Lecture 5. Membrane transport of ions and small molecules

Outline

I. Overview of transmembrane transport
II. channels
III. Transporters
IV. ATP-pumps
V. Membrane potential

Ions are distributed differently inside and outside a cell

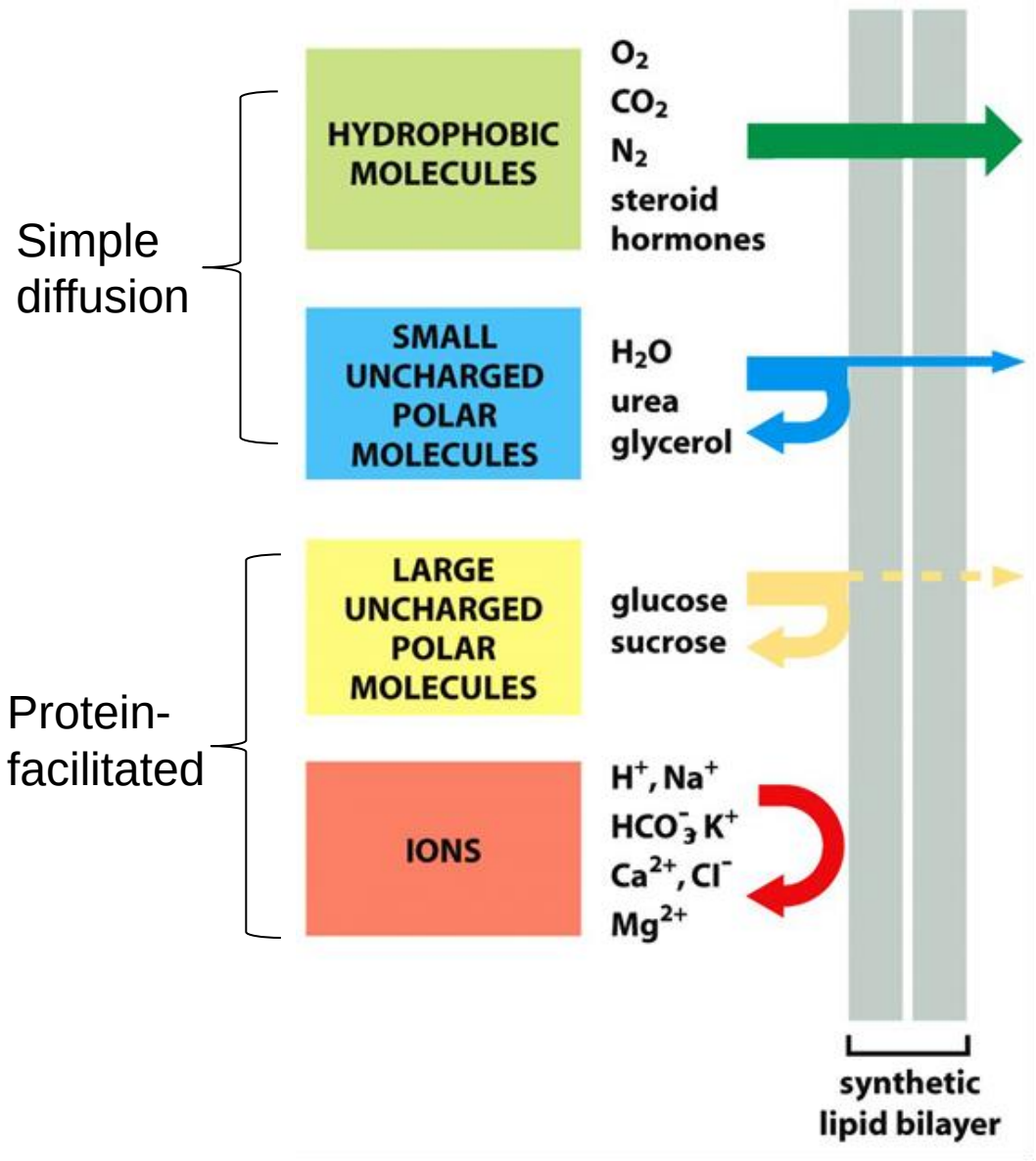
Table 11–1 A Comparison of Ion Concentrations Inside and Outside a Typical Mammalian Cell

COMPONENT	INTRACELLULAR CONCENTRATION (mM)	EXTRACELLULAR CONCENTRATION (mM)
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5	1–2
Ca^{2+}	10^{-4}	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions*		
Cl^-	5–15	110

*The cell must contain equal quantities of positive and negative charges (that is, it must be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cell constituents are negatively charged (HCO_3^-, PO_4^{3-}, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions. There is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, but both are mostly bound to proteins and other substances and, for Ca^{2+}, stored within various organelles.

Inside the cells, higher K+, but lower Na+ and Cl-
as compared to outside of a cell

Lipid bilayer only is impermeable to ions



Lipid bilayer, due to its strong hydrophobicity, only allow hydrophobic molecules, and some small uncharged polar molecules, to pass freely.

Some small molecules like water Can be channeled, too, to increase its diffusion rate.

Simple diffusion

1. No membrane protein facilitated.
2. Relatively slow.
3. Apply to gases, small uncharged molecules. e.g., O2, CO2, urea, ethanol

Three factors influence diffusion rate:

(1). The concentration gradient across the membrane.
(2). Hydrophobicity
(3). Size of molecules

Questions:

1. Diethylurea will diffuse more rapidly than urea?

Partition coefficient (the ratio of concentration of a substance between oil and water) for:

diethylurea: K=0.01
urea: K =0.0002

There are two major classes of membrane transport proteins

- Transporter
- Channels

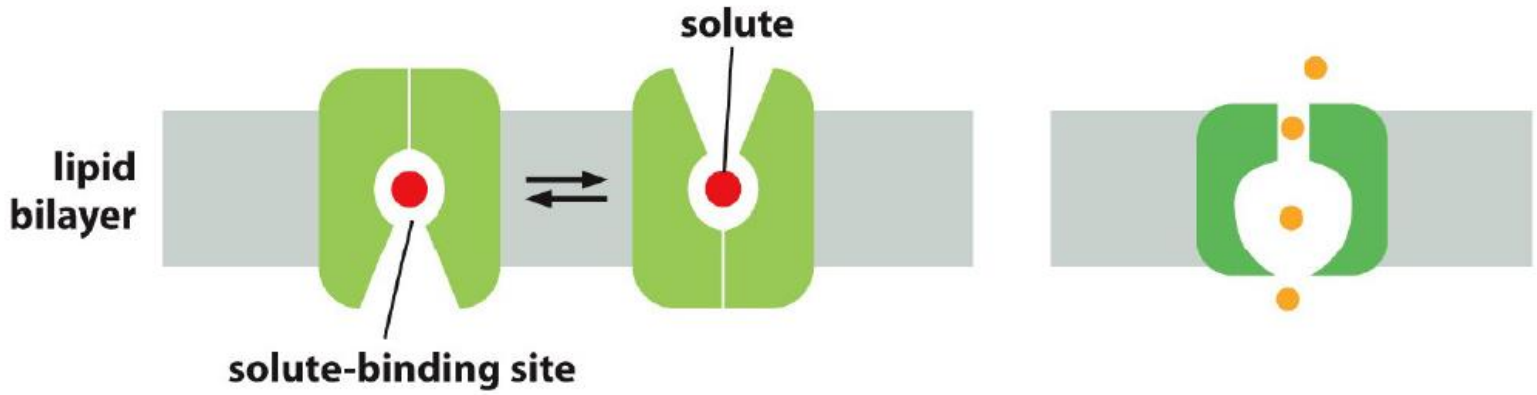
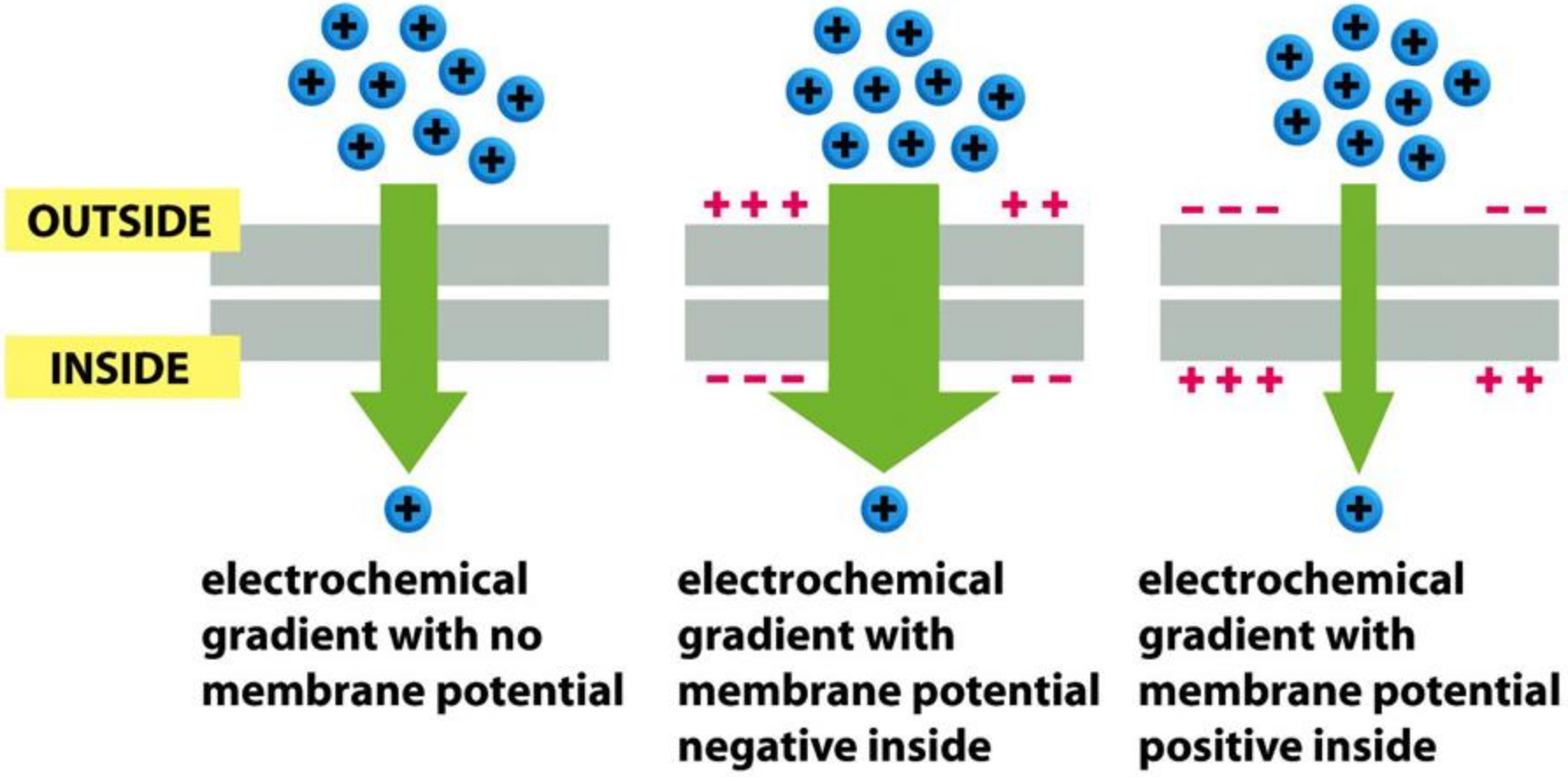


Figure 11-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

An important factor in molecule transport: electrochemical gradient



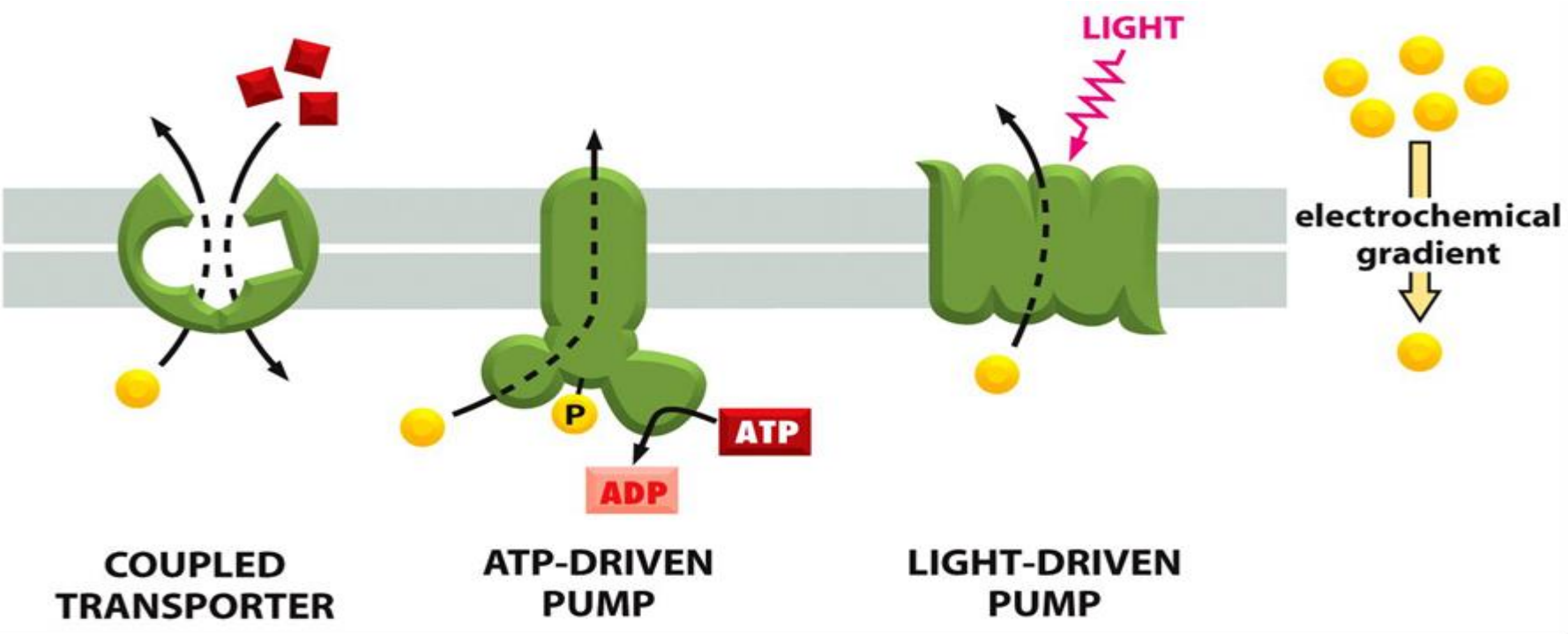
It consists of molecule gradient and electric potential between inside and outside Of membrane.

Passive transport and active transport

Passive transport: solute crosses membrane down the electrochemical gradient.

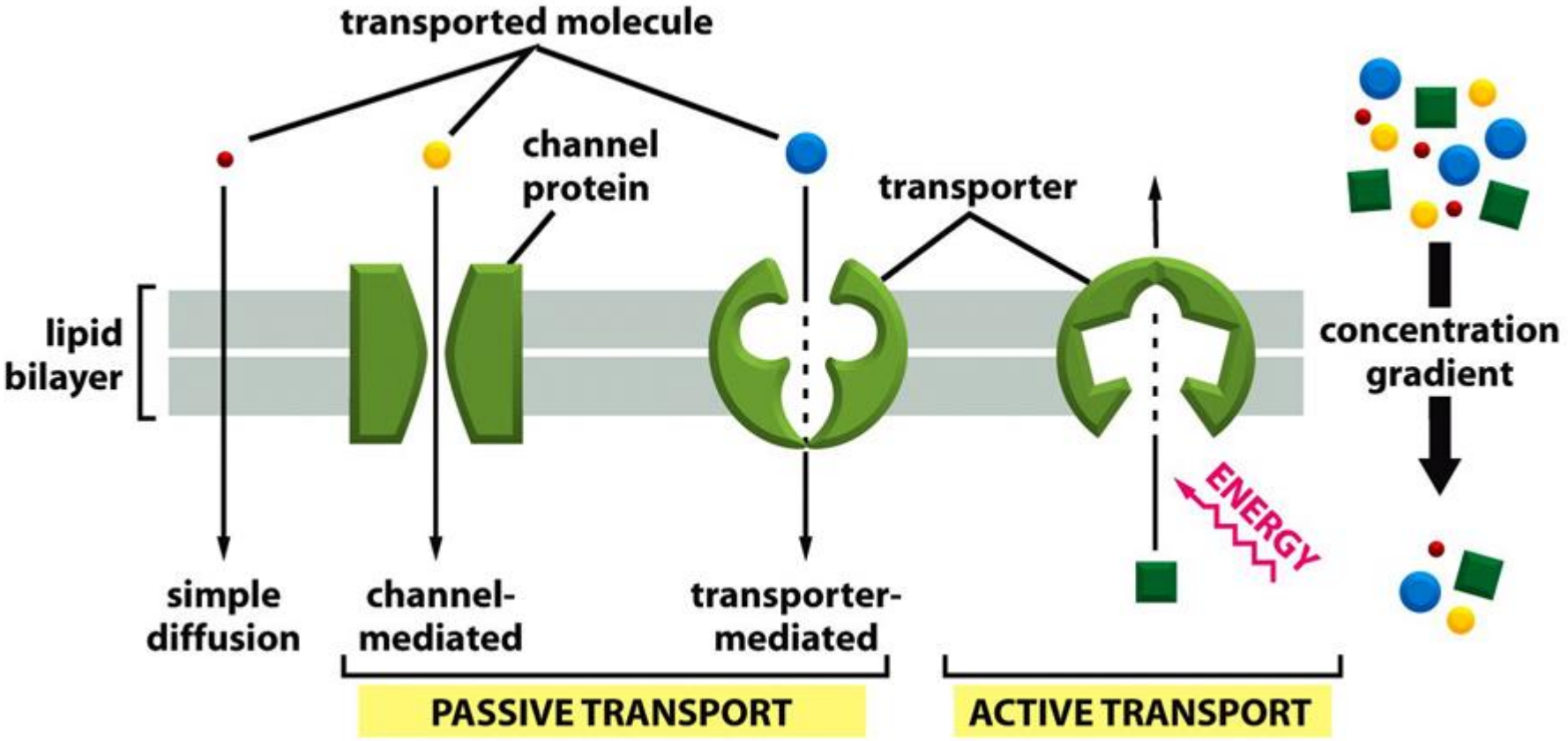
Active transport: solute crosses membrane against the electrochemical gradient.

Three ways of active transport



Coupling an energetically favorable transport to an energetically unfavorable transport

Most molecules are transported facilitated by proteins in three types



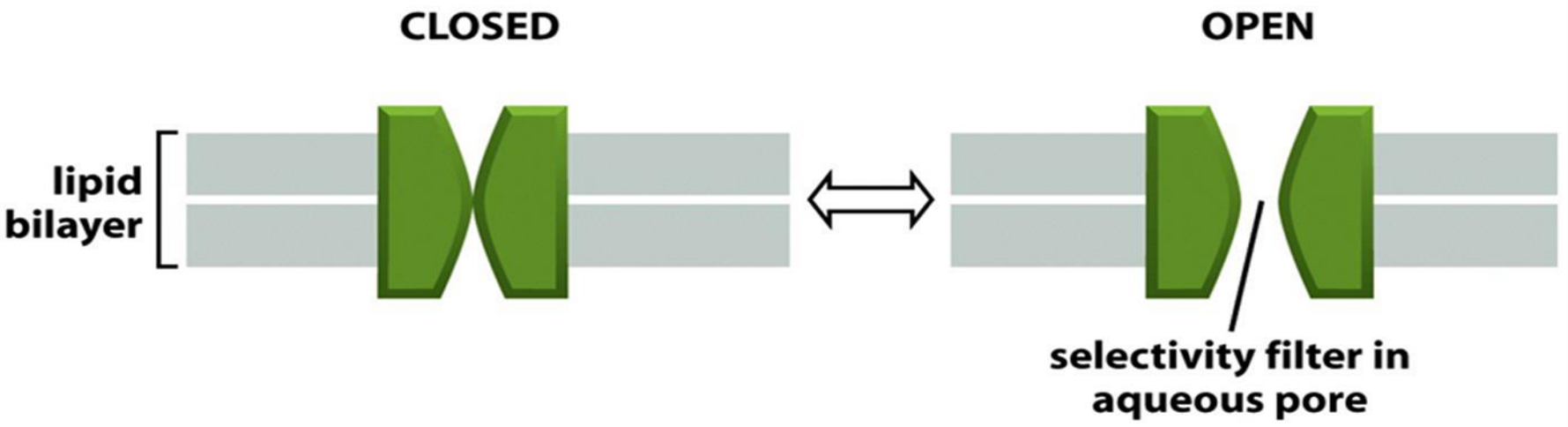
A comparison for these three different transport ways

1. Channels: move molecules down its gradient, less binding affinity, 10^7-10^8ions/sec
2. Transporters: bind molecules tightly, undergo conformational changes, 10^2-10^4 ions/sec.
3. Energy-driven pumps: move molecules against its gradient, need energy (ATP or light), 10^0-10^3 ions/sec.

1. Channels

1. Also called facilitated transport, a passive transport
2. Channels are selective to molecules to be transported.
3. No conformational change
4. Less binding affinity
5. Move ions at a rate of 10^7-10^8/sec

6. Can be divided into two types:
   A. nongated channels: open most of the time
   B. gated channels: open and close in response to signals



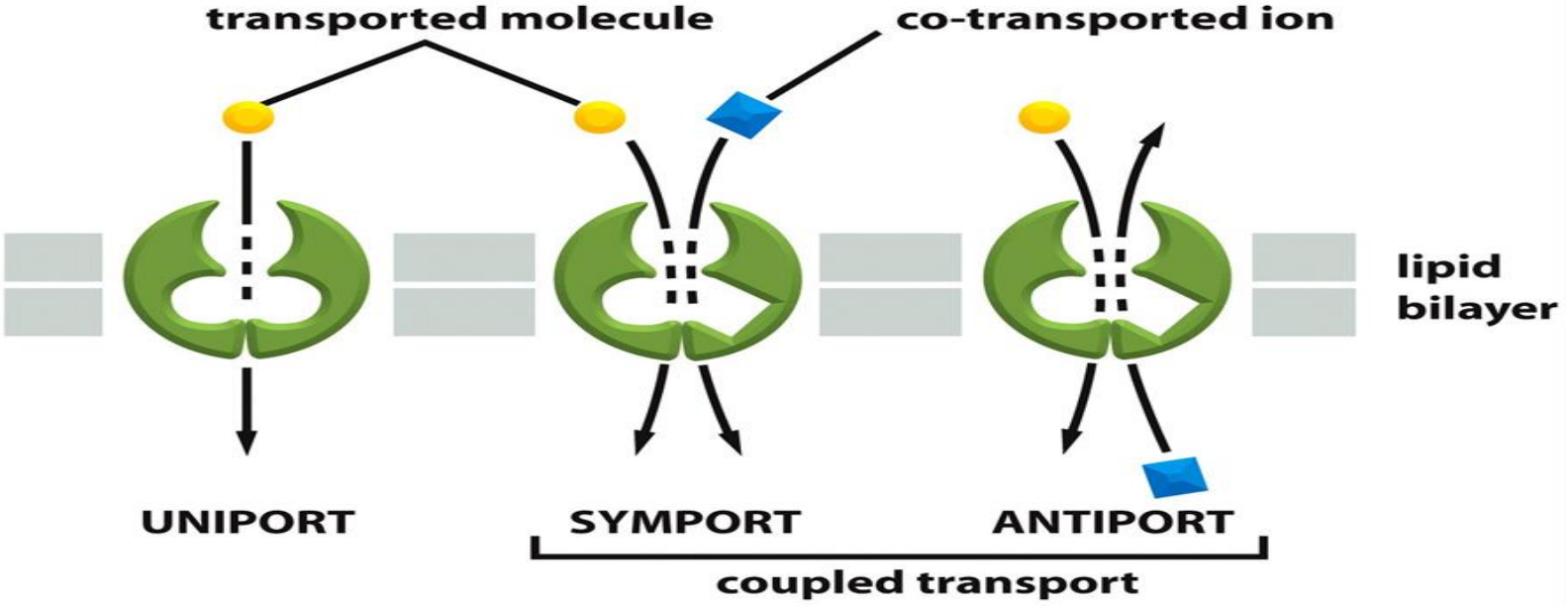
2. Transporters

1. Undergo conformational changes
2. Bind molecules more tightly
3. Pump molecules at a rate of 10^2-10^4/sec
4. Further divided into 3 types:

Uniport: transport a single type of molecule down its gradient.

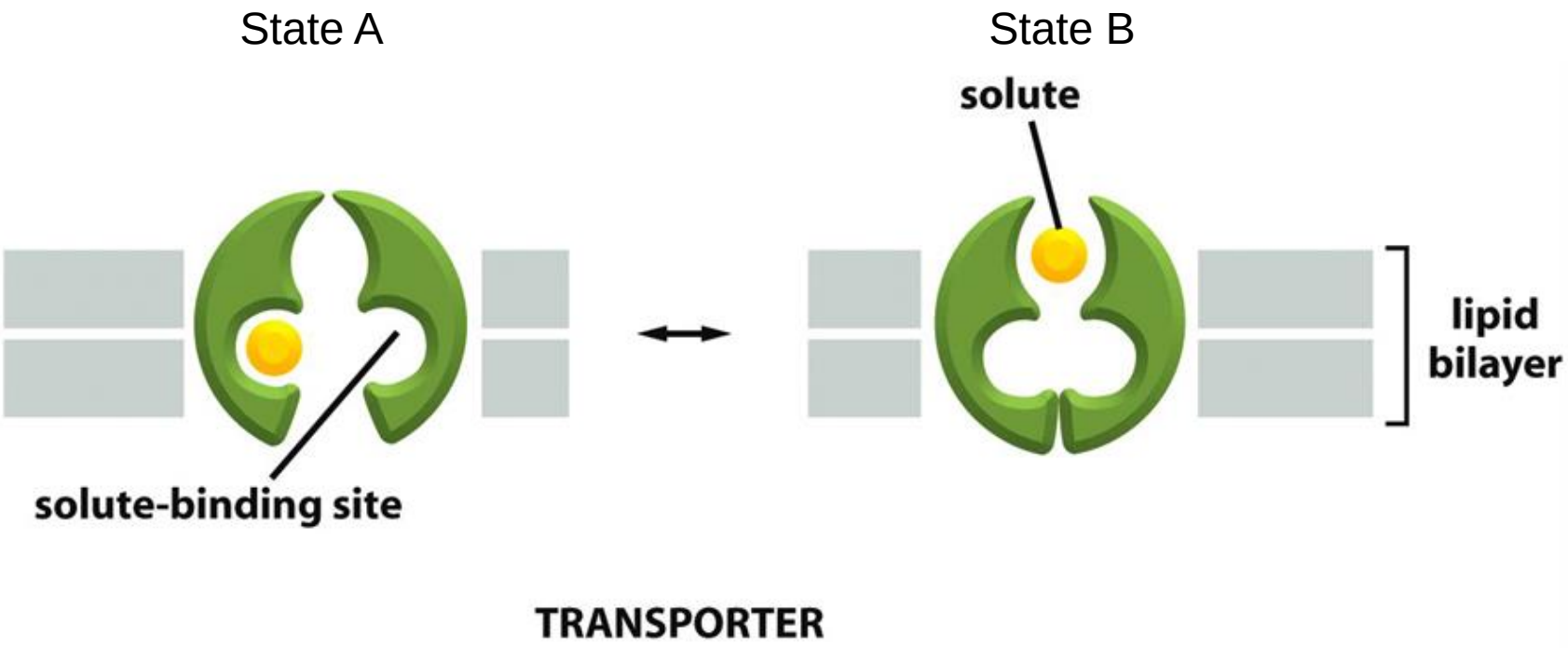
Symport: couple the movement of one type of molecule against its gradient in the same direction

Antiport: couple the movement of one type of molecule against its gradient in the opposite direction



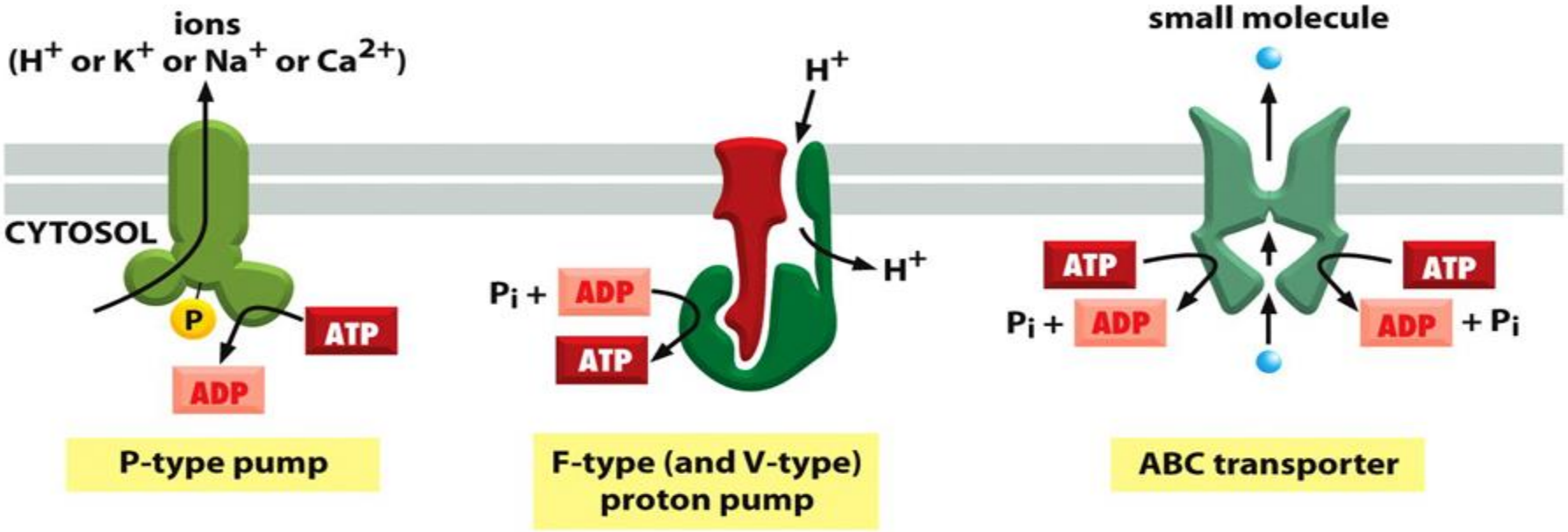
Symport and antiport are also called “co-transpor”

Transporters have conformational changes



3. ATP-pumps

- One of the active transporters
- Has ATPase activity
- Couple ATP hydrolysis with transport
- Can be divided into 3 types:
    A. P-type
    B. F-type
    C. ABC transporter



II. Channels

- unlike transporters, channel proteins form hydrophilic pores across membranes
- gap junctions are found in virtually all animals forms
- ion channels are ion-selective and fluctuate between open and closed states
- channels are not be coupled to an energy source

1. channels- aquaporin
2. ion channel (K+ channel)

Cells must maintain appropriate solute concentration to avoid osmotic swelling

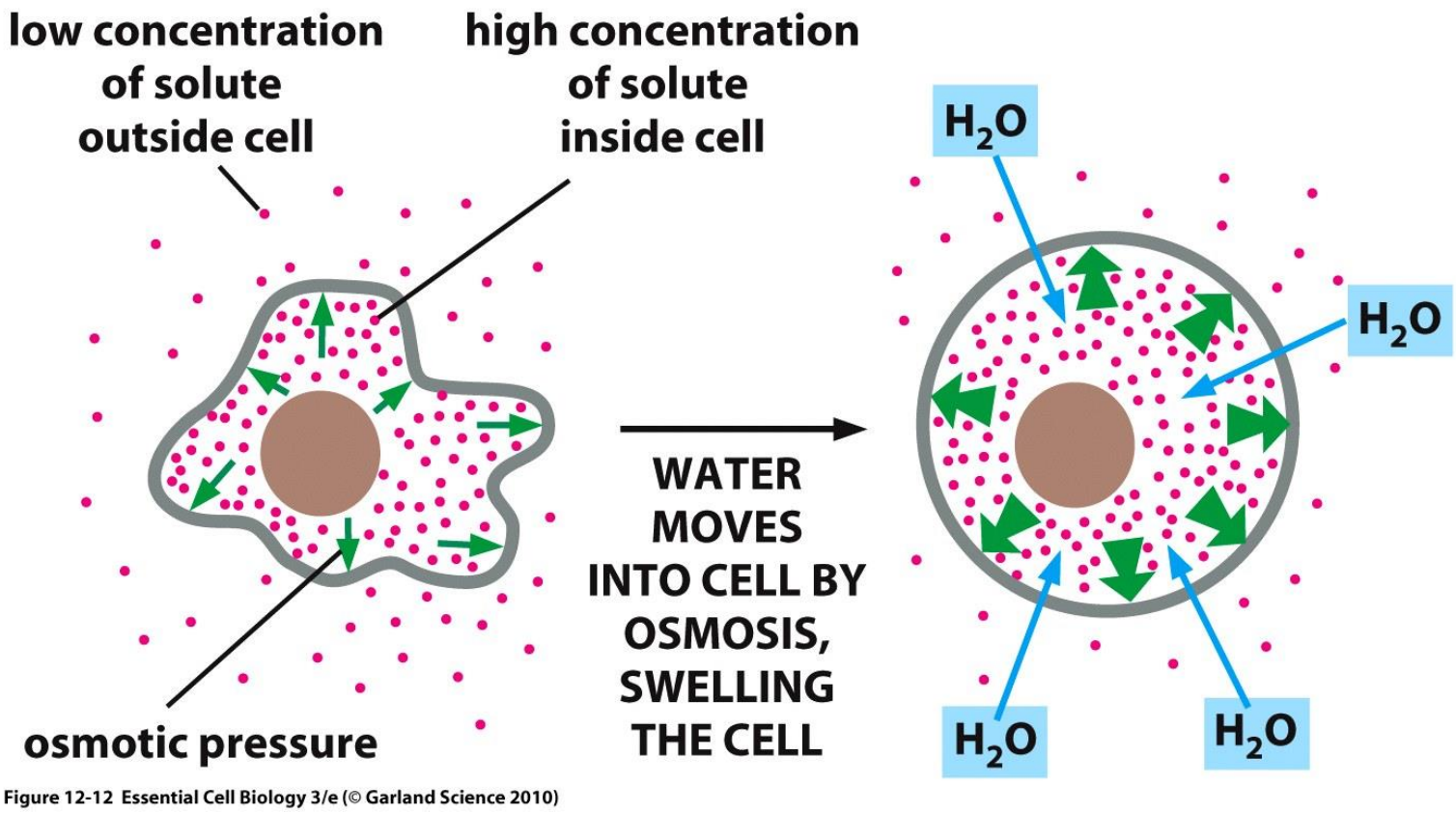
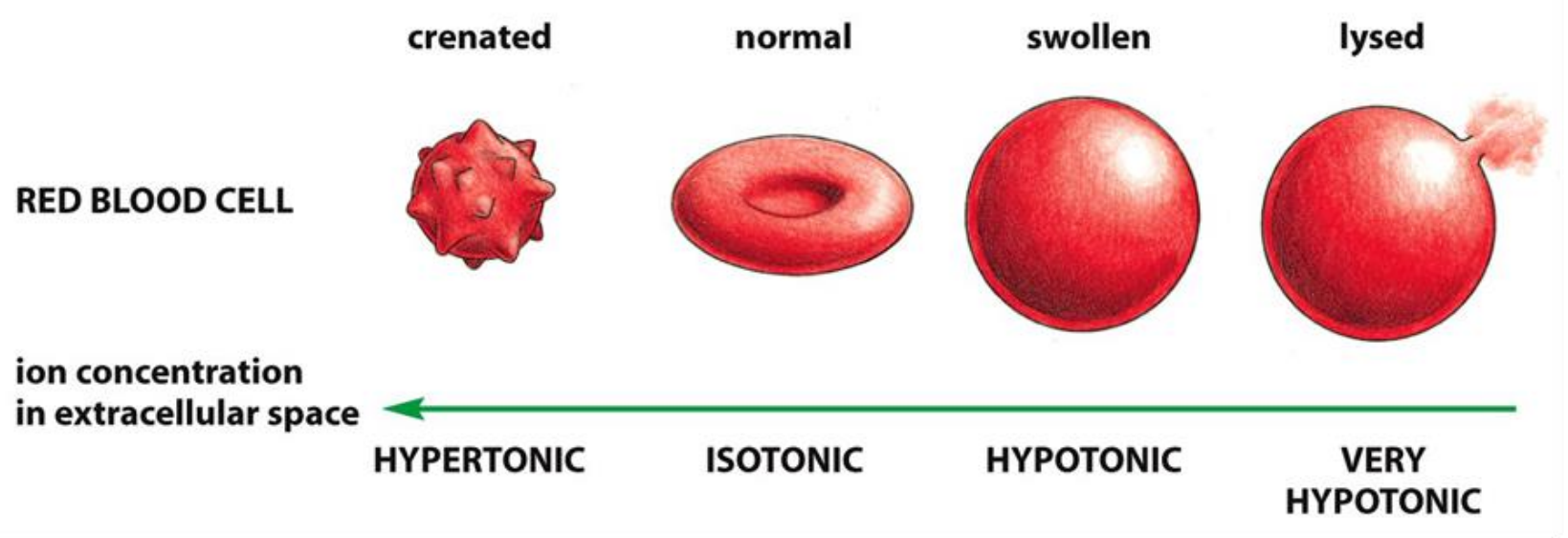


Figure 12-12 Essential Cell Biology 3/e (© Garland Science 2010)



Question: Why don't the cells of fresh-water animals burst in water?

Cells have different ways to keep cell from osmotic swelling

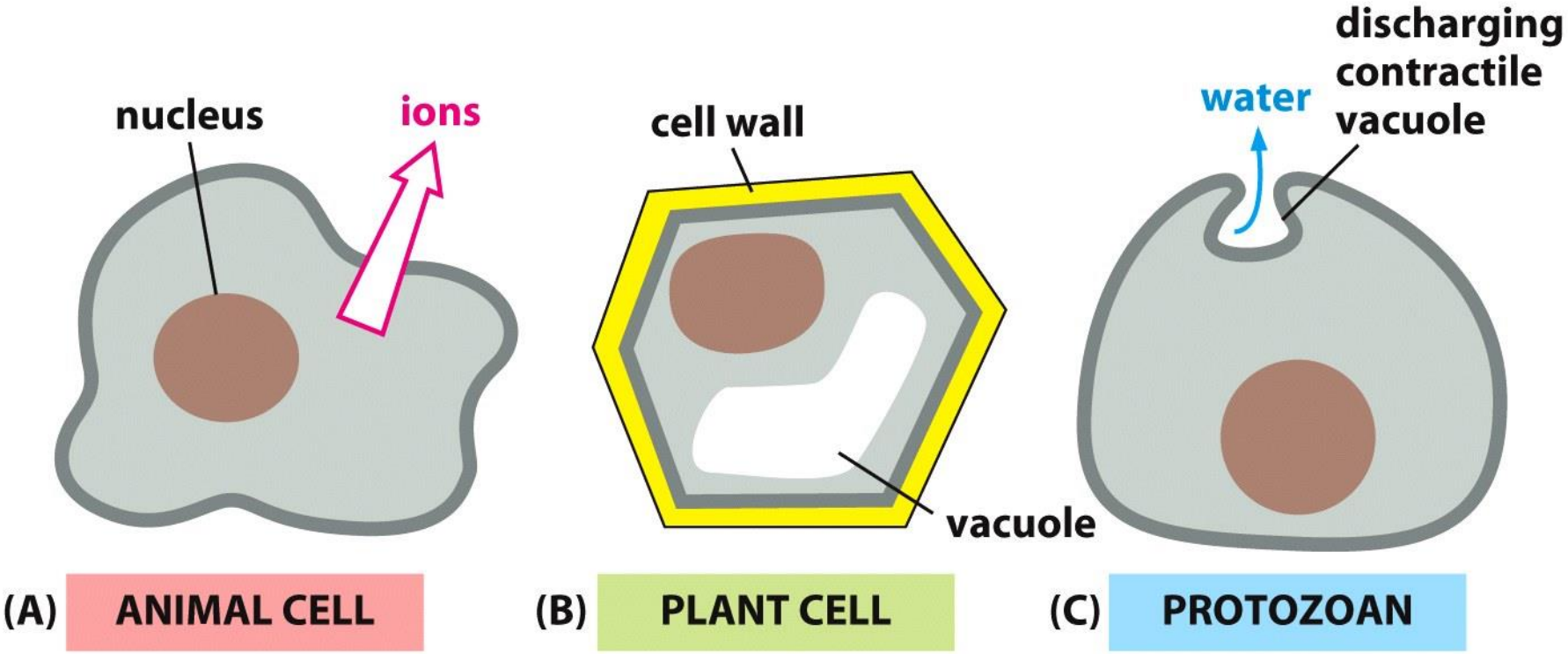
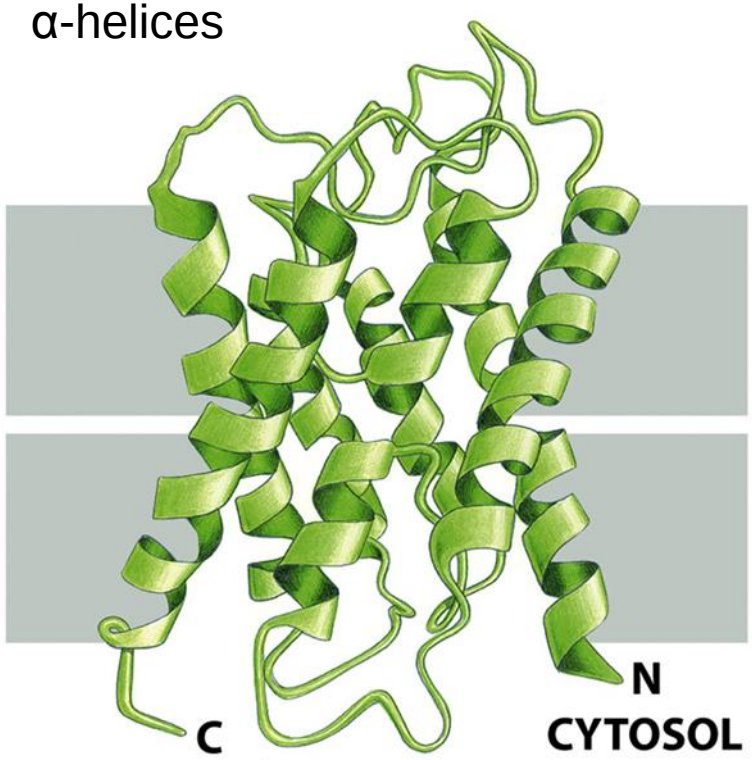


Figure 12-13 Essential Cell Biology 3/e (© Garland Science 2010)

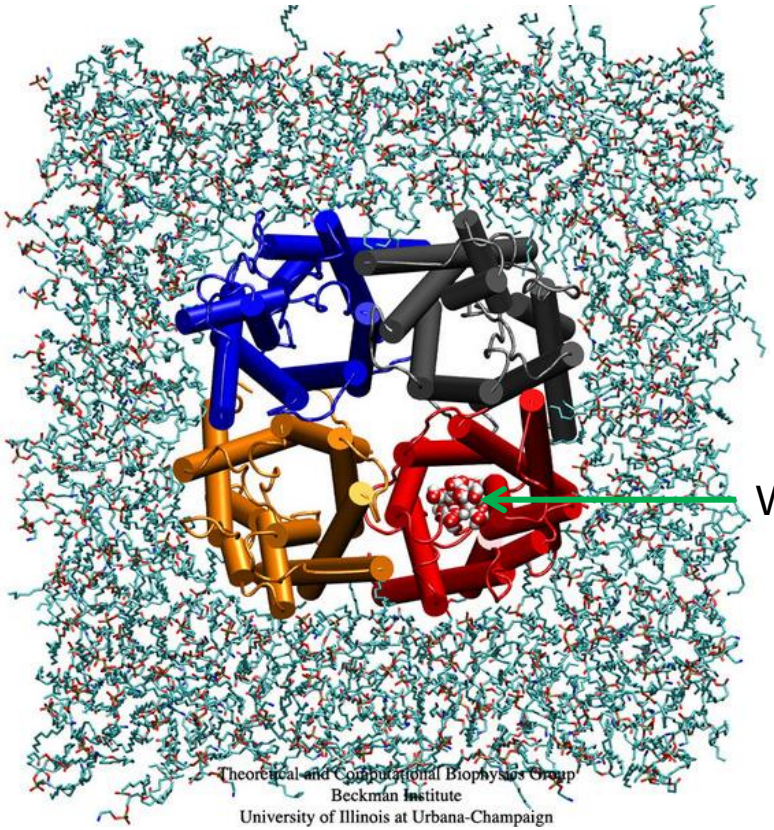
1. Aquaporins increase the water permeability of cell membranes

Abundant in cells that Transport water quickly, esp., kidney epithelial cells, and Erythrocytes.

Monomer has 6 transmembrane α-helices

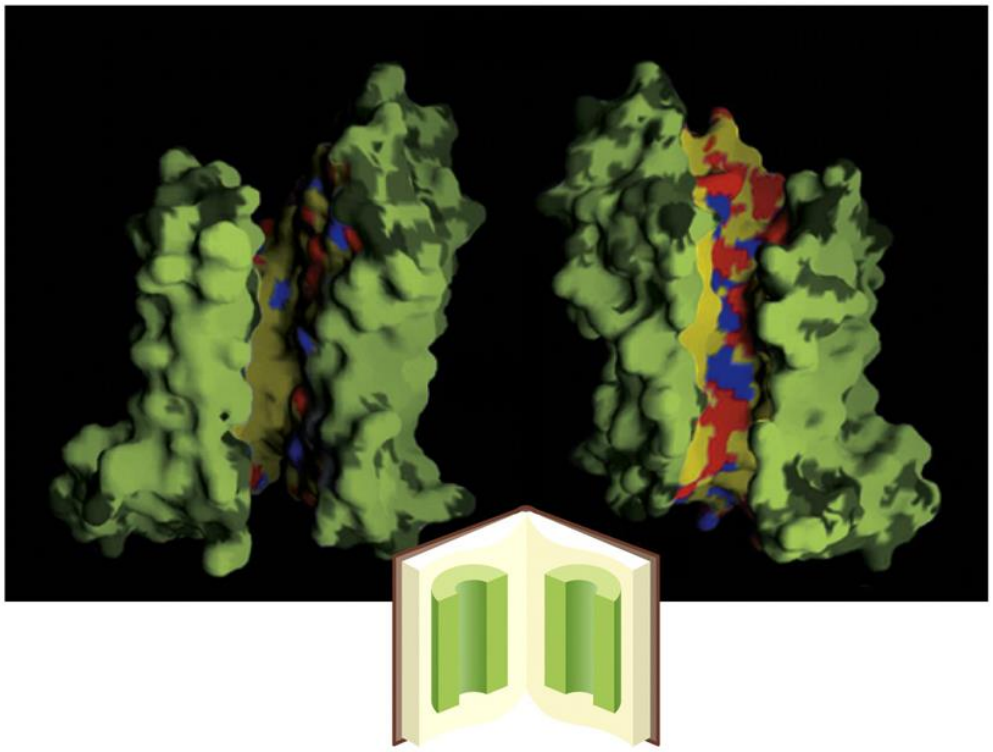


Aquaporin is tetrameric protein



How does aquaporin transport water?

A view by cutting the monomer in two halves

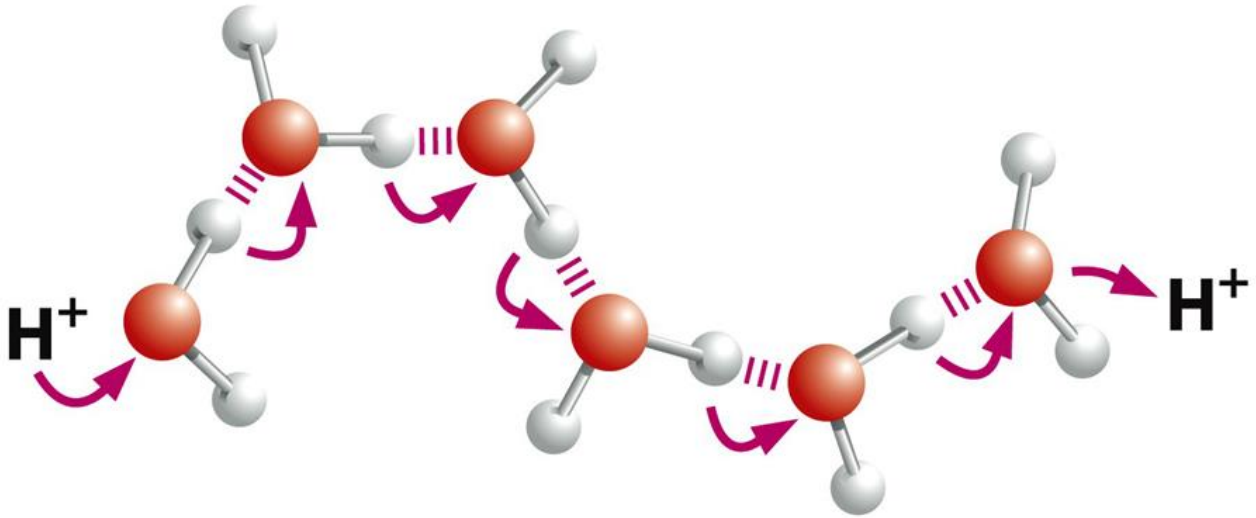


By aligning water through its
Hydrophilic side by hydrogen-bonding,
And prohibiting hydrogen-bonding in
The other hydrophobic side,
It can align the water in single file.

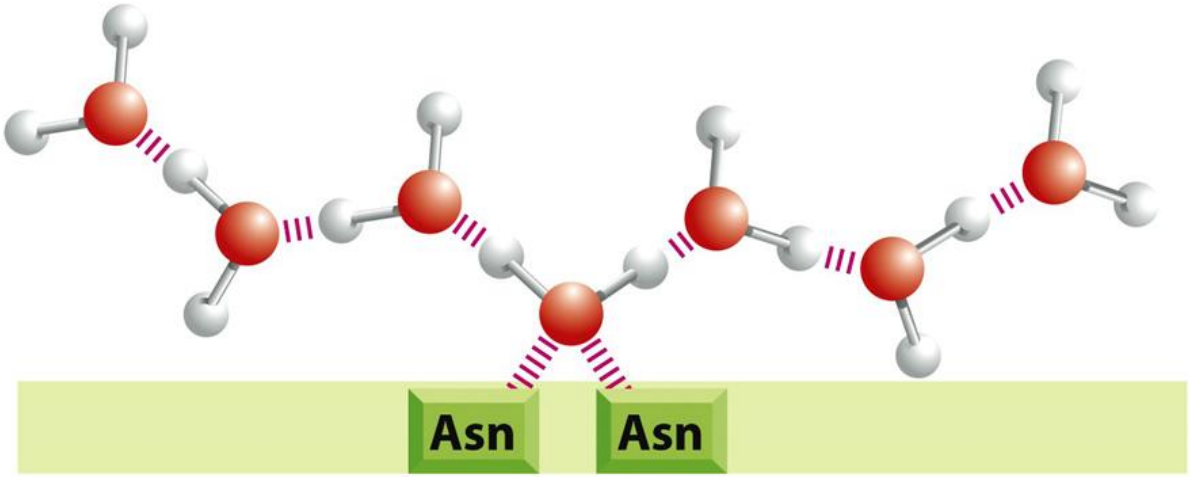
Blue and red : hydrophilic residues
Yellow: hydrophobic residues
Green: irrelevant to water channeling

How does aquaporin prohibit H+ to pass?

Normal proton relay



proton relay stops
In aquaporins

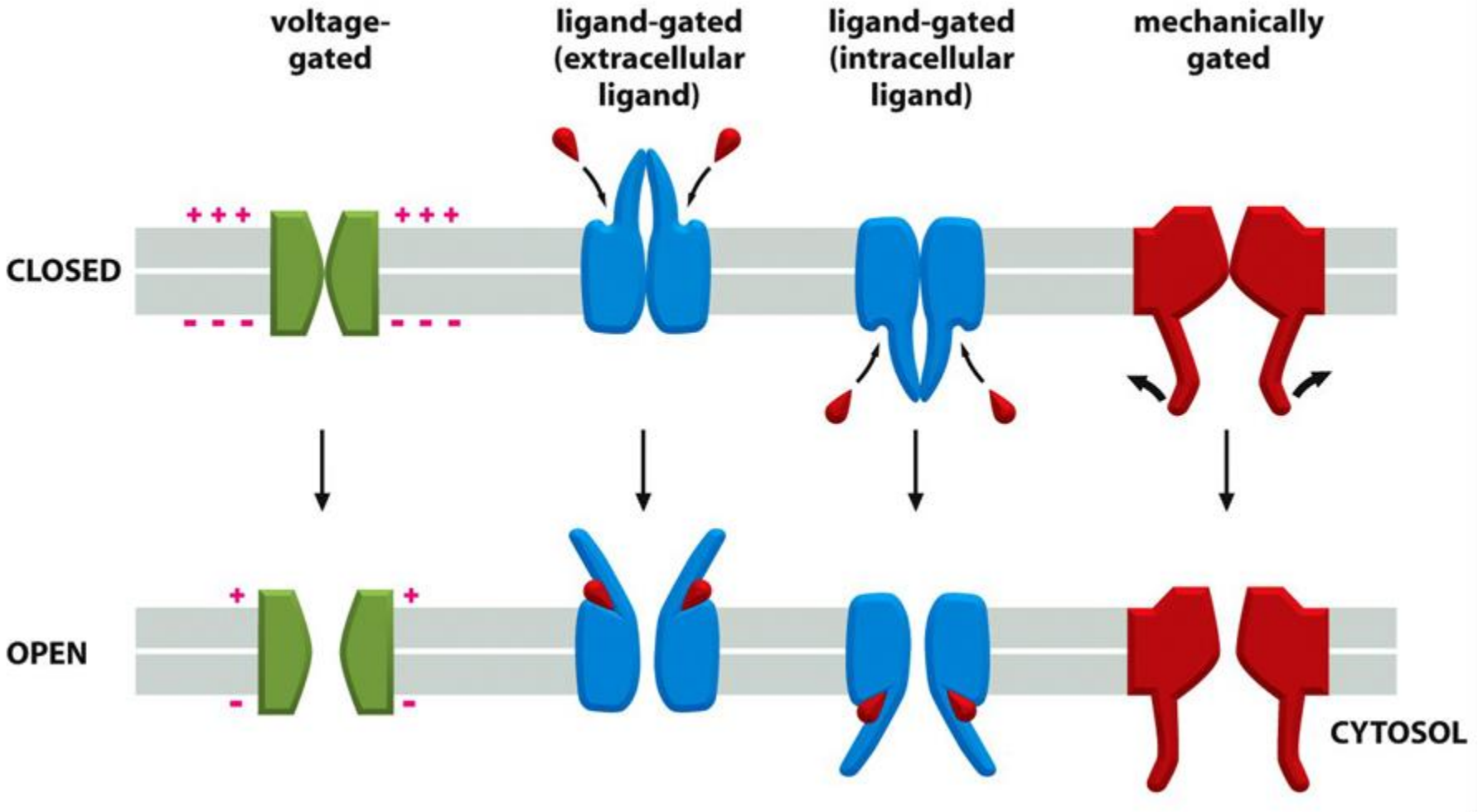


2. Ion channels

1. Highly selective, >100 types have been described thus far
2. Transport ions down the gradient.
3. Tight control in its open and close forms.
4. Transport with a high efficiency.
5. Are important for muscle and nerve cell function

The gating of ion channels

(pulling by cytoskeleton)



Mechanical gated channel is found in the hair cell of the ear

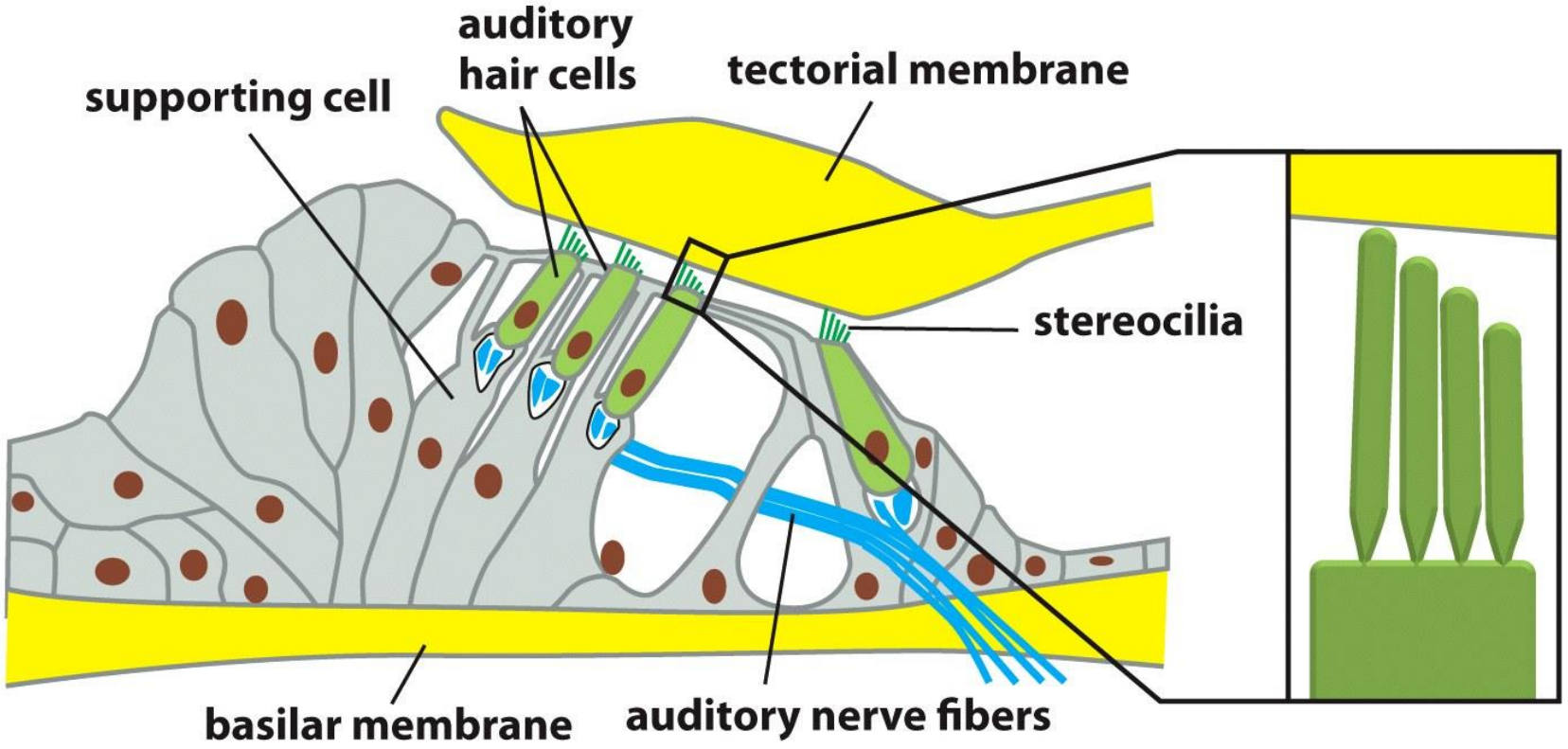
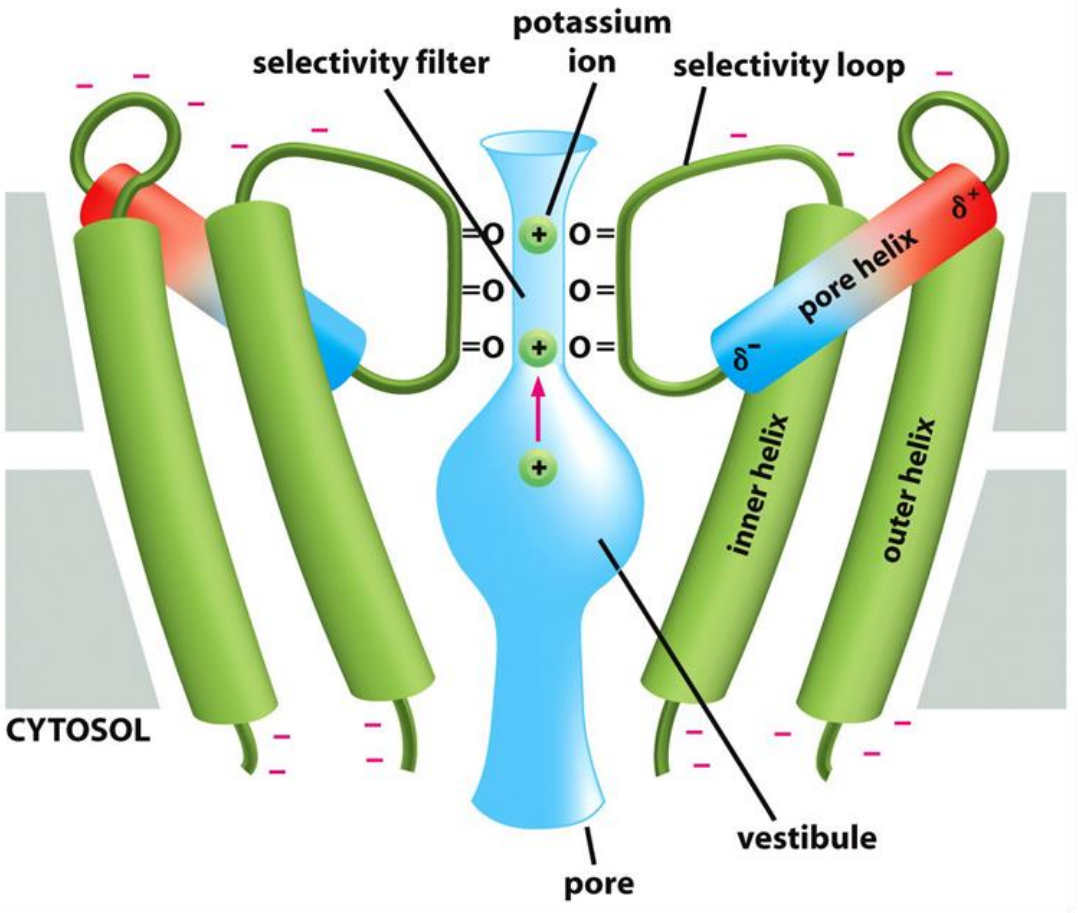
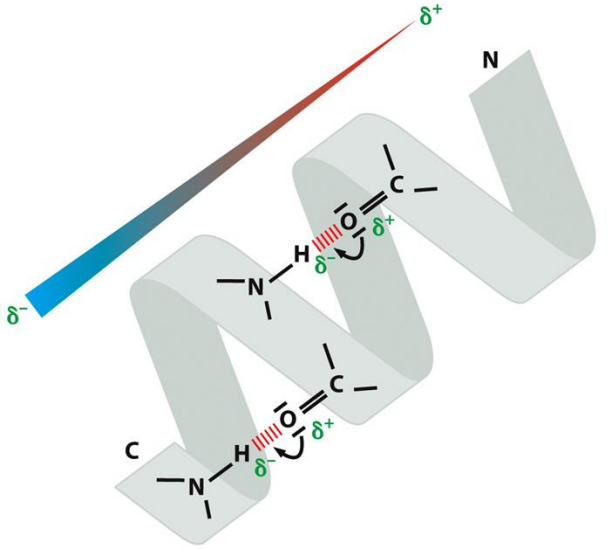


Figure 12-26a Essential Cell Biology 3/e (© Garland Science 2010)

Example: Bacterial K+ channel

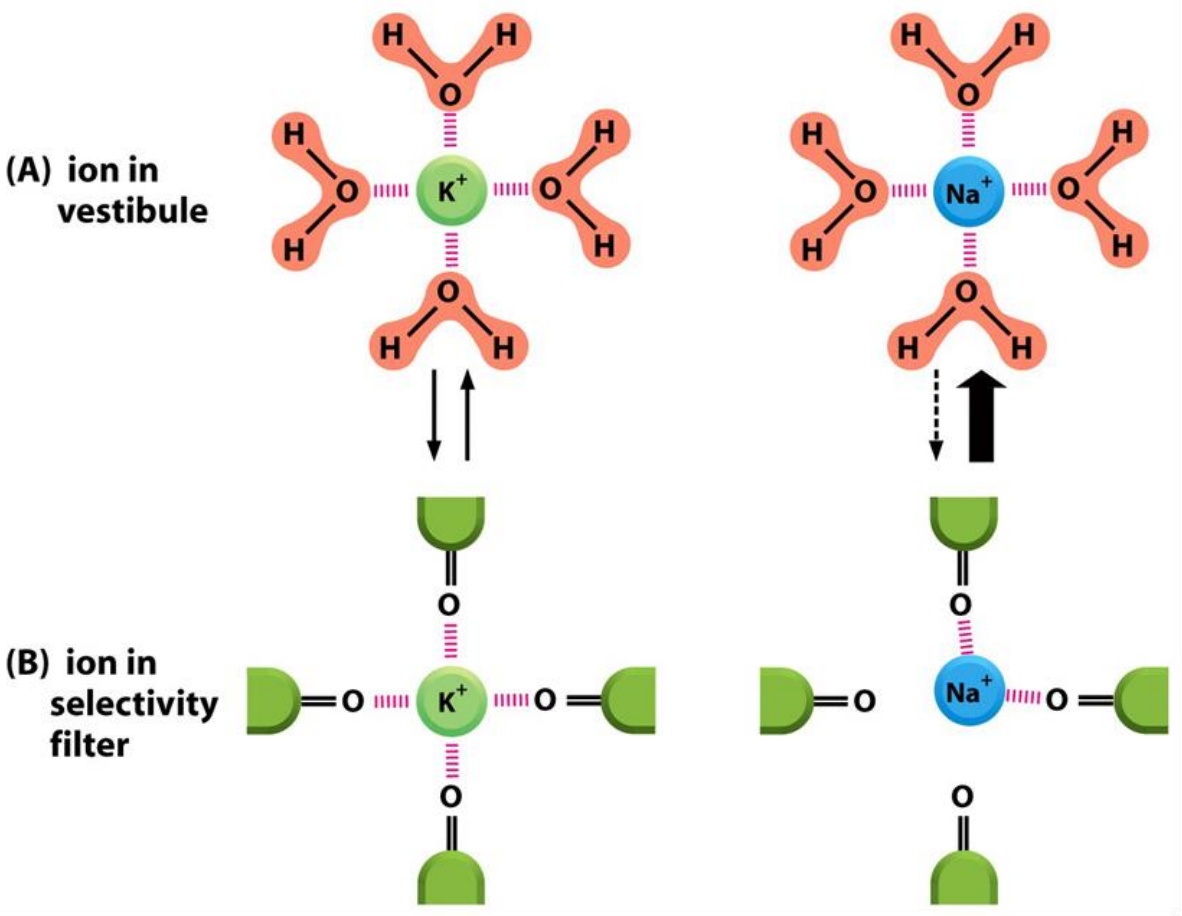
Conduct K+ 10,000 fold better than Na+



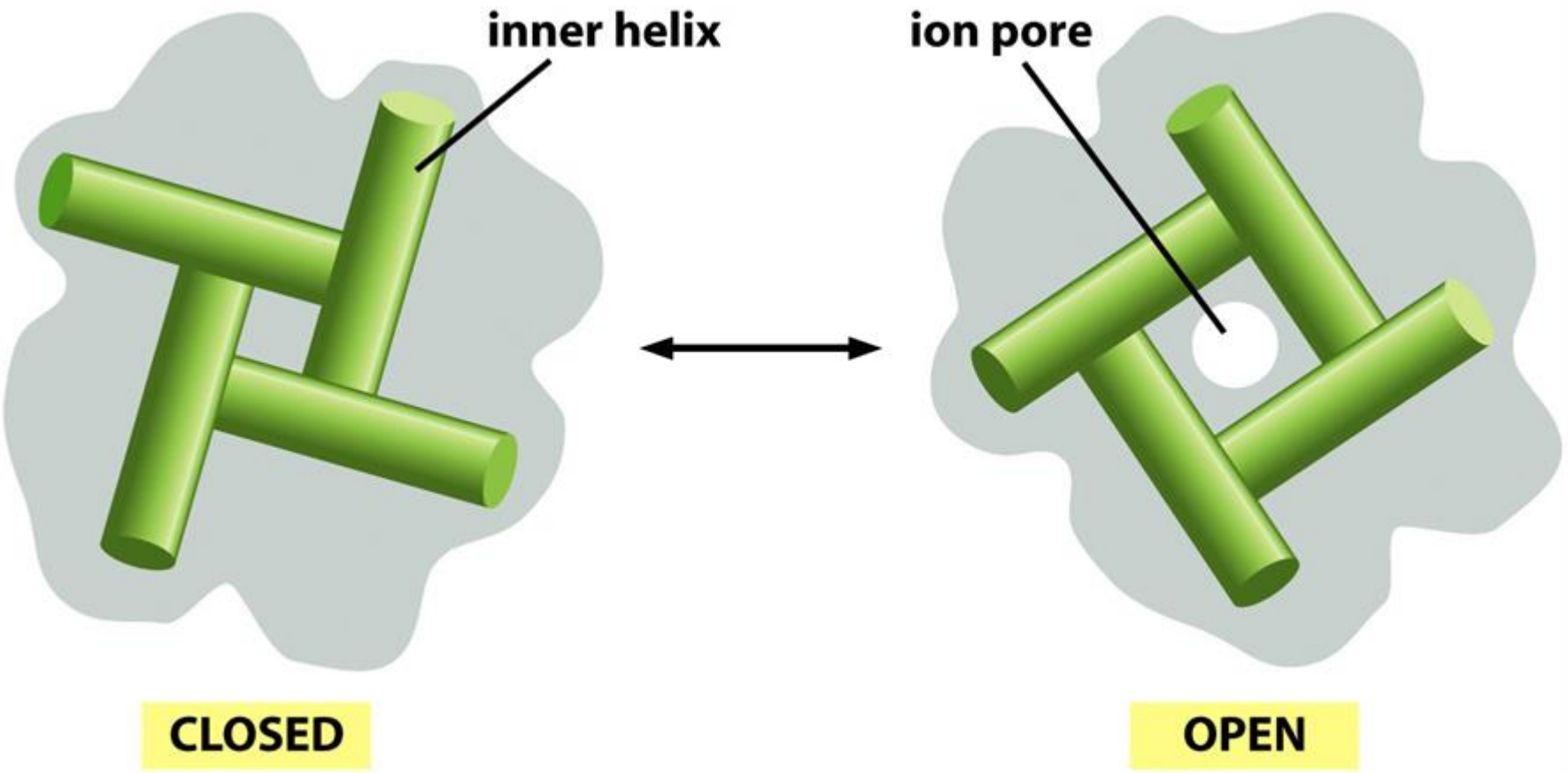


polarity in pore helix

Specificity for K+ due to the selectivity filter



Model for gating of a bacterial K+ channel



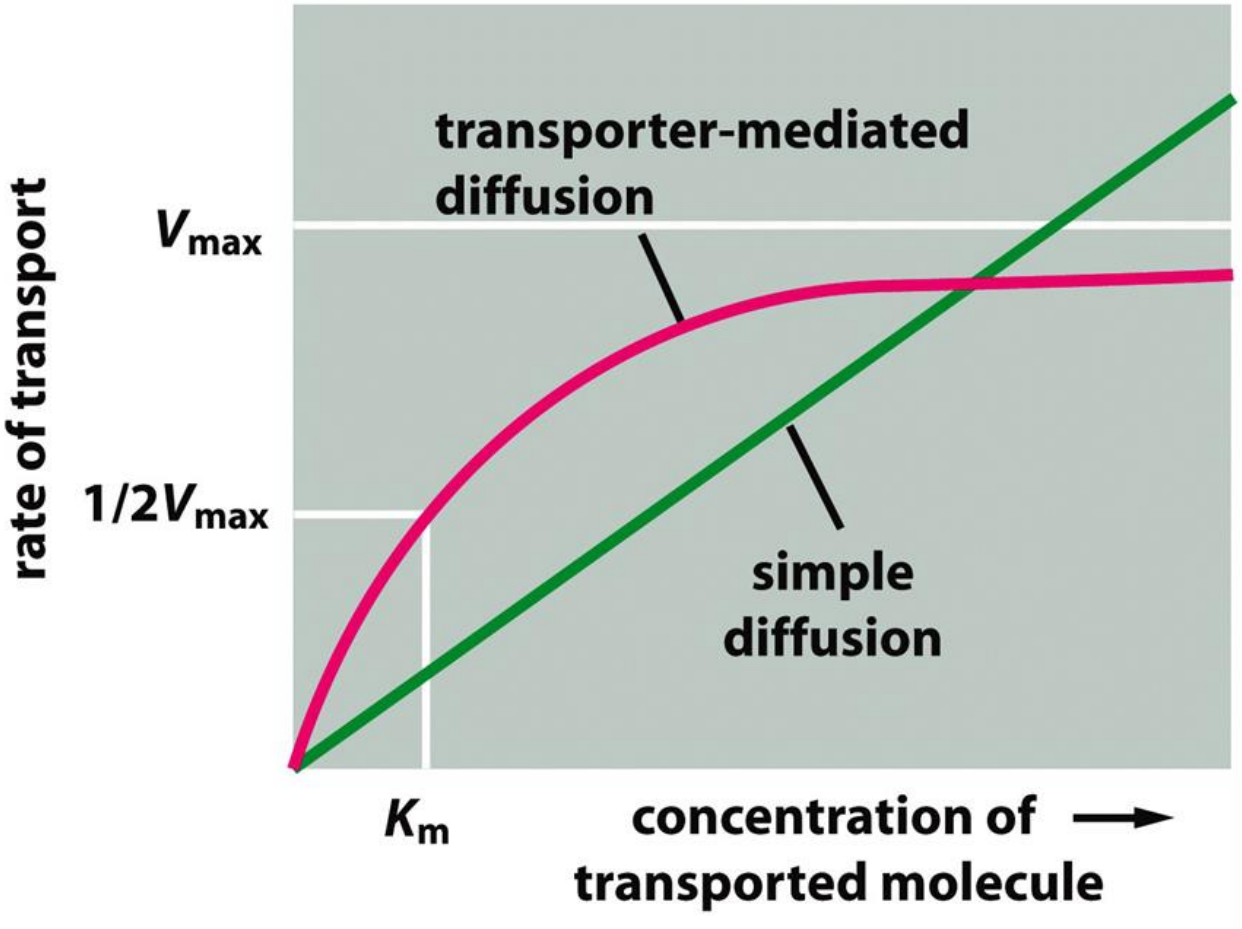
III. Transporters

1. Uniporter – GLUT1
2. Symporter- lactose permease
3. Antiporter :
   - Na+/H+ exchanger
   - Na+ driven Cl-/HCO3-exchanger
   - Na+ independent Cl-/HCO3- exchanger
4. Transcellular transport

Features of transporters

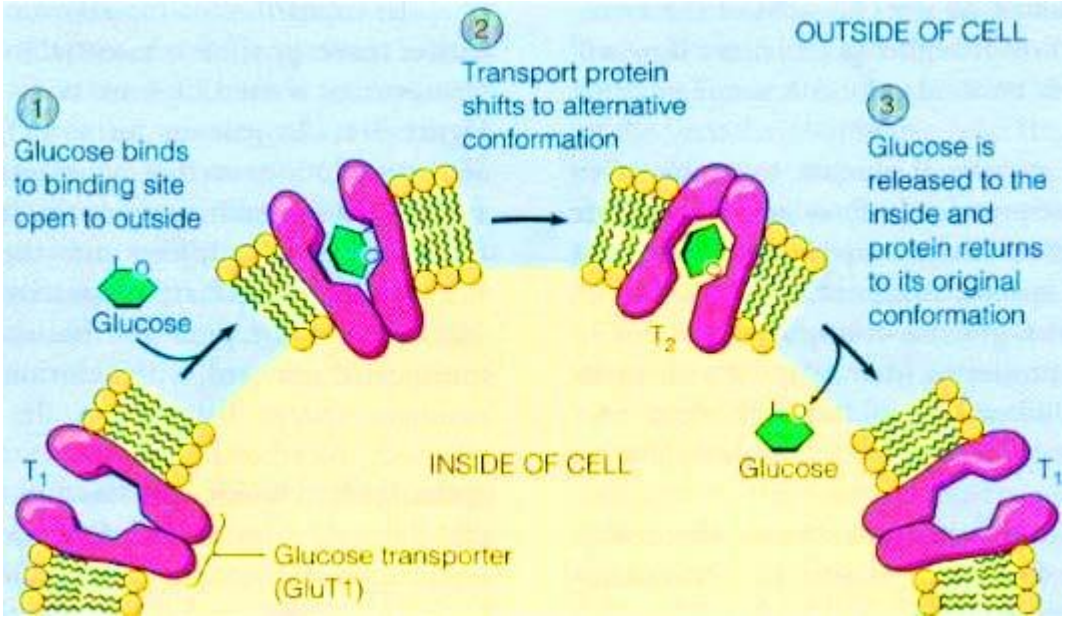
1. There are limited number of transporter in cell membrane
2. Each transporter has one or more specific binding sites for its solute
3. Each transporter has a *V*max, which is characteristic of a carrier
4. Each transporter has a characteristic affinity, *K*m
5. Binding and transport is reversible.
6. Binding can be competitively or non-competitively inhibited.
7. Transport is specific. Only one single or a group of closely related molecules will be transported.
8. Transport rate is higher than simple diffusion
9. Hydrophobicity of solutes does not matter.

Binding and transporting resembles enzyme kinetics



1. Uniporter- GLUT1

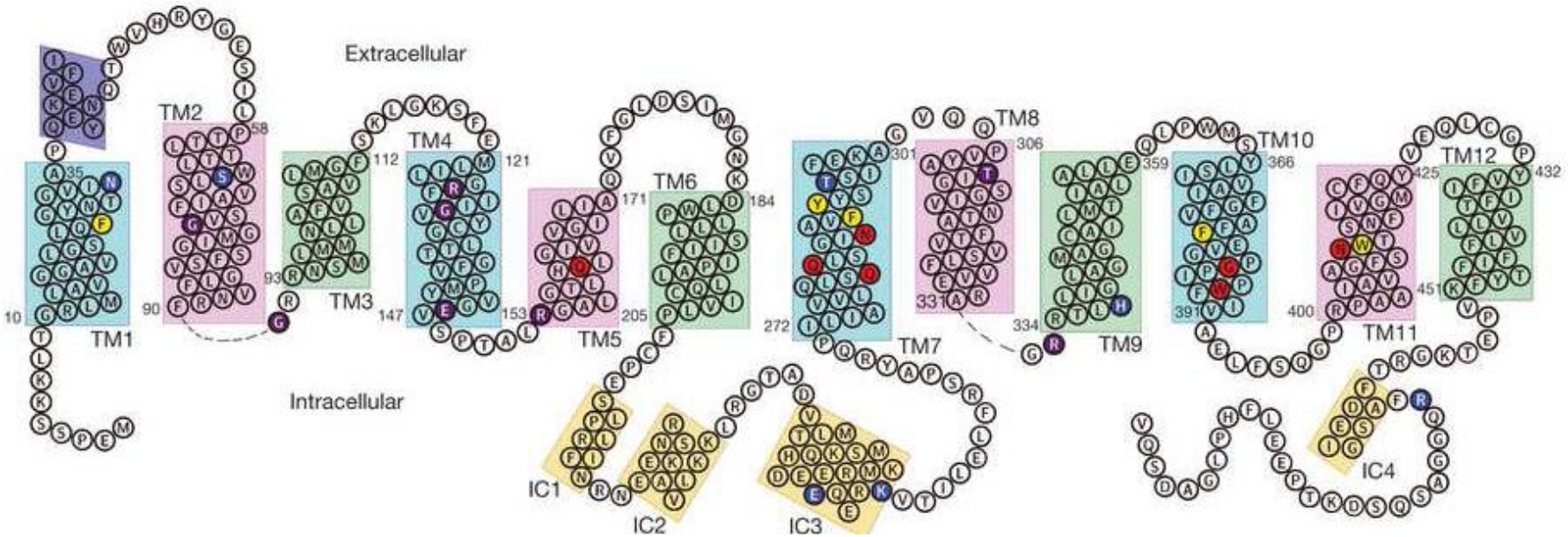
GLUT 1 is glucose transporter, transport glucose from extracellular space into cells



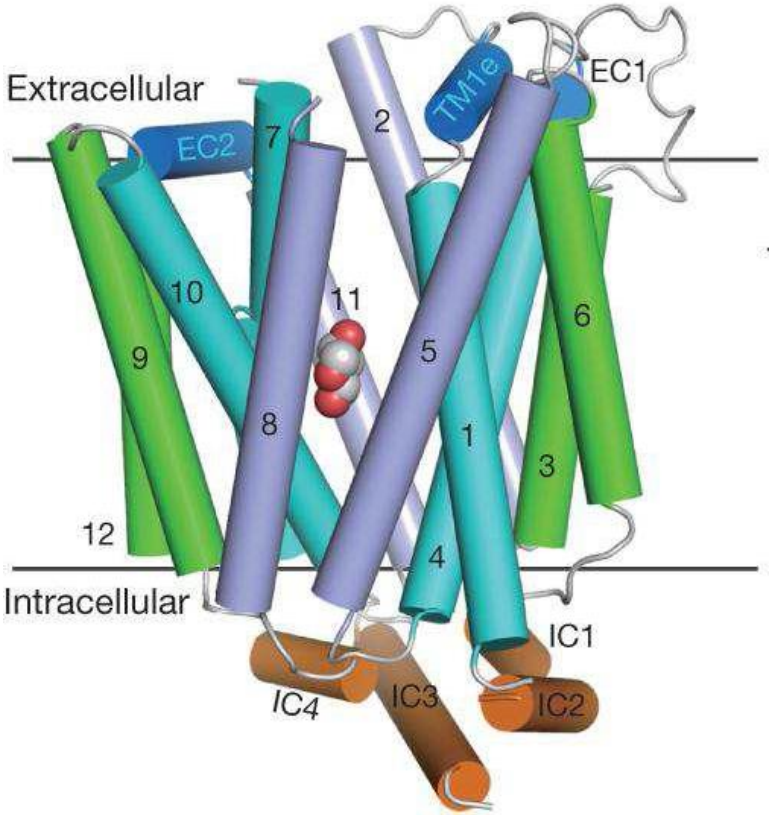
Undergoes conformational change

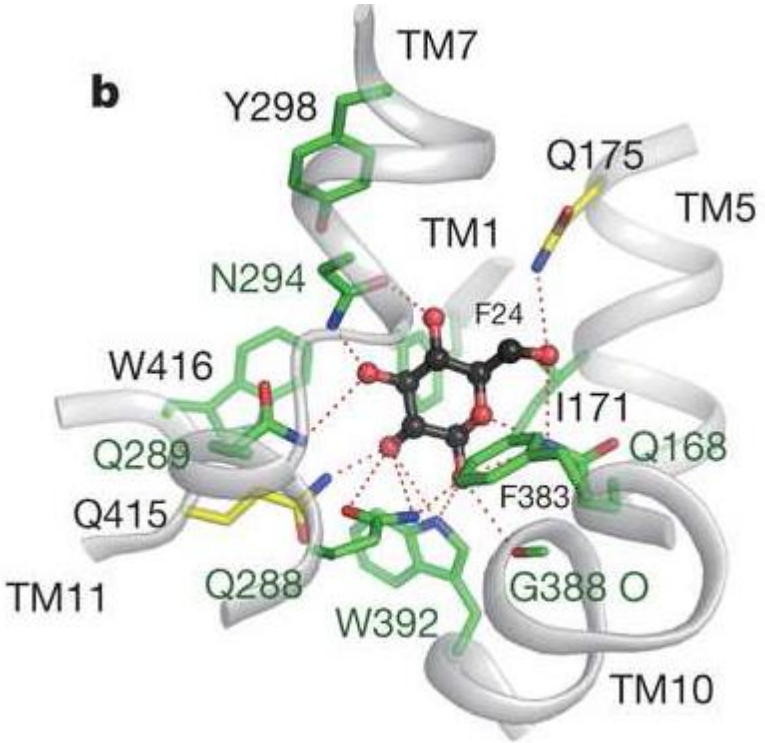
GLUT1 transporter

1. Abundant in erythrocytes
2. Is quite specific for D- glucose (Km-1.5mM) than for D-mannose (Km-20mM)
3. And D-galactose (Km-30mM)
4. Right after transport inside cells, Glucose is phosphorylated into Glu-6-P
   Maintaining a sufficient gradient for rapid transport of Glucose inside cells.
5. Has 14 homologs in human genome, differential tissue expression.



Structure of GLUT1





Sun, Nature, 2012

2. Symporter

Energy stored in electrochemical gradients

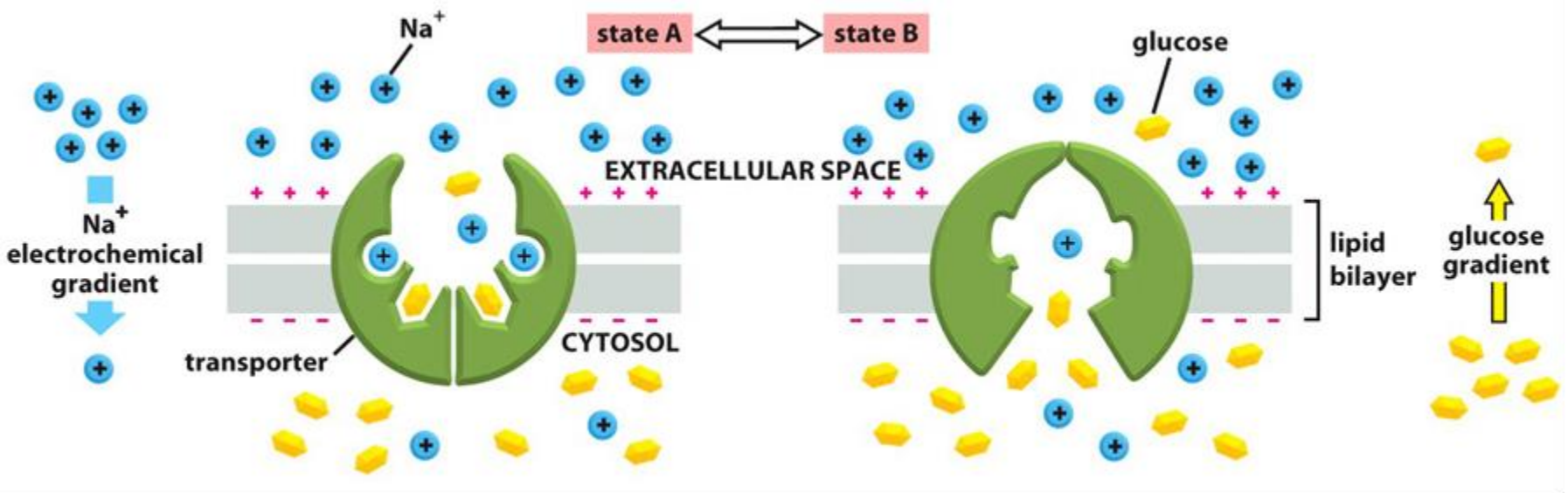
Symporter couple the Na+ or H+ gradient in transport

1. Eukaryotic cells: Na+ as a primary co-transporting ion

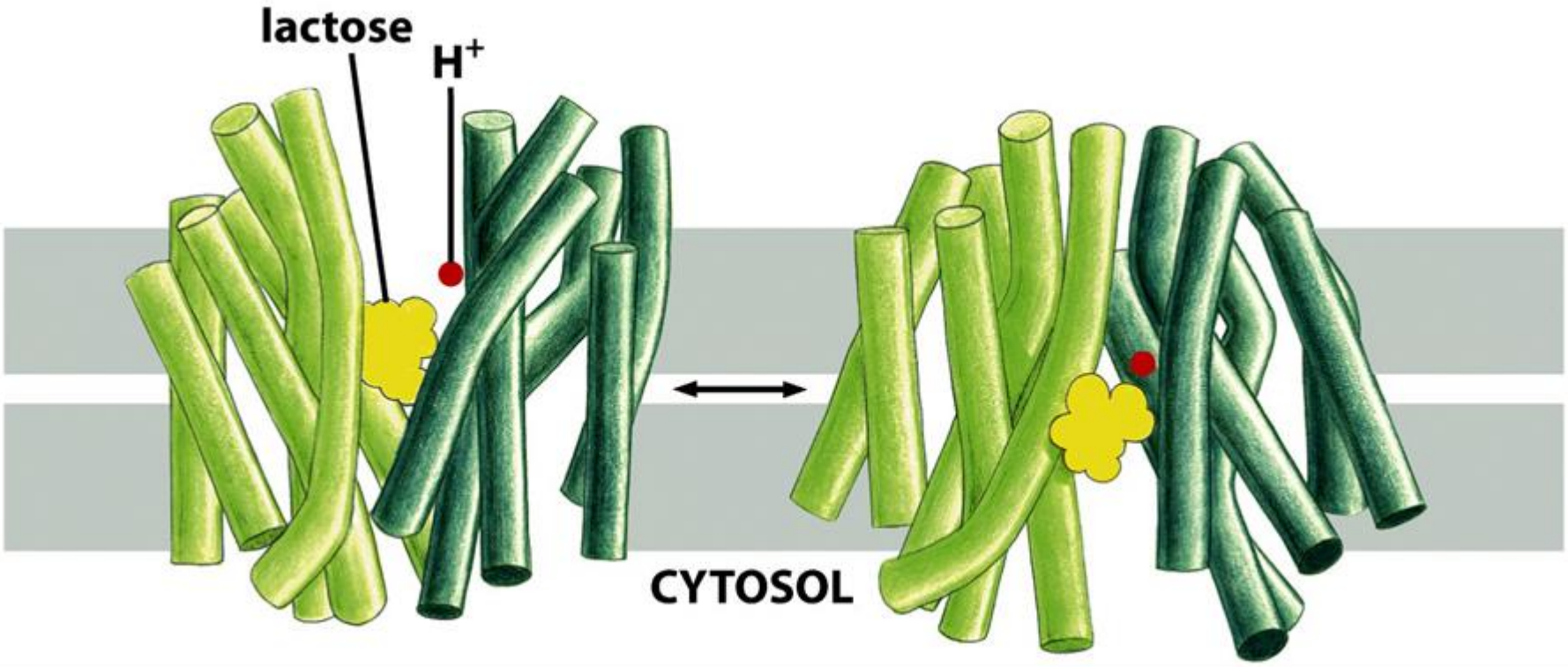
2. Bacteria and yeast or animal cell subcellular organelles:
   H+ as a primary co-transporting ion

Example :

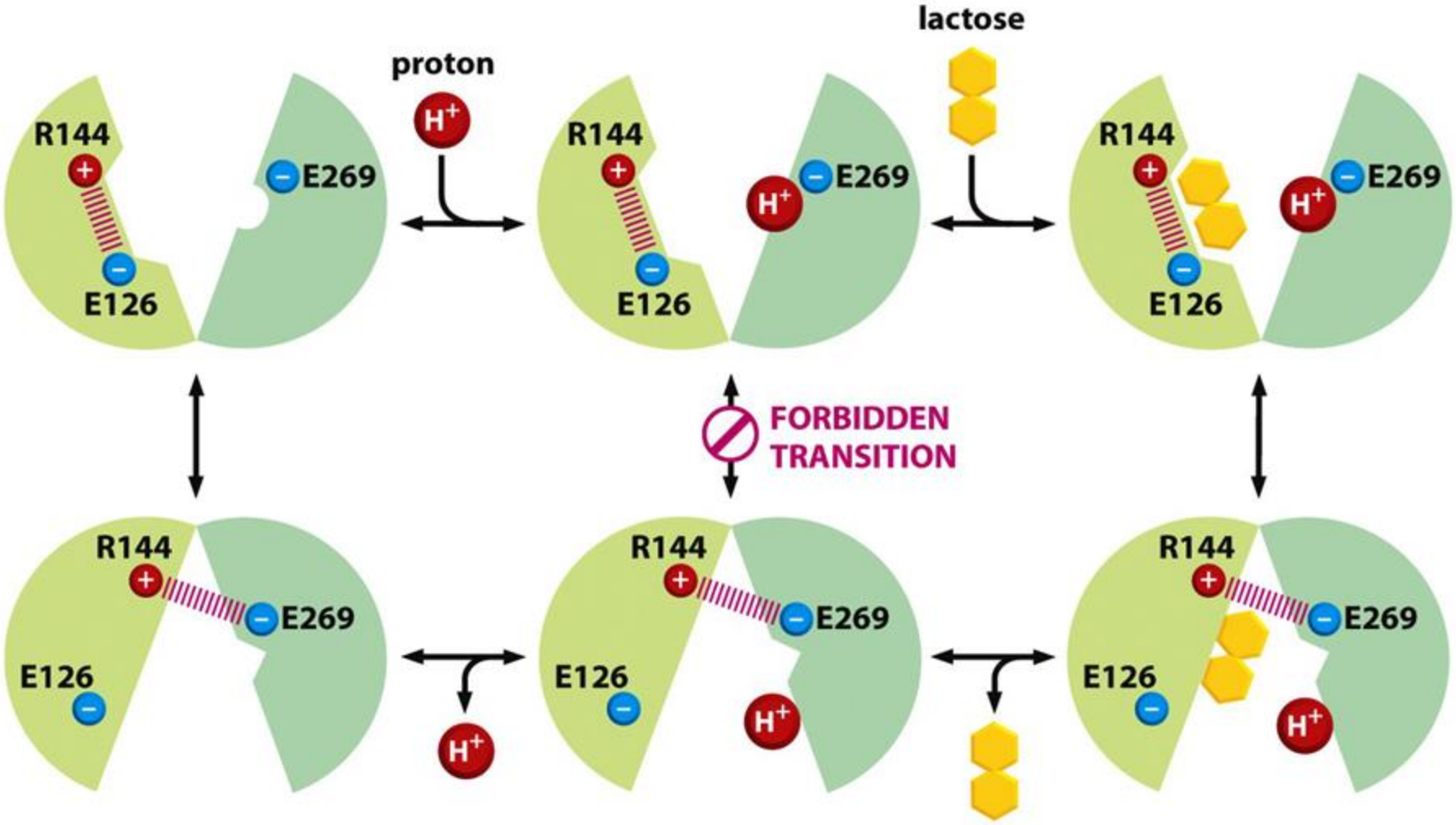
Glucose transport can be driven by Na+ gradient



Lactose permease--- use H+ to symport in *E. coli*



Lactose permease transport- at molecular level



3. Antiporter- a way for cells regulate pH

Cells has nice control in pH:
Cellular pH ~ 7.2;
while lysosome pH~5.0

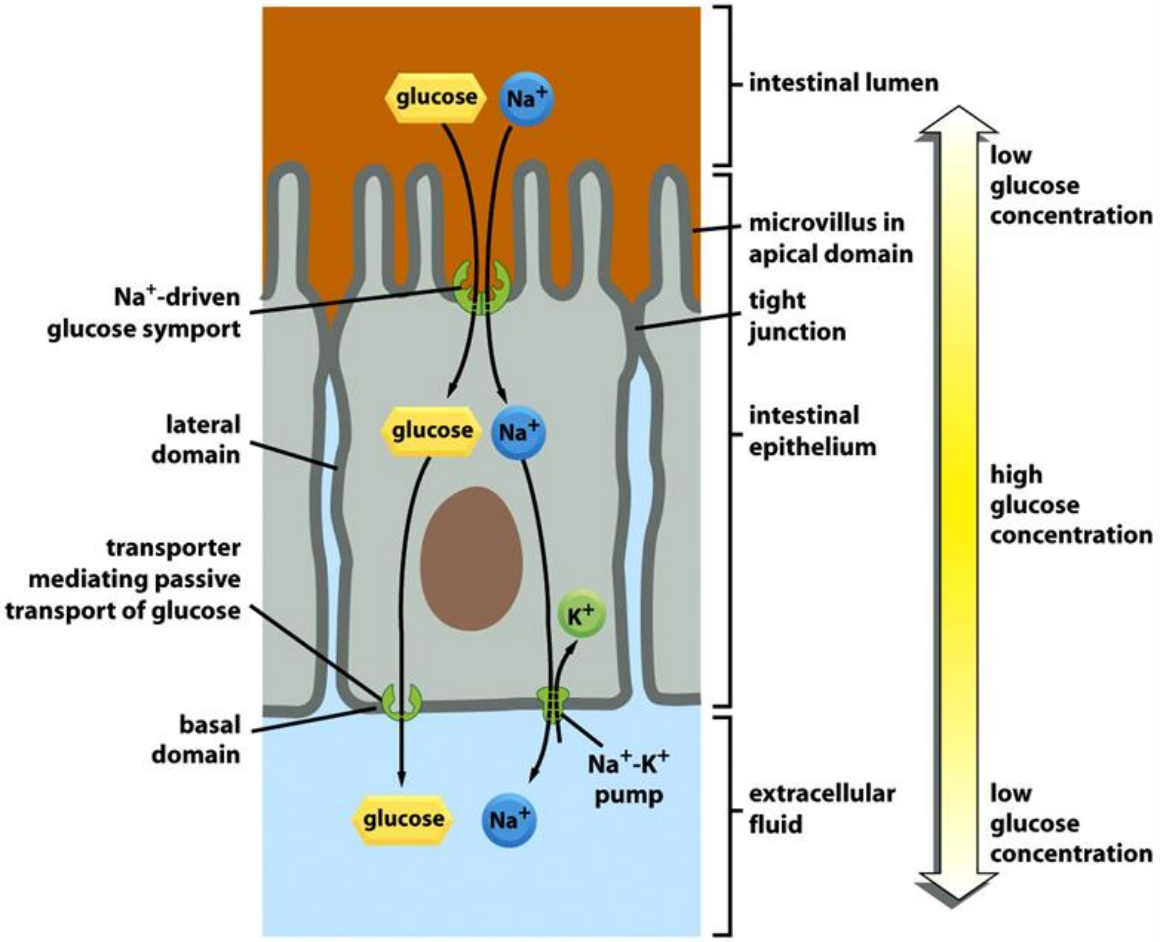
(1). Na+/H+ exchanger: Coupled influx of Na+ and efflux of H+
its activity is increased by cytosolic low pH

(2). Na+-driven Cl-/HCO3- exchanger:
2-fold effective than Na+/H+
coupled influx of NaHCO3 and efflux of HCl
its activity is increased by cytosolic low pH

(3). Na+-independent Cl-/HCO3- exchanger:
coupled efflux of NaHCO3 and influx of HCl
its activity is increased by cytosolic high pH

4. Transcellular transport

Transcellular transport: solute is transferred from one cell to the other cell.
Reason: Transporters are distributed nonuniformly in the plasma membrane



IV. ATP pumps

One or more ATP binding sites on the cytosolic portion of these transporters

1. P-type:
   - Ca2+ pump
   - Na+-K+ pump
2. ATP-synthase (F-type)
3. V-type
4. ABC transporters

P-type, F-type and V-type transport ions, as do some AB transporters, but most ABC transporters transport small molecules such as :

amino acids
sugars
peptides
lipids
drugs, etc.

A diagram comparing different ATP pumps

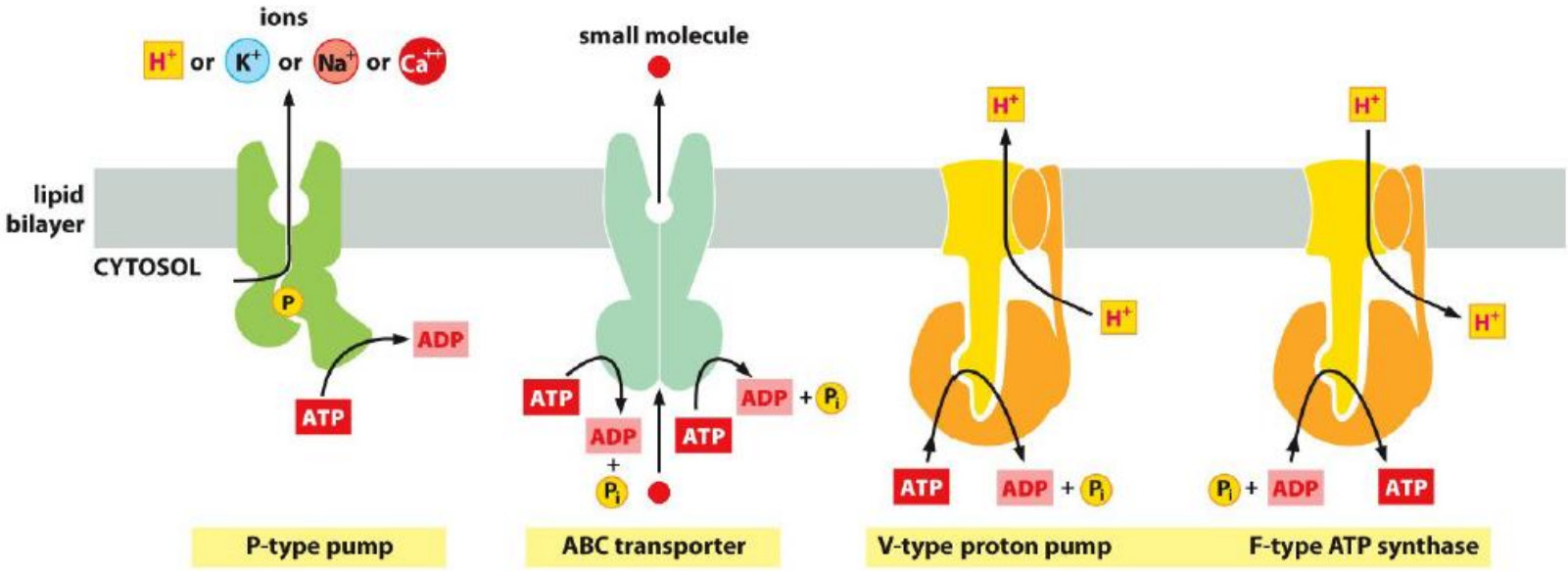


Figure 11-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

1. P-type pumps

(1). Structurally and functionally related multipass transmembrane protein
(2). They phosphorylate themselves during the pumping cycle.
(3). Many of them are responsible for setting up and maintaining gradients of Na+, K+, H+ and Ca2+ across cell membranes, include:

H+ pump in plants and fungi
Na+/K+ pump in higher organism
H+/K+ pump in stomach
Ca2+ pump all eukaryotic cells

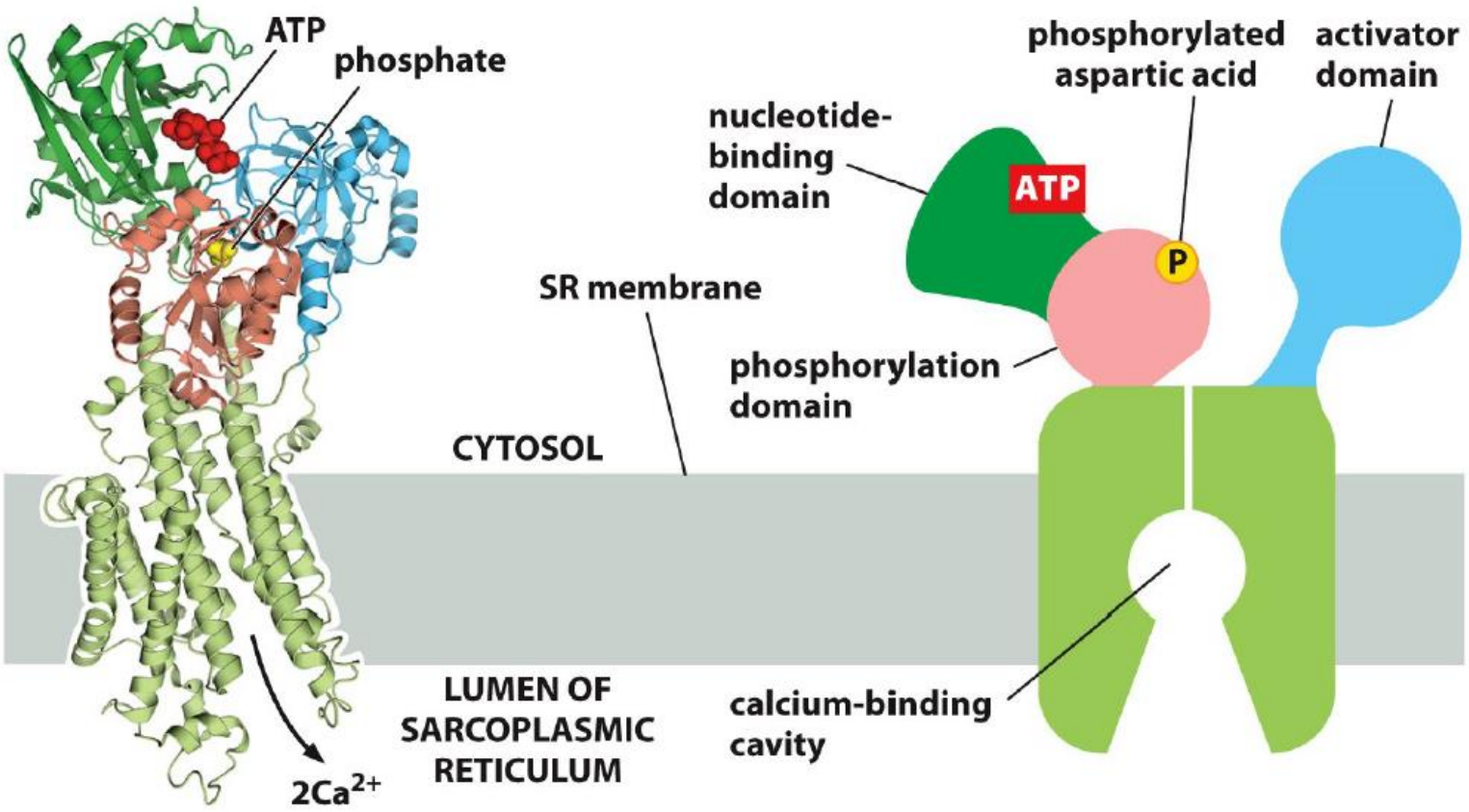
Example 1. Ca2+ pump or Ca2+ ATPase

Ca2+ is an important messenger in signal transduction,

What is the cytosolic concentration of Ca2+? How about extracellular?

1. Locate on endoplasmic reticulum of cells
2. Take up 90% of the membrane protein in sarcoplasmic reticulum of skeletal muscle cells
3. 10 transmembrane α-helices

Ca2+ pump can be phosphorylated to induce conformational change



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The pumping cycle of Calcium pump

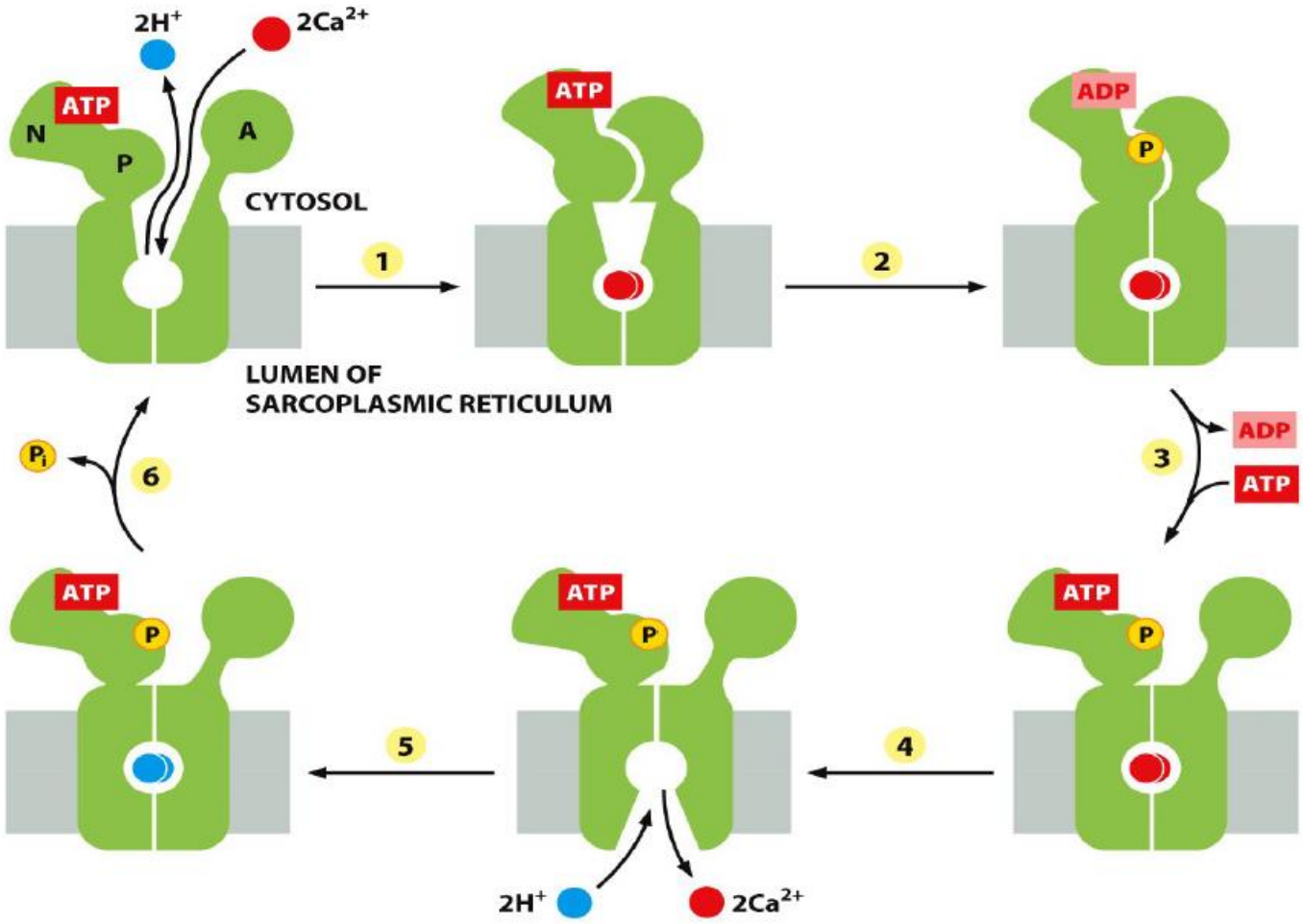


Figure 11-14 Molecular Biology of the Cell 6e (© Garland Science 2015)

Example 2: Na+/K+ pump (Na+/K+ ATPase)

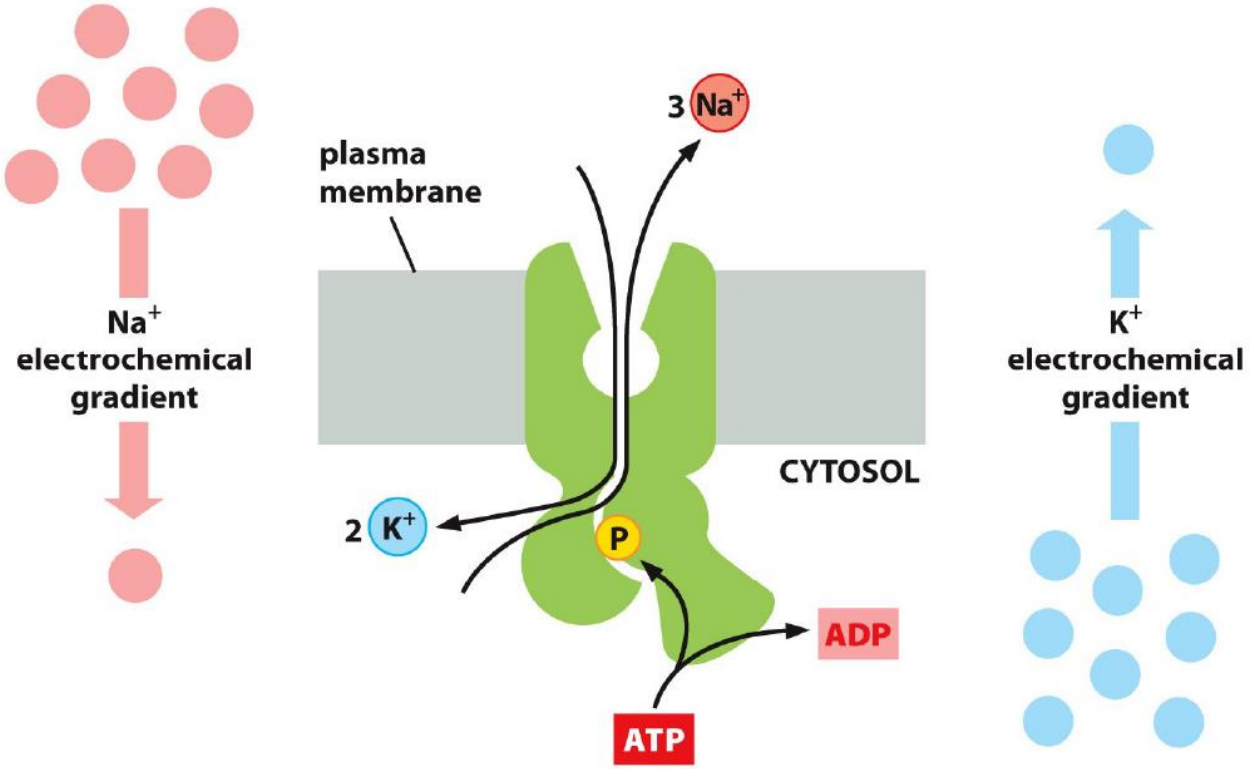
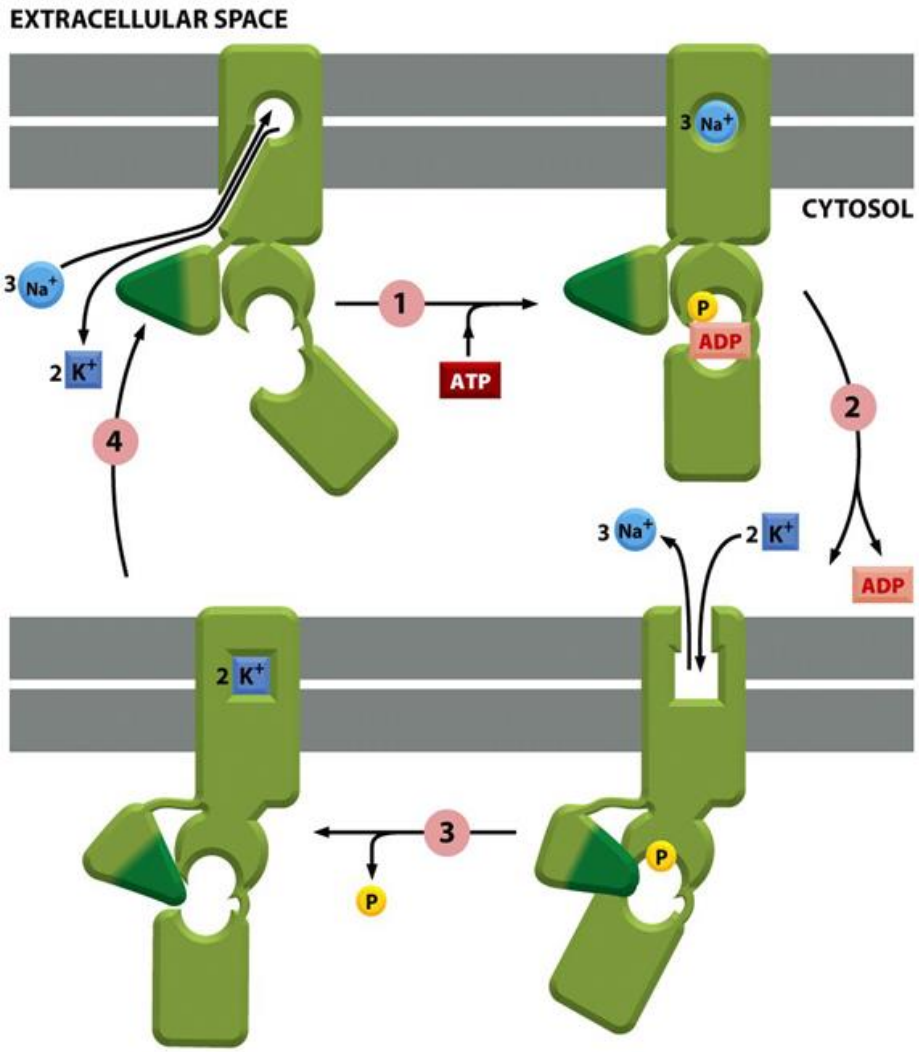


Figure 11-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

Na+/K+ pump

1. It is a tetramer of subunit composition α2β2
2. For each ATP hydrolysis, 3 Na+ ions are pumped outside and 2 K+ ions are pumped inside, both against their gradients.
3. Structurally homologous to Ca2+ pump,
4. It has the conserved Asp as phosphorylation site
5. Phosphorylation of Asp is Na+ dependent.
6. Dephosphorylation of Asp is K+-dependent.

Cycle of Na+/K+ pump



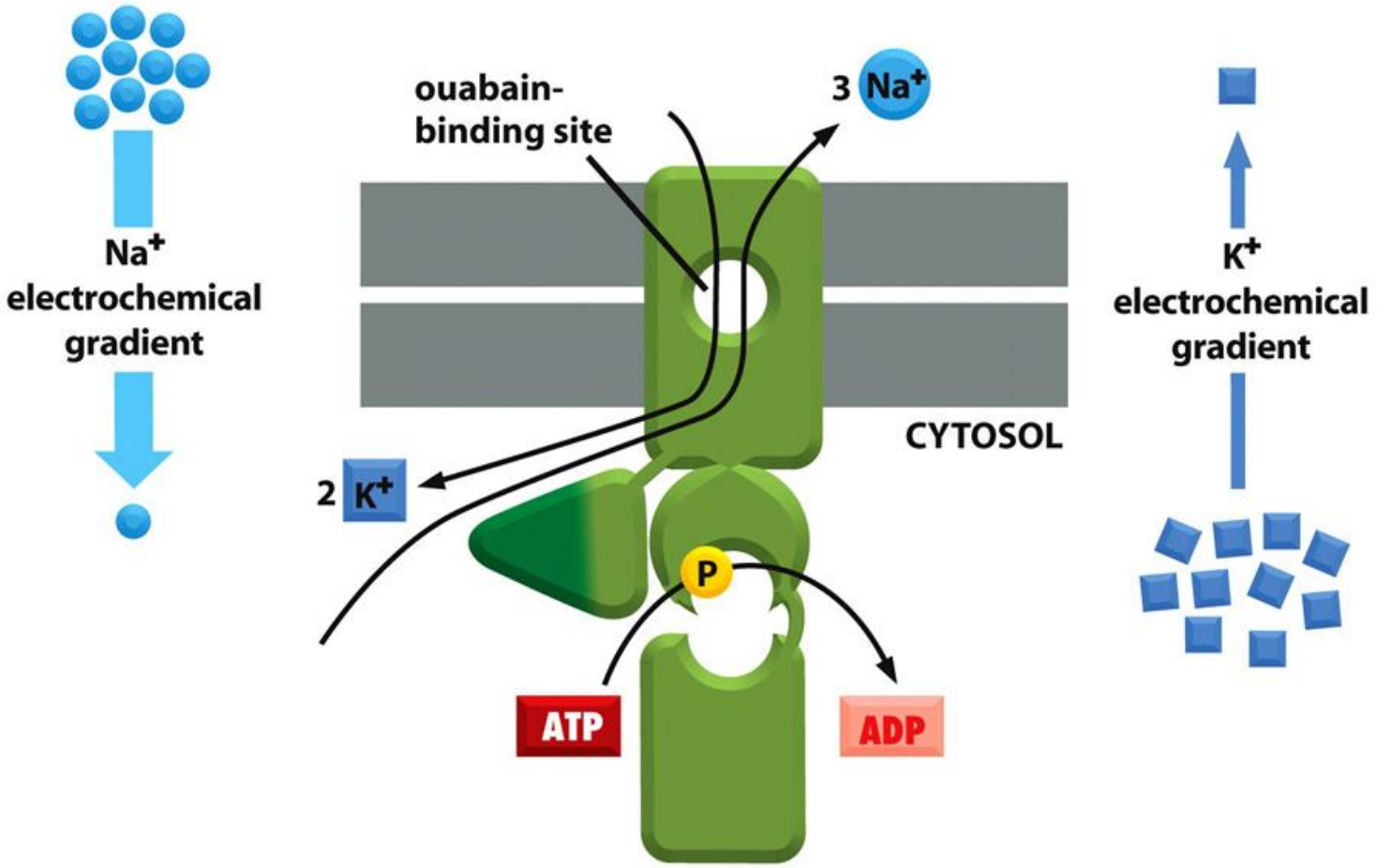
First step: in the cyotsolic surface
The transporter has high affinity for
Na+ ions and low affinity for K+ ions

Second step: ATP binding and hydrolysis
Cause conformational change and
Release of 3 Na+ ions outside.

Third step: Now phosphorylated transporter
Has high affinity for K+ and low affinity
For Na+ ions in the exoplasmic surface.

Fourth step: K+-dependent dephosphorylation
causes conformational change and release of
K+ inside.

Certain drugs target Na+/K+ pump

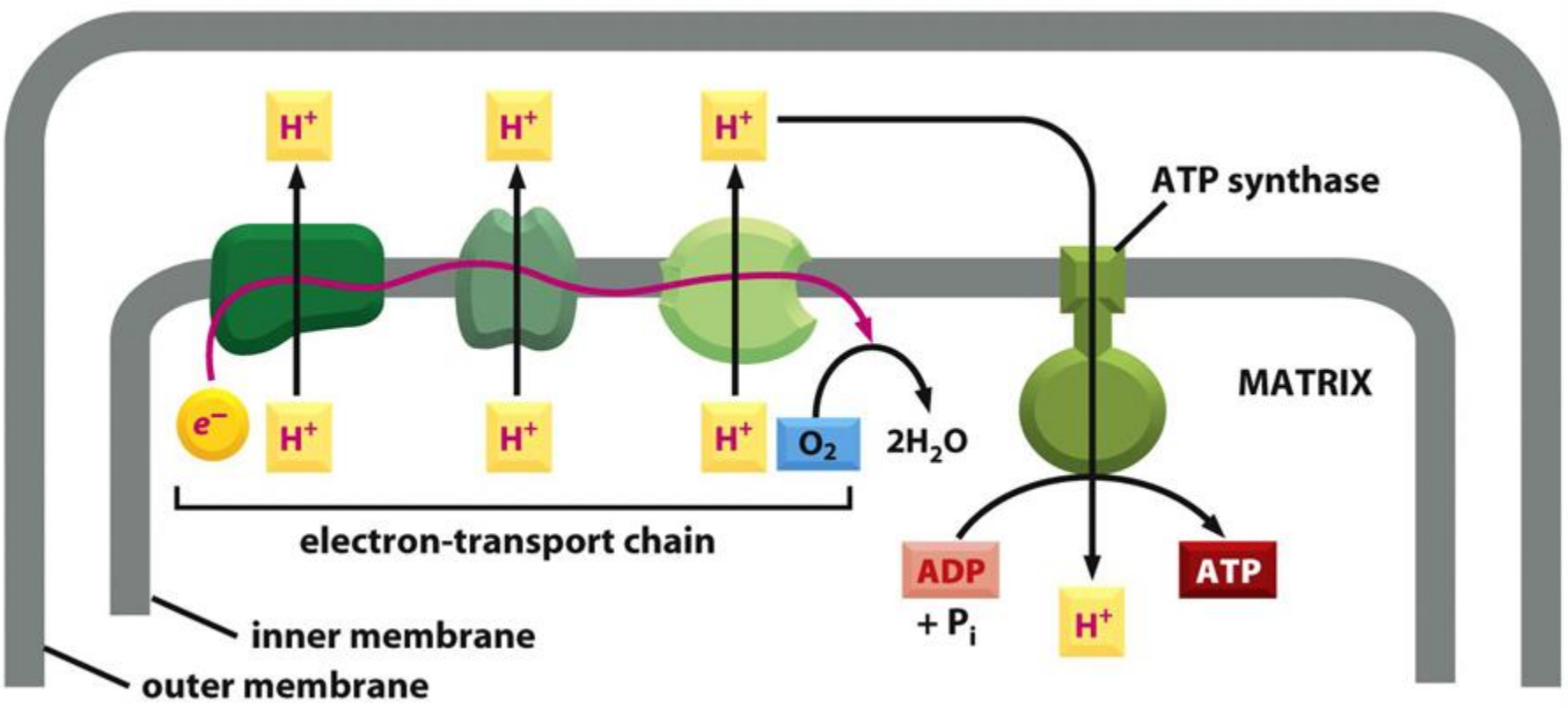


Ouabain and digoxin bind to exoplasmic domain and specifically inhibits ATPase activity.

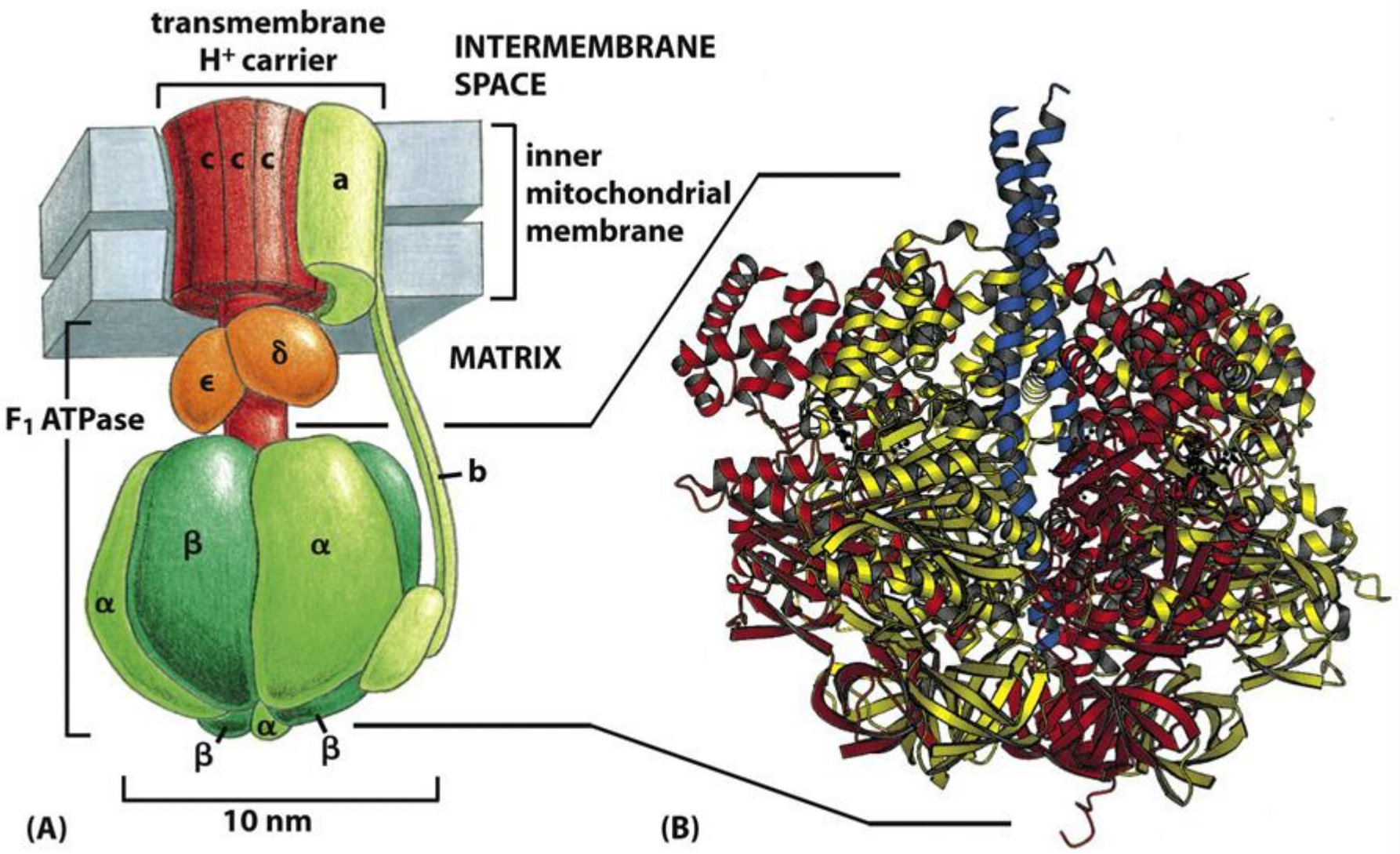
2. F-type pumps-ATP synthase

1. Turbine –like proteins, constructed from multiple different subunits.
2. Found in the plasma membrane of bacteria, the inner membrane of mitochondria, and the thylaloid membrane of chloroplast.
3. Called ATP synthase, they use the H+ gradient across the membrane to drive the synthesis of ATP from ADP.
4. NOT controlled by phosphorylation.

Proton gradient drives ATP synthesis

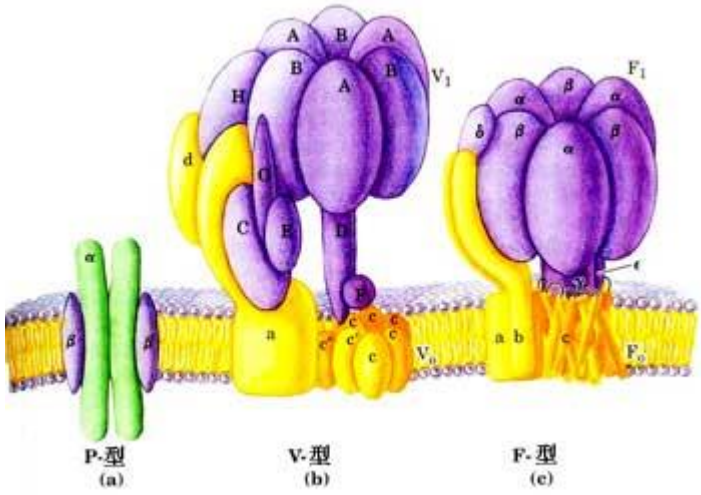


Crystal structure of ATP synthase



V- type transporter

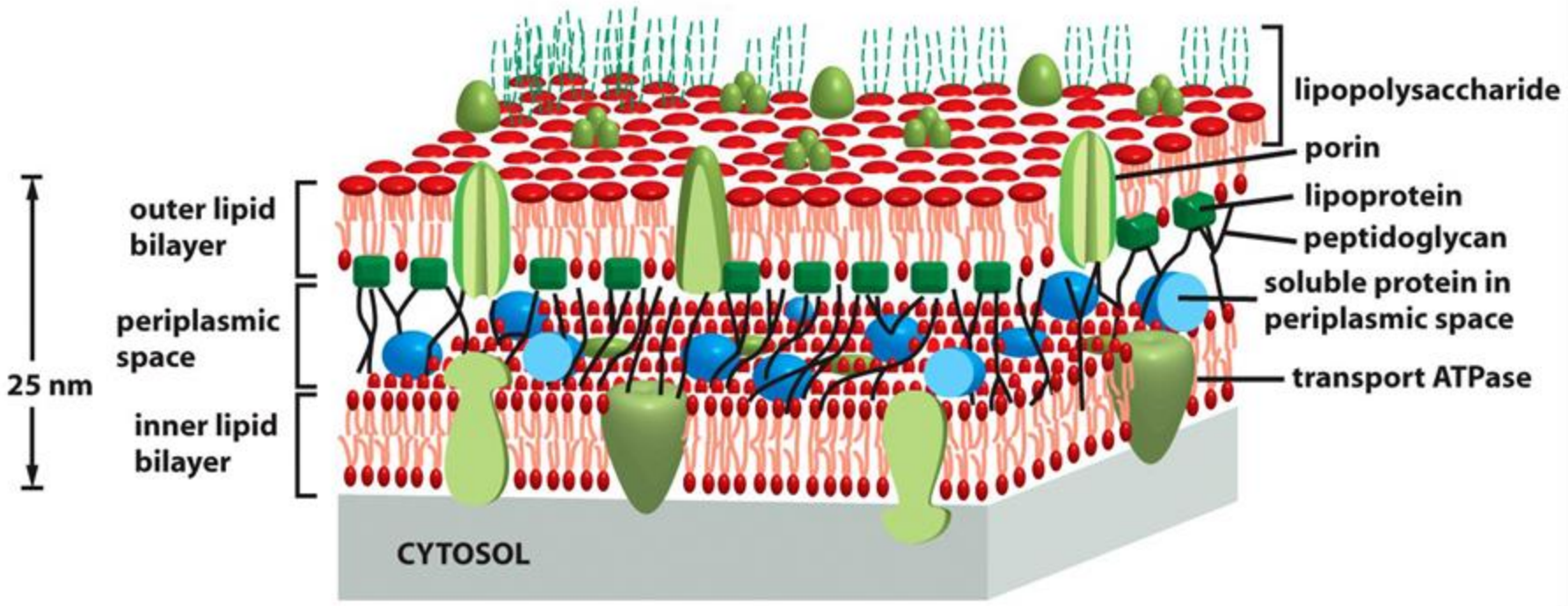
1. Transport only protons, but without ATP synthase
2. Present in lysosomes, endosomes, plant vacuoles.
3. Pump in protons in the organelle to maintain low pH.
4. Structurally similar to F-type transporters.



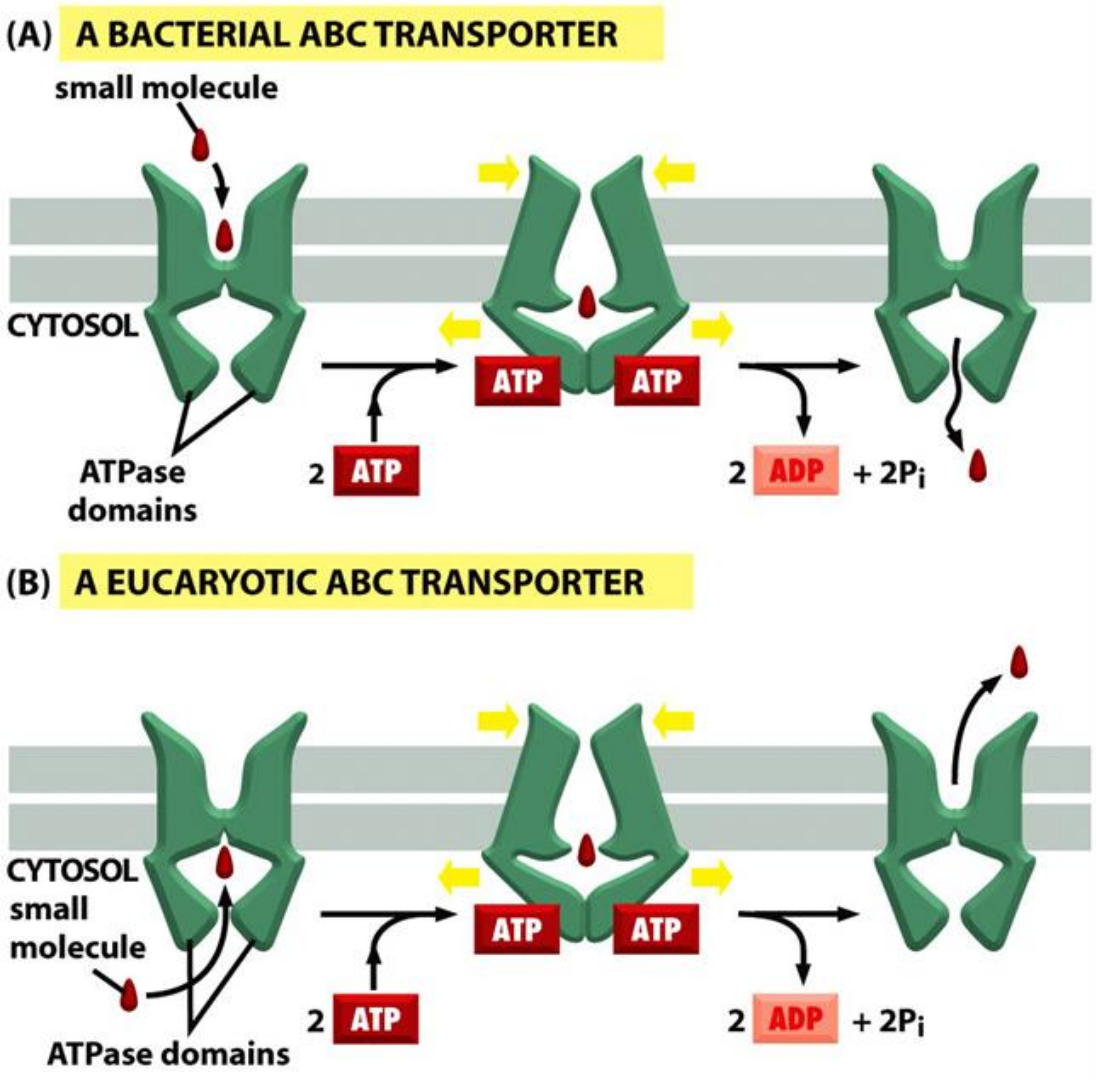
3. ABC transporters

1. Pump small molecules across cell membranes.
2. Contain two transmembrane domain and two cytosolic ATP binding domain (ATP-cassette)
3. ATP binding leads to dimerization of ATP–cassettes.
4. ATP hydrolysis leads to the dimer dissociation.
5. Conformational changes leads to transport of small molecules.
6. In E. coli, take up 5% of all bacteria genes
7. They are clinically important.

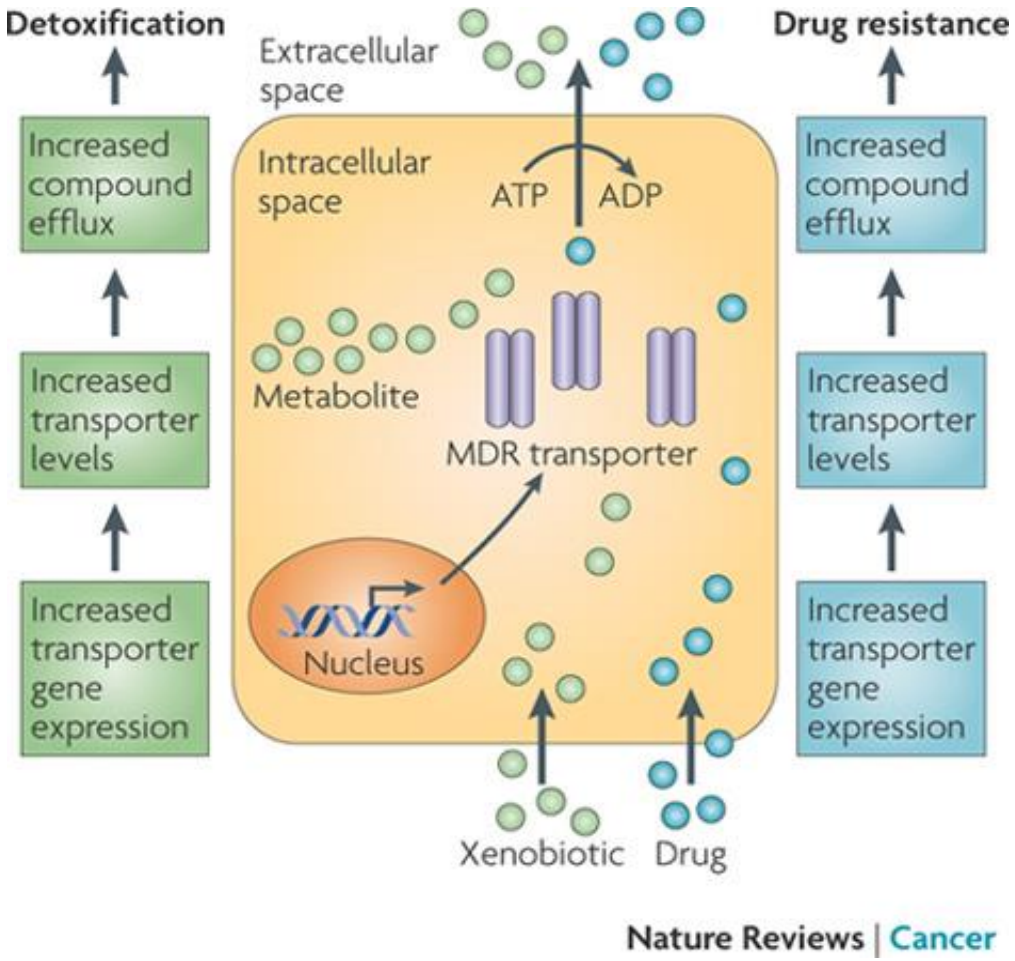
ABC in E. coli


lipopolysaccharide
porin
lipoprotein
peptidoglycan
soluble protein in periplasmic space
transport ATPase
outer lipid bilayer
periplasmic space
inner lipid bilayer
25 nm
CYTOSOL

Typical ABC transporters



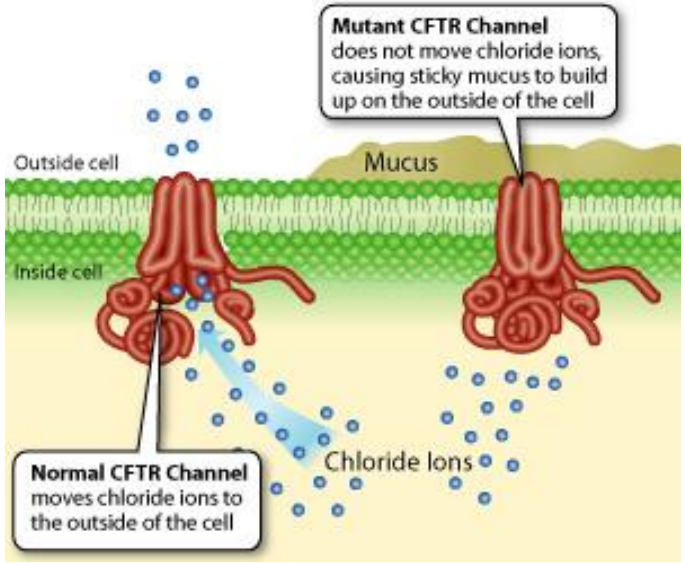
Example 1: Multidrug resistance (MDR) protein --- the first eukaryotic ABC transporter identified



Cancer cells with overexpression for MDR are resistant to cancer drugs

Example 2: Cystic fibrosis transmembrane conductance regulator (CFTR)

CFTR is an ABC transporter
CFTR regulates open and close of Cl- channel
Mutation in CFTR leads to cystic fibrosis



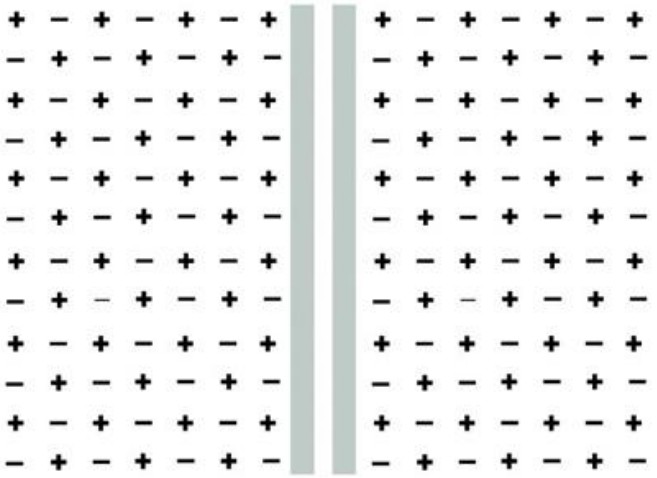
Example 3: Chloroquine resistance in malaria-causing *Plasmodium falciparum*

The protozoa has enhanced level of chloroquine ABC transporters and therefore has resistance to choloquine.

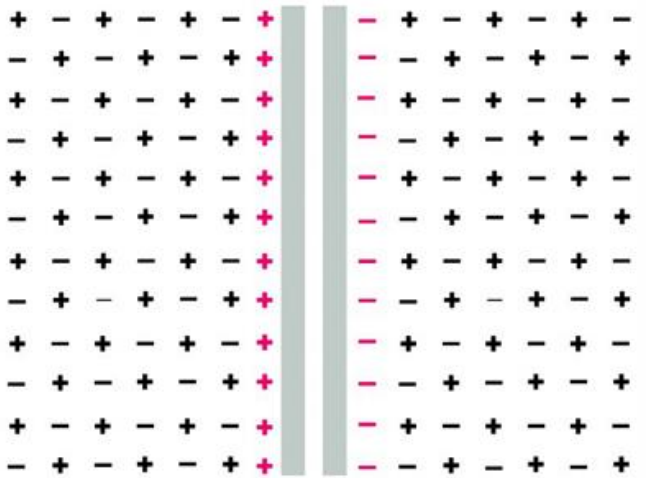
V. Neuron electric potential

1. Membrane potential
2. K+ channel and membrane potential
3. action potential
4. Transmitter-gated ion channels
5. Chemical synapses
6. Transmitter-gated ion channels as drug targets

1. Membrane potential



exact balance of charges on each side of the membrane; membrane potential = 0



a few of the positive ions *(red)* cross the membrane from right to left, leaving their negative counterions *(red)* behind; this sets up a nonzero membrane potential

Membrane potential:

Difference in the electrical charge on the two side of the membrane

Resting membrane potential:

Electric charge difference in the equilibrium conditions when there is no net flow of ions across the plasma membrane.

usually between -20mV to -120mV

2. K+ channel is an important factor in causing membrane potential

Na+/K+ pumps leaves a charge difference in and out of membrane
With high K+ inside and low K+ outside

K+ channels pump in and out to balance the difference.

Due to high K+ concentration inside the cell,
each K+ that is pump out by K+ channel will leave a negative charge inside
Which will result in a membrane potential.

3. Action potential---traveling wave of electrical excitation

a depolorization signal on the plasma membrane.

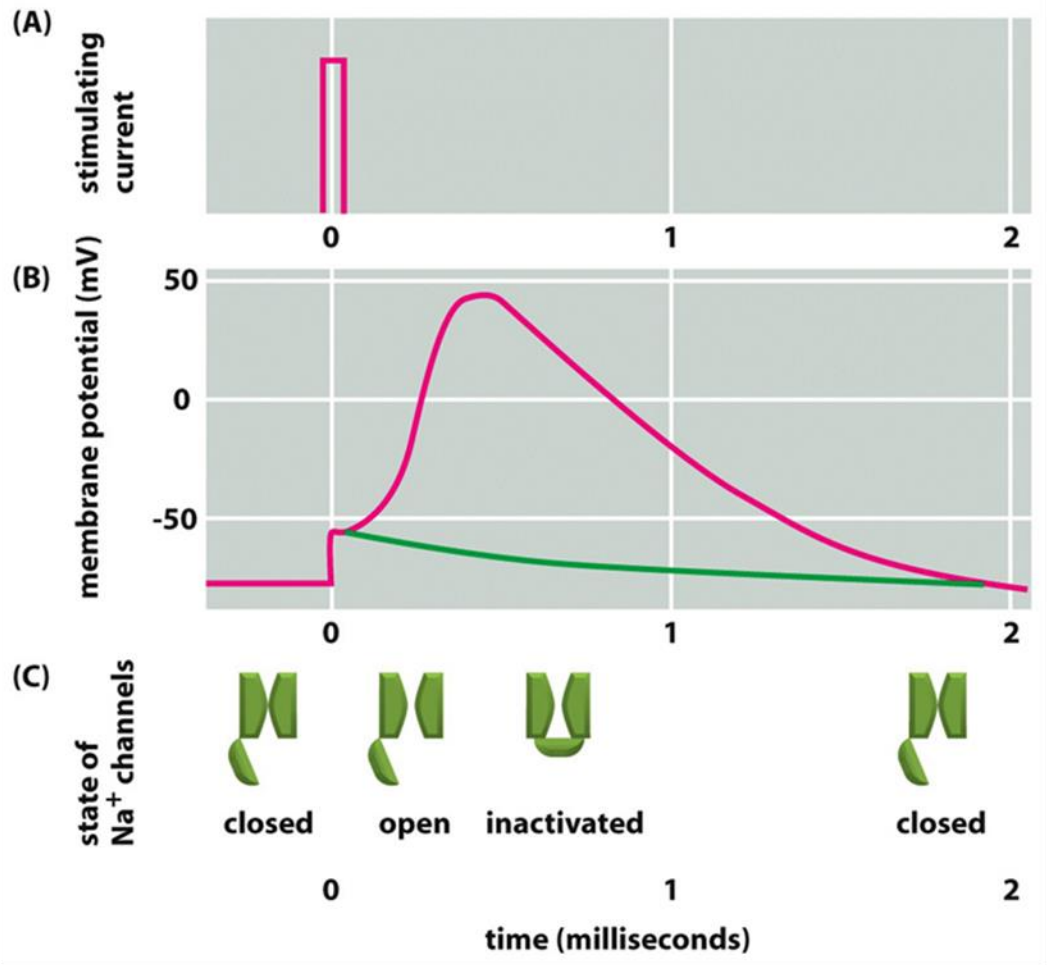
Voltage-gated Na+ channels open, cause influx of Na+

Opening of more Na+ channels, positive feedback

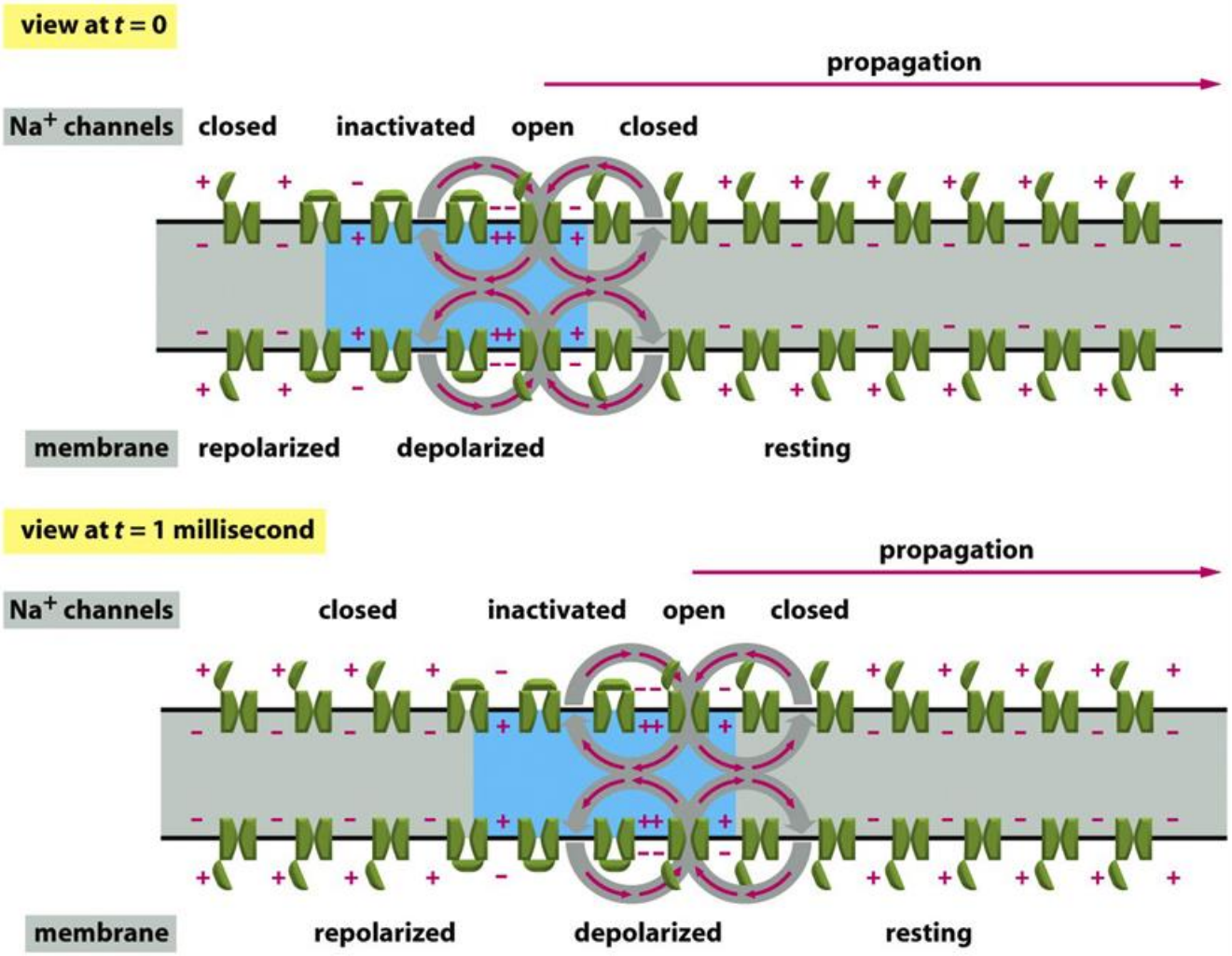
When electrochemical driving force is zero,
Na+ channels becomes inactivated, K+ open

Voltage-gated cation channels generate action potentials in electrically excitable cells

1. Nerve cells
2. Muscle cells

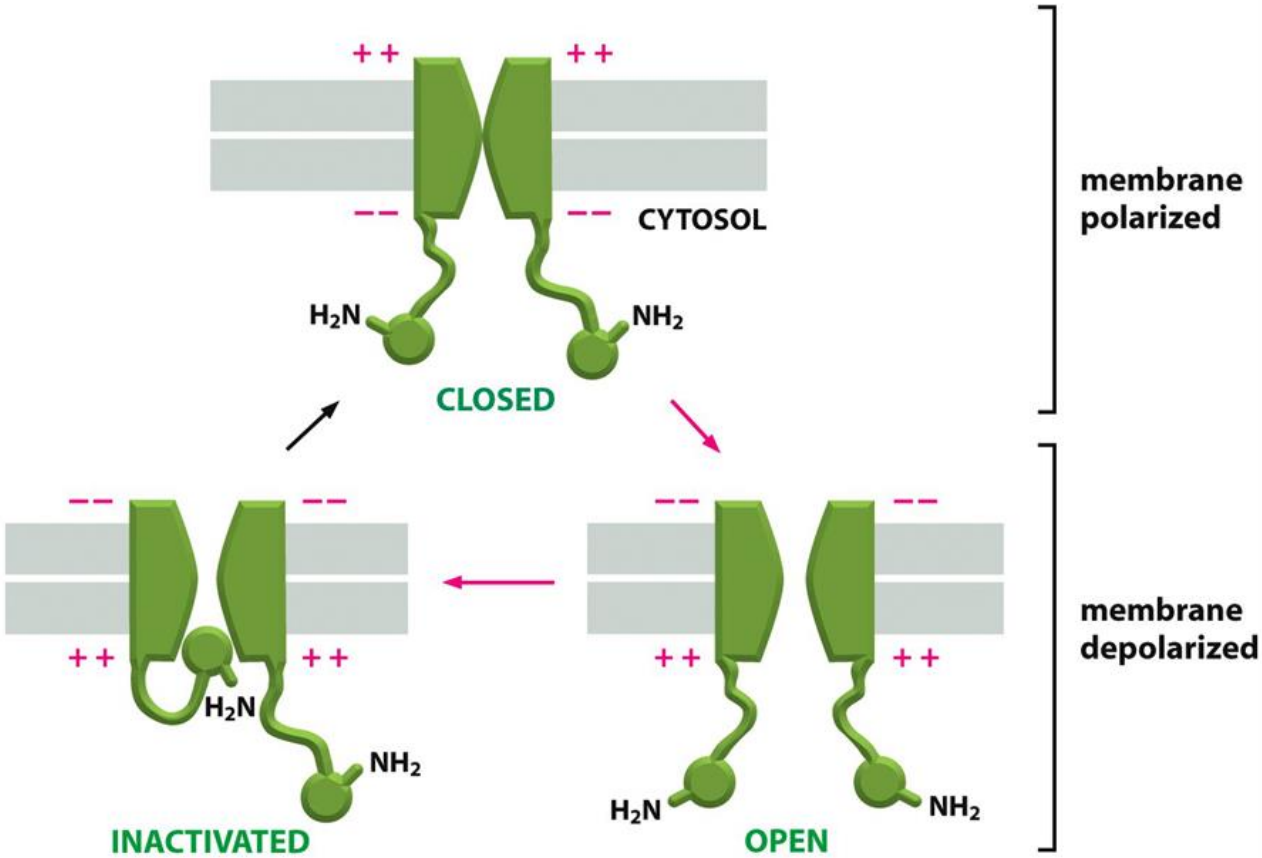


The propagation of an action potential along an axon

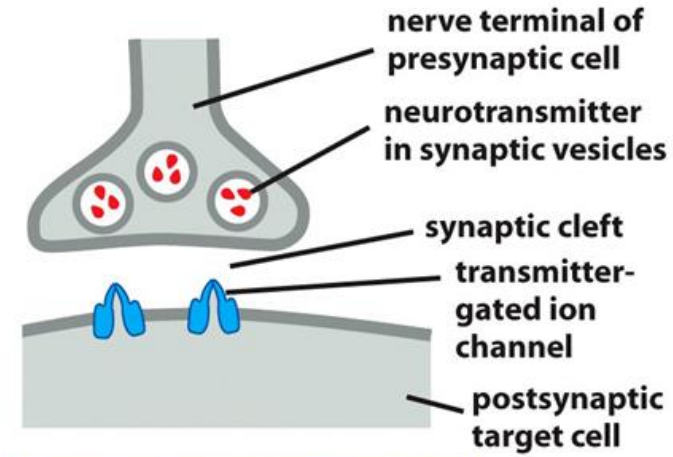


Voltage gated K+ channel brings back the membrane potential

Slower kinetics
Rapidly causes efflux of K+ ions
Has inactivated state



4. Transmitter-gated ion channels convert chemical signals into electrical ones at chemical synapses



5. Types of chemical synapses

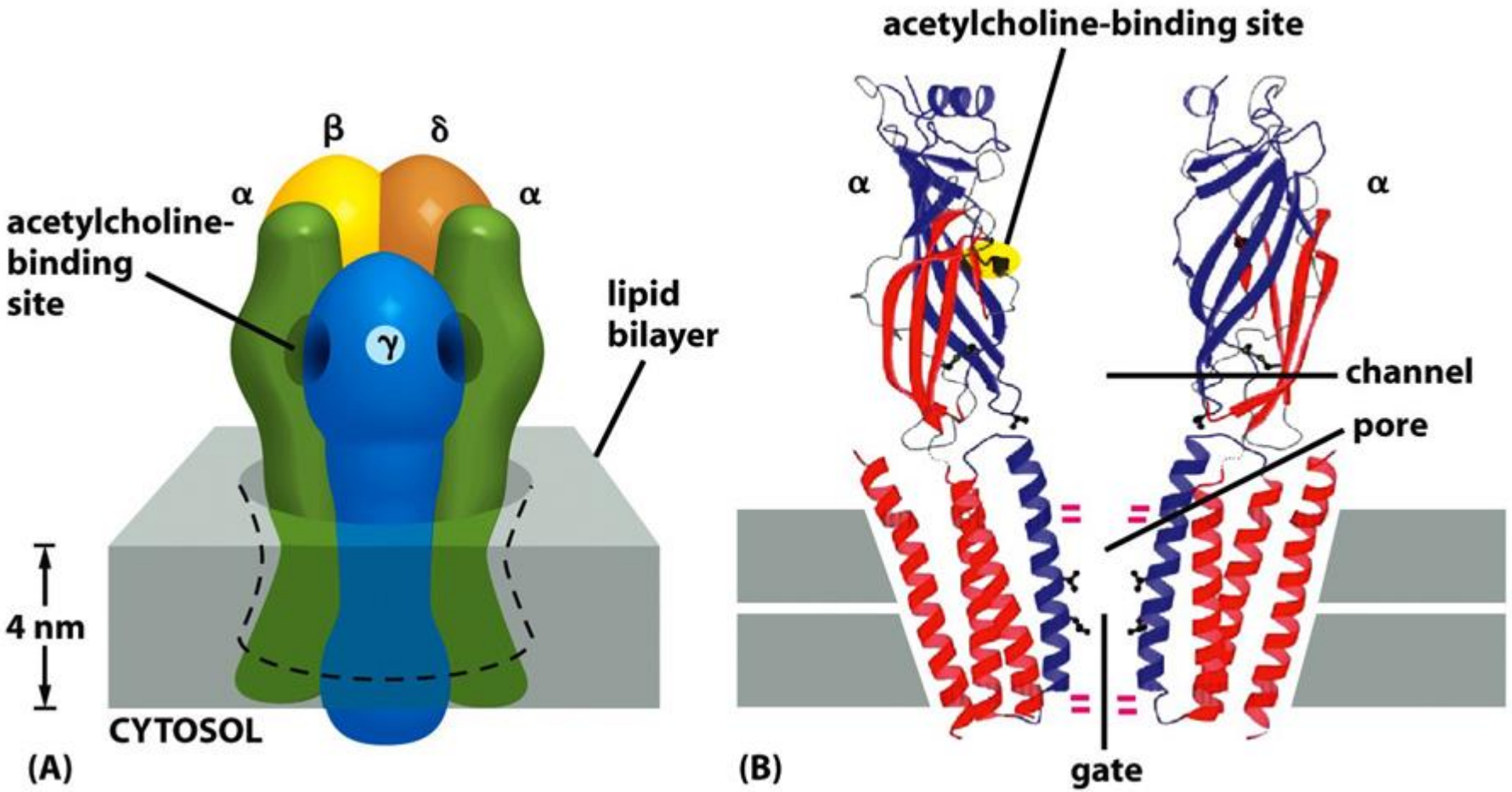
Excitatory neurotransmitters: open cation channels, causing influx of Na+ and firing of action potential

Inhibitory neurotransmitters: open Cl- or K+ channels. Delays action potential.

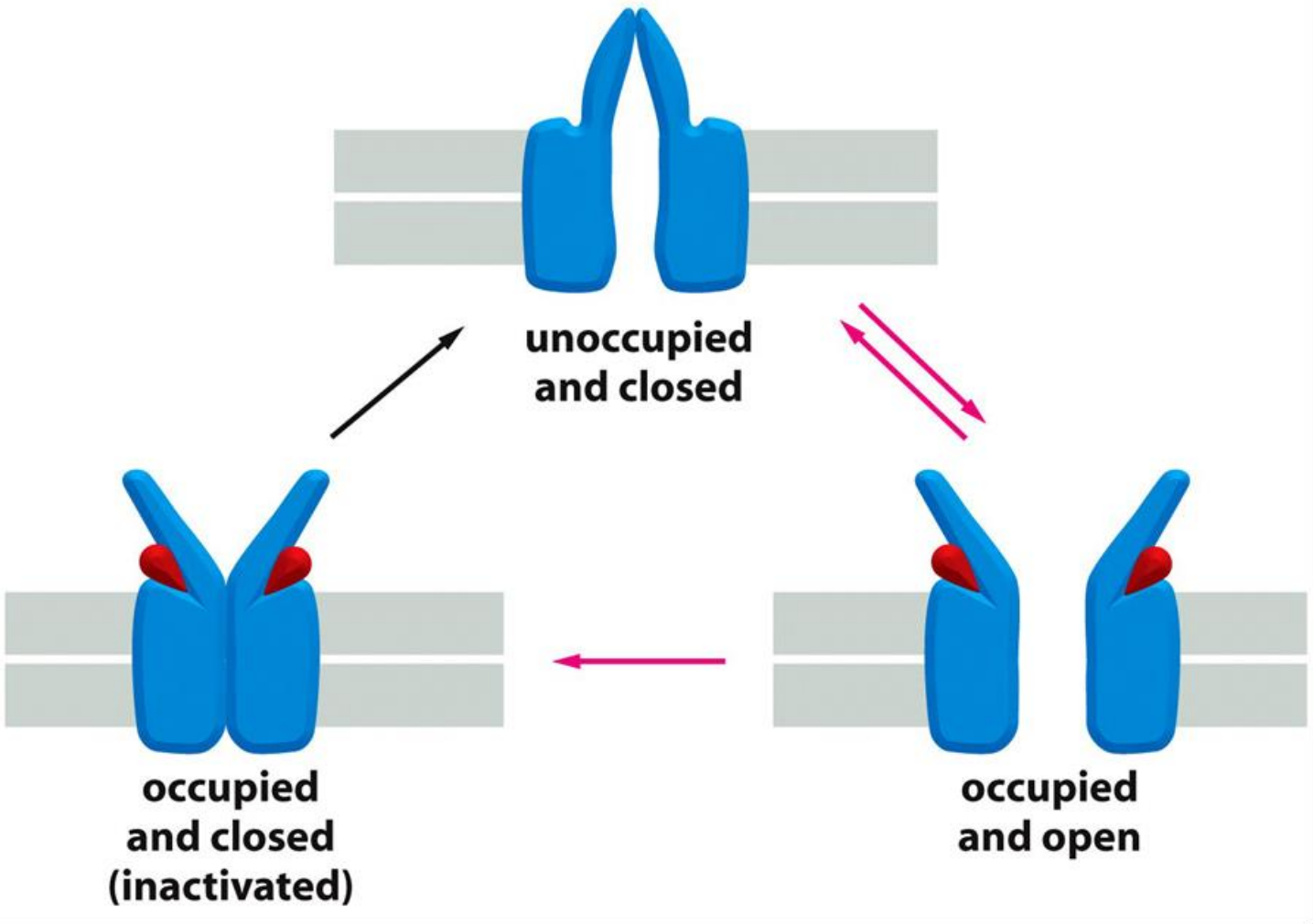
Usually : excitatory tranmitters--- acetylcholine, glutamate, serotonin

inhibitory trnasmitters--- γ-aminobutyric acid (GABA), glycine

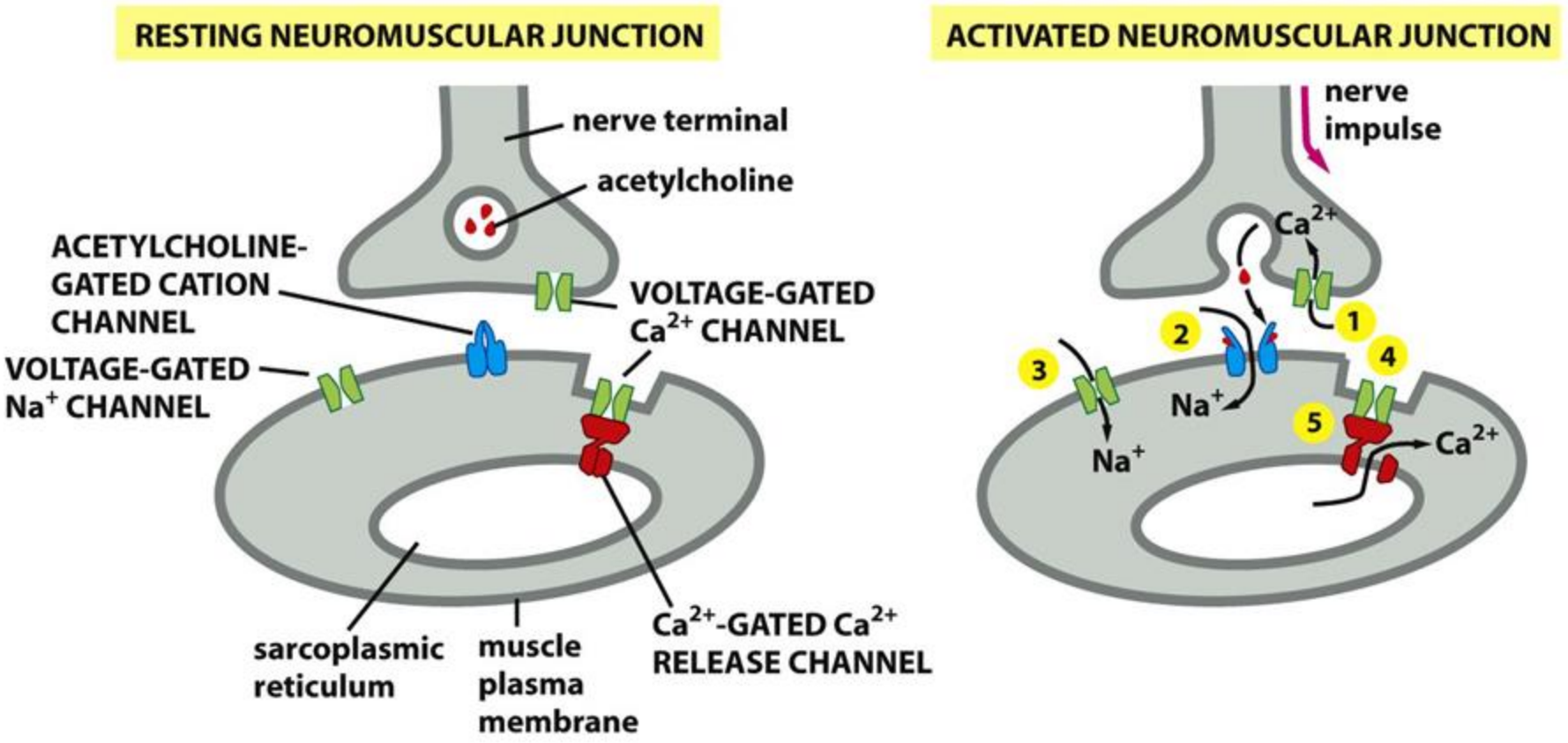
Acetylcholine receptor ---the first purified ion channel



Three states of acetylcholine receptor



Neuromuscular transmission



6. Transmitter-gated ion channels as Drug targets

1. Snake toxin--- bind to acetylcholine receptor and inhibit it.
2. Curare- acetylcholine receptor
3. Barbiturates, tranquilizers such as valium, librium- bind to GABA receptors
4. Prozac- inhibit uptake of serotonin
5. Many drugs to treat insomnia, anxiety, depression, schizophrenia , etc.