Membranes

Every living **cell** is surrounded by a membrane

1. Barrier between the cell and the external environment
2. Barrier between cell compartments
3. Selectively passes some molecules
4. Communication between cell and external medium
5. Molecular sensor

All membranes contain lipids, proteins and other molecules. The protein:lipid ratio varies greatly:

- The inner mitochondria membrane is 76% protein.
- The myelin membrane is only 18% protein.

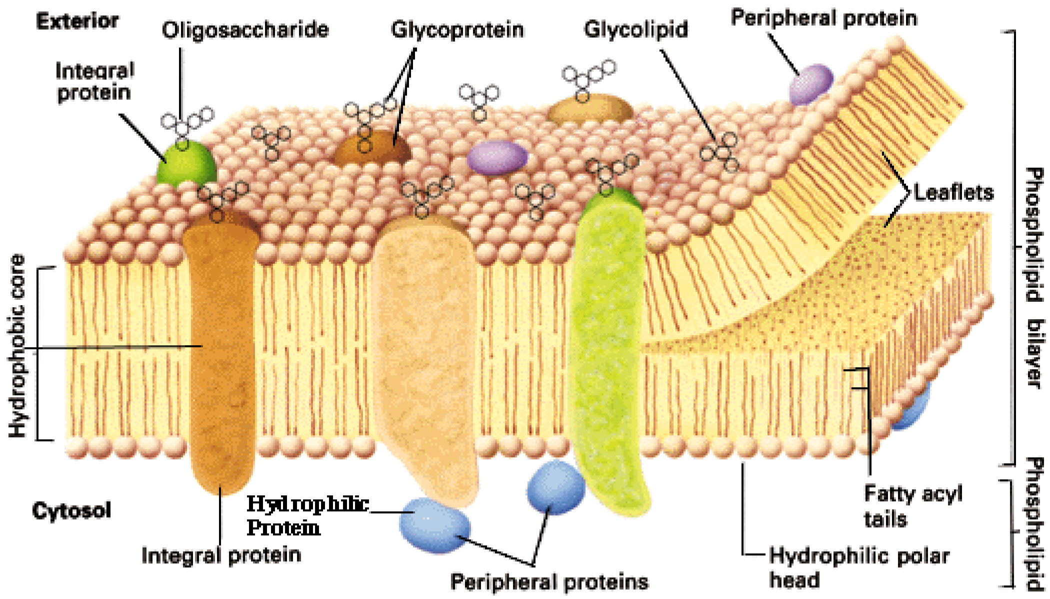
Because of its high phospholipid content, myelin can electrically insulate the nerve cell from its environment. The lipid composition varies among different membranes. All membranes contain a substantial proportion of phospholipids, predominantly phosphoglycerides, which have a glycerol backbone.

The composition chemical composition of membranes is not exactly determined by the genetic material, although the genetic material codes for the enzymes that synthesize lipids. Also, the assembly of the membrane proceeds along different routes than the assembly of proteins and the replication of DNA. The membrane composition depends on some extent on the diet.

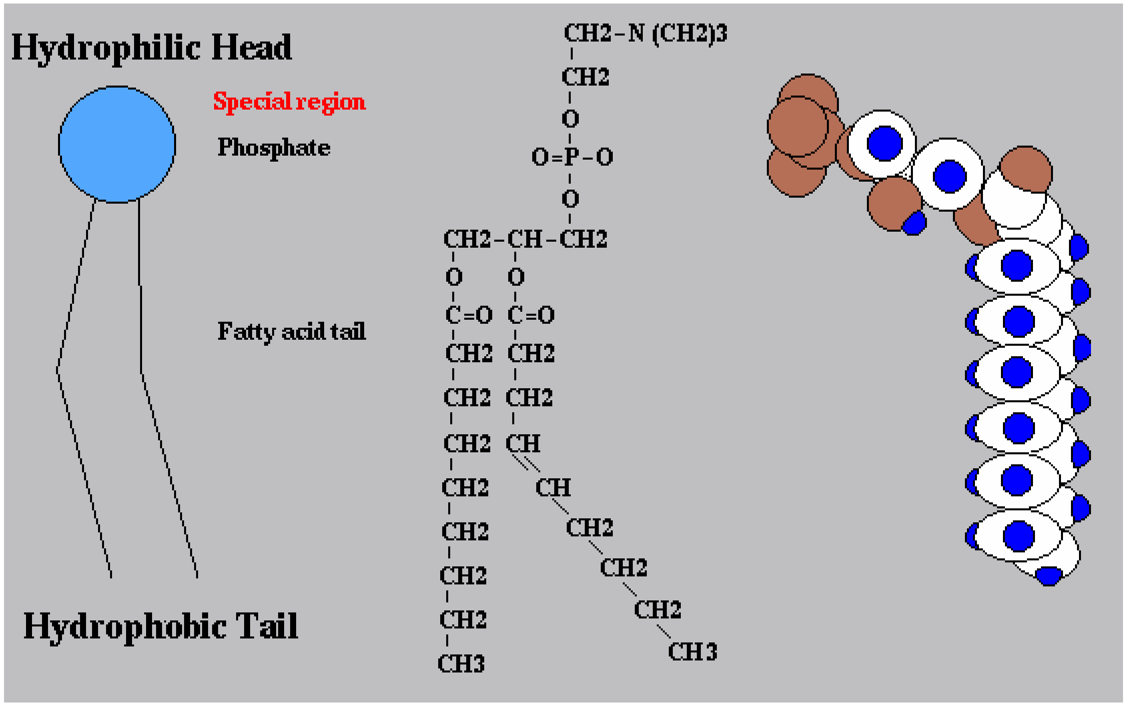
Self assembly is determined by the **Hydrophobic/Hydrophilic** character of the membrane components

A very simplistic idea of what these characteristics are is:

Hydrophilic and hydrophobic are, respectively, the like and dislike. Hydrophilic areas of a phospholipid or a protein are 'attracted' to water, and hydrophobic regions are repelled by water.

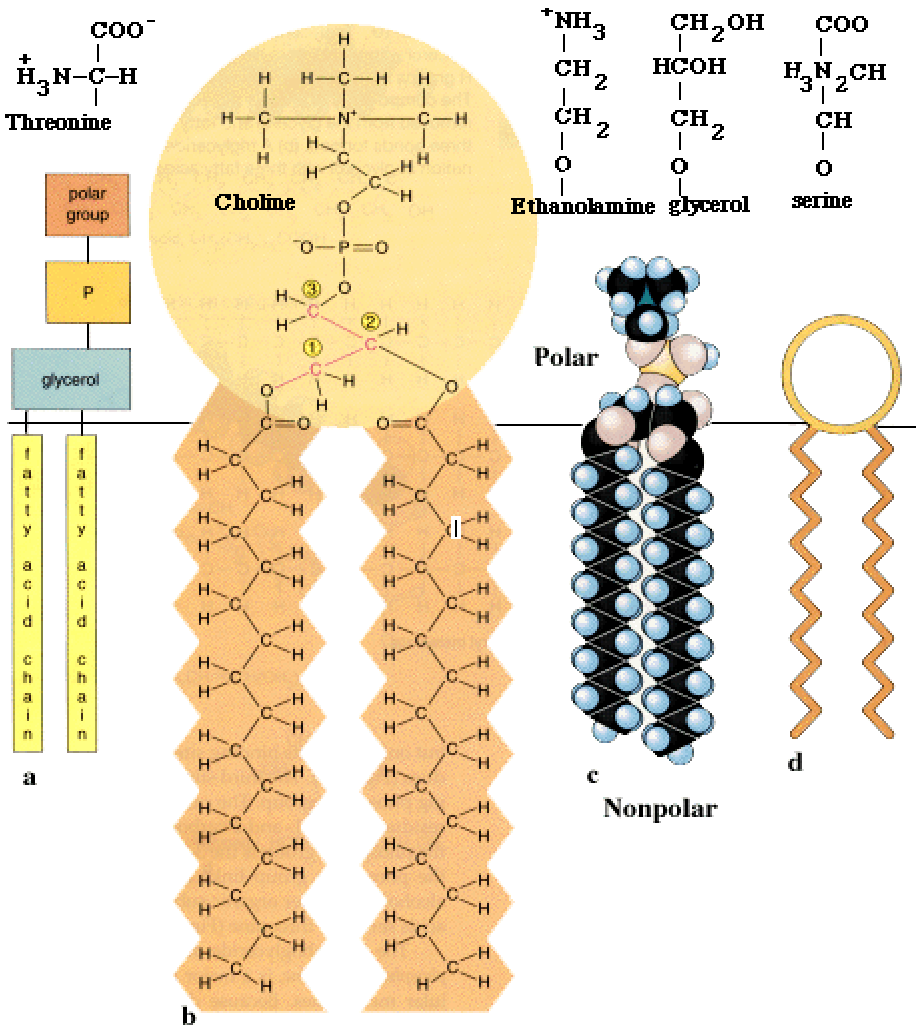

Exterior
Oligosaccharide
Glycoprotein
Glycolipid
Peripheral protein
Integral protein
Leaflets
Phospholipid bilayer
Hydrophobic core
Cytosol
Hydrophilic Protein
Integral protein
Peripheral proteins
Fatty acyl tails
Hydrophilic polar head
Phospholipid

Phospholipids are made up of a hydrophilic head and a hydrophobic tail. The head group has a 'special' region that changes between various phospholipids. This head group will differ between cell membranes [types of cells] or different concentrations of specific 'head groups'. The fatty acid tails could also differ, but there is always one saturated and one unsaturated 'leg' of the tail.


Hydrophilic Head
Special region
Phosphate
Fatty acid tail
Hydrophobic Tail
CH2-N (CH2)3
CH2
O
O=P-O
O
CH2-CH-CH2
O O
C=O C=O
CH2 CH2
CH2 CH2
CH2 CH
CH2 CH
CH2 CH2
CH2 CH2
CH2 CH2
CH3 CH3

Phospholipids contain 2 fatty acids one saturated and one unsaturated (shown by the double bond) that are linked to a glycerol.

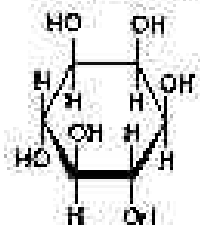
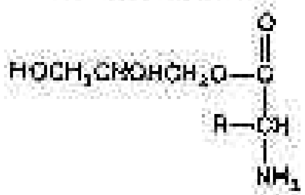
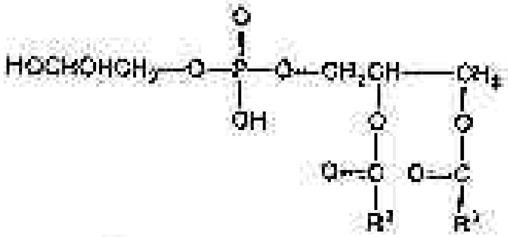
Phosphatidyl choline is a major lipid component of cellular membranes. Because different fatty acids may bind at the 1 and 2 carbons of the glycerol residue in phospholipids of this type, phosphatidyl choline is actually a family of closely relate molecules differing in the particular fatty acids present.


Threonine
Choline
Ethanolamine
glycerol
serine
polar group
P
glycerol
fatty acid chain
fatty acid chain
a
b
Polar
c
Nonpolar
d

The colored blocks represent the arrangement of subunits in phospholipids (a). Structure (b) represents the formula for phosphatidyl choline, a common membrane phospholipid. (c) is the space-filling model of phosphatidyl choline and (d) is a diagram widely used to depict a phospholipid molecule. The circle represents the polar end of the molecule and the zigzag lines the nonpolar carbon chains of the fatty acid residues.

Table 4-3

Alcohol forms of polar X groups found in major phosphoglycerides

Phosphoglyceride	Alcohol component
Phosphatidyl ethanolamine	$HOCH_2CH_2NH_2$
Phosphatidyl choline	$HOCH_2CH_2N(CH_3)_3$ Lecithin
Phosphatidyl serine	$HOCH_2CHNH_2COOH$
Phosphatidyl inositol	
Phosphatidyl glycerol	$HOCH_2CHOHCH_2OH$
Phosphatidyl-3'-O-aminoacyl glycerol	 $HOCH_2CHOHCH_2O-\overset{\overset{O}{\Vert}}{C}-\underset{\underset{NH_2}{\vert}}{CH}-R$
Cardiolipin	 $HOCHOHCH_2-O-\underset{\underset{OH}{\vert}}{\overset{\overset{O}{\vert}}{P}}-O-CH_2\underset{\underset{O=\underset{\underset{R^1}{\vert}}{C}}{\vert}}{\underset{\vert}{CH}}\ ---\ \underset{\underset{O=\underset{\underset{R^2}{\vert}}{C}}{\vert}}{\underset{\vert}{CH_2}}$

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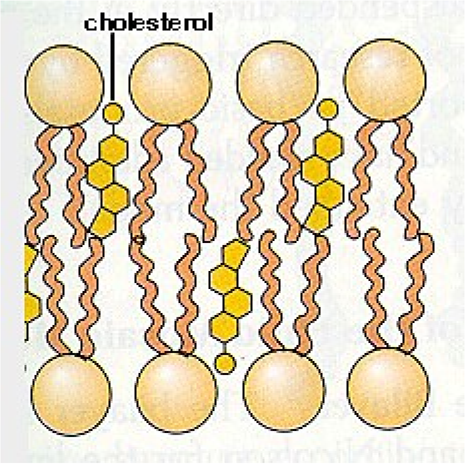
[a] The site of condensation with phosphate is indicated by the colored hydrogen atom.

Cholesterol:

Cholesterol and its derivatives constitute another important class of membrane lipids, the steroids. The basic structure of steroids is the four-ring hydrocarbon shown. Cholesterol is the major steroid constituent of animal tissues; other steroids play more important roles in plants. Although cholesterol is almost entirely hydrocarbon in composition, it is amphipathic because it contains a hydroxyl group that interacts with water. Cholesterol is especially abundant in plasma membrane of mammalian cells but is absent fro most prokaryotic cells.

Cholesterol is a major component of cell membranes and serves many other functions as well. Cholesterol helps to 'pack' phospholipids in the membranes, thus giving more rigidity to the membranes. Also cholesterol serves diverse functions such as: it is converted to vitamin D (if irradiated with Ultra Violet light), modified to form steroid hormones, and is modified to bile acids to digest fats.

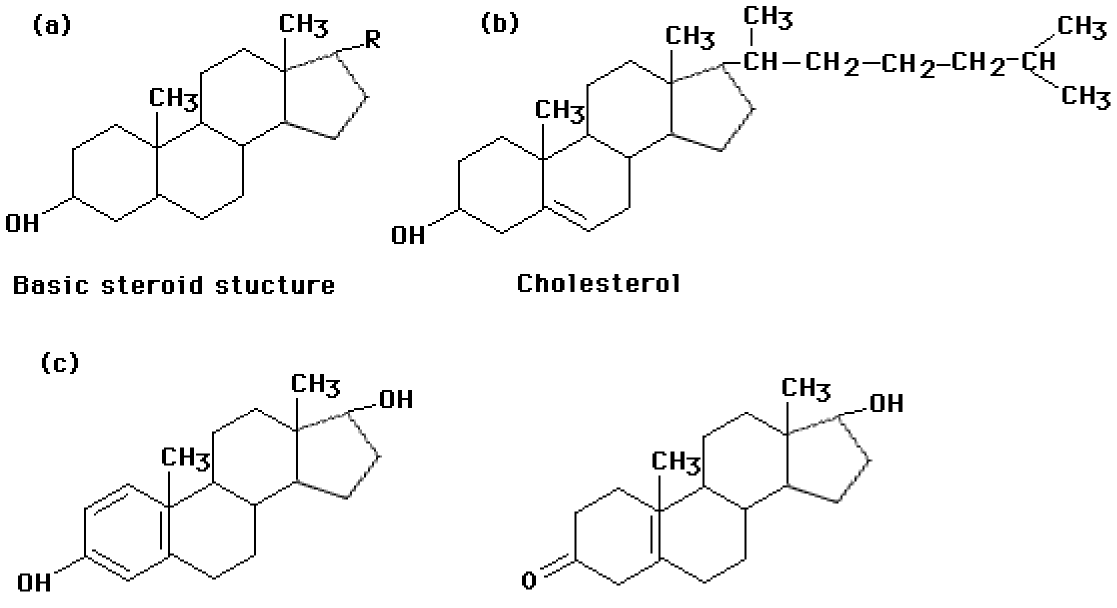
The cholesterol molecule inserts itself in the membrane with the same orientation as the phospholipid molecules. The figures show phospholipid molecules with a cholesterol molecule in between. Note that the polar head of the cholesterol is aligned with the polar head of the phospholipids. Figure modified from *Alberts et al. Molecular Biology of the Cell, Garland Publishing, N.Y., 1994, Third Edition, Figure 10-9; or Wolfe S.L., Molecular and Cellular Biology, Wadsworth Publishing Company, 1993 (figure below).*



Cholesterol molecules have several functions in the membrane:

- They immobilize the first few hydrocarbon groups of the phospholipid molecules. This makes the lipid bilayer less deformable and decreases its permeability to small water-soluble molecules. Without cholesterol (such as in a bacterium) a cell would need a cell wall.

- Cholesterol prevents crystallization of hydrocarbons and phase shifts in the membrane.


(a)
CH3
R
CH3
OH
(b)
CH3
CH3
CH–CH2–CH2–CH2–CH
CH3
CH3
CH3
OH
(c)
CH3
OH
CH3
OH
CH3
OH
CH3
O

Basic steroid stucture

Cholesterol

Estradiol

Testosterone

Fig. (a) Basic structure of the steroids. (b) Cholesterol (c) Estradiol, a mammalian female sex hormone. (d) Testostorone, a mammalian male sex hormone

Nearly all fatty acyl chains found in the membranes of eukaryotic cells have:

- an even number of carbon atoms (usually 16, 18, or 20).
- Unsaturated fatty acyl chains normally have one double bond

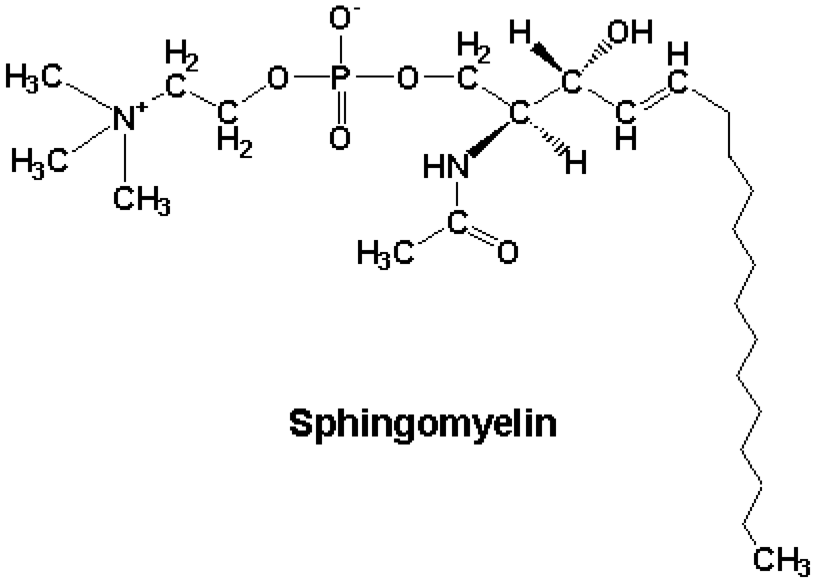
- but some have two, three ore four. In general, all such double bonds are of the cis configuration:

A cis double bond introduces a rigid kink in the otherwise flexible straight chain of a fatty acid.

A minor difference among phospholipids concerns the charge carried by the polar head groups at neutral pH. Some phosphoglycerides (phospatidylcholine, phosphatidylethanolamine) have no net electric charge (but they have a dipole!); others (9 phosphatidylglycerol, cardiolipin, phosphatidyserine) have a net negative charge. A few rare phospholipids carry a net positive charge at neutral pH.

Nonetheless, the polar head groups in all phospholipids can pack together into the characteristic bilayer structure. Sphingomyelin and glycolipids are similar in shape to phosphoglycerides and can form bilayer with them.

All membrane phospholipids are amphipatic, having both hydrophilic and hydrophobic portions. Sphingomyelin, phospholipid that lacks a glycerol backbone, also is commonly found in plasma membranes. Instead of a glycerol backbone it contains sphingosine, an amino alcohol with a long unsaturated hydrocarbon chain. A fatty acyl side chain is linked to the amino group of sphingosine by an amide bond to form a ceramide. The terminal hydroxyl group of sphingosine is esterified to phosphocholine, thus the hydrophilic head of sphingomyelin is similar to that of phosphatidyl choline.



Sphingomyelin

Semi-permeable/Osmosis

The membranes of cells are a fluid, they are semi-permeable, which means some things can pass through the membrane through osmosis or diffusion. The rate of diffusion will vary depending on: size, polarity, charge and concentration on the inside of the membrane versus the concentration on the outside of the membrane. When something is permeable it means that something can spread throughout, like (*The perfume is permeating the room.*). Here is a list of some molecules and how they relate to passing through the membrane without assistance, in other words, through osmosis:

Hydrophobic Molecules:

O_2 - Oxygen
$N_{\underline{2}}$ - Nitrogen
benzene

Small uncharged Polar Molecules:

H_2O - Water
urea
glycerol
$C0_2$ - Carbon Dioxide

Large Uncharged Polar Molecules:

Glucose
Sucrose

Ions:

H^+ - Hydrogen ion
Na^+ - Sodium ion
K^+ - Potassium ion
Ca^{2+} - Calcium ion
Cl^- - Chloride ion

Various substances will pass through the membranes at varying rates through osmosis.

Membrane proteins:

One role of proteins in cells is for transport of molecules/ions into or out of cells. Three methods of doing this are through active, facilitated or passive transport. Other roles are in cell recognition, receptors, cell to cell communication. There is more information on membrane proteins and other proteins in later sections.

Phospholipid Bilayer the Basic Structural Unit of membranes

Despite the variable compositions of biological membranes, the basic structural unit of virtually all biomembranes is the phospholipid bilayer. This bilayer is a sheet like structure composed of two layers of phospholipid molecules whose polar head groups face the surrounding water and whose fatty acyl chains form a continuous hydrophobic interior and 3 nm thick. Each phospholipid layer in this lamellar structure is called a leaflet. The major driving force for the formation of phospholipid bilayers is hydrophobic interaction between the fatty acyl chains of glycolipid and phospholipid molecules. Van der Waals interactions among the hydrocarbon chains favor close packing of these hydrophobic tails. Hydrogen bonding and electrostatic interactions between the polar head groups and water molecules also stabilize the bilayer. Micelles are generally not formed by phospholipids in aqueous solutions, since the fatty acyl chains in sphingomyelins, glycolipids, and all phosphoglycerids are too large to fit into the interior of a micelle.

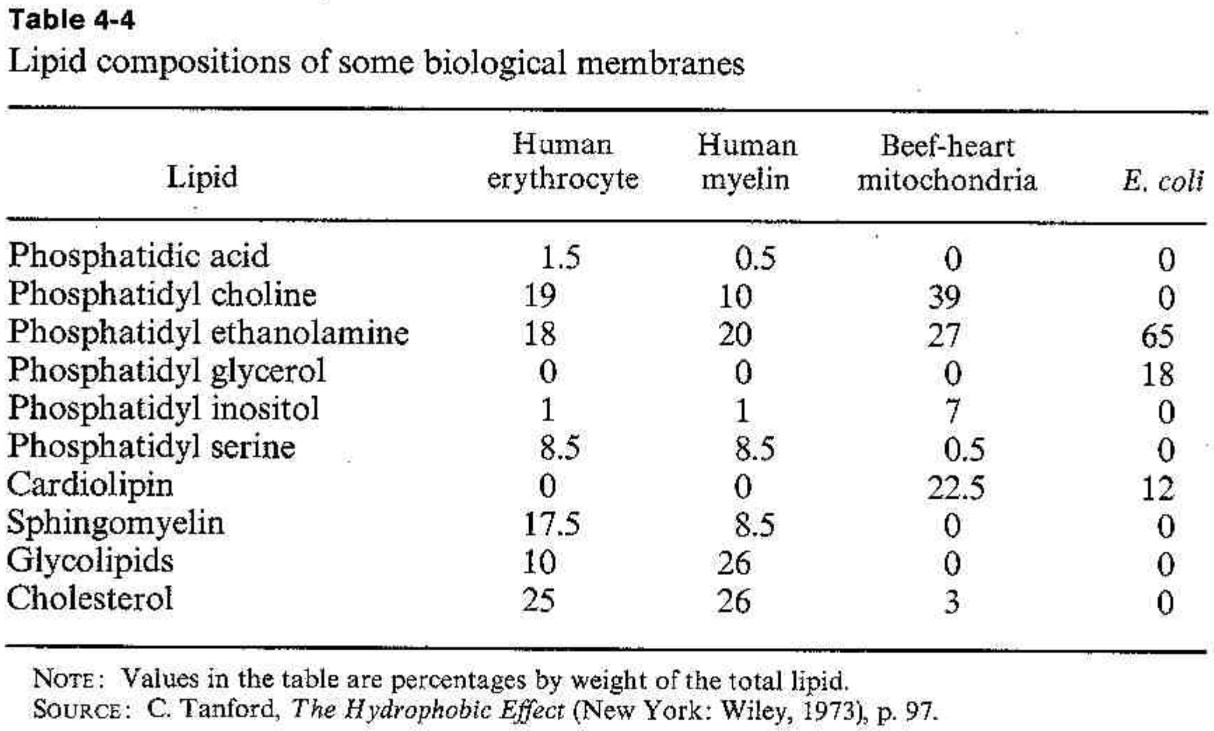


Table 4-4

Lipid compositions of some biological membranes

Lipid	Human erythrocyte	Human myelin	Beef-heart mitochondria	*E. coli*
Phosphatidic acid	1.5	0.5	0	0
Phosphatidyl choline	19	10	39	0
Phosphatidyl ethanolamine	18	20	27	65
Phosphatidyl glycerol	0	0	0	18
Phosphatidyl inositol	1	1	7	0
Phosphatidyl serine	8.5	8.5	0.5	0
Cardiolipin	0	0	22.5	12
Sphingomyelin	17.5	8.5	0	0
Glycolipids	10	26	0	0
Cholesterol	25	26	3	0

NOTE: Values in the table are percentages by weight of the total lipid.
SOURCE: C. Tanford, *The Hydrophobic Effect* (New York: Wiley, 1973), p. 97.

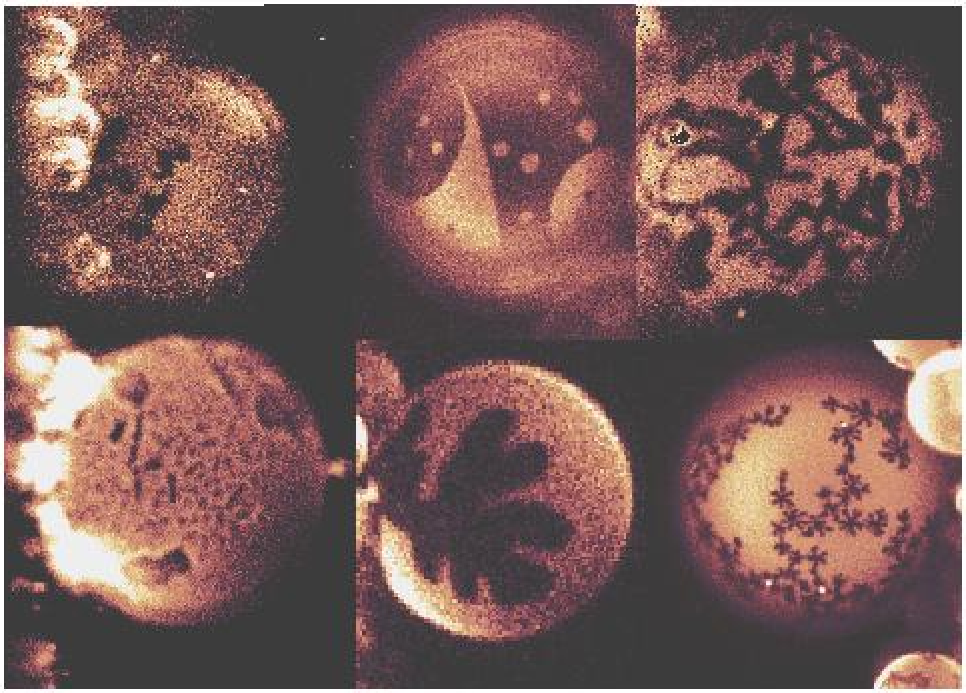
Two-dimensional movement of molecules in a membrane

In both pure phospholipid bilayers and in natural membranes, thermal motion permits phospholipid and glycolipid molecules to rotate freely around their long axes and to diffuse laterally within the membrane leaflet. Because such movements are lateral or rotational, the fatty acyl chains remain in the hydrophobic interior of the membrane. In both natural and artificial membranes, a typical lipid molecule exchanges places with its neighbors in a leaflet about 10^7 times per second and diffuses several micrometers per second at 37°C. Thus a lipid could travel 1 mm in a bacterial cell membrane in only 1 s and of an animal cell in about 20 s.

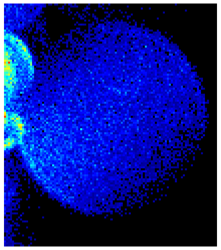
In pure phospholipid bilayers, phospholipids do not migrate, or flip-flop, from one leaflet of the membrane to the other. In some natural membranes, however, they occasionally do so, catalyzed somehow by one or more membrane proteins. Energetically, such movements are extremely unfavorable, because the polar head of a phospholipid must be transported through the hydrophobic interior of the membrane.

The mobility of membrane lipids can be measured by electron-spin resonance spectroscopy. In this technique, synthetic phospholipids containing a nitroxide group attached to a fatty acyl chain are introduced into otherwise normal phospholipid membranes.

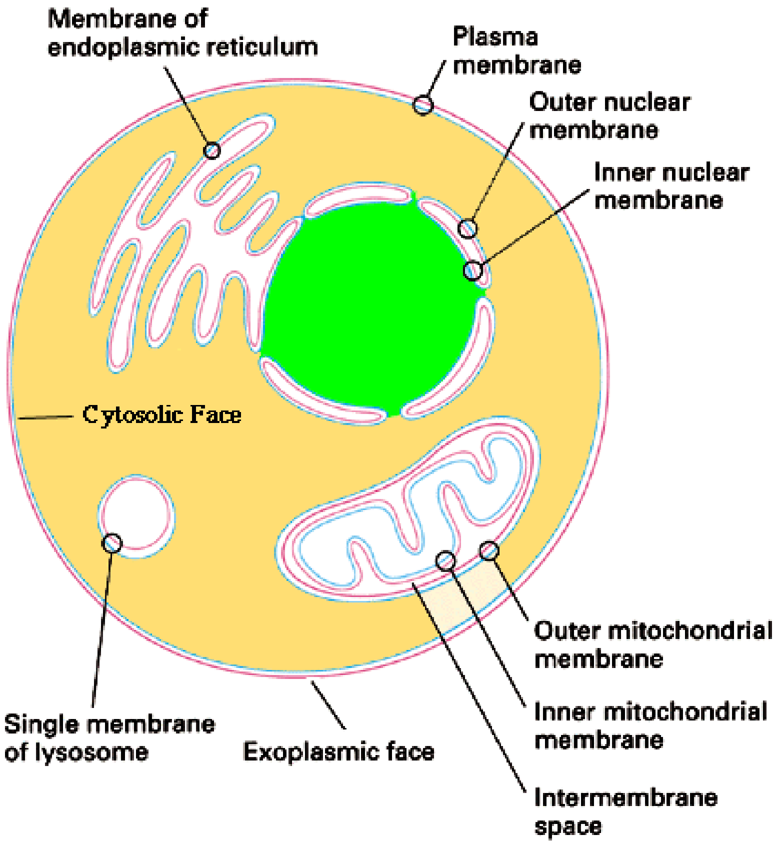
Two systems of pure phospholipid bilayer are liposomes and planar bilayers. Liposomes are spherical vesicles up to 1-10 μm in diameter consisting of a phospholipid bilayer that encloses a central aqueous compartment. They are formed by mechanically dispersing phospholipids in water.



GUVs displaying fluid/fluid or gel/fluid phase coexistence - Top (left to right): Brush border membranes lipid extract (Fo/Fd), DOPC/Sphingomielin/cholesterol 1:1:1 mol (Fo/Fd), DLPC/DPPC 1:1 mol. Bottom (left to right): Bovine lipid extract surfactans (BLES) (G/F), DPPE/DPPC 7:3 mol (G/F), PLFE lipid extracts from archibacteria. For further details see Bagatolli and Gratton, 2001, J. of Fluorescence 11(3):141-160 and Nag et al. 2002, Biophys. J. 82:2041 . Abbreviations are Fo/Fd = fluid ordered-fluid disordered, G/F= gel-fluid



Planar bilayers are formed across a hole in a partition that separates two aqueous solutions.


Membrane of endoplasmic reticulum
Plasma membrane
Outer nuclear membrane
Inner nuclear membrane
Cytosolic Face
Single membrane of lysosome
Exoplasmic face
Outer mitochondrial membrane
Inner mitochondrial membrane
Intermembrane space

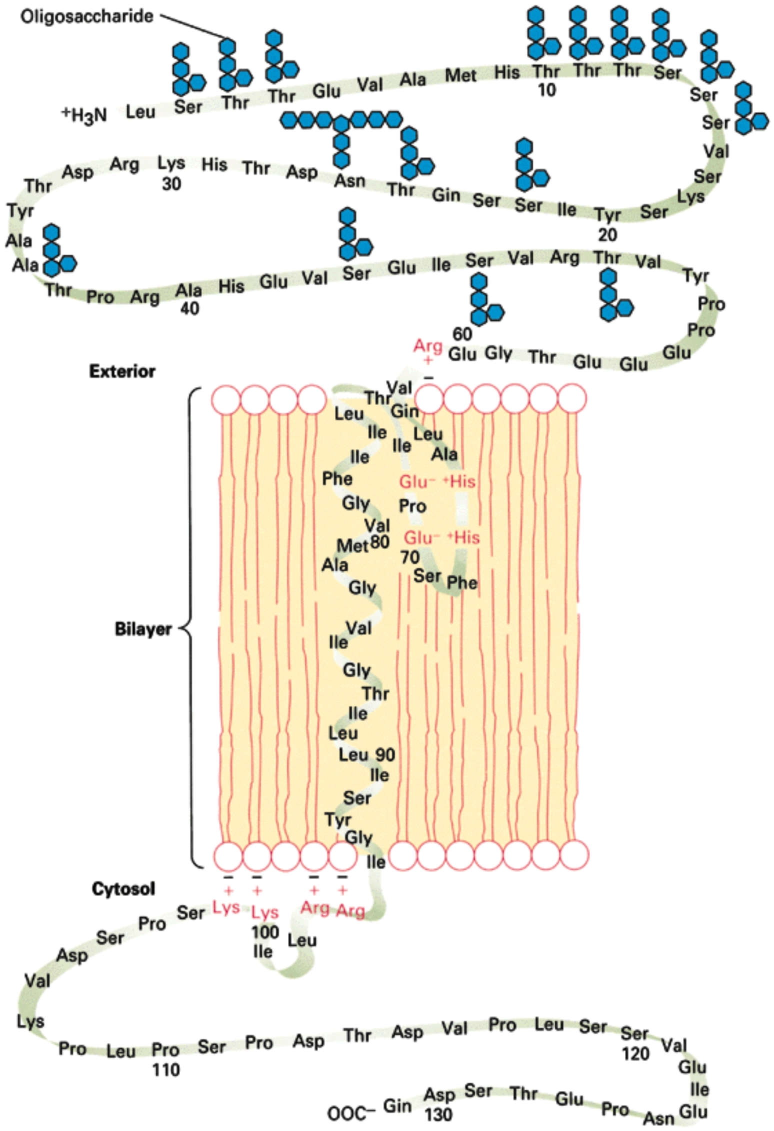
When a suspension of liposomes or a planar bilayer composed of a single type of phospholipid is heated, it undergoes an abrupt change in physical properties over a very narrow temperature range. This phase transition is due to increased motion about the C-C bonds of the fatty acyl chains, which pass from a highly ordered, gel-like state to a more mobile fluid state. During the gel-to fluid transition, a relatively large amount of heat is absorbed over a narrow temperature range which is the melting temperature of the bilayer. In general, lipids with short or unsaturated fatty acyl chains undergo phase transition at lower temperatures than lipids with long or saturate chains.

Biomembranes

One piece of evidence that the phospholipid bilayer structure is common to all biomembranes is that, as detailed above, many of the physical properties of pure phospholipid bilayers are similar to those of natural cellular membranes. Another is that either a single species of phospholipid, or a mixture of phospholipids with a composition approximating that found in natural membranes, spontaneously forms either planar bilayers or liposomes when dispersed in aqueous solutions.

All Integral Proteins Bind Asymmetrically to the Lipid Bilayer

Each type of integral membrane protein has a single, specific orientation with respect to the cytosolic and exoplasmic faces of a cellular membrane. All molecules of any particular integral membrane protein, such as glycophorin, lie in the same direction. This absolute asymmetry in protein orientation give the two membrane faces their different characteristics. In contrast to phospholipids, proteins have never been observed to flip-flop across a membrane. Such movement would be energetically unfavorable; it would require a transient movement of hydrophilic amino acid and sugar residues through the hydrophobic

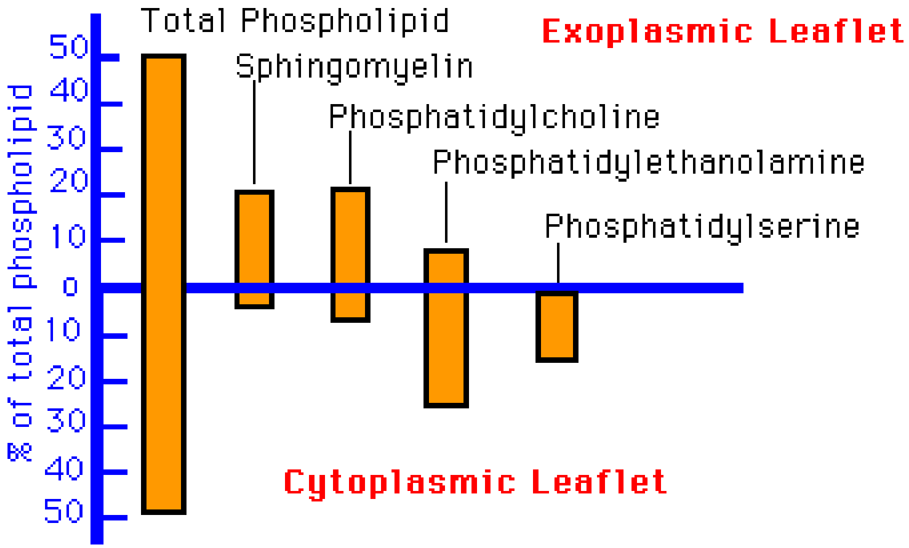


interior of the membrane. The asymmetry of membrane proteins is established during their biosynthesis and maintained throughout the proteins lifetime.

Membrane asymmetry is most obvious in the case of membrane glycoproteins and glycolipids. In the plasma membrane, all the O- and N-lined oligosacharides in glycoproteins and all of the oligosaccharides in glycolipids are on the exoplasmic surface. In the endoplasmic reticulum, they are found on the interior, or lumenal, membrane surface.

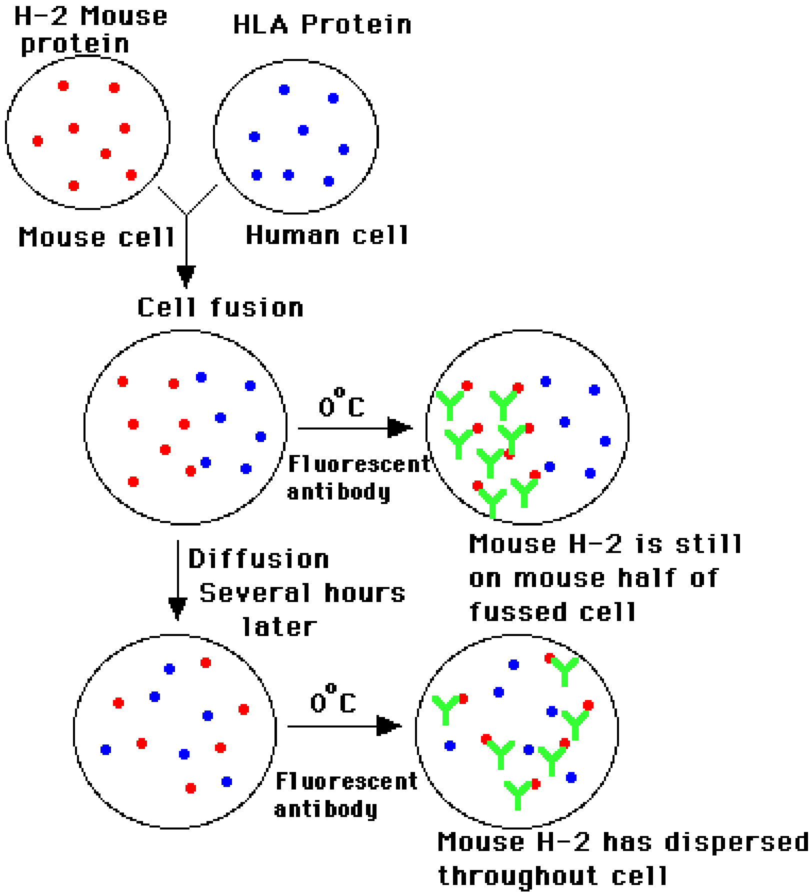
Membrane Leaflets Have Different Lipid Compositions

The lipid compositions of the two membrane leaflets are quite different in all the plasma membranes analyzed thus far. In the human erythrocyte and in a line of canine kidney cells grown in culture, all of the glycolipids, and almost all of sphingomyelin and phosphatidyl choline are found in the exoplasmic leaflet. In contrast, lipids with neutral or negative polar head groups, such as phosphatidyl ethanolamine and phosphatidyl serine are preferentially located in the cytosolic leaflet. The relative abundance of a particular phospholipid in the two leaflets of a plasma membrane can be determined based on its susceptibility to hydrolysis by phospholipases added to the cell exterior. Phospholipases cannot penetrate the cytosolic face of the plasma membrane.

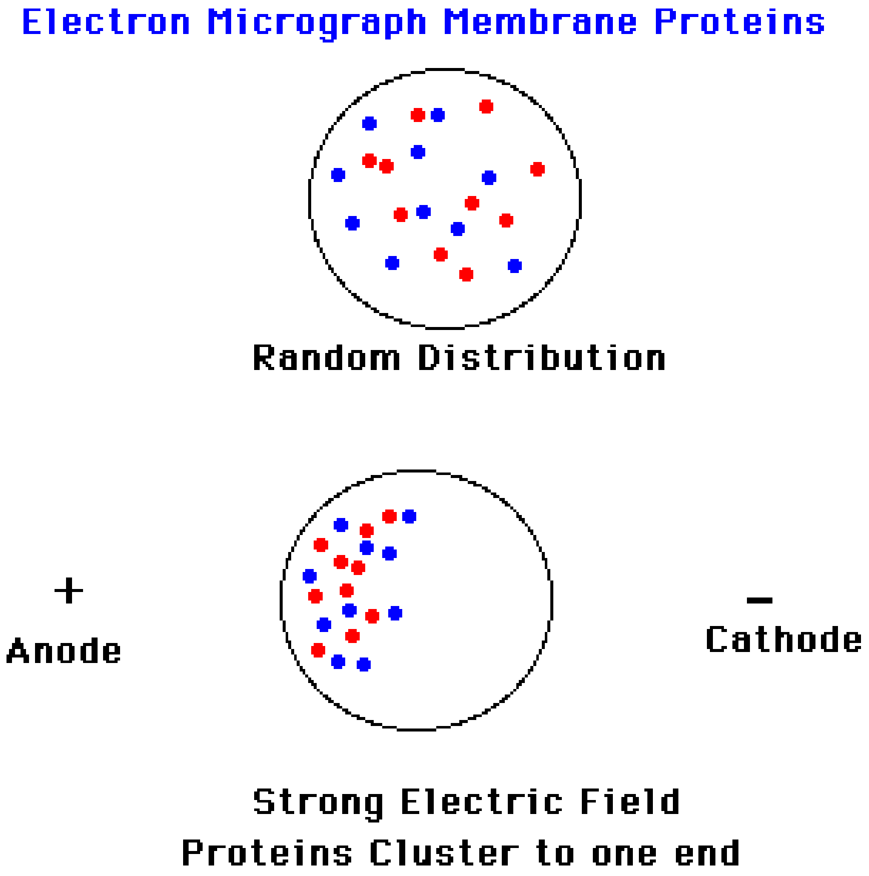


Most Integral Proteins and Lipids Are Laterally Mobile in Biomembranes

Proteins and lipids are laterally mobile within one leaflet. Two different cells can be fused for the purpose of observing the movement of their distinct surface proteins within the membrane. Immediately after fusion, the mouse and human antigens are grouped in separate areas, but they quickly diffuse throughout the cell. Soon after fusion both halves are equally fluorescent, demonstrating the most of the surface proteins were not rigidly held in place on the original mouse and human cells. According to this concept, a fluid mosaic model of the membrane has been developed.


H-2 Mouse protein
HLA Protein
Mouse cell
Human cell
Cell fusion
0°C
Fluorescent antibody
Mouse H-2 is still on mouse half of fussed cell
Diffusion
Several hours later
0°C
Fluorescent antibody
Mouse H-2 has dispersed throughout cell

Electron micrographs revealing mobility of protein particles in Freeze-fractured vesicles prepared from the inner mitochondrial membrane. Integral protein particles are visible as protuberances that are randomly distributed in the surface of a free-fractured vesicle. After the vesicle was subjected to a strong electric field and the rapidly frozen, all the particles clustered at one end, showing that the particles can move laterally within the membrane plane in response to a voltage gradient. Their rate of movement is similar to that of many other integral membrane proteins.


Electron Micrograph Membrane Proteins
Random Distribution
+
Anode
–
Cathode
Strong Electric Field
Proteins Cluster to one end