

CHAPTER II. BIOMEMBRANES

A. Membrane composition : isolation, composition

I. Isolation of plasma membrane fractions

A model cell: the erythrocyte. This cell type allows for easy isolation of the plasma membrane because there are no organelles or nucleus.

Isolation Method

- Plasma is removed by centrifugation to recover only red blood cells (RBCs);
- A hypotonic medium is added, a buffer with a lower salt concentration than that of the cell, so water moves according to the law of osmosis (from the less concentrated to the more concentrated);
- Water entering the RBC causes a rupture of the plasma membrane of this cells, which then empties its intracellular content;
- Centrifugation is performed to remove the medium and recover only the membranes of the isolated RBCs. Once the plasma membranes are isolated, their chemical composition is studied.

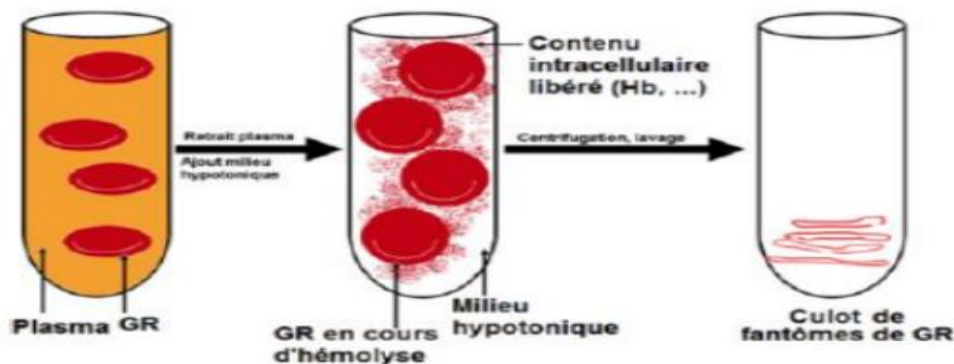


Figure II.1. Isolation of plasma membrane fragments from mammalian erythrocytes [3].

II. Chemical composition of the plasma membrane

II.1. Definition

The plasma membrane is a thin, essential barrier that surrounds the cytoplasm of a cell, thus separating the extracellular and intracellular environments. It is composed of a lipid bilayer in which proteins are inserted asymmetrically and heterogeneously. In electron microscopy, a trilamination of the membrane is observed: a clear leaflet of 3 nm surrounded by 2 dark leaflets of 2.5 nm each; the total thickness is therefore about 8 nm.

II.2. Membrane composition

Membranes are composed (by dry weight) of 40% lipids, 52% proteins, and 8% carbohydrates. Taking into account the difference in weight between these classes of molecules, there are 50 lipid molecules per protein molecule.

II.3. Diversity of membrane lipids

Lipids are the major components of the plasma membrane and all biological membranes. For each organelle membrane, the proportion of different types of lipids is different, which gives them specific functional properties. Within the membrane, lipids are present in various forms:

- **Phospholipids:** are the predominant lipids in biological membranes. They have a hydrophilic phosphorylated head and a tail formed by two aliphatic chains of saturated or unsaturated fatty acids (amphipathic molecules).

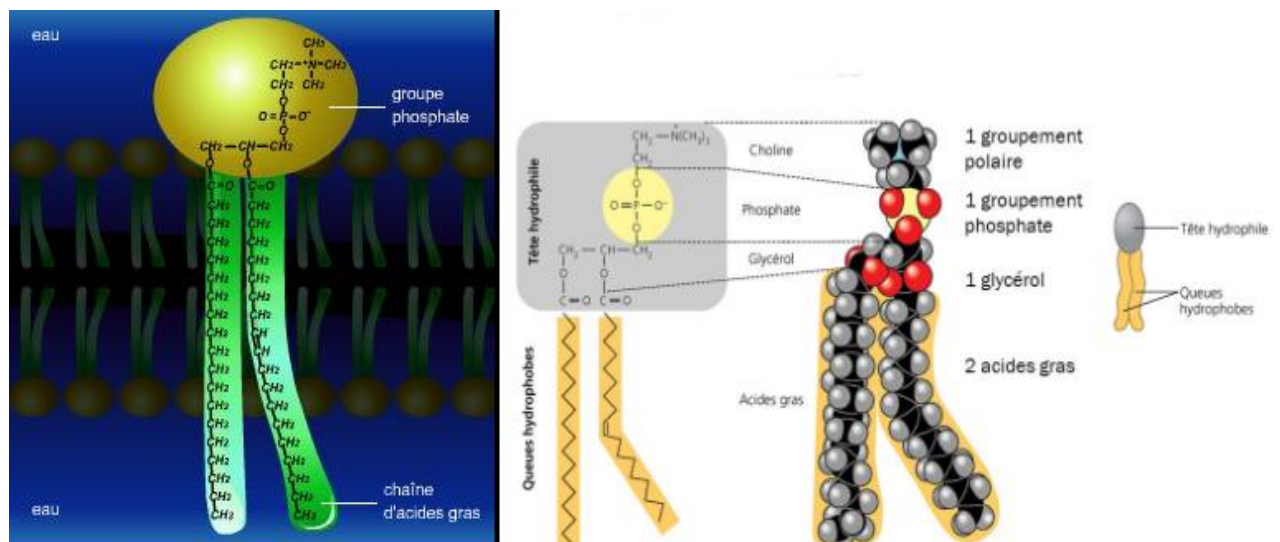


Figure II. 2. Schematic representation of a phospholipid within a lipid bilayer [4].

- **Glycolipids:** lipids whose oligosaccharide antenna is oriented toward the extracellular environment. Glycolipids play a role in molecular recognition at the surface of cell membranes. Some groups of glycolipids : Galactolipid, Glycosphingolipid, and Glycosylphosphatidyl inositol.

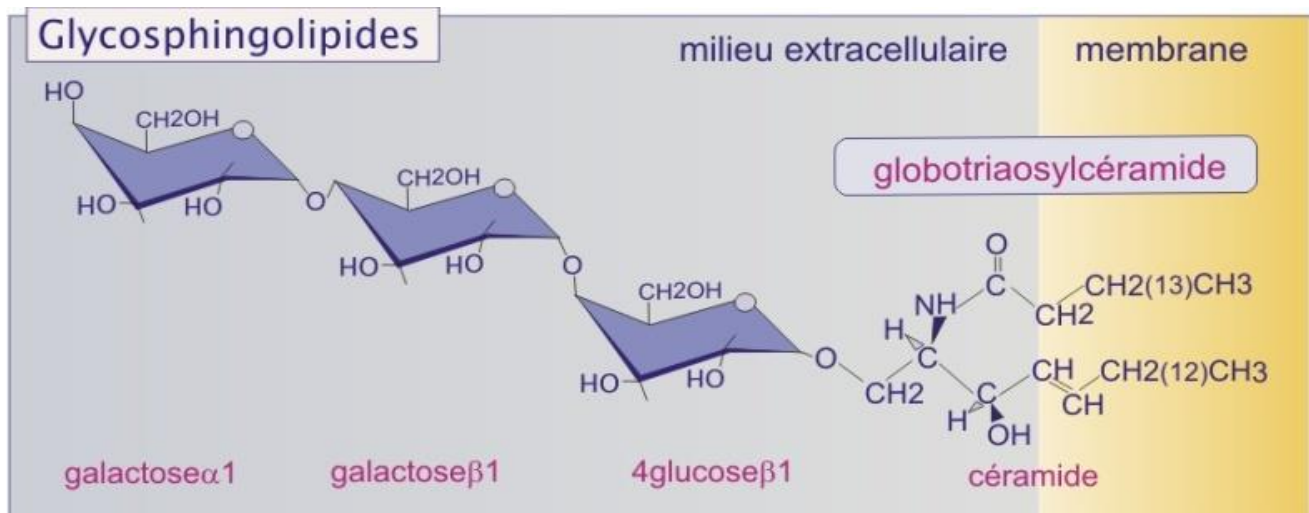


Figure II. 3. Membrane glycolipid [5].

- **Cholesterol** : A steroid-type lipid. Cholesterol is only present in the membranes of animal cells. Indeed, it is absent in plant cells and bacteria. It is composed of a hydrophobic polycyclic steroid nucleus, a hydrophobic tail, and a hydrophilic alcohol function, thus amphiphilic. It represents about a quarter of the membrane lipids and influences membrane fluidity.

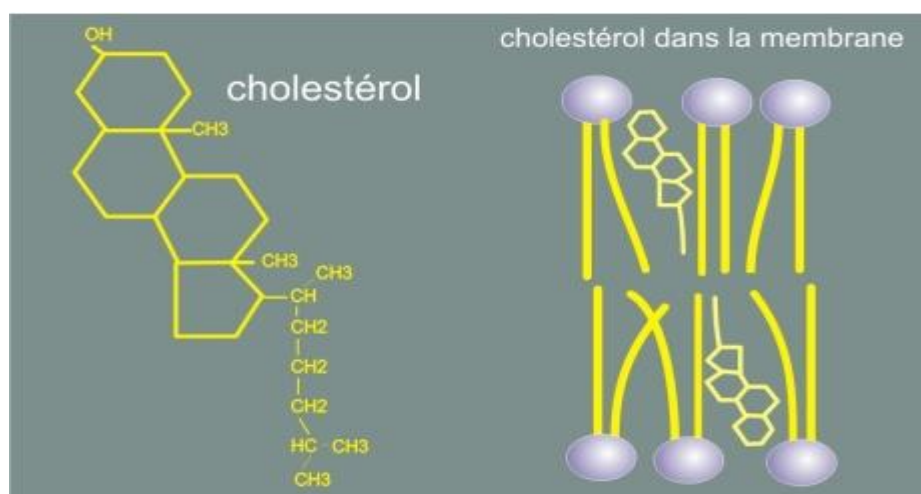


Figure II. 4. Cholesterol [5].

II.4. Diversity of membrane proteins

Many proteins are inserted into the lipid bilayers. They play a major physiological role there and are largely responsible for the characteristic activities of membranes: transport, recognition, communication, adhesion. Proteins are associated with the membrane in different ways. Proteins can either be integrated into the membrane (intrinsic or transmembrane) or attached to the membrane (extrinsic or peripheral) thru electrostatic interactions or anchors (lipid and glycolipid)

and constantly move within the membrane. We can group membrane proteins into two different classes:

II.4.1. Intrinsic proteins

Intrinsic proteins are very tightly bound to the lipid bilayer and can only be detached by the action of detergents or organic solvents that disrupt the lipid bilayer organization. They can be subdivided into two categories:

- **Transmembrane proteins:** with one or more transmembrane segments. They interact through non-covalent, but high-energy bonds with lipids via a domain rich in hydrophobic amino acids (phenylalanine, leucine, valine, tryptophan...).
- **Lipid-anchored proteins:** located outside the lipid bilayer, either on the extracellular face or the cytoplasmic face, but covalently bonded to a lipid molecule found inside the lipid bilayer.

II.4.2. Extrinsic proteins

Peripheral proteins are located entirely outside the lipid bilayer, either on the cytoplasmic side or on the extracellular side, but are associated with the membrane by non-covalent bonds.

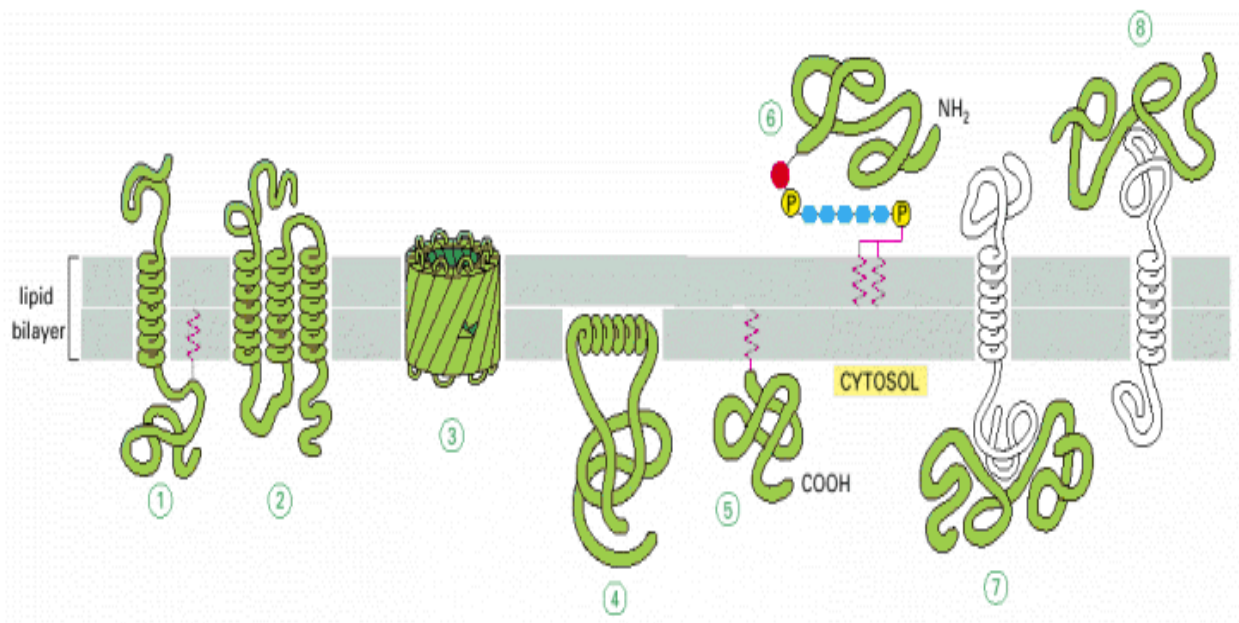


Figure II. 5. Different modes of protein association with the lipid bilayer [6]. Transmembrane proteins. (1) crosses the double layer in the form of an alpha helix. (2) crosses the double layer in the form of multiple alpha helices. (3) crosses the double layer in the form of a beta sheet barrel. Proteins associated with only one side of the membrane. (4) anchored by an amphiphilic helix.

(5) covalent bond with a fatty acid (cytoplasmic). (6) covalent bond with glycosyl-phosphatidyl-inositol or GPI. (7 and 8) non-covalent interaction with other membrane proteins.

II.5. Diversity of membrane carbohydrates

Carbohydrates are the third main component of plasma membranes. In general, they are always found on the outer surface of cells and are bound either to proteins (forming glycoproteins) or to lipids (forming glycolipids). These carbohydrate chains can be composed of 2 to 60 monosaccharide units and can be straight or branched (forming the glycocalyx). They have very important and varied physiological roles:

- Certain families of glycoproteins are involved in the adhesion of cells to each other and to the extracellular matrix;
- The antigens of blood groups A and B, present on the surface of red blood cells, are glycolipids derived from sphingomyelin;
- Galactocerebroside is the main glycolipid in myelin, which is found around certain axons.

Table II.1. The components of the plasma membrane [7].

Component	Location
Phospholipids	Phospholipids Main component of the membrane
Cholesterol	Nestled between the hydrophobic tails of phospholipid membranes
Integral proteins	Embedded in the lipid bilayer; may or may not extend across both layers
Peripheral proteins	On the inner or outer surface of the lipid bilayer, but not integrated into its hydrophobic core
Carbohydrates	Attached to proteins or lipids on the extracellular side of the membrane (formation of glycoproteins and glycolipids).

B. Biomolecular architecture of membranes

I. The lipid bilayer

Electron microscopy of thin membrane sections labeled with osmium tetroxide, which binds strongly to the polar head groups of phospholipids, reveals the bilayer structure. Two thin dark

lines (the complexes whose head groups are labeled) separated by a uniform clear space around 2 nm (the hydrophobic tails).

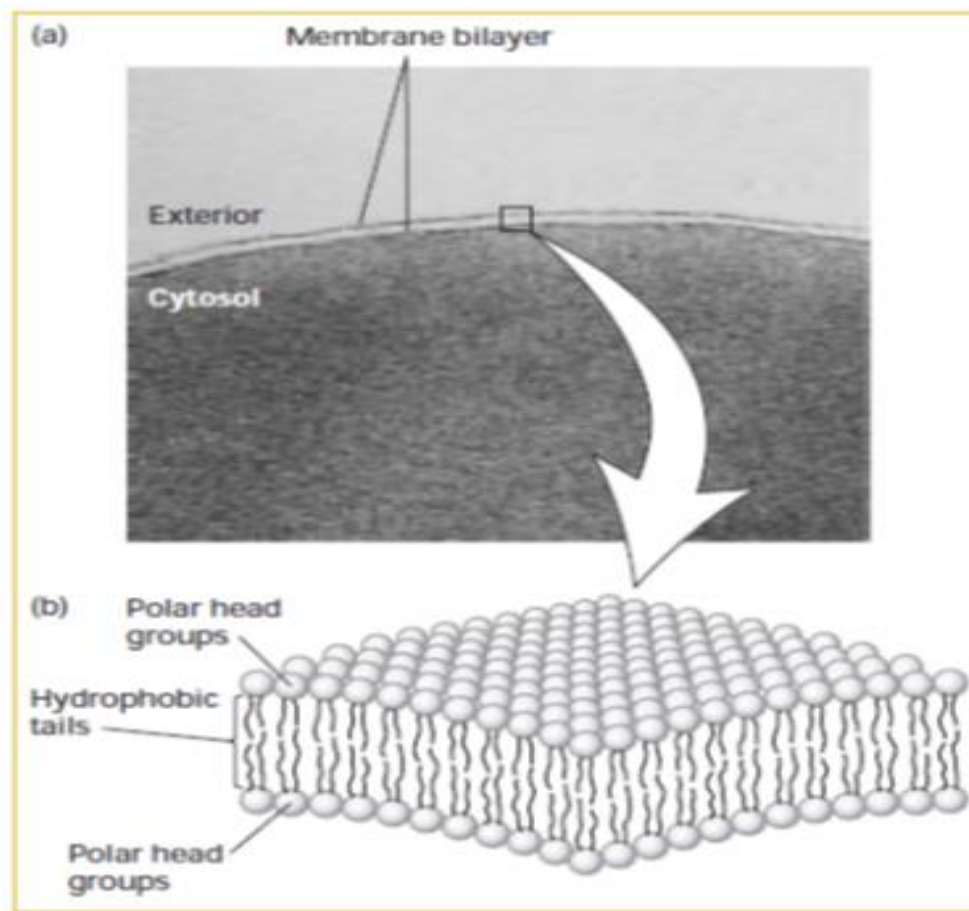


Figure II. 6. The bilayer structure of biomembranes [8]. (a) corresponds to an electron micrograph of a thin section of a membrane. The characteristic “railroad track” appearance of the membrane indicates the presence of two polar layers, which correspond to the bilayer structure of phospholipid membranes. (b) is a schematic interpretation of the phospholipid bilayer in which the polar groups face outward to protect the hydrophobic fatty acid tails from water. Hydrophobic and Van der Waals interactions between the fatty acid tails are responsible for the assembly of the bilayer.

II. The « fluid mosaic » membrane model

In the 1970s, Singer and Nicolson developed the “fluid mosaic” model, which suggests that the plasma membrane is composed of a fluid lipid bilayer hosting various membrane proteins. The lipids are organized into a bilayer made up of phospholipids and cholesterol, in which there are several proteins (intrinsic and peripheral).

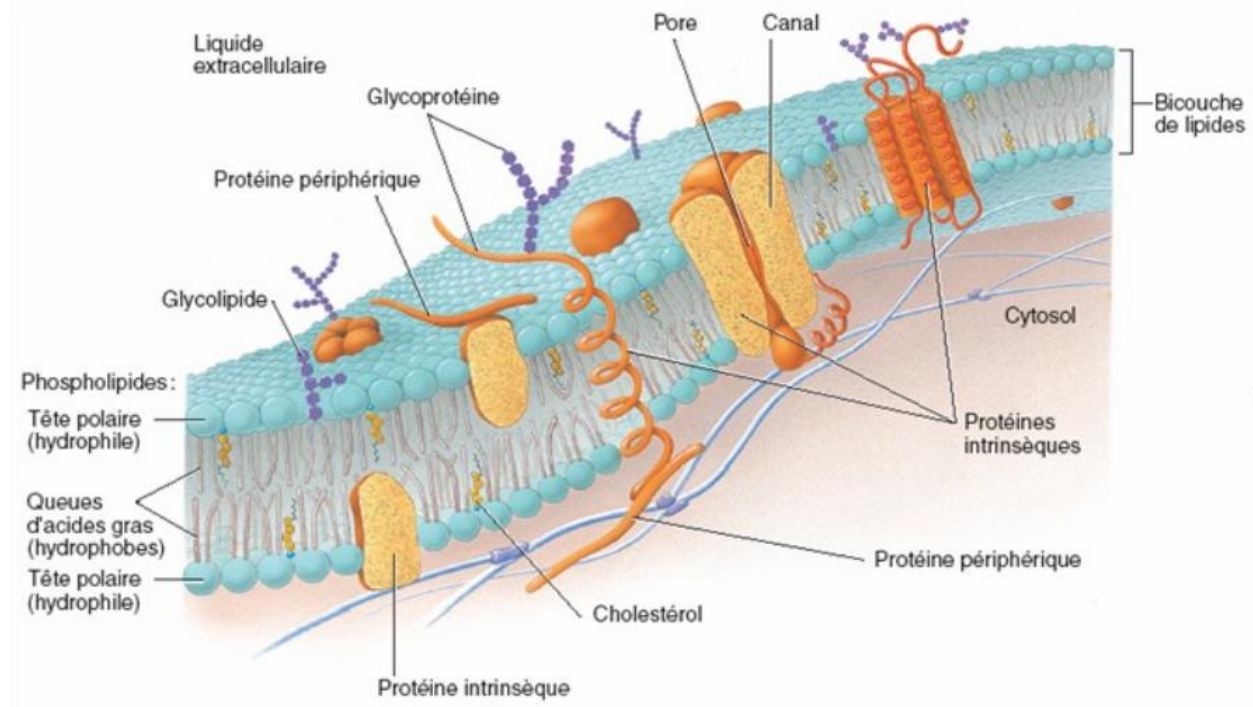


Figure II. 7. Fluid mosaic plasma membrane model [9].

III. Characteristics of the mosaic model

1. **The membrane is mosaic:** it is made up of different elements juxtaposed together: two layers of lipids in which globular proteins are inserted.
2. **The membrane is fluid:** these are quasi-fluid structures in which the lipids and integrated proteins are capable of translational movements within the entire bilayer.

Factors affecting the fluidity of the PM:

- **Temperature :** an increase in temperature causes an acceleration of movement..
 - **The amount of cholesterol:** cholesterol strengthens the solidity and rigidity of the membrane and accounts for up to 50% of the total lipids in the membrane.
 - **Fatty acid composition:** the shorter and more unsaturated the carbon chains of the fatty acids, the more fluid the membrane.
 - **Number of proteins:** proteins decrease membrane fluidity.
3. **The membrane is asymmetrical :** All biological membranes are made up of layers with different lipid compositions, except for cholesterol, which is found in equal amounts in both layers and can easily switch from one to the other.
 - The inner layer is characterized by phosphatidylserine (amphoteric) and phosphatidylethanolamine (negative charge).

- The outer layer is characterized by sphingomyelin (negative charge), phosphatidylcholine (negative charge), and glycolipids.

The asymmetry of the lipids thus leads to an asymmetry in the overall charge of each layer. There is also an asymmetry in the proteins present in the phospholipid bilayer; these proteins help to characterize the properties of the membrane, whether on the intracellular or extracellular side.

The greatest asymmetry is found in carbohydrates, as all carbohydrate motifs are located on the outer leaflet of the plasma membrane, forming what is known as the glycocalyx.

IV. Self-assembly of lipids

When membrane lipids are in an aqueous phase, they can organize themselves in several different ways:

- **Monolayers:** Monolayers these are single-molecular layers whose hydrophilic heads face the aqueous medium and whose hydrophobic tails face the lipid medium.
- **Micelles:** Micelles these are formations in the form of round droplets, where in an aqueous medium the hydrophilic heads are directed towards the outside of the sphere and the hydrophobic tails are directed towards the inside (in a lipid medium the conformation is reversed). They are obtained following treatment of the plasma membrane with detergents.
- **Lipid bilayers:** the polar heads are directed outward, in contact with the aqueous medium. The nonpolar tails are directed toward the center, where they interact hydrophobically with each other and are protected from the aqueous medium by the polar heads.
- **Liposomes:** these are small spherical vesicles bounded by a double lipid layer and filled with an aqueous medium. Liposomes are currently used in therapeutics to encapsulate medicinal substances.

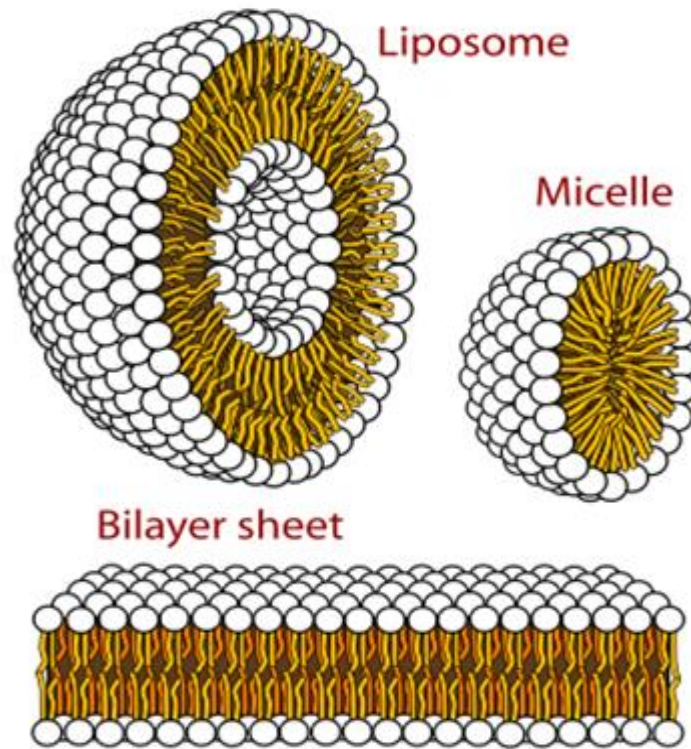


Figure II. 8. Membrane assembly [10].

V. Lipid movements

Membrane lipids exhibit three types of movement:

- Lateral diffusion within the lipid bilayer at high speed (a lipid can change places with its neighbor 7×10^8 times per second);
- Rotation of lipids in place is also common;
- Passage from one layer to another is rarer, occurring less than once a week (a phenomenon known as lipid flip-flop, which involves a specialized protein called flippase and consumes energy). Cholesterol passes easily from one layer to another.

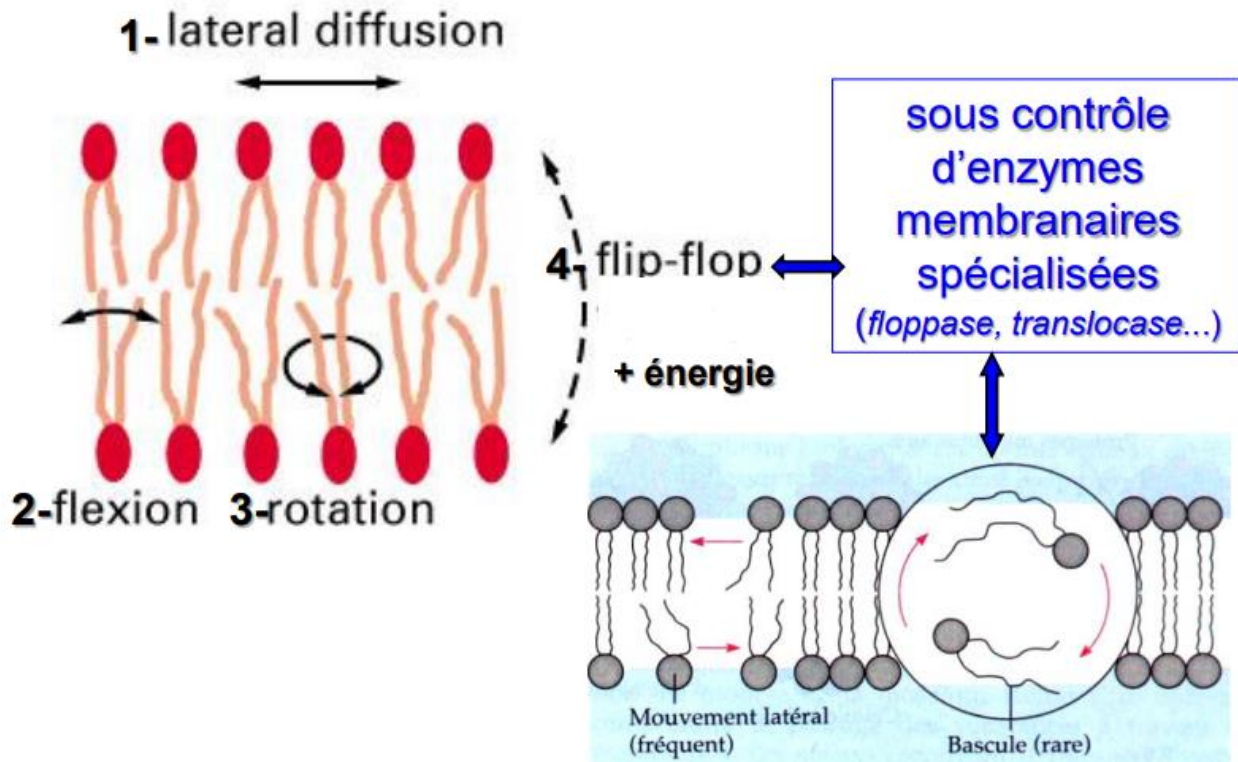


Figure II. 9. Types of membrane lipid movement [11].

VI. Protein movements

The rotation of proteins in place is comparable to that of lipids.

- The flip-flop phenomenon does not occur in membrane proteins;
- The most important phenomenon for cell physiology is the lateral diffusion of certain proteins;
- The movements of certain transmembrane proteins may be limited or prohibited by mechanisms that may also be associated, such as their anchoring to the cytoskeleton by extrinsic proteins on the cytosolic side, their interaction with the components of the extracellular matrix, or interaction with other proteins of the same type in the membrane or with molecules carried by two cells in contact or joined together.