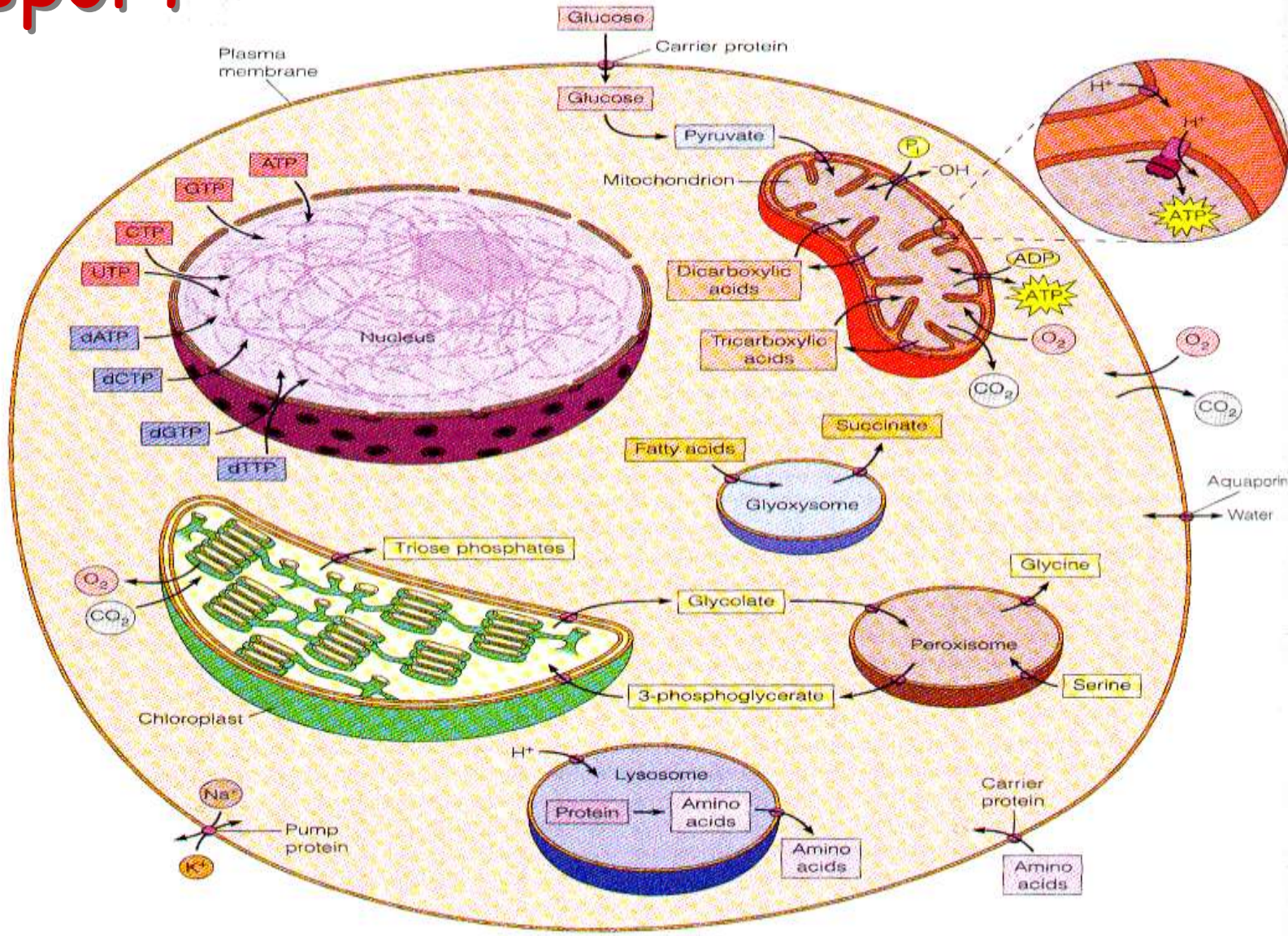
Membrane Transport



NAME OF TEACHER	**DR ROLEE SHARMA**
EMAIL ID	**roleesharma@csjmu.ac.in, roleesh@gmail.com**
DESIGNATION	**Professor**
UNIVERSITY NAME	**C.S.J.M. University, Kanpur**
COLLEGE NAME	**C.S.J.M. University, Kanpur**
STREAM NAME	**BIOLOGY**
FACULTY NAME	**SCIENCE**
DEPARTMENT NAME	**BIOSCIENCES**
SUBJECT NAME	**CELL BIOLOGY**
COURSE	**M.Sc. BIOCHEMISTRY / LIFE SCIENCE**
COURSE DURATION	**2 Years**
SUBTOPIC NAME	**Transport across Biological Membranes**
CONTENT TYPE	**PDF**
SEARCH KEYWORD	**Transport, membranes, carriers, channels, pumps**

Transport across Biological Membranes

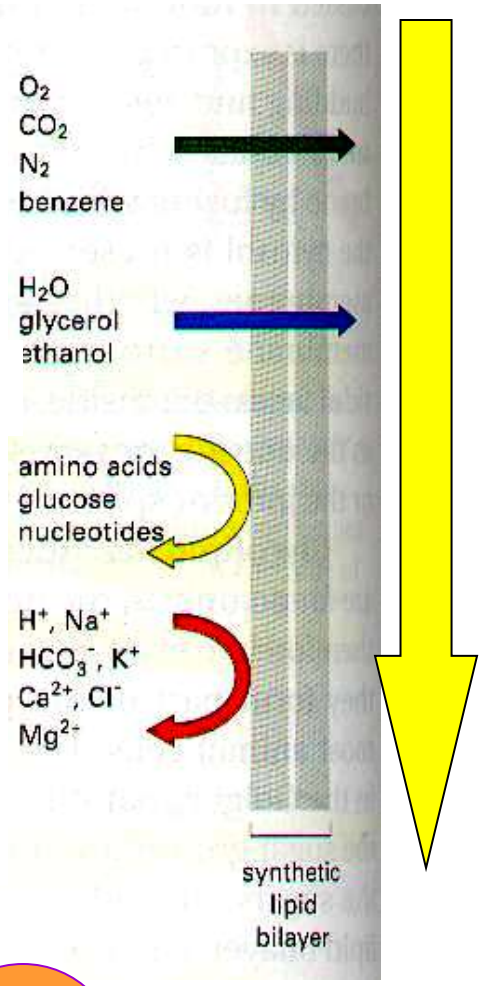
Objectives:

To acquaint the students about:

1. Membrane permeability phases;
2. Diffusion
3. Role of Transport proteins –facilitated
    - Channel proteins
    - Carrier proteins
4. Active Vs. Passive transport
5. Kinds of pumps in membrane
6. Transcellular transport

1. Lipid bilayers are *selectively* permeable

- small, nonpolar
- small uncharged, polar
- larger uncharged, polar molecules
- ions



Decreasing permeability

Size – polarity – ions

The Permeability of the Lipid Bilayer

- **Hydrophobic molecules**
  - Are lipid soluble and can pass through the membrane rapidly
- **Polar molecules**
  - Do not cross membrane rapidly
- **Ions**
  - Do not cross the membrane at all

Transport processes

Solutes – dissolved ions and small organic molecules

i.e., Na^+, K^+, H^+, Ca^{++}, Cl^-,
sugars, amino acids, nucleotides

Three transport processes:

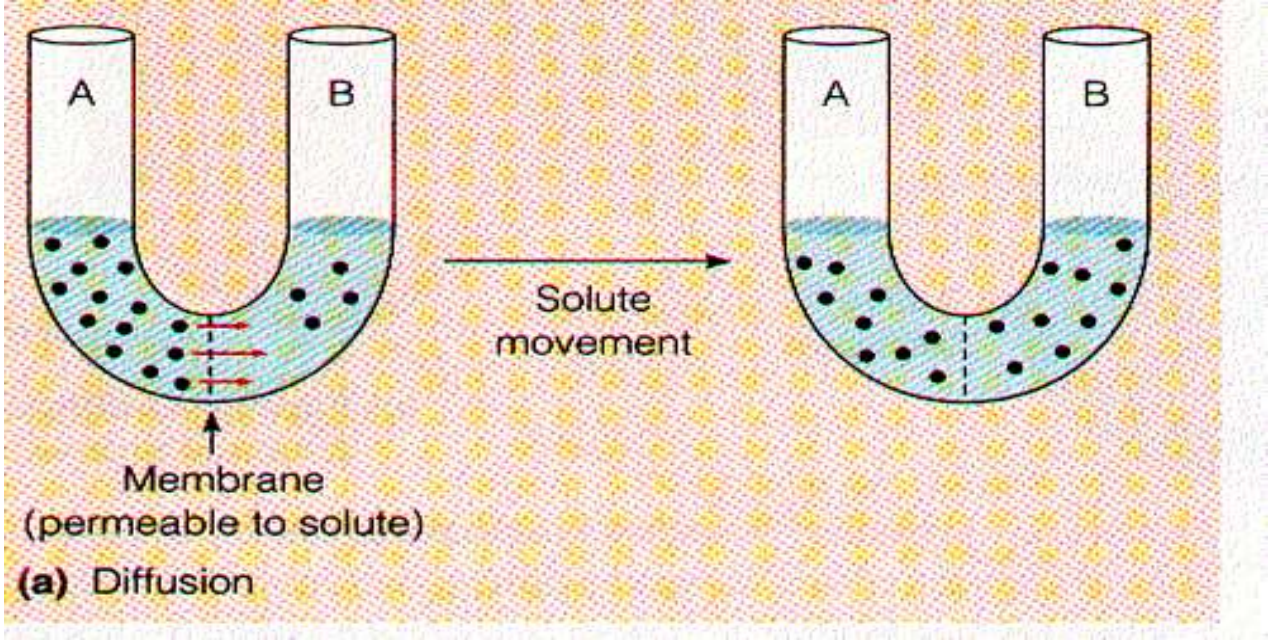
a. Simple diffusion – directly thru membrane

Req Carrier prot

b. Facilitated diffusion (passive transport)

c. Active transport – requires energy

Simple Diffusion:



- Tendancy of a material to spread out
- Always moves *toward* equilibrium

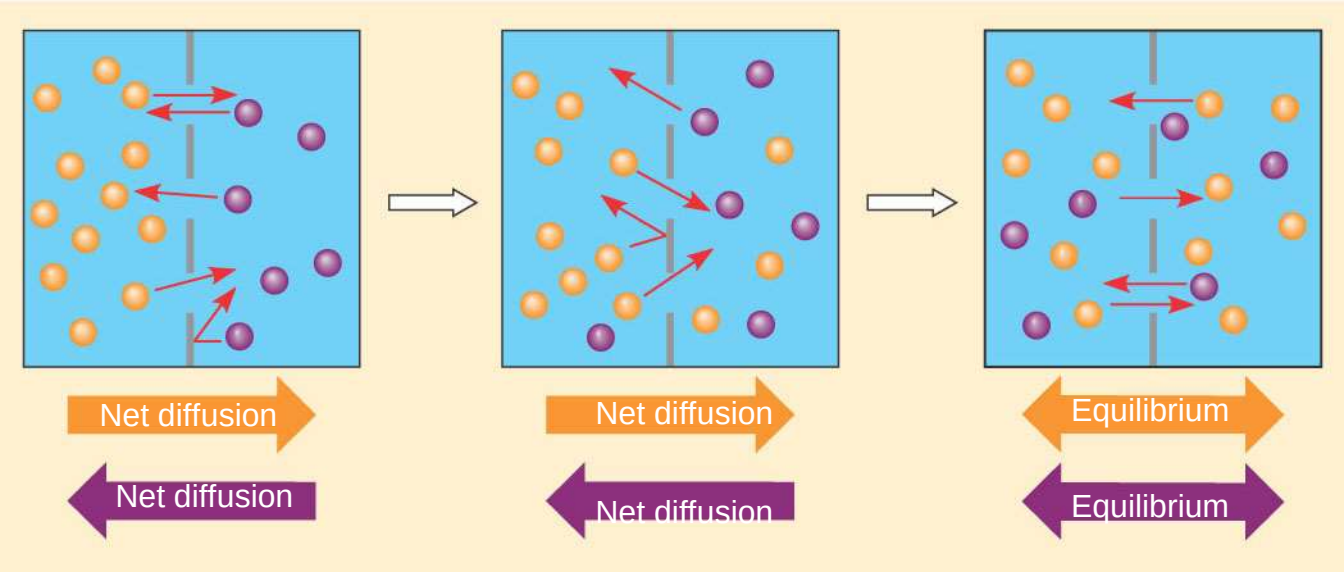


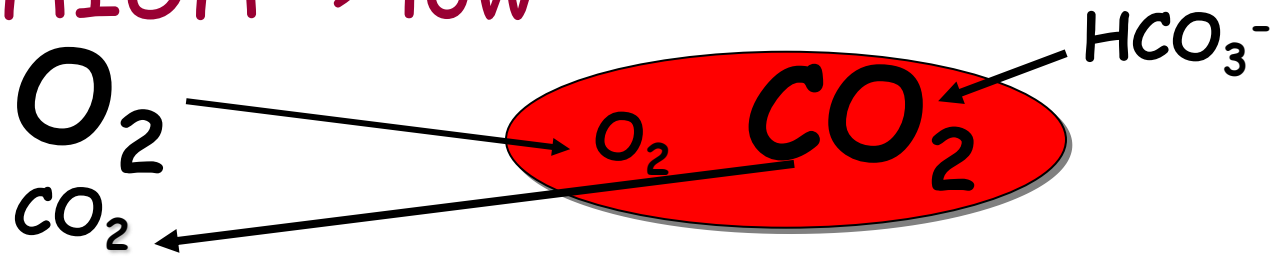
Figure 7.11 B

simple diffusion example:

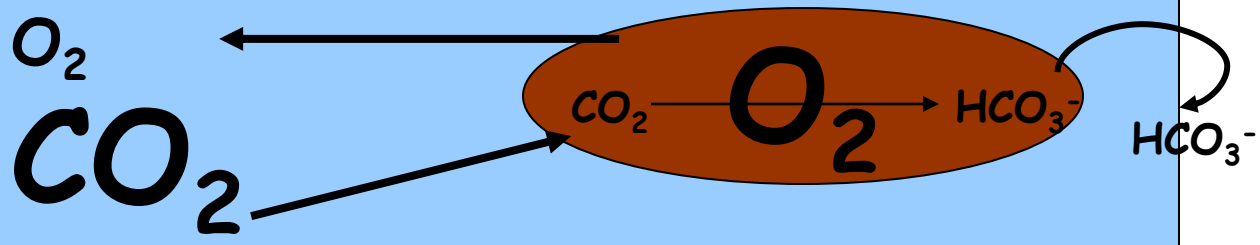
Oxygen crossing red cell membrane

HIGH -> low

Lungs



Tissues



Driving force: concentration gradient

Trying to even out concentration

H_2O transport: diffusion from area with low [solute] to one with high [solute]

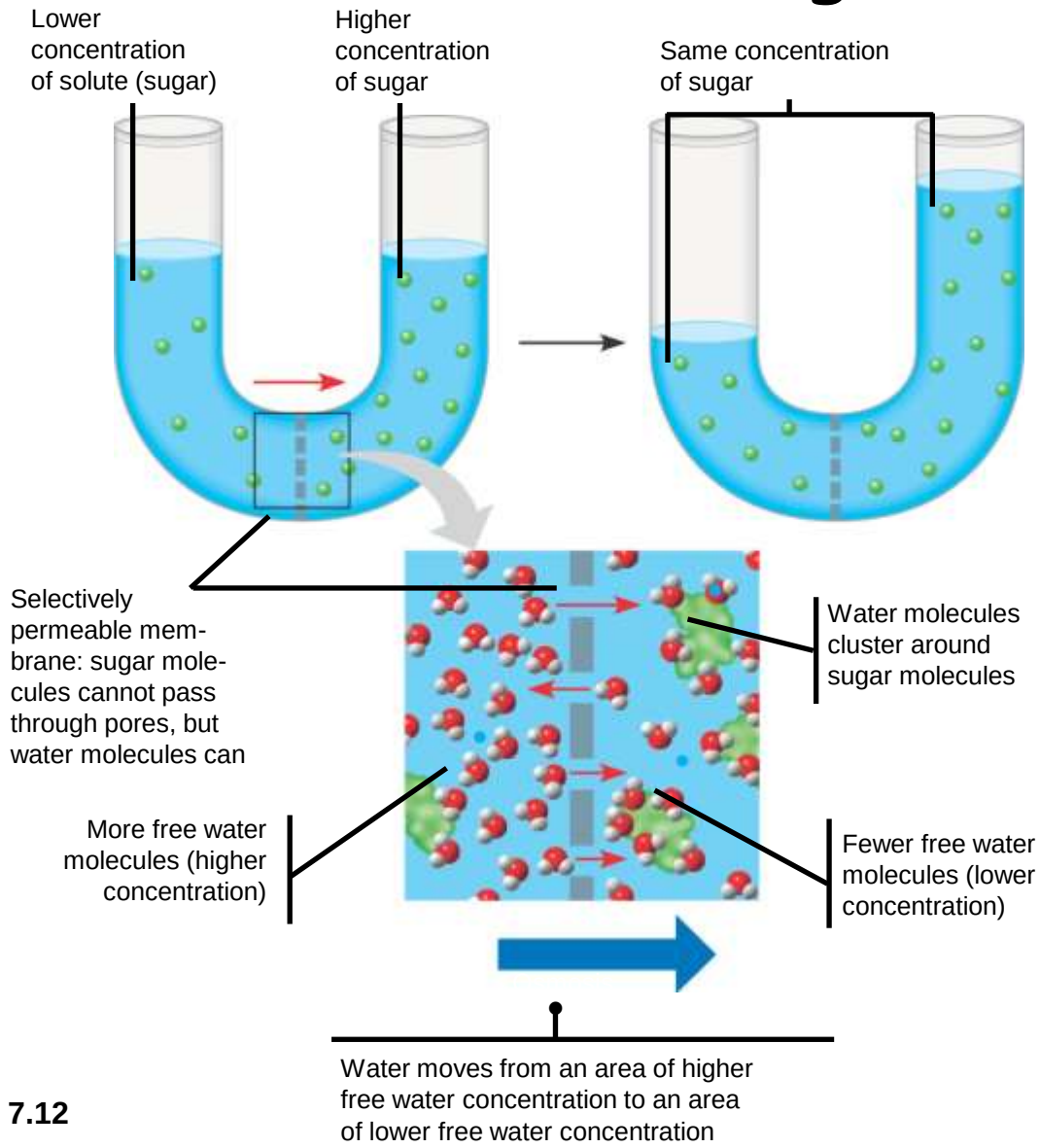


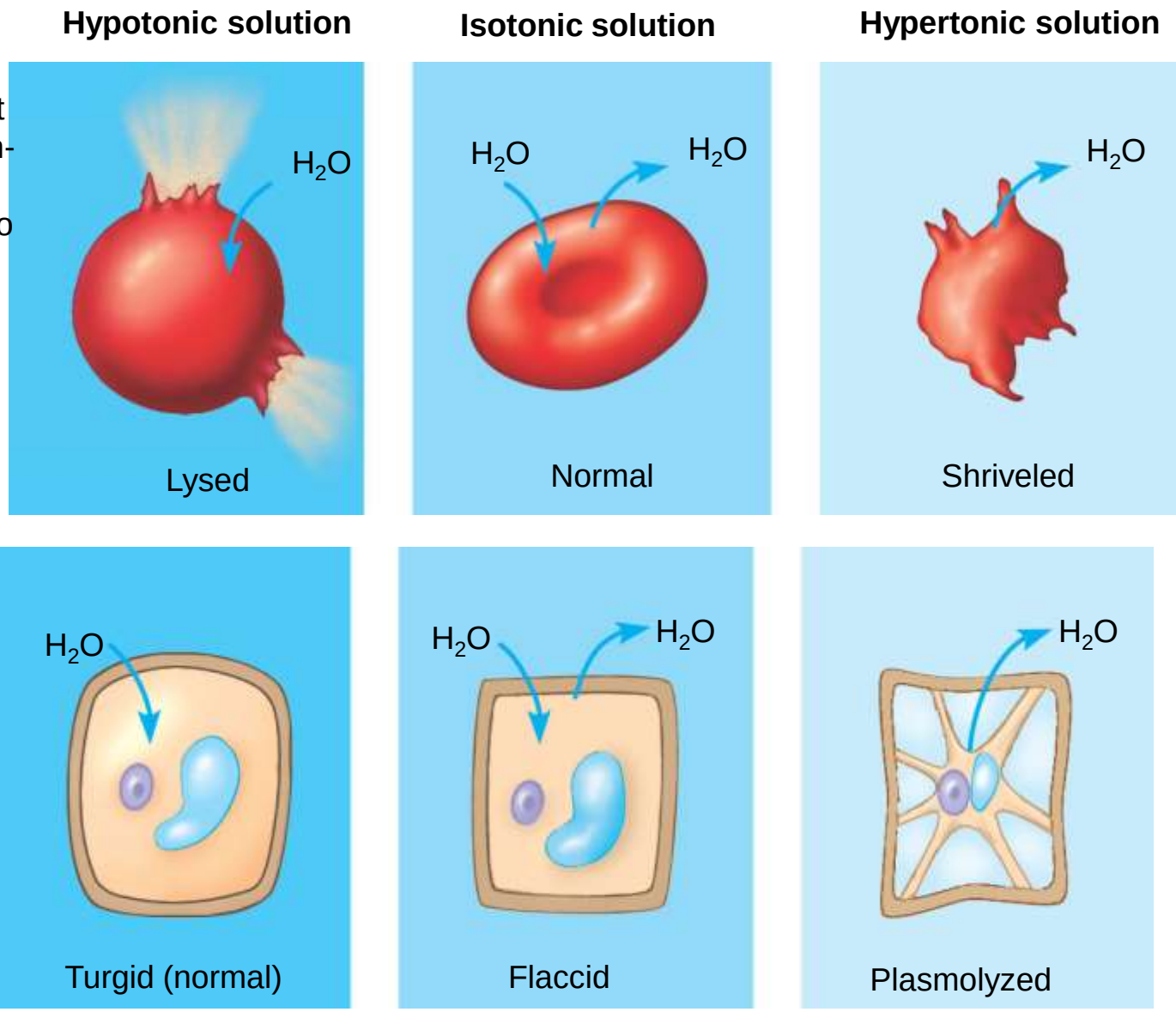
Figure 7.12

Osmosis

Diffusion of water

Impermeable Solutes

Animal cells – pump out ions
Plants, bacteria – cell walls



(a) Animal cell. An animal cell fares best in an isotonic environment unless it has special adaptations to offset the osmotic uptake or loss of water.

Figure 7.13

(b) Plant cell. Plant cells are turgid (firm) and generally healthiest in a hypotonic environment, where the uptake of water is eventually balanced by the elastic wall pushing back on the cell.

…but most things are too large or too polar to cross at reasonable rates using simple diffusion

Facilitated diffusion: protein-mediated movement down a gradient

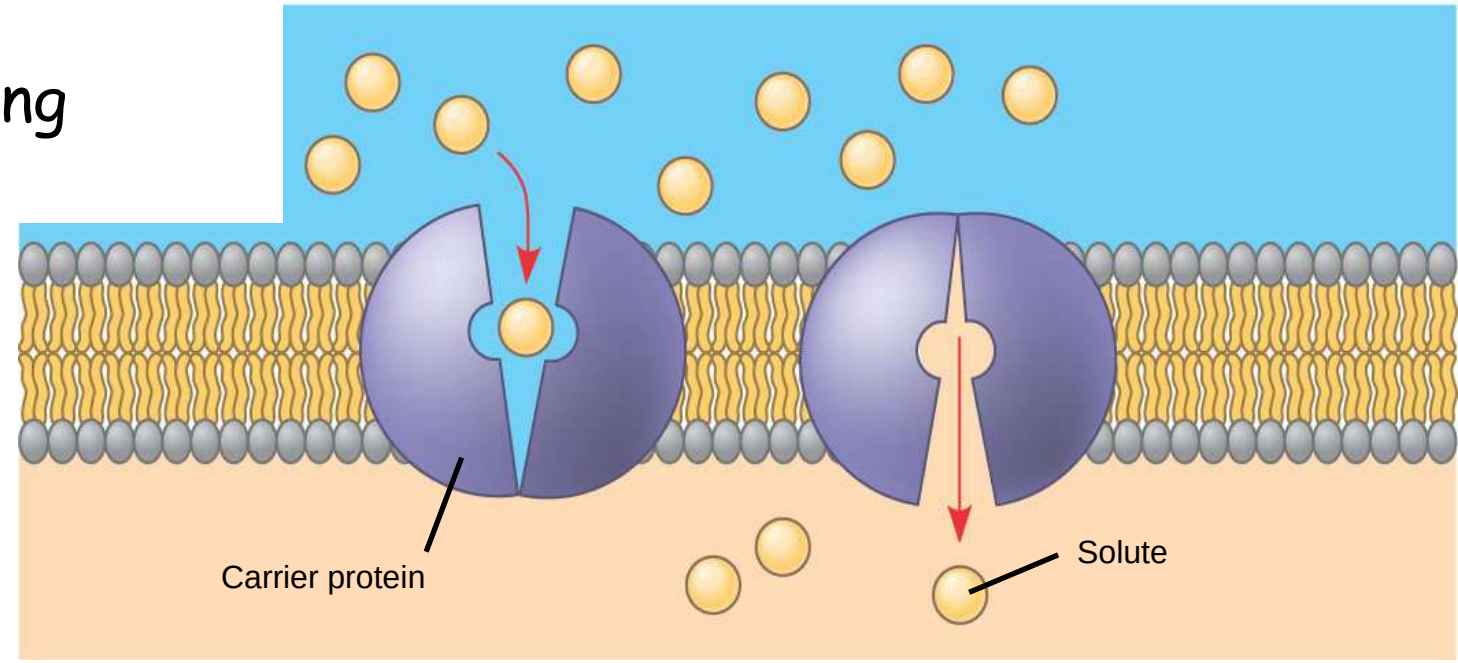
Transmembrane transport proteins

Transmembrane transport proteins

allow *selective* transport of hydrophilic molecules & ions

1. carrier protein

Bind solute,
conformational change,
release
Selective binding



(b) A carrier protein alternates between two conformations, moving a solute across the membrane as the shape of the protein changes. The protein can transport the solute in either direction, with the net movement being down the concentration gradient of the solute.

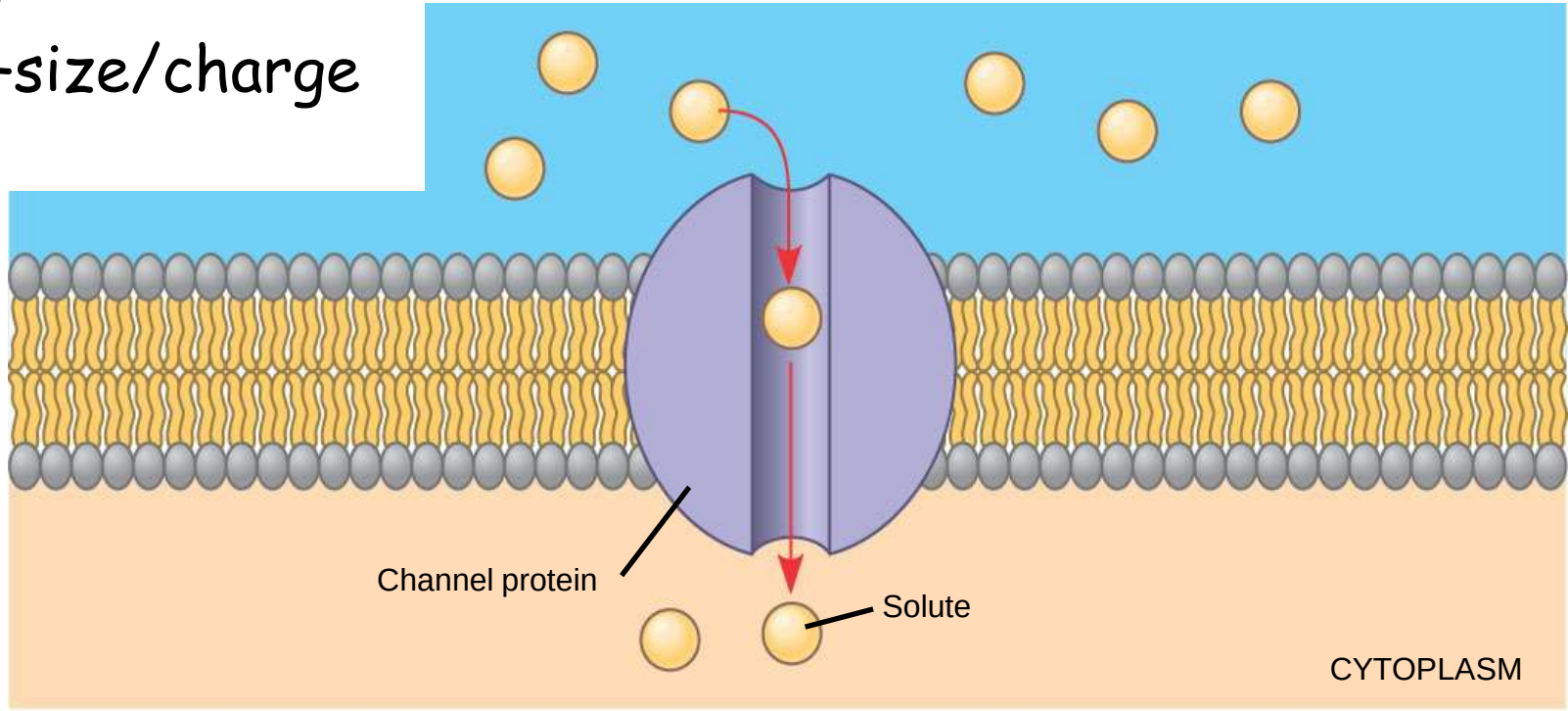
Figure 7.15

Transmembrane transport proteins

allow *selective* transport of hydrophilic molecules & ions

2. channel protein

aqueous channel
hydrophilic pore
very rapid
selective –size/charge

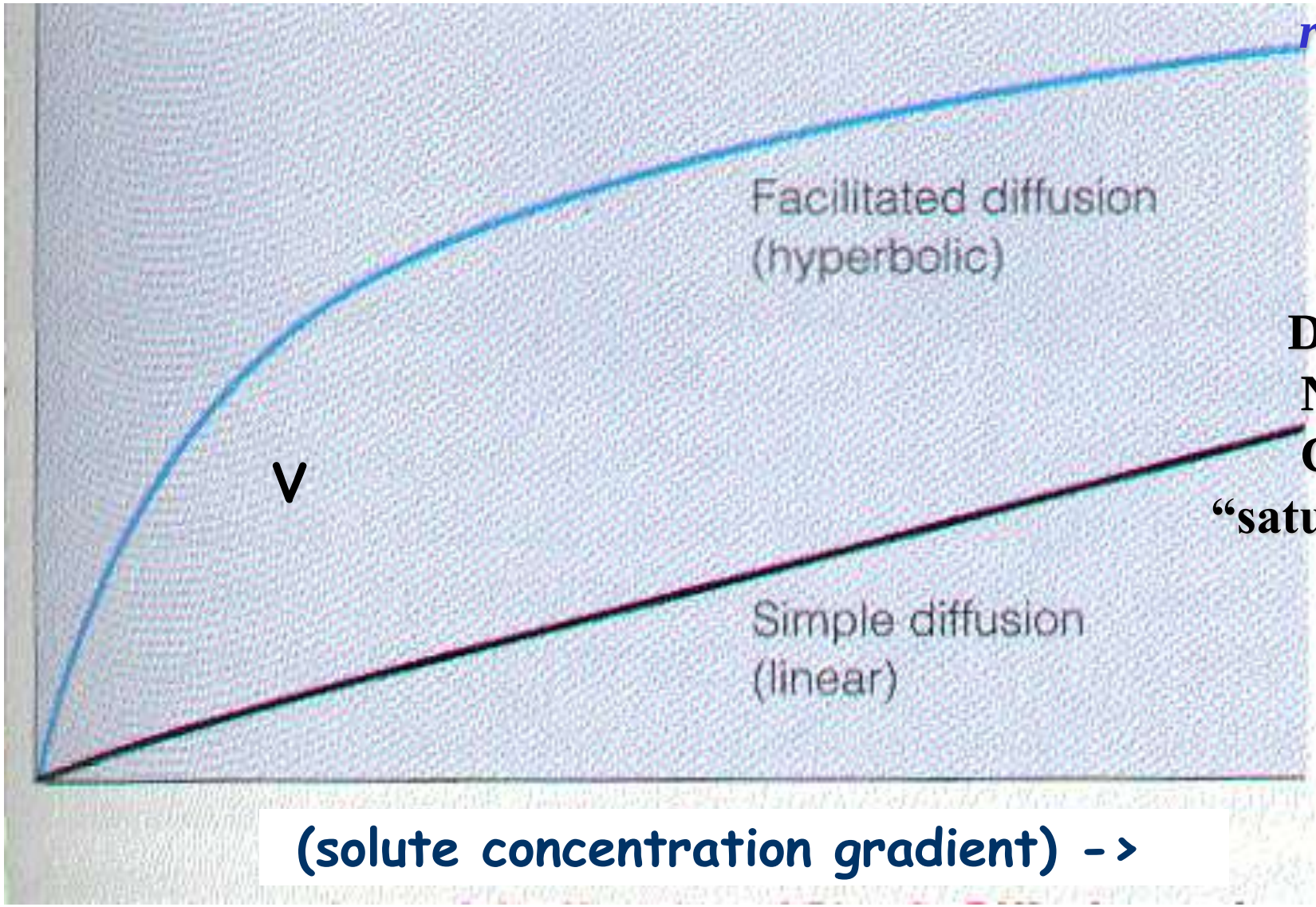


(a) A channel protein (purple) has a channel through which water molecules or a specific solute can pass.

Figure 7.15

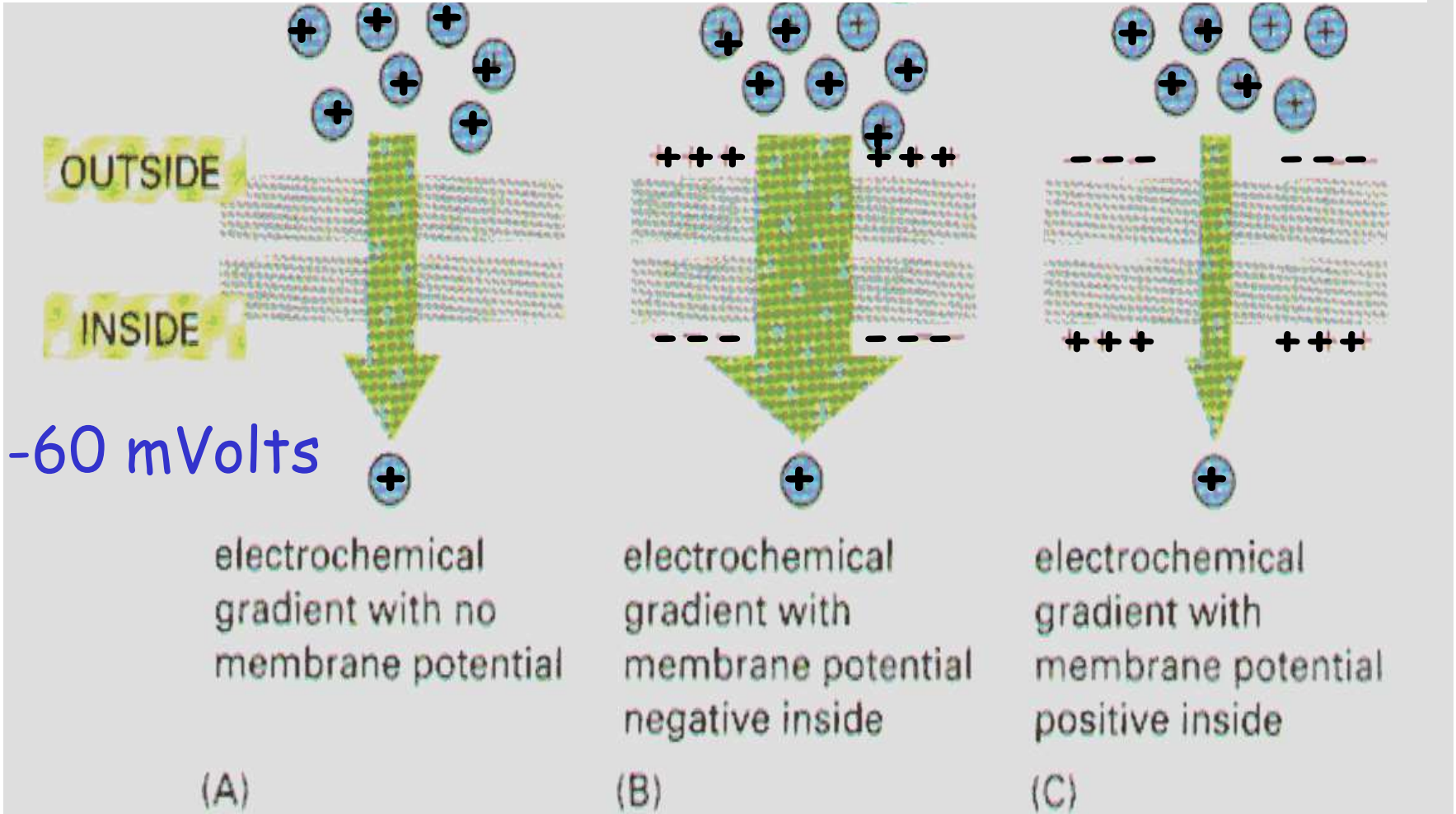
Kinetics of simple vs facilitated Diffusion

Gets "saturated"
Maximum rate



For CHARGED solutes (ions): net driving force is the electrochemical gradient

- has both a concentration + charge component;
- Ion gradients can create an electrical voltage gradient across the membrane (membrane potential)

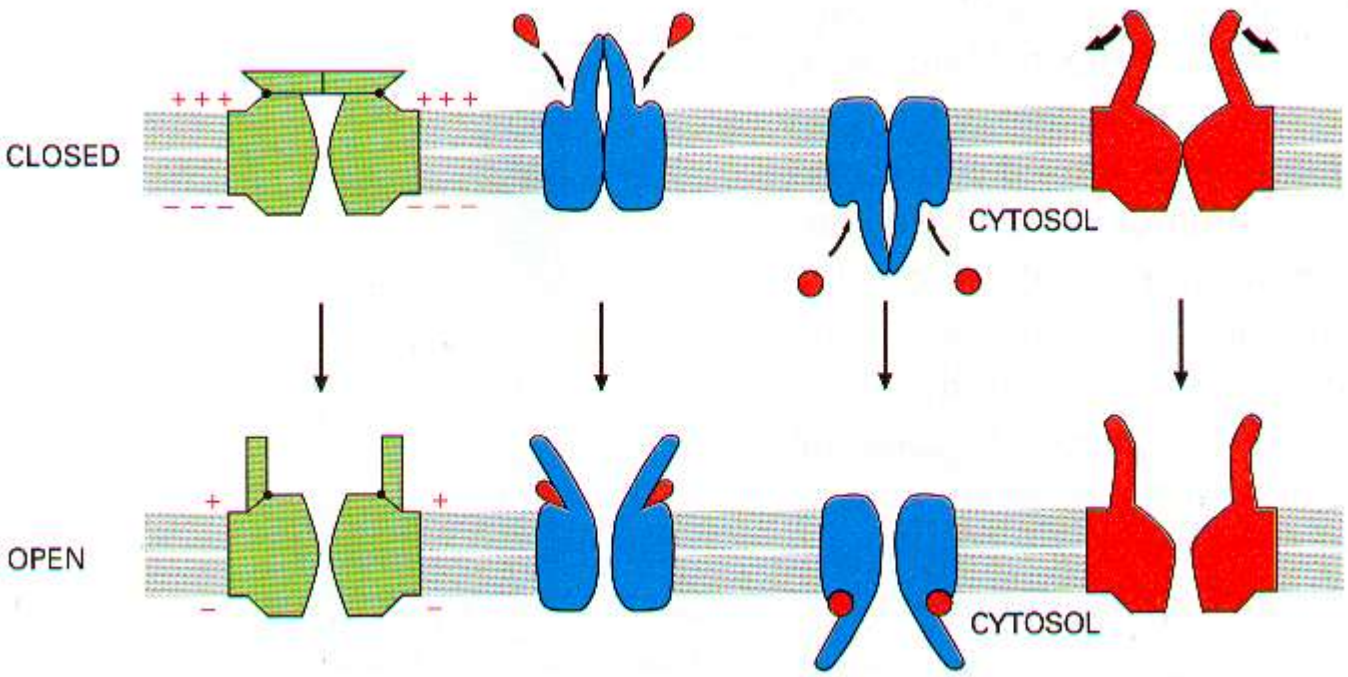


Channel Proteins:

facilitate passive transport

Ion channels: move ions down an electrochemical gradient; gated

"keys"



Voltage Ligand Mechanosensitive

Ion Channels

Protein ion channels:

-are passive, facilitated transport systems

-require a membrane protein

-typically move ions very rapidly from an area of HIGH concentration to one of lower concentration

Three basic properties of ion channels:

- To conduct ions rapidly
- Exhibit high selectivity: only certain ion species flow while others are excluded
- Conduction be regulated by processes known as gating, i.e. ion conduction is turned on and off in response to specific environmental stimuli

Ion Channels Have Very High Turnover Ratios

Carrier	Substrate Turnover (s^{-1})
Valinomycin	3×10^4
Na-K-ATPase	5×10^2
Ca-ATPase	2×10^2
Glucose transporter	$0.1\text{-}1.3 \times 10^4$

Channel	Substrate Turnover (s^{-1})
Na-channel (V)	7×10^6
Ca-channel (V)	1.9×10^6
K-channel (Ca, V)	$0.2\text{-}3 \times 10^7$
ACh receptor	2.3×10^7

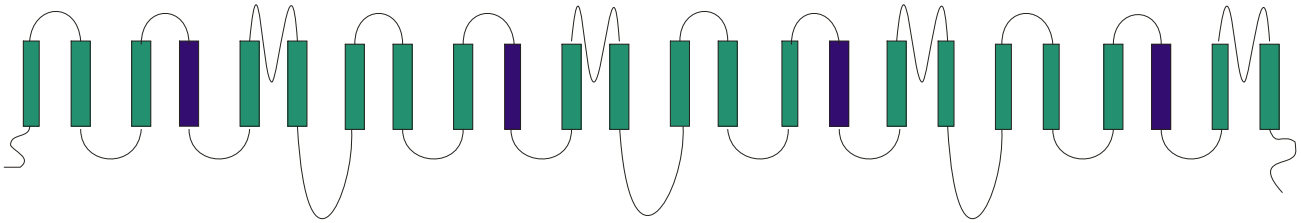
As a comparison, the turnover ratio (maximum number of processed substrate molecules per active site, per second) serves as a good evidence for the physical concept of pore. The turnover rates for some known carriers or active transporters are compared to those of several ion channels

Also …,

Very few ions are needed to generate a sizable transmembrane potential in cells

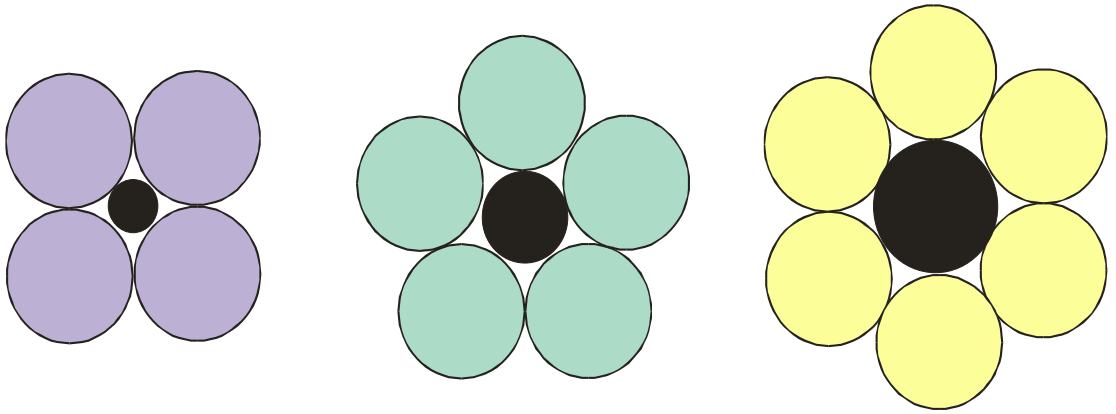
Unifying Themes in Ion Channel Structure

Polytopic Membrane Proteins



Oligomeric Arrangement
With Intrinsic Symmetry

Pore Size Correlates with the
Number of Subunits



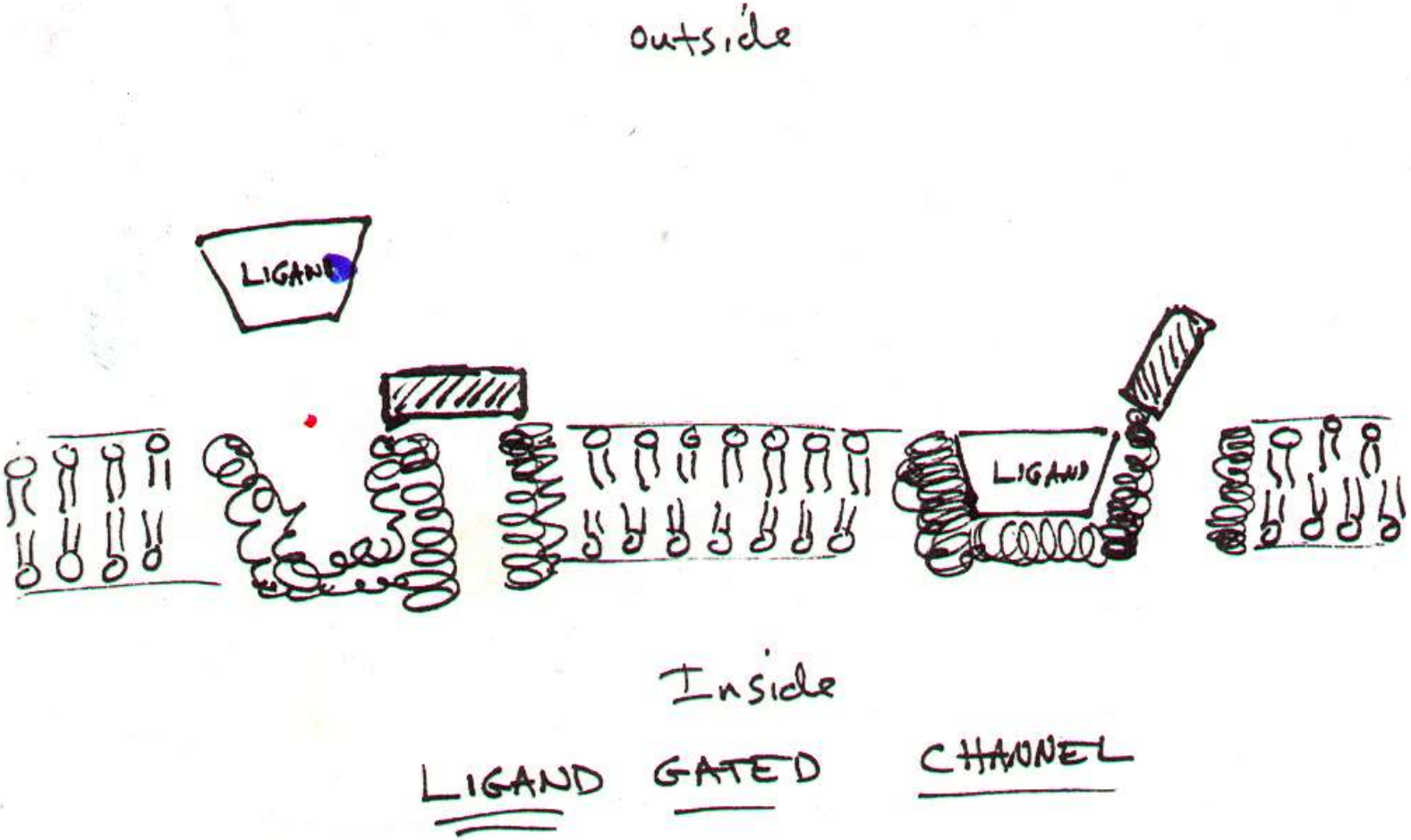
- Voltage-Dependent (Na^+, K^+, Ca^{++})
- Glutamate Receptors

- Ligand-Gated (Ach, Gly, GABA, 5-HT)
- Mechanosensitive

- Connexins (Gap Junctions)

Ligand-gated ion channel

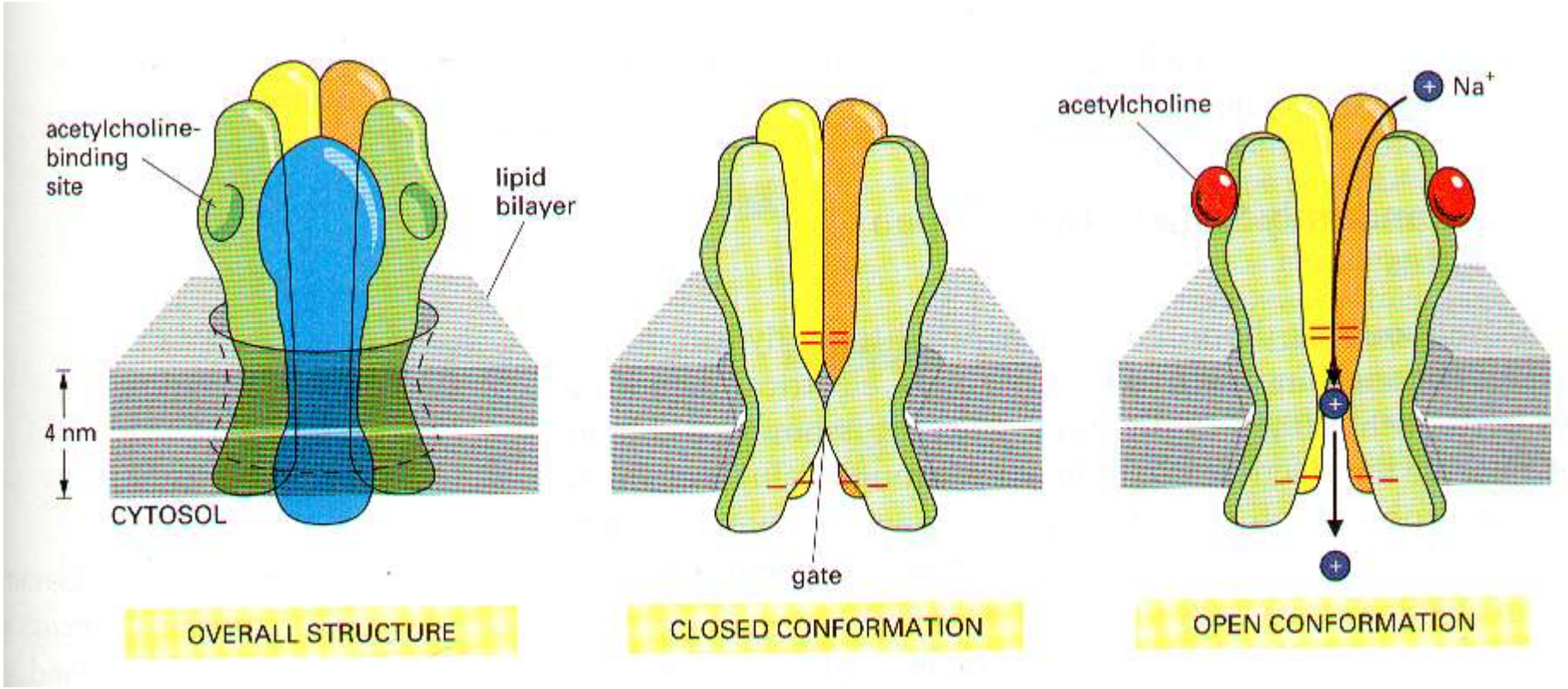
"Wastebasket model" – step on pedal & lid opens



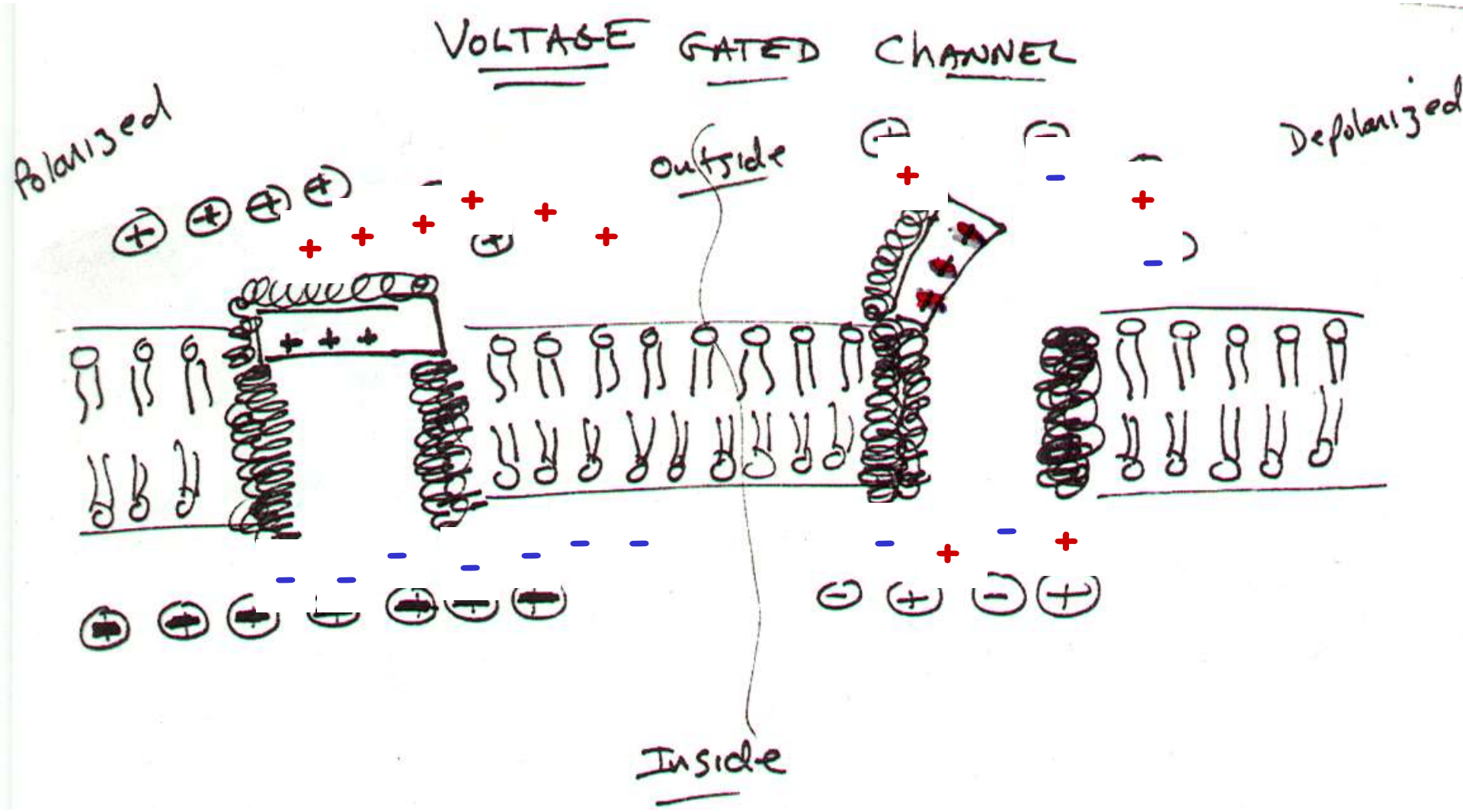
Ligand-gated

example: ligand-gated ion channel

"Key" - acetylcholine

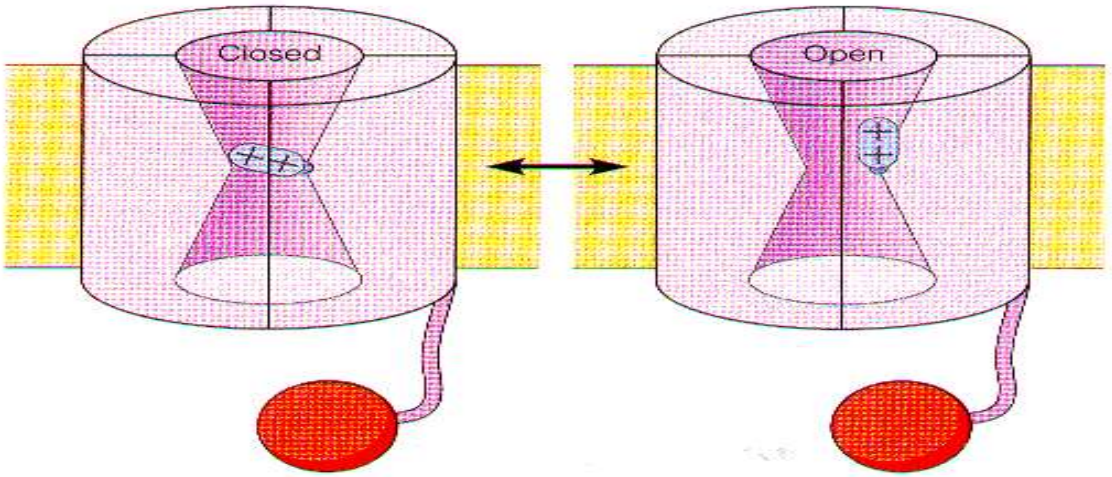


Voltage-gated channels

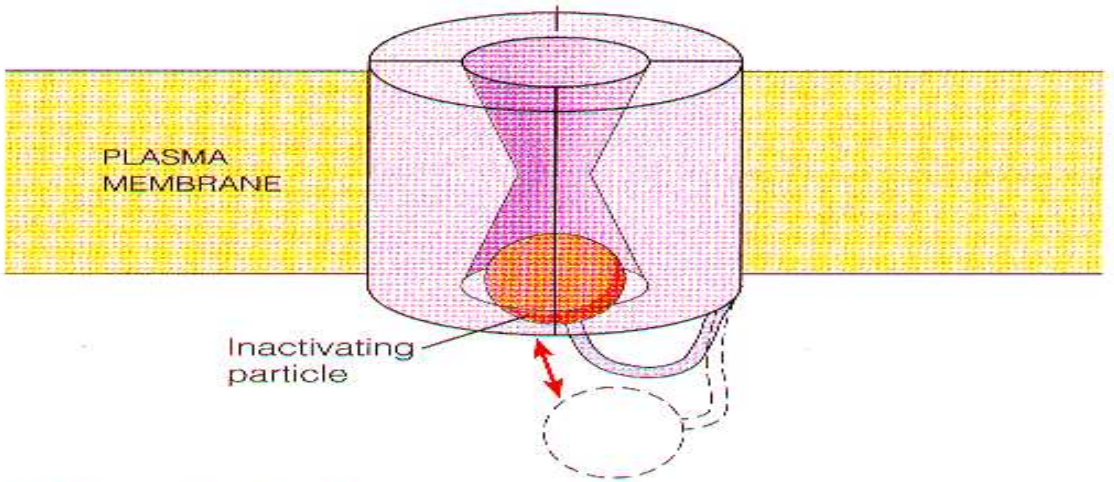


Note: channels are passive, facilitated transport systems

Example of voltage-gated ion channel



(b) Channel gating



(c) Channel inactivation

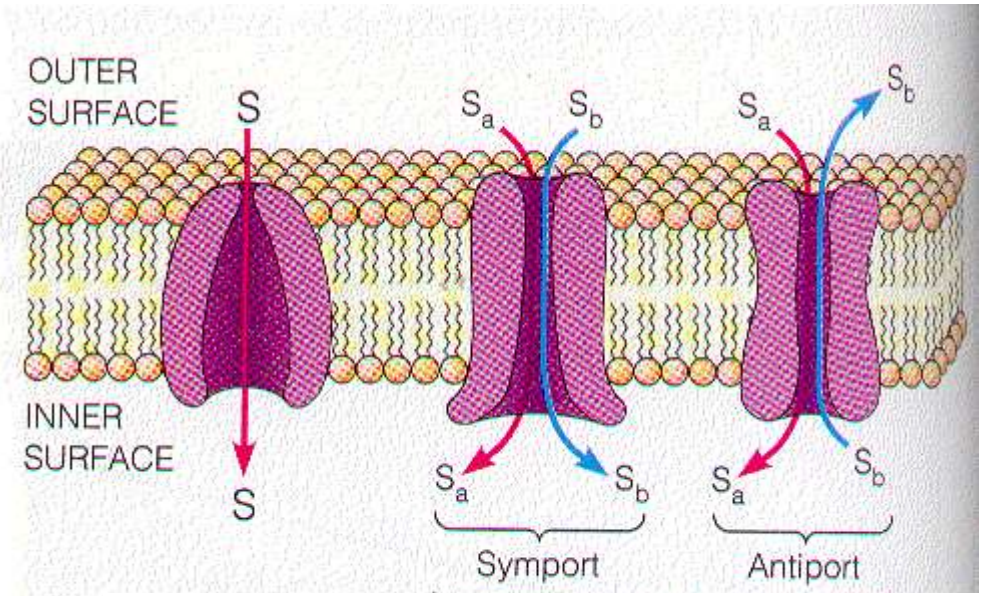
Transporters:
These are membrane transport proteins that transport ions and molecules across cell membranes, usually one or a few at a time with an average rate of about 10^2 – 10^4 molecules.

•The binding of substrate molecules to the transporters is specific and it results in conformational change in the protein that allows only the bound substrate molecules to be transported across the membrane.

•Transporter proteins can further be grouped into three types: uniporters, antiporters and symporters. The **uniporters** transport only one molecule at a time down a concentration gradient across the membrane, e.g., uniporters that transport glucose (*GLUT1* transporter) or amino acids into mammalian cells.

Carrier proteins

Transport solute across membrane by binding it on one side, undergoing a conformational change and then releasing it to the other side.



(a) Uniport **(b) Co-transport**

- facilitate 3 types of movements

Uniport – single molecule transport.

Symport – When two molecules - the transported molecule and co-transported ion move in the same direction, the process is called symport.

Antiport – when two molecules move simultaneously in opposite directions, the process is called antiport.

Carrier Proteins can mediate either:

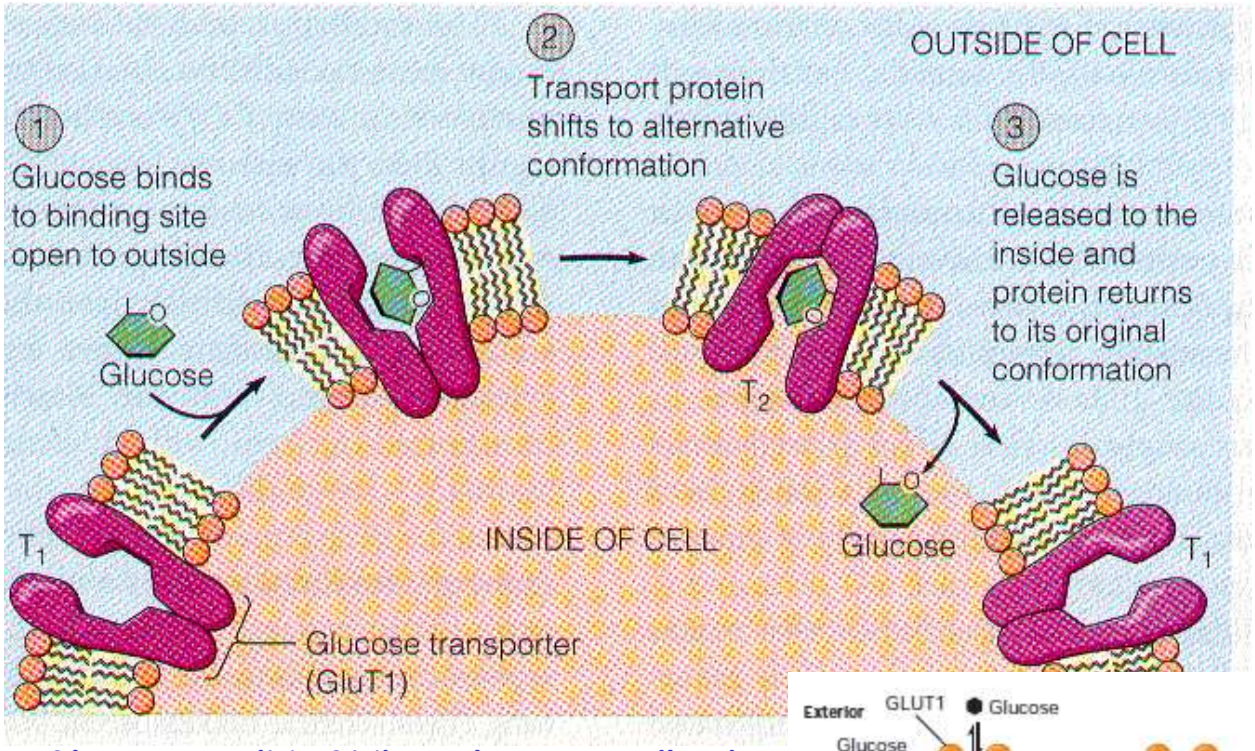
1. Passive transport (driving force -> concentration/electrochemical gradient)

OR

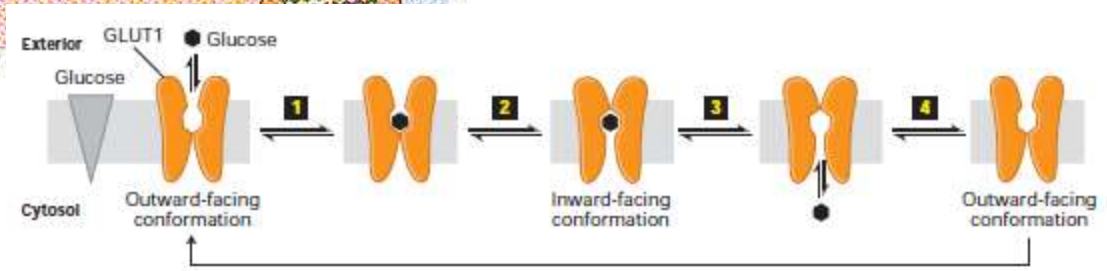
2. Active transport (*against a gradient; unfavorable*), requires energy input

Note: channel proteins mediate only passive transport

Glucose transporter GluT1 : carrier-mediated facilitated diffusion



$Glucose_{out}$ (HIGH)->$glucose_{in}$ (low)



Uniport transport by GLUT1. In one conformation, the glucose-binding site faces outward; in the other, the binding site faces inward. Binding of glucose to outward-facing site (1) triggers a conformational change in transporter that results in binding site's facing inward toward cytosol (2). Glucose then is released to inside of the cell (3). Finally, the transporter undergoes reverse conformational change, regenerating the outward-facing binding site (4). If the concentration of glucose is higher inside the cell than outside, the cycle will work in reverse (step 4 and step 1), resulting in net movement of glucose from inside to out.

ACTIVE TRANSPORT

- Carrier proteins move solute *against* its concentration gradient
- Requires energy, usually in form of ATP hydrolysis or a favorable gradient established by use of ATP

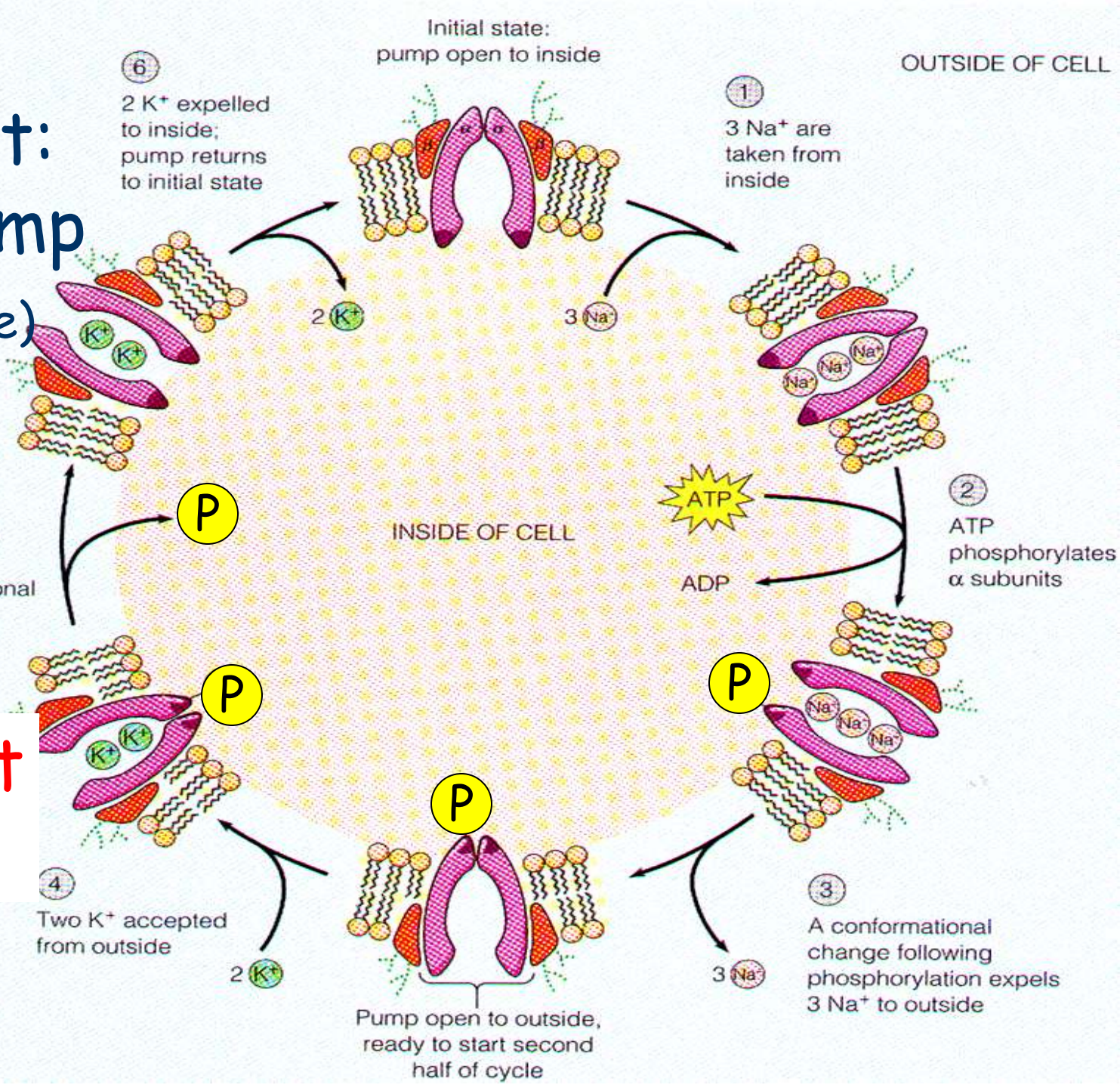
- Such carrier proteins are called as 'pumps', for example - Na^+/K^+ pump (Na^+/K^+-ATPase) that exports 3 Na^+ out of cell in exchange for intracellular import of 2 K^+ at the expense of ATP. Na^+/K^+ pump is responsible for creating an electrochemical gradient (high external Na^+ concentration).

-

- **ATP-powered pumps** are ATPases or protein pumps that utilize the energy generated from ATP hydrolysis to transport small molecules or ions across membrane against a concentration gradient. The process is referred to as active transport.
- These are responsible for maintaining low calcium (Ca^{2+}) and sodium (Na^+) ion concentrations inside compared to outer medium in most animal cells. They also maintain a low pH inside lysosomes in animal cells, in plant cell vacuoles and in the lumen of the stomach.
- There are **4 classes of ATP-powered pumps: P, F, V and ABC (ATP-binding cassette) classes**.
- ***P, F, and V classes transport ions only while the ABC class transports small molecules***

Active transport: Na^+K^+ Pump

(Na^+K^+ ATPase)



3 Na^+ out

2 K^+ in

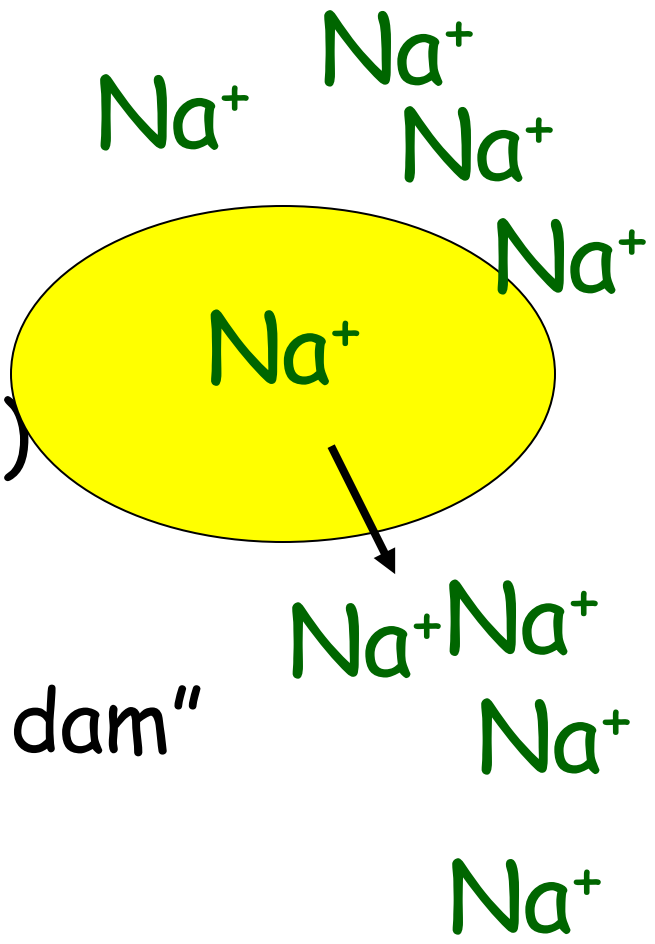
ATP!

The Na^+/K^+ Pump:

"bilge pump"

Creates an electrochemical gradient (high external [Na^+])

potential energy
- like "storing water behind a dam"



uses ~1/3 of cell's ATP!!

Active transport

- Carrier proteins move solute <u>against</u> its concentration gradient

- Requires energy, usually in form of ATP hydrolysis

- Such carrier proteins are called as 'pumps', for example - Na^+/K^+ pump (Na^+/K^+-ATPase) that exports 3 Na^+ out of cell in exchange for intracellular import of 2 K^+ at the expense of ATP. Na^+/K^+ pump is responsible for creating an electrochemical gradient (high external Na^+ concentration).

- **<u>ATP-powered pumps</u>** are ATPases or protein pumps that utilize the energy generated from ATP hydrolysis to transport small molecules or ions across membrane against a concentration gradient. The process is referred to as active transport.
- These are responsible for maintaining low calcium (Ca^{2+}) and sodium (Na^+) ion concentrations inside compared to outer medium in most animal cells. They also maintain a low pH inside lysosomes in animal cells, in plant cell vacuoles and in the lumen of the stomach.
- There are **4 classes of ATP-powered pumps: P, F, V and ABC (ATP-binding cassette) classes**.
- ***P, F, and V classes transport ions only while the ABC class transports small molecules***

1. **P-class**: Structurally, the P-class ion pumps consists of two identical catalytic α subunits that contain an ATP-binding site. Most of them also have two smaller β subunits with regulatory functions.

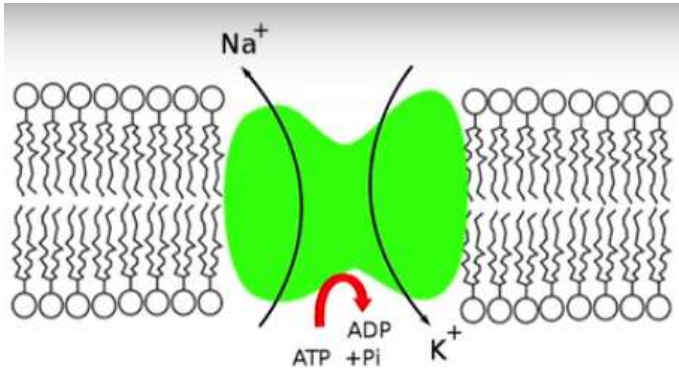
- These pumps are called “P” class since the transport by these pumps is associated with the *phosphorylation of at least one α subunit* through which the ions are believed to move through.
- Examples of this class includes

  -the **Na^+/K^+ ATPase** in animal cell PM (maintains low cytosolic Na^+ and high cytosolic K^+ concentrations by exporting 3 sodium ions for every 2 potassium ions imported),

  -Ca^{2+} ATPases pumps of PM and specialized ER of muscle cells called the sarcoplasmic reticulum (pumps Ca^{2+}ions out of the cytosol across the plasma membrane or into the endoplasmic reticulum from cytosol across the sarcoplastic reticulum),

  -protons transports found in acid-secreting cells of the mammalian stomach that pumps out H^+ and pumps in K^+ ions as well as the

  -H^+ pump in membranes of plant, fungal, and bacterial cells that generates and maintains the membrane electric potential.

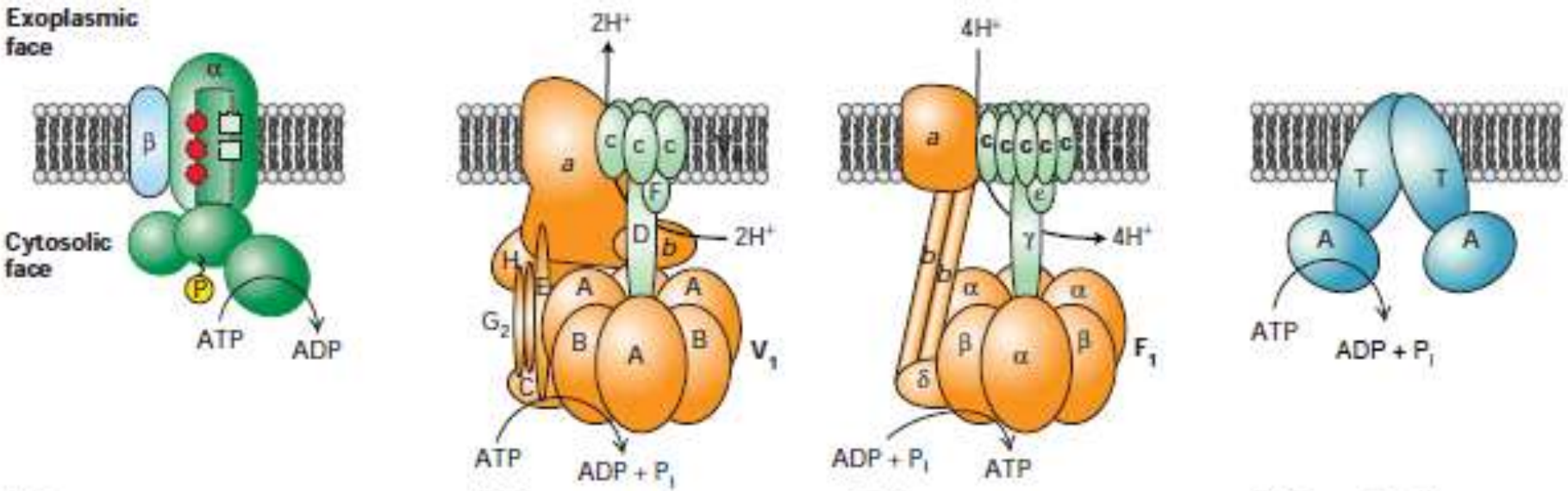


2, 3. F-class and V-class ion pumps: The proteins of these two classses are structurally similar but are unrelated. Both F- and V-class pumps have many different transmembrane and cytosolic subunits and they transport only protons without involving a phosphoprotein intermediate.
Examples of V-class pumps include the proton pumps of plant vacuolar membranes, lysosomes and other acidic vesicles that maintain low pH inside the vacuole or vesicles.

On the other hand the **F-class pumps**, also commonly called ***ATP synthases***, are found in the membranes of mitochondria, chloroplasts and bacterial plasma membranes, where they power the synthesis of ATP from ADP and Pi by pumping protons from the exoplasmic to the cytosolic face of the membrane down its electrochemical gradient.

4. ABC (*A*TP-binding cassette) superfamily: This class of pumps have a general structure consisting of four "core" domains: two transmembrane (T) domains that form a passageway for transporting molecules across the membrane and two cytosolic ATP-binding (A) domains.

Each ABC transport protein transport a specific single substrate or a group of substrates such as ions, sugars, amino acids, phospholipids, peptides, polysaccharides, or even proteins, drugs.



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)

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

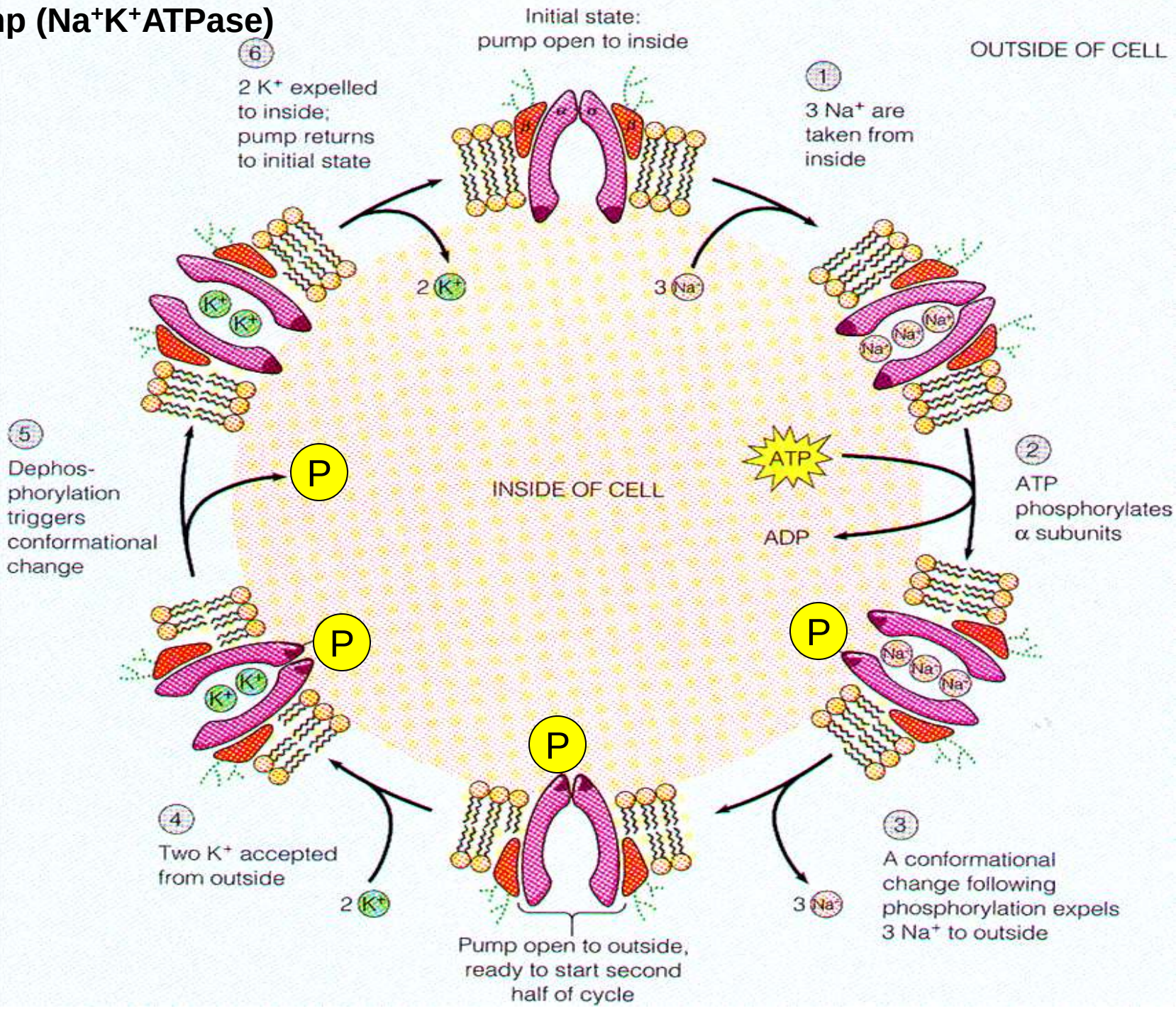
ABC superfamily

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

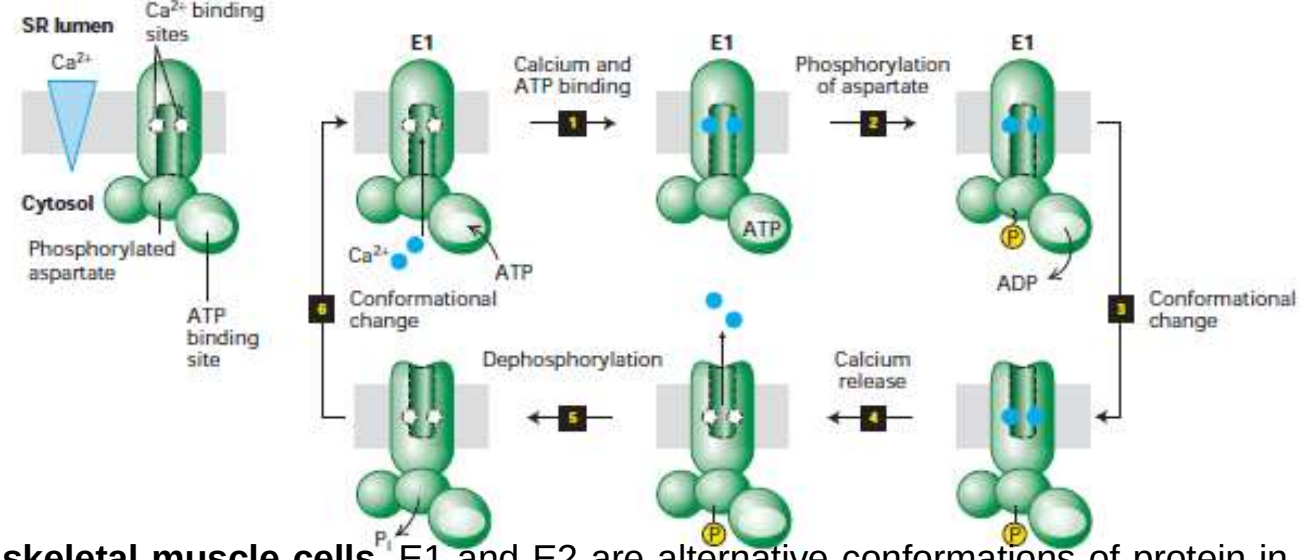
Mammalian plasma membranes (transporters of phospholipids, small lipophilic drugs, cholesterol, other small molecules)

<u>The four classes of ATP-powered transport proteins</u>. P-class pumps are composed of a catalytic subunit, which becomes phosphorylated during transport. A subunit, present in some of these pumps, may regulate transport. **F-class** and **V-class** pumps do not form phosphoprotein intermediates and transport only protons. V-class pumps couple ATP hydrolysis to transport of protons against a concentration gradient, whereas F-class pumps normally operate in the reverse direction to utilize energy in a proton concentration or electrochemical gradient to synthesize ATP. All members of the large **ABC superfamily** of proteins contain two transmembrane (T) domains and two cytosolic ATP-binding (A) domains, which couple ATP hydrolysis to solute movement.

Na^+K^+ Pump (Na^+K^+ATPase)



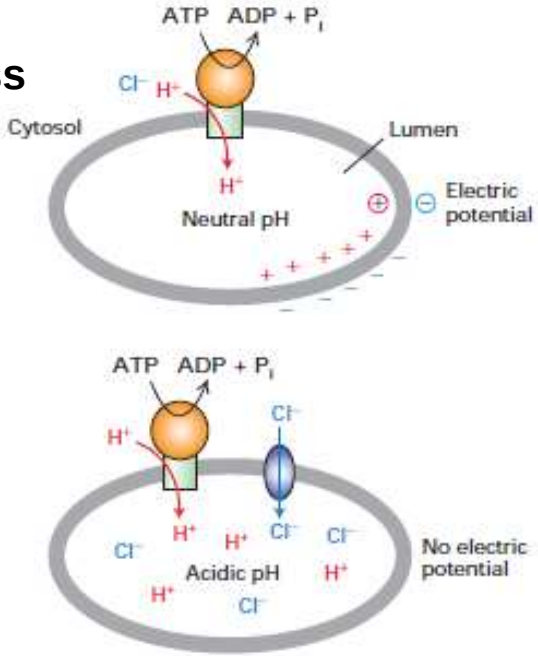
Muscle Ca^{2+}-ATPase Pumps Ca^{2+} Ions from the Cytosol into the Sarcoplasmic Reticulum



Ca^{2+}-ATPase in SR membrane of skeletal muscle cells. E1 and E2 are alternative conformations of protein in which Ca2-binding sites are accessible to cytosolic and exoplasmic faces, respectively. ATP hydrolysis is coupled with transport of Ca^2 ions across membrane. ~P indicates high-energy acyl phosphate bond; –P indicates low-energy phosphoester bond. Since Ca^2 affinity for cytosolic-facing binding sites in E1 is 1000-fold greater than for exoplasmic-facing sites in E2, this pump transports Ca2 unidirectionally from cytosol to SR lumen.

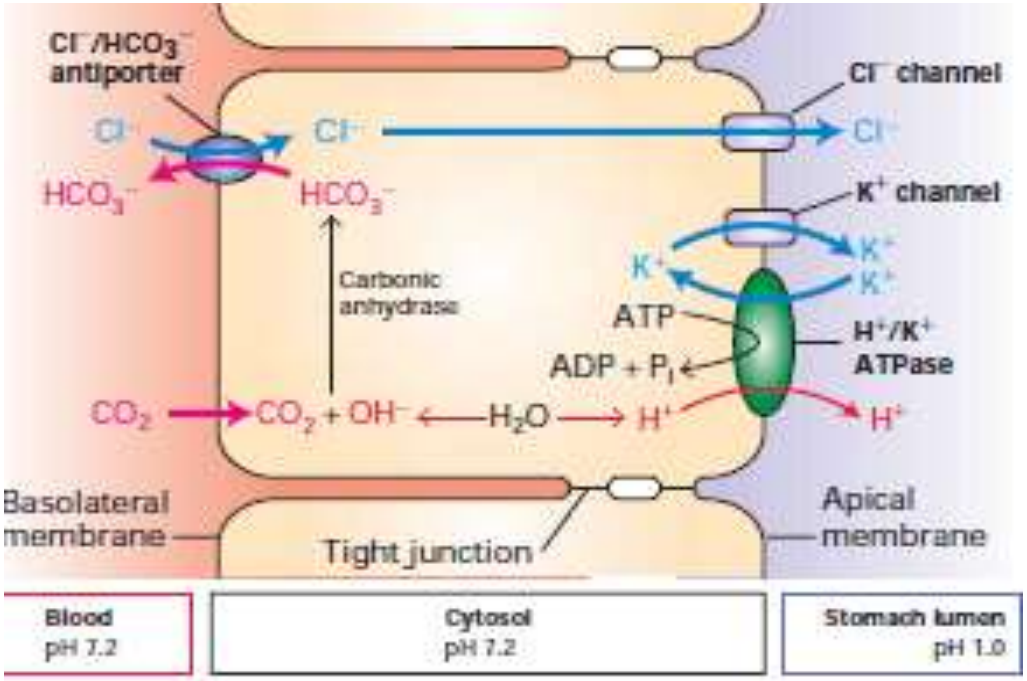
V-Class H ATPases Pump Protons Across Lysosomal and Vacuolar Membranes

All V-class ATPases transport only H^+ ions. These proton pumps, present in the membranes of lysosomes, endosomes, and plant vacuoles, function to acidify the lumen of these organelles. The pH of the lysosomal lumen can be measured precisely in living cells by use of particles labeled with a pH-sensitive fluorescent dye. After these particles are phagocytosed by cells and transferred to lysosomes, the lysosomal pH can be calculated from the spectrum of the fluorescence emitted. Maintenance of the 100-fold or more proton gradient between the lysosomal lumen (pH ≈4.5–5.0) and the cytosol (pH ≈7.0) depends on ATP production by the cell.



Effect of proton pumping by V-class ion pumps on H concentration gradients and electric potential gradients across cellular membranes. H^+ pumping generates an electric potential across membrane, luminal-side positive, but no significant change in intraluminal pH. Cl channels allow passive transport of anions following H^+, resulting in accumulation of H^+ (low luminal pH).

Parietal Cells Acidify the Stomach Contents While Maintaining a Neutral Cytosolic pH



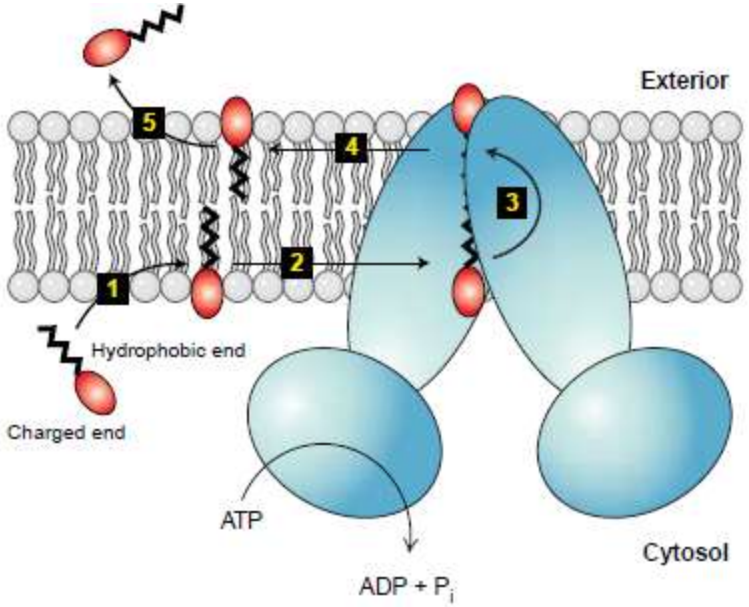
Acidification of the stomach lumen by parietal cells in gastric lining. Apical membrane of parietal cells contains an H/K ATPase (a P-class pump) and Cl and K channel proteins. The basolateral membrane contains an anion antiporter that exchanges HCO3 and Cl ions. The combined operation of these 4 different transport proteins and carbonic anhydrase acidifies the stomach lumen while maintaining the neutral pH and electroneutrality of the cytosol.

About 50 ABC Small-Molecule Pumps Are Known in Mammals

Discovery of the first eukaryotic ABC protein to be recognized came from studies on tumor cells and cultured cells that exhibited resistance to several drugs with unrelated chemical structures. Such cells eventually were shown to express elevated levels of a *multidrug-resistance (MDR) transport protein* known as *MDR1.*

This protein uses the energy derived from ATP hydrolysis to *export a large variety of drugs from* the cytosol to the extracellular medium. The *Mdr1 gene is* frequently amplified in multidrug-resistant cells, resulting in a large overproduction of the MDR1 protein.

Most drugs transported by MDR1 are small hydrophobic molecules that diffuse from medium across the plasma membrane, unaided by transport proteins, into the cell cytosol, where they block various cellular functions. Two such drugs are colchicine and vinblastine, which block assembly of microtubules. ATP-powered export of such drugs by MDR1 reduces their concentration in cytosol. As a result, a much higher extracellular drug concentration is required to kill cells that express MDR1 than those that do not.

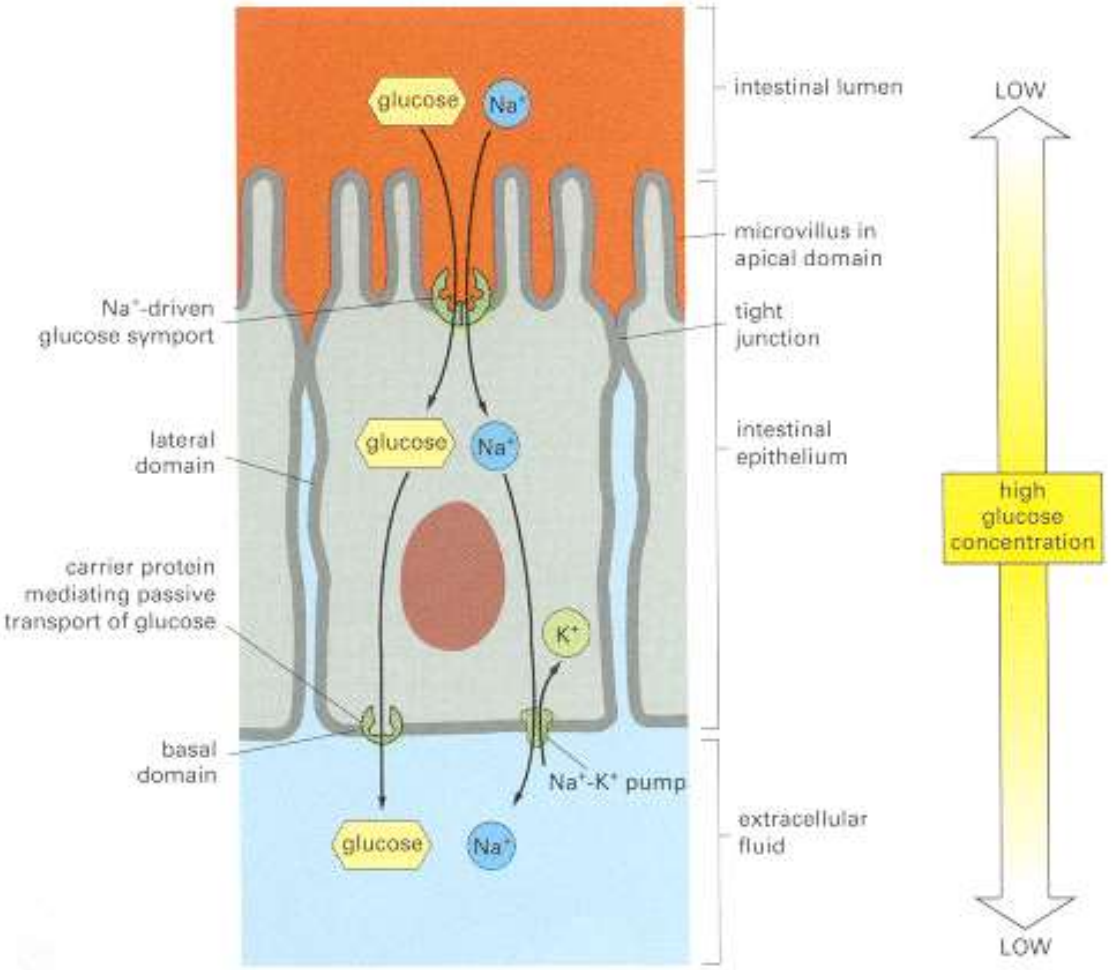


Flippase model of transport by MDR1 and similar ABC proteins.

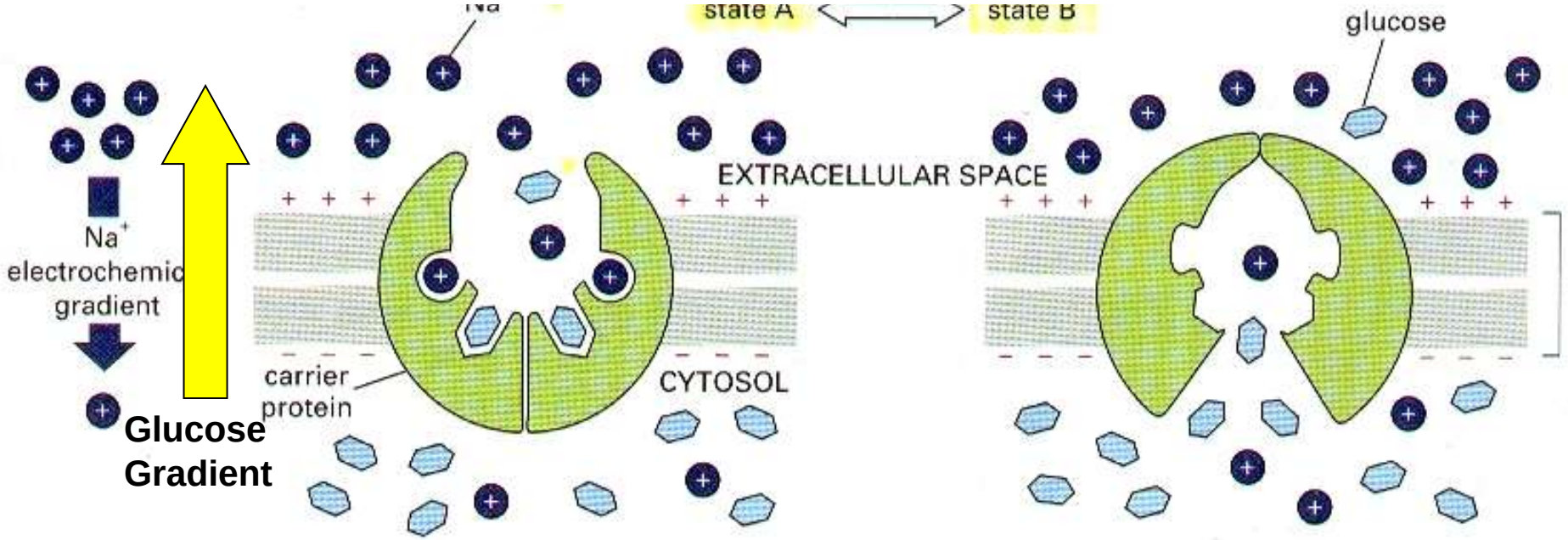
•On other hand, the antiporters and symporters carry out the transport of a type of ion or molecule “uphill” against its concentration gradient (which is usually energetically unfavorable) coupled with the transport of another ion or molecule “downhill” its concentration gradient (which is an energetically favourable reaction).

•Although these transporter proteins are often referred to as “*secondary active transporters*”, they do not involve hydrolysis of ATP during the transport of molecules. Therefore, these transporters are also commonly called **cotransporters** since they are capable of transporting two different solutes simultaneously.

•Such type of transport is also called **secondary active transport** since the *cotransporters use the energy stored in an electrochemical gradient unlike the ATP pumps that use energy from hydrolysis of ATP*.



Example of indirect active transport: Na^+ gradient drives other transport (Na^+ glucose <u>symport)</u>



Coupled transport

Transport protein oscillates between 2 conformations, A is open to outside; binding of Na+ induces a conformational change that increases the binding affinity for glucose.

Na-Linked Symporters Import Amino Acids and Glucose into Animal Cells Against High Concentration Gradients

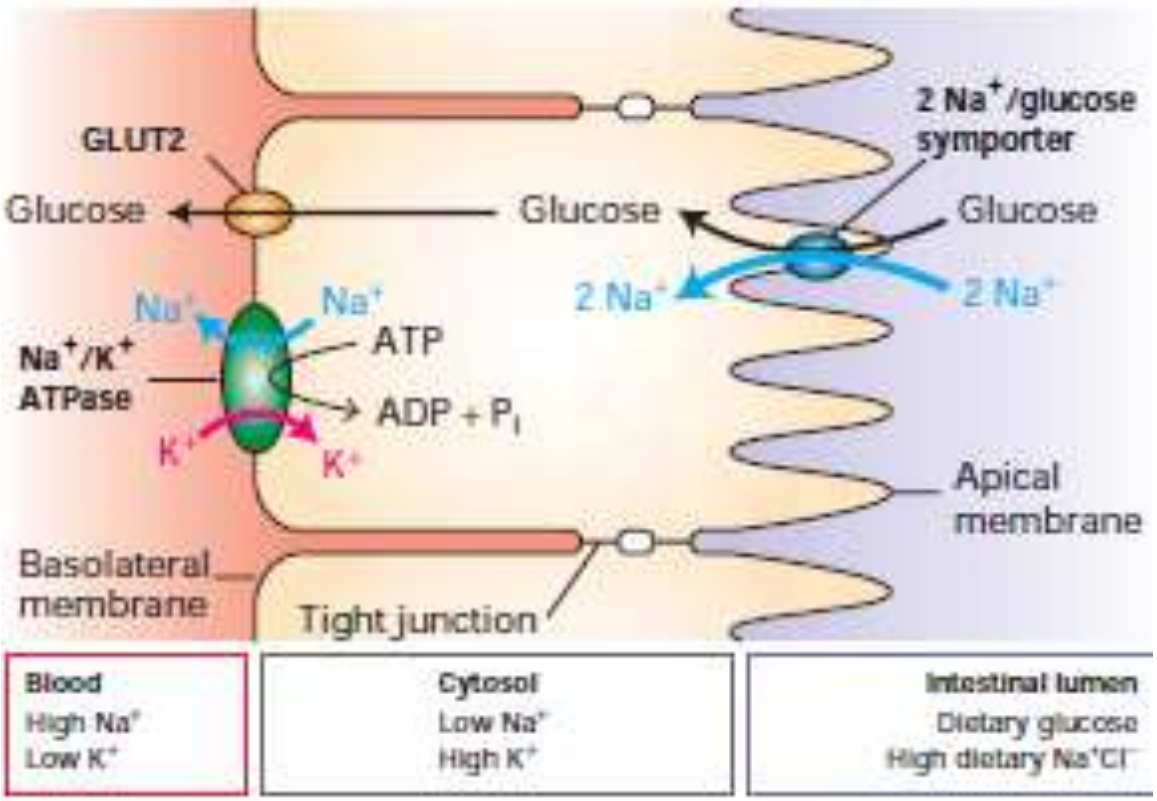
Transcellular Transport:

Such type of transport usually ***takes place across a layer of cells and occurs through carrier or channel molecules present on the luminal and antiluminal sides of the cells.***

Transcellular transport may be facilitated diffusion or active transport. In transcellular facilitated diffusion, the membranes on opposite sides of the cell usually have similar carriers and solutes transported along their concentration gradients.

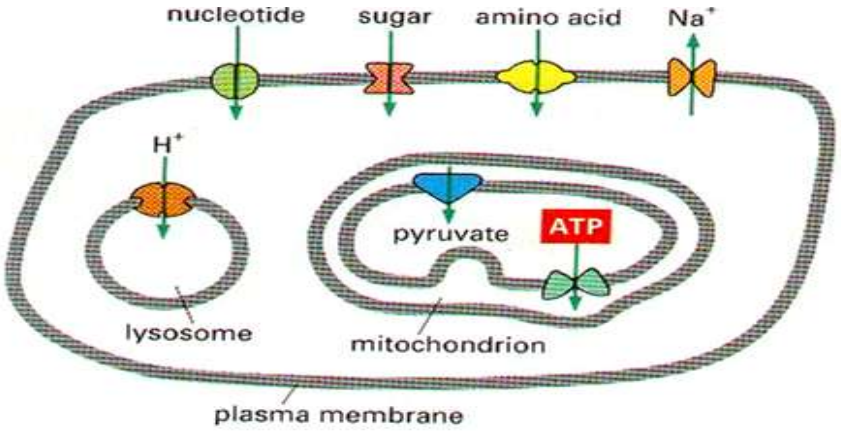
While in transcellular active transport, **active transport proteins** are present only on one side of the cell and the other side of the cell usually lacks active transport system giving different properties to the plasma membranes of the two surfaces of a cell have, called cellular polarity.

Such type of arrangement leads to the accumulation of solutes within the cell by active transport through the transport proteins on one side and the solutes leaves the cell from the opposite side of the cell through a **channel or facilitated transport**.

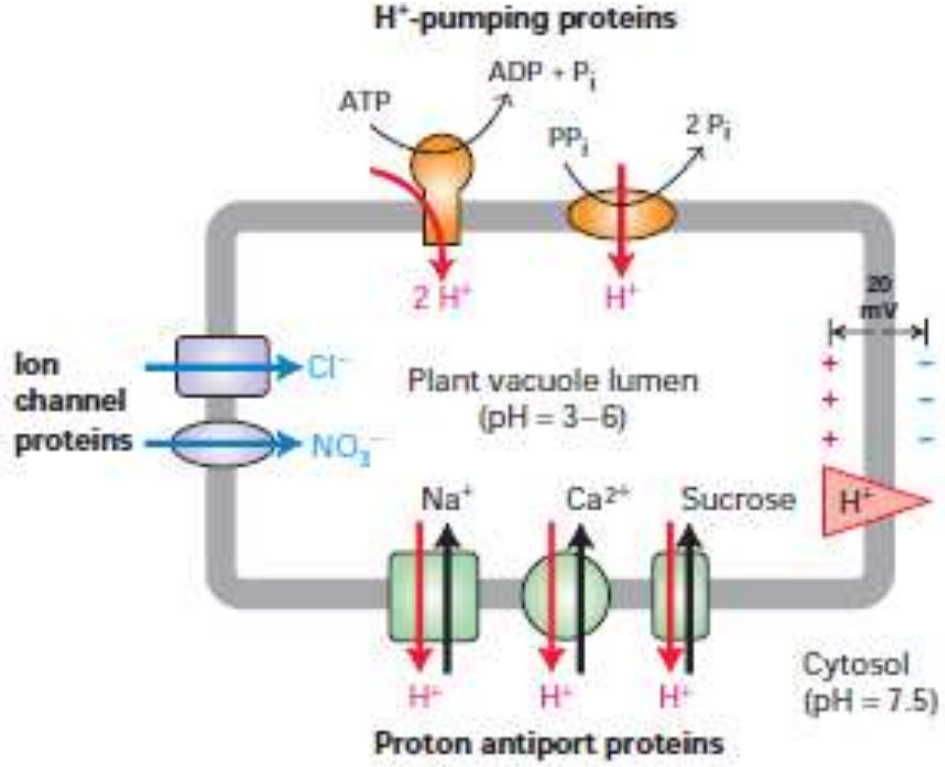


Transcellular transport of glucose across an intestinal epithelial cell depends on nonuniform distribution of transport proteins in cell's PM. Glucose is transported from the intestinal lumen to extracellular fluid (from where it passes into blood). Glucose is pumped into the cell through apical domain of membrane by Na+-powered glucose symport, and passes out of the cell (down its concentration gradient) by passive transport mediated by a different glucose carrier protein in the basal and lateral membrane domains. The Na+ gradient driving the glucose symport is maintained by a Na+ pump in the basal and lateral PM, which keeps the internal concentration of Na+ low. Adjacent cells are connected by impermeable tight junctions, which prevent solutes from crossing the epithelium between cells, allowing a concentration gradient of glucose to be maintained across cell sheet, and also serve as diffusion barriers within the PM.

Each membrane has its own characteristic set of transporters



Numerous Transport Proteins Enable Plant Vacuoles to Accumulate Metabolites and Ions



Na/K ATPase Maintains the Intracellular Na and K Concentrations in Animal Cells

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

Comparison of mechanisms for transporting Ions and small molecules across membranes

	Transport Mechanism			
Property	**Passive Diffusion**	**Facilitated Diffusion**	**Active Transport**	**Cotransport***
Requires specific protein	−	+	+	+
Solute transported against its gradient	−	−	+	+
Coupled to ATP hydrolysis	−	−	+	−
Driven by movement of a cotransported ion down its gradient	−	−	−	+
Examples of molecules transported	O_2, CO_2, steroid hormones, many drugs	Glucose and amino acids (uniporters); ions and water (channels)	Ions, small hydrophilic molecules, lipids (ATP-powered pumps)	Glucose and amino acids (symporters); various ions and sucrose (antiporters)

*Also called *secondary active transport*.

Summary:

Passive transport

Simple diffusion

No protein

HIGH to low conc

favorable

Facilitated diffusion

channel protein carrier protein

HIGH to low conc

favorable

Active transport

carrier protein

low to HIGH conc

Unfavorable

Add energy

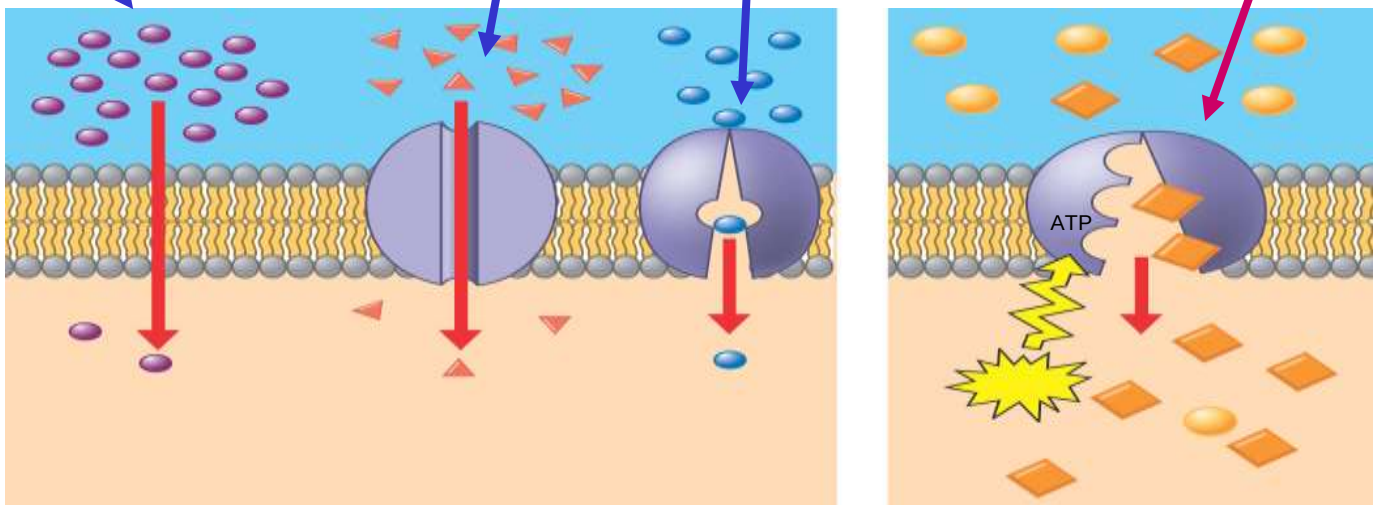


Figure 7.17

Disclaimer

All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline.

References

- Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell*. New York: Garland Science, 2002.

- Lodish, Harvey F. *Molecular Cell Biology.* 5th ed. New York: W.H. Freeman, 2003.

- Cooper, G.M., The Cell: A Molecular Approach, 4th edition, Sunderland (MA): Sinauer Associates; 2000.

- NPTEL Swayam online platform.