Toppersnotes
Unleash the topper in you

CSIR-NET

Council of Scientific & Industrial Research

LIFE SCIENCE

VOLUME – 4

CELLULAR ORGANIZATION, CELL COMMUNICATATION & CELL SIGNALING

SIERRA INNOVATIONS

CONTENT

BIOMEMBRANE

All cells and cell organelles are made up of a basic structure that is called as biomembrane.eg. Endoplasmic reticulum and Golgi apparatus. All membranes consist of three substances: - Lipids, Proteins, Carbohydrate.

The ratio of lipids and proteins depends on the function of cell. Every cell has a different combination of protein, lipid and carbohydrate as follows:

S.No.	Membrane of different organelles	Protein	Lipid	Carbohydrate
		(in percentage by weight)		
1.	Mouse liver	42	53	5
2.	Amoeba	53	43	4
3.	Human erythrocyte	48	44	8
4.	Mitochondrial inner membrane	78	22	0
5.	Myelin	19	78	3

The biomembrane is made up of phospholipids. It is impermeable for water-soluble molecules, ions and biological molecules. Membranes are formed by a lipid bilayer of phospholipids. Phospholipids are made up of a polar heads (water) and fatty acid tails (oil). Polar heads are hydrophilic and tails are hydrophobic.

Natural phospholipids fatty acids have one or more double bonds. It causes kink formation in the interior of the membrane and make the hydrocarbon chains difficult to be packed together. The polar head region is variable in charge and fatty acids (R) chains also vary in length from Cię to Czas well as in their degree of unsaturation. i.e. double bond.

Biomembrane Components:

Biomembrane has mainly three components.
(1) Proteins (2) Lipid (3) Carbohydrate

Proteins

All biomembranes consist of proteins, glycolipids steroids and cholesterol. Due to the presence of different types of proteins in biomembranes, the cell may vary in its functions. Membrane receptor proteins transfer the signals between the cells internal and external environments. Transport proteins involve in transport of molecules across the membrane. Many types of proteins are present in membrane. Some are attached to the surface of membrane and many proteins are embedded in the membrane, that's why the cell membrane is not smooth, it seems to be rough. Membrane enzymes may play a crucial roles in many cellular activities like oxidoreductase, transferase or hydrolase. Cell adhesion molecules gives a platform

to the cells to identify and interact with each other. For eg. Protein involved in immune response. There are mainly four types of proteins, found in the biomembrane.

(A) Intrinsic or Integral protein
(B) Extrinsic or Peripheral protein
(C) Protein anchor proteins
(D) Lipid anchor proteins

Integral Proteins:

These proteins are embedded in the membrane. These are attached permanently in a bio membrane. These can be separated only by using nonpolar solvents, denaturing agents and detergents. Integral proteins can be single pass, double pass and multi-pass proteins. The protein which crosses single time a membrane and having a-helix structure is called a single pass. If a proteins a-helix structure passes twice in the membrane, it is called a double pass integral proteins and if a protein passes multiple time in the membrane, it is termed as multi-pass intrinsic protein.

- ***Helix bundle proteins***: Present in all types of biomembranes
- **β-Barrel proteins (Porins):** Porins are beta-barrel proteins that cross a cellular membrane and act as a pore, through which molecules can diffuse. They are large enough to allow passive diffusion. i.e. they act as channels that are specific to different types of molecules. Porins are present in the other membrane of gram negative bacteria such as E.Coli, mitochondria, chloroplast and some gram-positive bacteria of the group Mycoleta.

E.Coli has double membrane, the cell wall (outer membrane) & plasma-membrane (inner membrane). The outer membrane are more permeable than inner due to this porins channels, which form aqueous channels through the lipid bilayer. The outer membrane protect the intestinal bacterium from harmful agents (eg-antibiotics, bile salts & proteases) but permits the uptake and disposal of small hydrophilic molecules including nutrients and waste products. It also allows passage of disaccharides and other small molecules as well as phosphate.

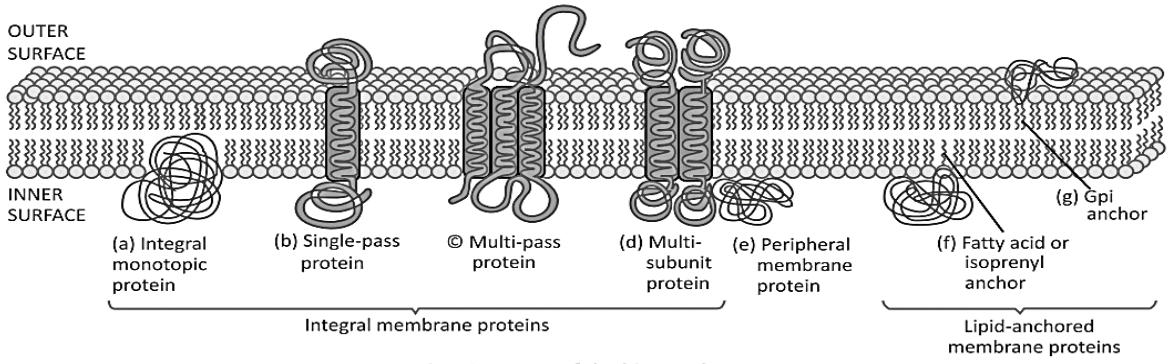


Fig. : Structure of the biomembrane

Integral monotopic proteins:

These proteins attach to only one side of biomembrane and do not span the whole way across.

Other examples of integral proteins are glycophorins and band 3 proteins.

(A) **Glycophorin: -** It is one of the example of integral proteins in red blood cells. It is rich in sialic acid. This acid gives a very hydrophilic charged coat to RBC. This makes them to circulate without disturbing the other cells. These are distributed in 5 types. Glycophorin A, Glycophorin B, Glycophorin Cand Glycophorin E.

(B) **Band - 3 -** It is 14 transmembrane protein. This gene protein is encoded by the SLC4A1 (Solute carrier family 4 member Gene in humans. This is called as Band 3 anion transport protein or anion exchanger 1 (AE1). Many activities like protein anchor, transporter activity, protein and ankyrin binding, inorganic anion exchanger activity, regulation of intracellular pH, chloride transport, sodium transport, anion transport are occured by this protein.

(C) **Peripheral membrane proteins or extrinsic proteins:** These proteins are temporarily attached to the biomembrane or to the integral proteins by a non-covalent, electrostatic or hydrophobic interactions. These can be dissociated by a polar reagent treatment. Integral and peripheral proteins may be post-translationally modified, with the addition of glycosyl phosphotidylinositol (GPI) prenyl chains and fatty acid. Which may be anchored in the lipid bilayer. Examples of these proteins are ankyrin and band 4.1.

(i) **Ankyrin:** Ankyrins are the mediator protein between spectrin and integral protein band 3. Thus it links between the plasma membrane and cytoskeleton. These have binding sites for b-subunit of spectrin and at least 12 families of integral membrane protein. This linkage plays a very important role in the integrity of plasma membrane, ion channels, ion exchangers and ion transporters in the plasma membrane.

(ii) **Band 4.1:** This protein is associated with the cytoskeleton and encoded by the EPB41 gene in the human. This protein plays a very crucial role in maintaining the structure and skeleton of erythrocytes.

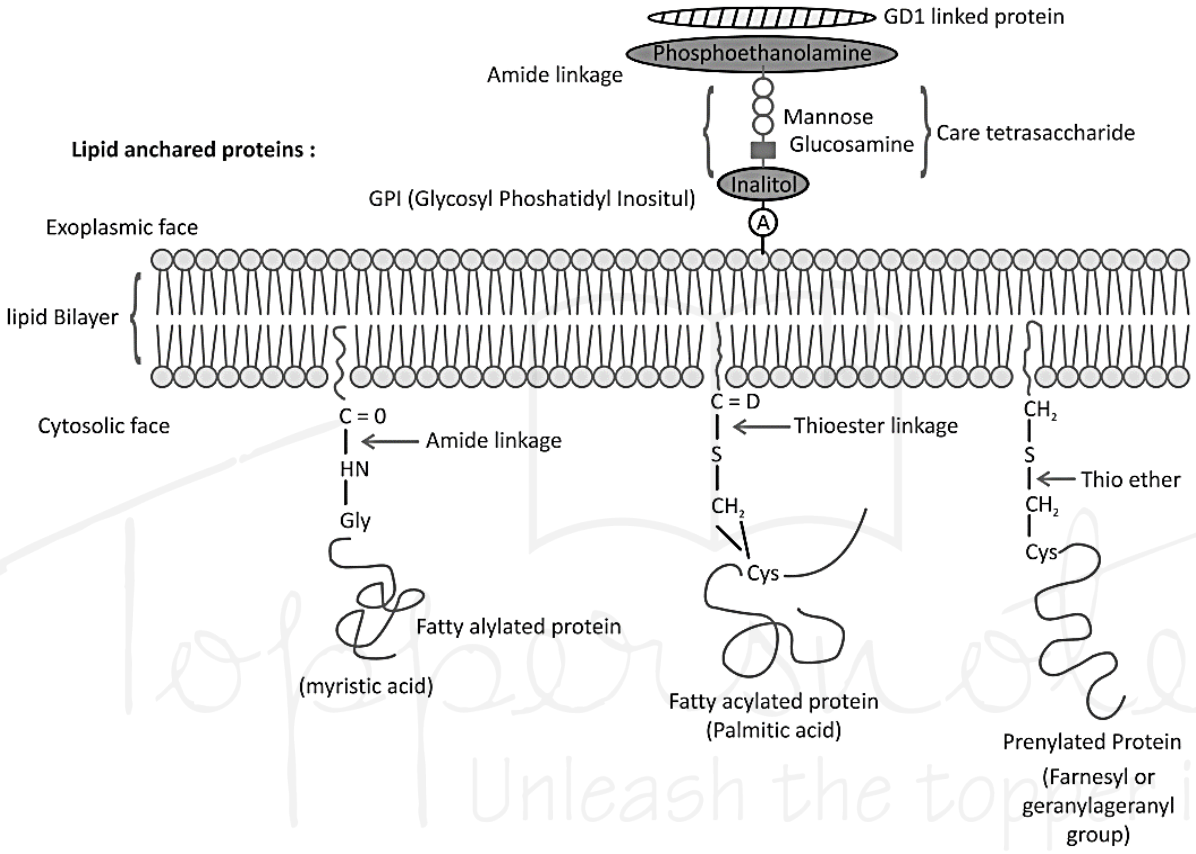
Protein anchor proteins:-

These type of proteins are involved in Apoptosis. These are water-soluble proteins but these can aggregate and associate irreversibly with the lipid bilayer and become reversibly or irreversibly membrane associated.

Lipid-anchor proteins:

LAP is also known as lipid-linked proteins. They are located on the surface of the cell membrane. They are covalently attached to lipids. These proteins are embedded

within the cell membrane. These proteins are linked to a certain fatty acid like myristate and palmitate. These anchors the protein to the cell membrane this protein to the cell membrane. These play an important role in protein functions. Lipids The involvement of phospholipids, cholesterol and proteins occurs in the fluidity of the membrane. Lipid packaging and its components influence the fluidity of the membrane. The fluidity of the membrane is crucial for membrane functions. The membrane behaves as two-dimensional fluids.



Membrane proteins which are covalently bound to lipid molecules are lipid linked membrane proteins. They form covalent attachment with thru classes of lipids

Isoprenoid groups
CEx-farnesyl (C_{15})
Geranylgeranyl (C_{20})
↓
Attales to a cys residue at the C-terminal by thioethes linkage

Fatty aeyl group.
(Ex-myristic acid (14c)
Palmitic acid (10c)

→ Myristic acid molecules attaches to a protein through an amide linkage to the α-amino group of an N-terminal gly residue.

→ Palmitic acid molecules attach to a cys residue close to the Nor C terminal

Glycosylated phoephilipid
(Ex-GPI)

→ GPI is denirative of P1 in which insitol have a core tetrasacch axide which inalently bhended to phoxphoethanolamine

→ GP1 attaches to the C-terminal amino acid via amide inkage

Membrane Fluidity

The ratio of saturated and unsaturated fatty acids determines the fluidity in the membrane at cold temperature. Factors affecting the fluidity of biomembrane;

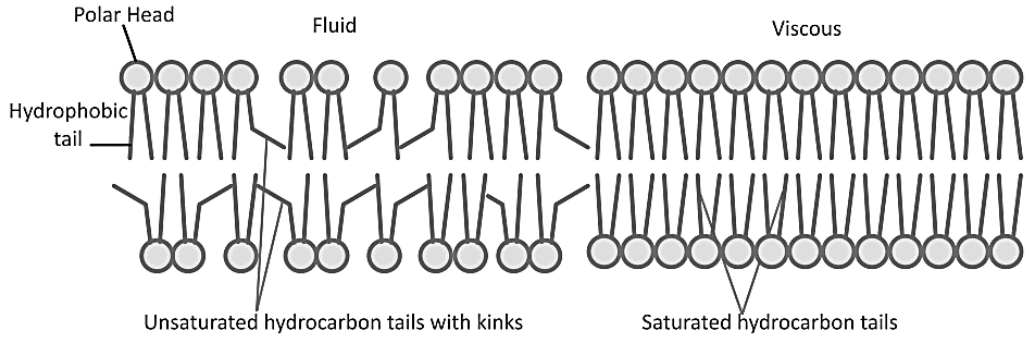


Fig. : Membrane fluidity

(a) Temperature: At low and high temperatures cholesterol increases and decreases the fluidity of membrane respectively. When fluidity increases, it prevent membrane lipids packing close together. Temperature decides what can enter or leave the cell. Cell functions it's best at normal physiological temperature, which is 98.6 degrees Fahrenheit in warm-blooded animals like the human.

(b) Lipid Composition: If saturated fatty acids are compressed by decreasing temperatures, they press in on each other, making a dense rigid membrane. If unsaturated fatty acids are composed, the kinks push adjacent phospholipid molecules away, which helps maintain fluidity in the membrane.

(c) The different form of fatty acid: Transform of fatty acid is more packed than the cis form of fatty acid. Thus trans fatty acid is solid and cis fatty acid is liquid.

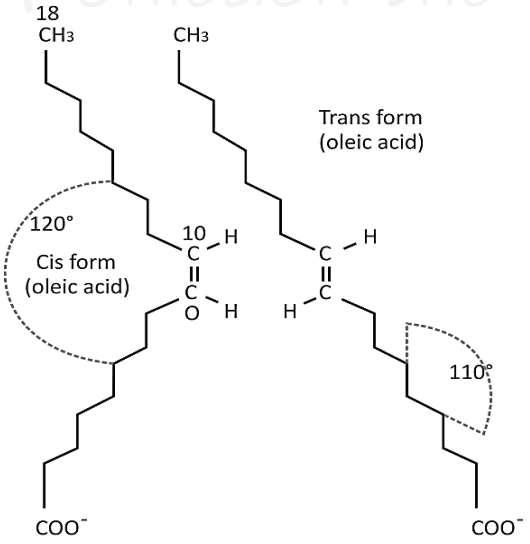


Fig. : Cis and trans form of fatty acid

(d) Cholesterol: Cholesterol serves as a buffer, preventing lower temperatures from inhibiting fluidity and preventing higher temperatures from increasing fluidity. Cholesterol helps to separate the phospholipids in our cell plasma membranes

at the high concentrations so that the chains of fatty acids cannot come together and crystalize. So, cholesterol prevents extremes whether too fluid or too firm in the consistency of the cell membrane.

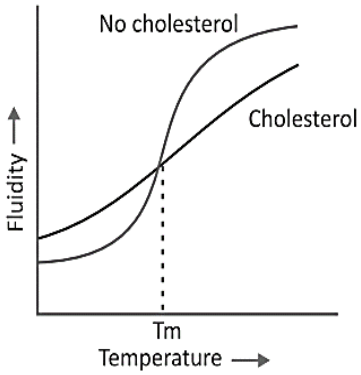


Fig. : Effect of Temperature and Cholesterol on Fluidity

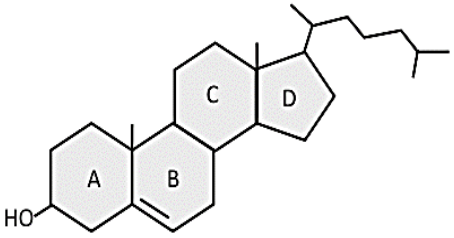


Fig. : Structure of Cholesterol

Lipid Rafts:

Some microdomains of cholesterol and sphingolipids from discrete membrane are known as lipid rafts. These clusters move laterally within the plasma membrane and get associated with specific membrane proteins. Two types of lipid rafts are (1) planar (Non-coveolar) (2) Caveolae. Caveolae are flask-shaped invagination of the plasma membrane that contains caveolin protein. Caveolin is an intrinsic cholesterol binding membrane protein. Although the function of lipid rafts remain to be fully understood, they play an important role in processes such as cell movement (Cholesterol trafficking) and the uptake of extracellular molecules by endocytosis as well as in cell signalling. The lipid composition of a bilayer also influences its thickness which in turn may play a role in localizing proteins to a particular membrane. It is found that sphingomyelin associates into a more gel-like & thicker bilayer than phospholipids do. Similarly, cholesterol and other molecules that decrease membrane fluidity increase membrane thickness. Because Sphingomyelin tails are already optimally stabilized, the extra addition of cholesterol has no effect on the thickness of a sphingomyelin bilayer. Lipid rafts are the centres of organizing for the assembly of signalling molecules, influencing membrane fluidity and membrane protein trafficking and regulating neurotransmission and receptor trafficking lipid rafts are more ordered and tightly packed than it's surrounding structure of biomembrane. Lipid rafts are present in plasma membranes and other parts of cells like Lysosomes and Golgi complex.

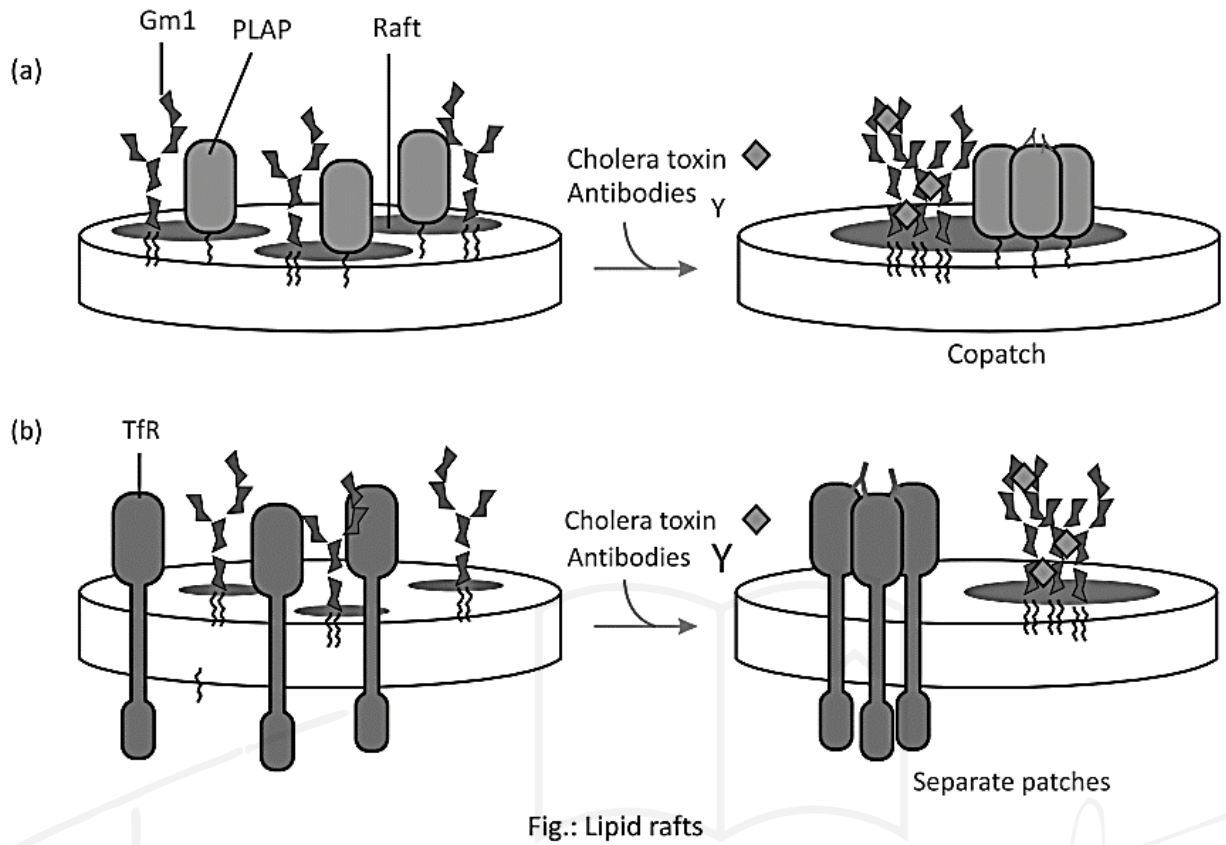


Fig.: Lipid rafts

TRANSPORT ACROSS A CELL MEMBRANE

The transport processes are critically important to all living cells because all living cells necessarily exchange the substances with their surrounding environment like nutrients, wastes, toxicant and the inorganic electrolytes are also able to pass through a living membrane.

Osmosis

Osmosis is the diffusion of water (solvent) molecules from a more dilute solution (high concentration of water) to more concentrated solution(less concentration of water) down the water potential through a semi-permeable membrane. This is a vital physical process found among all biological systems. Osmosis is a spontaneous process.

The pressure required to maintain the equilibrium where the net movement of solvent is zero is known as osmotic pressure. Osmotic pressure depends directly on the molar concentration of the solute. Osmoregulation is done by osmosis process. By osmoregulation organism can homeostat water and salt amounts in the body.

All type of cells maintaining a concentration gradient of all these various metabolites across their functional membranes. Such concentration gradients are

maintained by the membranes as a huge amount of potential energy. For example, Nat and K ions gradient across the membranes regulates the brain function, nerve impulses transmission, muscle contractions and all other normal functions of heart, kidney, liver, stomach and such other organs.

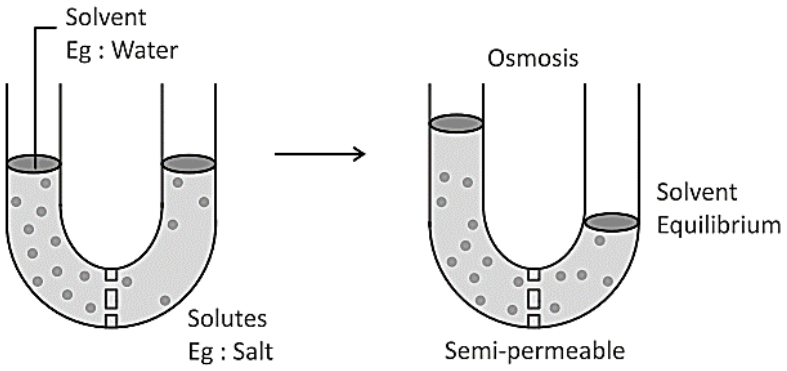


Fig. : Osmosis

The cell membrane is a bilayer of phospholipids that is nearly impermeable to the water-water soluble molecules and ions. But these substances are transported across the membrane at a high rate to fulfil the physiological and metabolic needs of a cell. This critical problem is solved with the help of some membrane's integral proteins that work as transporters. The transporting materials either diffuse through the channel formed by the proteins or they may be carried out or in by the carrier proteins.

The membrane transport processes are of two types based on according to the thermodynamics and the kinetics.
1. Passive transport
2. Active transport

Passive transport

Transport of ions or molecules from high concentration to low concentration without energy expenditure. Now LET'S TALK about why molecules move from high concentration to low concentration. The molecules move because of their own kinetic energy (K.E.=3/2 KT, where T is absolute Temperature). High concentration and high temperature allow more collision between the molecules.

Passive transport is also of two types, the first one is the simple diffusion or the second one is the facilitated diffusion.

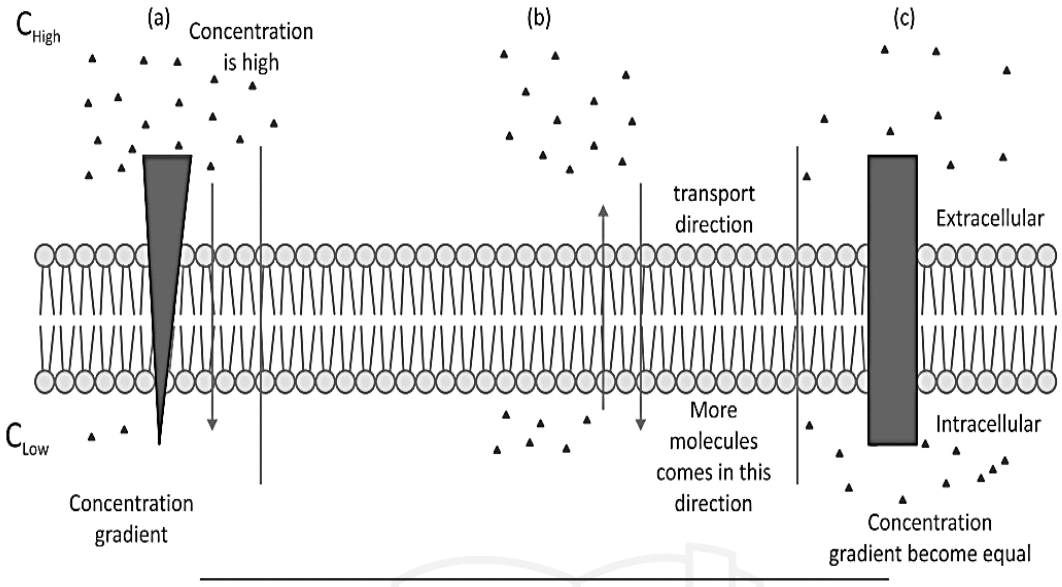


Fig. : Passive transport

Simple diffusion: Simple diffusion is the transport of ions or molecules from high concentration to low concentration without energy expenditure across a membrane of hydrophobic phospholipids and cholesterol bilayer.

This is the simplest transport process that has no need of any protein. Gases like O_2 N_2 and CO_2 and the small uncharged molecules like urea, ethanol can easily move by simple diffusion through the plasma membrane. Because the transportation of such uncharged molecules across the membrane is an entropic process, in which the movement of molecules will be continuous until the concentration of the same molecule will be equal on either side of the membrane.

C_{high} & C_{low} are the concentration of the molecules on the side one and side two respectively of a cell membrane. Here the free energy change on side 1 and side 2 is driven as:

$$\Delta G = -RT \ln \frac{[C_{High}]}{[C_{low}]}$$

$$\Delta G = -2.303\ RT \log \frac{[C_{High}]}{[C_{low}]}$$

The difference in the concentration (C_{high} - C_{low}) is termed as the concentration gradient and the ΔG is the chemical potential difference.

Passive transport of charged molecules

The movement of charged ions depends upon two factors (1) Electric (Voltage) Gradient-The difference in voltage across the membrane (2) Chemical (concentration) Gradient. The difference in concentration across the membrane. In combined form these two forces jointly named as Electrochemical gradient, that determines the energetically favourable direction of transport of a molecule. The movement of charged molecules is somewhat more difficult because the net charge they carry affects the ability of a membrane to permit the transport.

$$\Delta G = -RT \ln \frac{[C_{High}]}{[C_{Low}]} \pm ZF\Delta V$$

$$\Delta G = -2.303\ RT \log \frac{[C_{High}]}{[C_{Low}]} \pm ZF\Delta V$$

Here Z is the charge on the transported species, F is the Faraday's constant (the charge on 1 mole of electrons=96,485 coulombs/mol =96,485 joules/volt mol, because 1 volt = 1 joule/coulomb) and AV is the electric potential difference (in voltage difference) across the membrane.

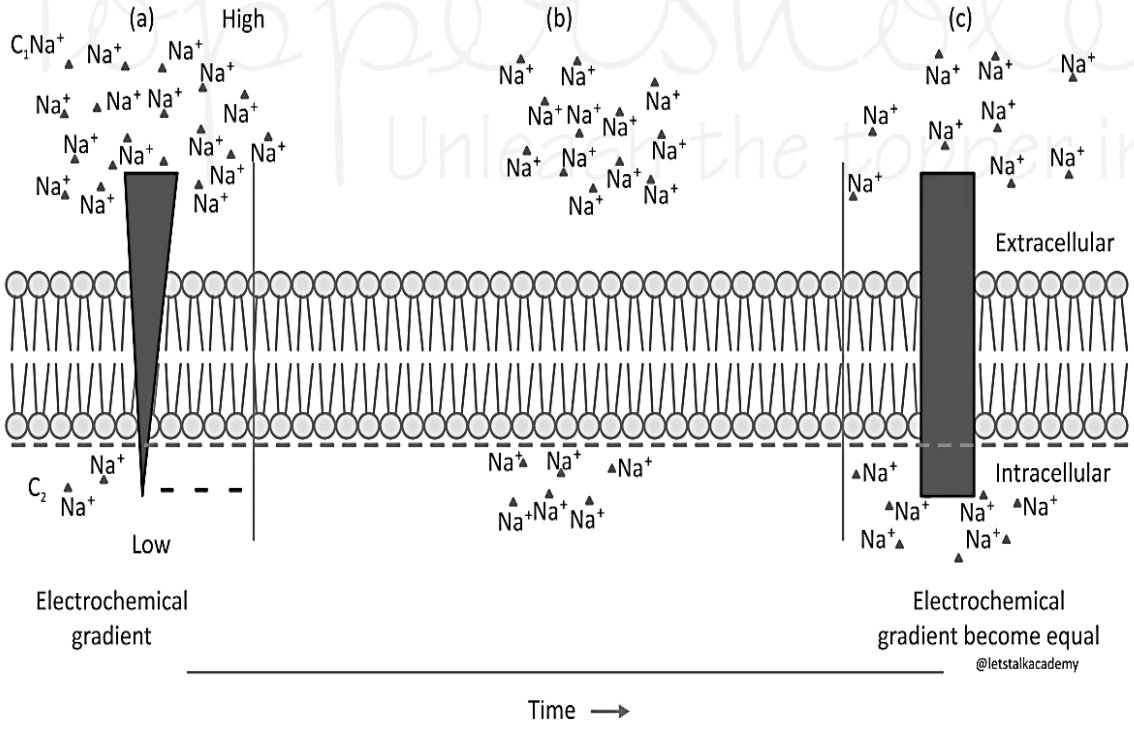


Fig. : Diffusion of ions depends upon electrochemical gradients.

In a case, if the sum of two terms on the right side of the equation is a negative number, then the transport of the ion from side one to side two would occur spontaneously.

Passive transport always depends on the following factors:

1. Substrate concentration gradient across the layer.
2. Hydrophobicity of the molecule to be transported.
3. Size and charge of the molecule.
4. Electric potential across the membrane.
5. Diffusion area and distances.

Hydrophobicity of a substance is measured by its partition coefficient K. K is the equilibrium for its partition between oil and water. The substance with high partition coefficient is more soluble in lipid. For example, diethyl urea having an ethyl group (CH_3CH_2O) attached to each nitrogen atom of urea, has a K of 0.01, whereas urea has a K of 0.0002. Diethyl urea is 50 times (0.01/0.0002) more hydrophobic than urea so it will diffuse approx 50 times faster than urea.

Facilitated Diffusion of uncharged molecule/polar molecule

Facilitated diffusion is a spontaneous process of passive diffusion of a molecule or an ion across living membranes with the help of specific membrane integral proteins, containing the multiple membranes spanning a helices. These proteins have two common features:

(i) They facilitate net movement of solutes only in the thermodynamically favourable situation (that is AG<0).

(ii) They show a measurable affinity and specificity for the transported solute.

These proteins also show the behaviour of saturation regarding their binding with solute molecules that experimentally distinguish the facilitated diffusion with simple diffusion.

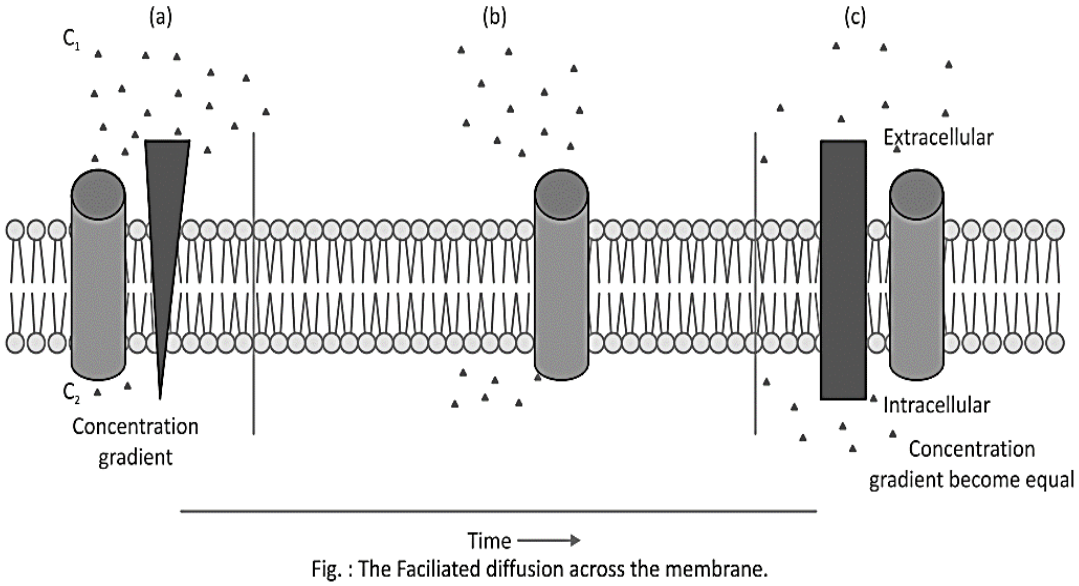


Fig. : The Faciliated diffusion across the membrane.

The curves shown below illustrate the major kinetic difference between simple or facilitated diffusion.

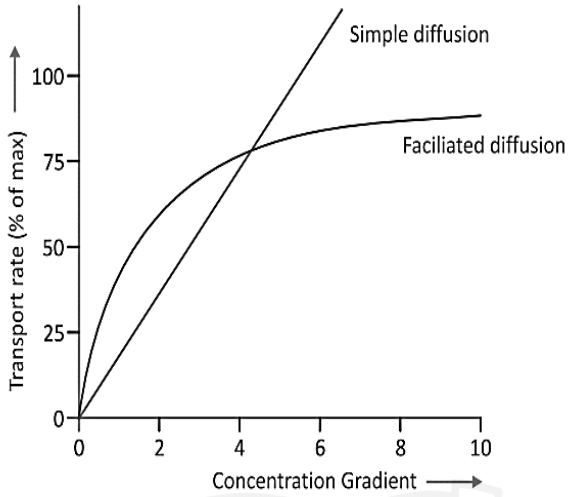


Fig. : Relation between transport rate and concentration gradient for simple diffusion and facilitated diffusion

Many proteins facilitating the diffusion by forming a protein-lined pathway through the membrane. They allow the movement of hydrophilic molecules without their contact with a hydrophobic interior of the membrane.

Transporters

The transporter proteins are also known as the carriers, they move a wide variety of ions or molecules. A carrier protein alternates between two conformations, likewise there are three types of transporters have been identified.

Transporter name	Mode of function	example
Uniporters	Transports a single type of a molecule down its concentration gradient.	GLUT 1 for glucose
Symporters (cotransporters)	Transports a couple of ions or molecules simultaneously in the same direction, one down its concentration gradient and other against its concentration gradient.	SGLT1 for glucose and Na^+
Antiporters	Transports a couple of ions or molecules simultaneously in the opposite direction, one down its concentration gradient and other against its concentration gradient.	Na^+/Ca^{+2} exchanger

The rate of facilitated diffusion by uniporters is very high than passive diffusion through a pure phospholipids bilayer and there is a maximum transport rate V_{max} that is achieved when the concentration gradient across the membrane is very high and each uniporter is working at a maximal rate. The transport is also specific because each uniporter transports only a single type of molecule or a single group of closely related molecules.

GLUT 1-GLUT 12 are the 12 glucose transporters expressed in humans and the structure of all GLUT isoforms are quite similar with 12 hydrophobic membrane-spanning a-helices. Many helices having the amino acids (e.g. serine, threonine, glutamine and asparagine) which can form hydrogen bonds with the OH group of glucose. All GLUT proteins transport glucose but their differential expression among the various body cells enables them to regulate glucose metabolism independently at different rates.

For instance, GLUT 1 and GLUT3 are found in erythrocytes and other cells to take up glucose continuously at high rates from the blood. GLUT 2 expressed in the liver have very high influx efficiency in the response of insulin and here the excess glucose is stored as a polymer, named glycogen. GLUT4 is expressed only in fat and muscle cells which respond to insulin by increasing their glucose uptake.

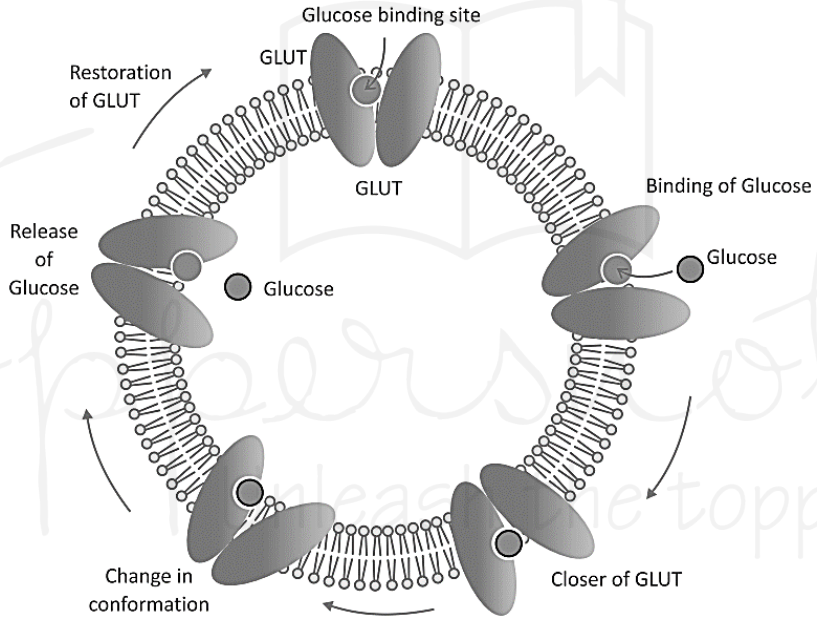


Fig. : Transport of glucose by GLUT.

The kinetics of glucose transport via GLUT1 uniporter is likely similar to an enzyme-catalyzed chemical reaction. If the substrate glucose, S, is present initially only on The outside of the membrane. In this case,

$$S_{out} + GLUT\ 1 \underset{}{\overset{K_m}{\rightleftharpoons}} S_{out} - GLUT\ 1 \overset{V_{max}}{\rightleftharpoons} S_{in} + GLUT\ 1$$

Here Sout-GLUT1 represents GLUT1 in the outward facing conformation of GLUT1 with bound glucose. As Michaelis Menten equation we can derive the following expression for V_0, the initial transport rate for Scatalyzed by GLUT1:

$$V_0 = \frac{V_{max} \times S}{K_m + S}$$

Here S is the concentration of Solute (the initial concentration of Sin = 0). V_{max} the highest transport rate when all molecules of GLUT1 bound with the substrate, occurs at an infinitely high substrate concentration outwards.

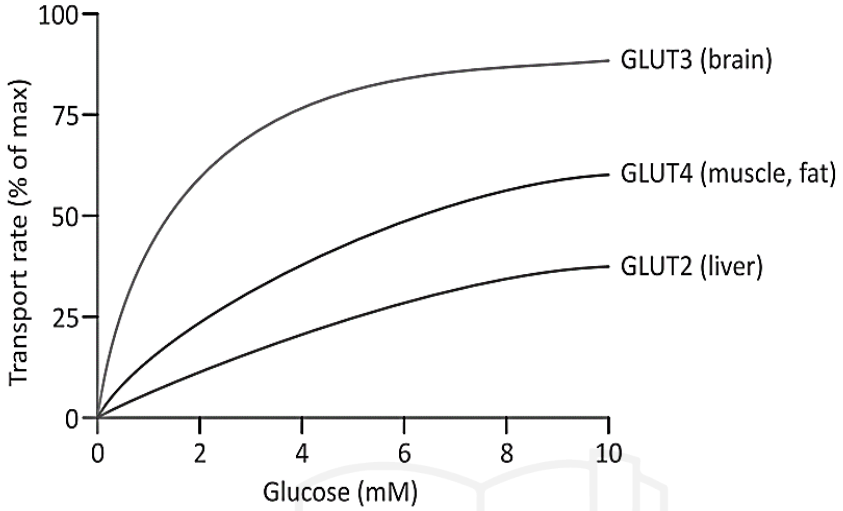


Fig. : Graph of Glucose transport rate via GLUT2, GLUT3 and GLUT4

The value of Km of GLUT 1 for D-glucose is 1.5mm, where approximately half of the total transporters have a glucose molecule bonded to the binding site and the greater the transport rate V_{max} will be half. Some isomers of glucose are also transported by GLUT1 which have much lower Km like D-mannose (20mm) and D-galactose (30mm). Thus GLUT1 is very specific with higher affinity for D-glucose.

GLUT	Primary site of expression	Affinity to glucose
GLUT -1	All foetal tissues, erythrocytes, brain	High affinity (+ + +)
GLUT -2	Liver, pancreas, intestine	Low affinity (+)
GLUT -3	Neurons, kidney, testis, placenta	Highest affinity (+ + + +)
G LUT -4	Adipose tissue, skeletal muscle, cardiac muscle	Moderate affinity (+ +)
GLUT -5	Small intestine, testis, kidney	Fructose uptake (+)
GLUT -6	Brain, spleen, leucocytes	Low affinity (+)

Facilitated Diffusion of ions by channel proteins

Channels are hollow membrane-bound proteins having a hydrophilic gateway. Channels are integral proteins embedded cell membrane. Ion channels control the movement of ions through the cell membrane

The channels are:

1. Selective for particular ions.
2. Involve in Facilitated diffusion of ions.
3. Regionally located at a specific site on a cell surface. Like Na channel at the soma of neurons.
4. Functionally unique.

Channel selectivity depends on various factors:

1. The charge on the ion-that is, whether it is positive or negative.

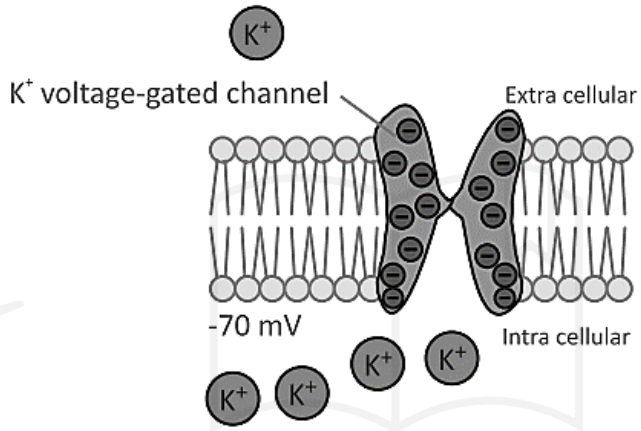


Fig. : The K^+ Channel

2. On the size of the ion.
3. On how much water the ion attracts and holds around it.

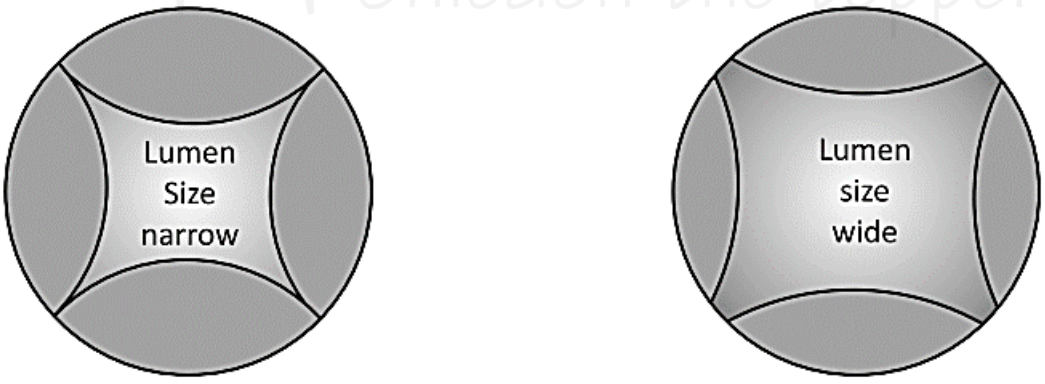


Fig. : K^+ Channel size is smaller than Na^+ channels

Types of Ion channels

- Ion channels are either active or passive: Passive Ion Channels are always open. Passive channels, also called leakage channels, are always open and ions pass through them continuously.