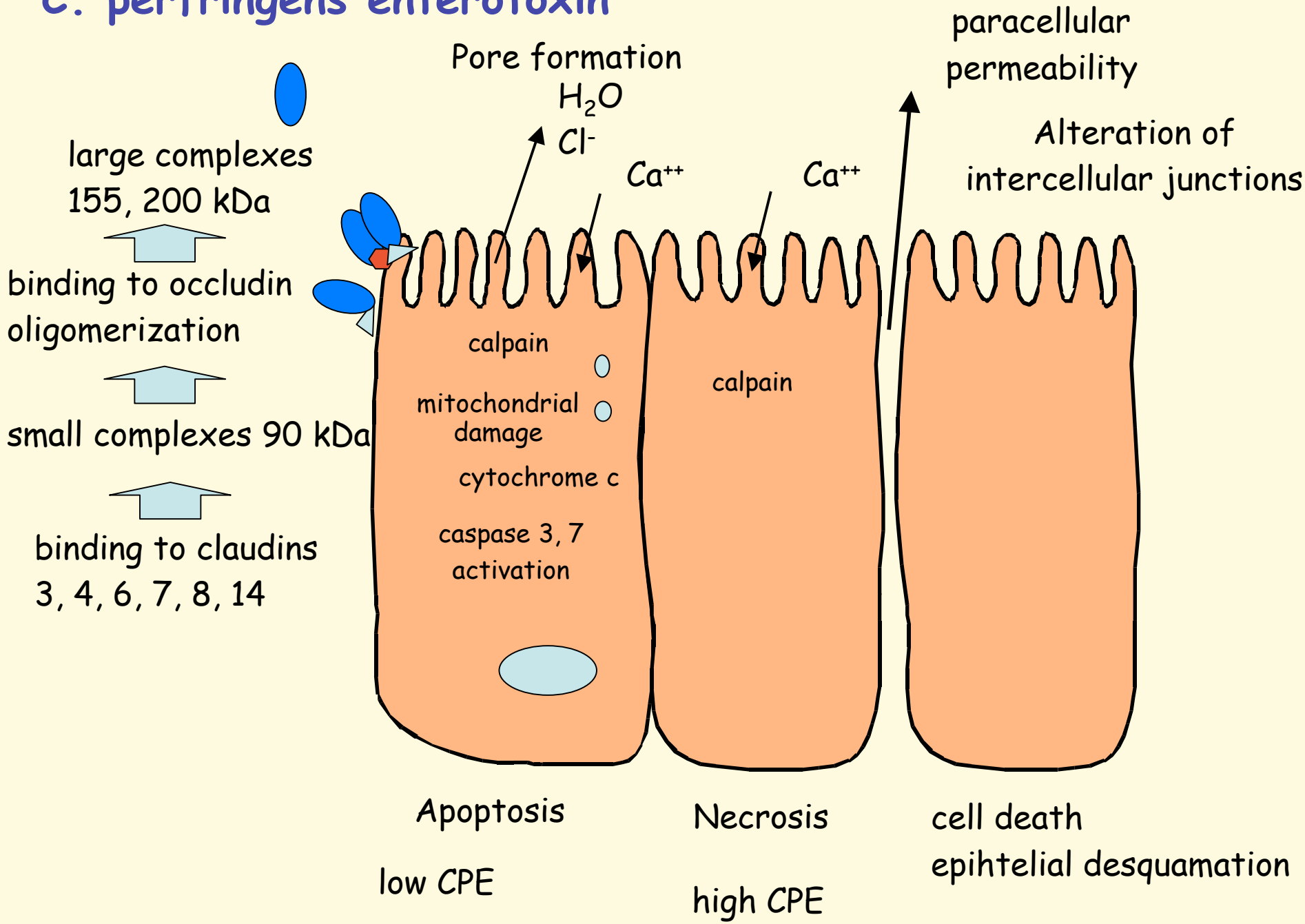
Multiplication and
sporulation in the intestine

Enterotoxin



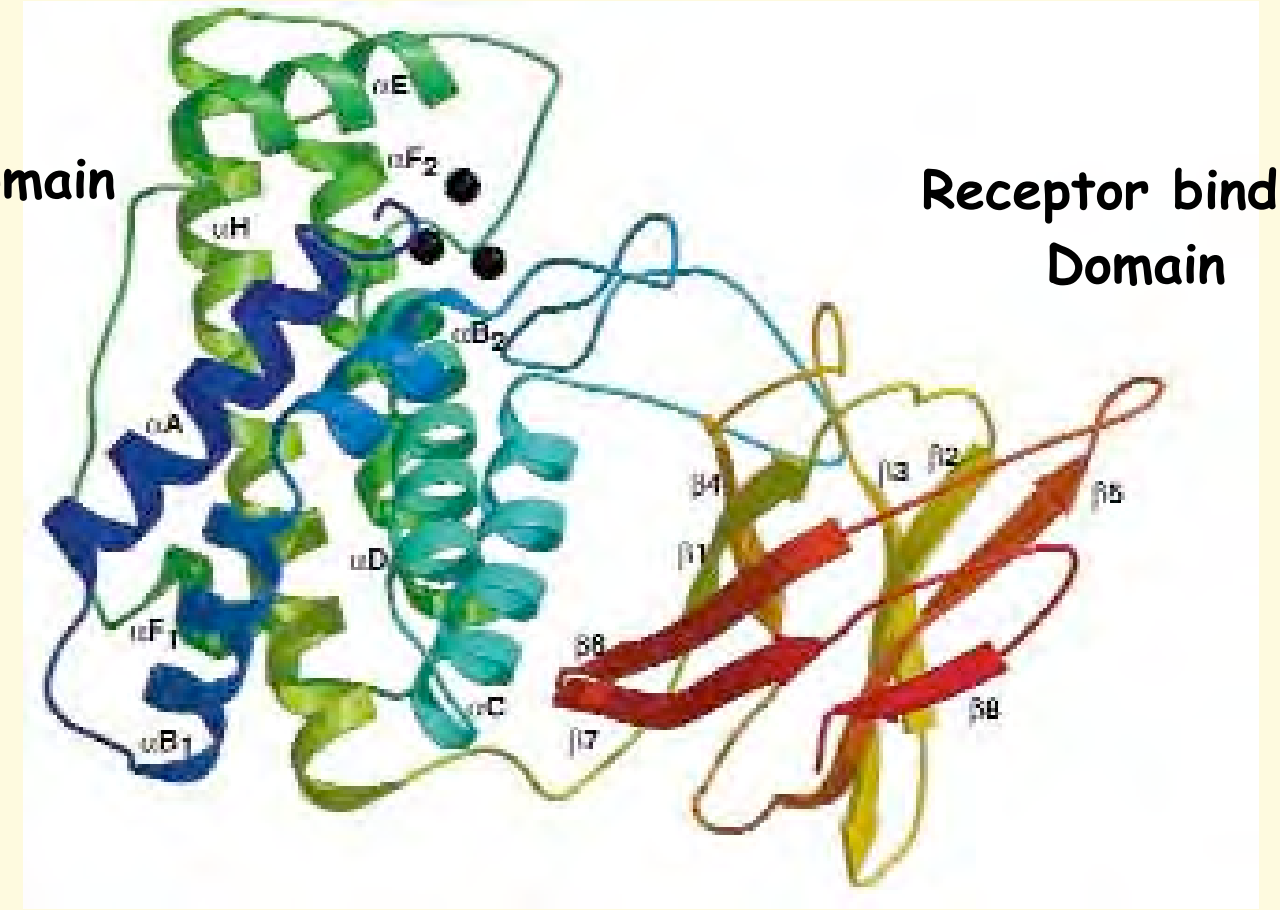
Toxi-infection
(diarrhea, abdominal pain)

C. perfringens enterotoxin



Membrane damaging Toxins, enzymatically active on cell surface

Catalytic domain



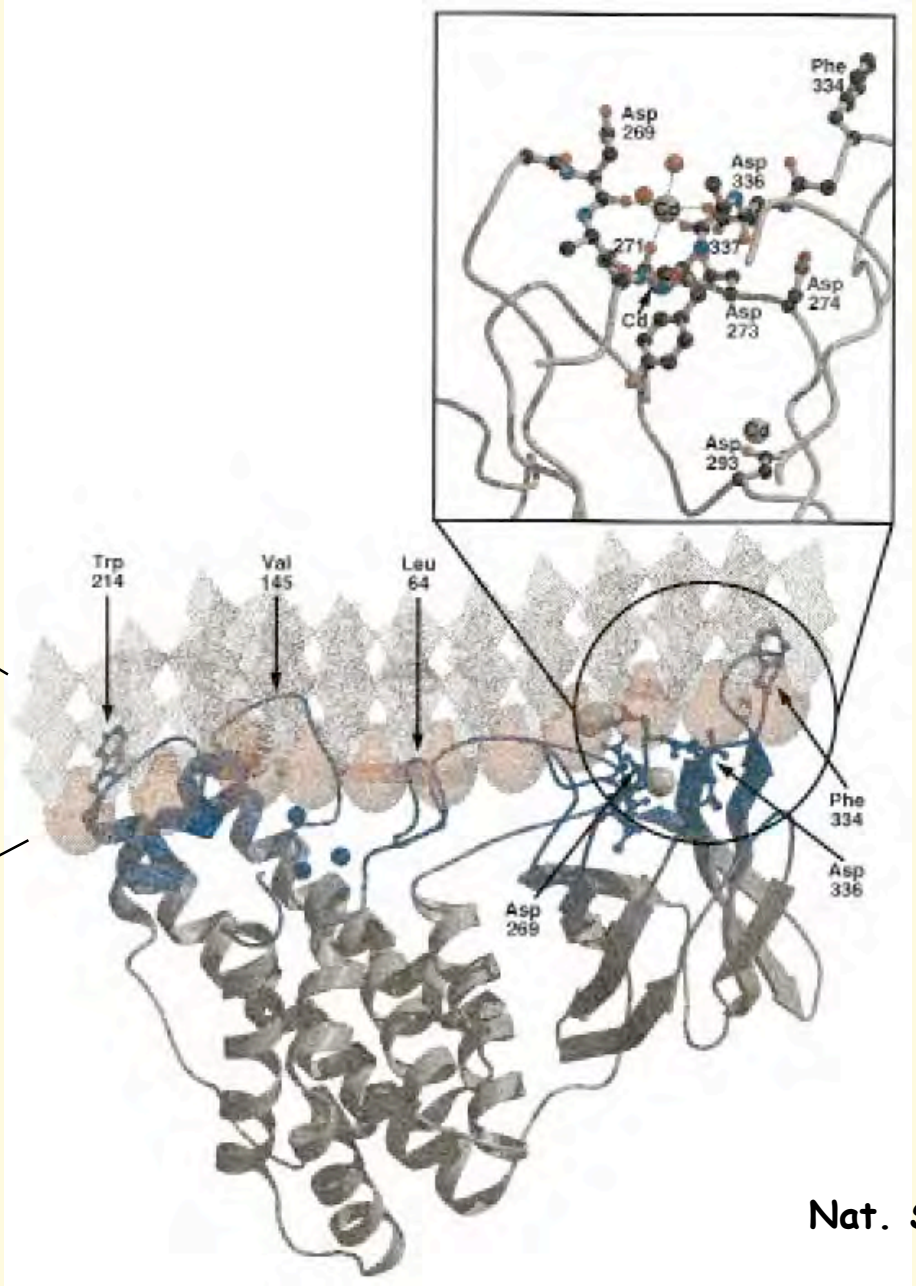
Receptor binding Domain

C. perfringens alpha Toxin

Nat. Struct. Biol. 1998, 5:738

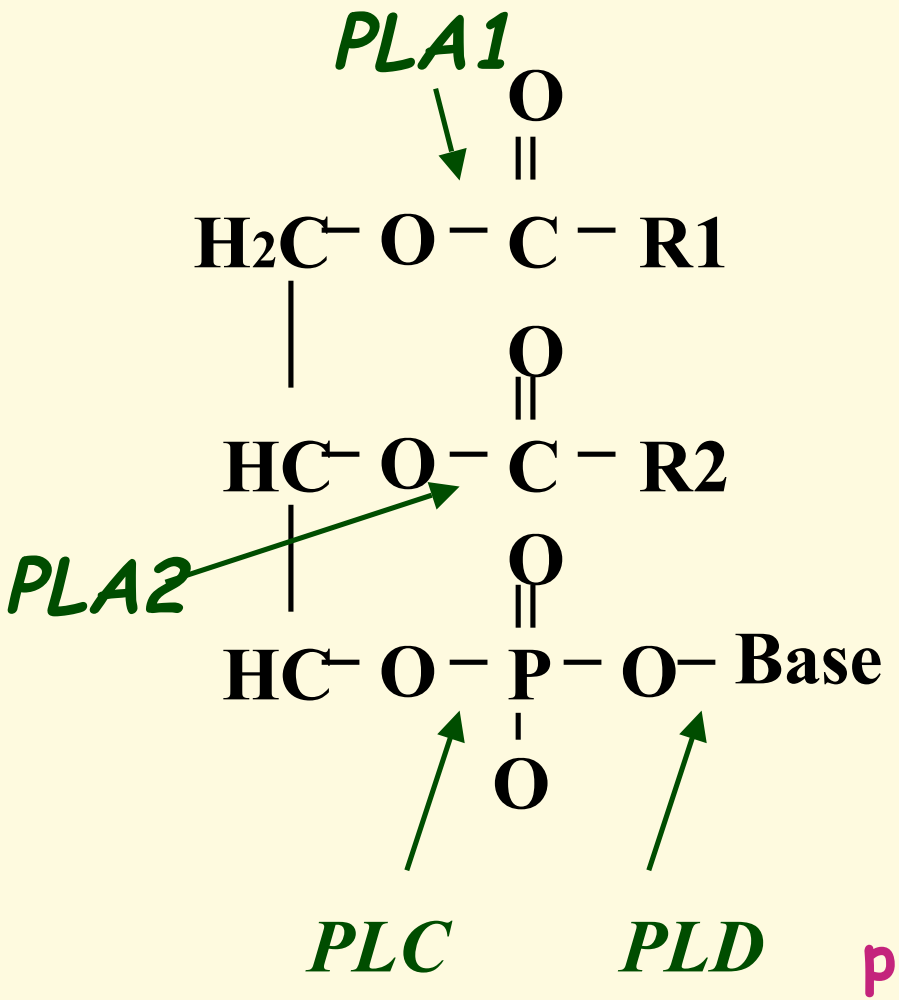
Fatty acids

Phospholipids



Nat. Struct. Biol. 1998, 5:738

Interaction of alpha toxin with membrane

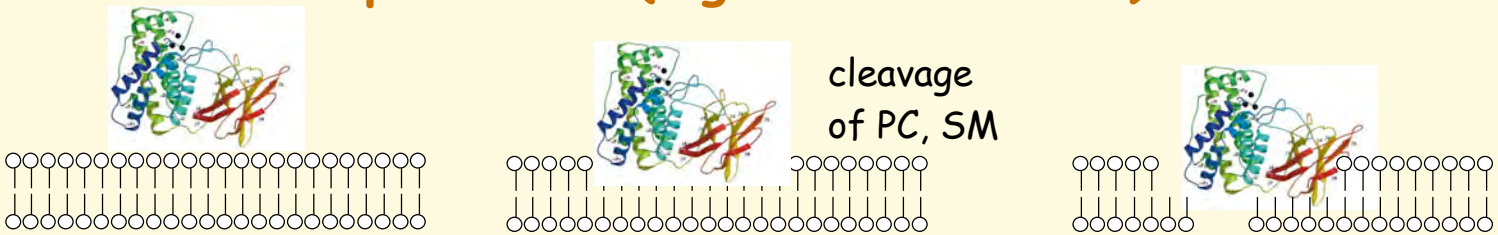


Lysophosphatidyl + R2

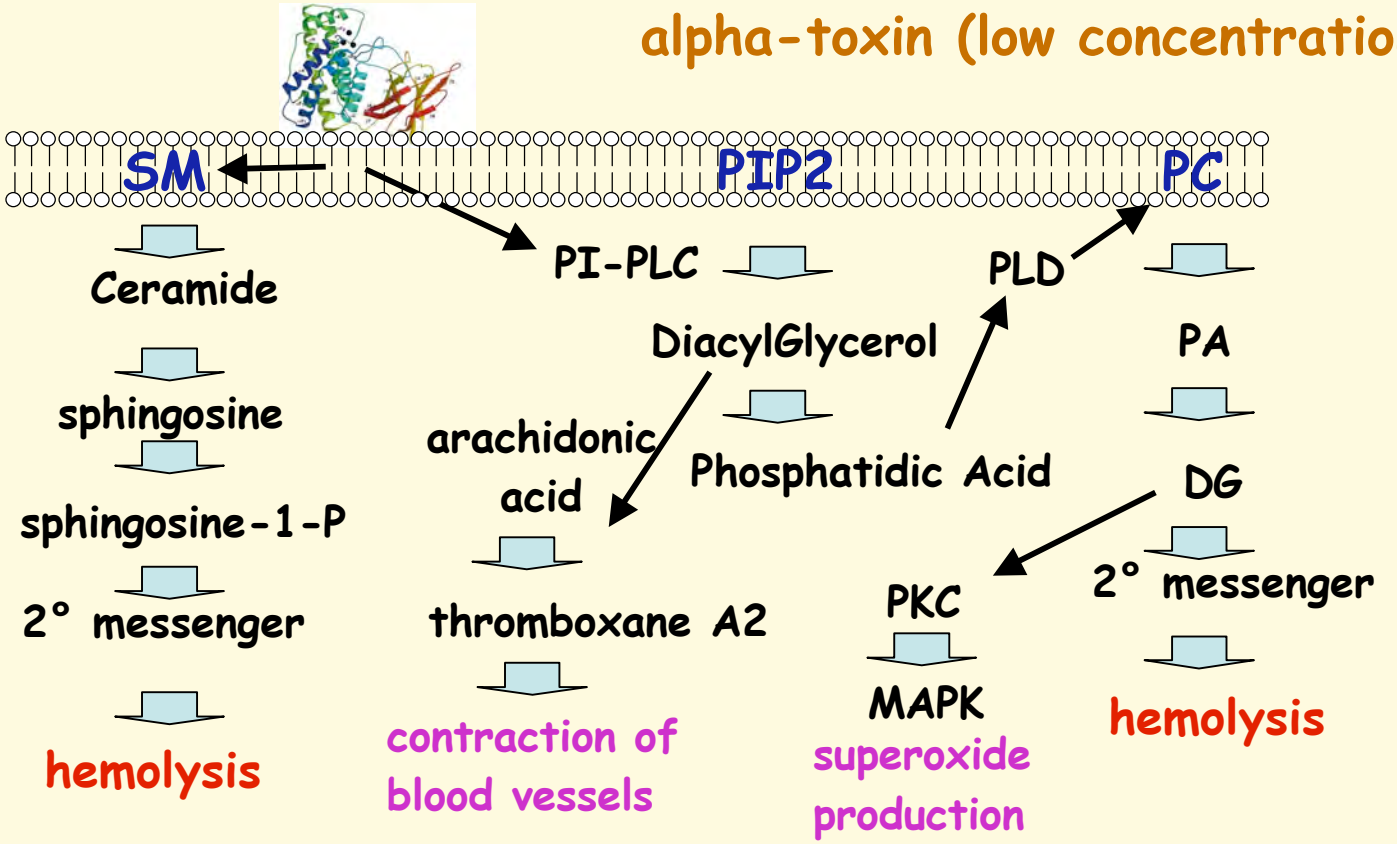
phosphatidic acid + base

DAG + phosphoryl choline

alpha-toxin (high concentration)



alpha-toxin (low concentration)



Intracellularly active Clostridial Toxins

Clostridial binary toxins

Iota family

C. perfringens Iota toxin	calf enterotoxemia
C. spiroforme toxin (CST)	rabbit enteritis
C. difficile CDT	additional virulence factor?

C2 family

C. botulinum C2 toxin	hemorrhagic enteritis (birds, cattle)

Large clostridial toxins

C. difficile toxins A and B	colitis
C. sordellii lethal toxin LT, hemorrhagic toxin HT	gangrene, toxic shock
C. novyi alpha toxin	gangrene

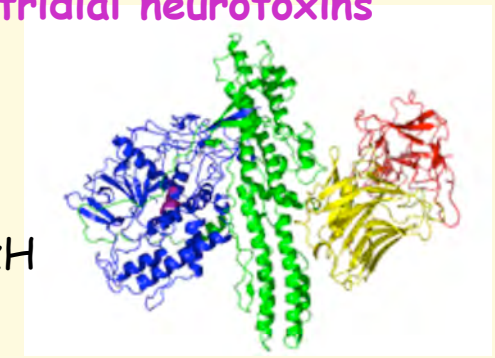
clostridial neurotoxins

Botulinum neurotoxin A to G	botulism
Tetanus neurotoxin	tetanus

Clostridial Toxins active intracellularly

Single chain toxins

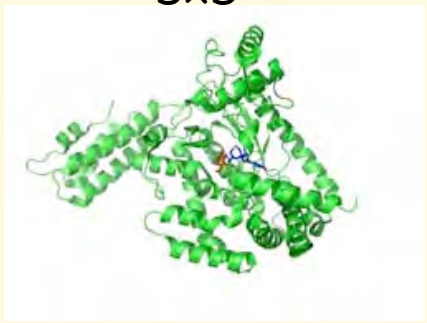
clostridial neurotoxins



HExxH

A or enzymatic Translocation Binding

DxD



N-ter ToxB

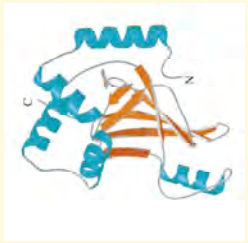
C-ter repeats

Large clostridial toxins

Clostridial binary toxins

Enzymatic component

catalytic site



C3 enzyme

binding components

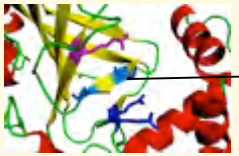
4 domain

Iota

enzymatic domain

catalytic site

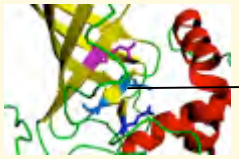
biglutamic motif



EYE

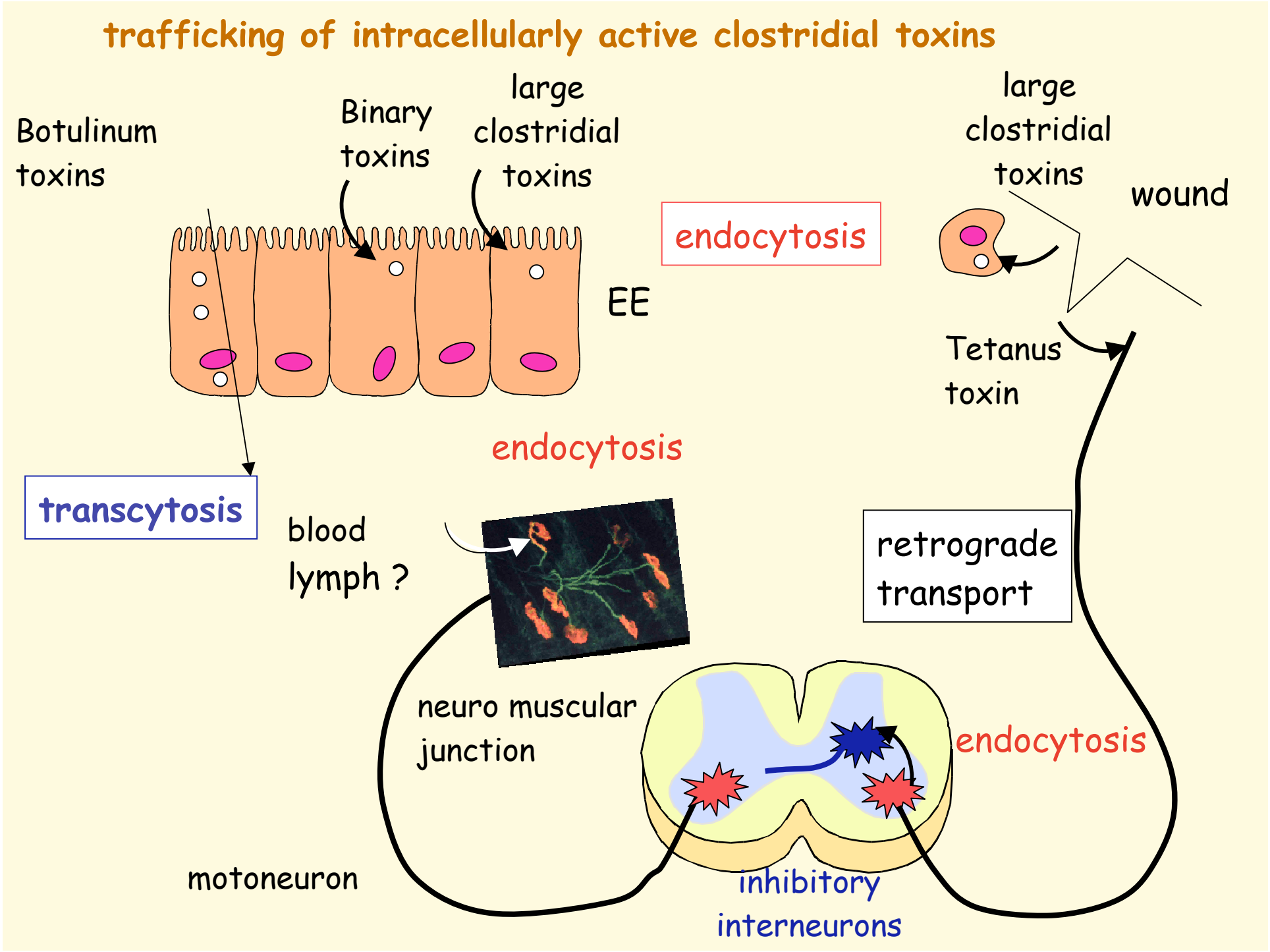
C3

duplication

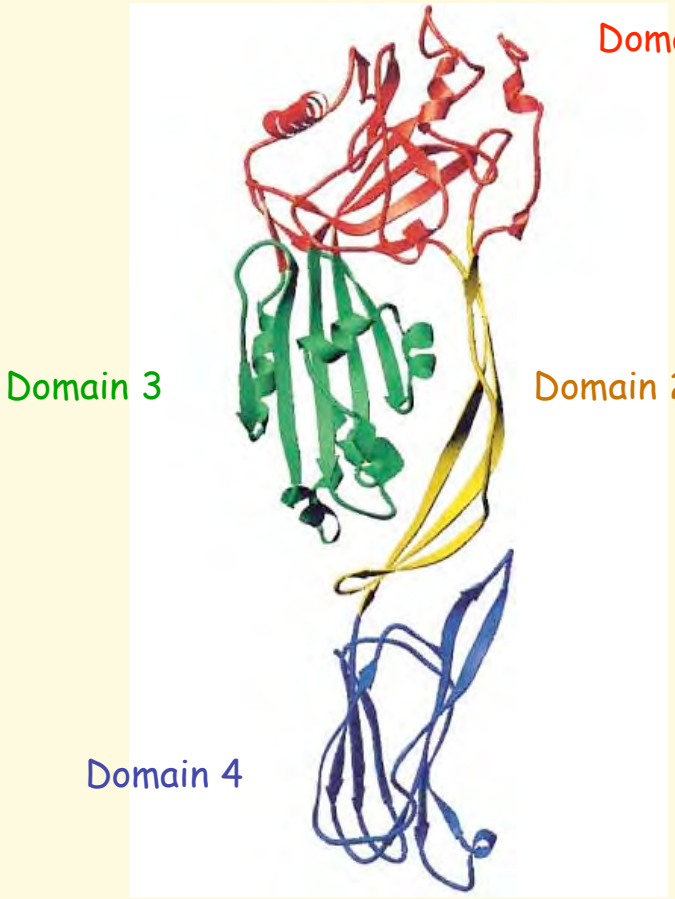


QLE

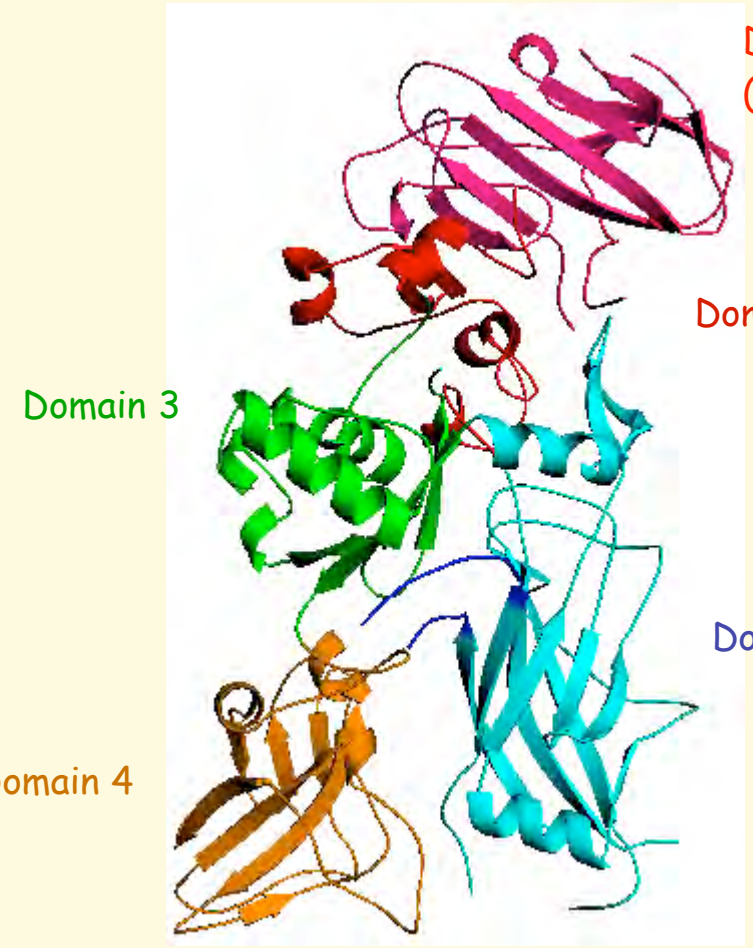
trafficking of intracellularly active clostridial toxins



Relatdness between β-pore forming toxins and binding components of binary toxins



Perfringolysin



PA C2-II

Binding components of binary toxins

- Only one β-hairpin from domain 3

- heptamerization

- pore formation at acidic pH
  (acidified endocytic vesicles)

- translocation of corresponding
  enzymatic component

PA T E H N E H S F F I G V A F / H S V G A V A D G S S G S

Ib T G S S G QNG F G I T Y H / N V V I A Y T N T S S T

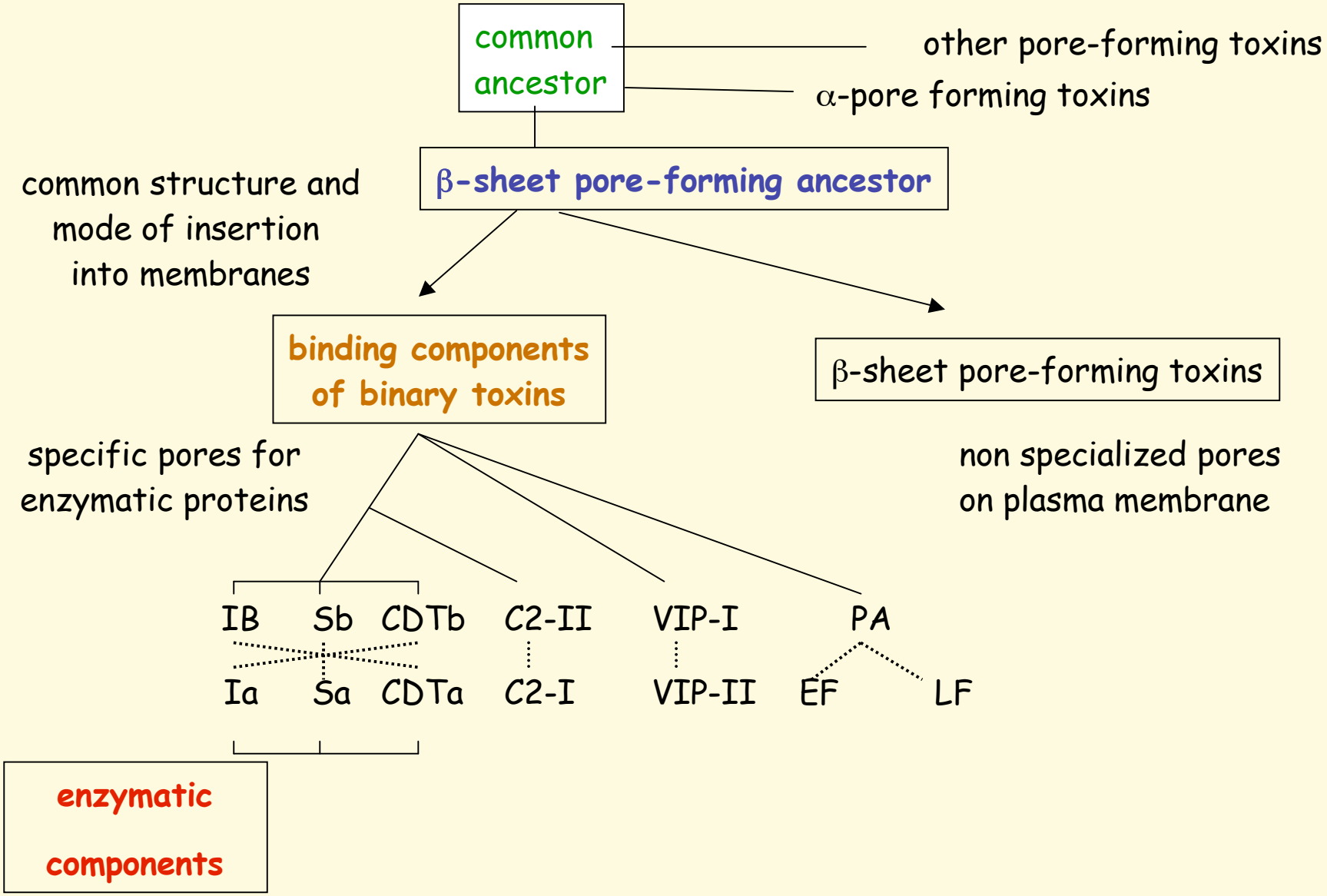
CSTb T G A N A QNG F G I T Y H / N A V I I Y T S T N S T

CDTb T G S N G QNG F A V T Y H / N A V V V Y T N T N S T

C2II T G E S S QLAGGIFPV F M A A Y H / N V A V G L S S S N S T

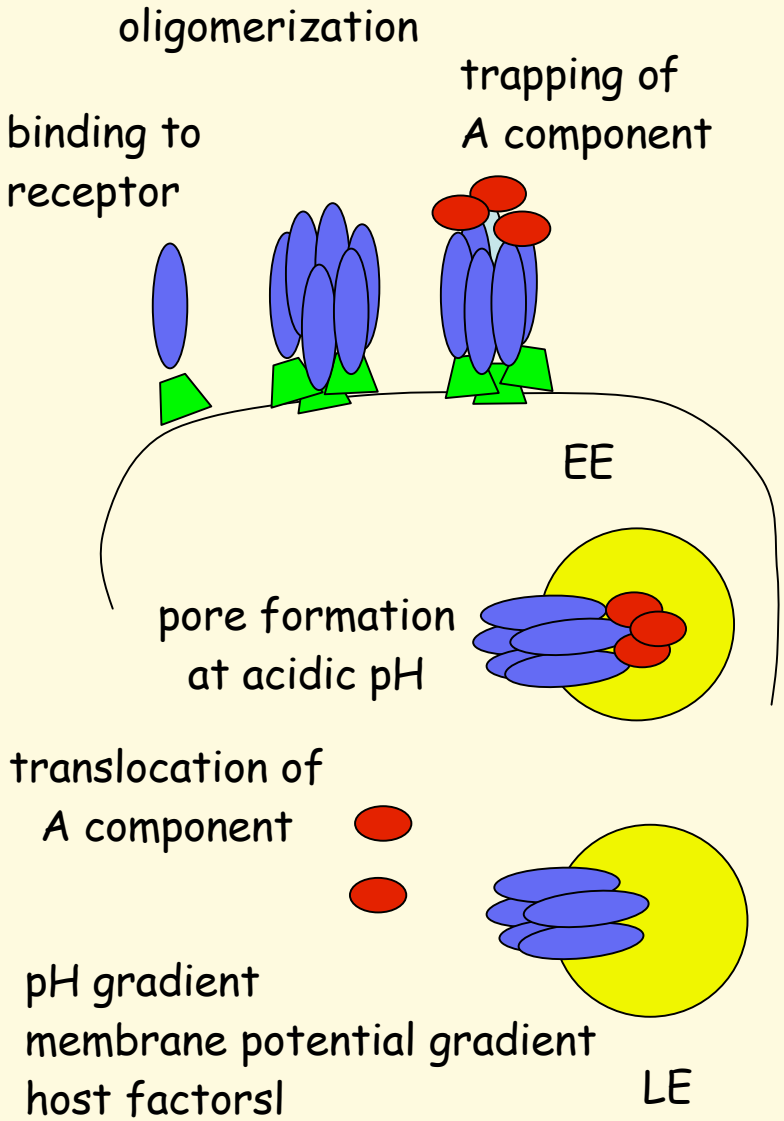
Epsilon T G S Q T KFTVPFNE T V L T Y F / H V T I A A G S T S S A

α-septicum K G K S K G EAIAD S V T F F A Q / I V T F V L K E S E N E

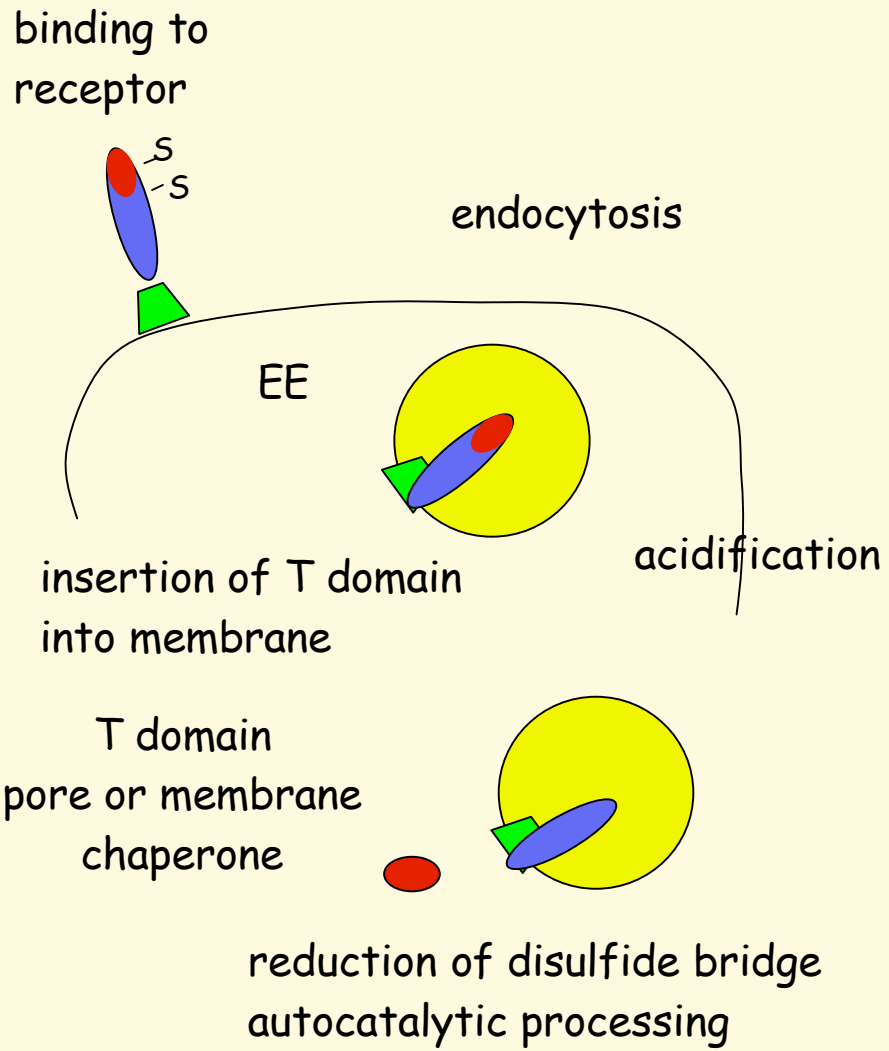

common ancestor
other pore-forming toxins
α-pore forming toxins
β-sheet pore-forming ancestor
common structure and mode of insertion into membranes
binding components of binary toxins
β-sheet pore-forming toxins
specific pores for enzymatic proteins
non specialized pores on plasma membrane
IB
Sb
CDTb
C2-II
VIP-I
PA
Ia
Sa
CDTa
C2-I
VIP-II
EF
LF
enzymatic components

Entry into cells

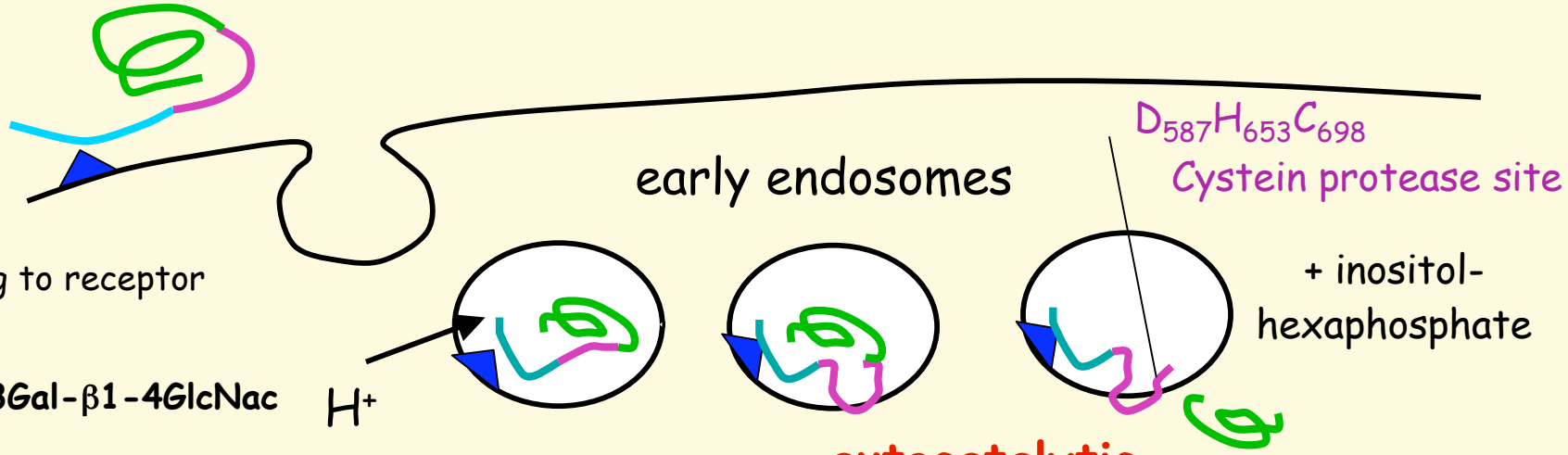
Binary toxins



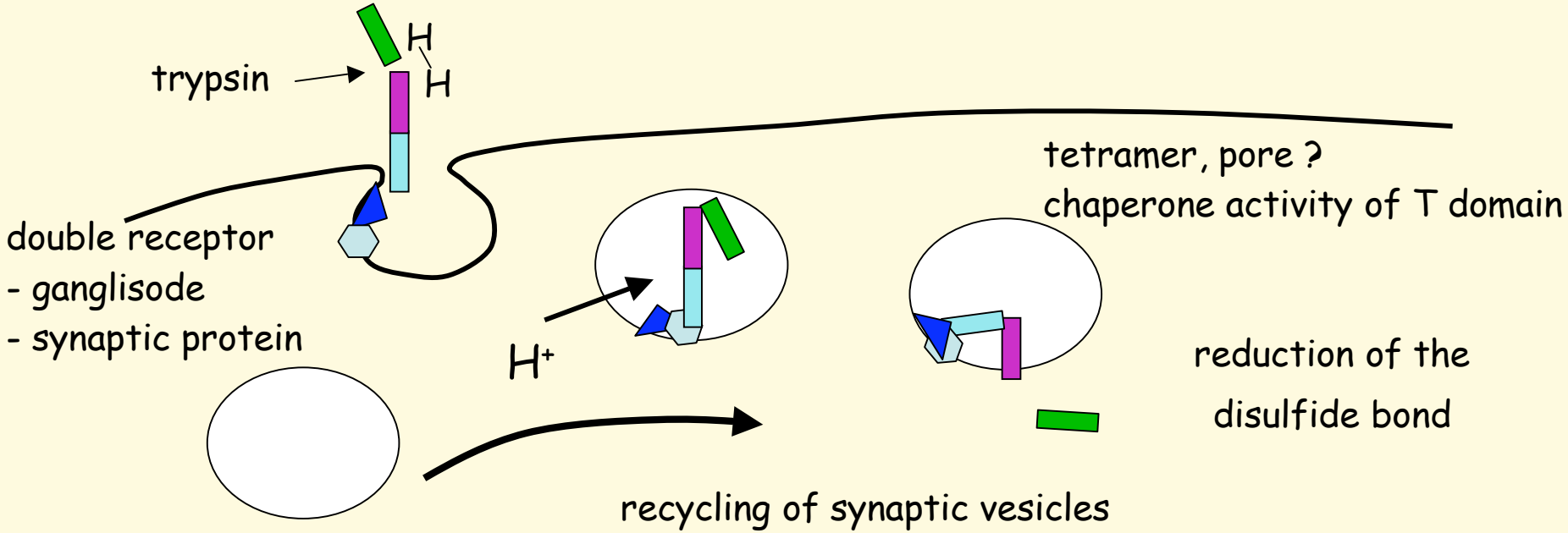
Single chain toxins



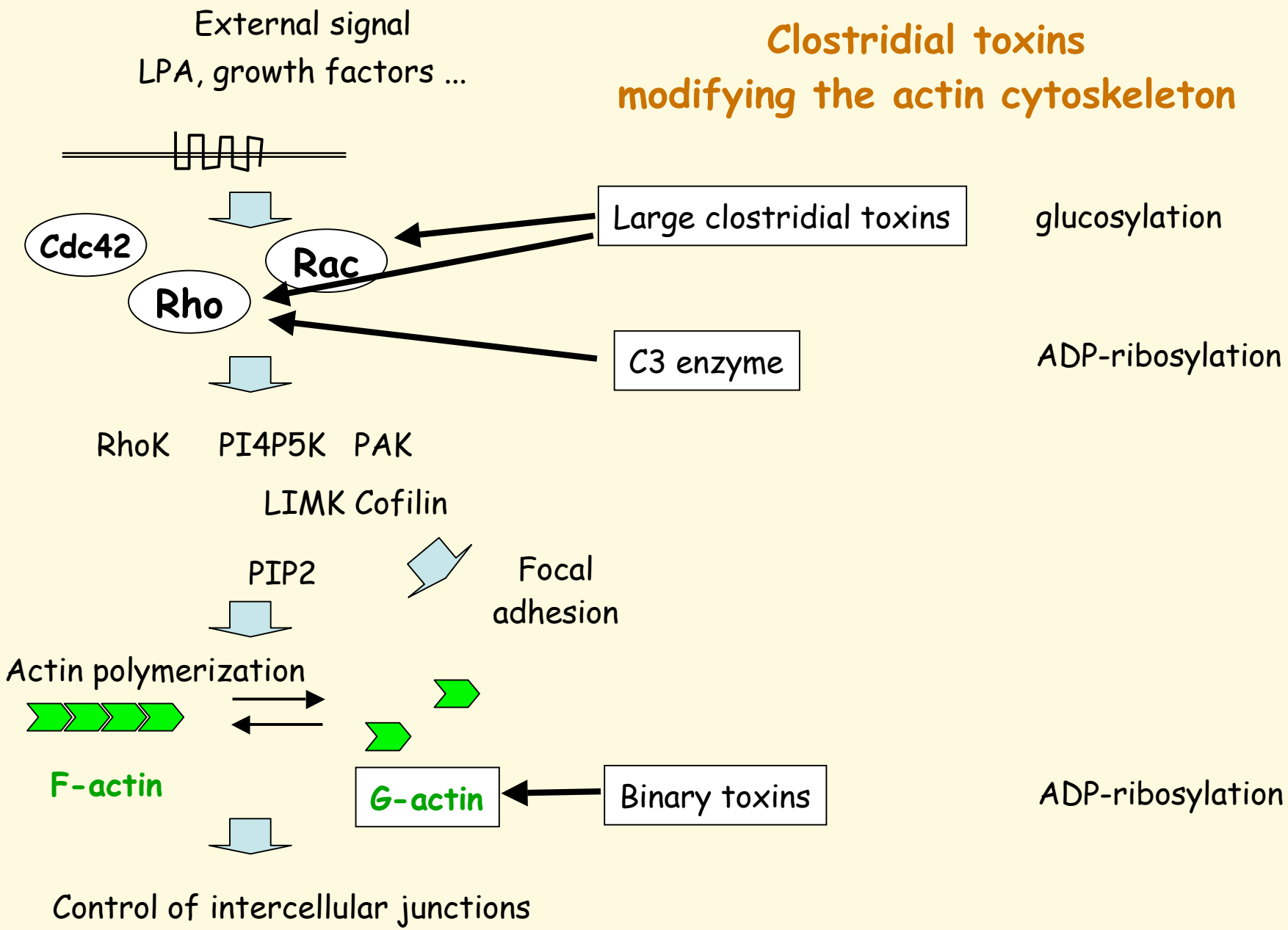
Entry of the large clostridial toxins into cells



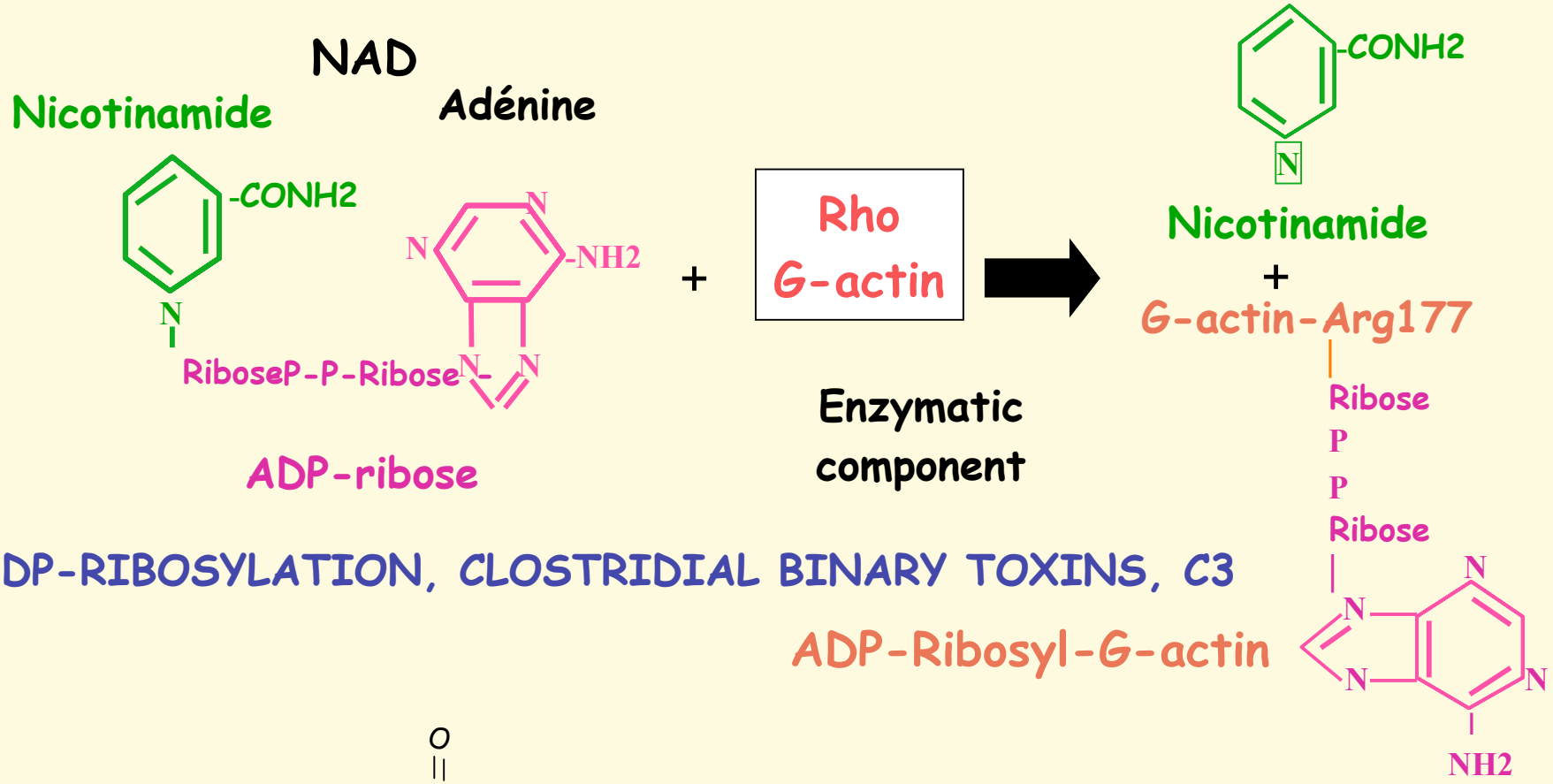
Entry of neurotoxins into neuronal cells



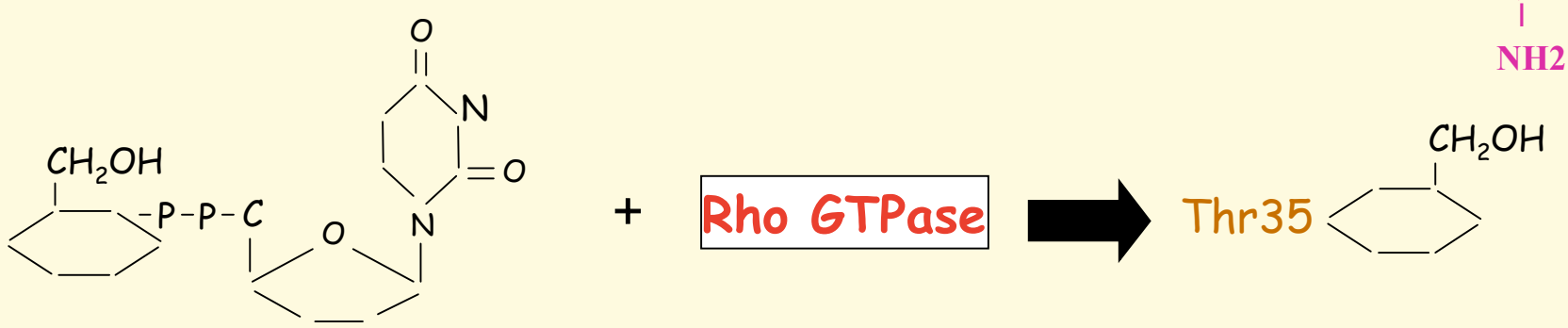
Clostridial toxins modifying the actin cytoskeleton



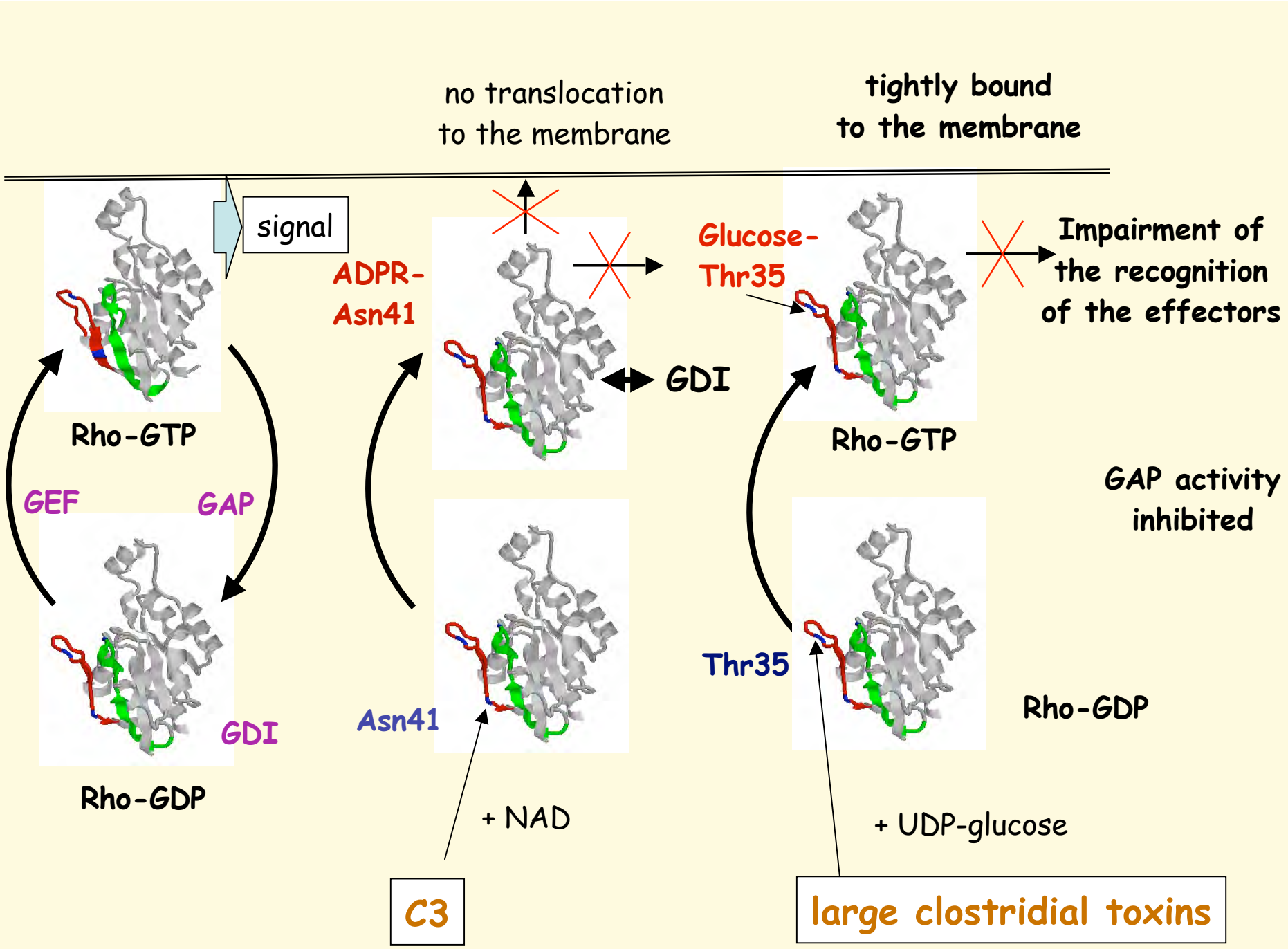
Enzymatic activity of clostridial toxins modifying the actin cytoskeleton



ADP-RIBOSYLATION, CLOSTRIDIAL BINARY TOXINS, C3



UDP-GLUCOSYLATION, LARGE CLOSTRIDIAL TOXINS


no translocation
to the membrane
tightly bound
to the membrane
signal
ADPR-
Asn41
Glucose-
Thr35
Impairment of
the recognition
of the effectors
GDI
Rho-GTP
Rho-GTP
GEF
GAP
GAP activity
inhibited
GDI
Asn41
Thr35
Rho-GDP
Rho-GDP
+ NAD
+ UDP-glucose
C3
large clostridial toxins

Modification of the actin cytoskeleton

- cell rounding
- alteration of intercellular junctions and increase in permeability of cell barriers
- loss of mobility
- loss of phagocytosis, endocytosis, exocytosis

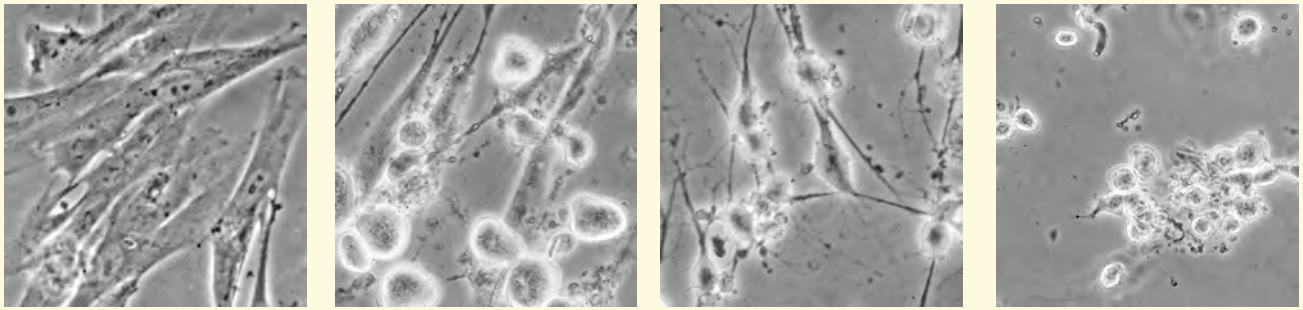
Rho-GTPase alteration

- cell-cycle arrest
- activation of gene transcription
- apoptosis

cell morphology changes

Ctrl | CDT | ToxB | LT82

Fibroblast cells

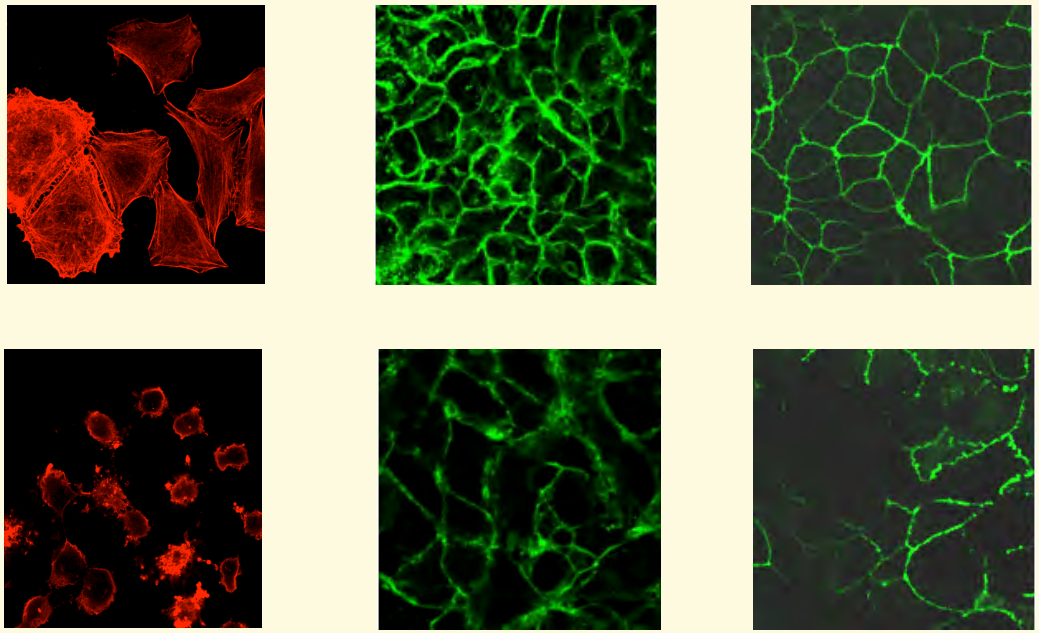


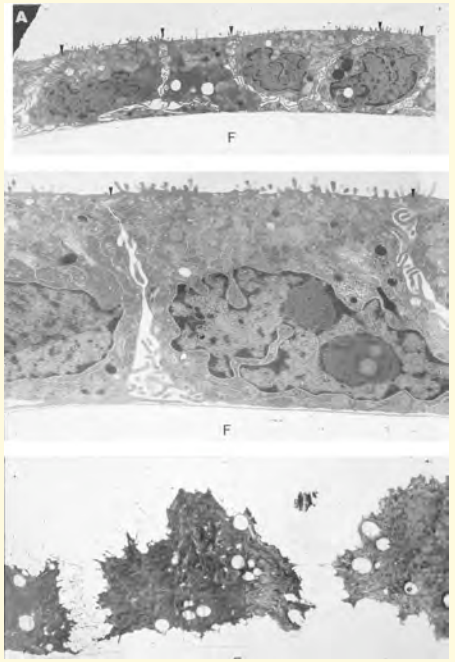
alteration of actin cytoskeleton and intercellular junctions

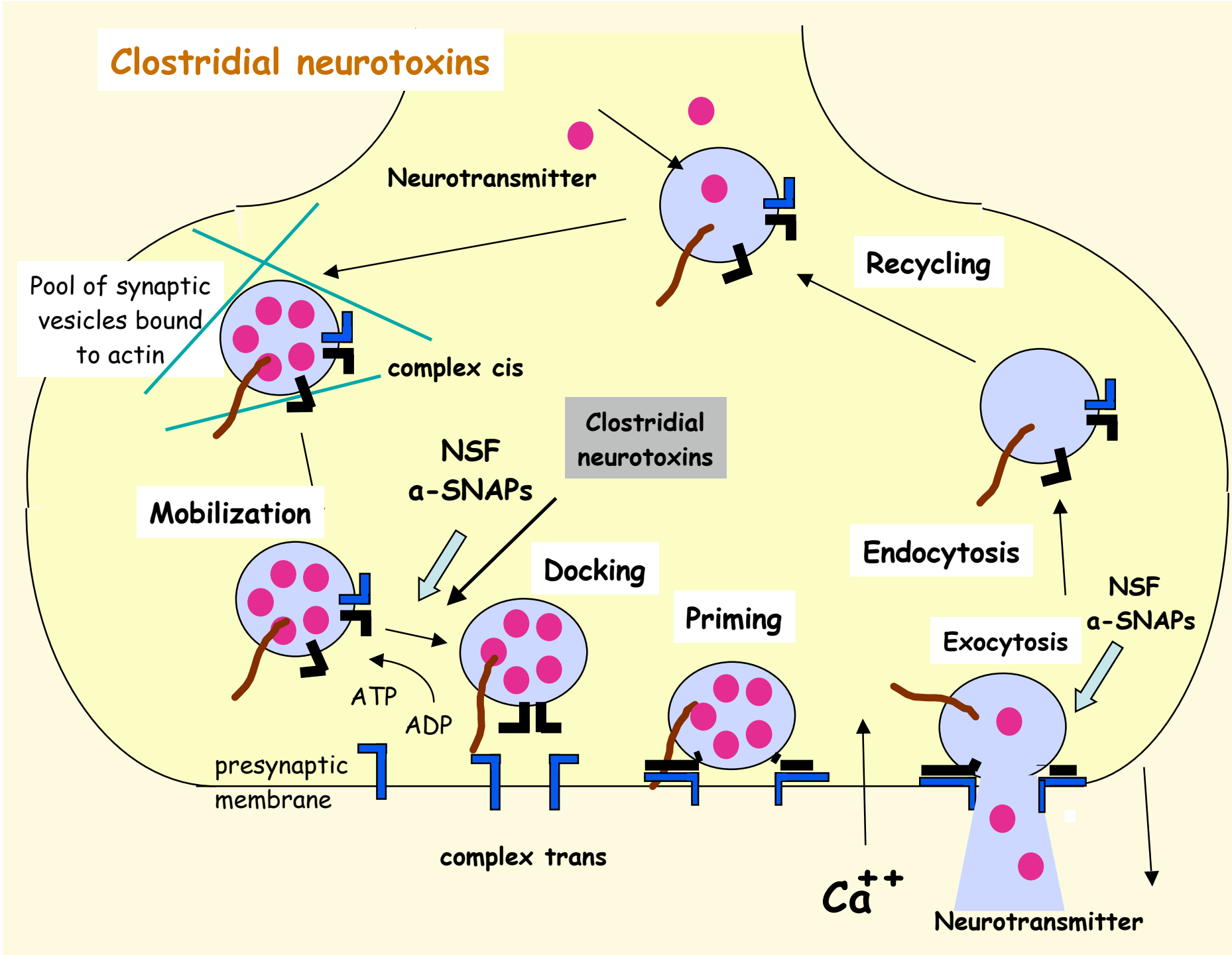
actin filaments | E-cadherin | ZO-1

Ctrl

ToxB

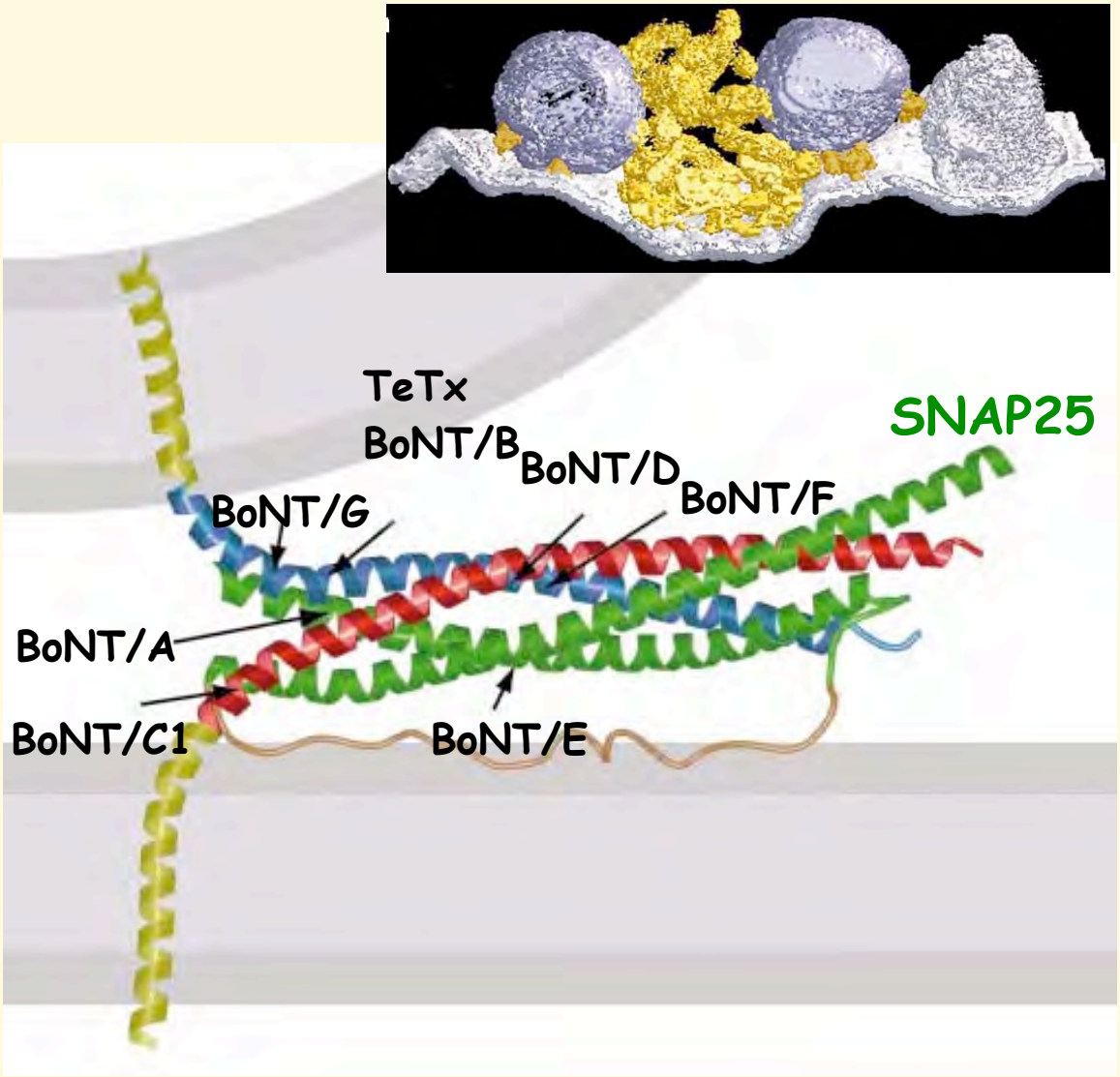


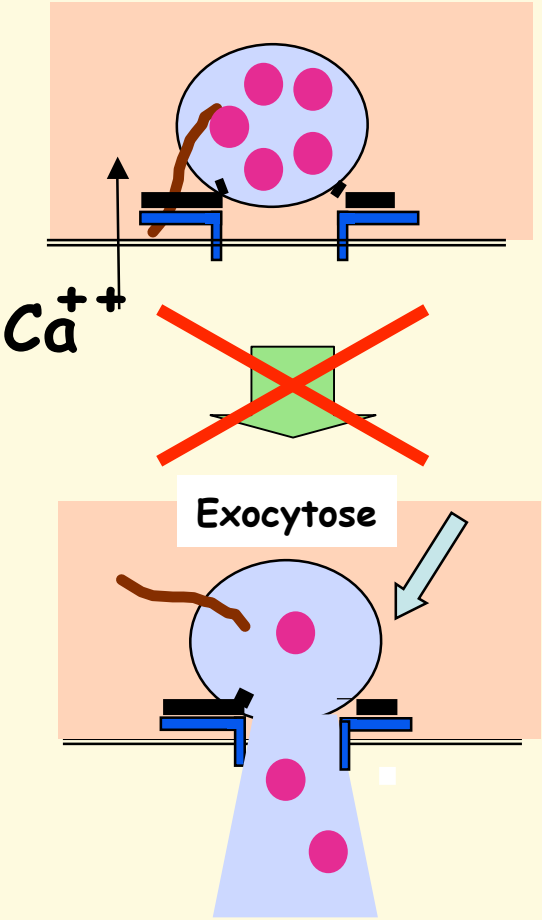



Clostridial neurotoxins
Neurotransmitter
Recycling
Pool of synaptic vesicles bound to actin
complex cis
Clostridial neurotoxins
NSF
a-SNAPs
Mobilization
Docking
Priming
Endocytosis
NSF
a-SNAPs
Exocytosis
ATP
ADP
presynaptic membrane
complex trans
Ca++
Neurotransmitter

Clostridial neurotoxins

Alteration of the vesicular traffic, metalloprotease activity





Why clostridial toxins are so potent?

Enzymatic activity of intracellularly active clostridial toxins
equivalent to that of regular enzymes

But, specific routing to target cells
membrane-damaging toxins to muscle, connective cells
but also cells involved in host defense
botulinum neurotoxin to neuromuscular junctions
tetanus toxin to central inhibitory interneurons via
a retrograde transport

Critical target for cell activity
- regulatory G-proteins controlling actin polymerization
- actin monomers
- specific proteins of the neuroexocytosis

10% cleavage of SNAP25 abolishes acetylcholine release
20% decrease in endplate potential prevents muscle fiber contraction
paralysis of 10% of diaphragma fibers causes asphyxia