NATIONAL UNIVERSITY OF PHARMACY
Department of Educational and Information Technologies

BIOPHYSICS, PHYSICAL METHODS OF ANALYSIS

Lecture 5

Transport of substances through biological membranes.

Plan of the Lecture

1. Membrane Structure.
2. Membrane Transport.
3. Membrane Permeability.
4. Fick’s Law of Diffusion.
5. Effects of Osmosis on Water Balance.
6. Passive Transport.
7. Active Transport.

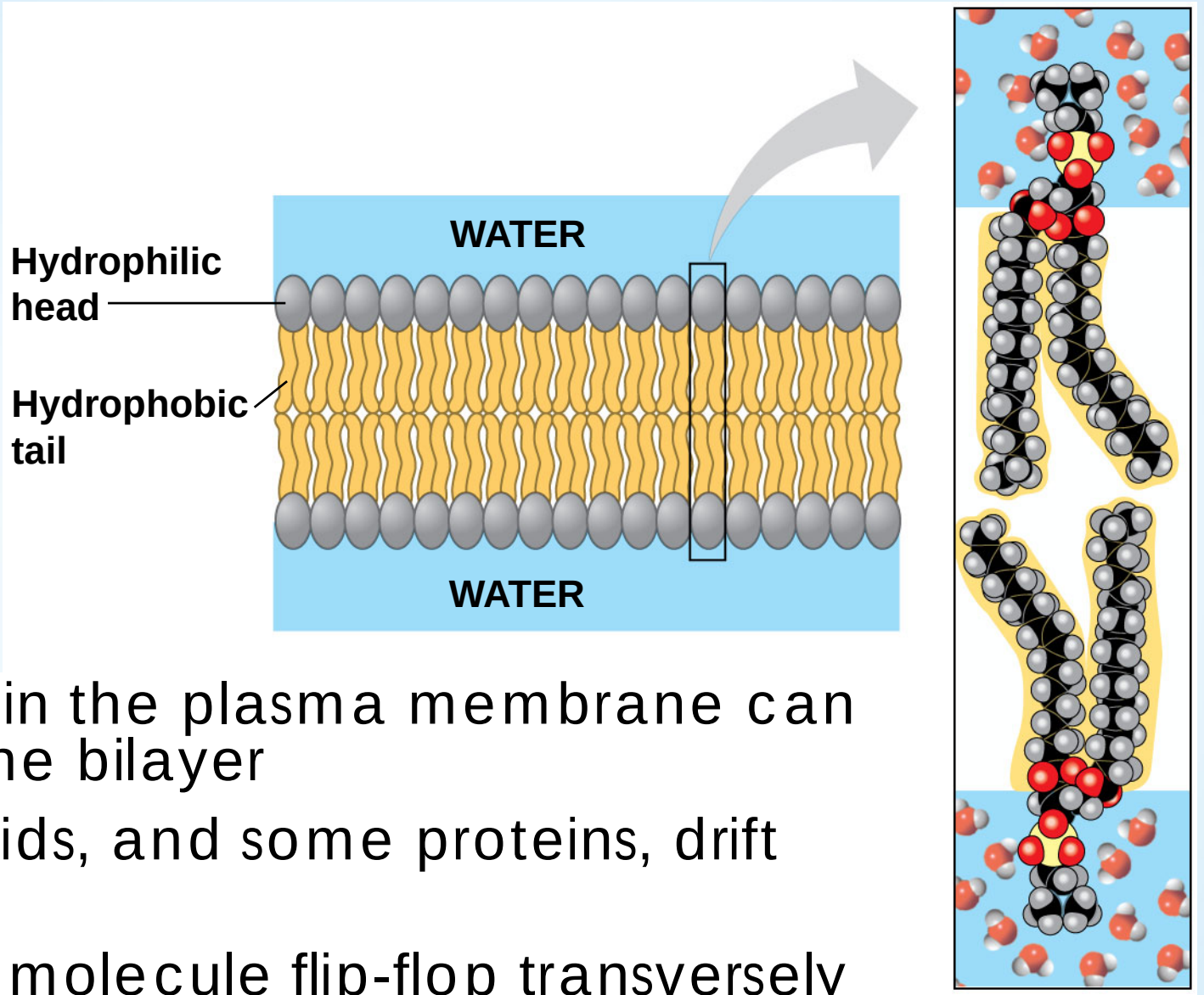
Purpose of the lecture is

- to form the basic information about the types of transport and the ability of substances to overcome the membranes barrier.

Transport of substances through biological membranes

- The plasma membrane is the boundary that separates the living cell from its surroundings
- The plasma membrane exhibits **selective permeability**, allowing some substances to cross it more easily than others
- Phospholipids are the most abundant lipid in the plasma membrane
- Phospholipids are **amphipathic molecules**, containing hydrophobic and hydrophilic regions
- The **fluid mosaic model** states that a membrane is a fluid structure with a “mosaic” of various proteins embedded in it

Membrane Structure



- Phospholipids in the plasma membrane can move within the bilayer
- Most of the lipids, and some proteins, drift laterally
- Rarely does a molecule flip-flop transversely across the membrane

Membrane Structure

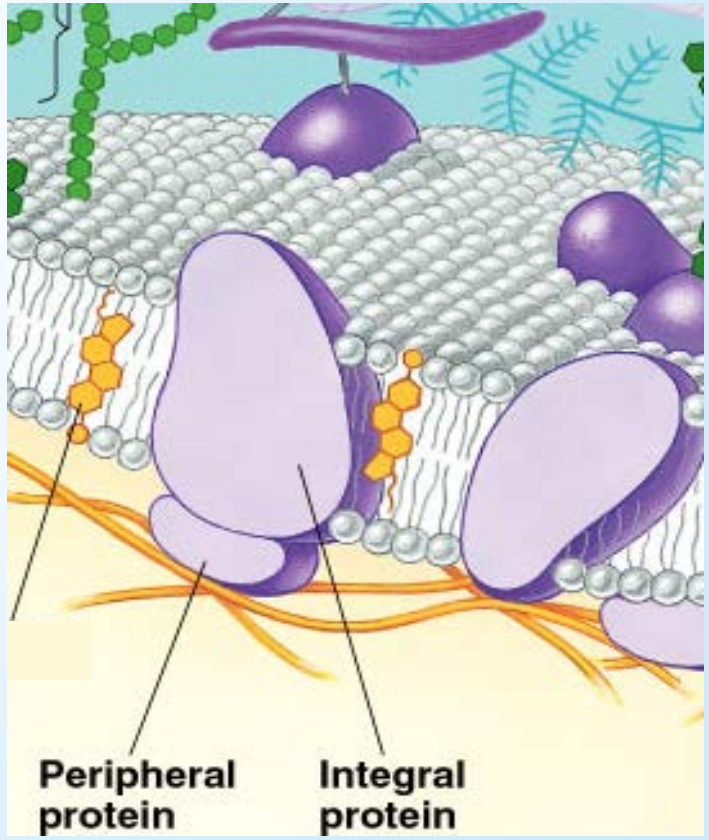
- Phospholipids arranged in a bilayer
- Globular proteins inserted in the lipid bilayer
- Fluid mosiac model – mosaic of proteins floats in or on the fluid lipid bilayer like boats on a pond

Cellular membranes have 4 components

1. Phospholipid bilayer
   - Flexible matrix, barrier to permeability
2. Transmembrane proteins
   - Integral membrane proteins
3. Interior protein network
   - Peripheral membrane proteins
4. Cell surface markers
   - Glycoproteins and glycolipids

Membrane Proteins

- Proteins determine most of membrane's specific functions
  - cell membrane & organelle membranes each have unique collections of proteins
- Membrane proteins:
  - peripheral proteins = loosely bound to surface of membrane
  - integral proteins or transmembrane proteins = penetrate into lipid bilayer, often completely spanning the membrane

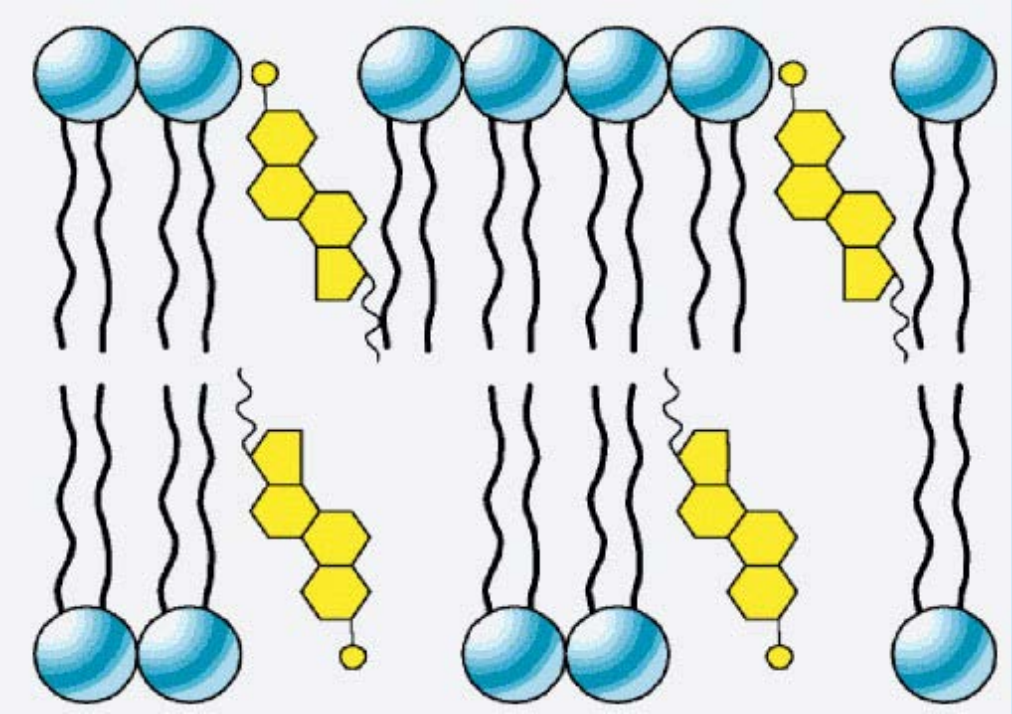


Membrane Protein Types

- Channel proteins – wide open passage
  - Ion channels – gated
  - Aquaporins – water only, kidney and plant root only
- Carrier proteins – change shape
- Transport proteins – require ATP
- Recognition proteins - glycoproteins
- Adhesion proteins – anchors
- Receptor proteins - hormones

Cholesterol

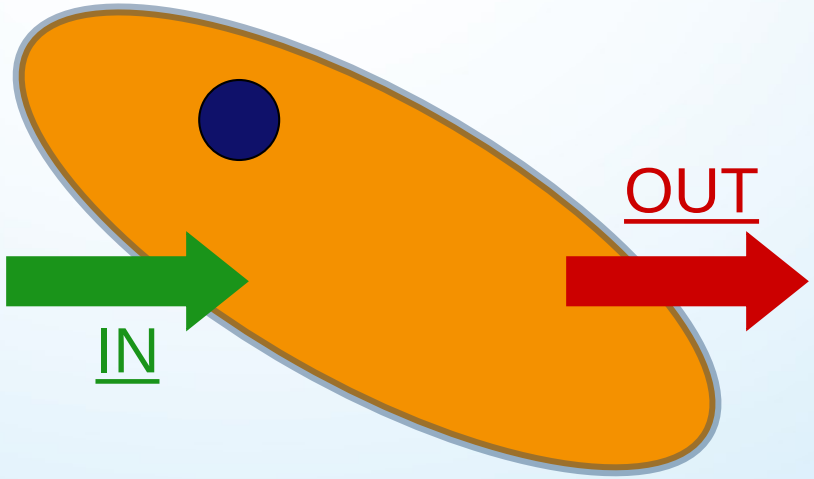
Provides stability in animal cells
Replaced with sterols in plant cells



Transport:

- **Across tissue barriers**
  - Gut Wall (absorption of nutrients)
  - Lung Alveoli (exchange of gases)
  - Capillary Beds (blood-tissue exchange of nutrients, gases, and waste products)
  - Nephrons in kidneys (urine formation)
  - Transcellular versus Paracellular
- **Across cell membranes**
  - Regulation of cell size
  - Regulation of cell electrical activity
  - Passive vs. Active Transport

IN
food
carbohydrates
sugars, proteins
amino acids
lipids
salts, O_2, H_2O



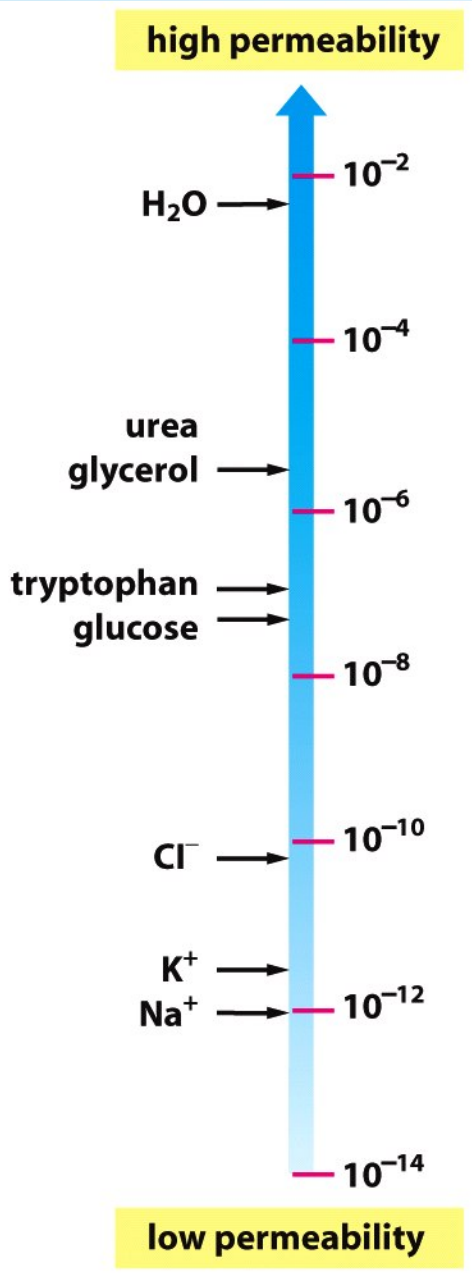
OUT
waste
ammonia
salts
CO_2
H_2O
products

Membrane Transport

- Requires:
  1. Permeability of the membrane
  2. A driving force
- Passive Transport
  - movement of particles *along* a gradient
  - does not require energy expenditure
- Active Transport
  - movement of particles *against* a gradient
  - requires energy expenditure

Membrane Permeability

- Size
  - the smaller the particle, the more permeable
  - small molecules (O_2, CO_2, H_2O) can
  - large molecules (protein, DNA) cannot
- Lipid Solubility
  - YES: non-polar molecules (O_2, cholesterol),
  - NO: charged atoms/molecules (Na^+, Cl^-, HCO_3^-), large polar molecules (glucose)



Permeability coefficients (in cm/sec) through synthetic lipid bilayers

Product of the concentration difference (in mol/cm^3) and permeability coefficient (in cm/sec) gives the flow of solute in moles per second per square centimeter of membrane

What determines permeability (P)?

$$P = kT/6\pi r\eta x$$

k = Boltzmann's constant
T = absolute temperature
r = radius of the solute
η = viscosity of the medium
x = thickness of membrane

Transport across membranes:

- **Passive Transport**
  - Diffusion, Osmosis
    - Solutes or water cross a membrane by following a concentration gradient.
  - Facilitated Diffusion
    - Carrier Molecule binds a solute and brings it across the membrane with no energy expended.
- **Active Transport**
  - Requires energy expenditure, and can go against a gradient.

The **fundamental laws** that govern the flow of ions through the cell membrane are:

- Ficks Law of Diffusion
- Ohms Law of Drift
- Space charge neutrality
- Einsteins Relation between diffusion and drift

Fick's Law of Diffusion

Fick's law relates the diffusion gradient of ions to their concentration.

$$\text{Ion Flux} = J_{\text{diffusion}} = -D\frac{\partial[C]}{\partial x}$$

- J_{diff}: Diffusion flux, measuring the amount of substance flowing across unit area per unit time (molecules/ cm^2/s)
- D: Diffusion coefficient (cm^2/s)
- [C]: Concentration of the ions (molecules/cm^3)

Space charge neutrality

Biological systems are overall electrically neutral; i.e., the total charge of cations in a given volume of biological material equals the total charge of anions in the same volume biological material

$$\sum_i z_i^C[C_i] = \sum_j z_j^A[C_j]$$

Ohms law of drift (Microscopic view)

Charged particle in the presence of external electrical field **E** experience a force resulting in their drift along the E field gradient

$$\text{Ion drift} = J_{\text{drift}} = -z[C]\mu\frac{\partial V}{\partial x}$$

- J_{drift}: Drift flux, measuring the amount of substance flowing across unit area per unit time (molecules/cm^{2}s)
- μ: electrical mobility of charged particle(cm^2/sV)
- [C]: Concentration of the substance (ions) (molecules/cm^3)
- z: Valence of ion

Einstein relation

It relates the diffusion constant (effect of motion due to concentration gradients) of an ion to its mobility (effect of motion due to electrical forces)

Basic idea is that the frictional resistance created by the medium is same for ions in motion due to drift and diffusion.

$$D = \frac{kT}{q}\mu = \frac{RT}{F}\mu$$

Diffusion: Fick's First Law

$$J_s = -PS(C_o - C_i)$$

J_s = solute flux; P = permeability coefficient;
S = surface area for diffusion;
$(C_0 - C_i)$ = concentration gradient of the solute

- Substances diffuse down their **concentration gradient**, the region along which the density of a chemical substance increases or decreases
- No work must be done to move substances down the concentration gradient
- The diffusion of a substance across a biological membrane is **passive transport** because no energy is expended by the cell to make it happen

What determines the rate of diffusion? There 4 factors:

1. **The steepness of the concentration gradient.** The bigger the difference between the two sides of the membrane the quicker the rate of diffusion.
2. **Temperature.** Higher temperatures give molecules or ions more kinetic energy. Molecules move around faster, so diffusion is faster.
3. **The surface area.** The greater the surface area the faster the diffusion can take place. This is because the more molecules or ions can cross the membrane at any one moment.
4. **The type of molecule or ion diffusing.** Large molecules need more energy to get them to move so they tend to diffuse more slowly. Non-polar molecules diffuse more easily than polar molecules because they are soluble in the non polar phospholipid tails.

Effects of Osmosis on Water Balance

- **Osmosis** is the diffusion of water across a selectively permeable membrane
- Water diffuses across a membrane from the region of lower solute concentration to the region of higher solute concentration until the solute concentration is equal on both sides

Described by van't Hoff Equation:

$$\pi = RT(\phi ic)$$

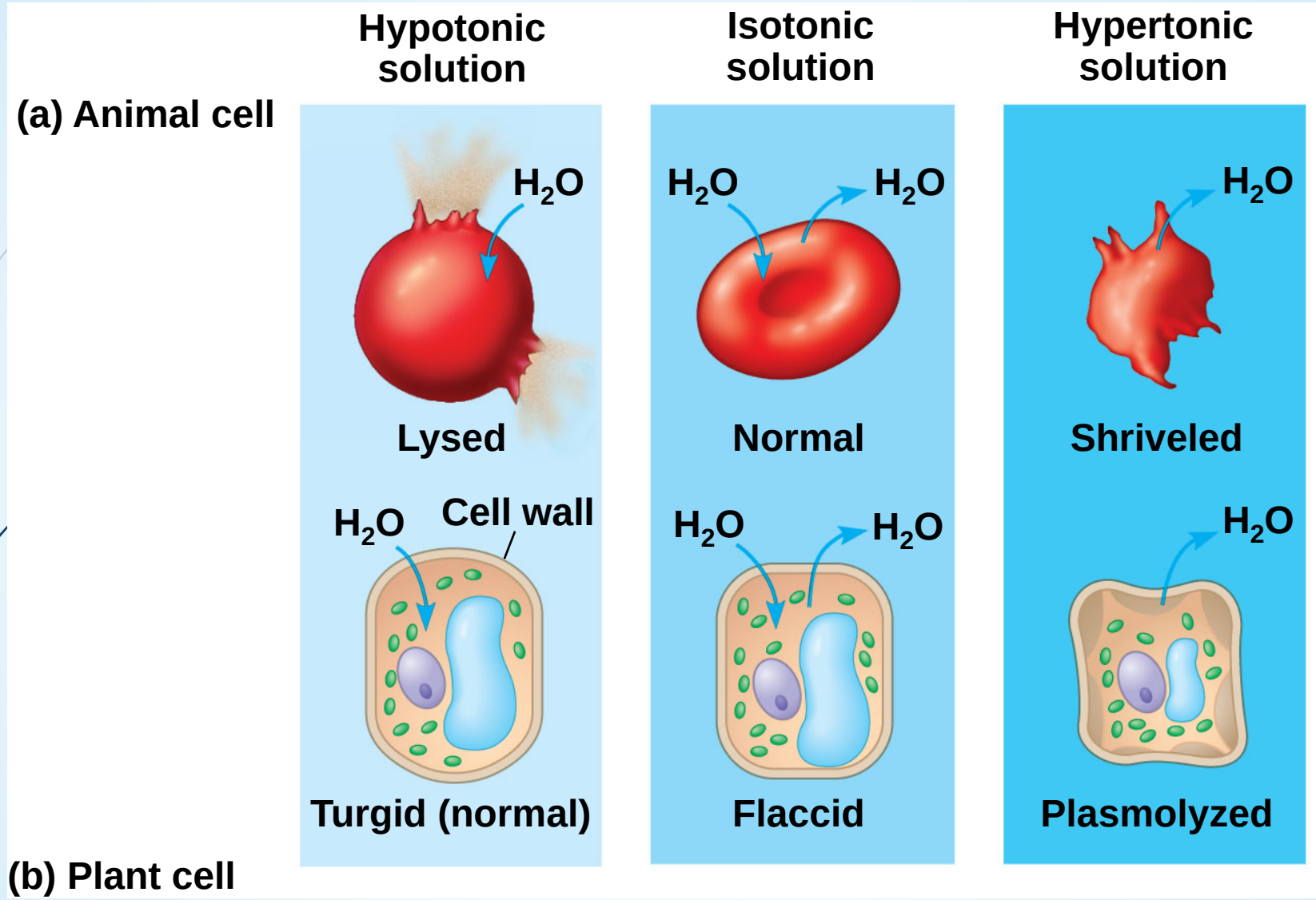
- π = osmotic pressure
- R = ideal gas constant
- T = absolute temperature
- ϕ = osmotic coefficient
- i = number of ions formed by dissociation of a solute molecule
- c = molar concentration of a solute
- (ϕic) = osmolarity of the solution

Water Balance of Cells Without Walls

- **Tonicity** is the ability of a surrounding solution to cause a cell to gain or lose water
- **Isotonic** solution: Solute concentration is the same as that inside the cell; no net water movement across the plasma membrane
- **Hypertonic** solution: Solute concentration is greater than that inside the cell; cell loses water
- **Hypotonic** solution: Solute concentration is less than that inside the cell; cell gains water

Hypertonic or hypotonic environments create osmotic problems for organisms

- **Osmoregulation**, the control of solute concentrations and water balance, is a necessary adaptation for life in such environments


Hypotonic solution
Isotonic solution
Hypertonic solution
(a) Animal cell
H_2O
Lysed
H_2O
H_2O
Normal
H_2O
Shriveled
(b) Plant cell
H_2O
Cell wall
Turgid (normal)
H_2O
H_2O
Flaccid
H_2O
Plasmolyzed

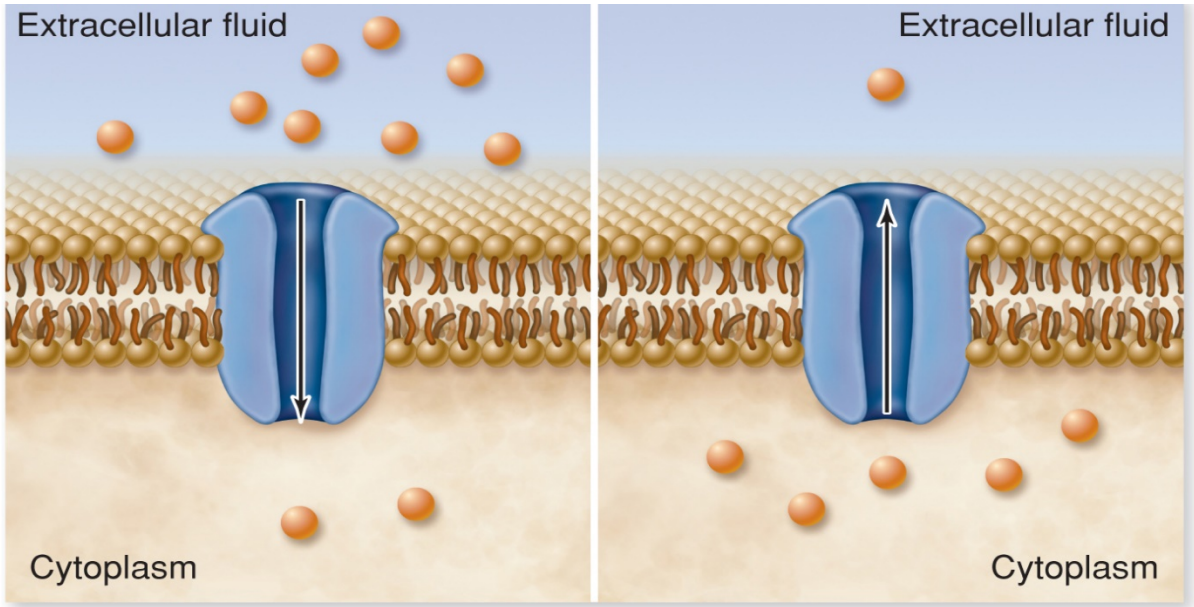
Osmosis

Facilitated Diffusion: Passive Transport Aided by Proteins

- In **facilitated diffusion**, transport proteins speed the passive movement of molecules across the plasma membrane
- Channel proteins provide corridors that allow a specific molecule or ion to cross the membrane
- Channel proteins include
    - Aquaporins, for facilitated diffusion of water
    - **Ion channels** that open or close in response to a stimulus (**gated channels**)
- Large polar molecules such as glucose and amino acids, cannot diffuse across the phospholipid bilayer. Also ions such as Na^+ or Cl^- cannot pass.
- These molecules pass through protein channels instead. Diffusion through these channels is called FACILITATED DIFFUSION.
- Movement of molecules is still PASSIVE just like ordinary diffusion, the only difference is, the molecules go through a protein channel instead of passing between the phospholipids.

Channel proteins

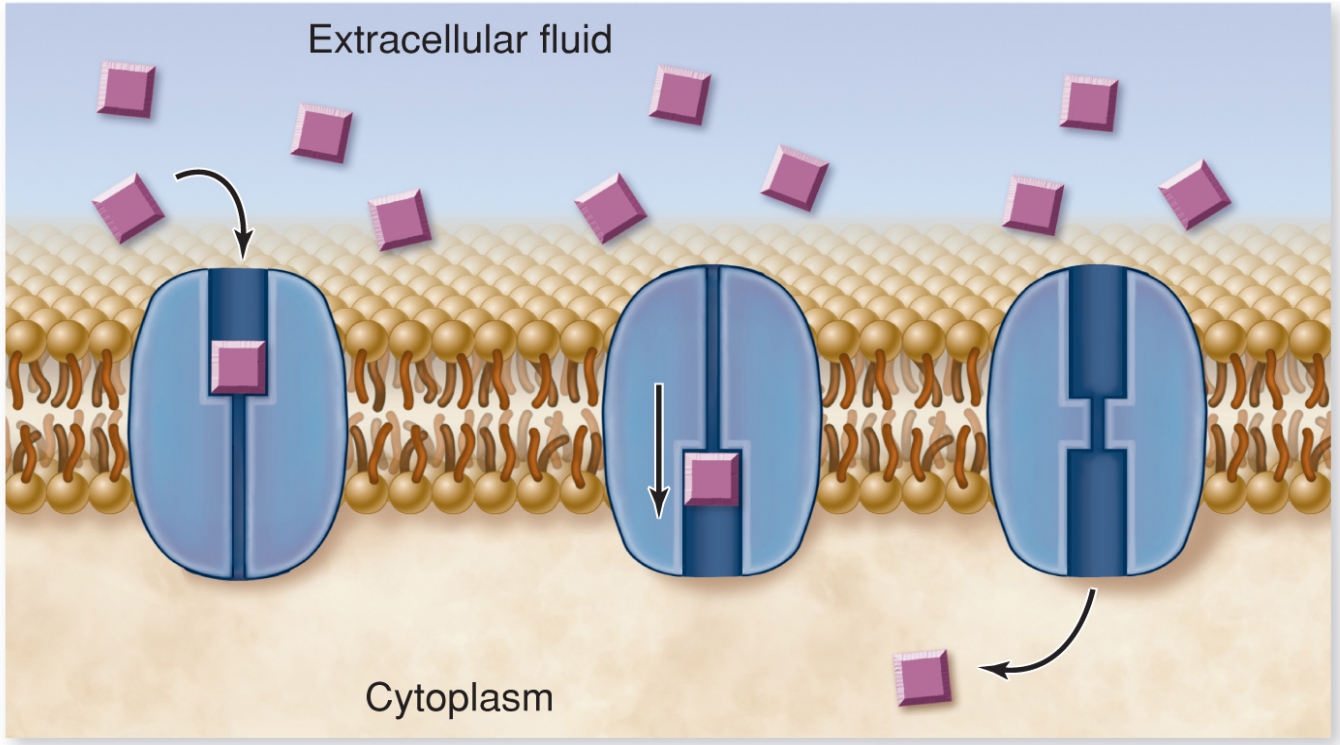
- Ion channels
  - Allow the passage of ions
  - Gated channels – open or close in response to stimulus (chemical or electrical)
  - 3 conditions determine direction
    - Relative concentration on either side of membrane
    - Voltage differences across membrane
    - Gated channels – channel open or closed



a.

Carrier proteins

- Can help transport both ions and other solutes, such as some sugars and amino acids
- Requires a concentration difference across the membrane
- Must bind to the molecule they transport
  - Saturation – rate of transport limited by number of transporters



b.

Nernst-Plank Equation

Nernst Plank equation governs the current generated from the flow of individual ions across the cell membrane.

Continuity Equation $$I = -(uz^2F[C]\frac{\partial V}{\partial x} + uzRT\frac{\partial [C]}{\partial x})$$

The time dependent Nernst Plank Equation: $$\frac{\partial I}{\partial x} = -\frac{\partial [C]}{\partial t}$$

$$\frac{\partial [C]}{\partial t} = \frac{\partial}{\partial x}(uz^2F[C]\frac{\partial V}{\partial x} + uzRT\frac{\partial [C]}{\partial x})$$

Nernst Equation

- Nernst equation is special case of NPE, where in the membrane potential is obtained as a function of concentration gradient across the cell membrane when the net current flow generated by the ion is zero.

$$V_m(I=0) = V_{\text{in}} - V_{\text{out}}$$
$$= \frac{RT}{zF} \ln\left(\frac{[C_{\text{out}}]}{[C_{\text{in}}]}\right)$$

Donnan Equilibrium Rule

- The membrane potential equals the reversal potential of all ions that can passively permeate through the cell membrane.
- Mathematically the Donnan Rule implies:

$$\left(\frac{[C_{\text{out}}^{+m}]}{[C_{\text{in}}^{+m}]}\right)^{1/m} = \left(\frac{[C_{\text{in}}^{-n}]}{[C_{\text{in}}^{-n}]}\right)^{1/n}$$

- The cytoplasm contains many large proteins, which typically have a negative charge. This influences how cation-anion pairs distribute across the membrane, creating an uneven distribution.
- These nondiffusible ions influence how diffusible ions distribute across a membrane, and is known as the **Gibbs-Donnan Effect**.

Steady state solution to NPE

- We can solve the NPE equation in steady state (ie no time dependence for concentrations). *A special case was seen through NE, wherein in addition to the membrane being in steady state, the membrane was in equilibrium (I=0)*.

$$I = zRTu\frac{\lambda(x_0)[C(x_0)] - \lambda(x)[C(x)]}{\int_{x_0}^{x}\lambda(x')dx'}$$

$$\lambda(x) = \exp\left(\frac{zF}{RT}V(x)\right)$$

Constant field assumption

- Most common assumption is charge density within the membrane is identically zero. This assumption leads to the following expression for V(x) with boundary conditions: V(x=0)=Vm and V(x=l)=0

$$V(x) = -\frac{V_m x}{l} + V_m$$

- We get the following expression for current:

where

$$I = PzF\zeta\left(\frac{[C_{in}] - [C_{out}]e^{-\zeta}}{1 - e^{-\zeta}}\right) \qquad \zeta = zV_mF/RT$$

Goldman Hodgkin Katz Model

- It is widely used model to predict the resting membrane potential V_m for nerve cells
- The formula for V_m is derived as a solution NPE with constant field assumption
- Further more it is assumed that ions flow across the cell membrane without interacting with each other
- For membrane that is permeable to M positive monovalent ions and N negative monovalent ions, the GHK formula for V_m in equilibrium conditions is:

$$V_m(I=0) = \frac{RT}{F} \ln \left(\frac{\sum_{i+}^{M} P_i[C_i]_{\text{out}} + \sum_{j-}^{N} P_j[C_j]_{\text{in}}}{\sum_{i+}^{M} P_i[C_i]_{\text{in}} + \sum_{j-}^{N} P_j[C_j]_{\text{out}}} \right)$$

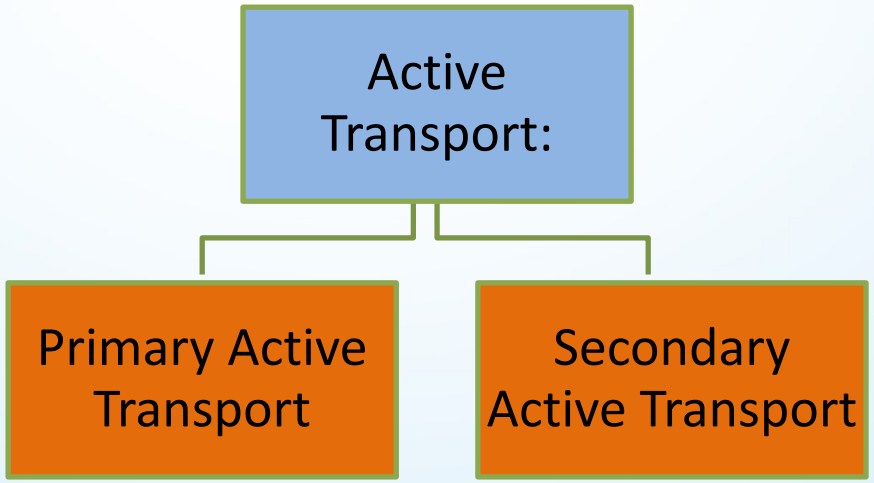
ACTIVE TRANSPORT

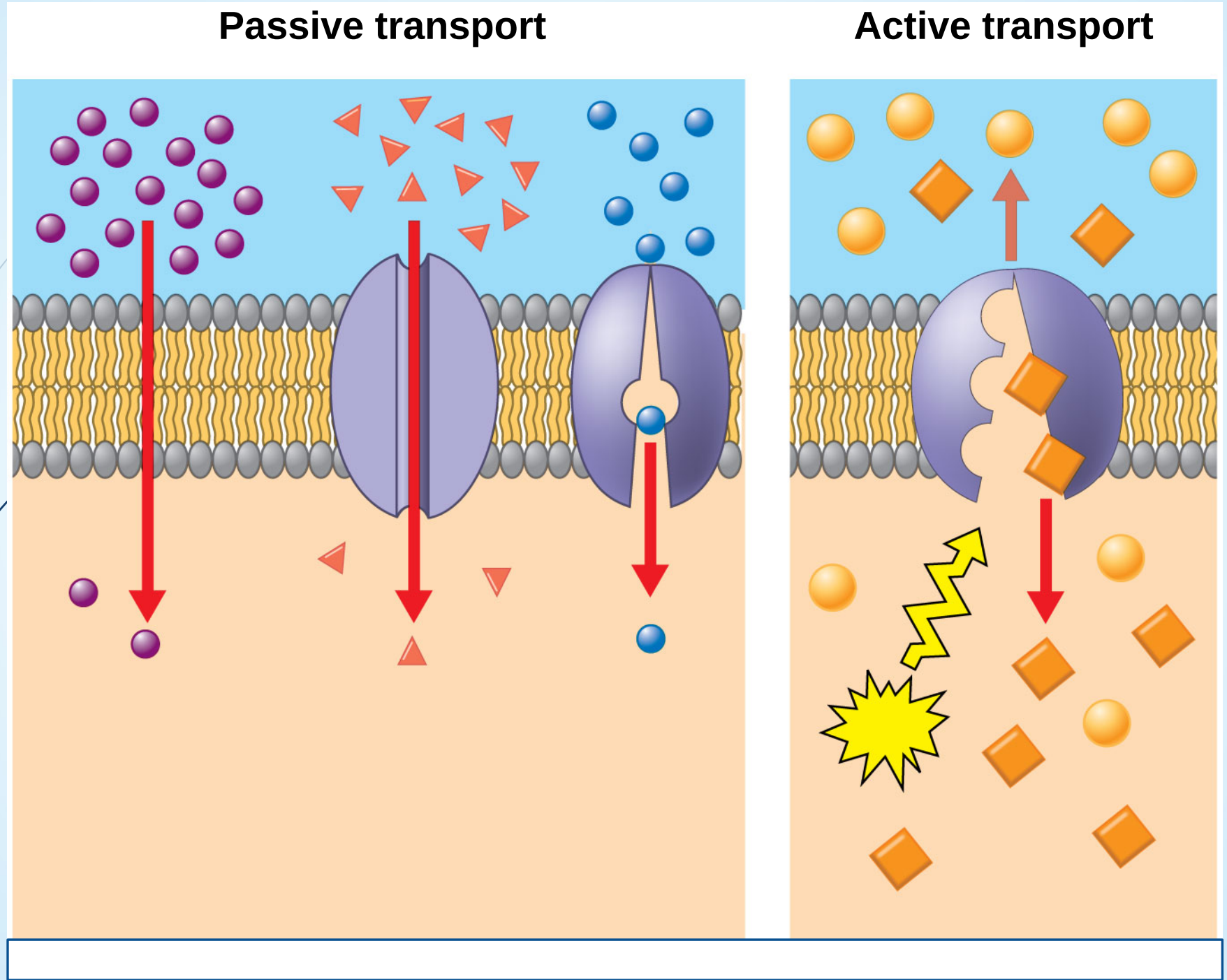
Definition:

Active transport is a carrier-mediated transport wherein molecules and ions are moved against their concentration gradient across a membrane and requires expenditure of energy.

The primary active transport carriers are termed as *pumps.*

Active transport is divided into 2 types according to the source of the energy used.




Passive transport
Active transport

Types of Active Transport:

Active transport is divided into 2 types depending on the source of energy used:

In **primary active transport**, the energy is derived directly from breakdown of adenosine triphosphate (ATP) or from some other high-energy phosphate compound.

In **secondary active transport**, the energy is derived secondarily from energy stored in the form of an ion concentration gradient between the two sides of a cell membrane, created originally by primary active transport. Thus, energy is used but it is “secondhand” energy and NOT directly derived from ATP.

In both instances, transport depends on *carrier proteins.*

However, in active transport, the **carrier protein** functions differently from the carrier in facilitated diffusion because it is capable of imparting energy to the transported substance to move it against the electrochemical gradient by acting as an enzyme and breaking down the ATP itself.

Primary Active Transport

- In primary active transport, energy in the form of ATP is required to change the affinity of the carrier protein binding site when it is exposed on opposite sides of plasma membrane.
- The carrier protein also acts as an enzyme that has **ATPase** activity, which means it splits the terminal phosphate from an ATP molecule to yield ADP and inorganic phosphate plus free energy.

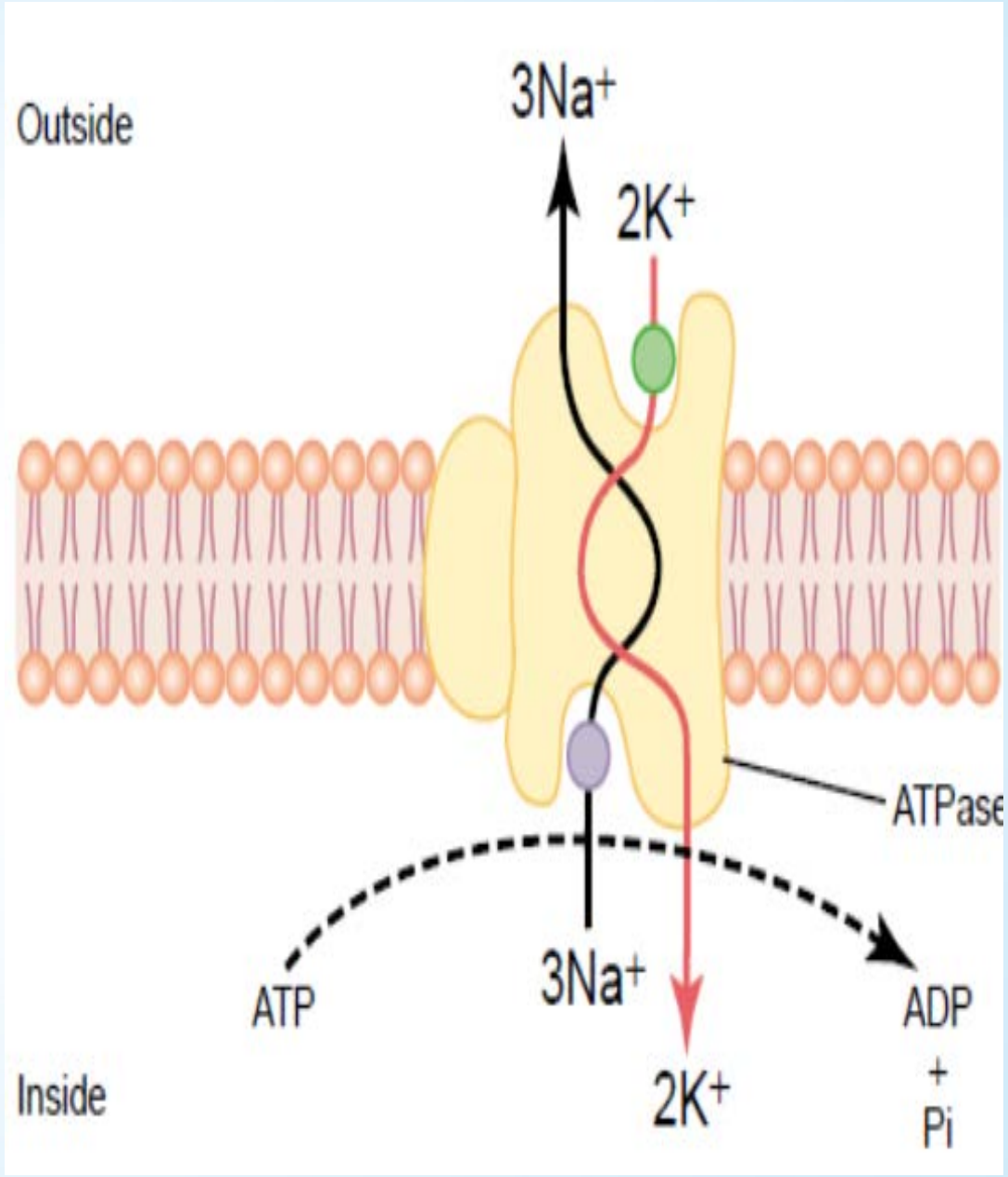
Examples:

1. Sodium-Potassium Pump.
2. Transport of Hydrogen ions: occurs at 2 places in the human body:
   - in the gastric glands of the stomach
   - In the kidneys

Na-K PUMP:

- The carrier protein in the pump has 2 subunits; a larger α subunit and a smaller β subunit. They perform the following functions:

1. **3 receptor sites** for binding **Na** ions on the portion of the protein that protrudes to the inside of the cell.
2. **2 receptor sites** for **potassium** ions on the outside.
3. The inside portion of this protein near the sodium binding site has **ATPase** activity.



FUNCTIONS OF SODIUM-POTASSIUM PUMP:

1. Control the ***Volume*** of each cell: It helps regulate cell volume by controlling the concentrations of solutes inside the cell and thus minimizing osmotic effect that would induce swelling or shrinking of the cell. If the pump stops, the increased Na concentrations within the cell will promote the osmotic inflow of water, damaging the cells.
2. ***Electrogenic*** nature of the pump: It establishes Na and K concentration gradients across the plasma membrane of all cells; these gradients are critically important in the ability of nerve and muscle cells to generate electrical signals essential to their functioning.
3. ***Energy*** used for Secondary active transport: The steep Na gradient is used to provide energy for secondary active transport.

SECONDARY ACTIVE TRANSPORT

Secondary active transport: is also called *coupled transport.* In secondary active transport, the downhill flow of an ion is linked to the uphill movement of a second solute either in the same direction as the ion **(co-transport)** or in the opposite direction of the ion **(co-transporter).**

The diffusion of Na^+ down its concentration gradient into the cell can then power the movement of a different ion or molecule against its concentration gradient. If the other molecule or ion is moved in the same direction as Na^+ (that is, into the cell), the coupled transport is called either *cotransport* or *symport.* If the other molecule or ion is moved in the opposite direction (out of the cell), the process is called either *countertransport* or *antiport.*

CO-TRANSPORT OR SYMPORT:

- The carrier protein has **two** binding sites: one for the solute being moved against its concentration gradient and one for Na.
- **Sites:** intestinal and kidney cells
- INTESTINAL CELLS: more Na+ is present in the intestinal lumen (ECF) than inside the cells (because Na-K pump moves the Na out of the cell keeping its intracellular conc. low).
- Because of this conc. difference, more Na binds to the carrier protein in the ECF.
- Binding of Na increases the affinity of the protein for Glucose which is present in low conc. In the ECF.
- When both Na and Glucose are attached to the carrier protein, it undergoes a conformational change and opens to the inside of the cell.
- Both Na & glucose are released to the inside of the cell: Na as there is low conc. & glucose as carrier proteins affinity for it decreases as Na is released.
- The released Na is quickly pumped out by the Na-K pump, keeping the levels of intracellular Na low.
- Thus, Na has been moved down its “downhill” while glucose is moved “uphill”.

SECONDARY ACTIVE TRANSPORT

- CO-TRANSPORT
  - Symport
  - Na moves downhill
  - Molecule to be co-transported moved in the same direction as Na, i.e. to the inside of the cell.
  - E.g. Na with glucose and amino acids.
  - Site: intestinal lumen and renal tubules of kidney.

- COUNTER TRANSPORT
  - Anti-port
  - Na moves downhill
  - Molecule to be counter-transported moves in the opposite direction to Na, i.e. to the outside of the cell.
  - E.g. Na with Calcium and Hydrogen ions.
  - Site: Na-Ca counter transport in almost all cells of the body and Na-H^+ in the proximal tubules of the kidney.

Control Questions

1. Membrane Structure.
2. Water Balance.
3. Channel proteins.
4. Nernst-Plank Equation.
5. Sodium-potassium pump.
6. Secondary active transport

Recommended literature:

Basic:

1. Vladimir Timanyuk, Elena Zhivotova, Igor Storozhenko. Biophysics: Textbook for students of higher schools / Kh.: NUPh, Golden Pages, 2011.- 576p.
2. Vladimir Timaniuk, Marina Kaydash, Ella Romodanova. Physical methods of analysis / Manual for students of higher schools/– Kharkiv: NUPh: Golden Pages, 2012. – 192 p.
3. Philip Nelson. Biological Physics. – W. H. Freeman, 1st Edition, 2007. – 600 p.
4. Biophysics, physical methods of analysis. Workbook: Study guide for the students of higher pharmaceutical educational institutions / Pogorelov S. V., Krasovskyi I. V., Kaydash M. V., Sheykina N. V., Frolova N. O., Timaniuk V. O., Romodanova E.O., Kokodii M.H. – Kharkiv., – 2018. – 130 p.
5. Center for distance learning technologies of NPhaU. Access mode: http://nuph.edu.ua/centr-distancijjnih-tehnologijj-navcha/

Support:

1. Eduard Lychkovsky. Physical methods of analysis and metrology: tutorial / Eduard Lychkovsky, Zoryana Fedorovych. – Lviv, 2012. – 107 p.
2. Daniel Goldfarb. Biophysics DeMYSTiFied. – McGraw-Hill Professional, 1st Edition, 2010. – 400 p.


Thanks for
your attention