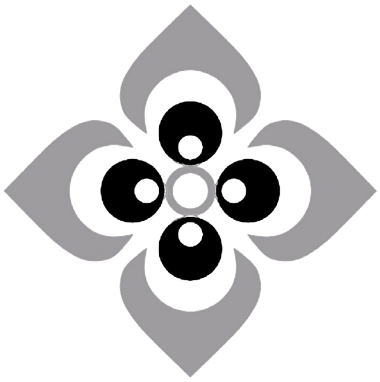

Consortium for Educational Communication

Module on

Biological Membranes

By

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Text

Introduction

The word 'membrane' comes from Latin word 'membrana' that means a skin. Todays word 'membran' has been extended to describe a thin flexible sheet or film, acting as a selective boundary between two phases because of its semi-permeable properties. A biological membrane or biomembrane is an enclosing or separating membrane that acts as a selective barrier, within or around a cell. The membranes are active structures surrounding cells and most eukaryotic organelles, such as endoplasmic reticulum, the Golgi apparatus, vacuoles, mitochondria, chloroplasts and the nucleus. It consists of a lipid bilayer with embedded proteins. Biological membrane, together with cytoskeleton, forms the structure of living cell. They are among the fundamental concepts upon which cellular biology is based. Regardless of their nature, prokaryotic or eukaryotic, membranes are integral to the structure and function of all cells.

The membranes define the external boundaries of cells and regulate the molecular traffic across that boundary; in eukaryotic cells, they divide the internal space into discrete compartments to segregate processes and components. They organize complex reaction sequences and are central to both biological energy conservation and cell-to-cell communication. The membranes are flexible, self-sealing, and selectively permeable to polar solutes. Their flexibility permits the shape changes that accompany cell growth and movement (such as amoeboid movement). With their ability to break and reseal, two membranes can fuse, as in exocytosis, or a single membrane-enclosed compartment can undergo fission to yield two sealed compartments, as in endocytosis or cell division, without creating gross leaks through cellular surfaces. Because membranes are selectively permeable, they retain certain compounds and ions within cells and within specific cellular compartments, while excluding others.

History of Biological Membranes

The term "membrane" as an invisible film that surround a cell and serves as a barrier between cell contents and the environment and at the same time as a semipermeable partition through which water and

some substances dissolved in it can pass, was first used independently by botanists von Mol and K. Von Negeli in 1855 for explanation of plasmolytic phenomena. In 1890, German physicist W. Ostwald drew attention to a possible role of membranes in bioelectrical processes. Between 1895 and 1902, E. Overton measured cell membrane permeability for many compounds and showed a direct relationship between the ability of these compounds to penetrate through membranes and their solubility in lipids. It was a clear indication that it is lipids who forms the film through which substances from surrounding solution pass to cell. In 1902, Yu Bernstein used the membrane hypothesis for explanation of the electric properties of living cells.

Gorter and Grendel (1925) showed that the area of the monolayer of lipids extracted from erythrocyte membranes is two times larger than the total area of erythrocytes. They extracted lipids from hemolysed erythrocytes with acetone, evaporated the solution on the surface of water, and measured the area of the formed monomolecular lipid film. The results of these investigations suggested that lipids in membrane are arranged as a bimolecular layer. This supposition was verified by investigations of the electrical parameters of biomembranes: high electrical resistance (approx. 107 Ohm $\geq m^2$) and high electrical capacitance (0.51 F/m^2). At the same time, there were experimental data that testified to the fact that biological membranes contained protein molecules as part of their composition. These contradictions in experimental results were removed by Danielli and Davson who proposed in 1935 the so-called 'Sandwich (Butterbrod/Bread-and-Butter) model of biological membranes' composition that had been used in membranology, though with some small variations, for almost forty years. Danielli and Davson postulated the presence of a protein layer attached to the polar head groups on either side of Gorter and Grendel's bimolecular layer of lipid sheet. The presence of protein contributes to the stability and strength of the lipid film comprising the membrane.

Structure of Biological Membranes

Several models have been proposed to explain the arrangement

and the structure of lipid and protein molecules in the plasma membrane. On the basis of its lipid permeability character, Overton in 1902 regarded it as a lipoidal membrane. Gorter and Grendel in 1925 regarded it as a lipid bimolecular layer. Thereafter, several important models were proposed to explain the physical and biological features of cell membranes, e.g. butter-sandwich model of Danielli and Davson (1935, 43); unit membrane model of Robertson (1953); micellar or mosaic theory of Hilleir and Hoffman (1953); protein crystal model of David Green and his co-workers; lipid-globular protein mosaic theory of Roderick and Capaldi (1974) and fluid-mosaic model of Singer and Nicholson (1972). The fluid-mosaic model of plasma membrane is most widely accepted and is thus described in detail.

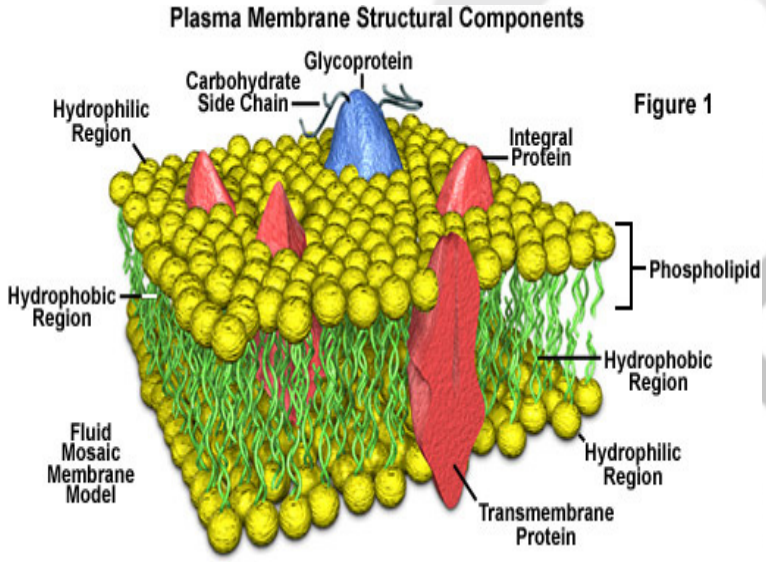


Figure 1

The Fluid Mosaic Model

It was proposed by S. J. Singer and G. Nicholson in 1972, and has been widely accepted. According to this model, a plasma membrane is a continuous lipid bilayer having integral protein molecules (Fig 1). The two are so dispersed that they form a mosaic pattern. The biological membranes are semi-fluid structures where lipid and protein molecules perform translational movements. The butter-sandwich model of Danielli-Davson assumes hydrophillic binding between protein and lipid molecules whereas fluid-mosaic model considers this to be hydrophobic. The membrane has globular proteins which may be intrinsic or extrinsic. Many intrinsic proteins and phospholipids of the membrane show amphipathy i.e., they have both hydrophilic and hydrophobic groups within the same molecule. The Nuclear Magnetic

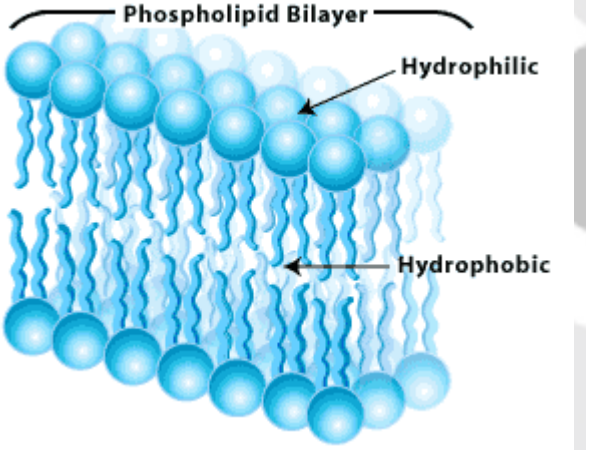
Resonance (NMR) and Electron Spin Resistance (ESR) studies show that the membrane is dynamic. The lipid tails show flexibility. The molecules can rotate or show flip-flop motion.

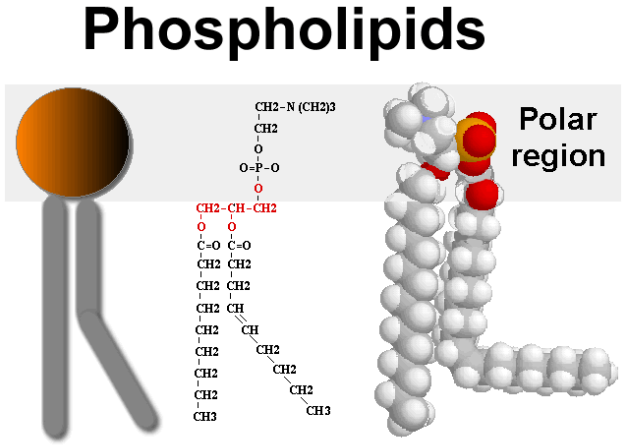
Singer and Nicolson proposed the proteins that are integral to the membrane are a heterogenous set of globular molecules, each arranged in an amphipathic structure, that is, with the ionic and highly polar groups protruding from the membrane in to the aqueous phase, and the nonpolar groups largely buried in the hydrophobic interior of the membrane. These globular molecules are partially embedded in a matrix of phospholipid. The bulk of the phospholipid is organized as a discontinuous, fluid layer, although a small fraction of the lipid may interact specifically with the membrane proteins. The fluid mosaic structure is therefore formally analogous to a two-dimensional oriented solution of integral proteins (or lipoproteins) in the viscous phospholipid bilayer solvent. In the fluid mosaic model, a mosaic of proteins floats in the fluid lipid bilayer like boats on a pond.

Composition of Biological Membranes

Depending on the cell type, function, and species, the thickness of biological membranes will vary from approximately 2 to 10 nanometers. Regardless of their source, all biological membranes have a number of structural characteristics in common. The main feature of all membranes is their phospholipid bilayer organization. The plasma membrane contains about 20% to 79% lipids whose constituents are mainly phospholipids, cholesterol and glycolipids. Their proportion varies in different cell membranes. The phospholipids which make up 55 and 75% of the total lipid content, consists chiefly of lecithin and cephalin. The remainder consists of sphingolipids (with an amino group) and glycolipids (conjugated with carbohydrates). Phospholipids are unique molecules in that one end of the molecule is polar or hydrophilic with the other end being nonpolar or hydrophobic. This type of molecule is referred to as amphiphilic that is having both hydrophilic and hydrophobic regions (Fig. 2 & 3). The nonpolar end of the lipid is composed of two fatty acids chemically bond to either a glycerol or serine molecule. The fatty acids generally range from fourteen to twenty-four carbon atoms in length. One of the fatty acids

is saturated whereas the other one is unsaturated with at least one *cis*-double bond. Furthermore, chemically bound to the glycerol or serine portion of the molecule is a single phosphate group to which the polar end of the phospholipid is attached. These polar ends can be ethanolamine, choline, serine, or to a lesser extent inositol. Four very common phospholipids found in membranes include: phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and sphingomyelin. The later phospholipid is the only one in which the fatty acids are chemically bound to a serine molecule rather than glycerol. The molecules are aligned in such a manner that the inner part of the membrane is composed of the hydrophobic ends of each fatty acid while the hydrophilic portion of each molecule either faces the exterior or interior portions of the cell. It should be noted that the arrangement of the phospholipids within the bilayer structure is not uniform. This asymmetric organization is due to differences in the chemical compositions of the inner and outer monolayer portions of the phospholipid bilayer. In addition to phospholipids, the membrane bilayer may also contain glycolipids, proteins, as well as cholesterol.





Since the molecules within the lipid bilayer are not physically attached to each other a certain degree of movement takes place within the membrane. Lateral diffusion within each monolayer occurs when membrane phospholipids exchange places. In addition, these molecules are free to rotate about their axis. Movement of their hydrophobic ends likewise takes place. This fluidity within the membrane is greatly dependent upon the composition of each

monolayer. The hydrocarbon chain length and the number of *cis*-double bonds within specific fatty acids both appear to influence the degree of fluidity within the membrane. The shorter the fatty acid length and the more double bonds within the fatty acid favour an increased fluidity within the membrane. Not only is cholesterol a major component of eukaryotic plasma membranes, but it also has a profound effect on the fluidity of the membrane. It is estimated that in eukaryotic cells the ratio of phospholipid to cholesterol molecules is on the order of one to two. Each amphiphilic cholesterol molecule has the same membrane orientation as the phospholipids. Its steroid ring structure inhibits the movement of adjacent phospholipids thereby decreasing both fluidity and membrane permeability.

Proteins are integral components in the composition of membranes. For example, seventy-six percent of the inner mitochondrial membrane is protein and twenty-four percent is phospholipid. The plasma membranes from human red blood cell, in comparison, contain forty-four percent proteins and forty-three percent phospholipids. The myelin from nerve fibers only contains about eighteen percent proteins with seventy-six percent being phospholipids. These membrane proteins participate in a number of fundamental roles including: receptors, enzymes, and as transport proteins. The two basic types of membrane proteins are integral and peripheral proteins. The integral proteins are those proteins which are tightly bound to the membrane. They represent more than 70% of the two protein types and penetrate the lipid layer wholly or partially. Their polar ends protrude from the membrane surface while nonpolar ends are embedded in the interior of the membrane. Usually they are insoluble in water solutions and detergents or organic solvents are required to separate them from the membrane. The integral proteins may be attached to the oligosaccharides to form glycoproteins or to phospholipids to form lipoproteins or or proteolipids. Common intrinsic proteins are rhodopsin found in retinal rod cells and cytochrome oxidase found in mitochondrial membranes. Another important glycoprotein is glycophorin. On the other hand, the peripheral proteins are loosely bound to only one side of the lipid bilayer. They are also called extrinsic

proteins and can be separated by addition of salts, are soluble in aqueous solutions and are usually free of lipids. They are bound to the surface membranes by electrostatic and hydrogen bond interactions. They form outer and inner layers on the lipid layer of plasma membrane. Common examples of extrinsic proteins are cytochrome C (found in mitochondria), acetylcholinesterase (in electroplax membranes) and spectrin (found in erythrocytes), which are easily removed in high salt solutions. Spectrin and actin, both associate to form microfilaments, providing a kind of skeletal support for the membrane. The manner in which a protein is associated with the membrane is indicative of its role. Transmembrane proteins are integral membrane proteins since they traverse the membrane from one side to the other (Fig. 4).

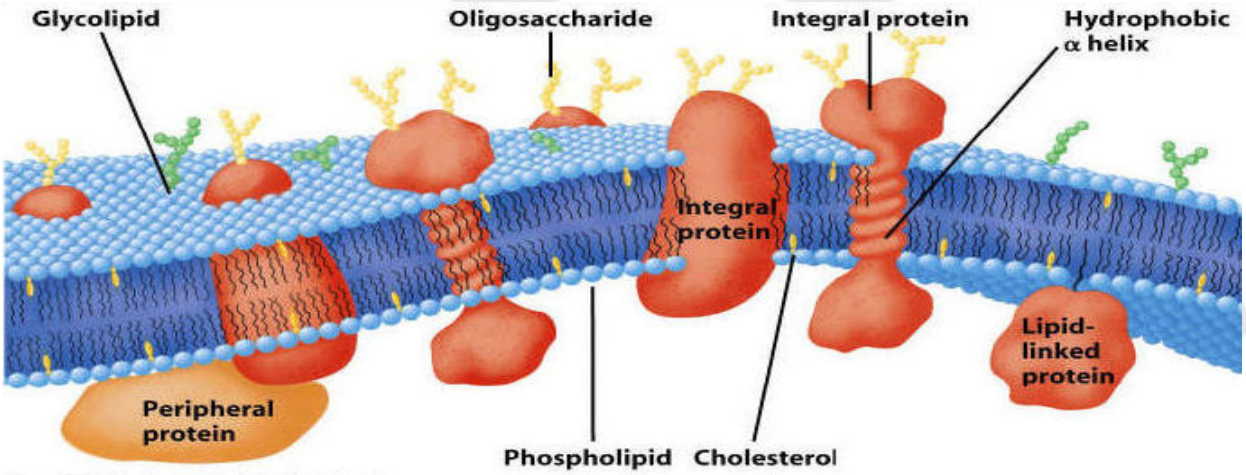


Figure 9-26 Fundamentals of Biochemistry, 2/e
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About 30 enzymes have been isolated from various plasma membranes. Those most constantly found are 5`-nucleotidase, Na^+-K^+-activated ATPase, alkaline phosphatase, adenyl cyclase, RNAase and acid phosphomonoesterase. Na^+-K^+ activated Mg^+ ATPase plays an important role in ion exchange and may also act as carrier protein or permease across the plasma membrane.

The presence of carbohydrates in the plasma membrane was suggested by Bell in 1962. The membranes of eukaryotic cells usually contain between 2% and 10% carbohydrate, in the form of glycolipids and glycoproteins. About 5% carbohydrates have been found in the plasma membrane of liver cells and RBC. Hexose, hexoamine, fucose and sialic acid are the commonest carbohydrates found in the membrane. The sialic acid is found in the form of glycolipids or gangliosides in the

plasma membrane of liver.

The salts are also present in cell membranes. Water present in cell membranes form part of the membrane structure as it does in all cell constituents.

Kinds of Membrane Proteins

The plasma membrane is a complex assembly of proteins enmeshed in a fluid array of phospholipid molecules (Fig. 5). This enormously flexible design permits a broad range of interactions with the environment, some directly involving membrane proteins. The cells interact with their environment through their plasma membranes in many ways. Some classes of membrane proteins of plasma membrane are:

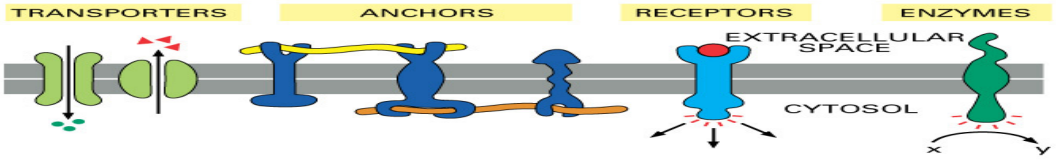


Figure 11-20 Essential Cell Biology, 2/e. (© 2004 Garland Science)

1. Transporters. Membranes are very selective, allowing only certain substances to enter or leave the cell, either through channels or carriers. In some instances, they take up molecules already present in the cell in high concentration.

2. Enzymes. Cells carry out many chemical reactions on the interior surface of the plasma membrane, using enzymes attached to the membrane.

3. Cell surface receptors. Membranes are exquisitely sensitive to chemical messages, detecting them with receptor proteins on their surfaces that act as antennae.

4. Cell surface identity markers. Membranes carry cell surface markers that identify them to other cells. Most cell types carry their own ID tags, specific combinations of cell surface proteins characteristic of that cell type.

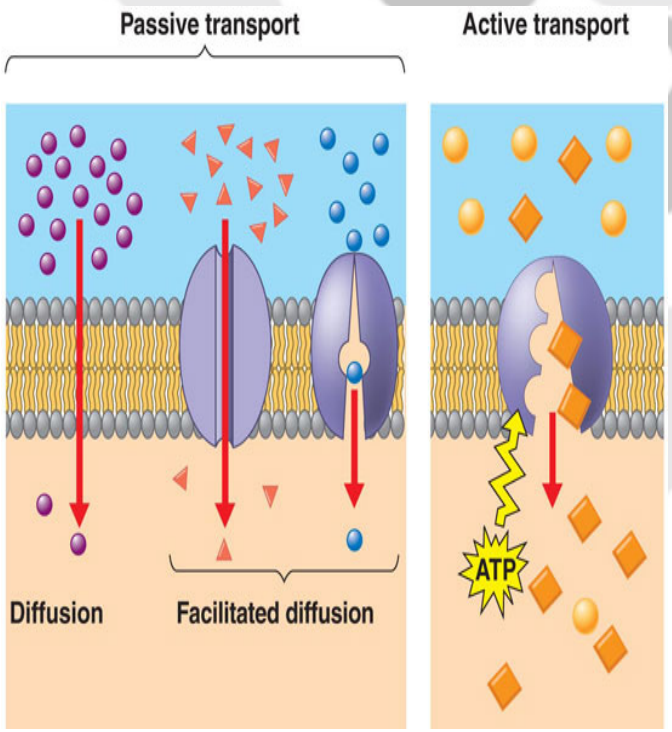
5. Cell adhesion proteins. Cells use specific proteins to glue themselves to one another. Some act like Velcro, while others form a

more permanent bond.

6. Attachments to the cytoskeleton. Surface proteins that interact with other cells are often anchored to the cytoskeleton by linking proteins.

Biological Membranes and the Transport of Molecules

One of the integral roles attributed to membranes is the regulation of materials into and out of the cell and/or membrane-bound organelles. Due to the amphiphilic nature of the phospholipids, the ability to traverse a membrane is a function of both the size and polarity of the molecule. Whereas very small nonpolar molecules are able to diffuse fairly rapidly across membranes, polar molecules experience a certain degree of difficulty. Uncharged polar molecules less than ninety daltons in size are capable of diffusing across membranes, whereas those greater than ninety daltons in size do not. One dalton is equivalent to 1.66×10^{-24} grams. Ions, however, are incapable of diffusing across the membrane regardless of their size. From this it is apparent that the movement of substances across biological membranes involves a number of different mechanisms. The major cellular transport mechanisms are passive transport and active transport.



Passive Transport

The process whereby substances move from one side of a

membrane to the other without the expenditure of energy is referred to as passive transport or diffusion. In this type of transport, the movement of an uncharged substance across the membrane is based solely on differences in concentration. This concentration gradient determines the direction of transport which is from an area of greater concentration to one of lesser concentration (Fig. 6).

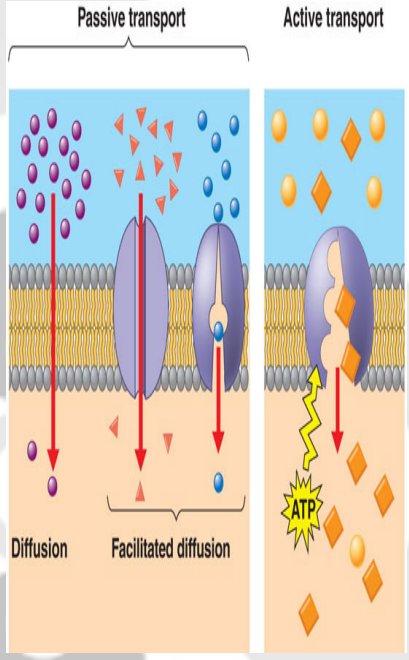
In addition to the phospholipids, proteins are important to the structural composition of membranes. Not only are there are a number of different functions attributed to membrane proteins, but there are also a number of different ways in which these proteins are associated with the membrane. Transmembrane proteins, whether single pass or multiple pass, span the membrane with portions of the protein on either side of the membrane. It is believed that one role of these transmembrane proteins is to mediate the transport of substances across the membrane. They can act either as carrier proteins or as channel proteins. A carrier protein functions by binding to a specific uncharged substance and then by undergoing a number of structural changes is able to transport that molecule across the membrane. Channel proteins form a channel through which polar molecules and/or ions can pass from one side of the membrane to the other. A discrete set of channel proteins, the ion channel proteins, form channels or pores through which ions of a specific size and charge can pass. It has been estimated that the rate of transport through these ion channels is on the order of one thousand times greater than the rate of transfer by way of any other type of carrier protein. To regulate the flow of ions through the ion channel protein, these transmembrane proteins have the ability to open or close the pore through which the ion is transported. These are referred to as voltage-gated channels.

The difference in the concentration of ions on either side of a membrane creates an electrochemical gradient or membrane potential. In general, the interior of a cell tends to have an overall negative charge with respect to its exterior environment. This potential difference favors the transport of positively charged substances into the cell. Therefore, the transport of charged molecules and/or ions

across a membrane is not only based on concentration differences, but it is also based on its membrane potential.

Active Transport

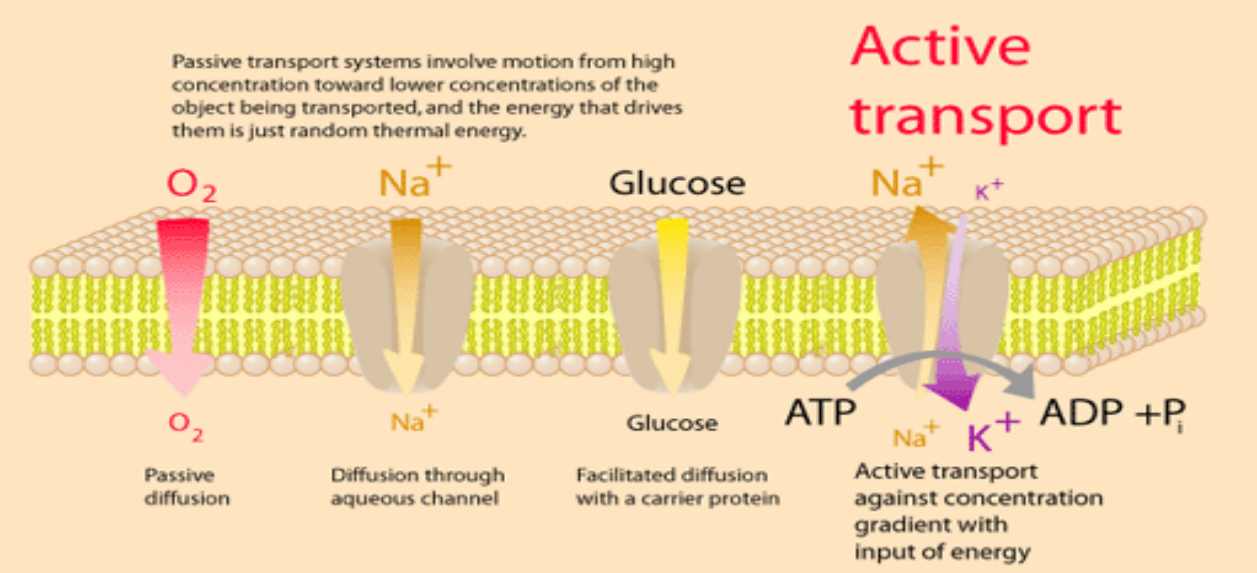
The transport of substances against a concentration gradient is known as active transport and requires both a carrier protein and an energy source (Fig. 7). These transmembrane carrier proteins have been classified as uniport, symport, and antiport carrier proteins. The uniport carriers transport a single molecule from one side of the membrane to the other. Both symport and antiport carrier proteins



involve the dependent transfer of two molecules and as such they are also referred to as co-transporters. Symport carriers transport two molecules in the same direction, such as amino acids and Na^+. Antiport carriers, in comparison, transport the two molecules in opposite directions, as in the case of Na^+ and K^+. The transport of macromolecules such as proteins and polysaccharides rely on vesicular transport. In this type of transport, membranous vesicles are formed around the substance or substances that are to be transported into or out of the cell or membrane bound organelle. Where endocytosis refers to vesicular transport into the cell, exocytosis is the vesicular transport of substances out of the cell. Two forms of endocytosis are: pinocytosis, and phagocytosis. The two are classified on the basis of the size of the transport vesicles. Pinocytosis involves vesicles up to 150 nm in diameter whereas in phagocytosis the vesicles formed are

in excess of 250 nm in diameter.

The energy required for the active transport of molecules across a membrane can come from the hydrolysis of adenosine triphosphate (ATP) or from specific ion gradients. Active transport can be classified into primary or secondary active transport. The energy released from the hydrolysis of ATP drives the primary active transport mechanism, whereas ion gradients drive the secondary active transport mechanisms. There are several different classes of primary active transport which include: P-class ion pumps, F-class ion pumps, V-class ion pumps, and the ATP-binding cassette or ABC superfamily. Included in the P-class are the Na^+ - K^+ ATPase, Ca^{2+} ATPase, and the H^+- K^+ ATPase pumps (Fig. 8). Although the F-class and V-class pumps are believed to transport only H^+, they are both more complex than the P-class pumps. Within the ABC superfamily there are approximately one hundred known transport proteins. All of these proteins share a number of similar characteristics. The ABC superfamily is responsible for the transport of a variety of substances ranging from simple inorganic ions up to and including some proteins.



Secondary active transport does not involve the energy released from the hydrolysis of ATP. Instead, it involves the transport of a substance against its concentration gradient with the concomitant transport of an ion along its electrochemical gradient. Secondary active transport may include either symport or antiport co-transporters and are responsible for the transport of such substances as amino acids

and sugars. An example of secondary active transport involving a symport co-transporter is glucose and Na^+. As the direct result of the Na^+ - K^+ ATPase pump, the diffusion of Na^+ into the cell along its electrochemical gradient provides the energy necessary for the transport of glucose into the cell against its concentration gradient. The transport of Na^+ into the cell and Ca^{2+} out of the cell is an example of secondary active transport involving an antiport co-transporter. Once again the energy needed to drive the reaction was obtained from the equilibrium established as a result of the Na^+ - K^+ ATPase pump.

Functions of biological membranes

In all living cells, biological membranes carry out the function of barrier that divides the cell from the environment and the internal cell volume into comparably isolated compartments. Partitions dividing cells into compartments are built of a double layer of lipid molecules (which is often called bilayer) and are practically impermeable for ions and polar water-soluble molecules. But this lipid bilayer includes numerous built-in protein molecules and molecular complexes one of those have/possess the properties of selective channels for ions and molecules, and others- those of pumps capable to pump/transfer actively ions through membrane. The barrier properties of membranes and working of membrane pumps cause irregular distribution of ions between the cell and extracellular medium, which lies in the basis of the processes of intracellular regulation and signal transfer in the form of electrical impulse between cells.

A second function, common for all membranes, is the function of mounting plate, or matrix on which there are proteins and protein groups that are disposed in a definite order and create systems of electron transfer, energy accumulation in the form of ATP, regulation of intracellular processes by hormones coming in from outside and intracellular mediations, recognizing of other cells and foreign proteins, light reception, mechanical effects, etc.

A flexible and elastic film which lay in the basis of all membranes also plays a definite mechanical function keeping the cell intact under mild mechanical loads and disturbances in upsets of osmotic balance between the cell and environment.

Common for all membranes functions of barrier for ions and molecules and matrix for protein groups are mainly provided by the lipid bilayer that has in principle the same structure in all membranes. Nevertheless, the set of proteins is unique for each membrane type which allows membranes to take part in carrying out various functions in different cells and cell structures.

Function of biomembranes in Food Technology

Biomembranes and rehydration

Dry phospholipid bilayers are known to unergo transient i.e. phase transitions and permeability changes in dry membrane during rehydration.

Liposomes, spherical bilayer vesicles, a good example of biomembrane functioning in food industry. Liposomes have been used to deliver food flavour, nutrients and more recently food antimicrobials that could aid in the protection of food products against growth of spoilage and pathogenic microorganisms.

Unique interaction of trehalose and biomembranes

Trehalose, a non-reducing diglucose sugar found in nature, confers to certain plant and animal cells the ability to survive dehydration for decades and to restore activity soon after rehydration. The interaction between trehalose and cell membranes or proteins is subject of recent research to elucidate the mechanisms in this unique behaviour of preservation.

Biomembranes and Pulsed Electric Field processing (PEF)

The ever increasing trend toward nutritionally qualified food has challenged food technology to produce fresh-like foods by replacing thermal treatments. PEF is used to kill or inactivate microorganisms based on the principle of membrane fluidity and disruption. PEF is successfully used in processing and facilitating juice extraction from fruits also based on structural properties of biomembranes.

Biomembranes and chill injury

Membranes associated disorders of plant (fruits and vegetables) including chilling, freezing and desiccation injuries.. Mitochondria and other cell organelles of plant cells exposed to low temperature and other abiotic and biotic stresses, produce superoxide/hydrogen

peroxide in the cells.. This superoxide/hydrogen peroxide can diffuse throughout cell causing peroxidation of membrane lipids which results In membrane disruption, increased permeability and metabolic disturbances and eventually the visible symptoms of chilling injury.