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BIOCHEMISTRY

[201]: CELL BIOLOGY

Unit 5
Biological Membranes and Membrane Transport

Prepared By

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CHEMICAL COMPOSITION OF BIOLOGIDCAL MEMBRANES:

The **cell membrane** (also known as the **plasma membrane** (**PM**) or **cytoplasmic membrane,** and historically referred to as the **plasmalemma**) is a biological membrane that separates the interior of all cells from the outside environment (the extracellular space) which protects the cell from its environment.

The cell membrane consists of a lipid bilayer, including cholesterols (a lipid component) that sit between phospholipids to maintain their fluidity at various temperatures. The membrane also contains membrane proteins, including integral proteins that go across the membrane serving as membrane transporters, and peripheral proteins that loosely attach to the outer (peripheral) side of the cell membrane, acting as enzymes shaping the cell.

The cell membrane controls the movement of substances in and out of cells and organelles. In this way, it is selectively permeable to ions and organic molecules.

In addition, cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signalling and serve as the attachment surface for several extracellular structures, 0including the cell wall, the carbohydrate layer called the glycocalyx, and the intracellular network of protein fibres called the cytoskeleton. In the field of synthetic biology, cell membranes can be artificially reassembled.

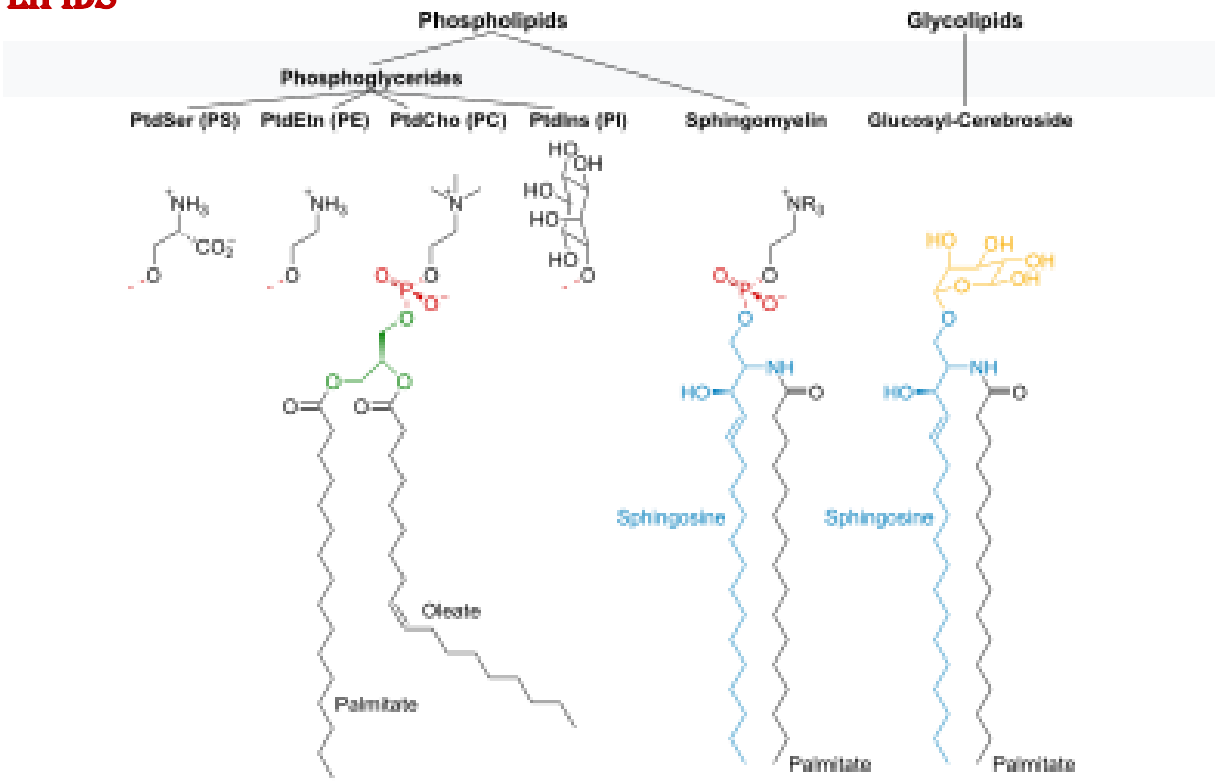
Cell membranes contain a variety of biological molecules, notably lipids and proteins. Composition is not set, but constantly changing for fluidity and changes in the environment, even fluctuating during different stages of cell development. Specifically, the amount of cholesterol in human primary neuron cell membrane changes, and this change in composition affects fluidity throughout development stages.

Material is incorporated into the membrane, or deleted from it, by a variety of mechanisms:

- Fusion of intracellular vesicles with the membrane (exocytosis) not only excretes the contents of the vesicle but also incorporates the vesicle membrane's components into the cell membrane. The membrane may form blebs around extracellular material that pinch off to become vesicles (endocytosis).
- If a membrane is continuous with a tubular structure made of membrane material, then material from the tube can be drawn into the membrane continuously.

- Although the concentration of membrane components in the aqueous phase is low (stable membrane components have low solubility in water), there is an exchange of molecules between the lipid and aqueous phases.

LIPIDS



Examples of the major membrane phospholipids and glycolipids: phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidyl inositol (PtdIns), phosphatidylserine (PtdSer).

The cell membrane consists of three classes of amphipathic lipids: phospholipids, glycolipids, and sterols. The amount of each depends upon the type of cell, but in the majority of cases phospholipids are the most abundant, often contributing for over 50% of all lipids in plasma membranes.

Glycolipids only account for a minute amount of about 2% and sterols make up the rest. In RBC studies, 30% of the plasma membrane is lipid. However, for the majority of eukaryotic cells, the composition of plasma membranes is about half lipids and half proteins by weight.

The fatty chains in phospholipids and glycolipids usually contain an even number of carbon atoms, typically between 16 and 20. The 16- and 18-carbon fatty acids are the most common. Fatty acids may be saturated or unsaturated, with the configuration of the double bonds nearly always "cis". The length and the degree of unsaturation of fatty acid chains have a profound

effect on membrane fluidity as unsaturated lipids create a kink, preventing the fatty acids from packing together as tightly, thus decreasing the melting temperature (increasing the fluidity) of the membrane.

The ability of some organisms to regulate the fluidity of their cell membranes by altering lipid composition is called homeoviscous adaptation.

The entire membrane is held together via non-covalent interaction of hydrophobic tails, however the structure is quite fluid and not fixed rigidly in place. Under physiological conditions phospholipid molecules in the cell membrane are in the liquid crystalline state. It means the lipid molecules are free to diffuse and exhibit rapid lateral diffusion along the layer in which they are present.

However, the exchange of phospholipid molecules between intracellular and extracellular leaflets of the bilayer is a very slow process. Lipid rafts and caveolae are examples of cholesterol-enriched microdomains in the cell membrane.

Also, a fraction of the lipid in direct contact with integral membrane proteins, which is tightly bound to the protein surface is called annular lipid shell; it behaves as a part of protein complex.

In animal cells cholesterol is normally found dispersed in varying degrees throughout cell membranes, in the irregular spaces between the hydrophobic tails of the membrane lipids, where it confers a stiffening and strengthening effect on the membrane.

Additionally, the amount of cholesterol in biological membranes varies between organisms, cell types, and even in individual cells. Cholesterol, a major component of animal plasma membranes, regulates the fluidity of the overall membrane, meaning that cholesterol controls the amount of movement of the various cell membrane components based on its concentrations.

In high temperatures, cholesterol inhibits the movement of phospholipid fatty acid chains, causing a reduced permeability to small molecules and reduced membrane fluidity. The opposite is true for the role of cholesterol in cooler temperatures. Cholesterol production, and thus concentration, is up-regulated (increased) in response to cold temperature.

At cold temperatures, cholesterol interferes with fatty acid chain interactions. Acting as antifreeze, cholesterol maintains the fluidity of the membrane. Cholesterol is more abundant in cold-weather animals than warm-weather animals. In plants, which lack cholesterol, related compounds called sterols perform the same function as cholesterol.

PHOSPHOLIPIDS FORMING LIPID VESICLES

Lipid vesicles or liposomes are approximately spherical pockets that are enclosed by a lipid bilayer.[24] These structures are used in laboratories to study the effects of chemicals in cells by delivering these chemicals directly to the cell, as well as getting more insight into cell membrane permeability.

Lipid vesicles and liposomes are formed by first suspending a lipid in an aqueous solution then agitating the mixture through sonication, resulting in a vesicle. By measuring the rate of efflux from that of the inside of the vesicle to the ambient solution, allows researcher to better understand membrane permeability.

Vesicles can be formed with molecules and ions inside the vesicle by forming the vesicle with the desired molecule or ion present in the solution. Proteins can also be embedded into the membrane through solubilizing the desired proteins in the presence of detergents and attaching them to the phospholipids in which the liposome is formed. These provide researchers with a tool to examine various membrane protein functions.

CARBOHYDRATES

Plasma membranes also contain carbohydrates, predominantly glycoproteins, but with some glycolipids (cerebrosides and gangliosides). Carbohydrates are important in the role of cell-cell recognition in eukaryotes; they are located on the surface of the cell where they recognize host cells and share information, viruses that bind to cells using these receptors cause an infection.

For the most part, no glycosylation occurs on membranes within the cell; rather generally glycosylation occurs on the extracellular surface of the plasma membrane. The glycocalyx is an important feature in all cells, especially epithelia with microvilli.

Recent data suggest the glycocalyx participates in cell adhesion, lymphocyte homing, and many others. The penultimate sugar is galactose and the terminal sugar is sialic acid, as the sugar backbone is modified in the Golgi apparatus. Sialic acid carries a negative charge, providing an external barrier to charged particles.

PROTEINS

TYPE	DESCRIPTION	EXAMPLES
Integral proteins or transmembrane proteins	Span the membrane and have a hydrophilic cytosolic domain, which interacts with internal molecules, a hydrophobic membrane-spanning domain that anchors it within the cell membrane, and a hydrophilic extracellular domain that interacts with external molecules. The hydrophobic domain consists of one, multiple, or a combination of α-helices and β sheet protein motifs.	Ion channels, proton pumps, G protein-coupled receptor
Lipid anchored proteins	Covalently bound to single or multiple lipid molecules; hydrophobically insert into the cell membrane and anchor the protein. The protein itself is not in contact with the membrane.	G proteins
Peripheral proteins	Attached to integral membrane proteins, or associated with peripheral regions of the lipid bilayer. These proteins tend to have only temporary interactions with biological membranes, and once reacted, the molecule dissociates to carry on its work in the cytoplasm.	Some enzymes, some hormones

The cell membrane has large content of proteins, typically around 50% of membrane volume These proteins are important for the cell because they are responsible for various biological activities. Approximately a third of the genes in yeast code specifically for them, and this number is even higher in multicellular organisms. Membrane proteins consist of three main types: integral proteins, peripheral proteins, and lipid-anchored proteins.

As shown in the adjacent table, integral proteins are amphipathic transmembrane proteins. Examples of integral proteins include ion channels, proton pumps, and g-protein coupled receptors. Ion channels allow inorganic ions such as sodium, potassium, calcium, or chlorine to diffuse down their electrochemical gradient across the lipid bilayer through hydrophilic pores across the membrane. The electrical behaviour of cells (i.e., nerve cells) are controlled by ion channels.

Proton pumps are protein pumps that are embedded in the lipid bilayer that allow protons to travel through the membrane by transferring from one amino acid side chain to another. Processes such as electron transport and generating ATP use proton pumps.

A G-protein coupled receptor is a single polypeptide chain that crosses the lipid bilayer seven times responding to signal molecules (i.e., hormones and neurotransmitters). G-protein coupled receptors are used in processes such as cell to cell signalling, the regulation of the production of cAMP, and the regulation of ion channels.

The cell membrane, being exposed to the outside environment, is an important site of cell–cell communication. As such, a large variety of protein receptors and identification proteins, such as antigens, are present on the surface of the membrane. Functions of membrane proteins can also include cell–cell contact, surface recognition, cytoskeleton contact, signalling, enzymatic activity, or transporting substances across the membrane.

Most membrane proteins must be inserted in some way into the membrane. For this to occur, an N-terminus "signal sequence" of amino acids directs proteins to the endoplasmic reticulum, which inserts the proteins into a lipid bilayer. Once inserted, the proteins are then transported to their final destination in vesicles, where the vesicle fuses with the target membrane.

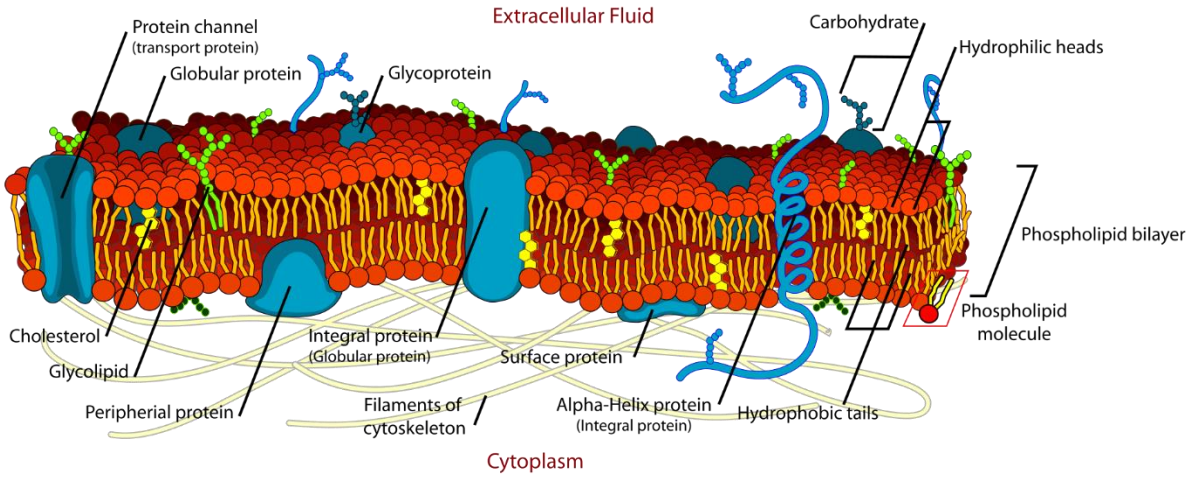
SINGER AND NICHOLSON MODEL OF PLASMA MEMBRANE STRUCTURE

The **fluid mosaic model** explains various observations regarding the structure of functional cell membranes. According to this biological model, there is a lipid bilayer (two molecules thick layer consisting primarily of amphipathic phospholipids) in which protein molecules are embedded. The lipid bilayer gives fluidity and elasticity to the membrane. Small amounts of carbohydrates are also found in the cell membrane.

The biological model, which was devised by SJ Singer and G. L. Nicolson in 1972, describes the cell membrane as a two-dimensional liquid that restricts the lateral diffusion of membrane components. Such domains are defined by the existence of regions within the membrane with special lipid and protein cocoon that promote the formation of lipid rafts or protein and glycoprotein complexes. Another way to define membrane domains is the association of the lipid membrane with the cytoskeleton filaments and the extracellular matrix through membrane proteins.

The current model describes important features relevant to many cellular processes, including: cell-cell signalling, apoptosis, cell division, membrane budding, and cell fusion. The

fluid mosaic model is the most acceptable model of the plasma membrane. Its main function is to separate the contents of the cell from the outside.



CONCEPT OF MEMBRANE ASYMMETRY

Additionally, the two leaflets of biological membranes are asymmetric and divided into subdomains composed of specific proteins or lipids, allowing spatial segregation of biological processes associated with membranes. Cholesterol and cholesterol-interacting proteins can concentrate into lipid rafts and constrain cell signalling processes to only these rafts.

Another form of asymmetry was shown by the work of Mouritsen and Bloom in 1984, where they proposed a Mattress Model of lipid-protein interactions to address the biophysical evidence that the membrane can range in thickness and hydrophobicity of proteins

LATERAL MOVEMENT AND FLIP FLOP MOVEMENT OF PHOSPHOLIPIDS AND PROTEINS IN BIOLOGICAL MEMBRANE

During the decade of 1970, it was acknowledged that individual lipid molecules undergo free lateral diffusion within each of the layers of the lipid membrane.[9] Diffusion occurs at a high speed, with an average lipid molecule diffusing ~2 µm, approximately the length of a large bacterial cell, in about 1 second.

It has also been observed that individual lipid molecules rotate rapidly around their own axis. Moreover, phospholipid molecules can, although they seldom do, migrate from one side of the lipid bilayer to the other (a process known as flip-flop). However, flip-flop might be enhanced by flippase enzymes. The processes described above influence the disordered nature of lipid

molecules and interacting proteins in the lipid membranes, with consequences to membrane fluidity, signalling, trafficking and function.

LIPID BILAYER

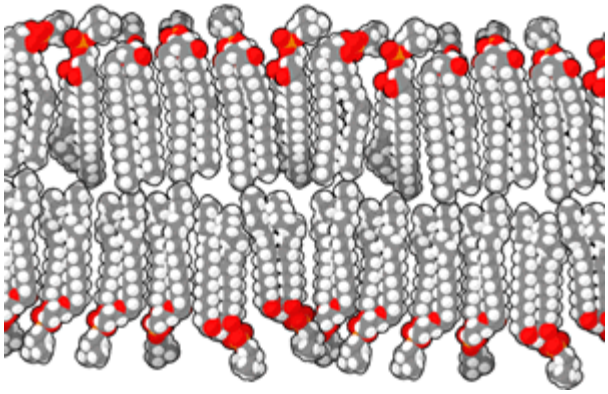
A lipid bilayer is a biological membrane consisting of two layers of lipid molecules. Each lipid molecule, or *phospholipid*, contains a *hydrophilic* head and a *hydrophobic* tail. The tail regions, being repelled by water and slightly attracted to each other, congregate together. This exposes the head regions to the outside, creating a barrier between two bodies of water. A lipid bilayer is the foundational part of all cellular membranes, typically completed with species-specific *integral proteins* and other functional aspects.

A lipid bilayer functions through the actions of *polarity*. The inside of the lipid bilayer is *non-polar*, while the heads are *polar* molecules and create hydrogen bonds with other polar molecules. This also means that polar molecules like water and ions cannot as easily cross through the nonpolar tail region of the lipid bilayer.

The cellular membranes of most organisms are created with lipid bilayer, as well as the *nuclear membrane* and various organelle membranes. The various functions of these membrane are then specified with a variety of proteins which allow or disallow certain substances to cross the membrane. In doing so, cells and individual organelles can create an ideal environment for biochemical reactions to occur, allowing them to stay in *homeostasis*.

STRUCTURE OF LIPID BILAYER

A lipid bilayer consists of two sheets of *amphiphilic phospholipids*, as seen in the image below. Amphiphilic describes a molecule which is part hydrophobic, part hydrophilic. There are often phosphorus atoms in the heads of the molecules, giving the heads polarity. The tails of the molecules are nonpolar and hydrophobic. In the image below, the polar parts of the molecules are marked in red.



As seen in the animation, the molecules are not stuck rigidly in place. In a single sheet, the molecules are actively moving around and past each other. In fact, a better analogy is that of people crammed in an elevator. They mostly stay put, but can slide past one another if someone needs to get off the elevator and is standing in the back. Put two of these layers together, and you have a lipid bilayer.

In living systems, a lipid bilayer is never by itself. It is associated with a number of surface and integral proteins, as well as extracellular and intracellular elements that have specific functions in the cell. An encompassing model of the entire *cellular membrane* is the *fluid mosaic model*, which assumes that proteins within the lipid bilayer act as icebergs within the sea, drifting around but not bound to anything. The specific properties of the protein and of the lipid bilayer keep them bound within the layers, but not stationary.

MEMBRANE FLUIDITY

In biology, **membrane fluidity** refers to the viscosity of the lipid bilayer of a cell membrane or a synthetic lipid membrane. Lipid packing can influence the fluidity of the membrane. Viscosity of the membrane can affect the rotation and diffusion of proteins and other bio-molecules within the membrane, there-by affecting the functions of these things.[1]

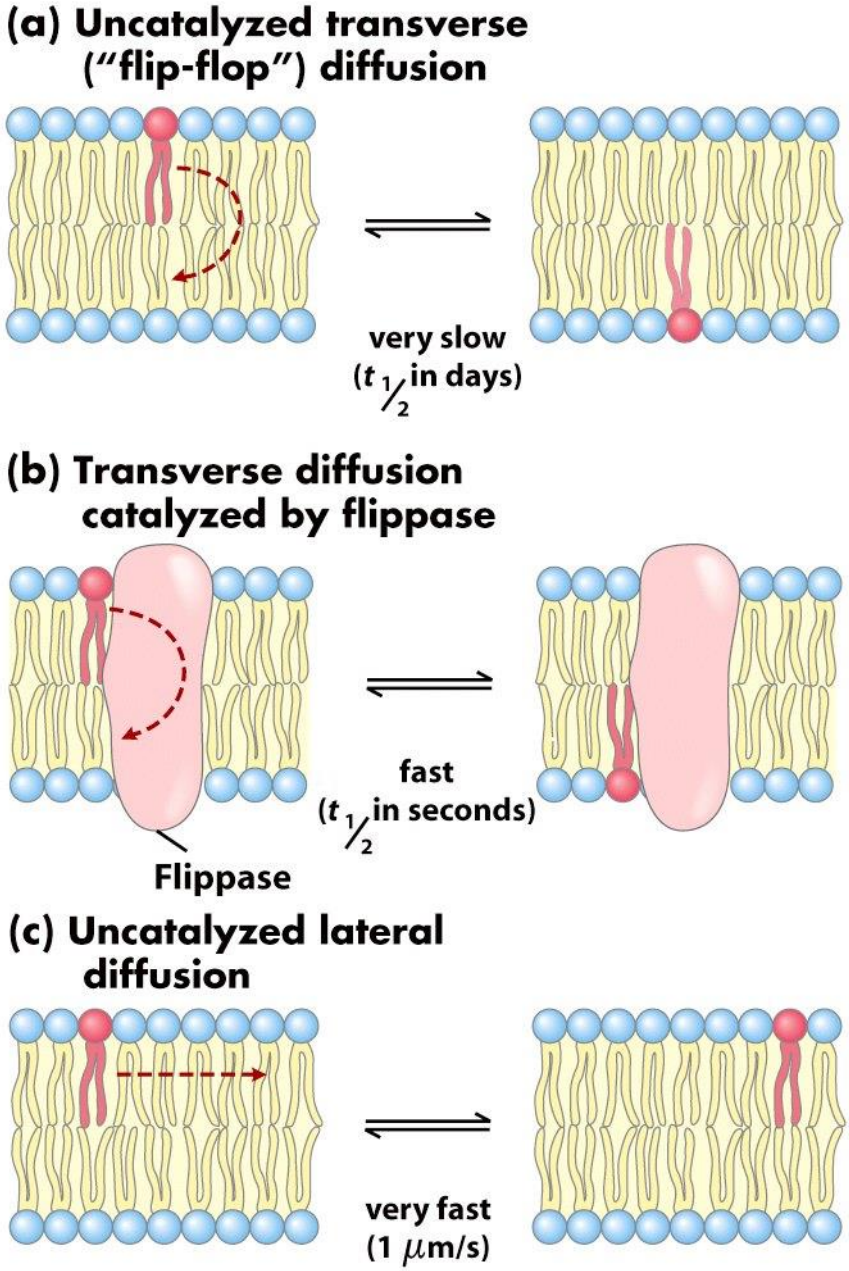
Membrane fluidity is affected by fatty acids. More specifically, whether the fatty acids are saturated or unsaturated has an effect on membrane fluidity. Saturated fatty acids have no double bonds in the hydrocarbon chain, and the maximum amount of hydrogen. The absence of double bonds decreases fluidity, making the membrane very strong and stacked tightly. Unsaturated fatty acids have at least one double bond, creating a "kink" in the chain. The double bond increases fluidity. Membrane fluidity is also affected by cholesterol. Cholesterol can make the cell membrane fluid as well as rigid.

FRAP EXPERIMENT

There is a good deal of evidence to support the fluid mosaic model of the plasma membrane:

1) Evidence supports of mosaic arrangement of proteins. Freeze fracture electron microscopy of the plasma membrane by Branton (1968) revealed the presence of bumps and depressions (7to 8 in diameter) which are randomly distributed. These were later shown to be trans membrane integral protein particles.

2) Evidence in support of fluid property of lipid bilayer. Mobility of membrane proteins due to fluid property of lipid bilaye was demonstrated by a classical experiment of D. frye and M.edidin. they fused two different types of cultured cells having different surface antigens. The cell fusion is achieved by the use of some fusogen

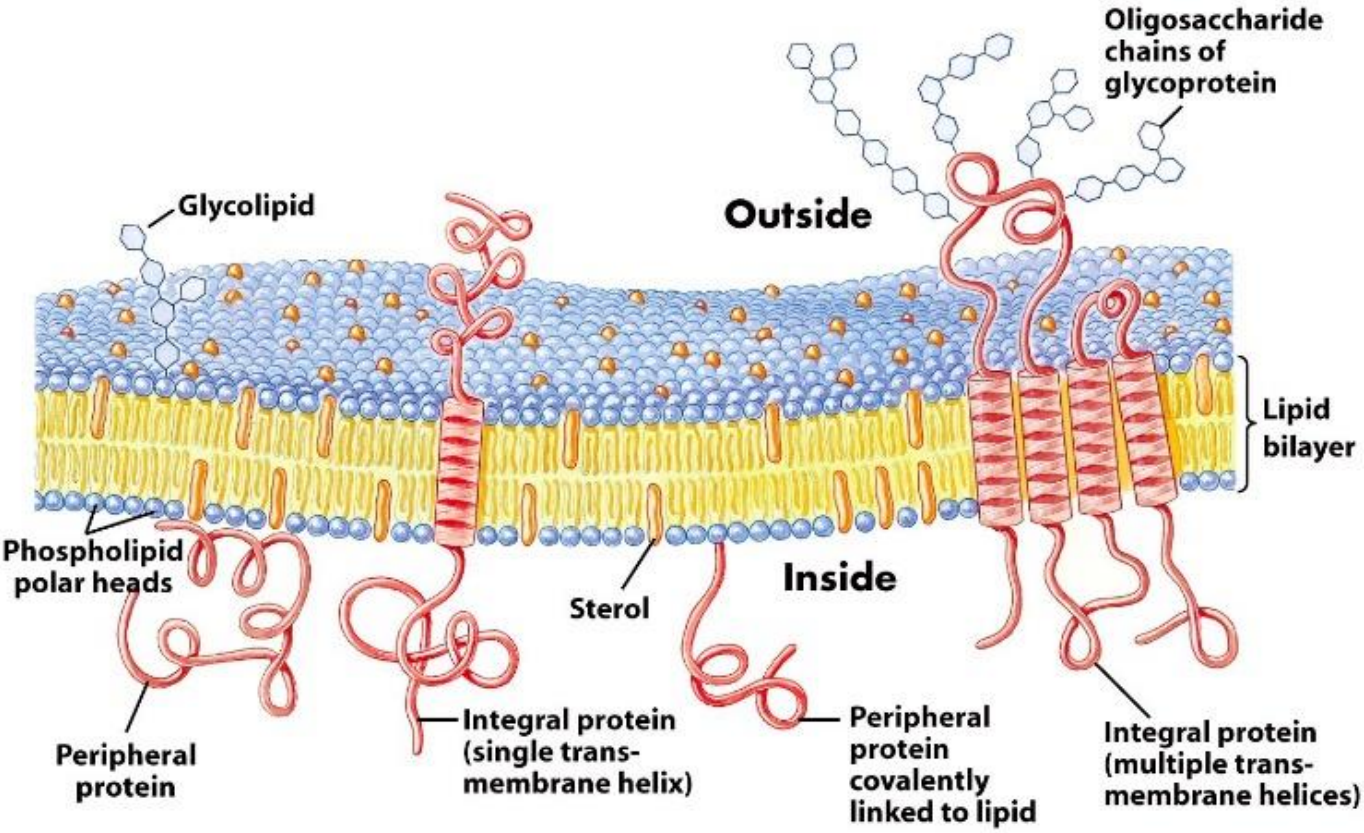


In a typical FRAP experiment, a fluorescent molecule is irreversibly bleached within a small region of interest (ROI) using high intensity laser light. Fluorescence then recovers as the population of bleached molecules is replaced by unbleached molecules from outside the ROI. Because the kinetics of this recovery reflect the underlying dynamics of the molecule of interest, FRAP experiments can tell us a great deal about the mobility of molecules within cells. Much has been gained from simple inspection of FRAP recovery curves, including determining the rates of local protein turnover, identifying immobile fractions, and demonstrating exchange between cellular compartments or lack thereof.

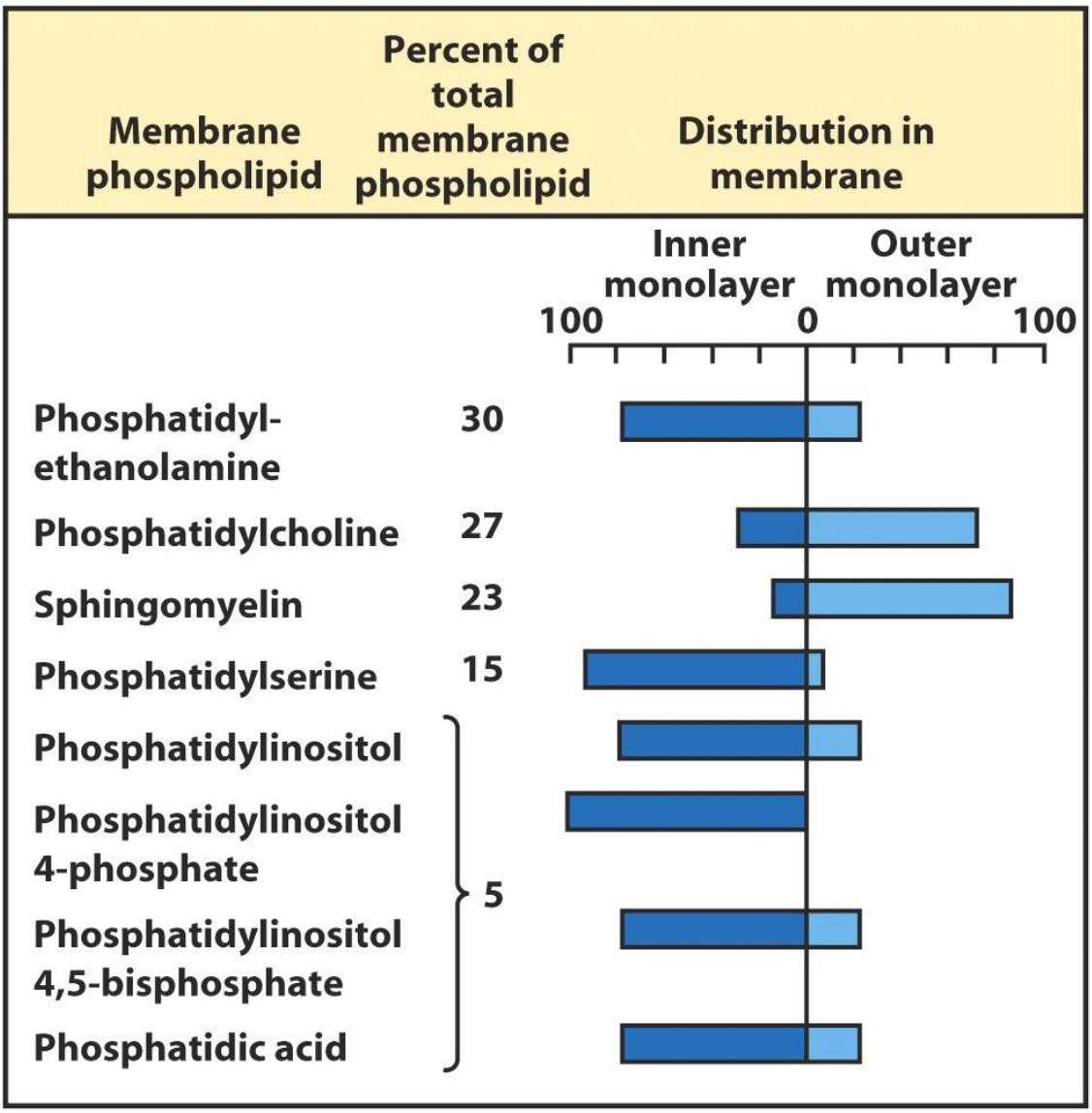
At the same time, because a molecule's dynamics within the cell will be determined by a combination of molecular mobilities, including both diffusion and bulk transport, and its binding interactions with components within the cell, FRAP experiments have the potential to allow measurement of key kinetic parameters, including the relevant rate constants.

Here we examine the case of FRAP analysis of a membrane-associated molecule. We consider a common case in which the mobility of this molecule within the cell is determined by three behaviours: reversible plasma membrane association, lateral diffusion in the membrane-associated state, and free, rapid diffusion in the cytoplasm. Analysis of FRAP recovery when binding reactions dominate is relatively straightforward. In such cases where binding can be uncoupled from the effects of diffusion, recovery should follow an exponential.

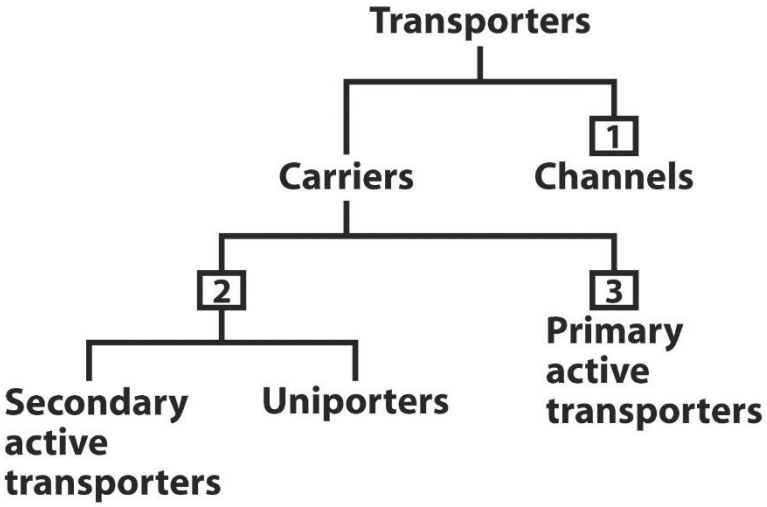
However, if diffusion contributes significantly to protein mobility at the relevant length and timescales, FRAP analysis is significantly more complicated. Notably, because recovery by diffusion is not spatially uniform across the bleached region, diffusion will change the shape of the bleached region over time, a phenomenon which itself provides evidence for lateral diffusion within cells.

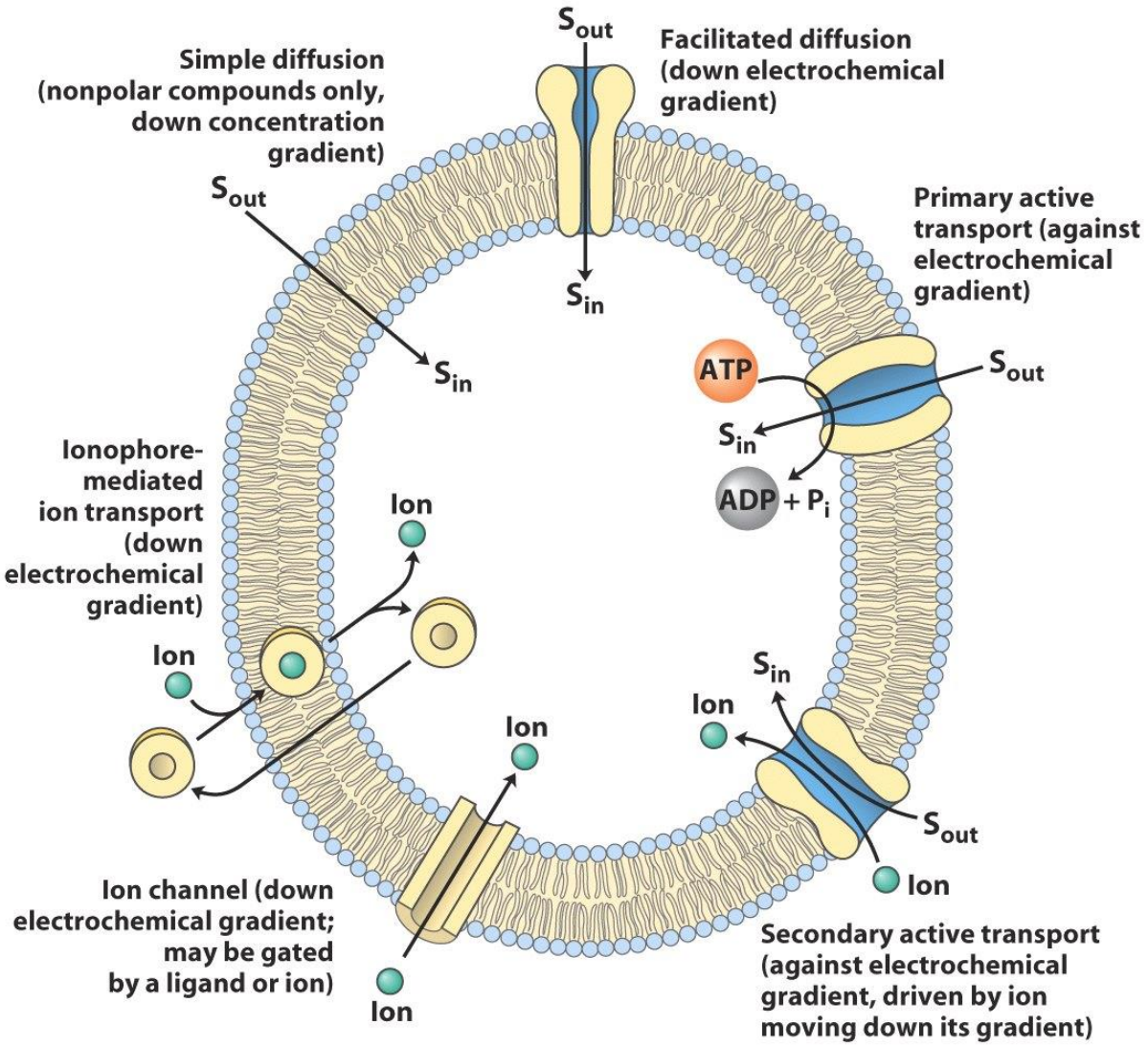


ASYMMETRIC DISTRIBUTION OF PHOSPHOLIPIDS BETWEEN THE INNER AND OUTER MONOLAYERS OF THE ERYTHROCYTE PLASMA MEMBRANE

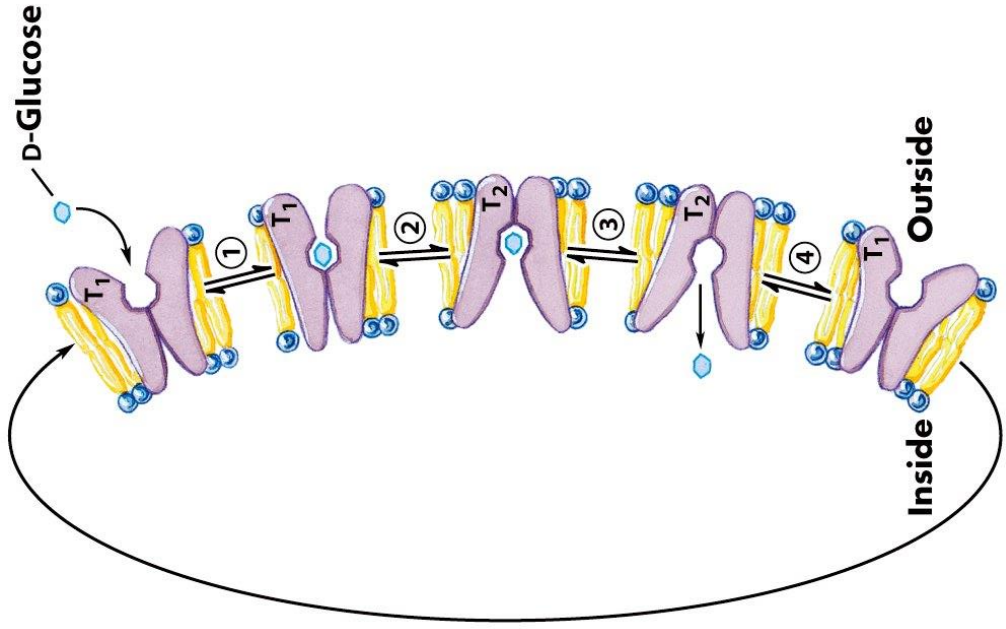


MEMBRANE TRANSPORTATION:

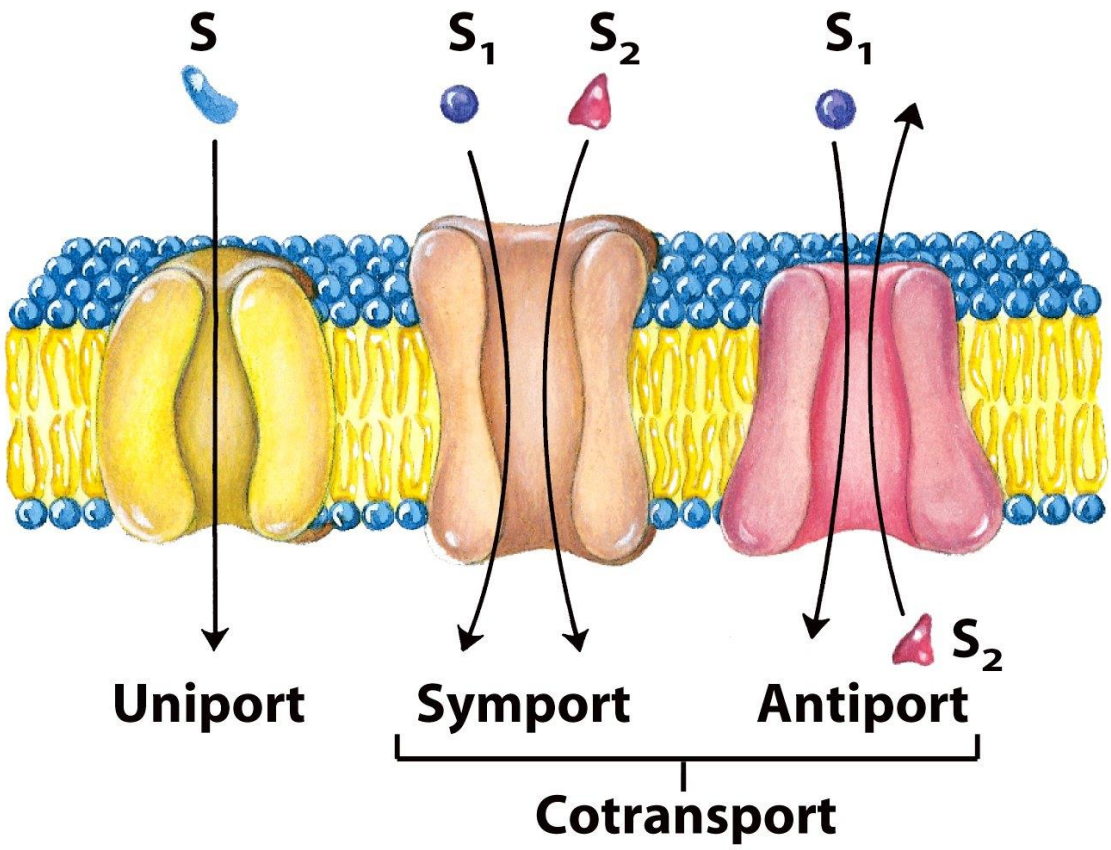




MODEL OF GLUCOSE TRANSPORT INTO ERYTHROCYTES BY GLUT1:



THREE GENERAL CLASSES OF TRANSPORT SYSTEMS:



POSTULATED MECHANISM OF Na^+ AND K^+ TRANSPORT BY THE Na^+K^+ ATPASE: