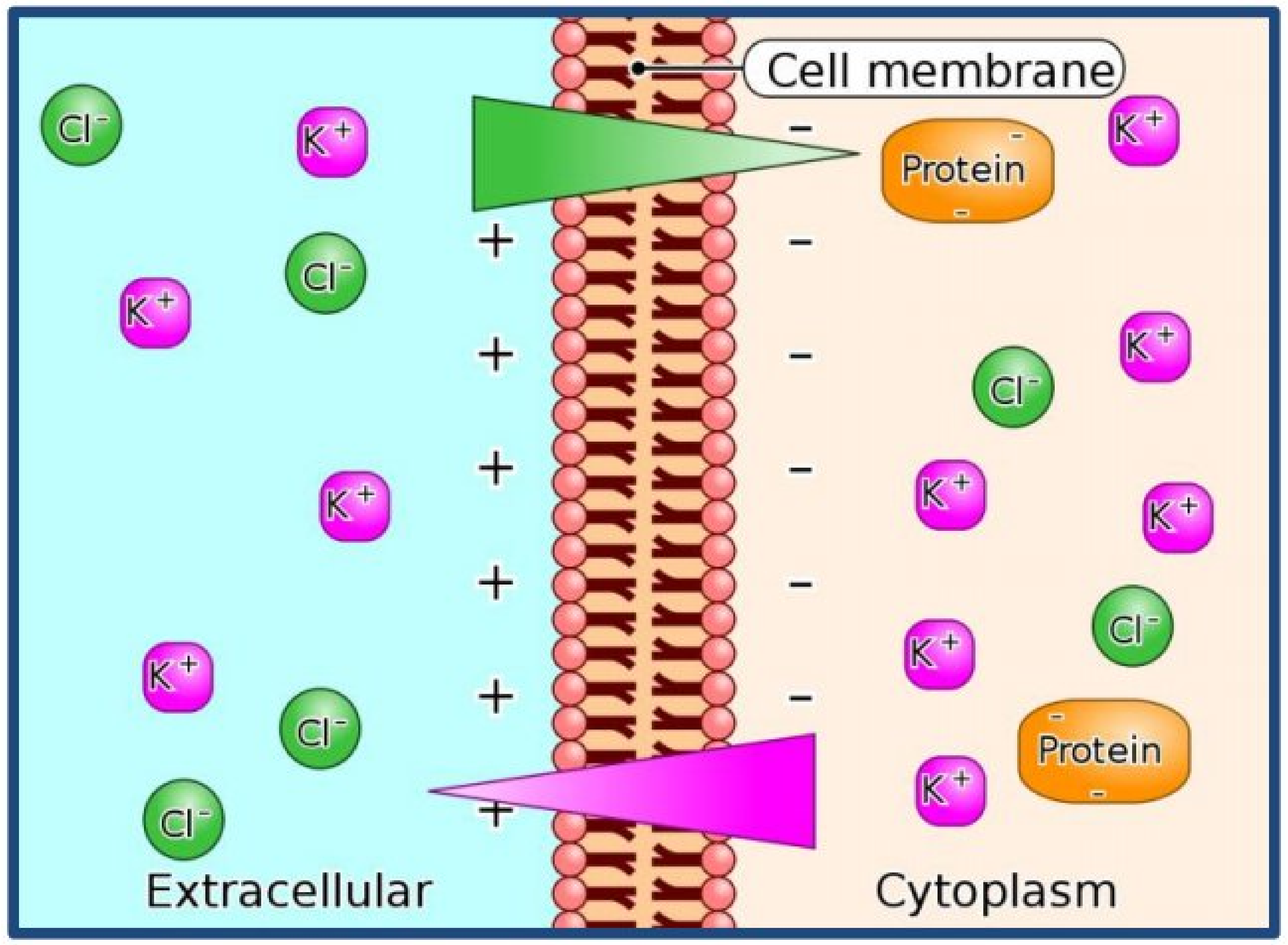
Structure of Biological Membranes & Transport Across Membranes and Donnan Membrane Equilibrium



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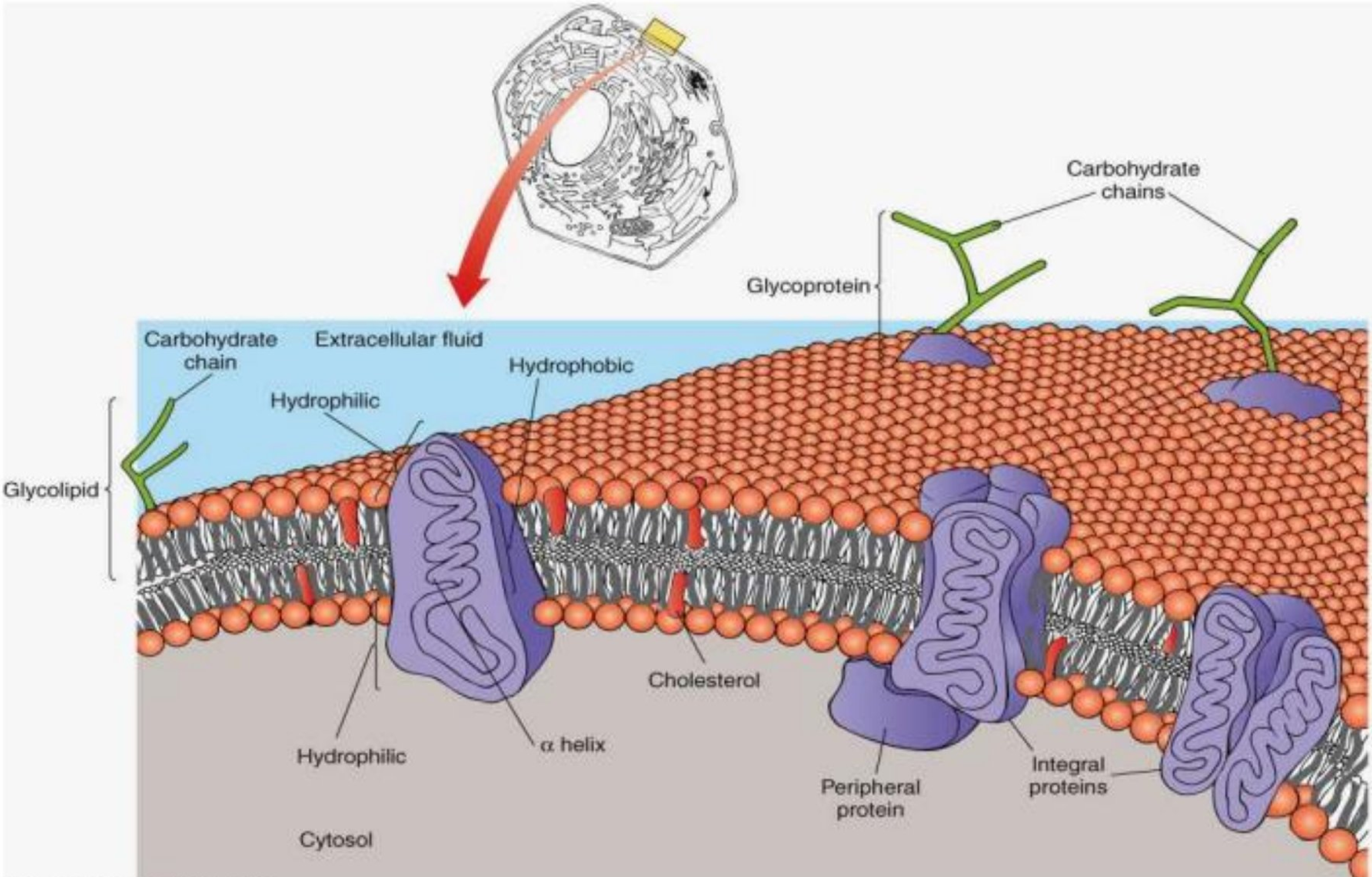
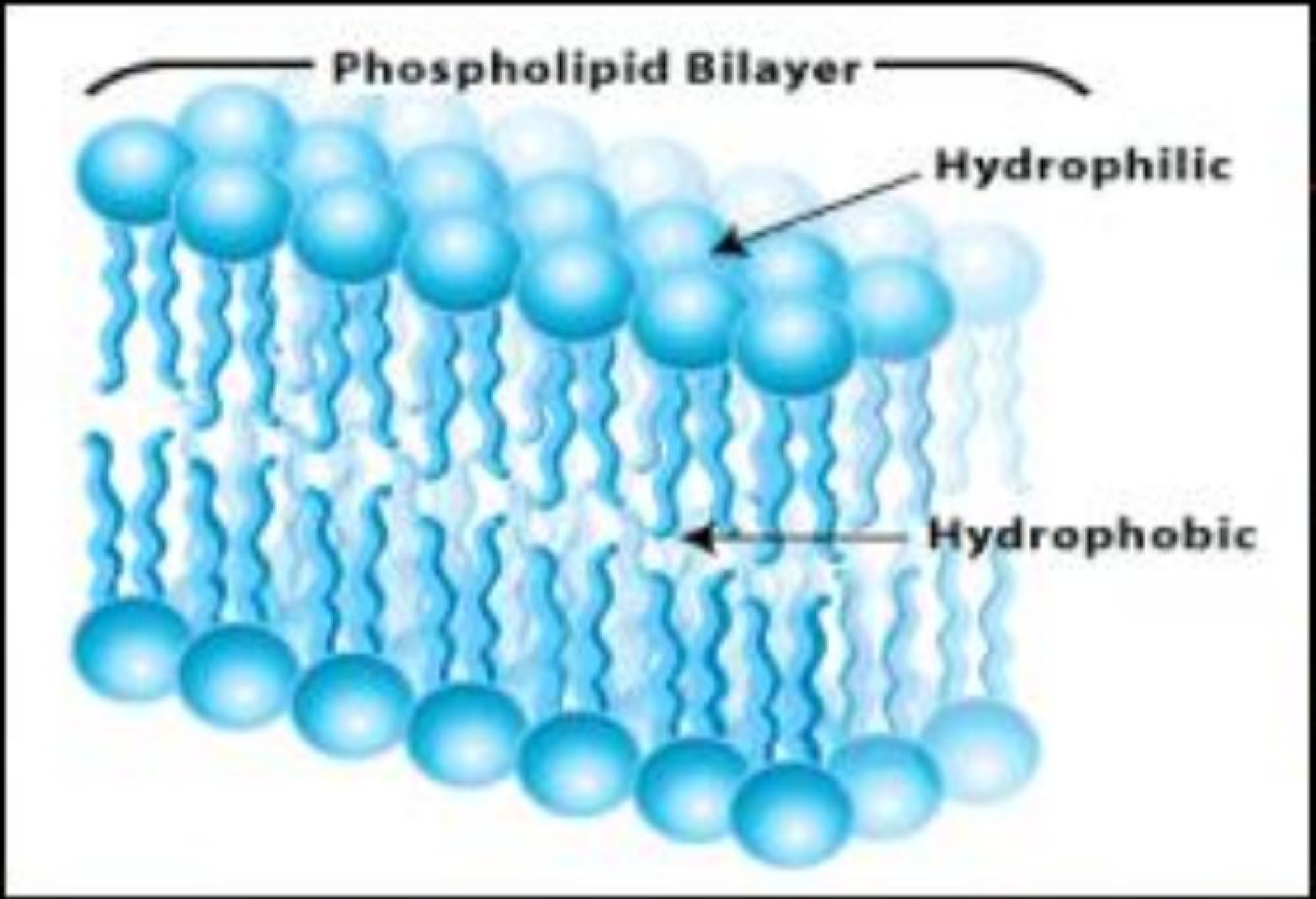
Structure of Biological Membranes

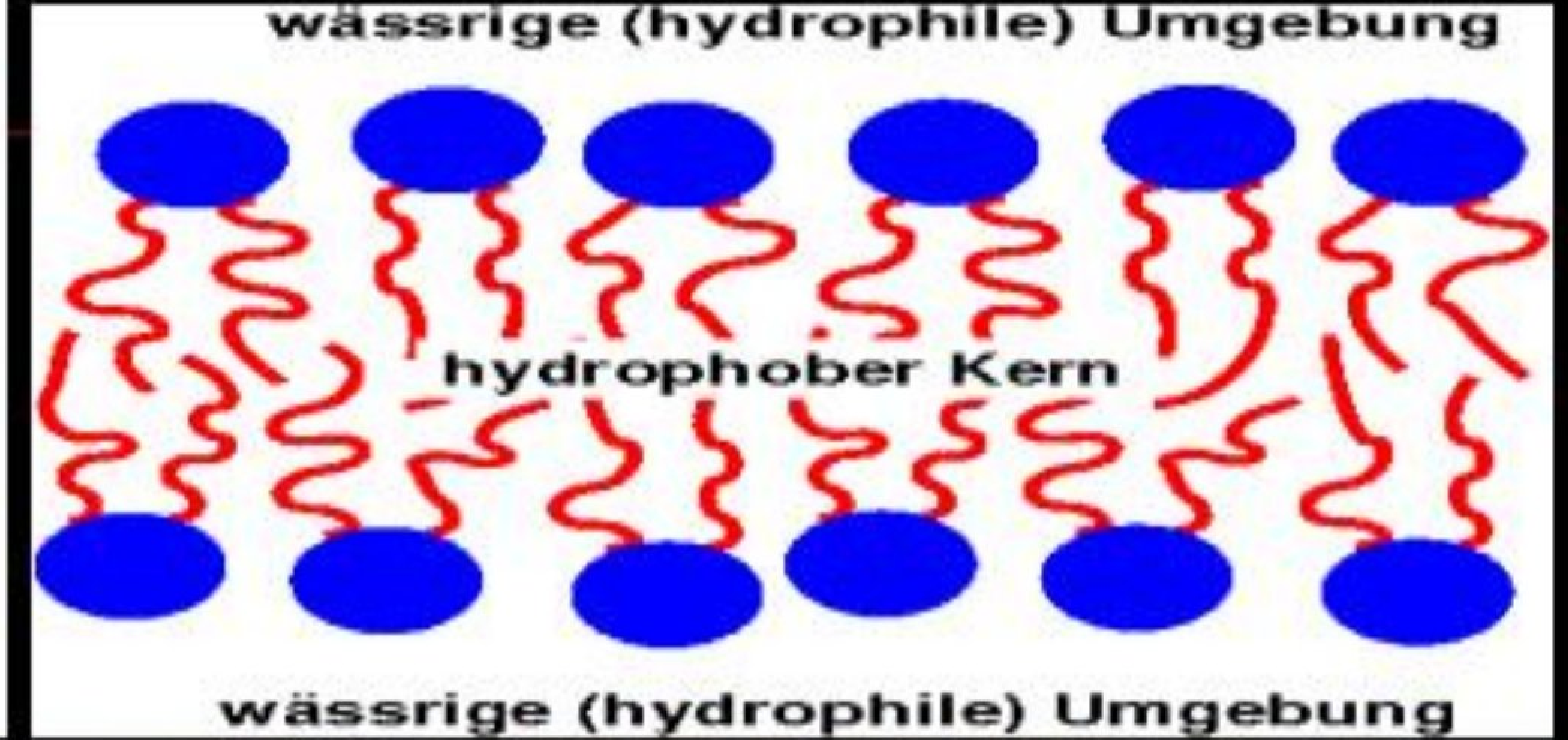
- Biological membranes are **thin sheet** like structure composed of **lipids, proteins and carbohydrates.**
- The ratio of proteins to lipids varies between different types of membranes. **Generally 40-50% proteins are present**. Myelin sheath has 80% lipids and 20% proteins. The inner membrane of mitochondria has 80% proteins and 20% lipids.
- Some membrane contains **carbohydrates up to 10%.** It is found on the external surface of the plasma membrane. They are attached to either **proteins as glycoproteins and lipids as glycolipids**.
- Many animal cell membranes have thick coating of complex polysaccharides referred to as **glycocalyx**.
- **Three main groups of amphipathic lipids are foun**d in the membrane viz **phospholipids, sphingolipids & cholesterol** in animal membranes**.**

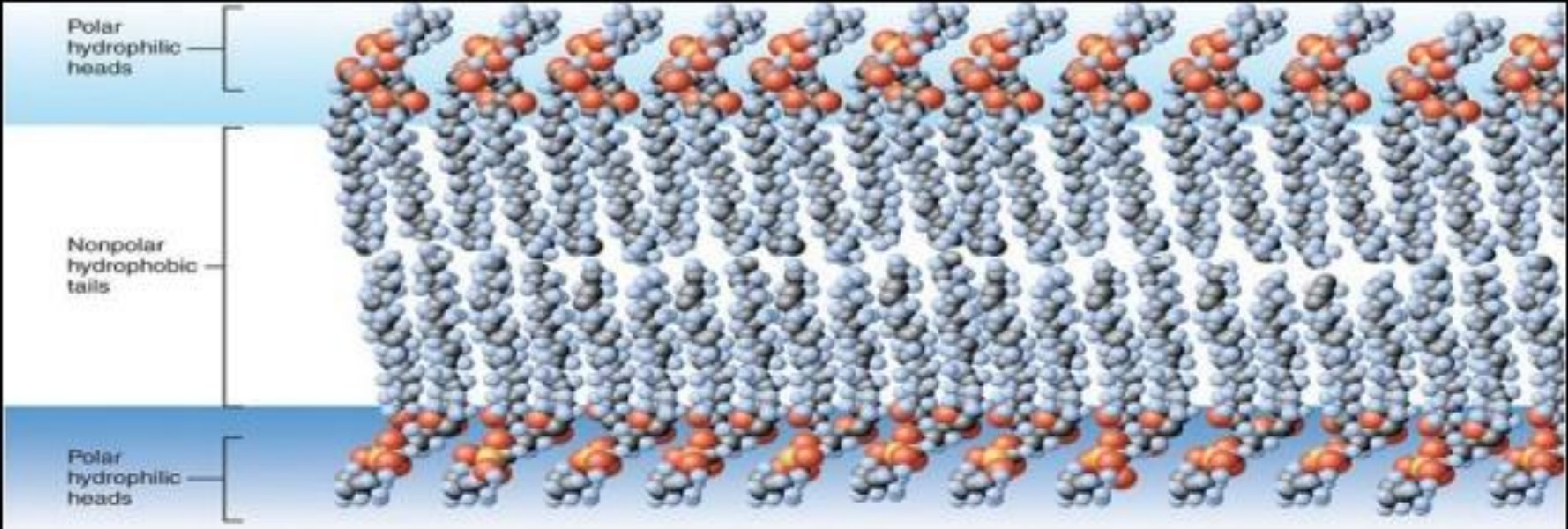
Detailed structure of the plasma membrane



Lipid bi-layer







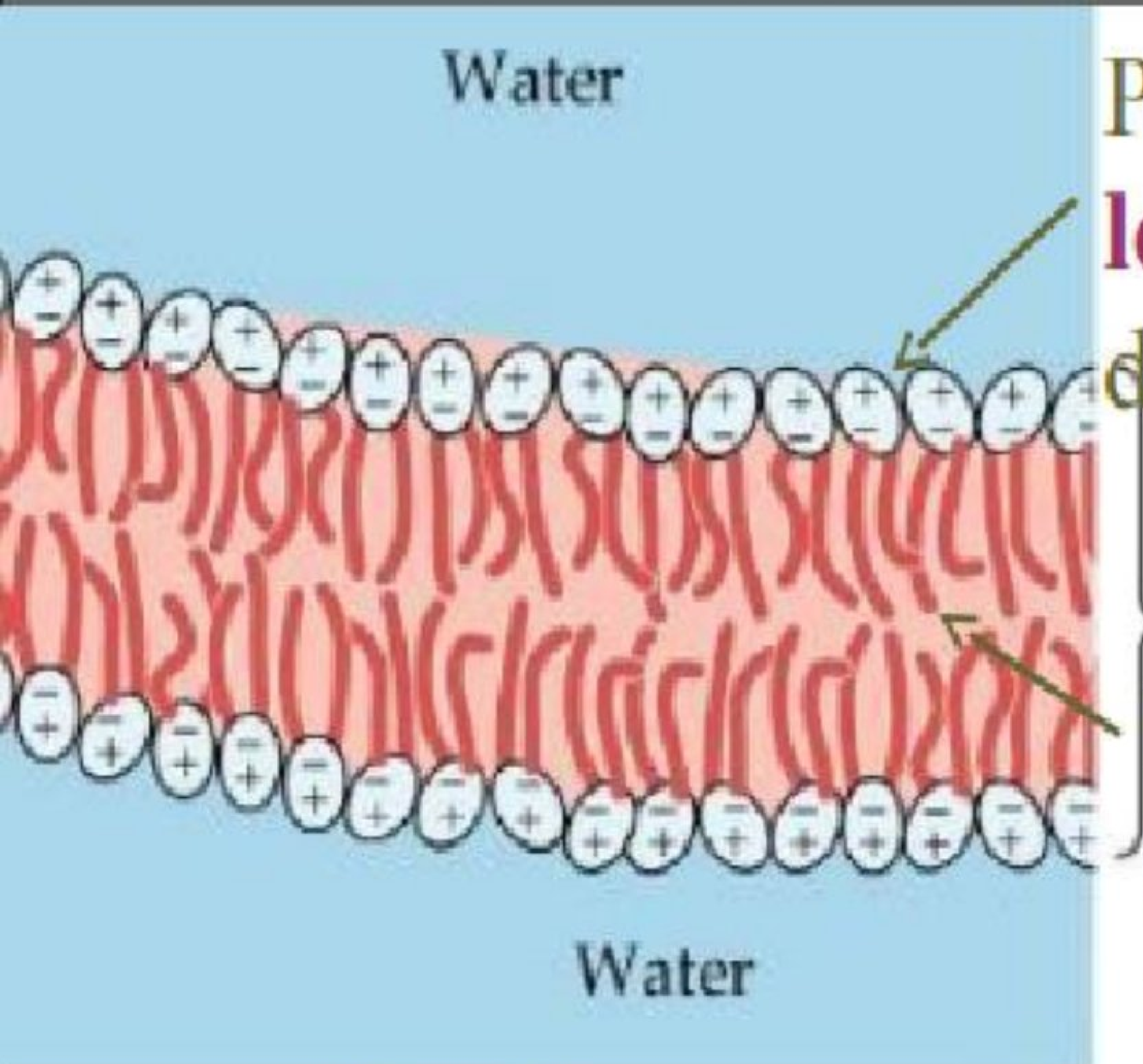
Fluid Mosaic Model

- A lipid bilayer model originally proposed for membrane structure in 1935 by Davson and Danielle has been modified. In 1972, S.J. Singer & G. Nicolson proposed that membrane **proteins are inserted** into the phospholipids bilayer.
- Fluid mosaic model proposed by **Singer and Nicolson** is a more recent and acceptable model for membrane structure.
- The biological membrane usually have a thickness of 5 – 8 nm. A membrane is essentially composed of a **lipid bilayer**.
- The **hydrophobic (nonpolar)** region of the lipids **(Fatty acid tails)** face each other at core of the bilayer while the **hydrophilic (polar)** region **(Phosphate group head)** face outward.

Fluid Mosaic Model

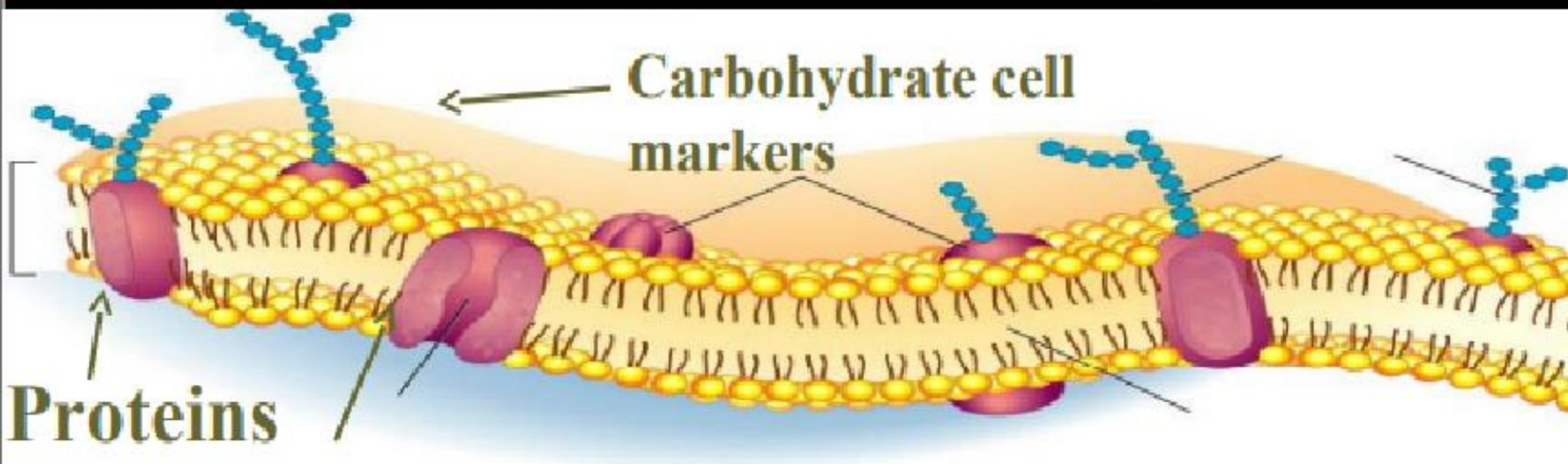
- **Extrinsic/Peripheral proteins membrane proteins** are **loosely** held to the surface of the membrane and they can be **easily separated** by mild treatment with solutions of high ionic strength e.g. cytochrome c of mitochondria.
- **Intrinsic/Integral membrane proteins (transmembrane proteins)** are **tightly** bound to the lipid bilayer and they can be separated only by the use of **powerful detergent or organic solvents** e.g. hormone receptors. These membranes contains high portions of hydrophobic amino acids, which can interact with hydrophobic regions of lipid bilayer.
- The membrane is asymmetric due to the irregular distribution of proteins.
- **Functions of membrane proteins** :
  - ❖ Localization of enzyme activity
  - ❖ Energy transduction
  - ❖ Facilitated transport
  - ❖ Receiving of extracellular signal

Fluid Mosaic Model of the cell membrane



Polar heads **love water** & dissolve.

Non-polar tails **hide from water**.



Functions of membranes

- It is the boundary between the cell interior and exterior and maintains the shape of the cell.
- It separates and protects the cell from the external environment.
- Transport of substances in and out of the cell. The lipid bilayer prevents the passage of hydrophilic ions and molecules. Controls what enters and exits the cell to maintain an internal balance called **homeostasis.**
- Cell-cell interaction.
- Signal transduction.

Fluidity of membrane

- The **degree of motion of the hydrocarbon chain within the lipid bilayer is called fluidity**. Several **factors** influence the fluidity in membranes.
- Long chain **Saturated fatty acids decrease** fluidity and permeability of membrane. They have straight tail, so that they are packed tightly.
- **Unsaturated fatty acids** present in the membrane phospholipids **increase** the fluidity and permeability of membrane. Greater the number of double bonds greater will be the fluidity and permeability. Unsaturated fatty acids exist in the **cis** form in the membrane, which makes kinked (twisted) tails. Membrane made of more unsaturated fatty acids has more kinks; hence, they are not highly packed and showing fluid nature.
- Presence of **cholesterol** prevents the movements of fatty acids and **reduces** fluidity. It prevents close packing of the hydrocarbon chains and thereby lowers the melting point
- Fluidity increase with **increase in temperature.**

Transport across the membranes

- The plasma membrane of the living cell not only covers the cytoplasm but also controls the **transport of water and solutes between the external and internal environments.**
- For the cell to survive it takes up the nutrients from outside and release the internal substances outside. This process occurs by means of transport either through cell membrane or through proteins in the lipid bilayer.
- It is highly selective filter, permits nutrients and leaves the waste products from the cell **(Selective permeability).**
- This transport process can be divided into two types -

1. Passive Transport
2. Active transport

TRANSPORT MECHANISMS

TRANSPORT

Active process

- Primary Transport
- Secondary Transport

Passive process

- Simple diffusion
- Facilitated diffusion
- Osmosis
- Bulk flow

Passive Transport

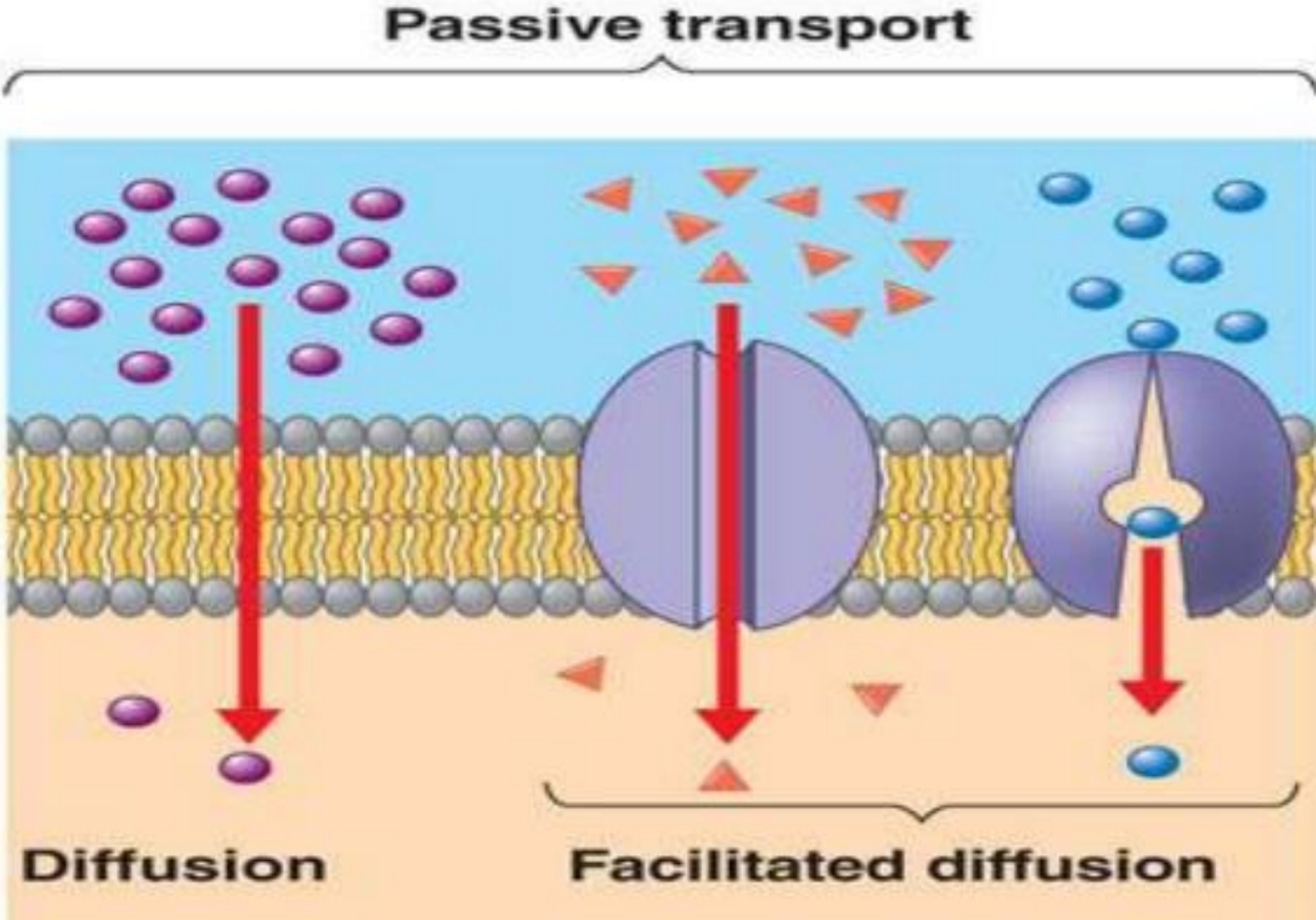
- Movements of molecules across the cell membrane **in response to a concentration gradient (downhill process)**.
- This type of transport may or may not be mediated by protein.
- The process requires **no metabolic energy.**
- Passive transport is of two types:
    - ❖ Simple diffusion
    - ❖ Facilitated diffusion

Simple Diffusion

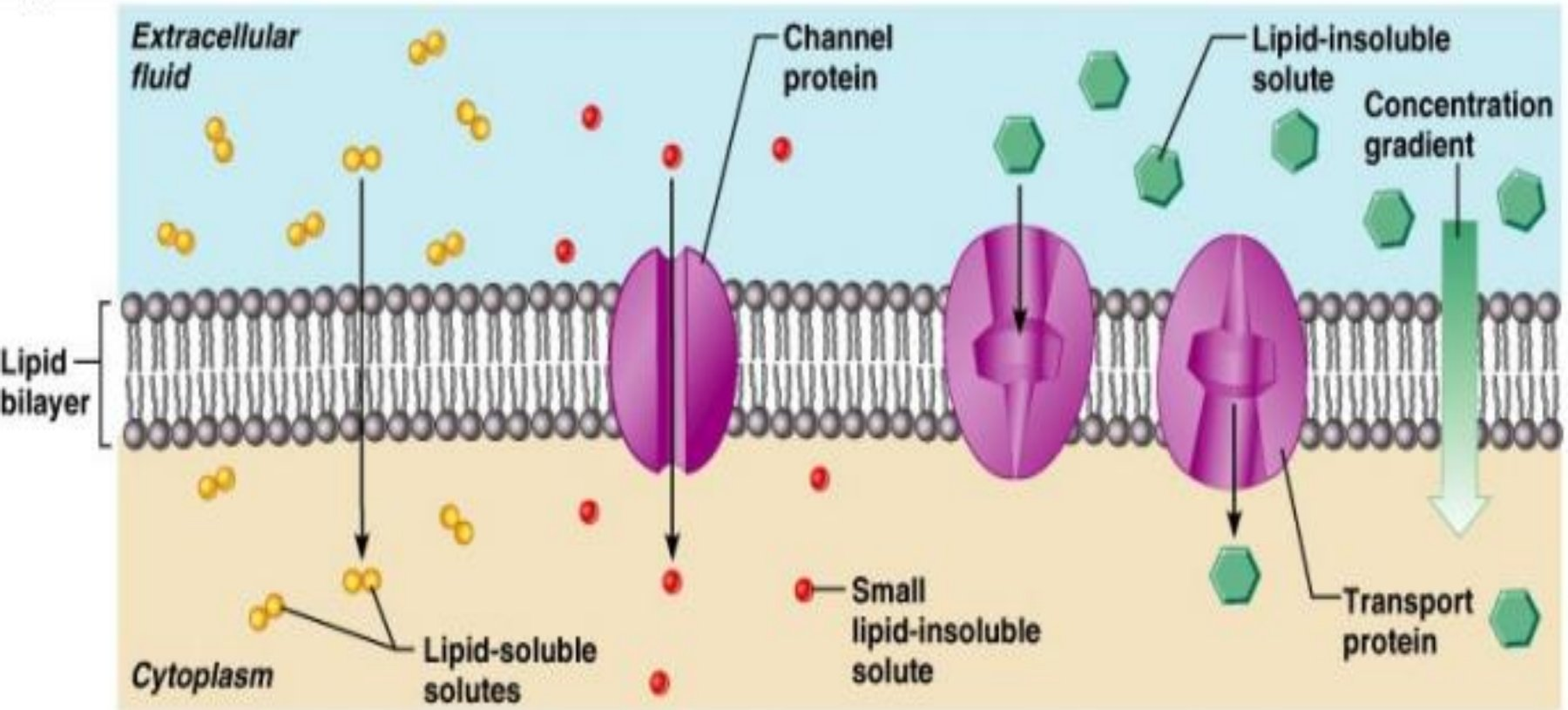
- A net movement (flux) of the solute from the region of **higher concentration to one of lower concentration is called as diffusion.** Diffusion occurs as **downhill process**. Higher the difference in concentration between two systems, the more rapid the rate of diffusion.
- Diffusion occurs either directly through the membrane or through membrane channels or through pores.
- Diffusion of molecules across the membrane depends on **charge, size and lipid solubility of the substan**ce. Smaller the molecules, greater the rate of diffusion and more the lipid solubility, more the rate of diffusion.
- Diffusion of ions depends on the **charge and concentration of the ions** inside and outside the membrane. Small, uncharged molecules (such as O_2, CO_2 and H_2O) and lipid soluble substances (Such as steroid hormones) cross membranes by simple diffusion.
- It does not exhibit saturation kinetics.

Facilitated Diffusion

- In facilitated diffusion, the movement of solute occurs through **a carrier protein known as transporters also known as translocase, permeases** .
- It is **faster than simple diffusion**. The process does **not require expenditure of energy**.
- This exhibits **saturation kinetics**.
- Specific carrier protein for the transport of glucose, galactose, leucine, phenylalanine etc. have been isolated and characterized.


Passive transport
Diffusion
Facilitated diffusion

Diffusion Through the Plasma Membrane

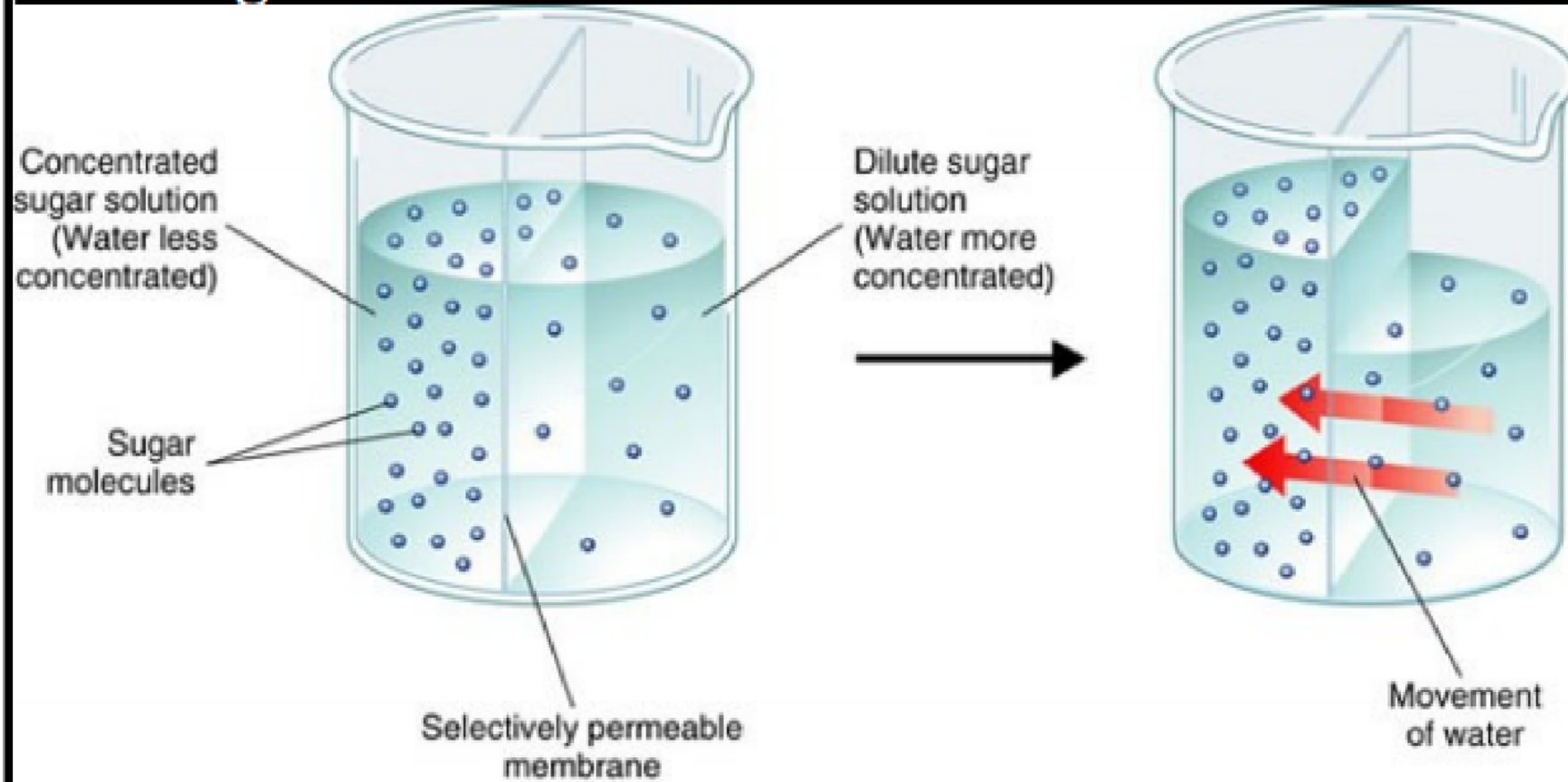


Biological Significance of Diffusion

- Absorption of **glucose** from the intestine into blood.
- **Exchange of oxygen and carbon dioxide** between air and blood.
- Exchange of anions like **chloride (Cl^-) for bicarbonate (HCO_3^-)** in red blood cells.
- Conduction of **nerve impulses** depends on the passive diffusion of ions.
- Transport of a substance into a cell by **facilitated diffusion** is an example of **uniport,** in which there is a transport of only single molecule in one direction. For example: glucose is transported into erythrocytes by a uniport mechanism.
- Another important **passive transport** system is **gated pores or channels**. These transport system is for specific ions and small molecules (pore is used for bacteria and channel is used for animals). These gates or channels open and close on receipt of a signal.

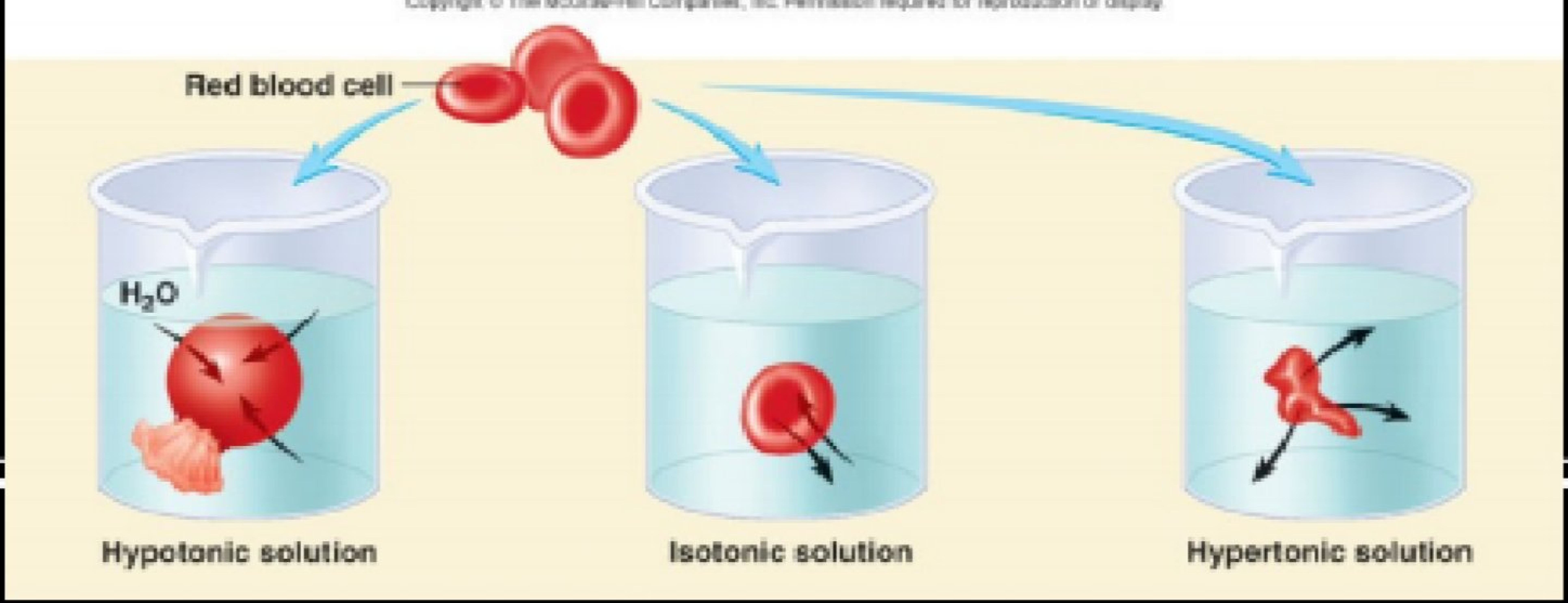
OSMOSIS

Osmosis is the process of moving water across a semi permeable membrane towards ion or solute rich region in a solution



Osmosis and Cells

- Important because large volume changes caused by water movement disrupt normal cell function
- Cell shrinkage or swelling
  - **Isotonic**: cell neither shrinks nor swells
  - **Hypertonic**: cell shrinks (crenation)
  - **Hypotonic**: cell swells (lysis)



Active Transport

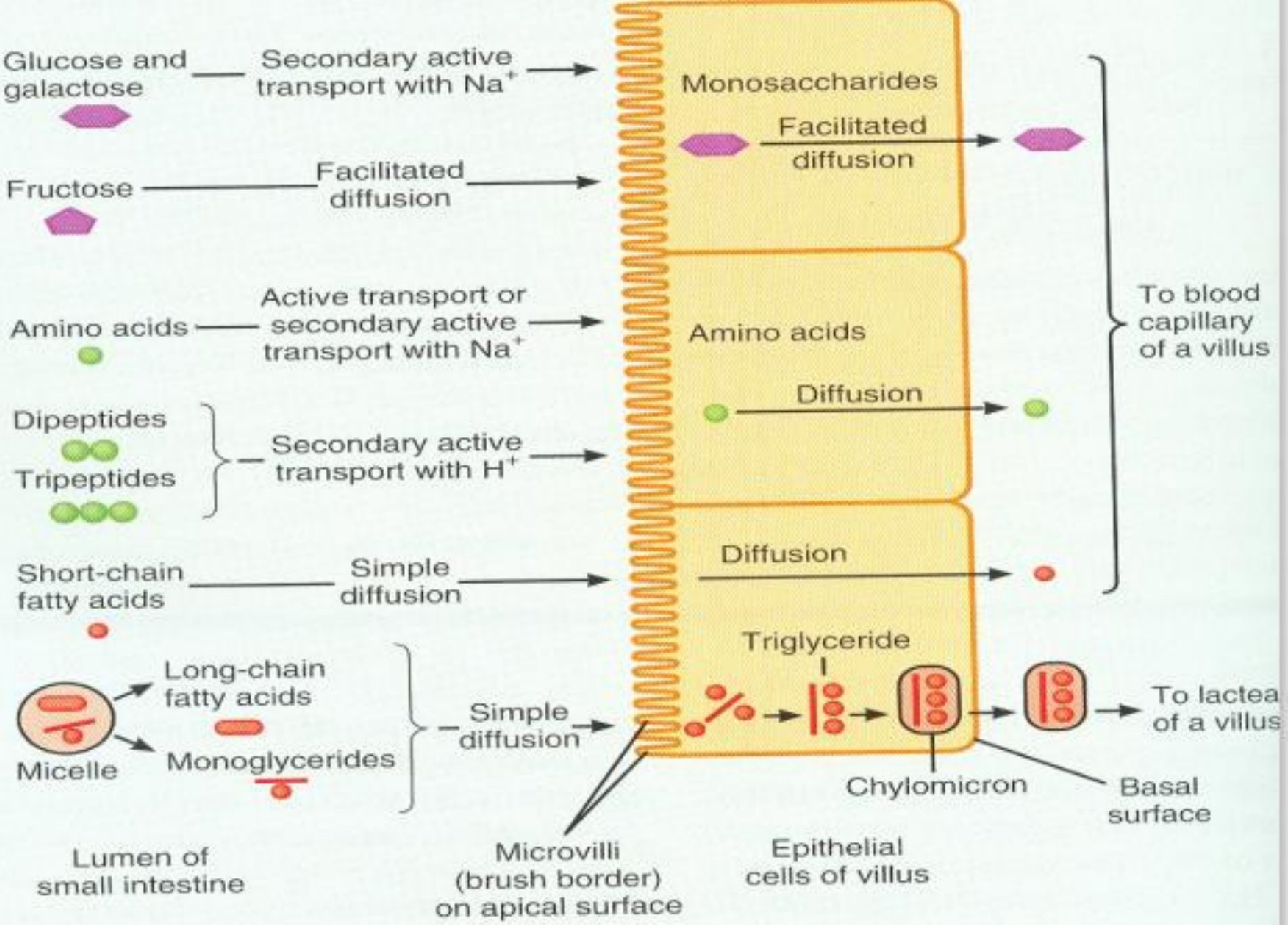
- In active transport, the **cell uses energy** (ATP) to transport the substances. This process requires **transport proteins.**
- The solutes are usually transported from an area of **lesser solute concentration to greater solute concentration**. Hence, this process is an **Uphill Process.**

Primary Active Transport

- The most important primary Active transport systems are ion pumps. It is powered by direct source of energy.
- The transport of **sodium and potassium** by sodium/potassium ATPase in the plasma membrane is an example. ATP provides energy for the transport. The concentration of sodium in the out side of the cell is higher and that in the interior of the cell is lower. It is responsible for maintaining the high K+ and low Na+ concentration inside the cell. **Ouabain inhibit Na+ -K+ ATPase pump**.
- Osmotic regulation (Acid – Base balance). Further Na+ and K+ gradients across plasma membrane are needed for the transmission of nerve impulses.

Secondary Active Transport

- It is driven by the concentration gradient. for example sodium moves into the cell where its concentration is lower, which facilitates the transport of amino acids or glucose into the cells.
- When two substances are **co-transported** from one side of the membrane to the other such as sodium and amino acid and sodium and glucose, the process is called **symport (same direction).**
- When the transport of a substance in one direction is coupled to the transport of another substance (**co-transport**) in the opposite direction the process is called **antiport.** For example: the transport of Cl^- and HCO_3^- and Exchange of H+ and Na+ in Renal tubule.



(a) Mechanisms for movement of nutrients through epithelial cells of the villi

Transport of Macromolecules/ Vesicular Transport

- **Endocytosis and Exocytosis**
- Cells need to import and export larger molecules that could not be transported via pores, channels or transport proteins. In eukaryotes, proteins and some other large substances are transported into and out of the cell by endocytosis and exocytosis respectively.
- In both cases transport is by the formation of specialized type of **lipid vesicles.**

Endocytosis

- It is the process by which **macromolecules** are engulfed by plasma membrane and brought into the cell within lipid vesicles.
- The receptor mediated endocytosis begins with the binding of macromolecules to specific receptor proteins in the plasma membrane of the cell. The membrane then invaginates forming a vesicle that contains the bound molecules. It fuses with the lysosome, which contains the hydrolytic enzymes. Inside of lysosome the endocytosed material and the receptor may be degraded.
- It is estimated that approximately 2% of the exterior surface of plasma membrane possesses characteristic **Coated-pits .**
- The pits can be internalized to form coated vesicles which contain an unusual protein called **Clathrin. (Receptor Mediated Endocytosis).**
- The uptake of LDL molecules by the cells is a good example of endocytosis.

Endocytosis

- There are two types of endocytosis - **Phagocytosis & Pinocytosis**
- **Phagocytosis (cell eating)-** involves the ingestion of large particles such as virus, bacteria, cells, or debris. Phagocytosis occurs only in specialized cells such as macrophages and granulocytes.
- **Pinocytosis (cell drinking)-** If the contents are liquid and smaller (protein) in size then the process is called **Pinocytosis (cell drinking).**

Exocytosis

- It is similar to endocytosis but the direction of transport is opposite. The release of macromolecules to the outside of the cells mostly occurs via the participation of Golgi apparatus.
- During exocytosis the materials to be secreted from the cell are enclosed in a vesicle, the vesicles then fuse with the plasma membrane releasing the vesicles contents into the extracellular space.
- The zymogens of **digestive enzymes** are exported from the pancreatic cell in this manner.
- The secretion of hormone e.g. Insulin usually occur by Exocytosis.

Active transport	Passive transport
Energy is utilised	No Energy is utilised
Movement of ions takes place against conc. gradient	Movement of ions takes place favouring conc. gradient
Specific carrier is required	No carrier is required
Cellular respiratory rate is ↑	No change
Enzymes are involved	No Enzymes are involved

Diseases due to loss of Membrane Transport Systems

- **Hartnup's disease** due to a decrease in the transport of neutral amino acids in the intestinal cells and renal tubules.
- **Cystinuria** characterized by increased excretion of cystine, lysine, arginine and ornithine. This results in the formation of renal cystine stones.
- **Decreased glucose uptake** in some individuals due to lack of the specific sodium-glucose transporter.
- **Renal reabsorption of phosphate** is decreased in vitamin D resistant rickets.

GIBBS-DONNAN'S MEMBRANE EQUILIBRIUM

- When membrane is freely permeable to ions (Na^+, Cl^-) and if the concentration of ions on both the sides is different, the ions freely diffuse to attain equal concentration.
- Gibbs-Donnan observed that the presence of a non-diffusible ion on one side of the membrane alters the diffusion of diffusible ions.
- The Gibbs-Donnan effect describes the unequal distribution of diffusible ions on either side of a semipermeable membrane or between the compartments which occurs in the presence of non-diffusible ions.

Consider the following Experiment

- The compartment A contains a solution of sodium Proteinate in which Na exist as Na^+ and protein as Pr^-.
- Sodium salt of protein is a colloidal solution, the proteinate PR^- is colloidal and is <u>not diffusible</u> through the membrane.
- The compartment B contains a solution of NaCl, in which both Na^+ and Cl^- are <u>diffusible</u>.

A	B
(90) Na^+	(90) Na^+
(90) Pr^-	(90) Cl^-

Membrane

- According to Donnan effect, the non-diffusible ion or ions on one side of the membrane influences the diffusion of diffusible ions, and both quality and quantity of diffusible ions will be influenced.
- In above situation, Na^+ ions can diffusible either way **but Cl^- ions can diffuse only to the left, i.e. to 'A' compartment containing non-diffusible Pr^- whereas Pr^- can not diffuse at all.**

- After equilibrium is attained, the following will be the situation in both compartments.
- In the side 'A', there will be more Na^+, as Na^+ ions have to balance now in addition to the existing non-diffusible Pr^-, the newly entered Cl^- to maintain electrical neutrality.
- On the other hand, in side 'B', Na^+ has to balance only Cl^- which are remaining after diffusion to 'A' side.
- Hence the concentration of Na^+ inside 'A' will be greater than that of Na^+ inside 'B' (some Na^+ will diffuse from 'B' to 'A').
  - Na^+ (A) > Na^+ (B)

A	B
(120)Na^+	(60) Na^+
(90) Pr^-	(60) Cl^-
Cl^- (30)	

Membrane

At equilibrium, the product of diffusible ions on either side of the membrane will be equal.

- **Thus, total ionic concentration inside (A) will be much greater than side (B).**
- The concentration of Cl^- ions in (A) should be much less than inside (B), i. e. Cl^- (A) < Cl^- (B)
- On the other hand, Cl^- inside (B) will be > side (A) i.e. Cl^- (B) > Cl^- (A)

Summary

- On the side in which non-diffusible ion is present, there is accumulation of oppositely charged diffusible ions, i.e. Na^+.
- In the other side of the membrane, the non-diffusible ions have made the accumulation of diffusible ions of the same, i.e. Cl^-.
- The total concentration of all the ions will be greater in which the non-diffusible Pr^- is present leading to osmotic imbalance between the two sides.

Summary

- When two solutions containing diffusible and non-diffusible ions are separated by a semipermeable membrane, diffusion takes place towards non-diffusible ions containing side.
- This also reduces the diffusion of like charged ions to that side. As a result, on the side which contains non- diffusible ions, diffusible counter-ions are more concentrated while the like charged diffusible ions concentrate more on the opposite side. **This is called as Gibbs- Donnan effect.**
- However, the total number of cations and anions are equal on both sides at equilibrium.

Biomedical Importance

Donnan's equillibrium has the following effects in the body:

1. Difference in the ionic concentrations of biological fluids / Proteins in Plasma and ISF: The lymph and interstitial fluids have lower concentration of inorganic cations (Na^+, K^+) and higher concentration of anions (Cl^-) compared to plasma. Proteins have much higher Concentration in the plasma than in Interstitial Fluids. This is due to impermeability of capillary walls to proteins. As per the Gibbs- Donnan effect, non-diffusible ions like protein in plasma enhance the outward diffusion of anions like Cl^{-} from blood vessel. This reduces the efflux of diffusible cations like Na^+. Therefore, there is lower concentration of Na^+ and higher concentration of chlorides is found in lymph and ISF as compared to plasma.

ISF/ tissue fluid is a solution that bathes and surrounds the cells of multicellular animals (interstitial spaces). It is the main component of the extracellular fluid, which also includes plasma and transcellular fluid.

Biomedical Importance

2. Osmotic pressure: Due to above, Na^+ ions are held back in plasma and hence increase the osmotic pressure.

3. Concentration of Na^+/ K^+ in renal glomerular filtrate: It is observed that slightly higher Cl^- conc. and slightly lower conc. of Na^+ or K^+ are found in renal glomerular filtrate than in the plasma.

4. Dialysis in renal failure : Donnan membrane equilibrium is the basic principle involved in the artificial means of purifying blood by dialysis in the patients of renal failure.

5. Resting Potential of membrane: Due to Cl^- and Na^+/ K^+ conc. unevenly distributed there is resting trans-membrane potential (-90 mV).

6. Membrane hydrolysis : The relative strength of H^+ and OH^- ions and, therefore, the acidic or alkaline nature on either side of a membrane, is influenced by the presence of non-diffusible ions. This phenomenon is referred to as membrane hydrolysis. Donnan membrane equilibrium explains the greater concentration of H^+ ions in the gastric juice.

Biomedical Importance

7. Concentration of Erythrocyte chlorides: Due to the Gibbs- Donnan effect, it is observed that the Cl^- conc. in erythrocytes is only 1/4th of its plasma conc.

8. pH of RBC: Due to retention of H+ as a result of the Gibbs- Donnan effect, pH of RBC is slightly **lower** that of plasma. Hemoglobin of RBC is negatively charged and, this causes accumulation of positively charged ions including H+. Therefore, the pH of RBC is slightly lower (7.25) than that of plasma (7.4).

9. Chloride shift or Hamburger phenomenon: It is due to the Gibbs- Donnan equilibrium.

10. Electrical Charges: Due to plasma proteins as anions the potential inside the vessel relative to its outside makes plasma slightly electronegative.

THANKS