Biochimie Membranaire

Introduction - Rôles et importance des membranes biologiques

1- Lipides et protéines membranaires / dynamique membranaire

2- Etude expérimentale des protéines membranaires

3- Principaux canaux et transporteurs: Etudes « structure-fonction »

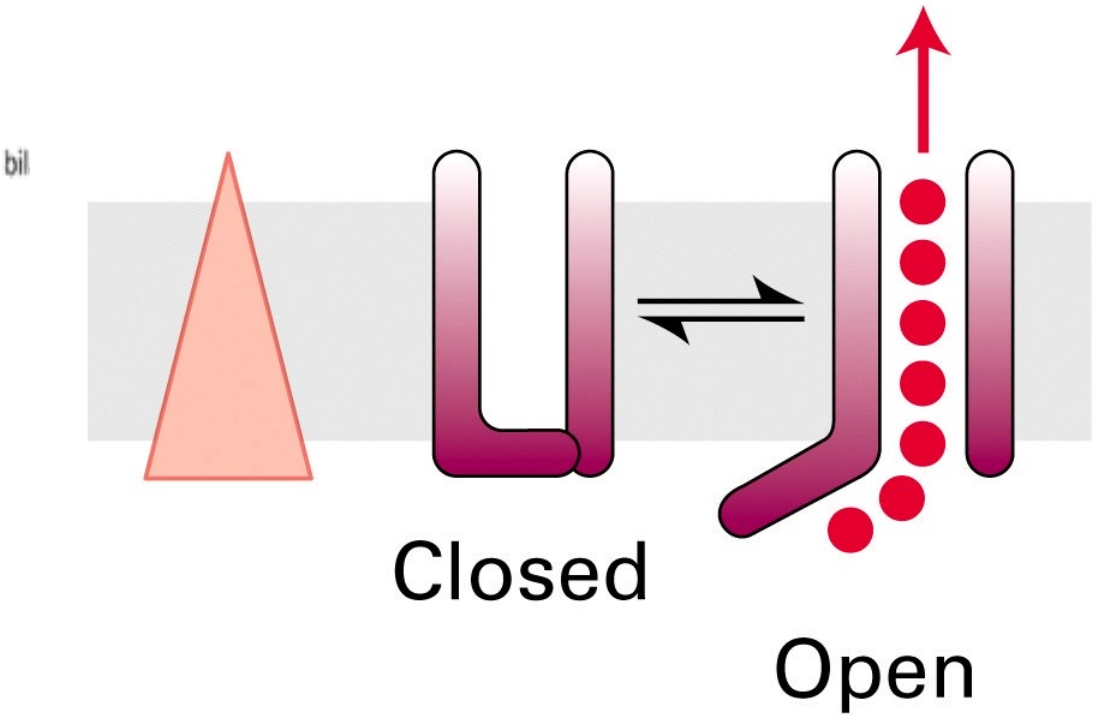
…….suite ……

Les canaux

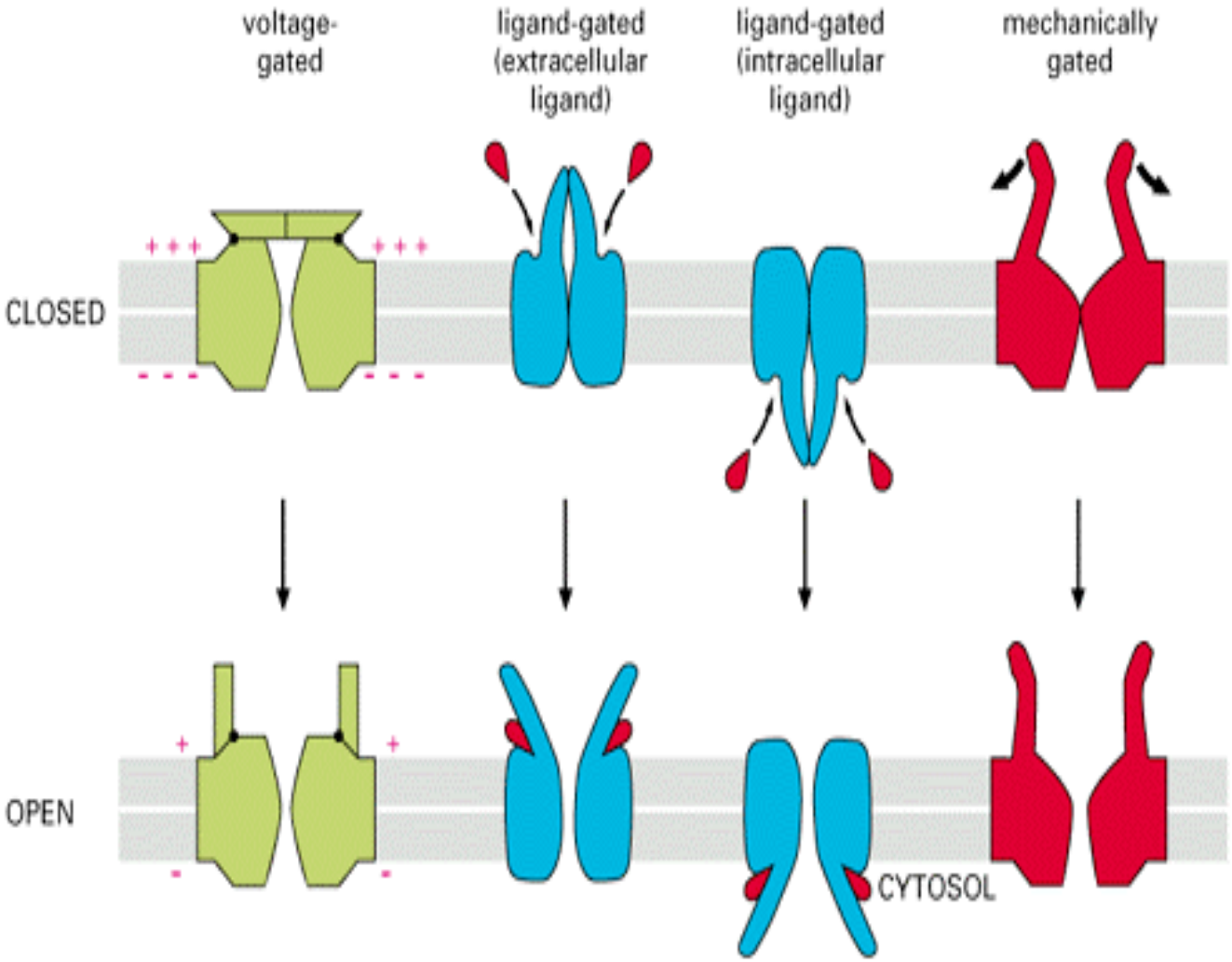
2

Ion channels

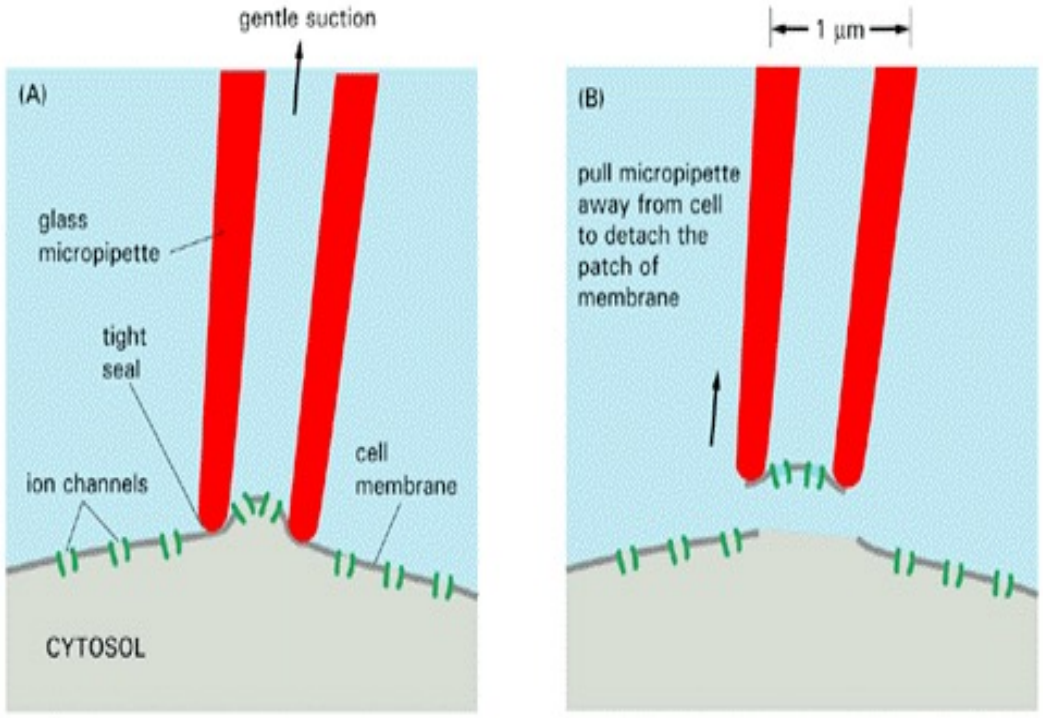
(10^7–10^8 ions/s)



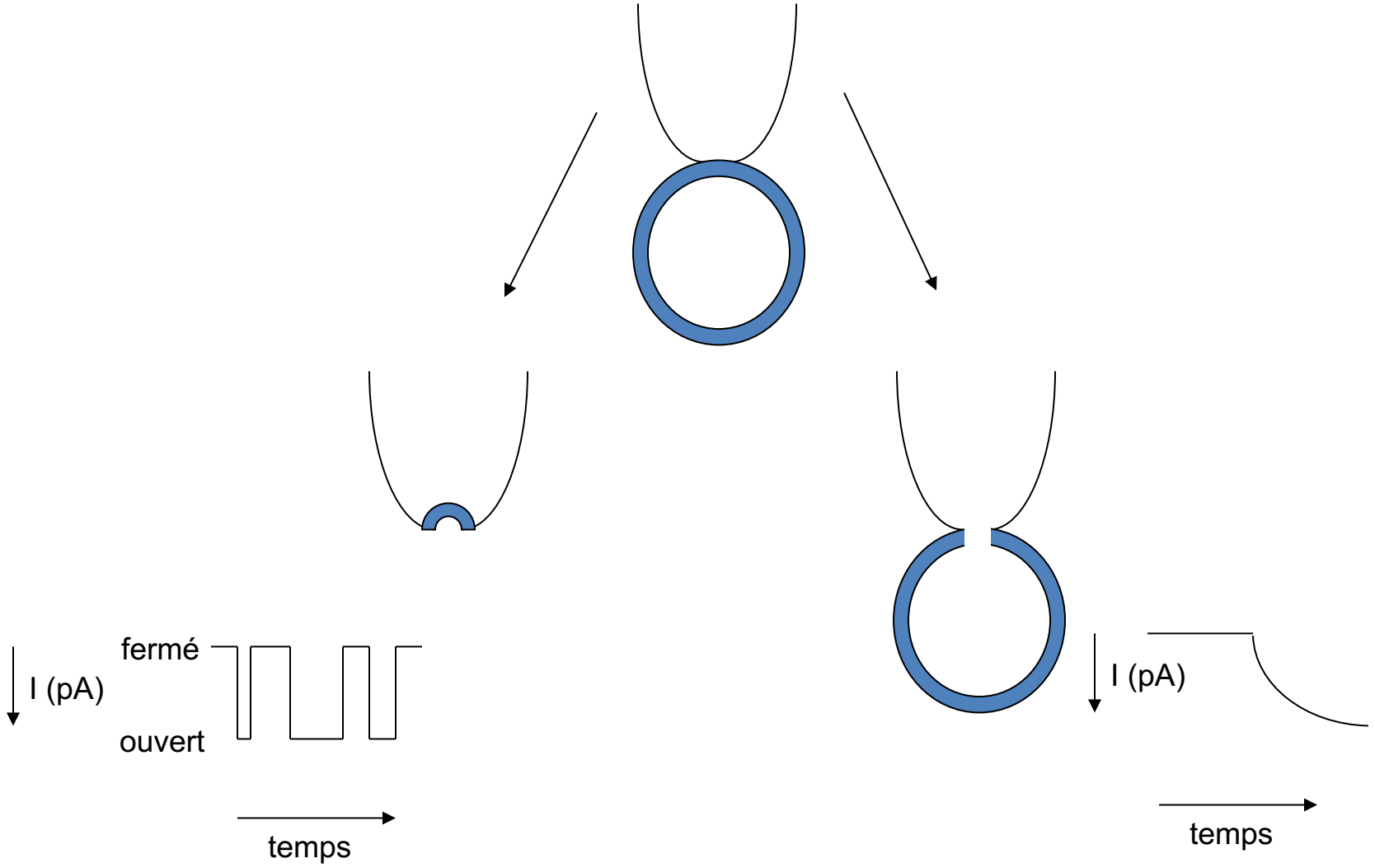
Les différentes catégories de canaux



Comment étudier un canal ?



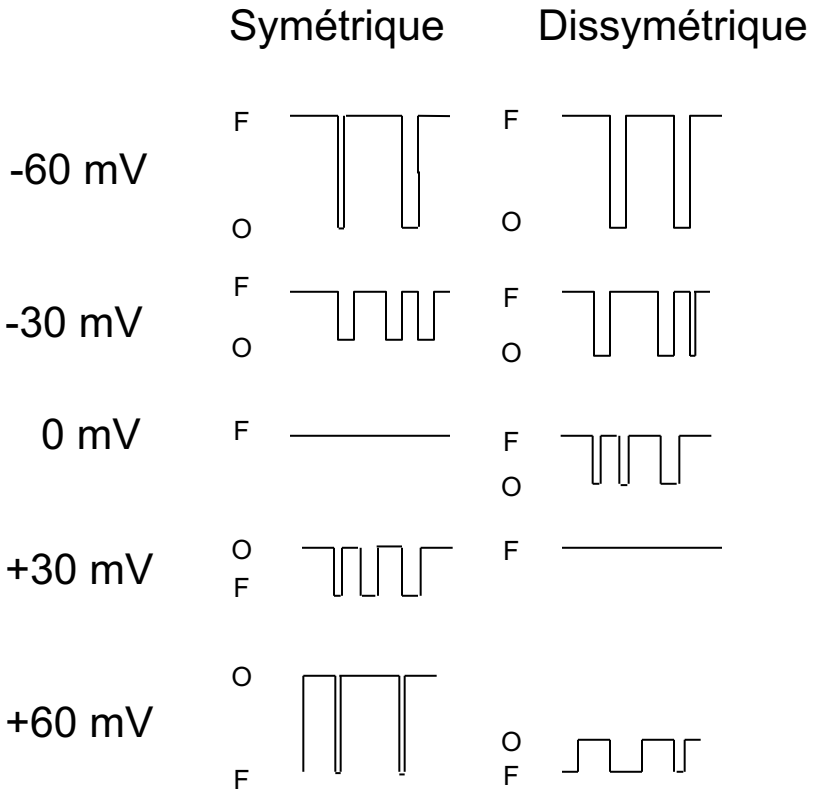
Canal isolé ou cellule entière

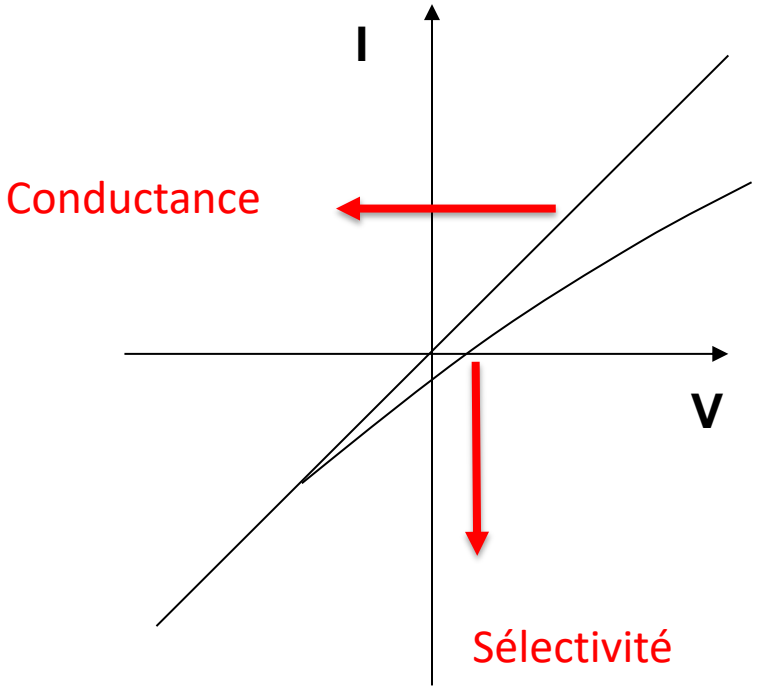


Courant unitaire

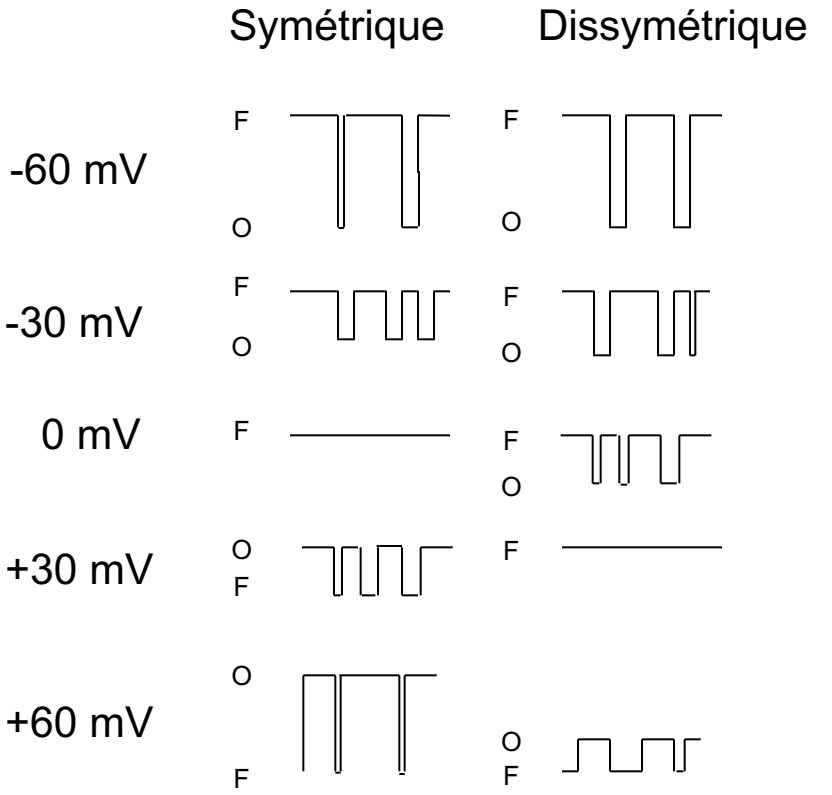
Courant global

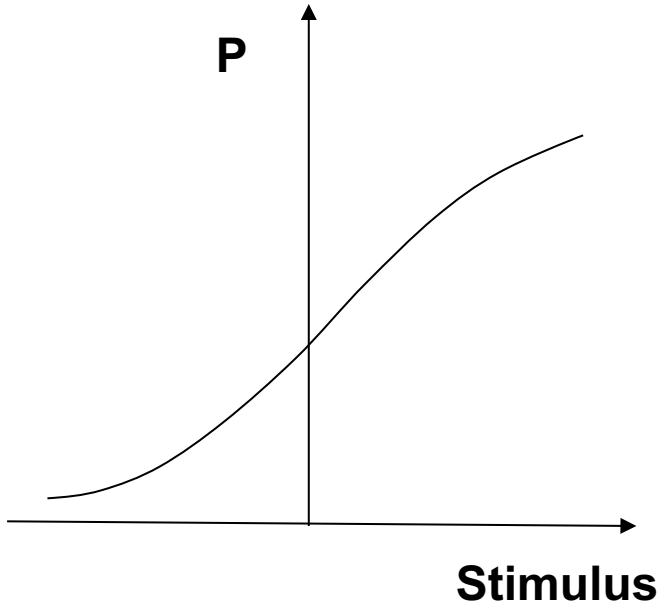
propriétés d’un canal





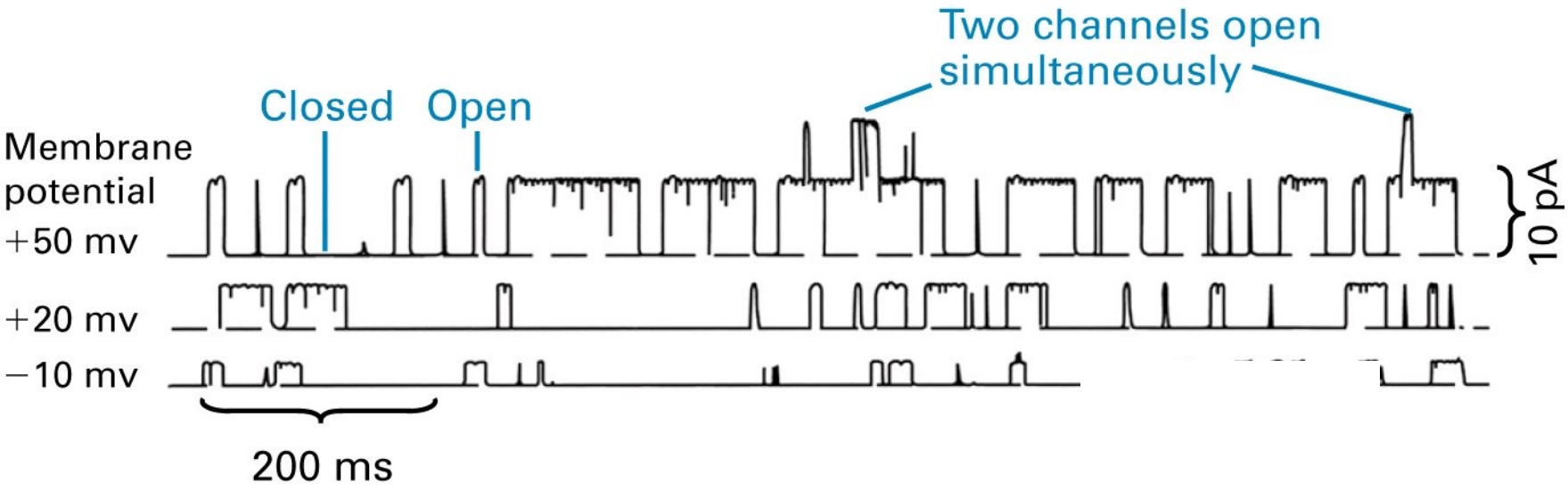
propriétés d'un canal

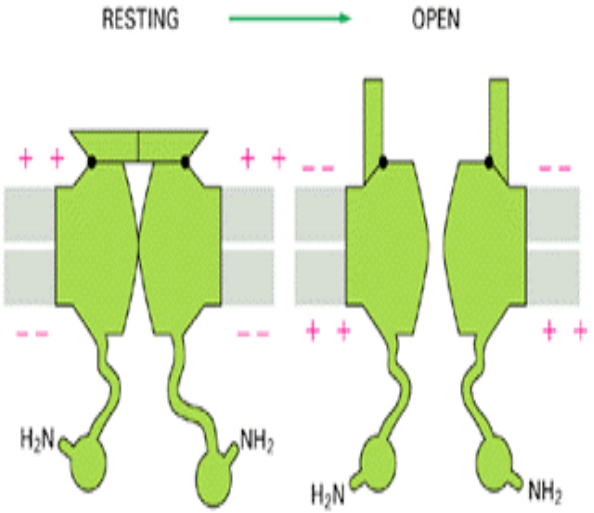




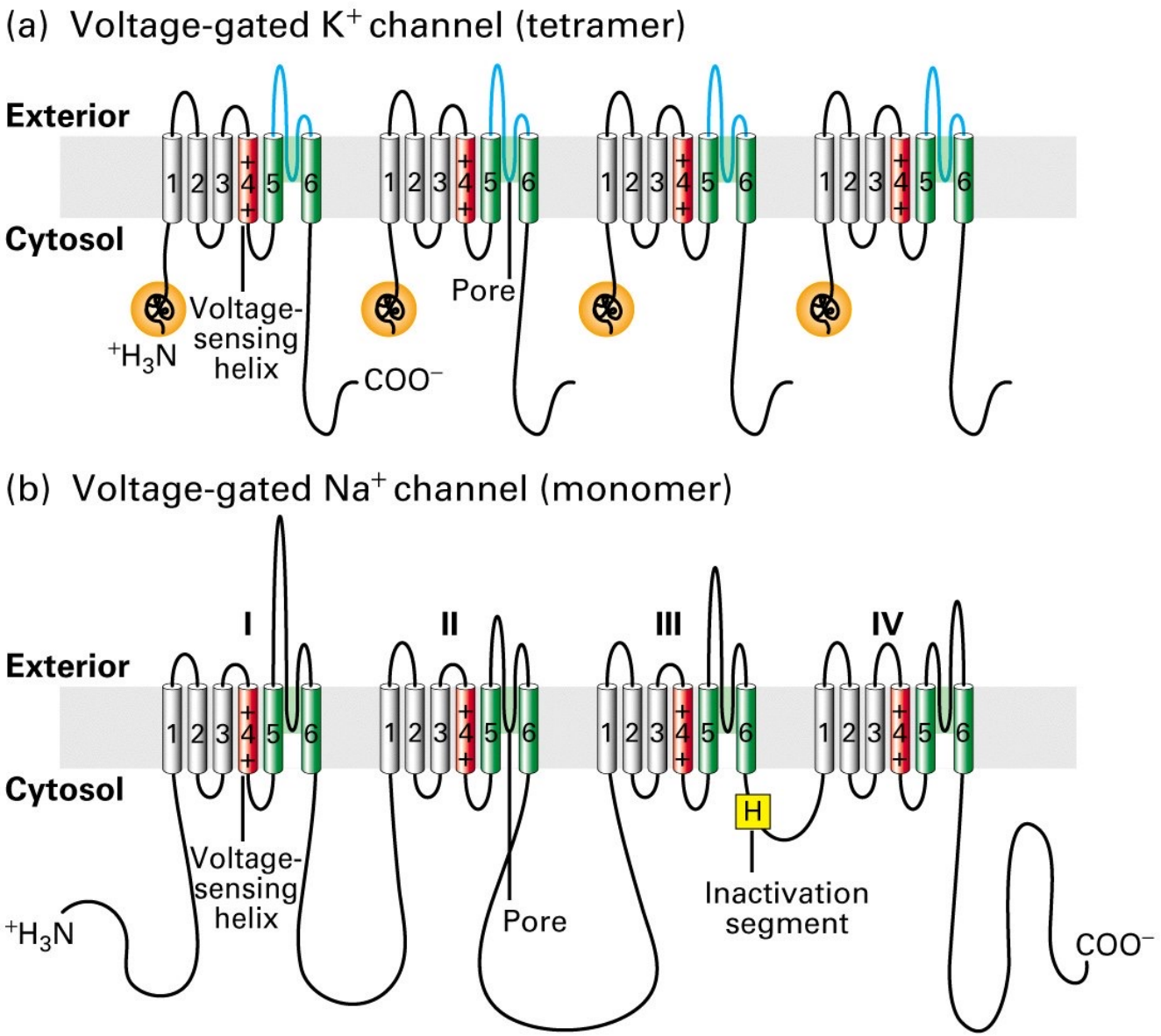
Probabilité d'ouverture

Canaux « voltage dépendants »

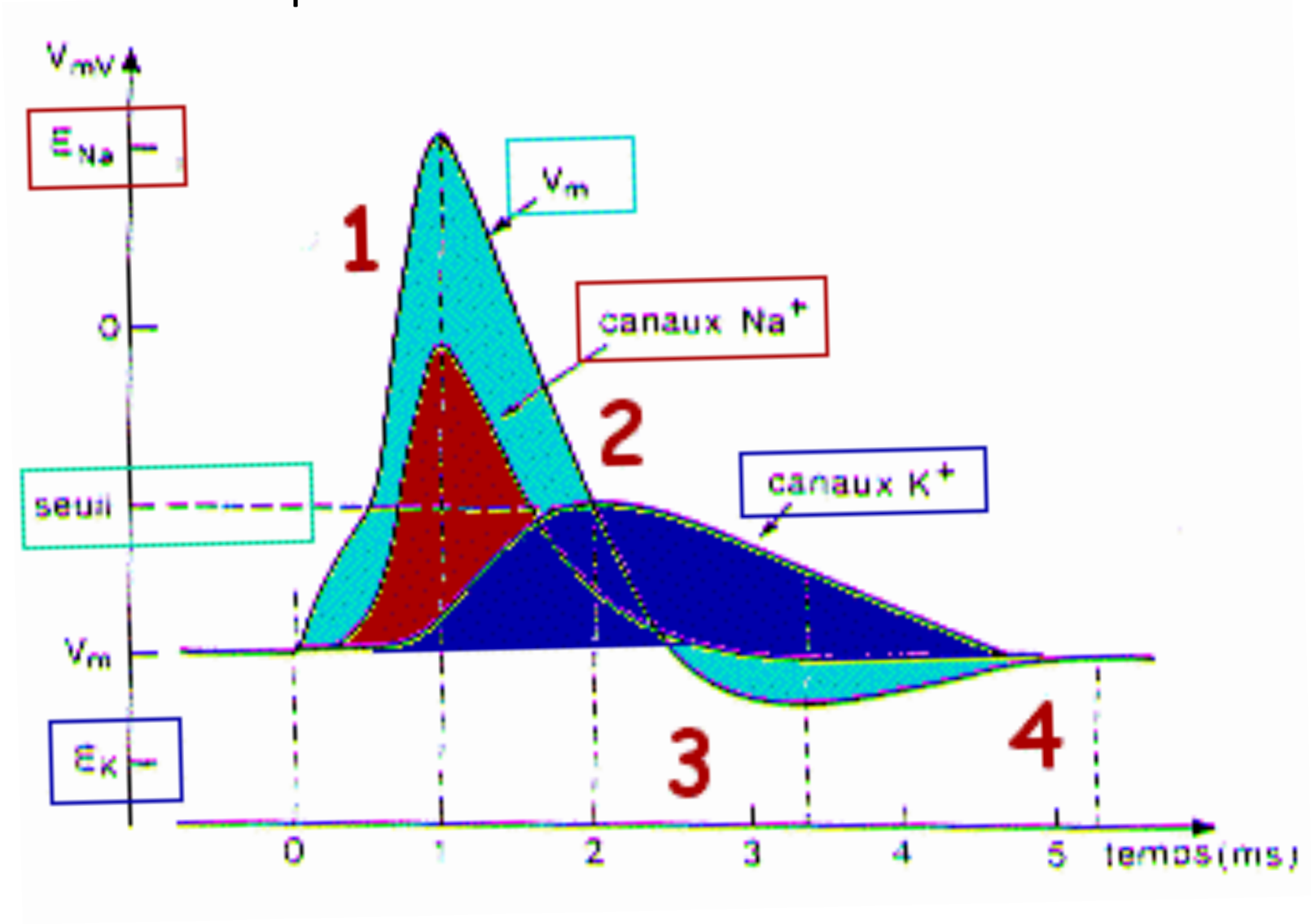




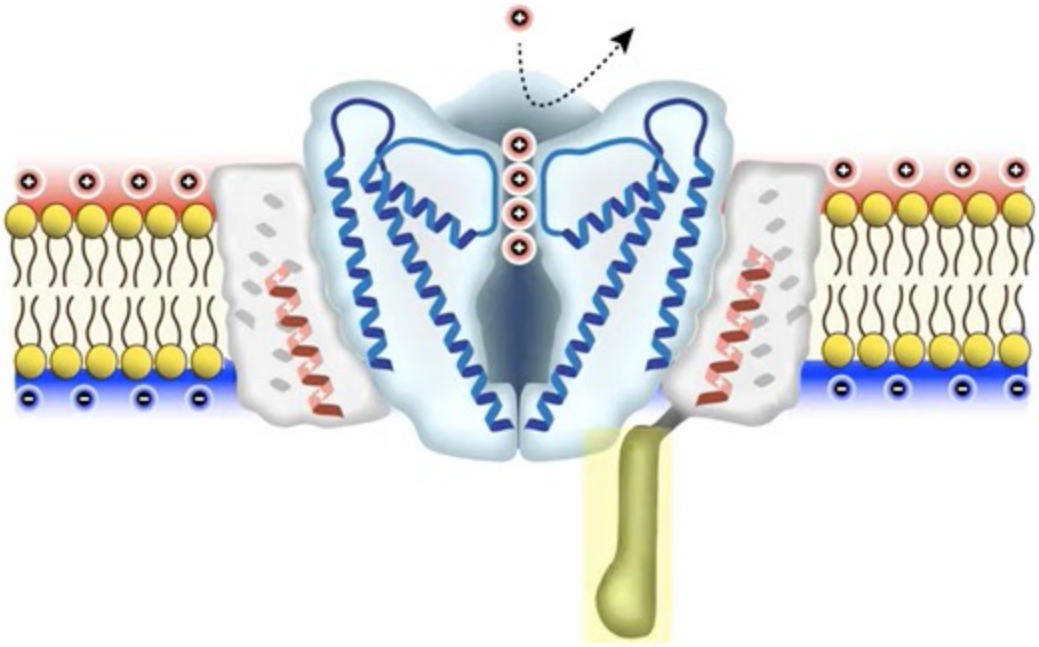
Une structure conservée



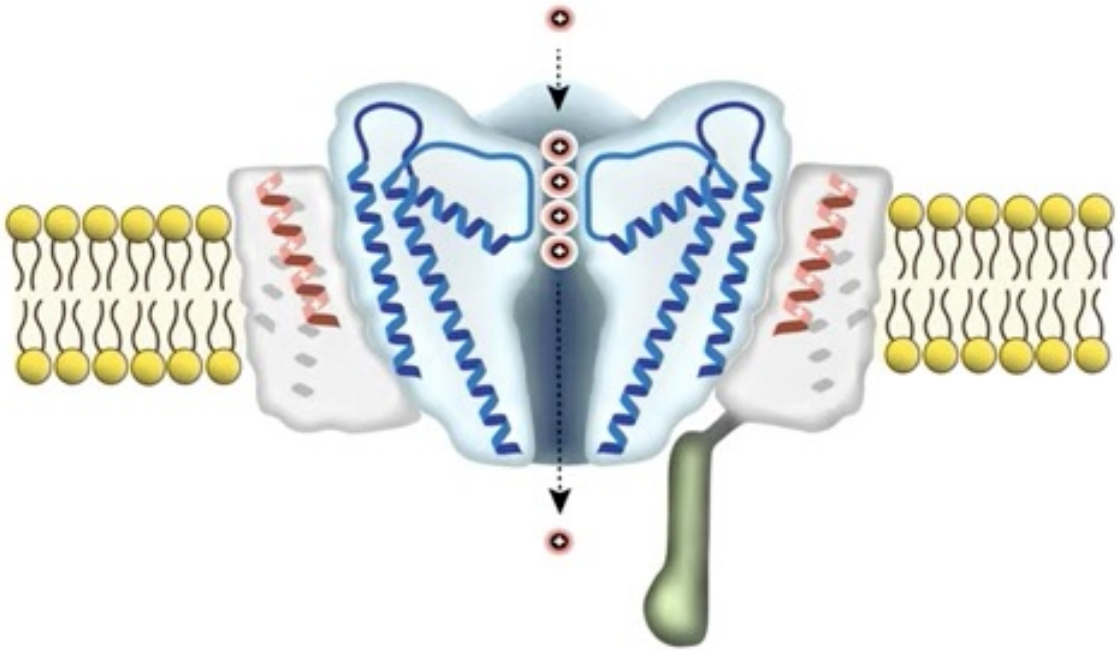
Les canaux voltage dépendants sont responsables
de la propagation du potentiel d'action:
Ils permettent de véhiculer une information

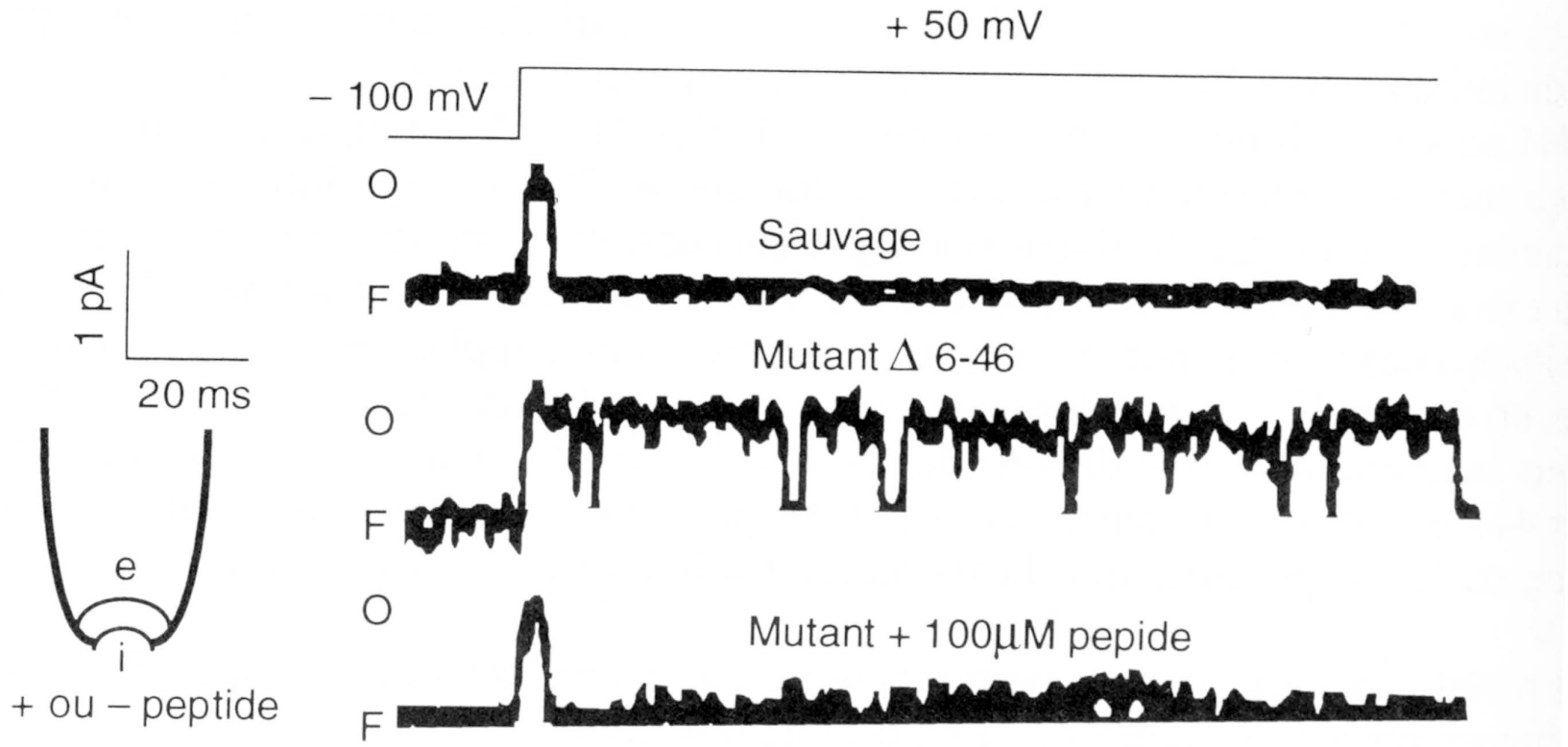


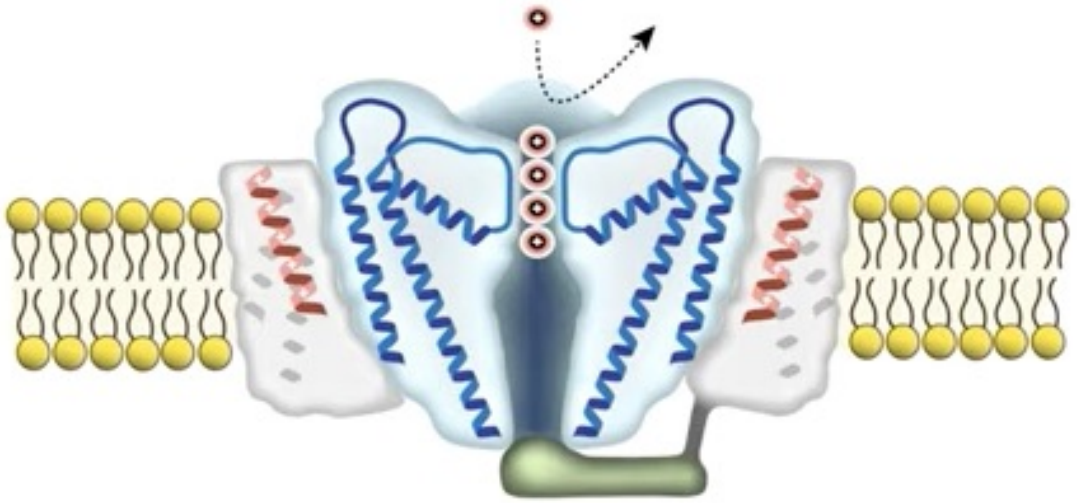
Closed



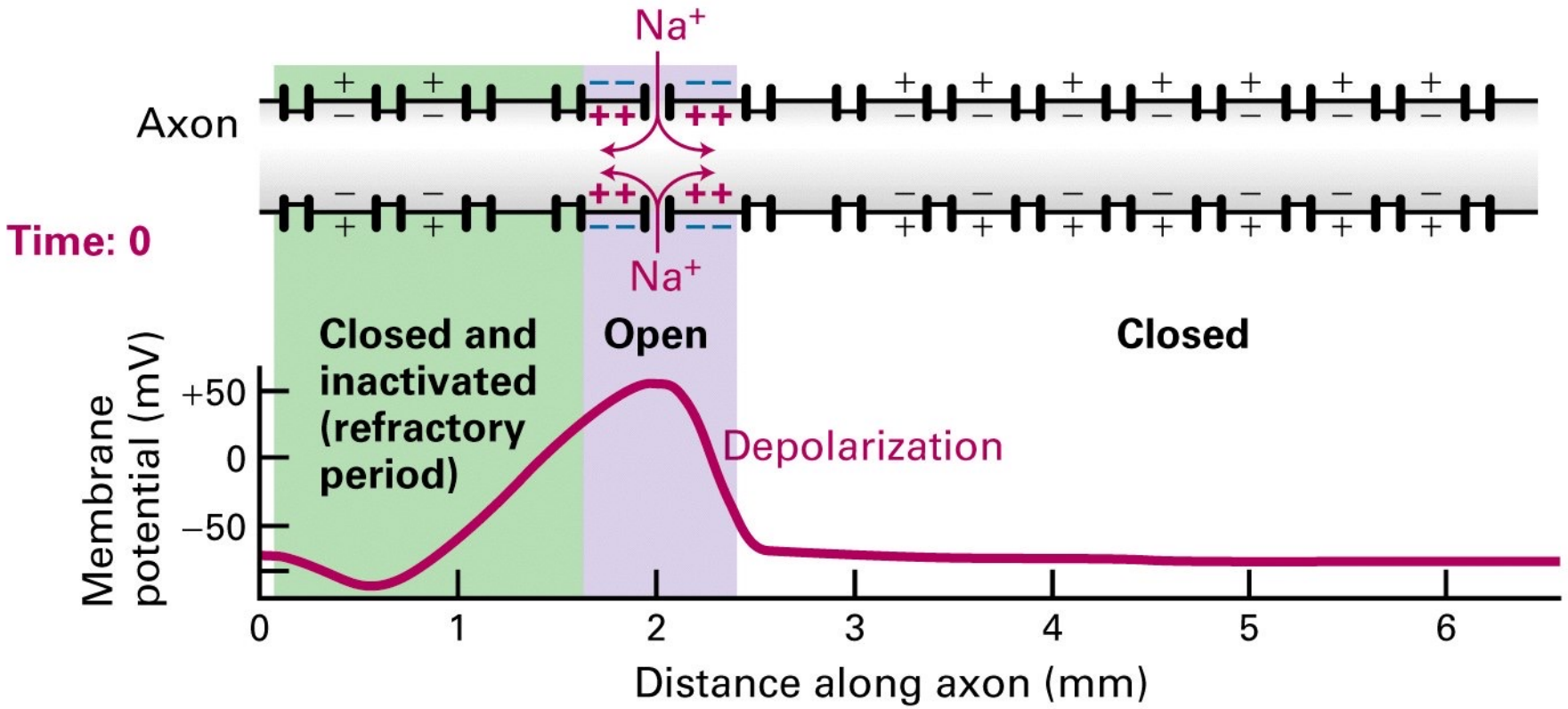
Open

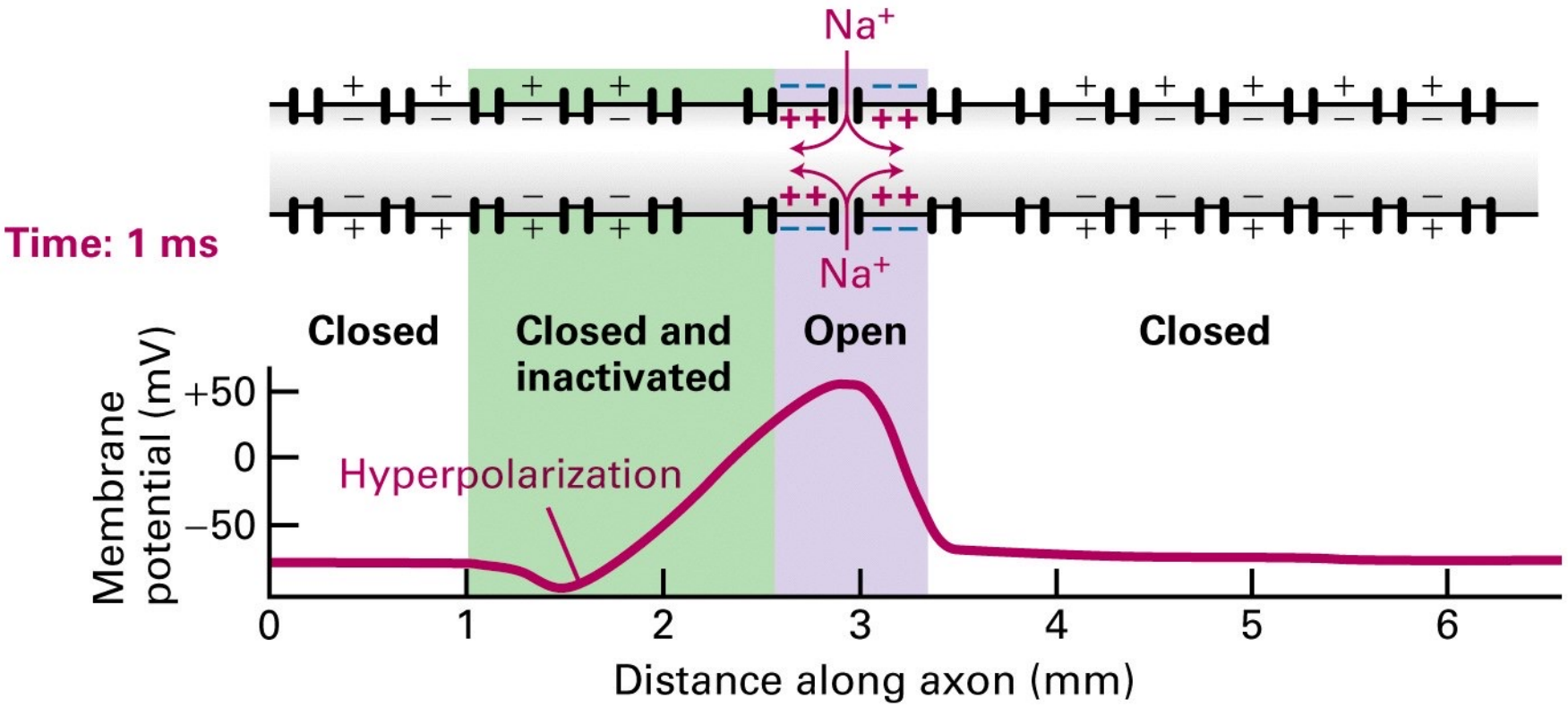


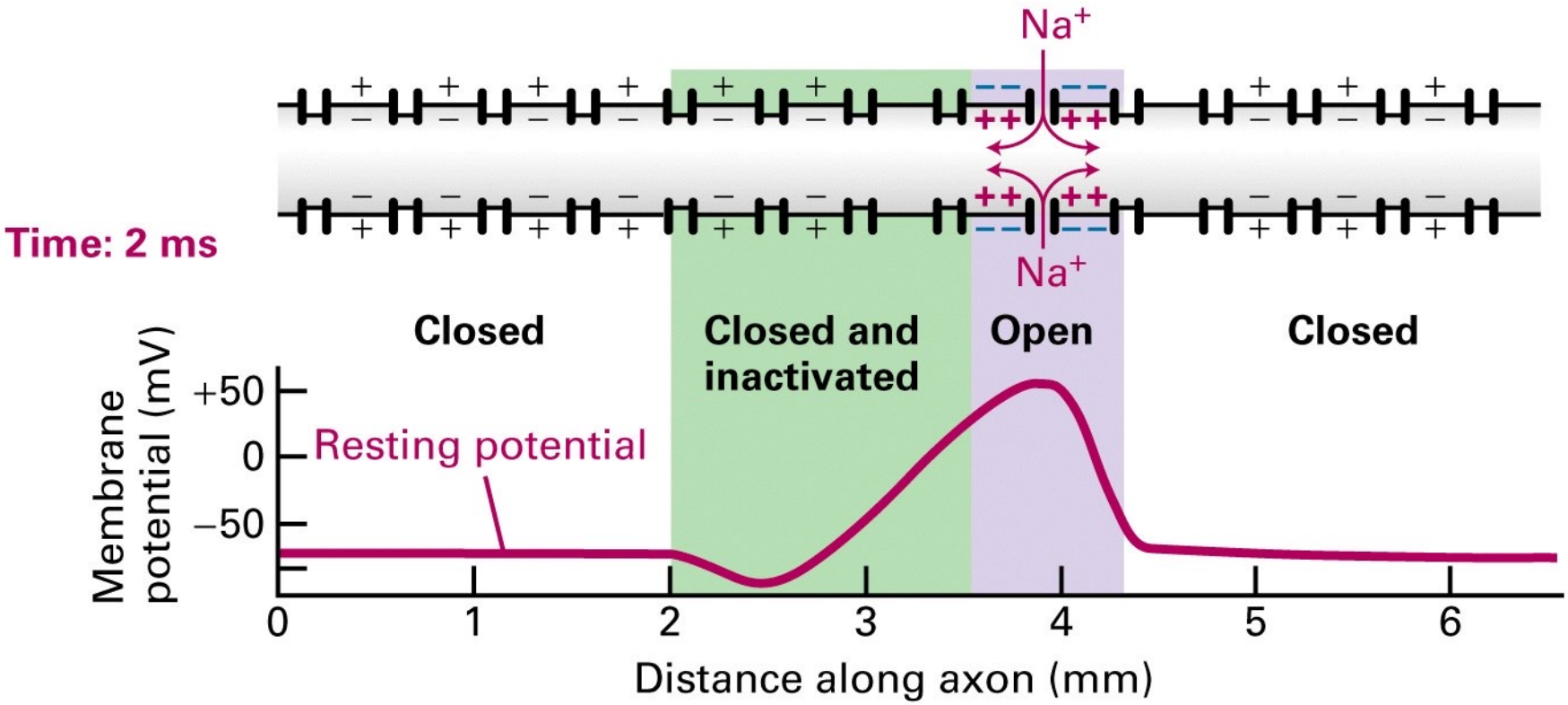

+ 50 mV
– 100 mV
O
F
Sauvage
1 pA
20 ms
Mutant Δ 6-46
O
F
e
i
+ ou – peptide
O
Mutant + 100µM pepide
F



Inactivated

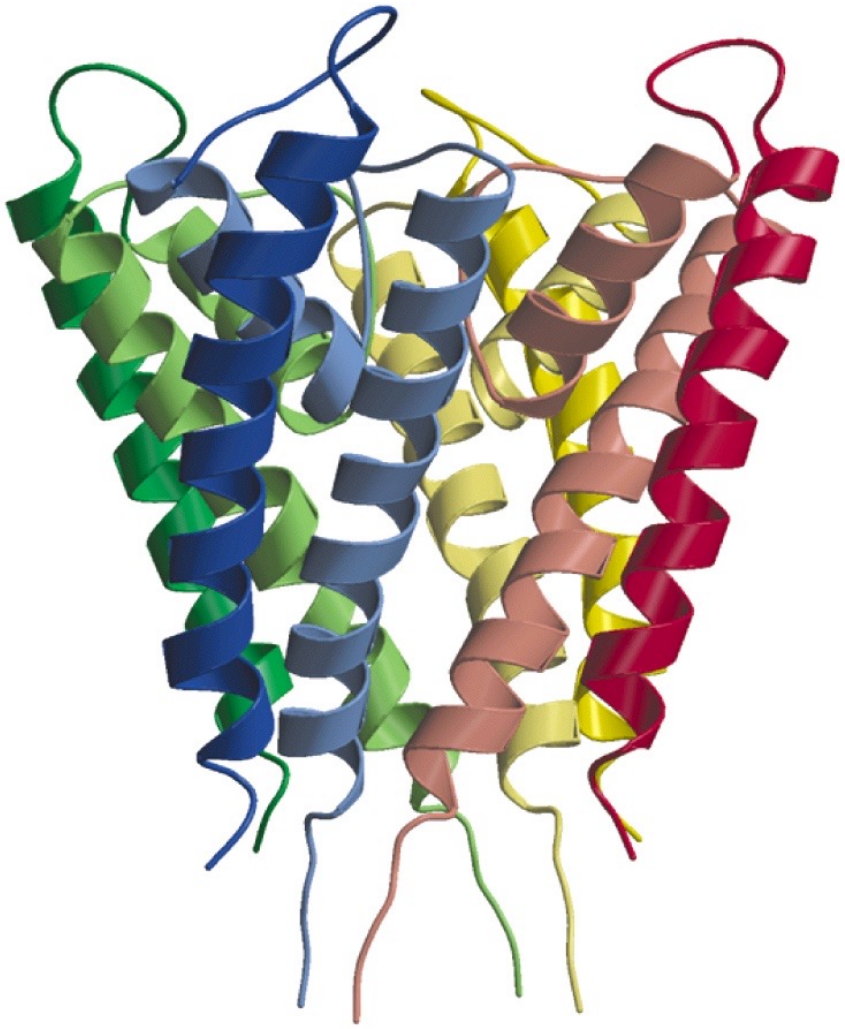

Na+
Axon
Time: 0
Na+
Closed and inactivated (refractory period)
Open
Closed
Depolarization
Membrane potential (mV)
+50
0
−50
0
1
2
3
4
5
6
Distance along axon (mm)

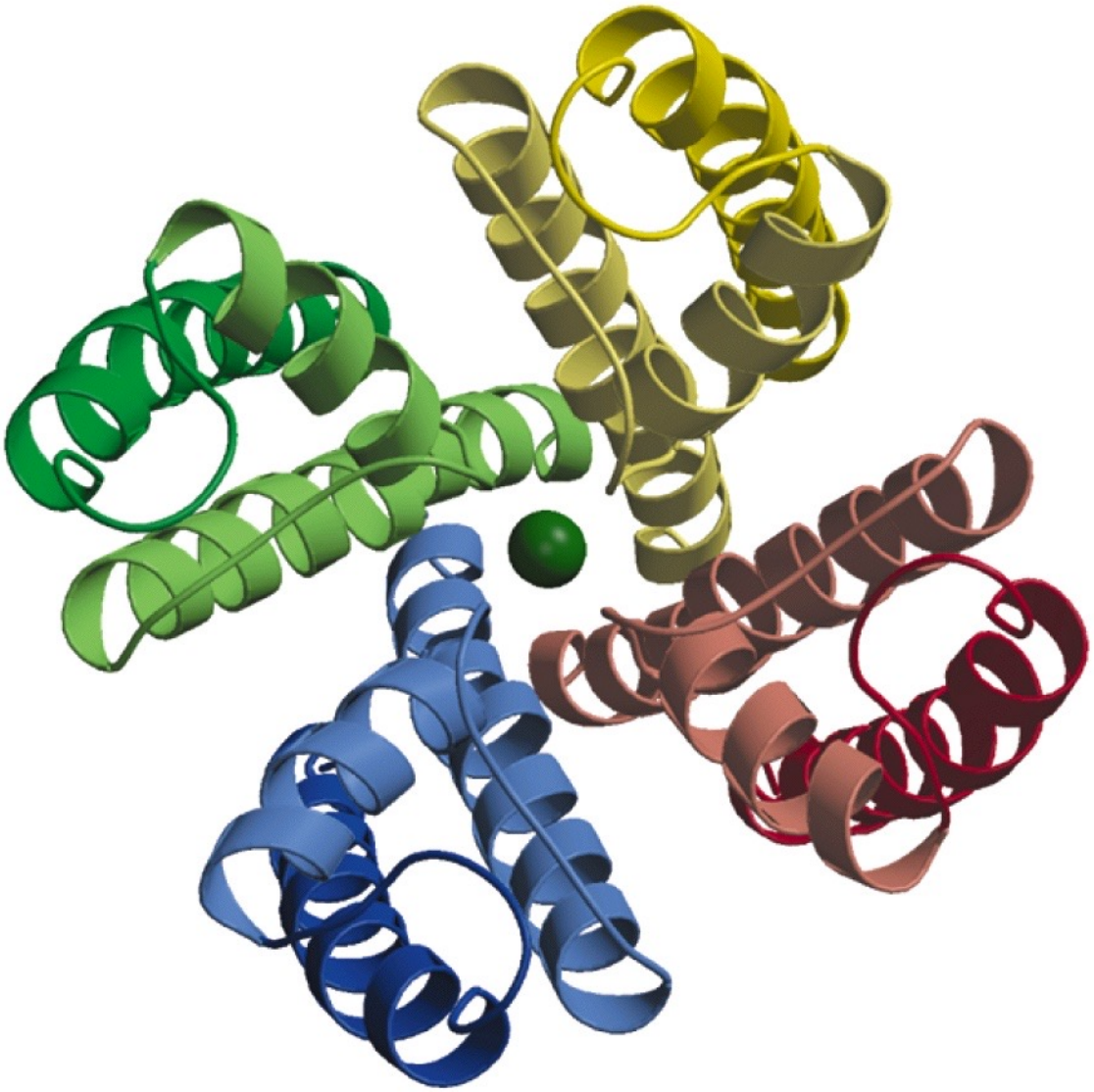

Na+
Na+
Time: 1 ms
Closed
Closed and inactivated
Open
Closed
Hyperpolarization
Membrane potential (mV)
+50
0
−50
0
1
2
3
4
5
6
Distance along axon (mm)


Time: 2 ms
Na+
Na+
Closed
Closed and inactivated
Open
Closed
Resting potential
Membrane potential (mV)
+50
0
−50
0
1
2
3
4
5
6
Distance along axon (mm)

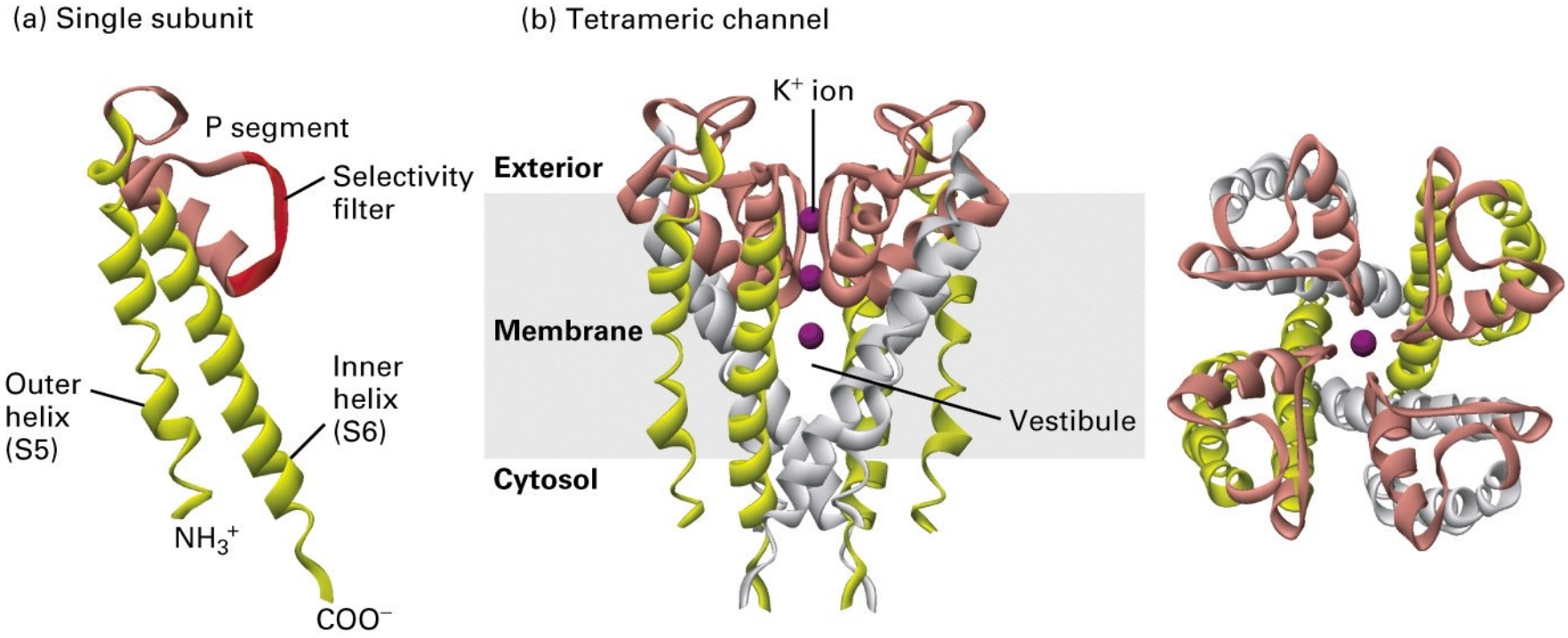
Mécanisme de sélectivité

Exemple du canal potassique KcsA

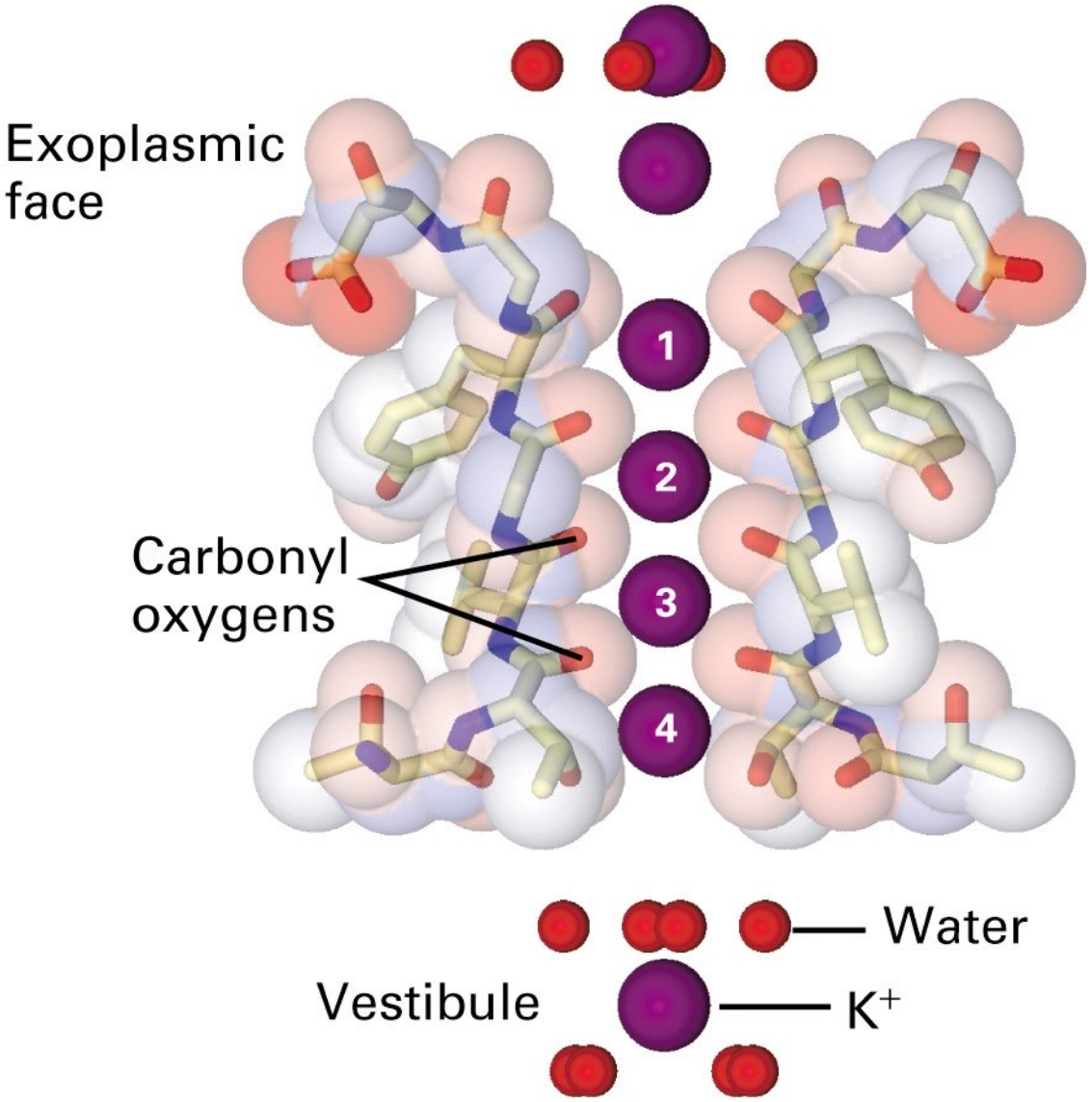




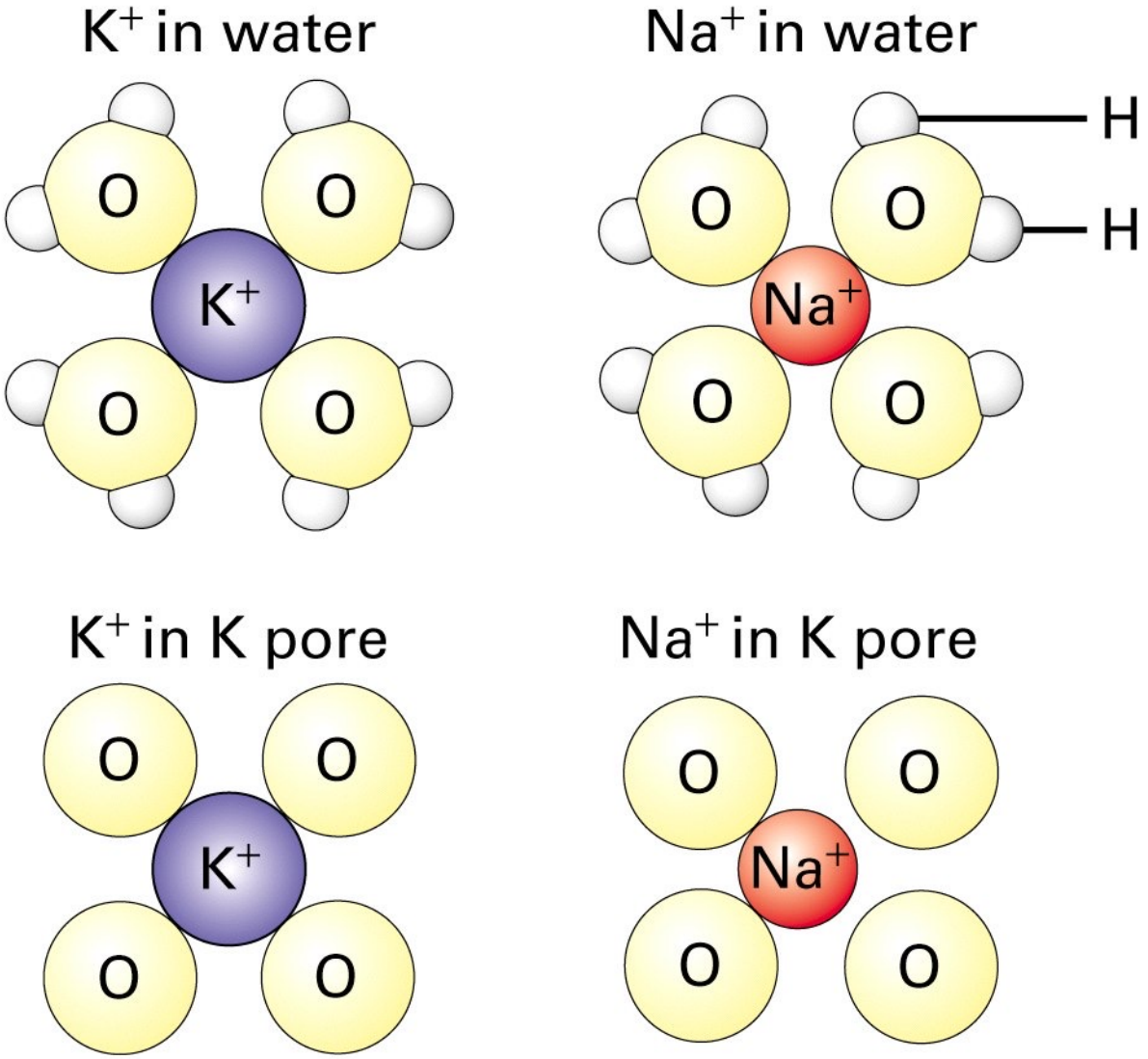
(b)


(a) Single subunit
(b) Tetrameric channel
P segment
Selectivity filter
Outer helix (S5)
Inner helix (S6)
NH_3^+
COO^-
K^+ ion
Exterior
Membrane
Cytosol
Vestibule

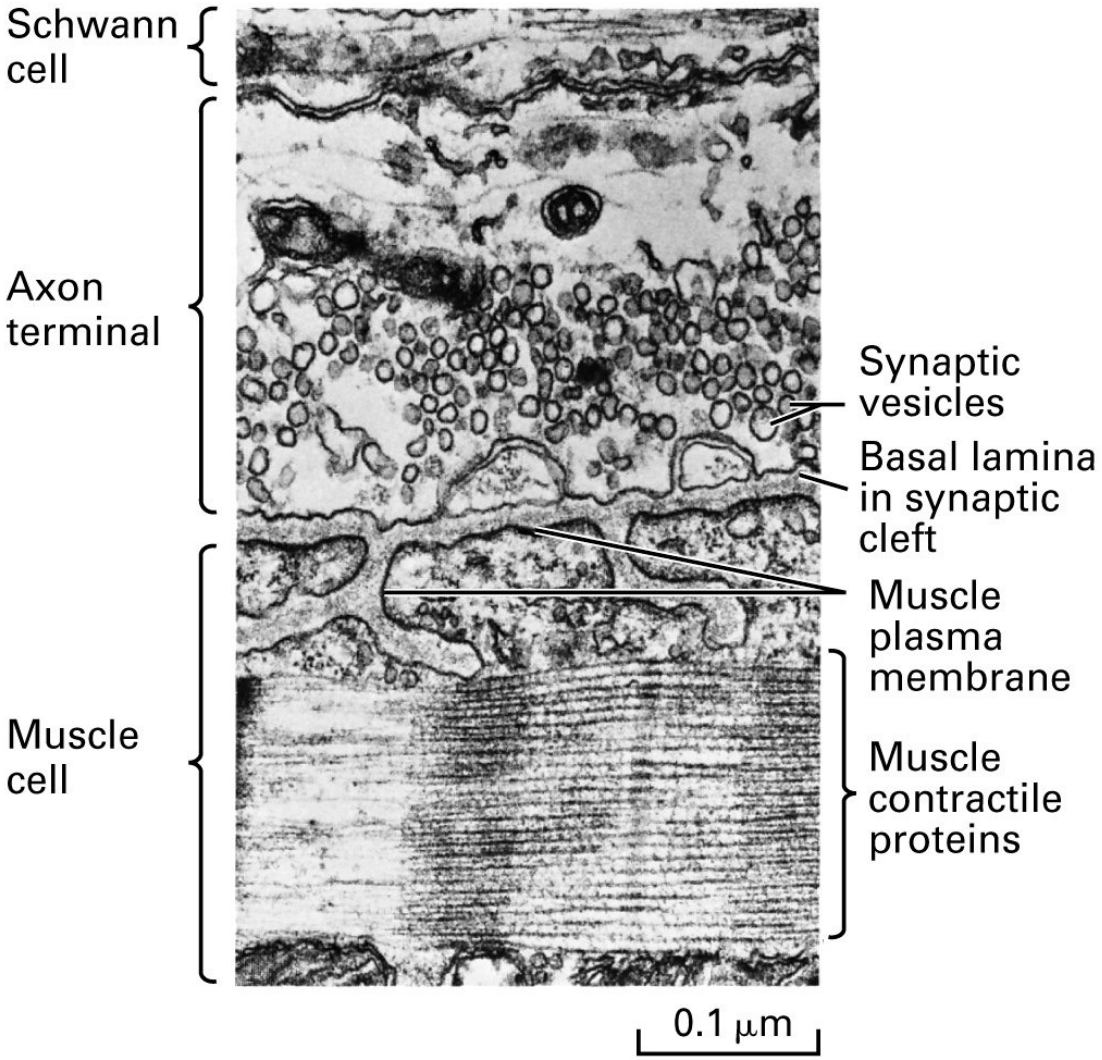
(b) K^+ ions in the pore of a K^+ channel (side view)

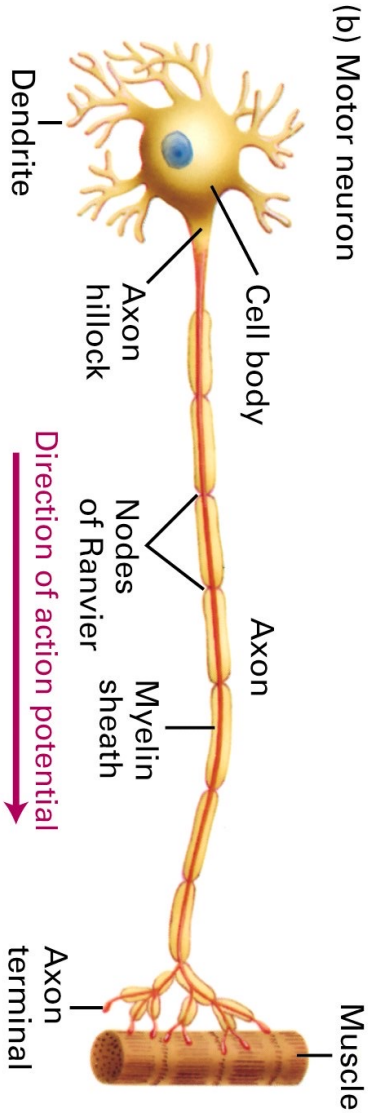


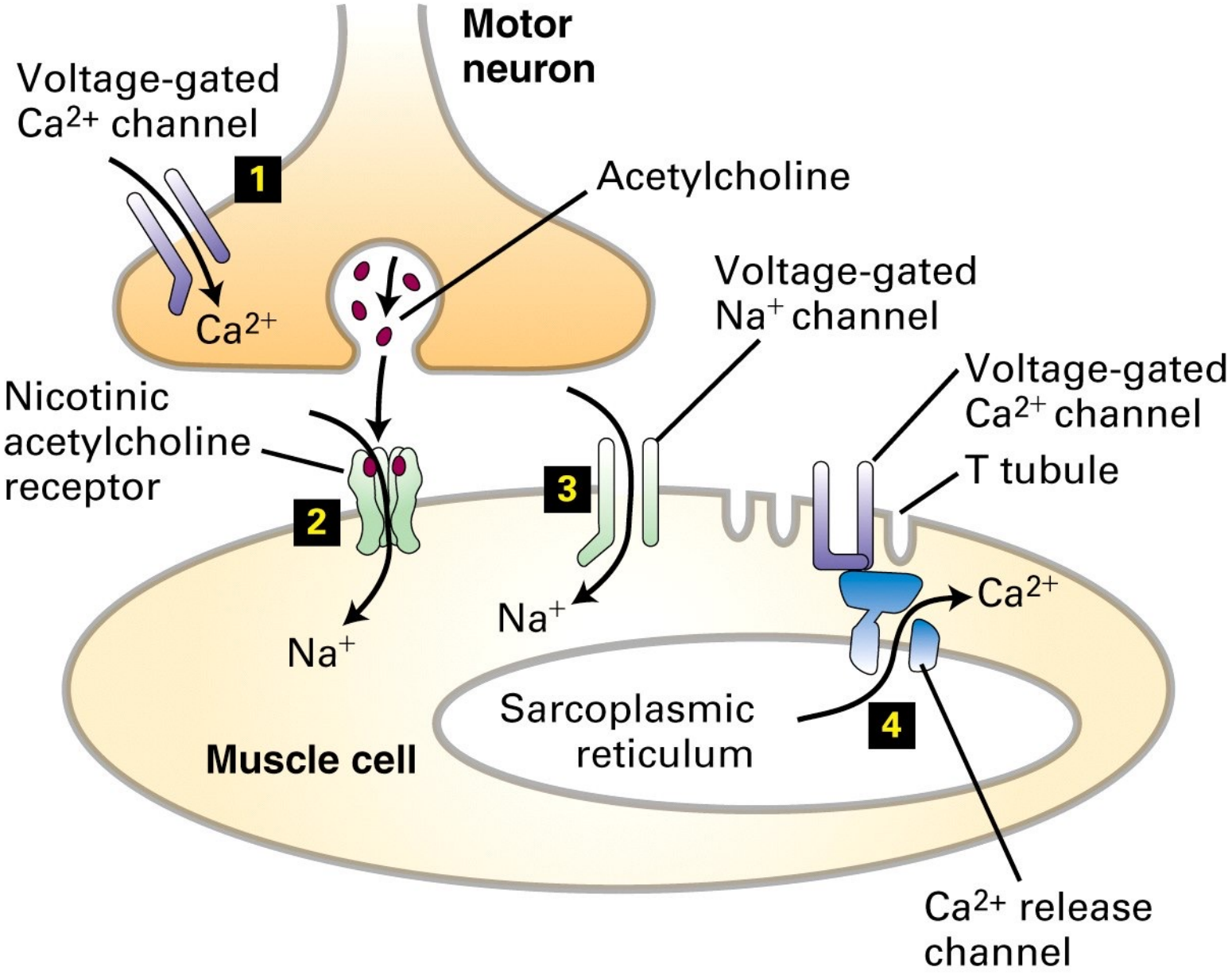
(a) K^+ and Na^+ ions in the pore of a K^+ channel (top view)

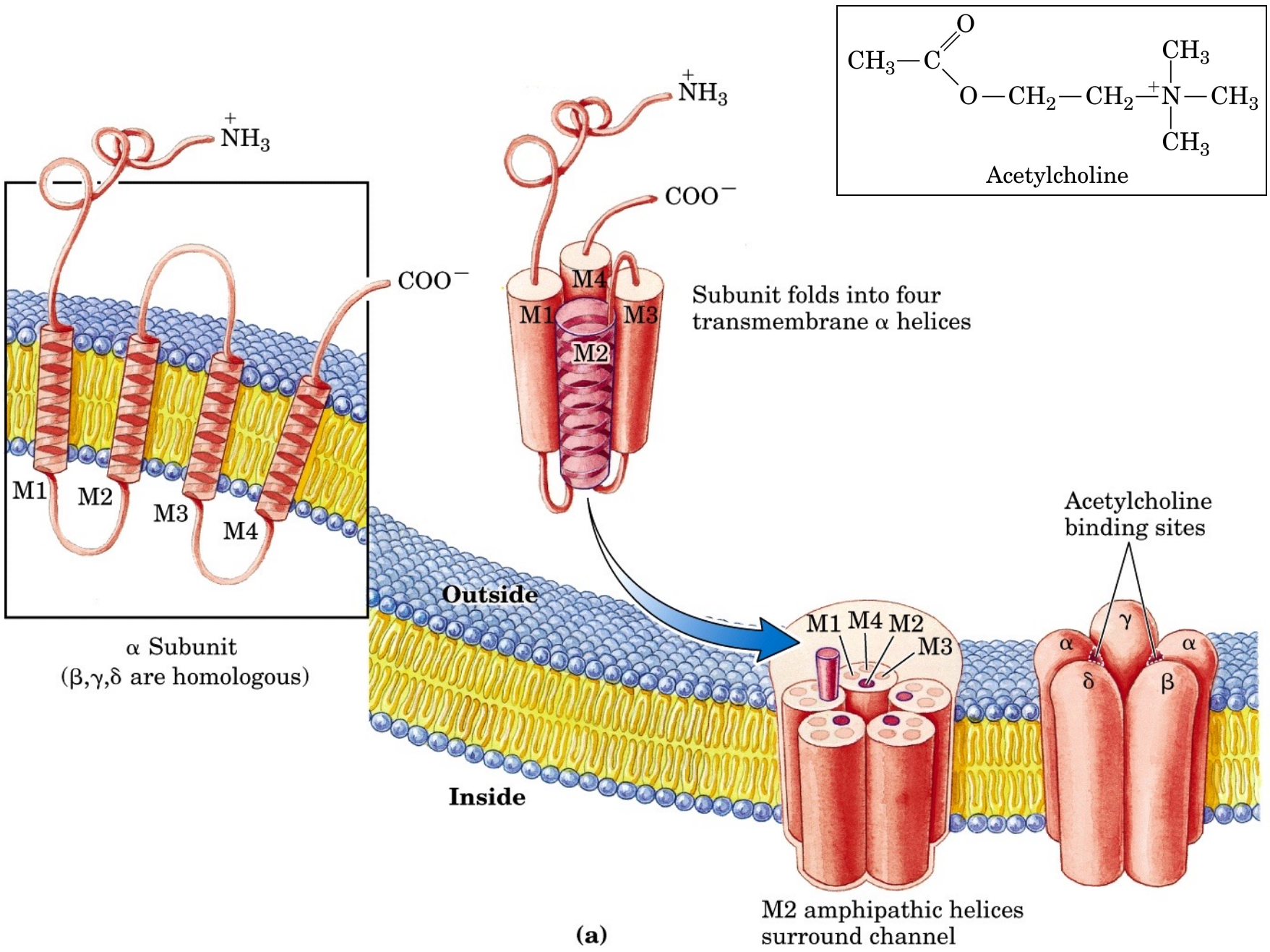


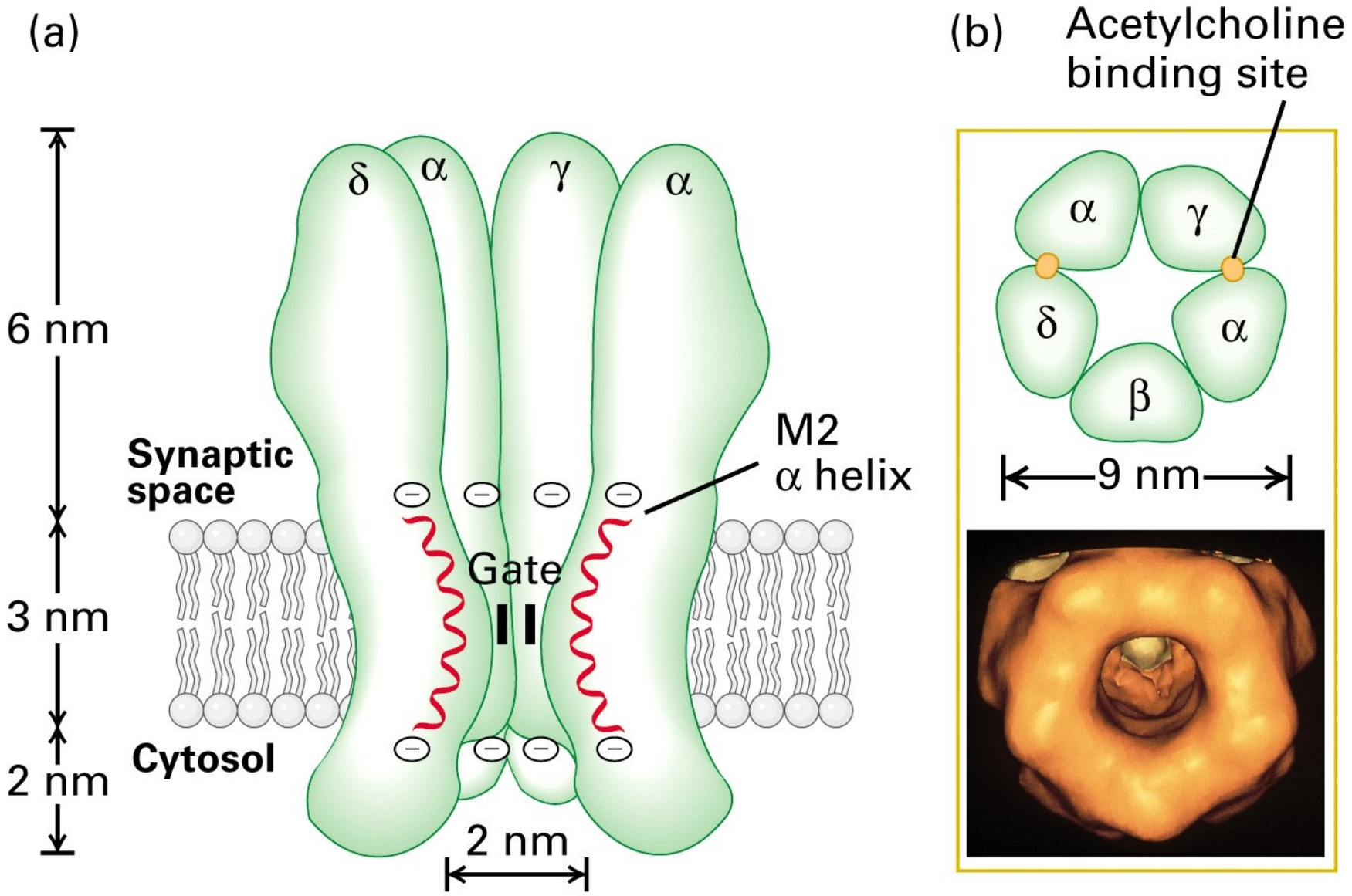
Canaux et transmission synaptiques



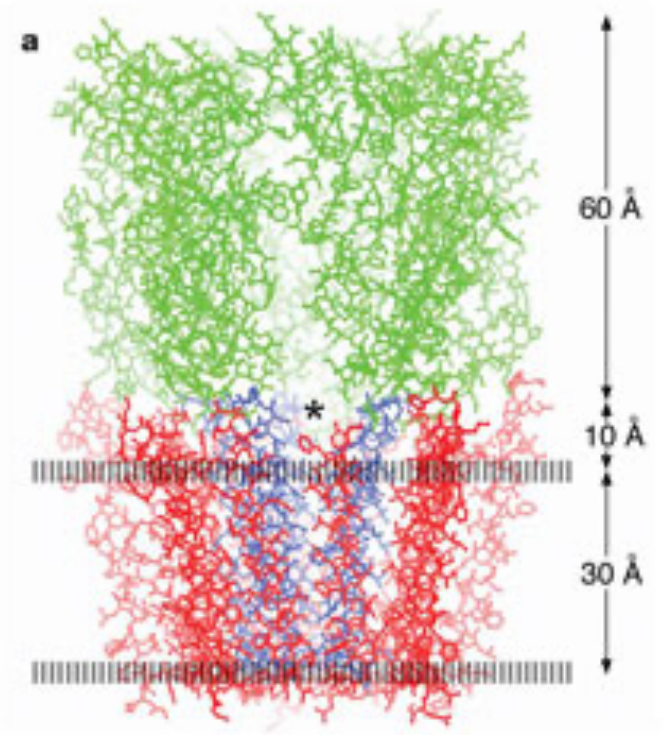


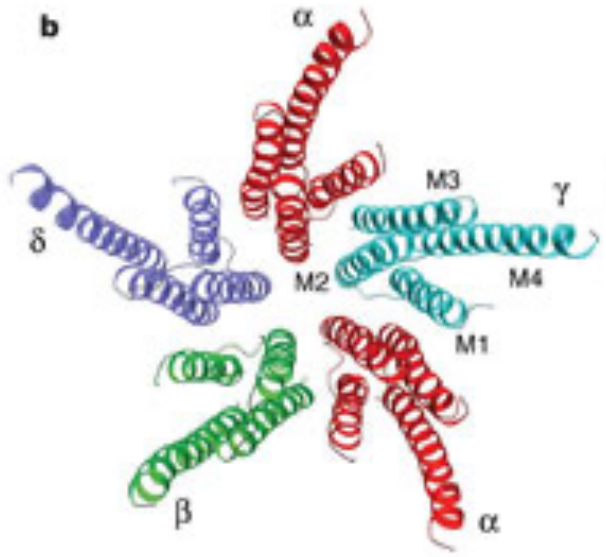

Motor neuron
Voltage-gated Ca^{2+} channel
1
Acetylcholine
Ca^{2+}
Voltage-gated Na^{+} channel
Voltage-gated Ca^{2+} channel
T tubule
Nicotinic acetylcholine receptor
2
3
Na^{+}
Na^{+}
Ca^{2+}
Sarcoplasmic reticulum
4
Muscle cell
Ca^{2+} release channel

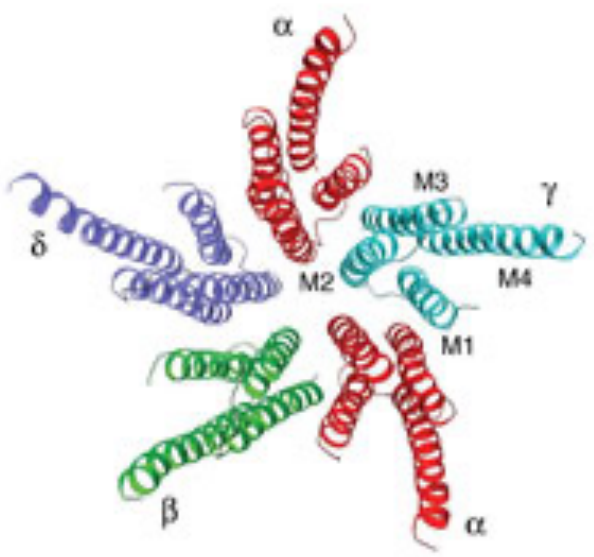

Acetylcholine
NH3+
COO−
M1
M2
M3
M4
α Subunit
(β,γ,δ are homologous)
Subunit folds into four
transmembrane α helices
Outside
Inside
Acetylcholine
binding sites
M1 M4 M2
M3
α
γ
α
δ
β
M2 amphipathic helices
surround channel
(a)

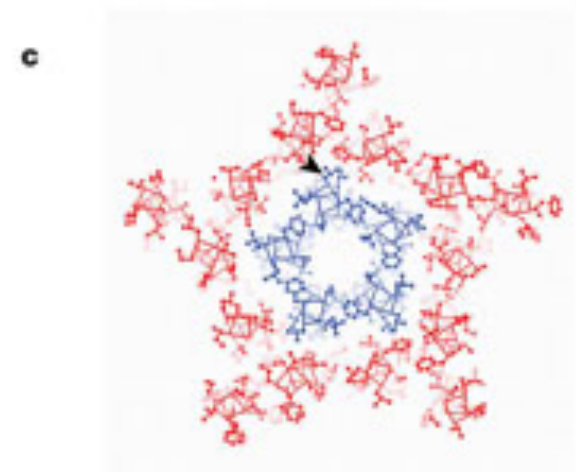

(a)
δ
α
γ
α
6 nm
3 nm
2 nm
Synaptic space
M2 α helix
Gate
Cytosol
2 nm
(b)
Acetylcholine binding site
α
γ
δ
α
β
9 nm

Structure du pore du récepteur nicotinique à l'acétylcholine (4 Å de résolution)





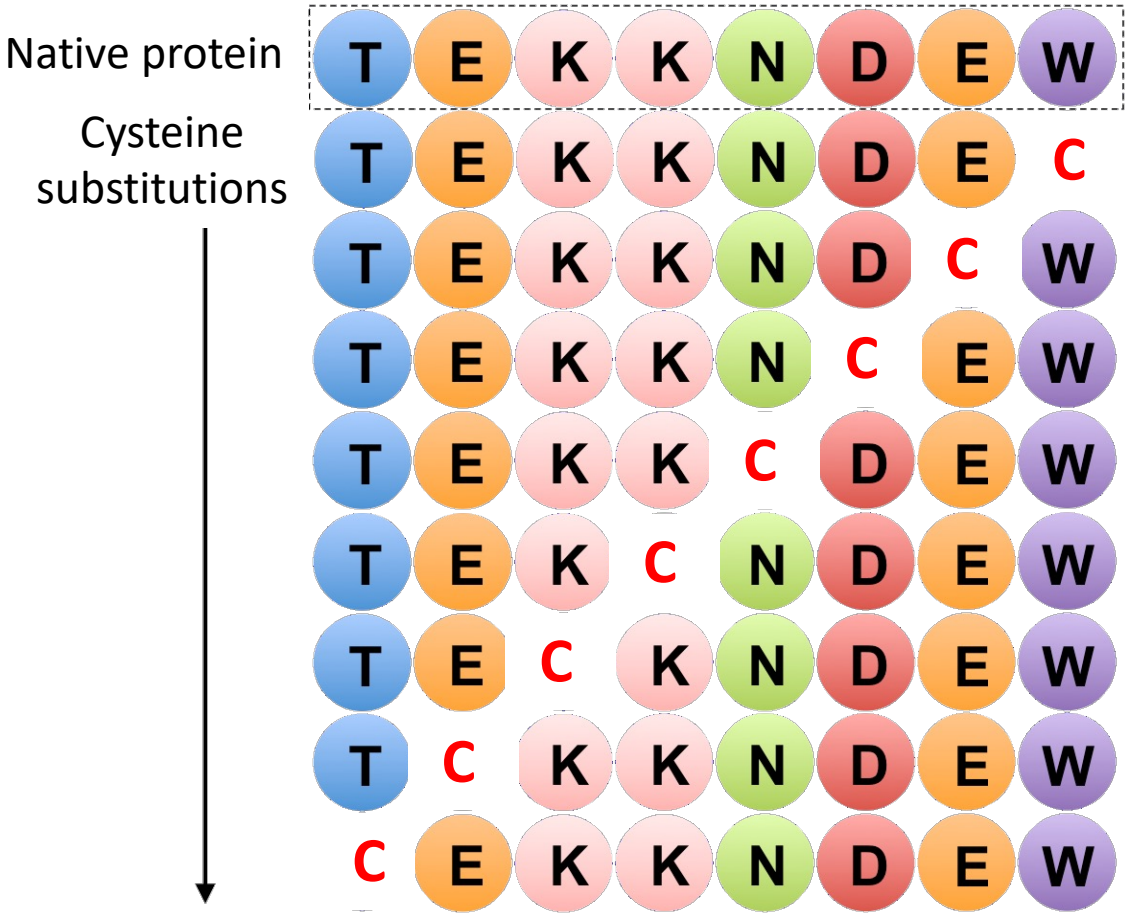




(Miyazawa et al (2003) *Nature*)

Comment étudier l'ouverture du canal ?

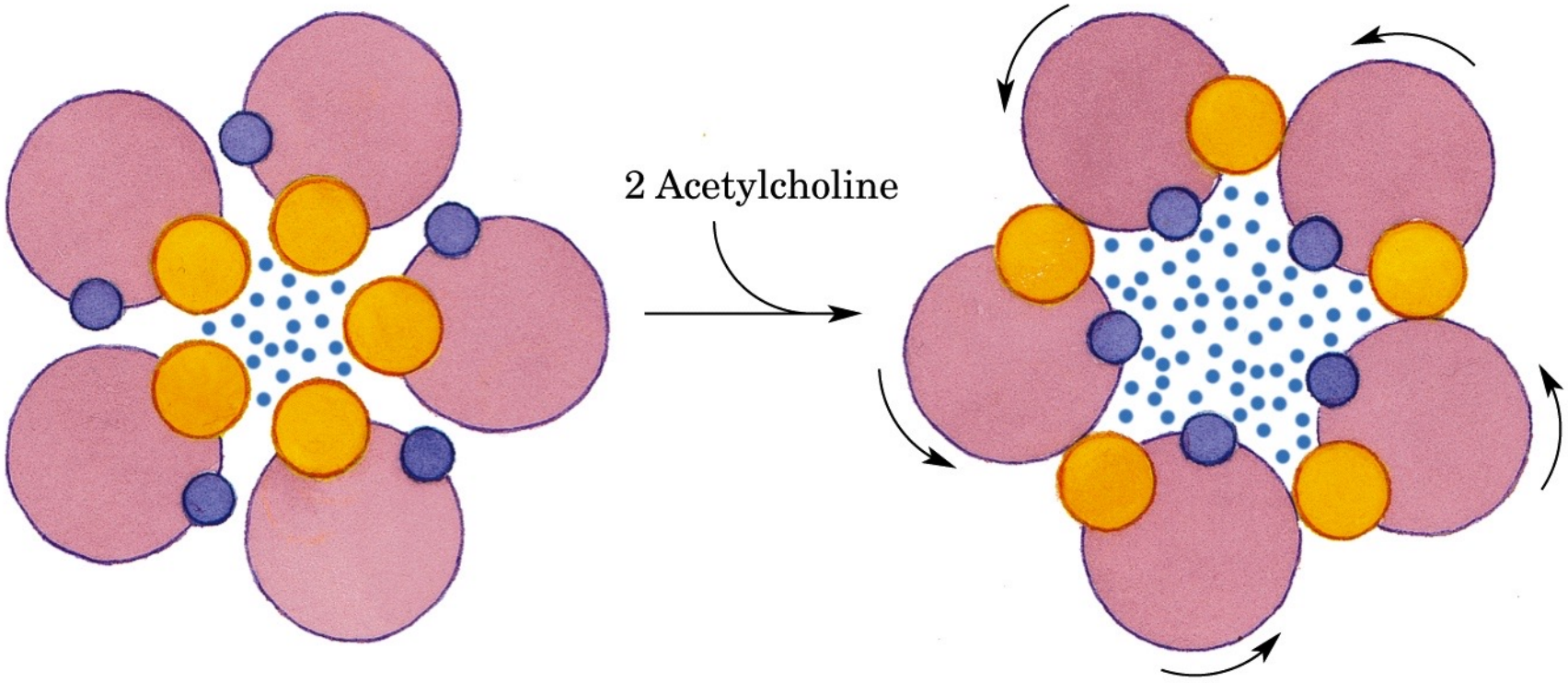
- Cysteine scaning mutagenesis (cf TD)



Bulky hydrophobic Leu side chains of M2 helices close the channel.

Binding of two acetylcholine molecules causes twisting of the M2 helices.

With receptor sites occupied, the M2 helices have smaller, polar residues lining the channel.

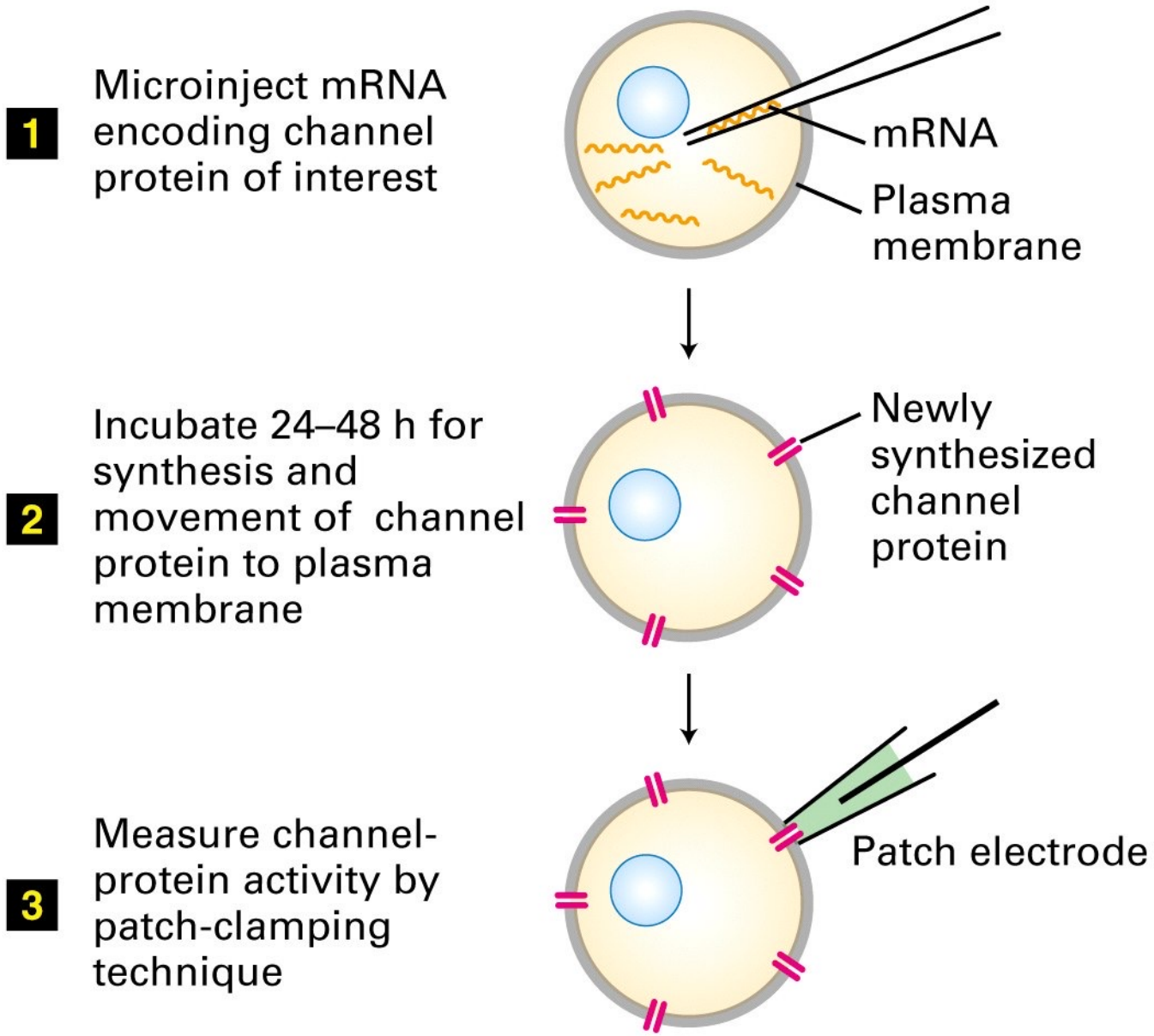


(b)

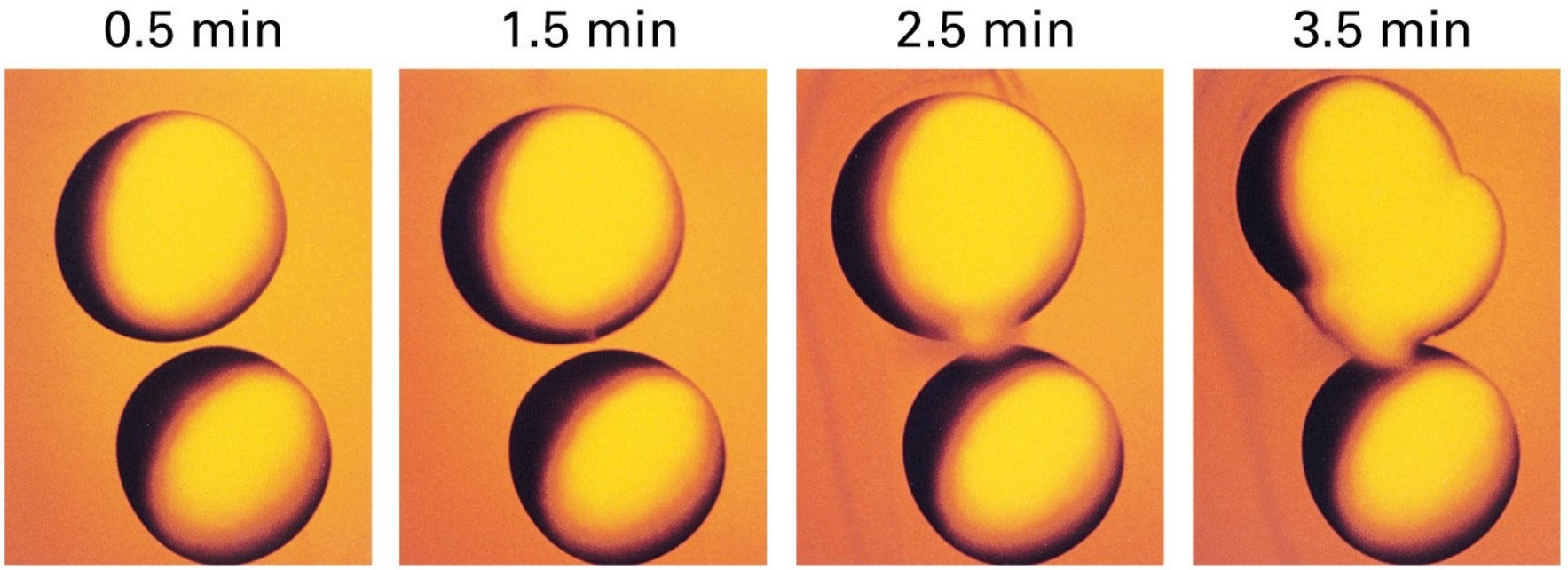
Canaux non ioniques

PORINES et ……..

…….AQUAPORINES

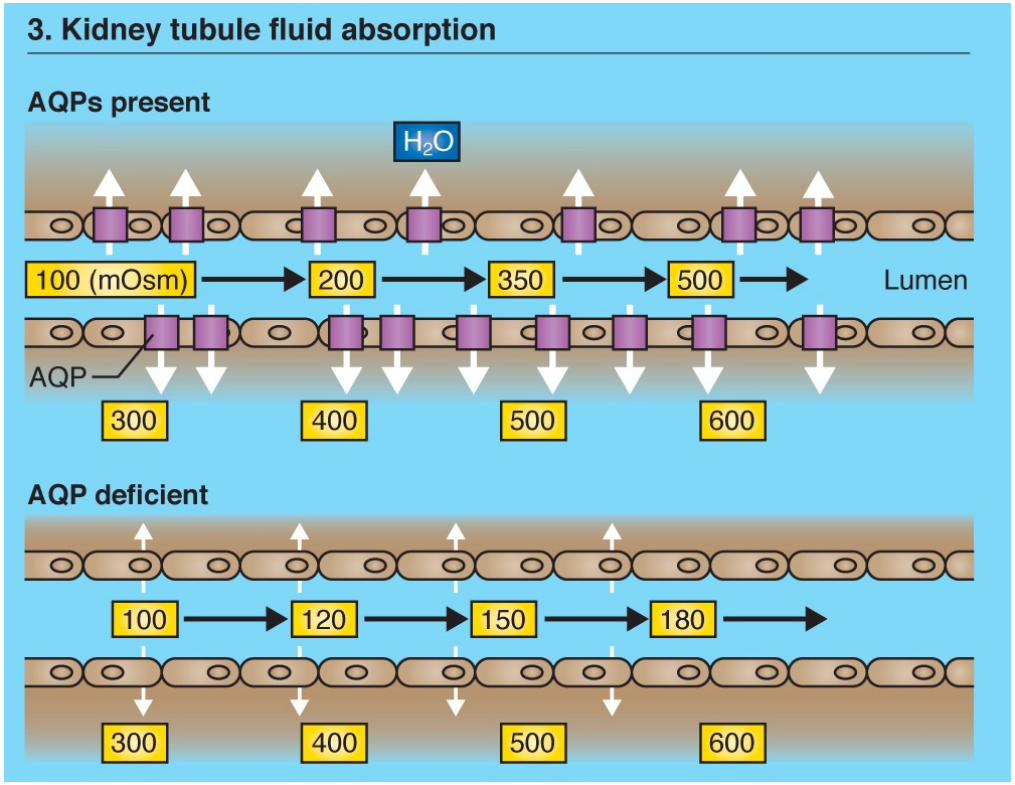

1
Microinject mRNA encoding channel protein of interest
mRNA
Plasma membrane
2
Incubate 24–48 h for synthesis and movement of channel protein to plasma membrane
Newly synthesized channel protein
3
Measure channel-protein activity by patch-clamping technique
Patch electrode

Il existe des canaux à eau



Transfert des ovocytes dans un milieu hypoosmotique

Les AQP ont un rôle important dans les tissus où l'absorption d'eau est importante



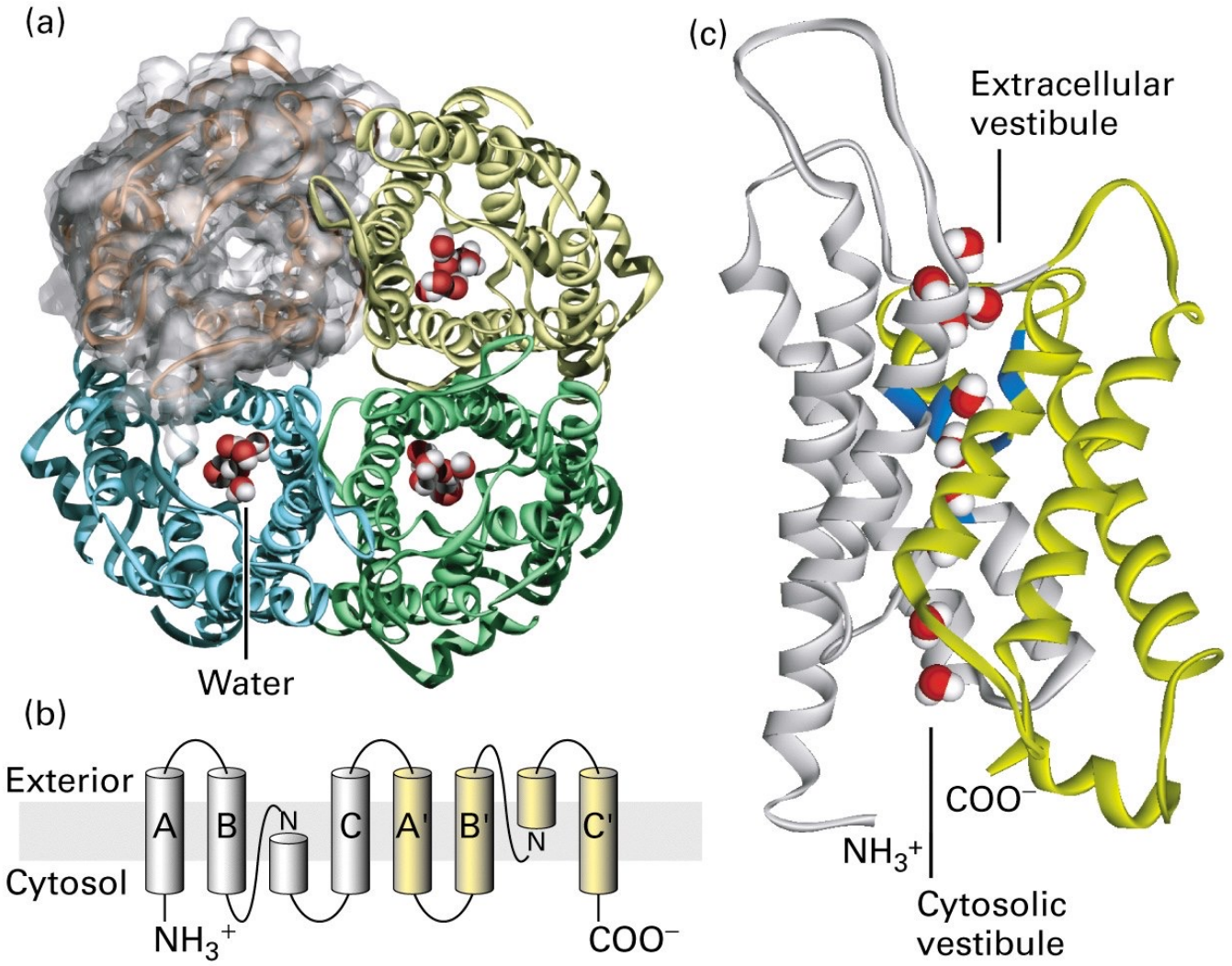
Augmentent perméabilité à l'eau
d'un facteur important (5 mini ou 50 maxi)
> 1000 canaux par μm^2

Régulation de AQP2

- Adressage de AQP2 vers le tube collecteur des reins est controlé par la vasopressine (regulation traffic endosomes, similaire à GluT4)

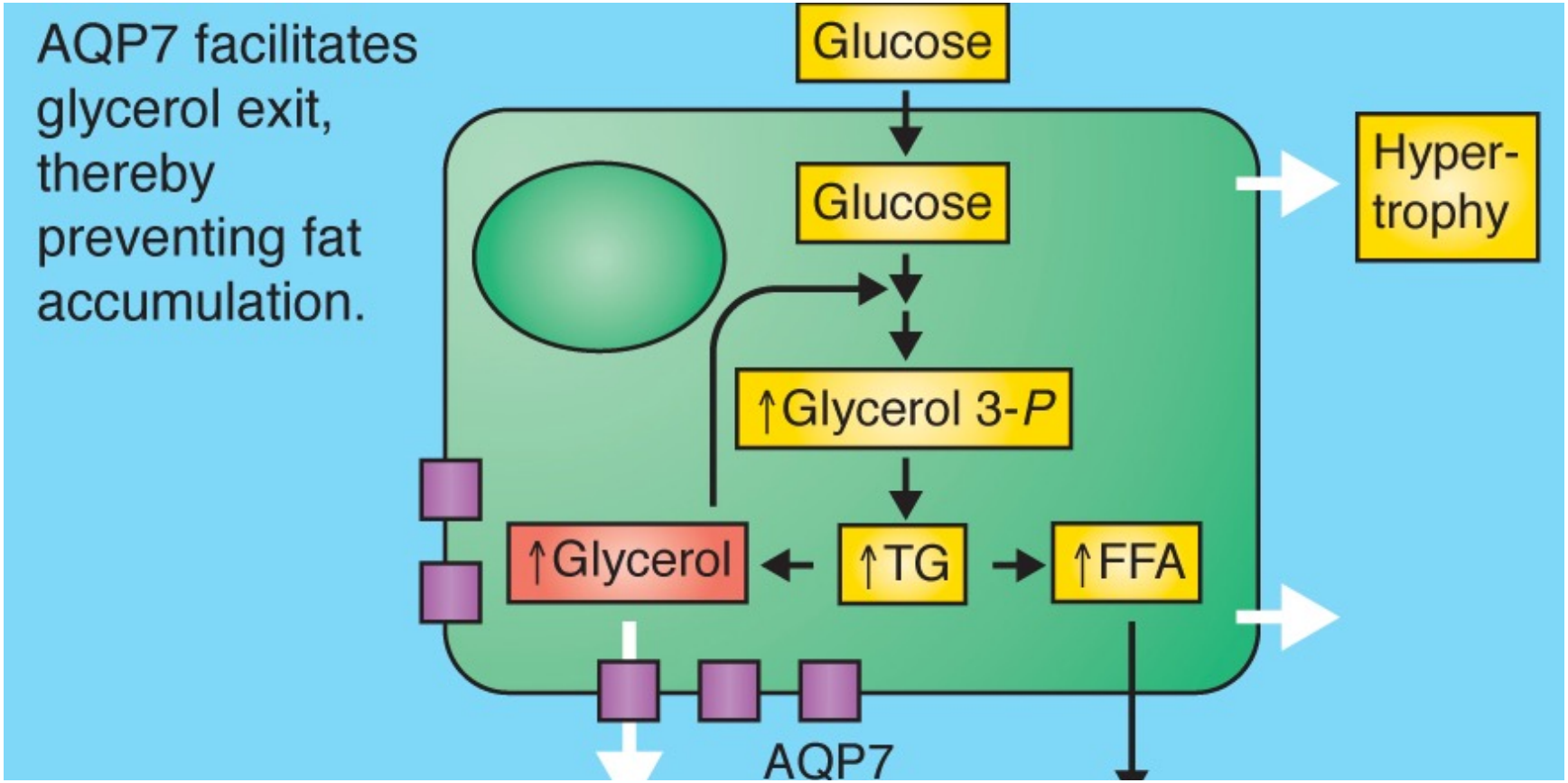
- Effet antidiuretique, donc rétention d'eau

Structure tetramérique / specifique de H_2O

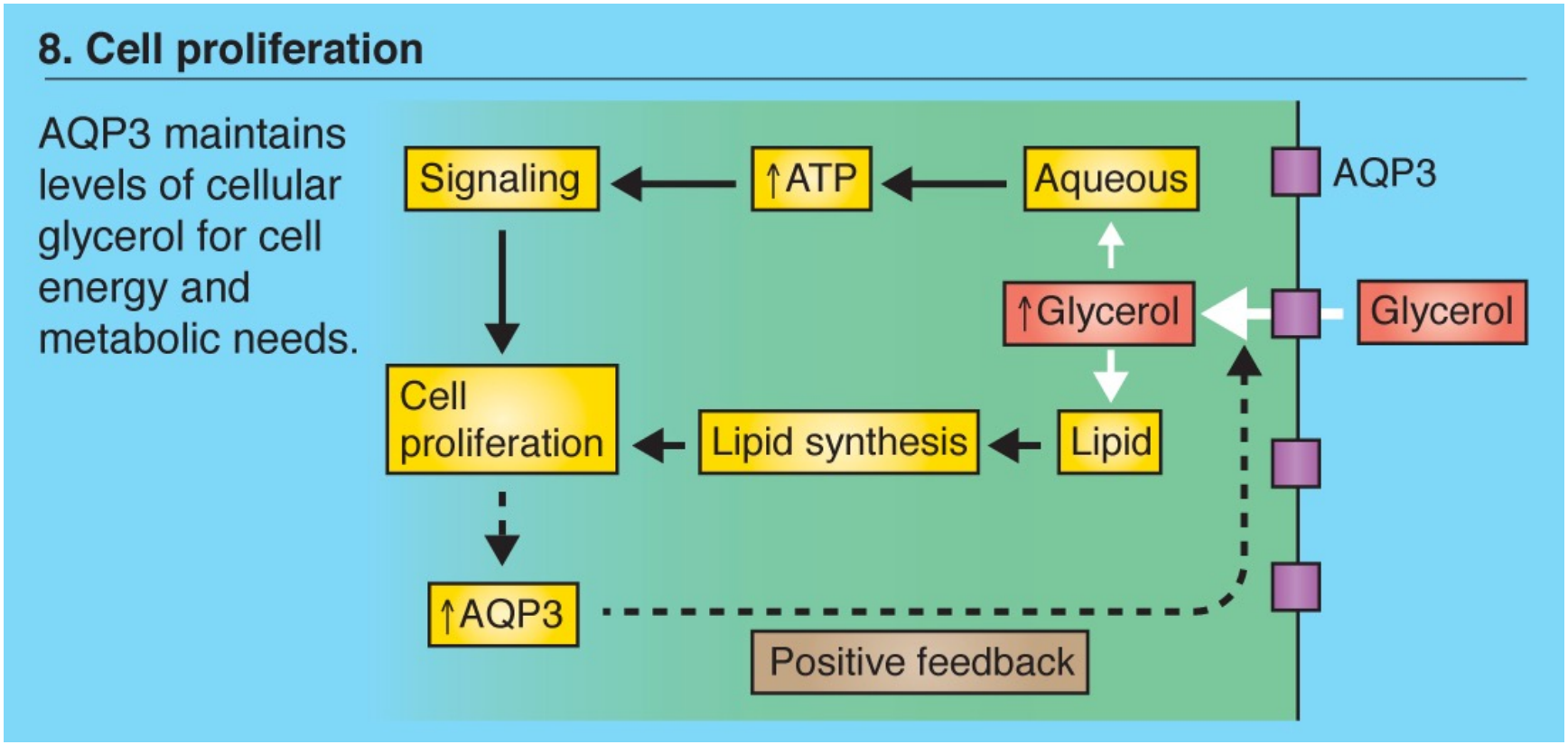


Les transporteurs de Glycerol font partie de la même famille que les AQP

Ils peuvent avoir un rôle dans les adipocytes…….



….. et dans la prolifération cellulaire



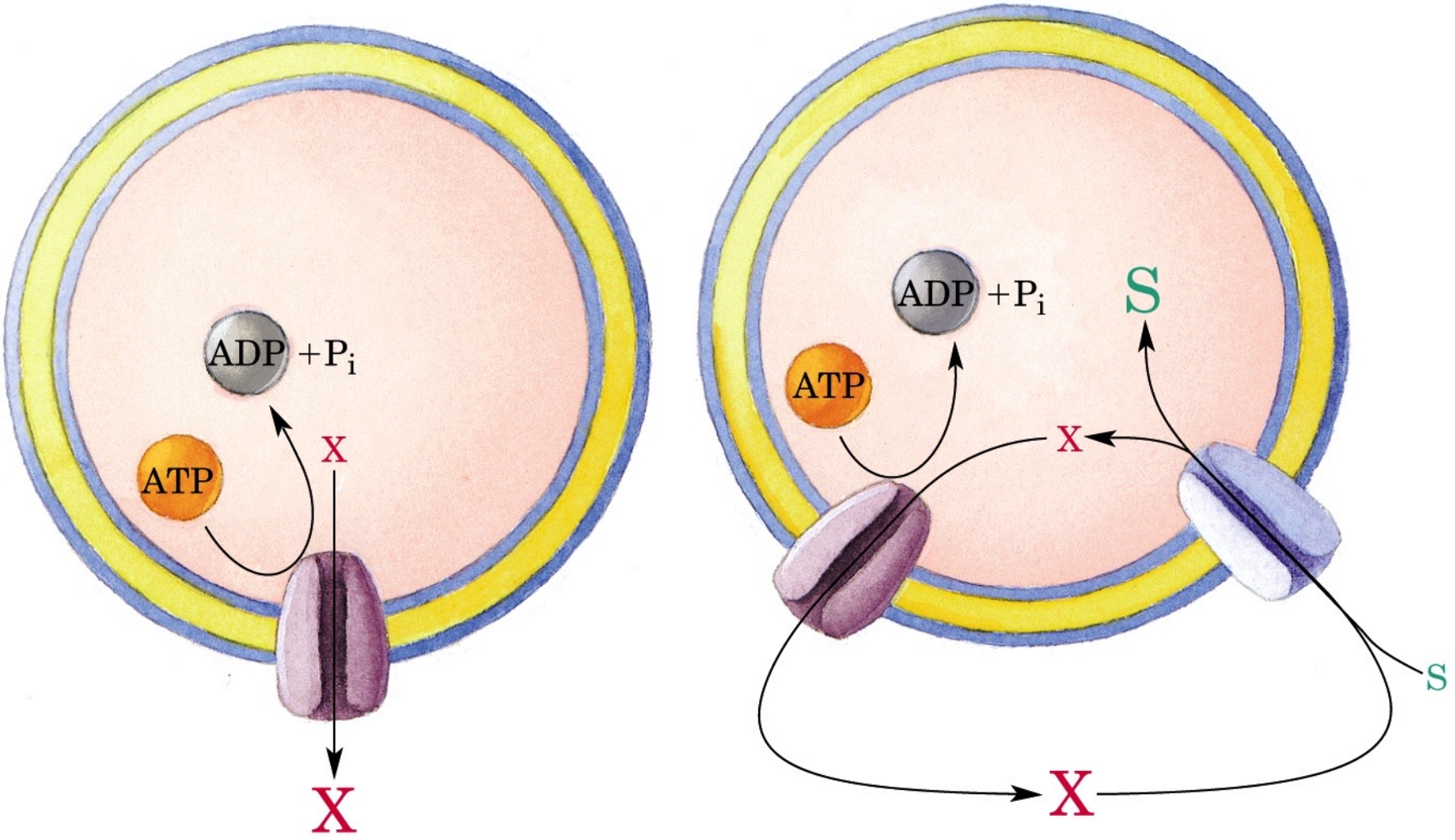
Biochimie Membranaire

Introduction - Rôles et importance des membranes biologiques

1- Topologie et Structure des protéines membranaires

2- Structure et fonction des canaux et transporteurs

3- Dynamique membranaire



Primary active transport
(a)

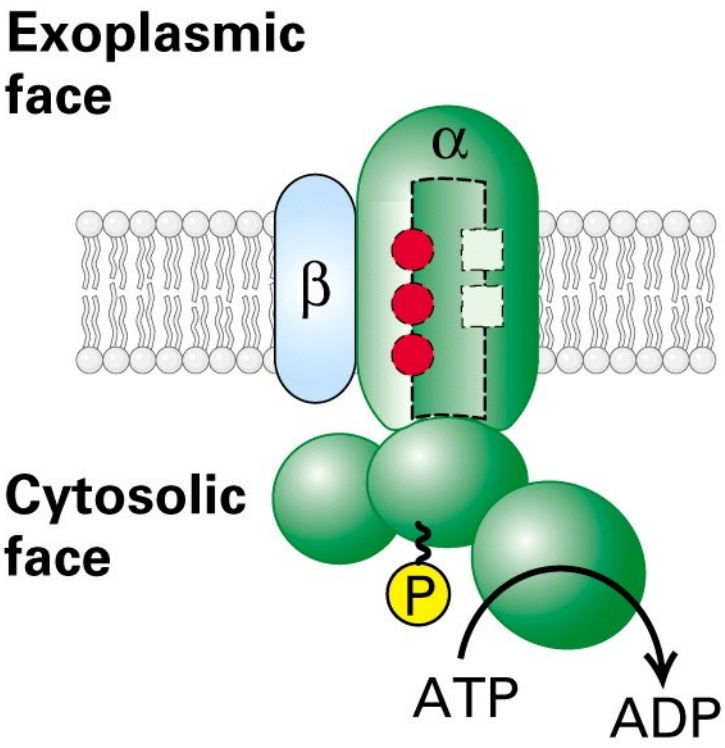
Secondary active transport
(b)

TABLE 7-2 Typical Intracellular and Extracellular Ion Concentrations

Ion	Cell (mM)	Blood (mM)
MAMMALIAN CELL (VERTEBRATE)		
K^+	139	4
Na^+	12	145
Cl^-	4	116
HCO_3^-	12	29
X^-	138	9
Mg^{2+}	0.8	1.5
Ca^{2+}	<0.0002	1.8

*The large nerve axon of the squid has been widely used in studies of the mechanism of conduction of electric impulses.

†X^- represents proteins, which have a net negative charge at the neutral pH of blood and cells.



P-class pumps

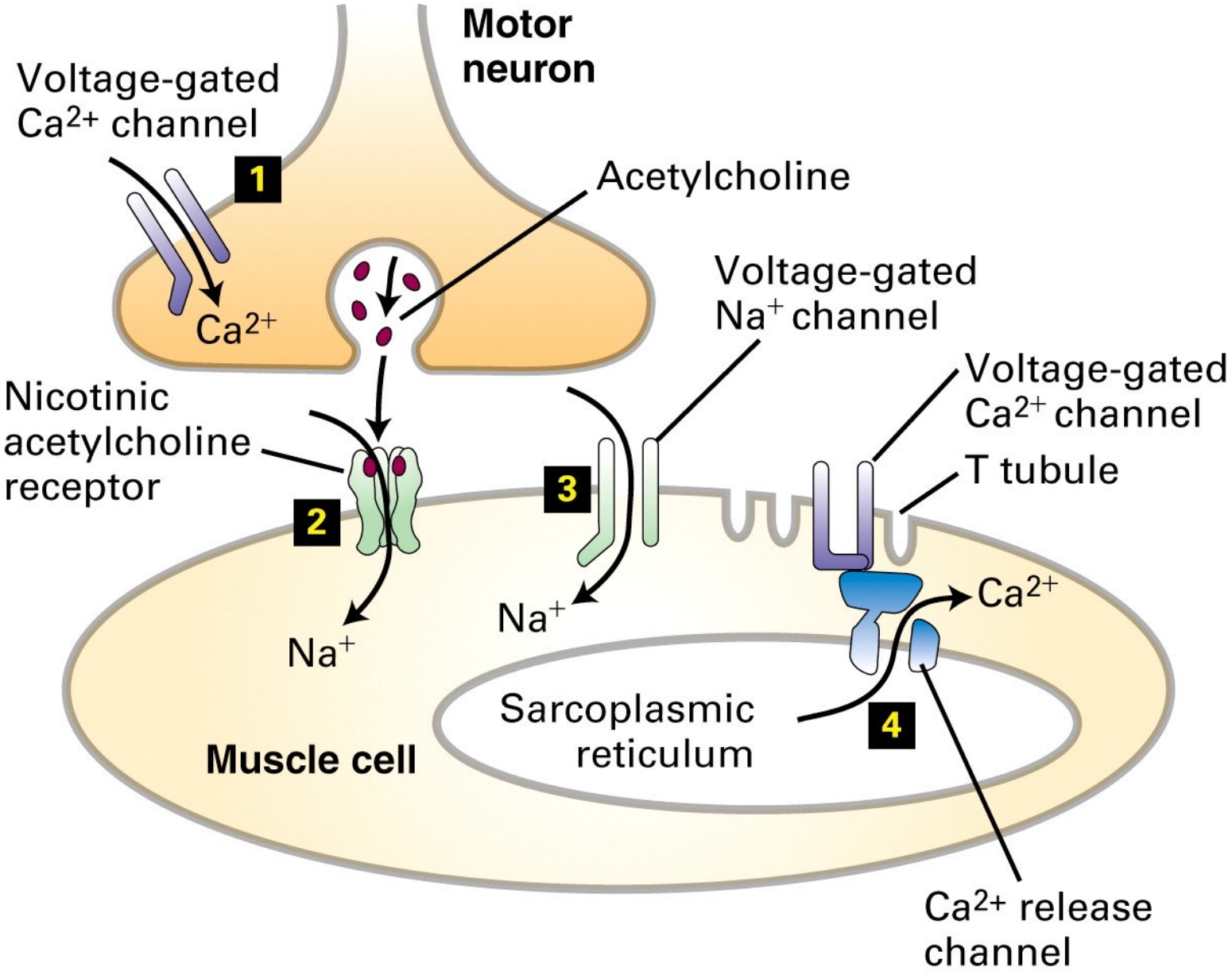
Plasma membrane of plants, fungi, bacteria (H^+ pump)

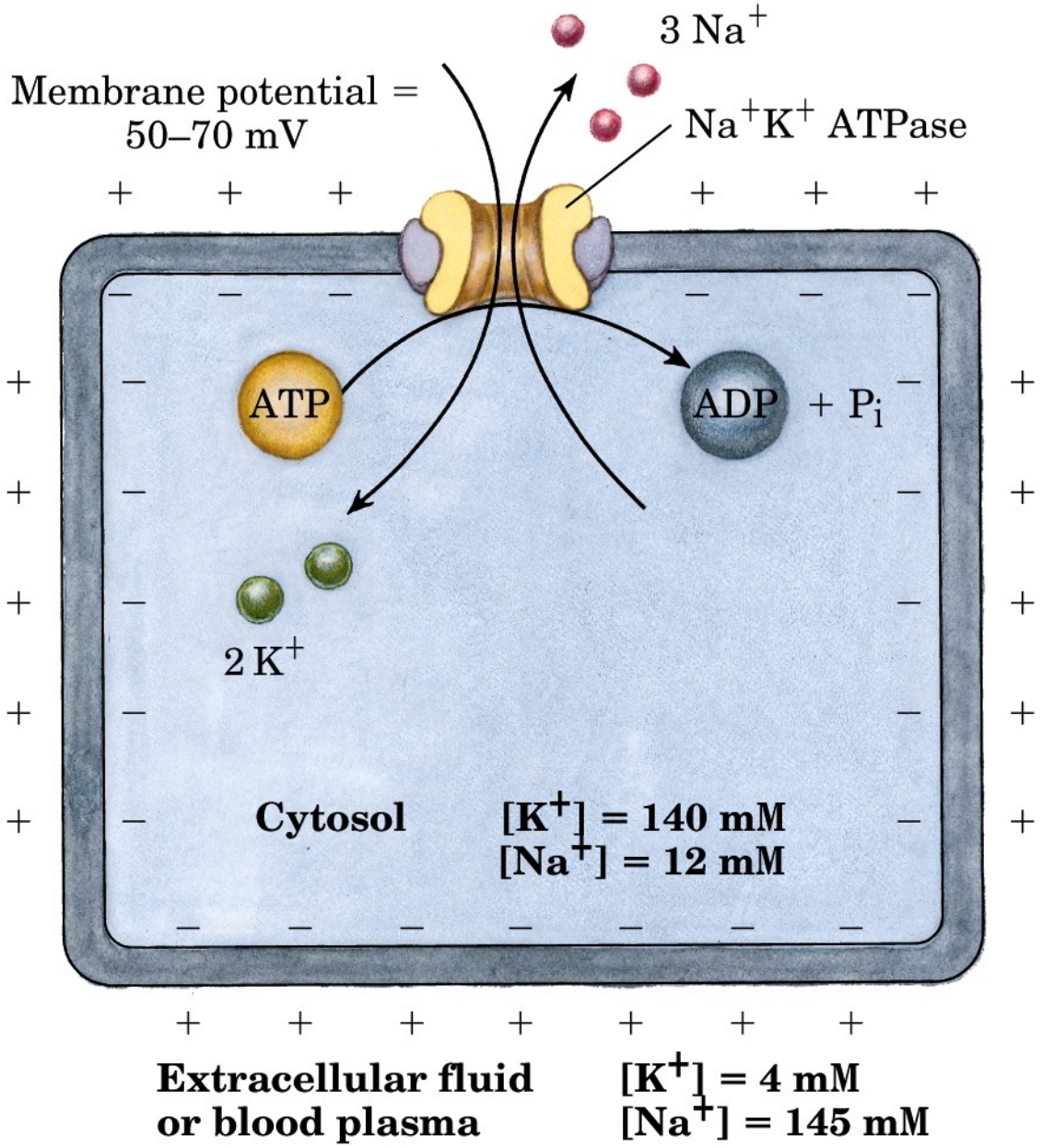
Plasma membrane of higher eukaryotes (Na^+/K^+ pump)

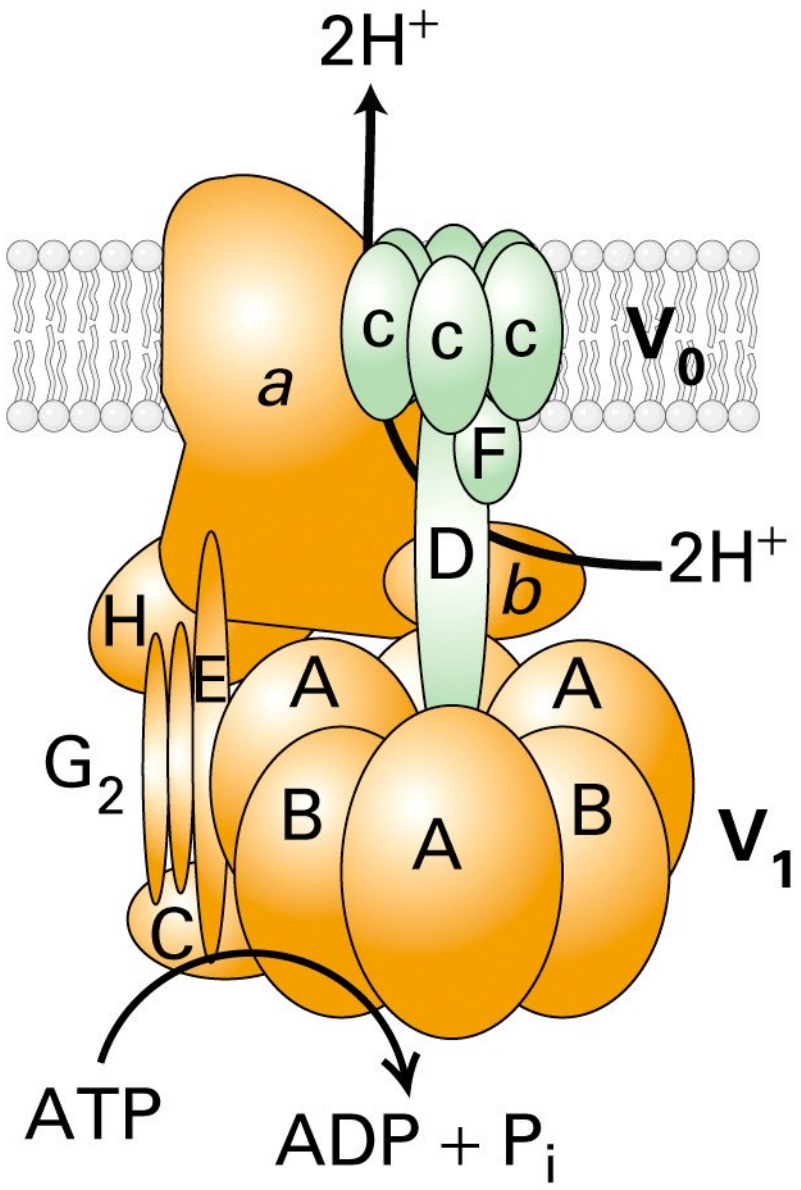
Apical plasma membrane of mammalian stomach (H^+/K^+ pump)

Plasma membrane of all eukaryotic cells (Ca^{2+} pump)

Sarcoplasmic reticulum membrane in muscle cells (Ca^{2+} pump)


Motor neuron
Voltage-gated Ca^{2+} channel
1
Acetylcholine
Ca^{2+}
Voltage-gated Na^{+} channel
Voltage-gated Ca^{2+} channel
T tubule
Nicotinic acetylcholine receptor
2
3
Na^{+}
Na^{+}
Ca^{2+}
Sarcoplasmic reticulum
4
Muscle cell
Ca^{2+} release channel


3 Na+
Membrane potential = 50–70 mV
Na+K+ ATPase
ATP
ADP + Pi
2 K+
Cytosol
[K+] = 140 mM
[Na+] = 12 mM
Extracellular fluid or blood plasma
[K+] = 4 mM
[Na+] = 145 mM

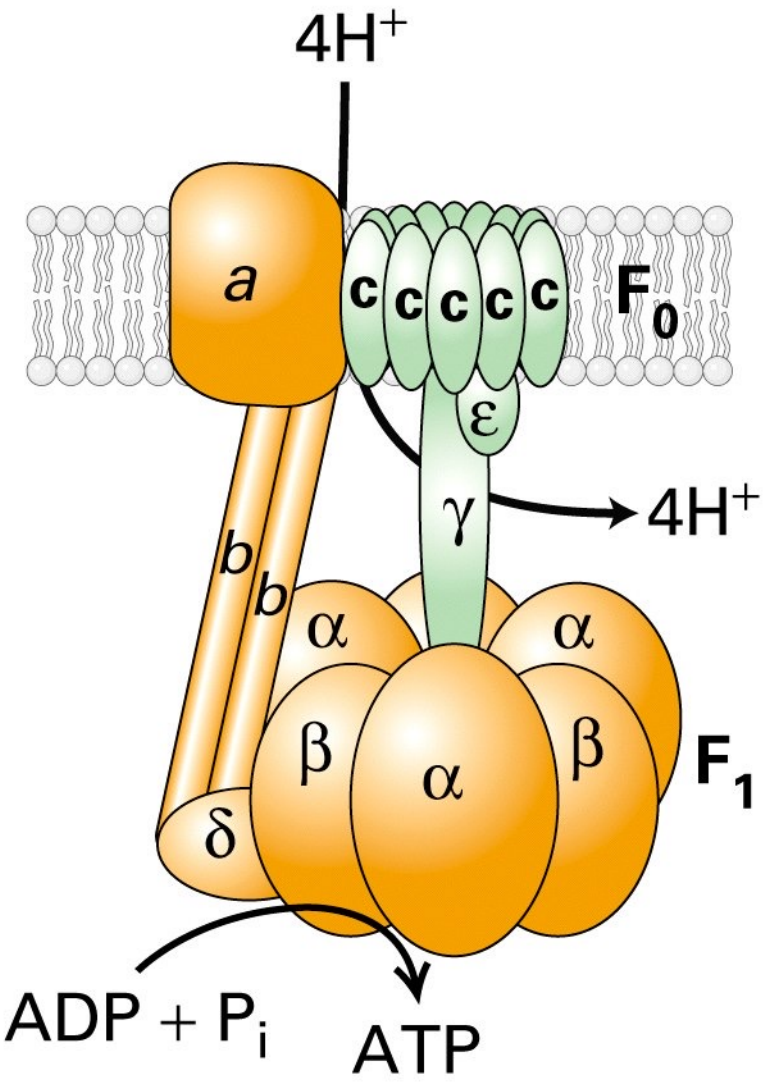


V-class proton pumps

Vacuolar membranes in plants, yeast, other fungi

Endosomal and lysosmal membranes in animal cells

Plasma membrane of osteoclasts and some kidney tubule cells

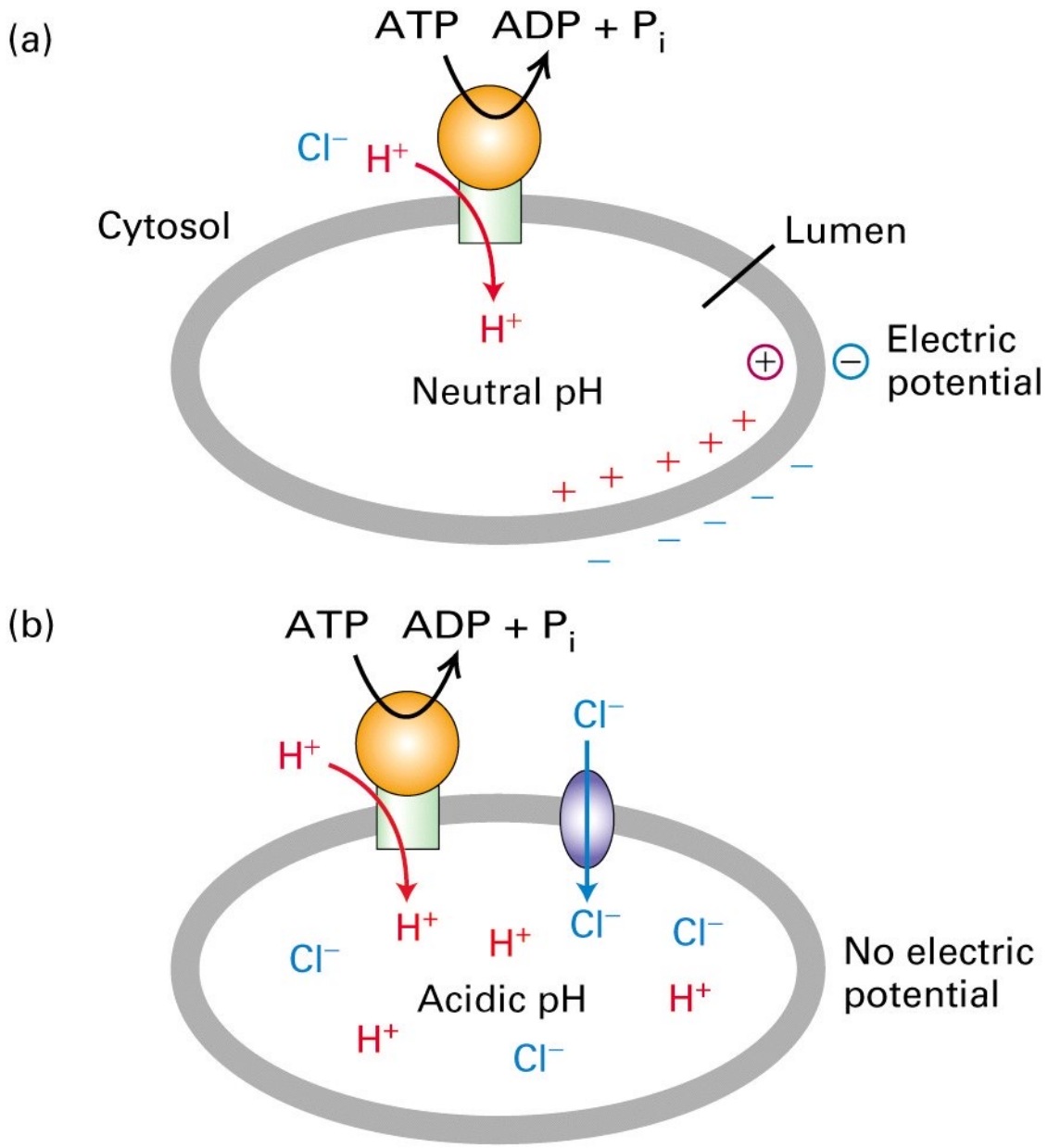


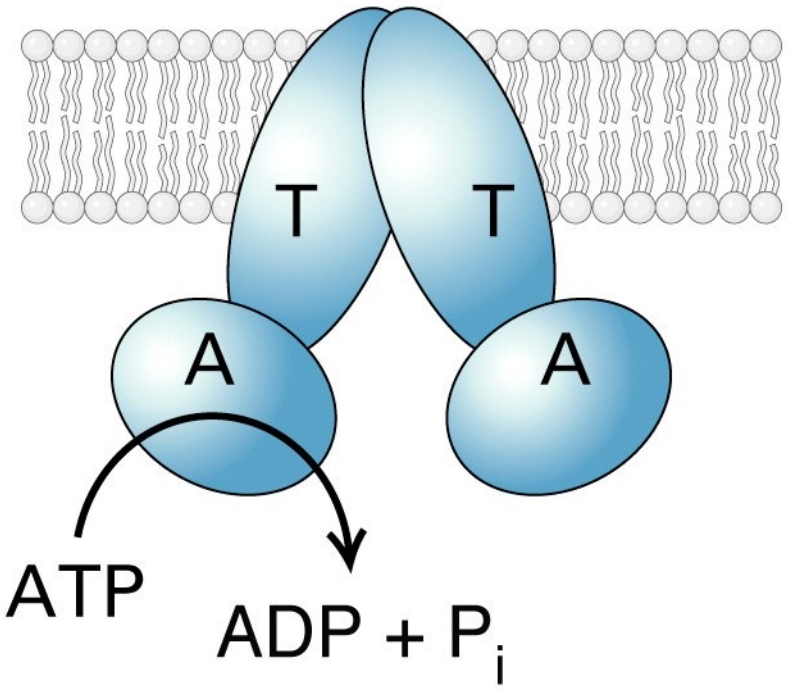
F-class proton pumps

Bacterial plasma membrane

Inner mitochondrial membrane

Thylakoid membrane of chloroplast


(a)
ATP
ADP + P_i
Cl^-
H^+
Cytosol
Lumen
H^+
Neutral pH
Electric potential
(b)
ATP
ADP + P_i
H^+
Cl^-
H^+
H^+
Cl^-
Cl^-
Cl^-
Acidic pH
H^+
H^+
Cl^-
No electric potential



ABC superfamily

Bacterial plasma membranes (amino acid, sugar, and peptide transporters)

Mammalian plasma membranes (transporters of phospholipids, small lipophilic drugs, cholesterol, other small molecules)

Topologie des ABC transporteurs

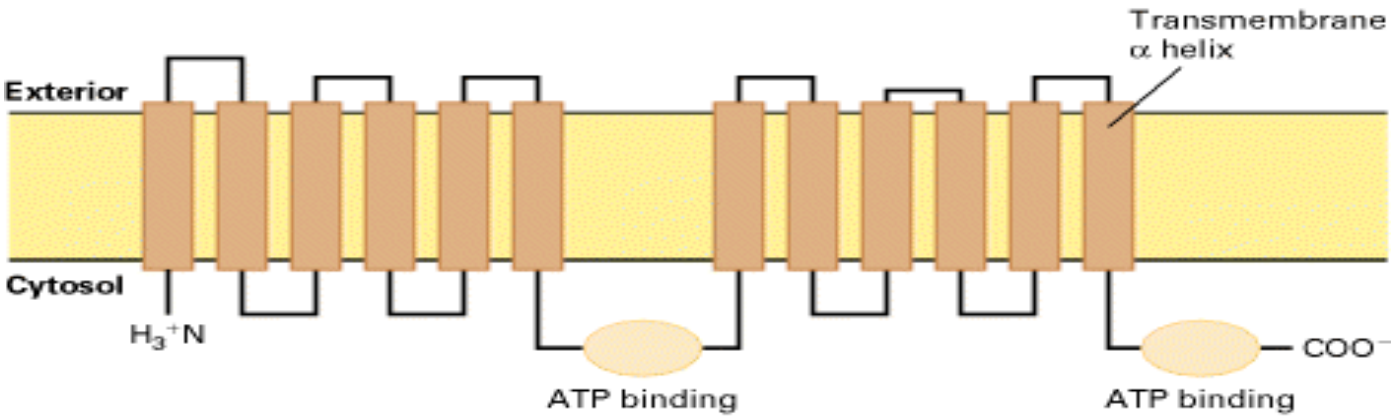


TABLE 18-2 Selected Human ABC Proteins

Protein	Tissue Expression	Function	Disease Caused by Defective Protein
ABCA1	Ubiquitous	Exports cholesterol and phospholipid for uptake into high-density lipoprotein (HDL)	Tangier's disease
ABCB1 (MDR1)	Adrenal, kidney, brain	Exports lipophilic drugs	
ABCB4 (MDR2)	Liver	Exports phosphatidylcholine into bile	
ABCB11	Liver	Exports bile salts into bile	
CFTR	Exocrine tissue	Transports Cl^- ions	Cystic fibrosis
ABCD1	Ubiquitous in peroxisomal membrane	Influences activity of peroxisomal enzyme that oxidizes very long chain fatty acids	Adrenoleukodystrophy (ADL)
ABCG5/8	Liver, intestine	Exports cholesterol and other sterols	β-Sitosterolemia

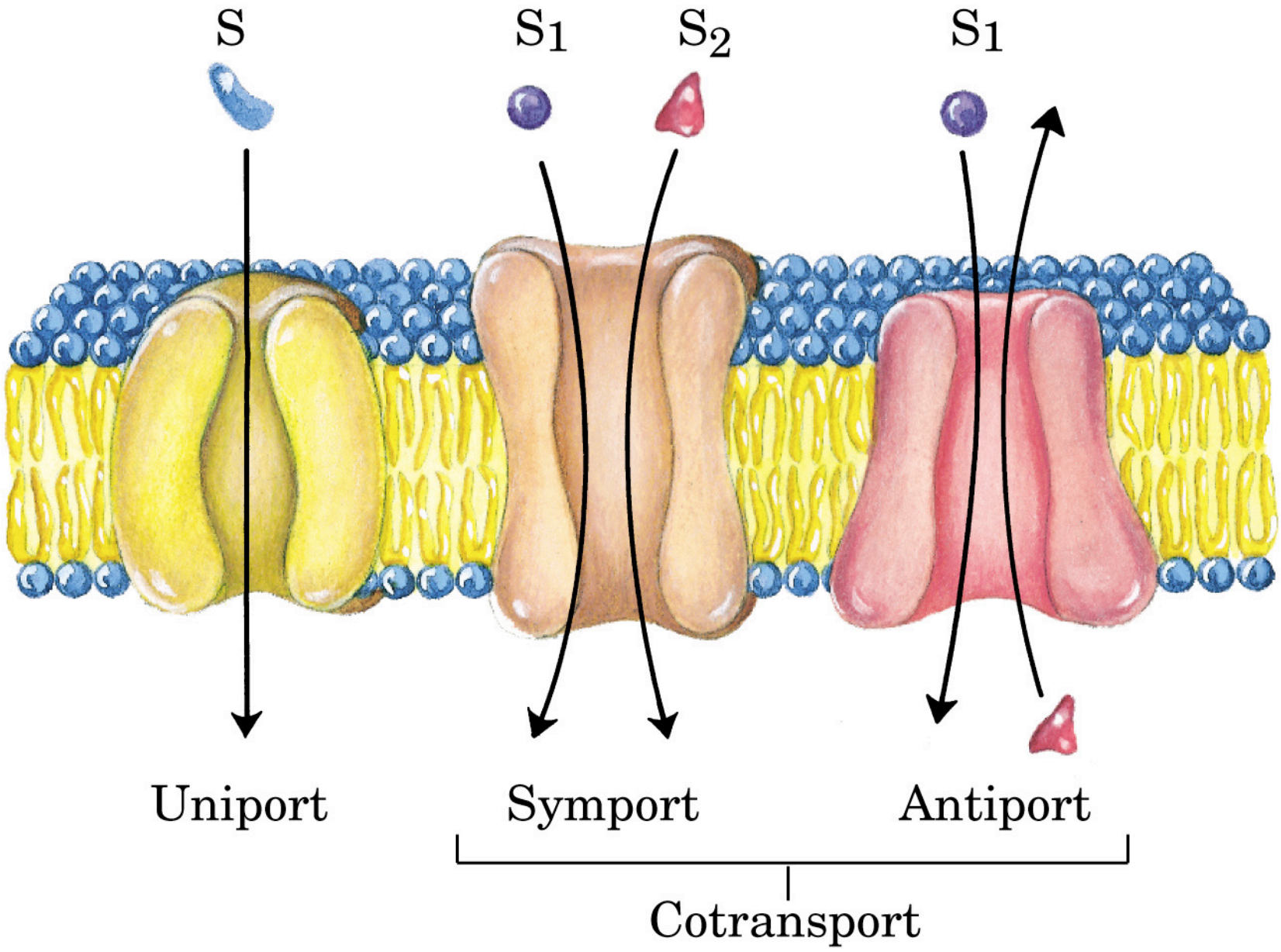
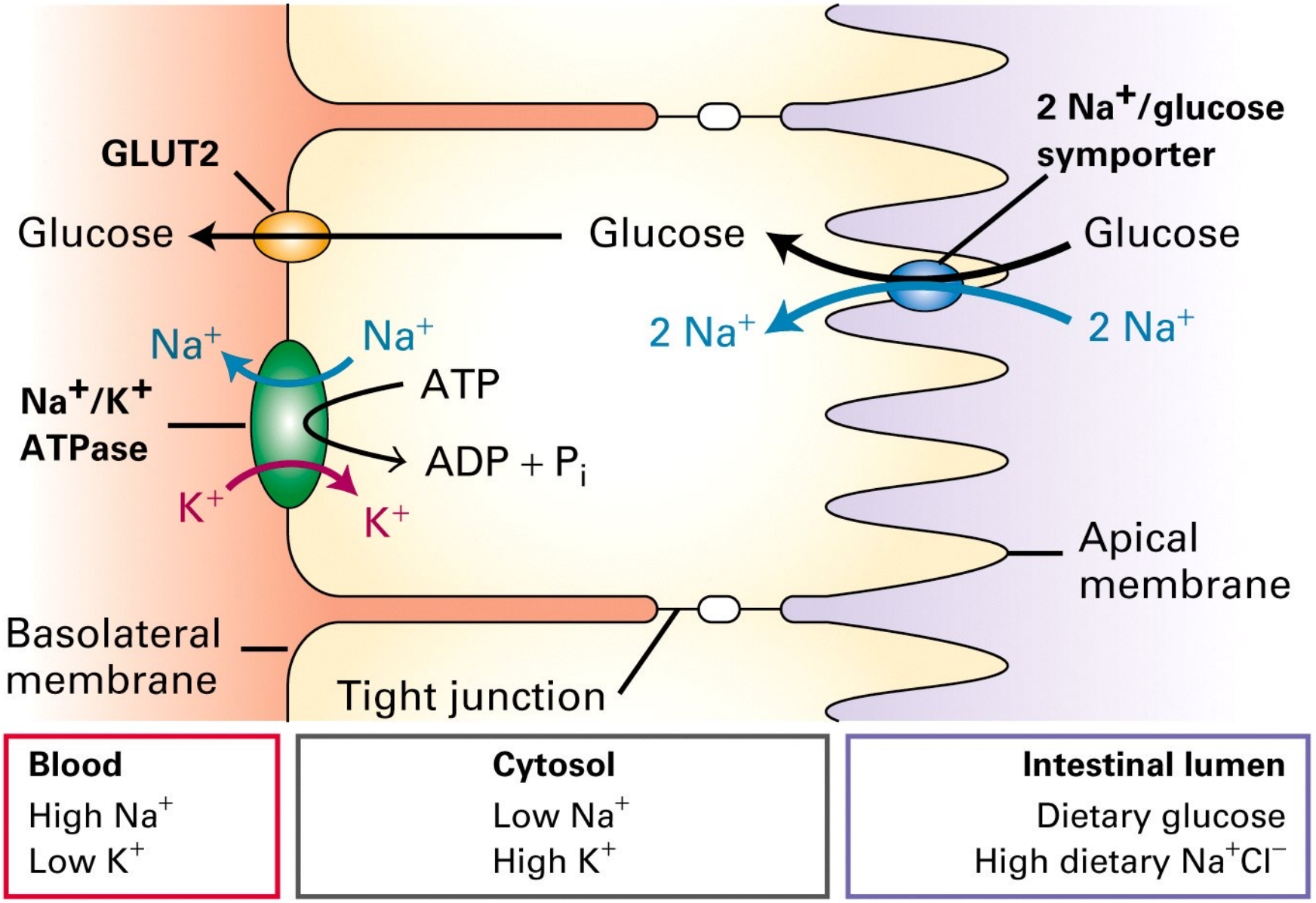
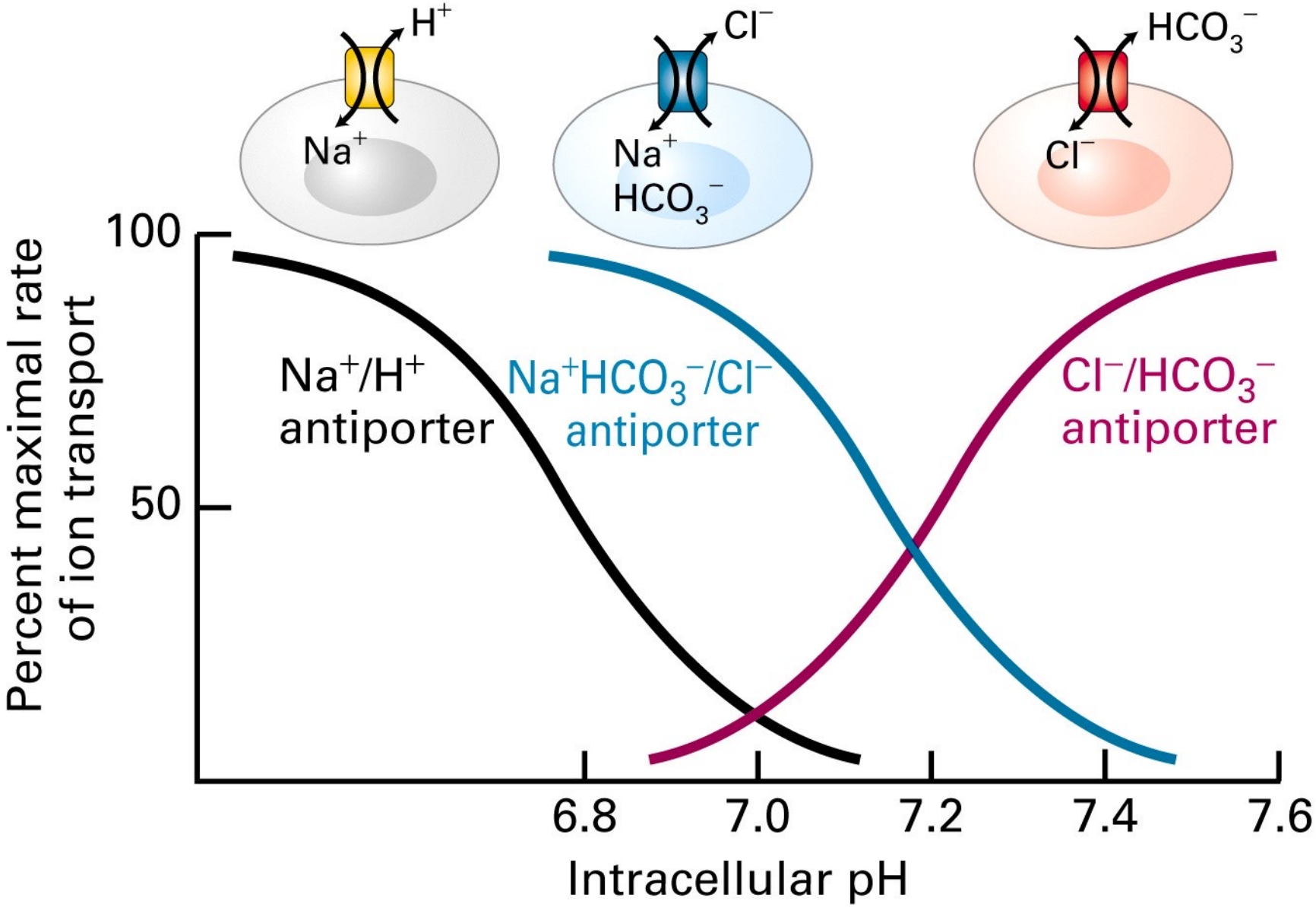

S
S1
S2
S1
Uniport
Symport
Antiport
Cotransport

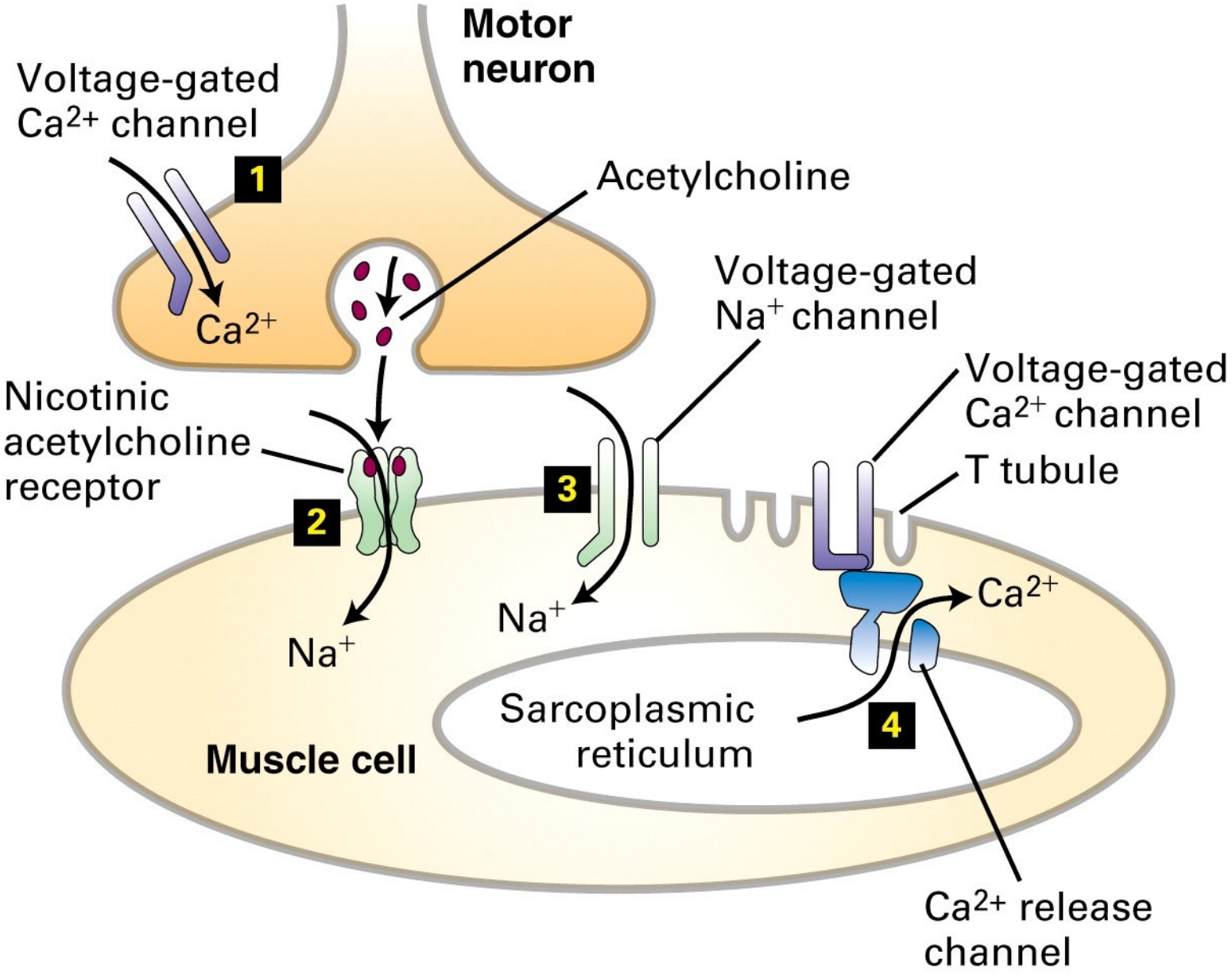
table 12–5

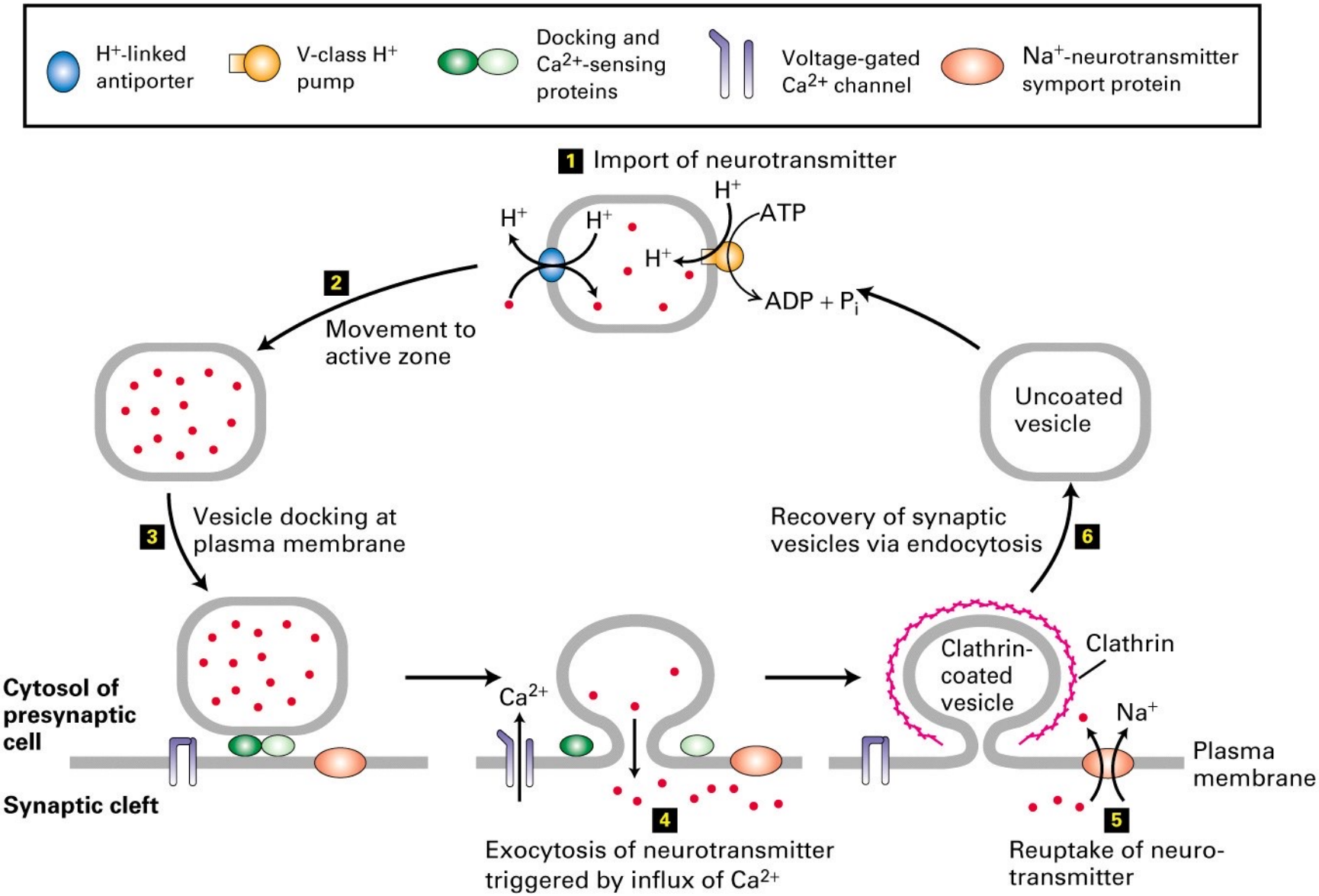
Cotransport Systems Driven by Gradients of Na^+ or H^+

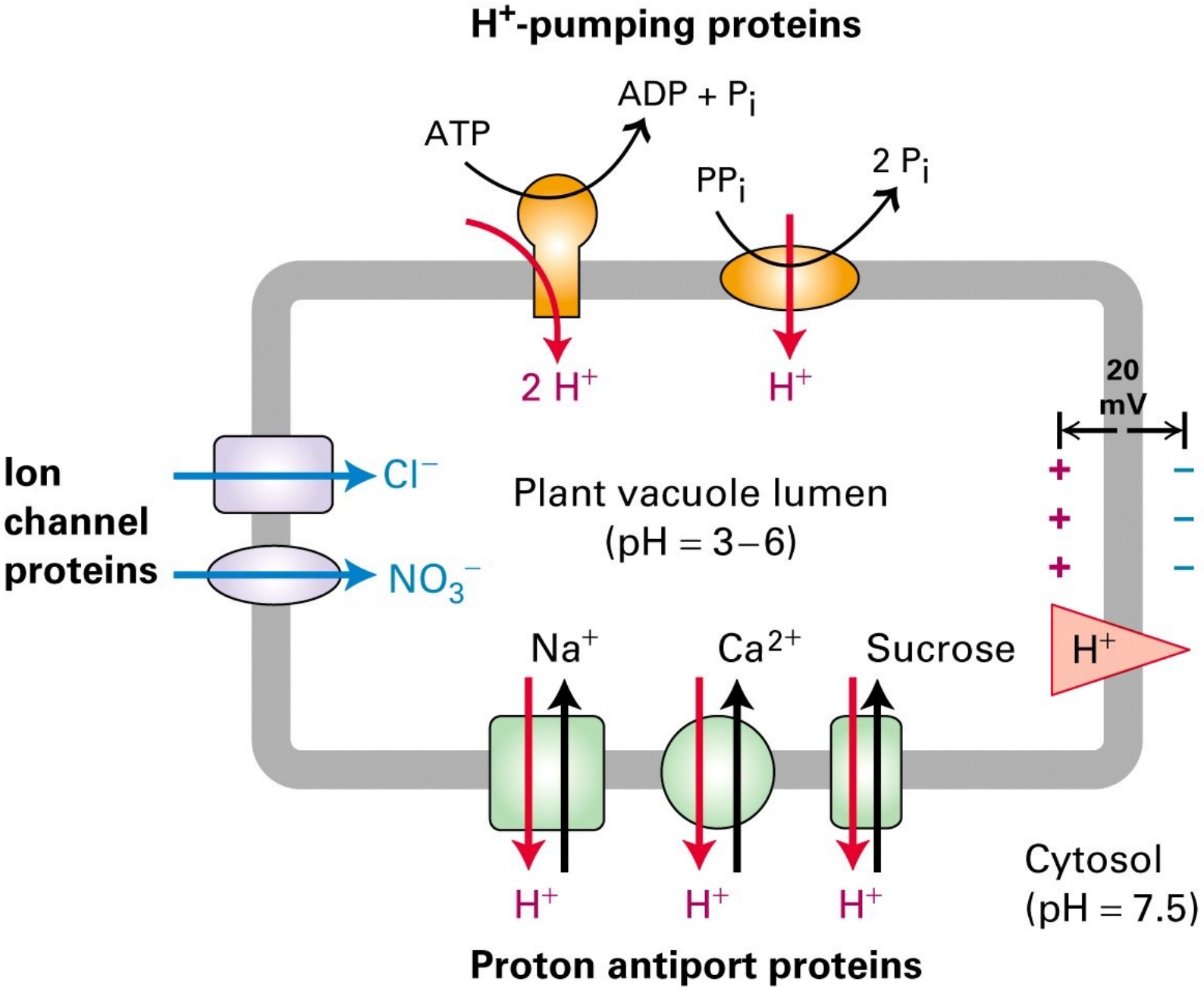
Organism or tissue	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transport
E. coli	*Lactose*	H^+	*Symport*
	Proline	H^+	*Symport*
	Dicarboxylic acids	H^+	*Symport*
Intestine, kidney of vertebrates	*Glucose*	Na^+	*Symport*
	Amino acids	Na^+	*Symport*
Vertebrate cells (many types)	Ca^{2+}	Na^+	*Antiport*
Higher plants	K^+	H^+	*Antiport*
Fungi (Neurospora)	K^+	H^+	*Antiport*


GLUT2
Glucose
Glucose
2 Na+/glucose symporter
Glucose
2 Na+
2 Na+
Na+
Na+
Na+/K+ ATPase
ATP
ADP + Pi
K+
K+
Apical membrane
Basolateral membrane
Tight junction
Blood
High Na+
Low K+
Cytosol
Low Na+
High K+
Intestinal lumen
Dietary glucose
High dietary Na+Cl−


H+
Na+
Cl−
Na+
HCO3−
HCO3−
Cl−
100
50
Percent maximal rate of ion transport
Na+/H+ antiporter
Na+HCO3−/Cl− antiporter
Cl−/HCO3− antiporter
6.8
7.0
7.2
7.4
7.6
Intracellular pH


Motor neuron
Voltage-gated Ca^{2+} channel
1
Acetylcholine
Ca^{2+}
Voltage-gated Na^{+} channel
Voltage-gated Ca^{2+} channel
T tubule
Nicotinic acetylcholine receptor
2
3
Na^{+}
Na^{+}
Ca^{2+}
Sarcoplasmic reticulum
4
Muscle cell
Ca^{2+} release channel


H+-linked antiporter
V-class H+ pump
Docking and Ca2+-sensing proteins
Voltage-gated Ca2+ channel
Na+-neurotransmitter symport protein
1 Import of neurotransmitter
H+
ATP
ADP + Pi
2 Movement to active zone
Uncoated vesicle
3 Vesicle docking at plasma membrane
Recovery of synaptic vesicles via endocytosis
6
Cytosol of presynaptic cell
Synaptic cleft
Ca2+
Clathrin-coated vesicle
Clathrin
Na+
Plasma membrane
4
Exocytosis of neurotransmitter triggered by influx of Ca2+
5
Reuptake of neuro-transmitter


H+-pumping proteins
ATP
ADP + Pi
PPi
2 Pi
2 H+
H+
20 mV
+
+
+
–
–
–
Ion channel proteins
Cl–
NO3–
Plant vacuole lumen (pH = 3–6)
Na+
Ca2+
Sucrose
H+
H+
H+
H+
Cytosol (pH = 7.5)
Proton antiport proteins

Aide pour Révisions

- Annales 2021-22 et 2022-23
- Exercices TD
- Séances questions le 11/10/2022
- Possibilité d’aide personnalisée sur RDV