The Plasma Membrane (1)

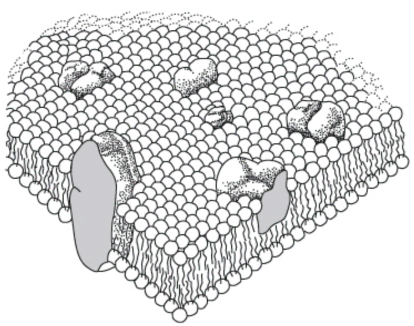
1972

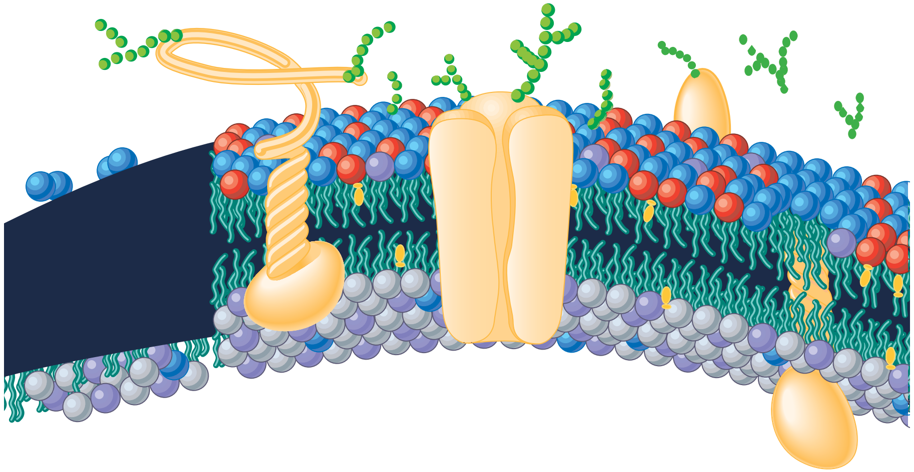
Singer and Nicholson

San Diego (USA)

FLUID MOSAIC
(dogma)

The state of membrane is fluid and lipids can move.
Proteins are localized in a mosaic, in a discontinuous way and they also can move.
Membranes are DYNAMIC



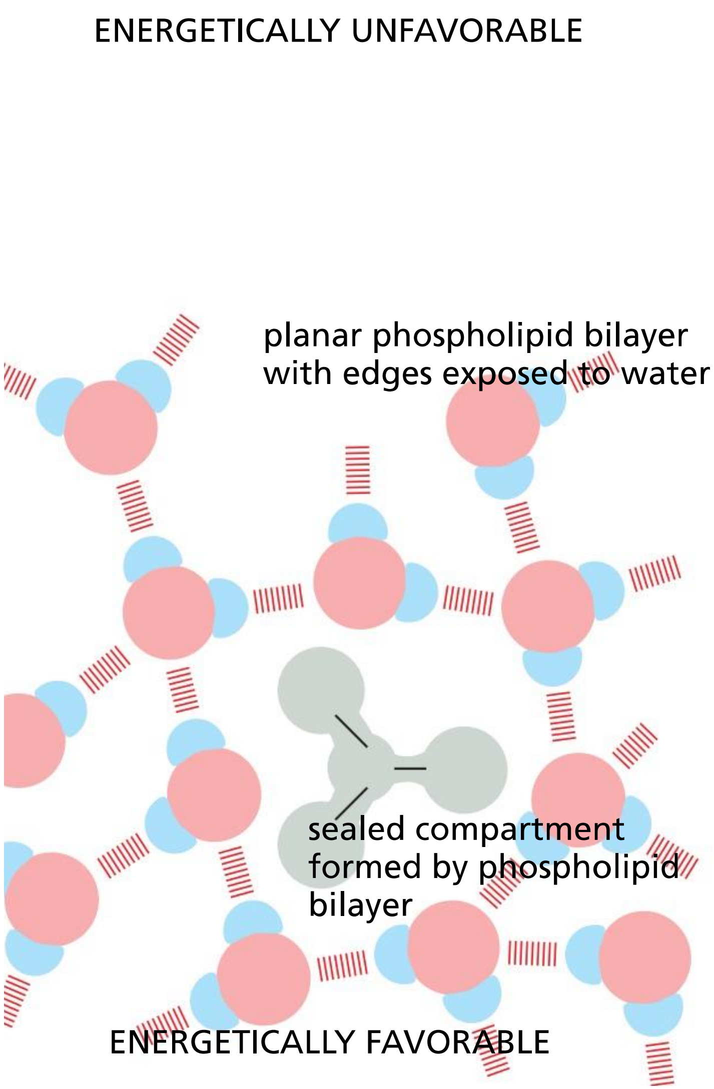


The plasma membrane (PM)

encloses each cell and defines its boundaries.

It is semipermeable therefore

the inner environment can be different compared to the external one.



Phospholipids are the skeleton of PM.

They stick together and form SEALED compartments avoiding the free and casual movement in and out of hydrosoluble molecules.

This is due to the propensity of the hydrophobic regions to escape water.

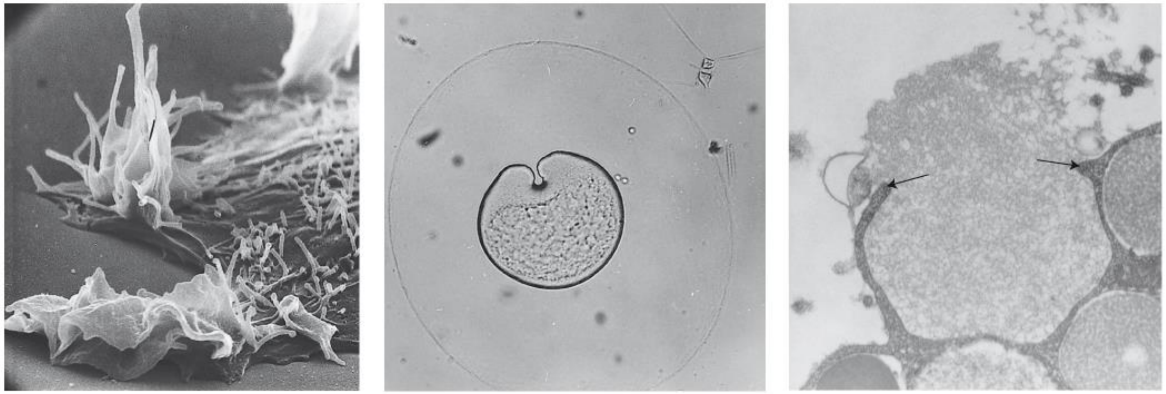
Membranes can also self-assemble

Never a free edge, therefore spheric or continuous systems

CELL MEMBRANES ARE FLUID, NOT RIGID

The composition of the membrane influences the physical state of it and some biological properties (e.g. chemical messengers).

Deformations, movement, division, membrane fusion of vesicles, inserted proteins.


(a) (b) (c)

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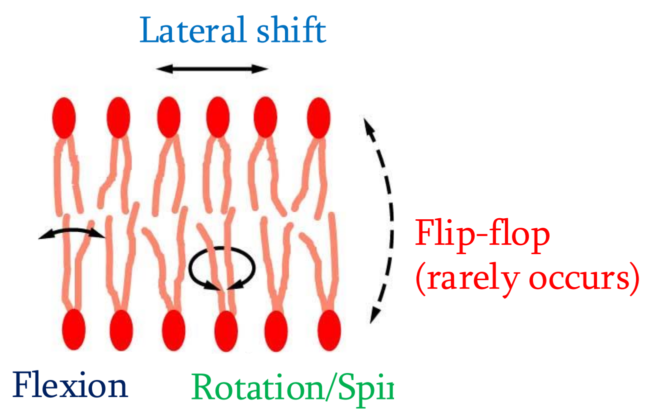
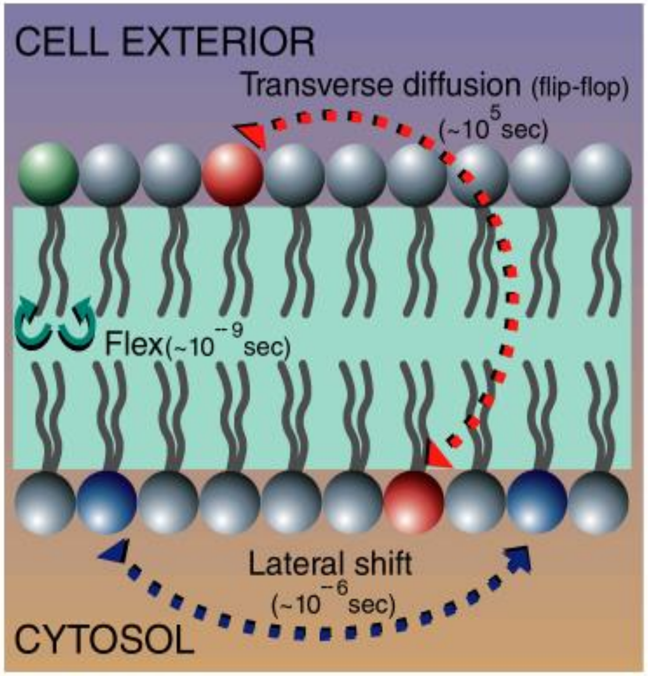
CELL MEMBRANES ARE FLUID, NOT RIGID

Lipids can move into membranes.

In different ways:

1. Rotation/Spin (around its on axis)
2. Lateral diffusion/shift in the same monolayer
3. Flip-flop/transverse diffusion (changing layers). This is RARE because energy costly (enzymes-TRANSLOCATORS like SCRAMBLASES and FLIPPASES)
4. Flex





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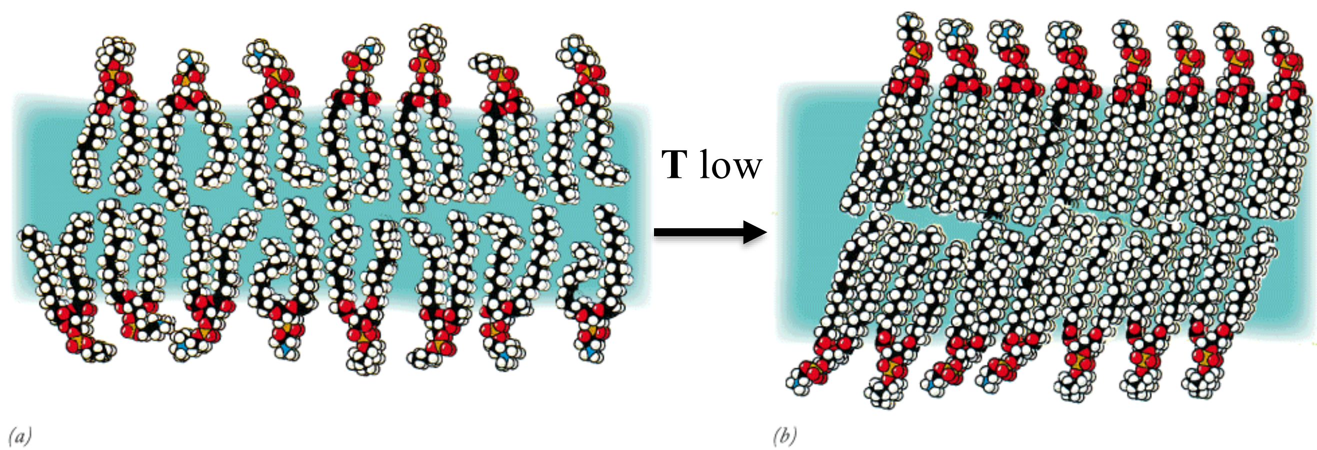
The fluidity of a lipid bilayers (the ability of lipids to move) depends on its own composition

Phase transition TEMPERATURE: to pass from a liquid state to a rigid (gel) crystalline state (freezing).



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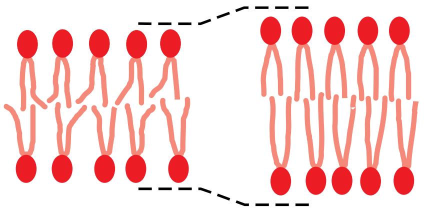
Fluidity (viscosity) depends on:

-Temperature

-Cis-double bonds (unsatured lipids): produce kinks wich reduce interactions and ability to stick, pack together, therefore they increase the fluidity and the phase transition T (needed to gelify) must be lower. VEGETABLE OIL and MARGARINE (hydrogenation).



-Length of hydrophobic tails: short tails reduce the tendency of hydrocarbon tail to interact to one another, whereas long tails interact better. Long tails make more rigid and less fluid.

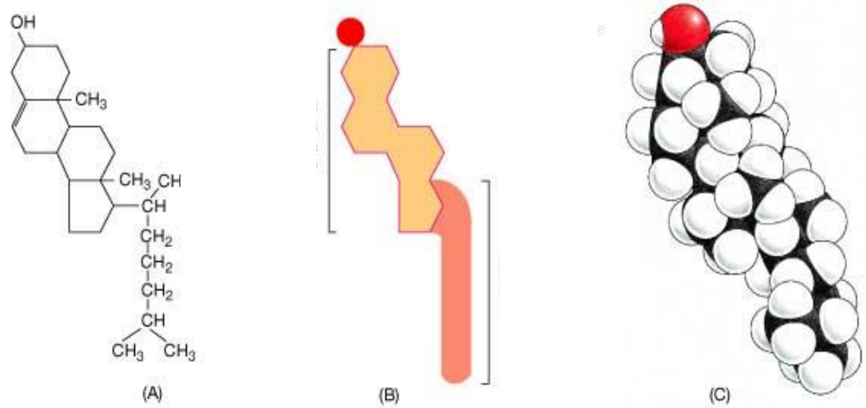
-Cholesterol. Its rings are plane and rigid and reduce the mobility of the upper part of hydrocarbon tails, partly immobilizes them and makes membrane less deformable and less permeable to small water-soluble molecules. It makes more rigids the membranes. But also avoid tails to get together and to crystallize (tend to increase stability of membrane and reduce permeability).

-Proteins. Higher rigidity.

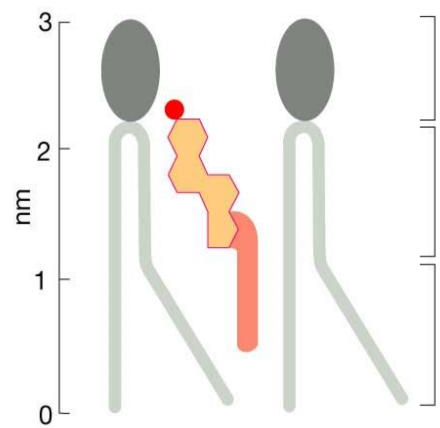
Cholesterol

Up to 1 molecule of cholesterol for every phospholipid molecule.

Cholesterol reduces the interactions between hydrocarbon chains and reduce their flexibility by making the PM more rigid but not crystallizing it, preserving it.



In procaryotes there is no cholesterol and the fluidity mostly depends on the lenght of chain and on the cis-double bonds or enzymes acting on double bonds. When the temperature falls, some bacteria synthetize more fatty acids with more cis-double bonds which counteract the decreased fluidity due to a low temperature.



Head
Polar
(Hydrophilic)

Ring strucrure
RIGID

Tail
Apolar
(Hydrophobic)
MORE FLUID REGION

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Plasma Membrane (PM) and Cell Membranes:

Half of Lipids
Half of Proteins
Few Carbohydrates

% very variable depending on the cell type and on the organelle

Lipids are the skeleton of membranes

% in weight
in various
Biological Membranes

	Proteins	Lipids	Carbohydrates	Proteins/Lipids ratio
Membrana plasmatica				
Eritrocita umano	49	43	8	1,14
Cellula epatica di mammifero	54	36	10	1,50
Ameba	54	42	4	1,29
Guaina mielinica dell'assone del nervo	18	79	3	0,23
Involucro nucleare	66	32	2	2,06
Reticolo endoplasmatico	63	27	10	2,33
Complesso di Golgi	64	26	10	2,46
Lamella di un cloroplasto	70	30	0	2,33
Membrana esterna mitocondriale	55	45	0	1,22
Membrana interna mitocondriale	78	22	0	3,54
Batterio gram-positivo	75	25	0	3,00

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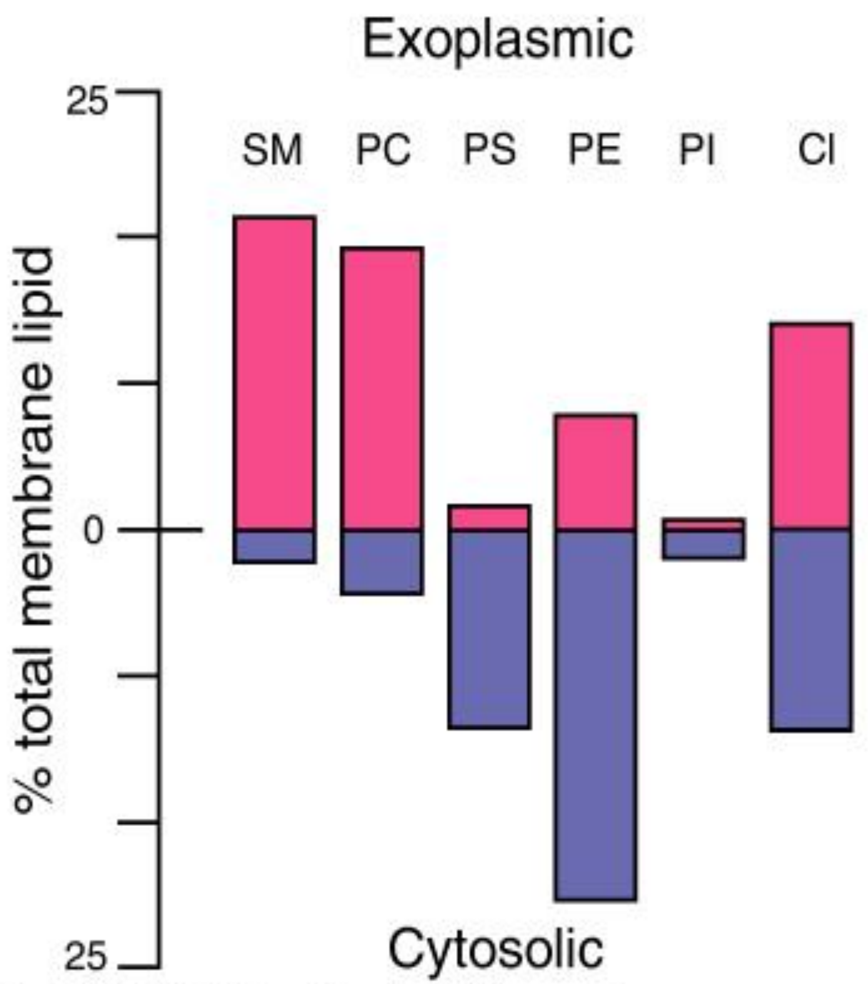
CELL MEMBRANES ARE ASYMMETRIC

Biological membranes are ASYMMETRIC



Found by using phospholipases

- SM: Sphingomyelin
- PC: Phosphatidylcholine
- PS: Phosphatidylserine
- PE: Phosphatidylethanolamine
- PI: Phosphatidylinositol
- Cl: Cardiolipin

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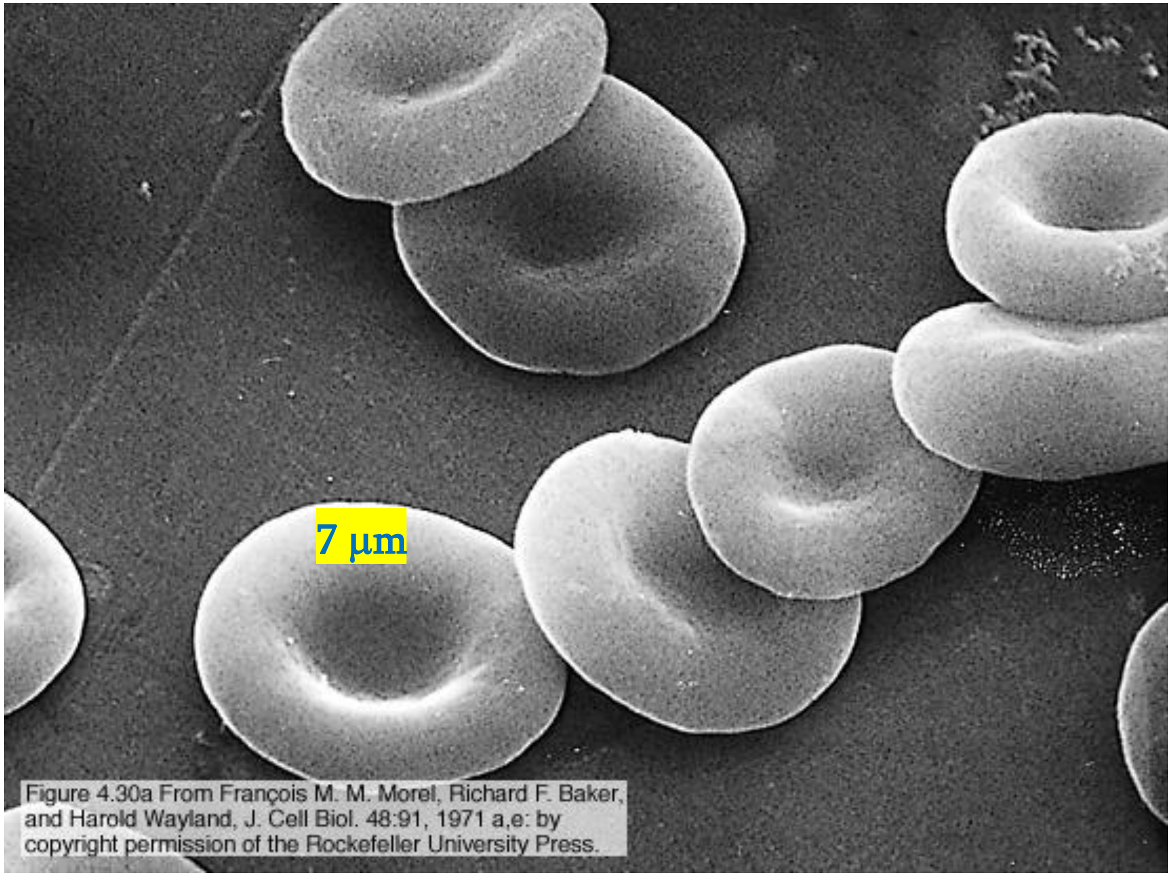
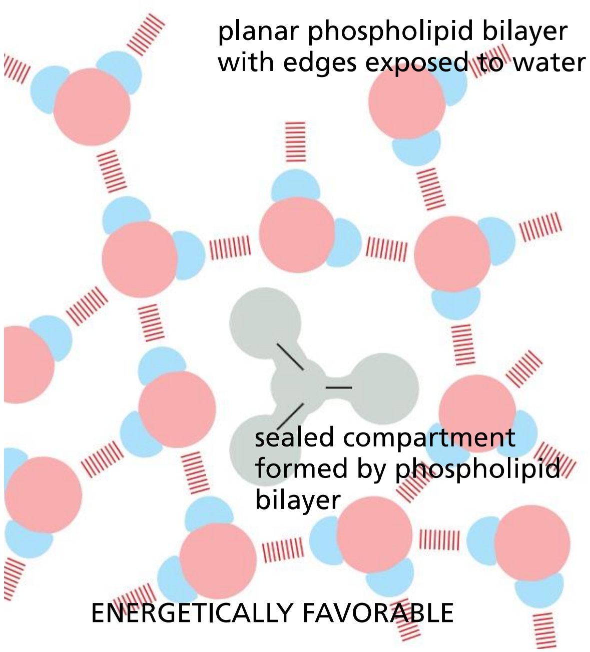
Demonstration of the ASYMMETRY of phospholipids in membranes

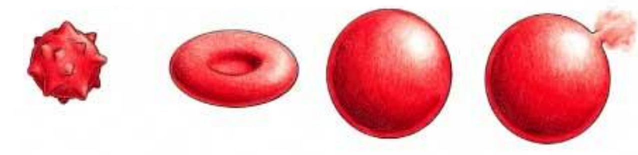


Mammal erythrocytes/red blood cells (scanning electron microscope)

Demonstration of the ASYMMETRY of phospholipids in membranes

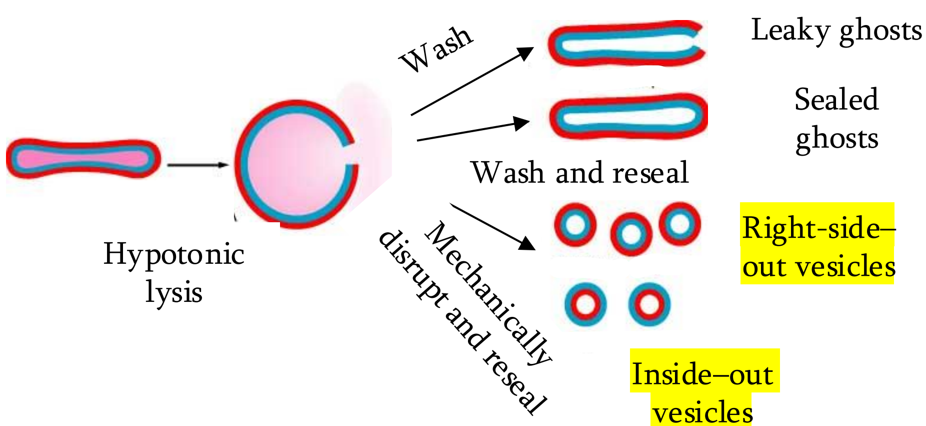


Hemolysis



Hypotonic

Sealing of fragments forming vesicles of 2 types in which the orientation of the membrane is different depending on the salt concentration.



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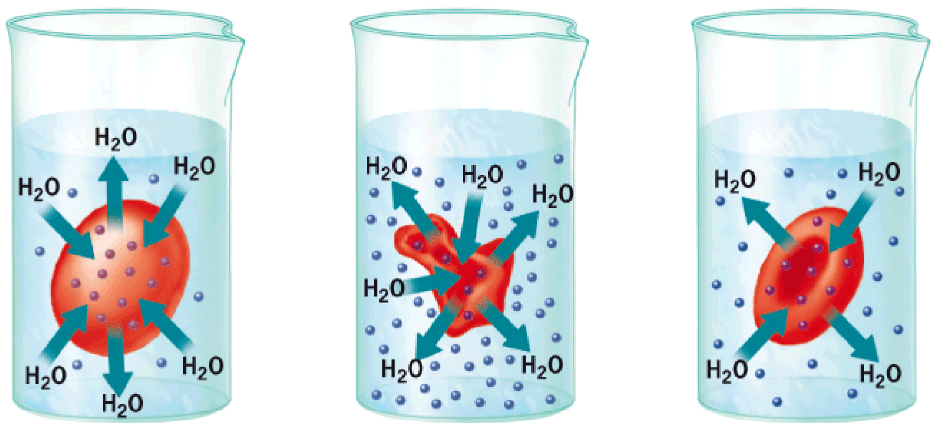
OSMOSIS

Hypotonic solution | Hypertonic solution | Isotonic solution



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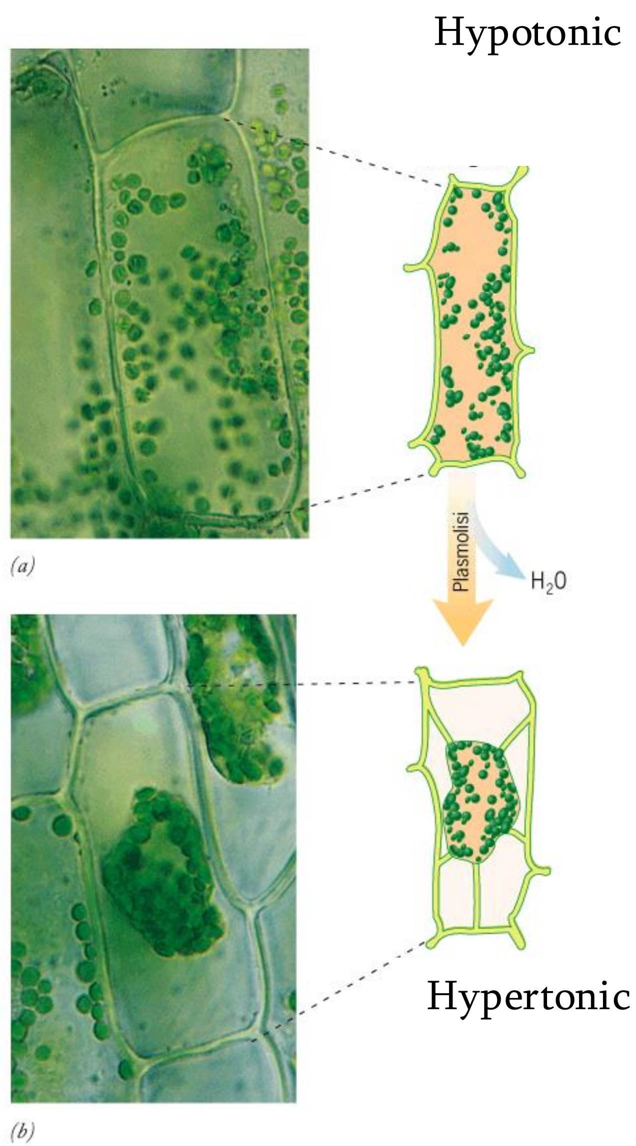
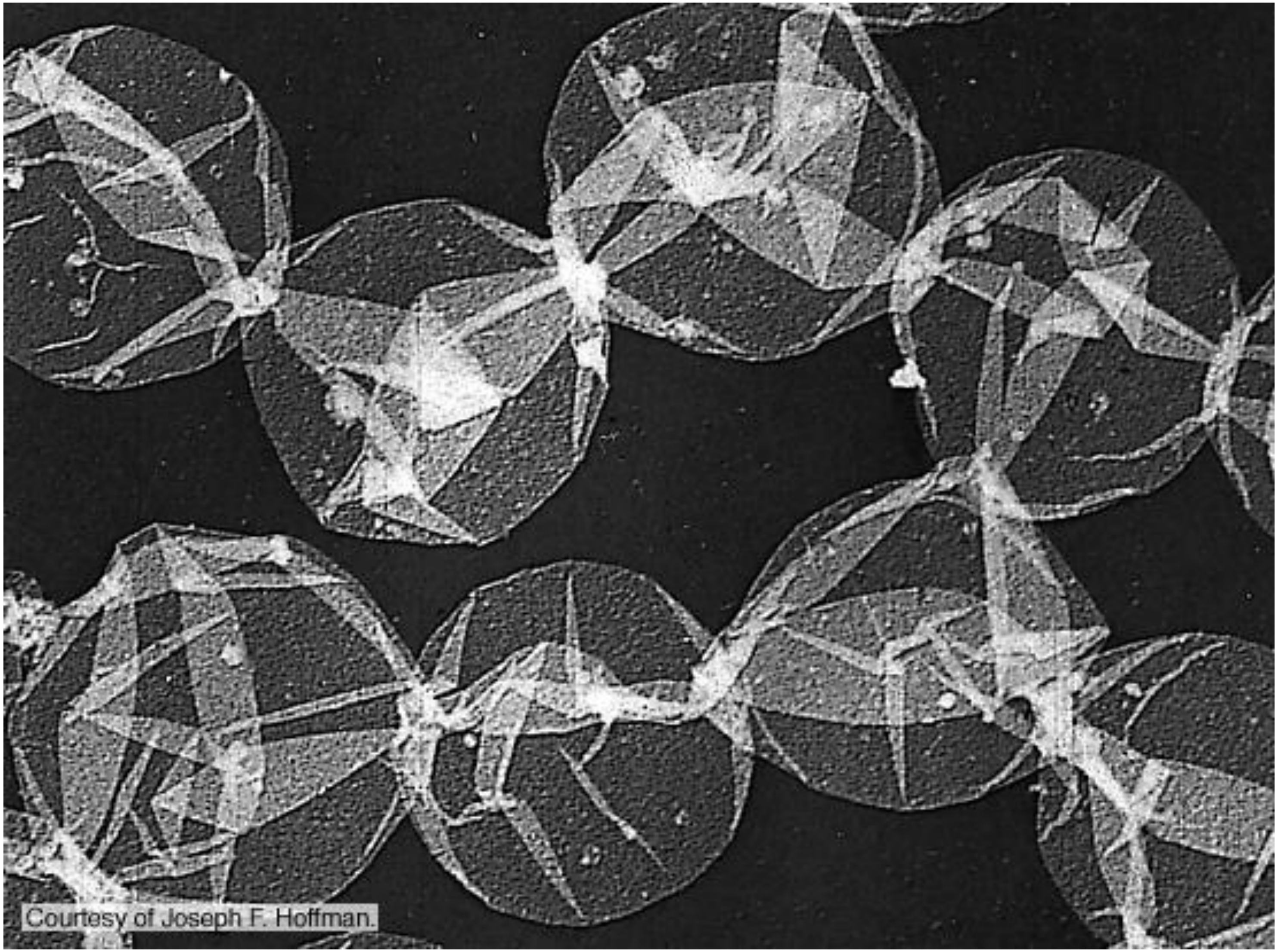
Pression of Turgor

Allows the support of a plant



"Ghosts" of erythrocytes (electron microscope)



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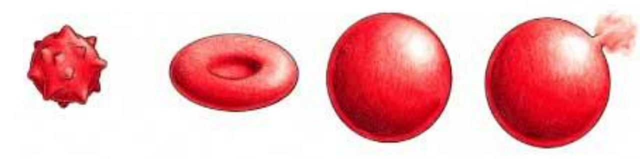
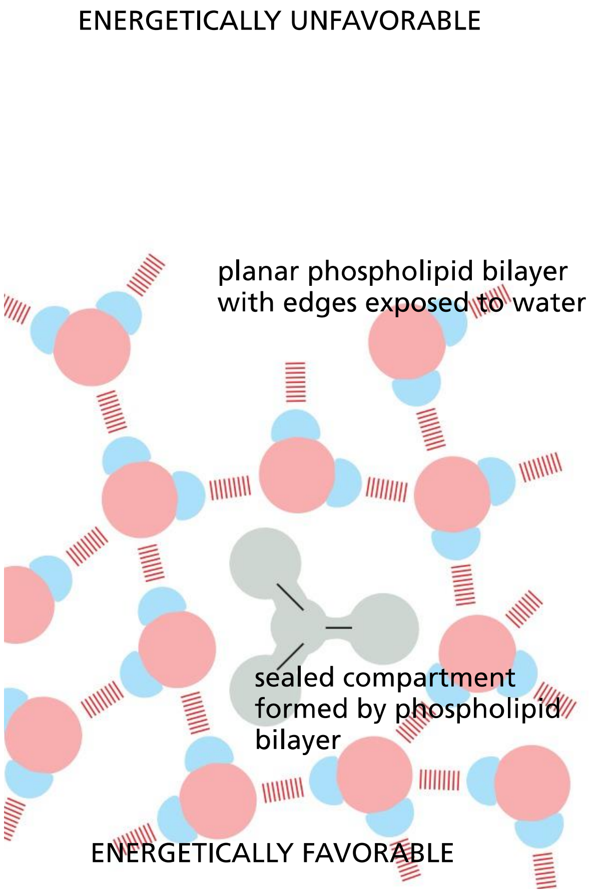
Hemolysis





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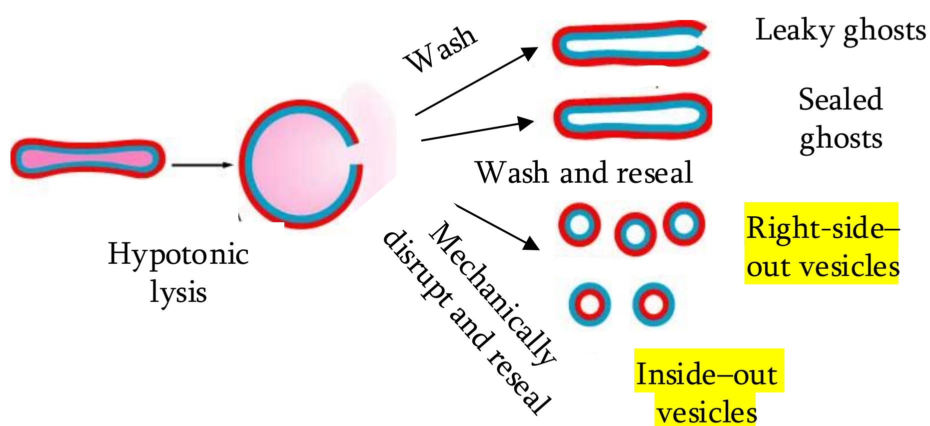

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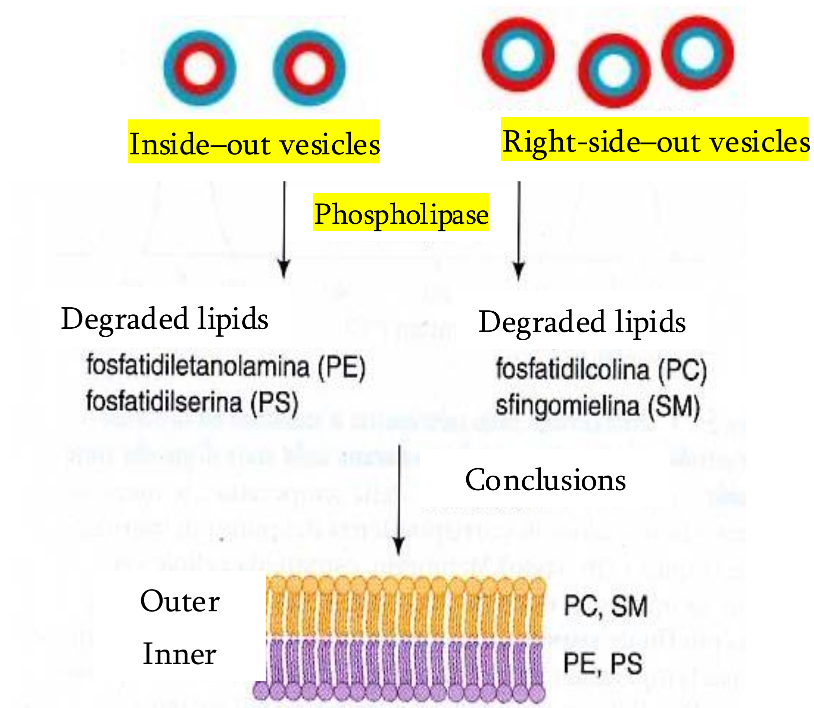

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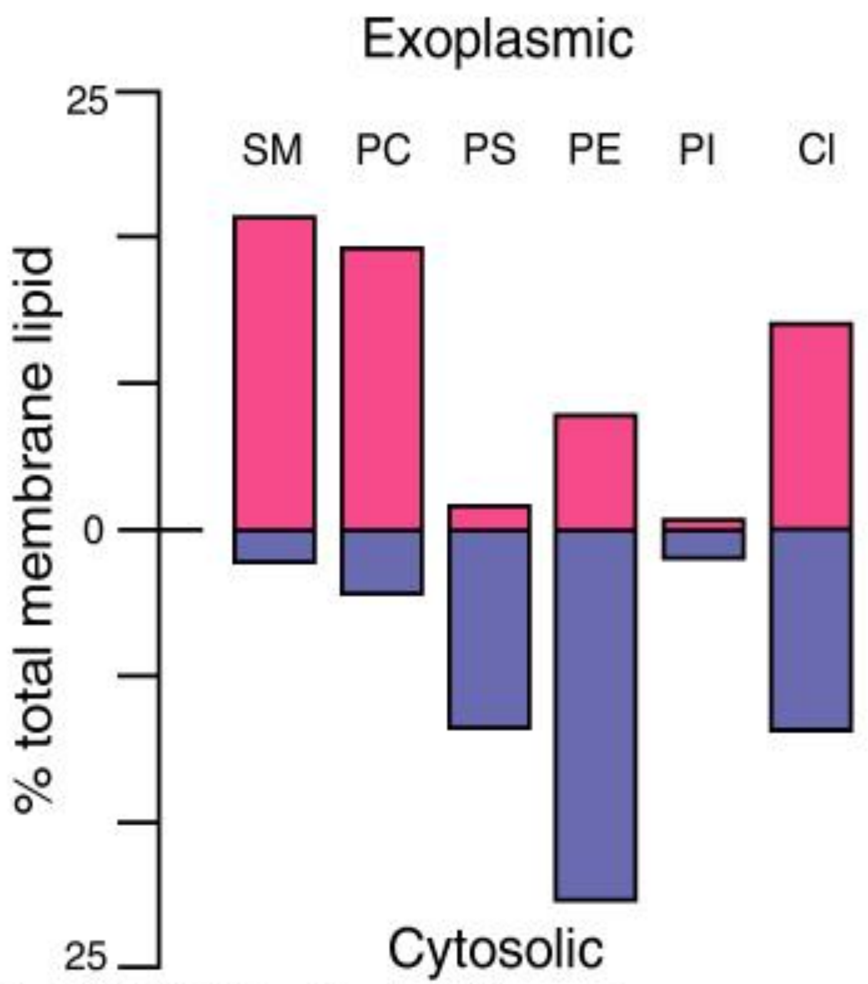
Biological membranes are ASYMMETRIC



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Membranes are formed by:

1.Lipids

- Phospholipids
- Sterols (e.g. cholesterol)

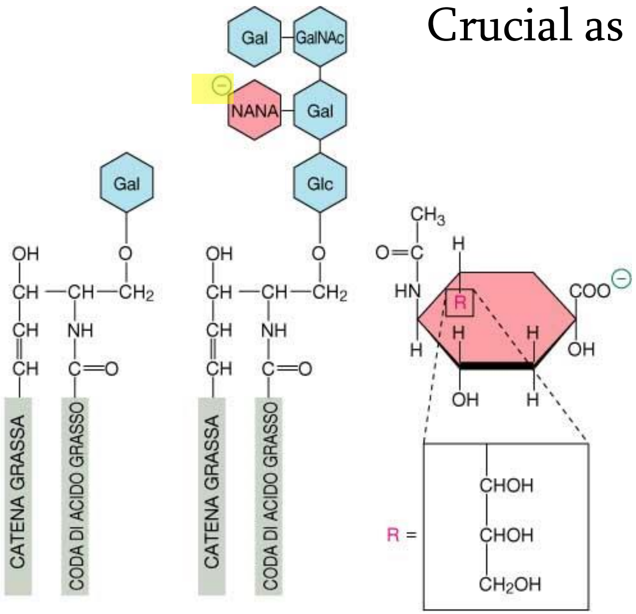
2. Proteins

3. Carbohydrates (outer layer-contribute to asymmetry) as short hydrophilic oligosaccharides (less than 15 various residues or just 1) covalenty bound to lipids or proteins

- Glycolipids (e.g. cerebrosides and gangliosides)
- Glycoproteins

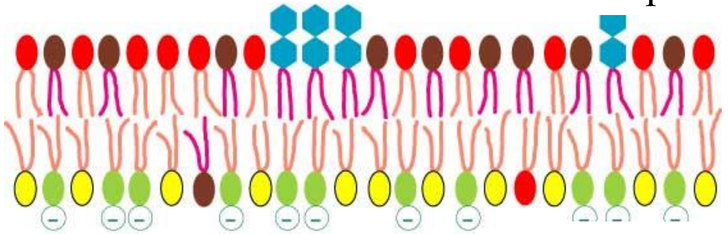
The PM asymmetry is also due to GLYCOLIPIDS which are present only in the outer layers of the PM
(5% of glycolipids)
or in the noncytosolic layer of other biological membranes.

Crucial as receptors, cell interactions.

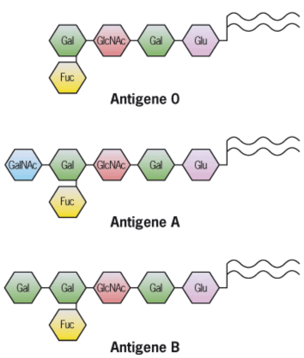


(A) galattocerebroside (B) ganglioside G_{M1} (C) acido sialico (NANA)
N-acetylneuraminic acid

Gangliosides into nerve cells (5-10%)



4 Blood groups
Different oligosaccharides associated to lipids or proteins of PM erytrocytes.
Due to different enzymes.
Genetic difference.



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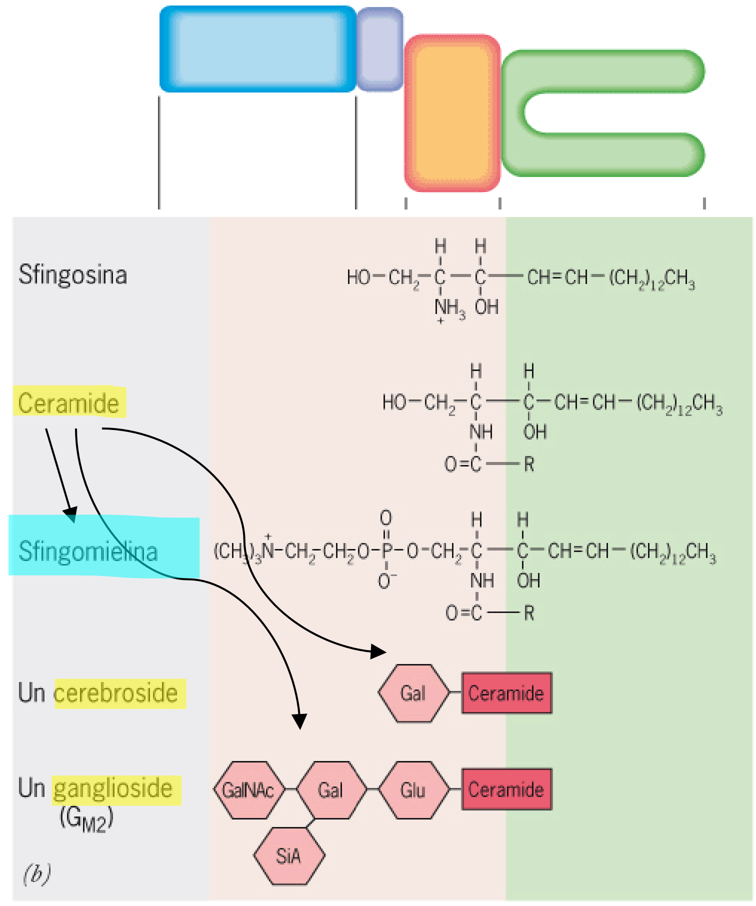
LIPIDS in the PM



They might form AGGREGATES thanks to H bond WITH ONE ANOTHER.

Glycolipids might
1-Protect from harsh conditions such as low pH and degrading enzymes
2-Elecrical effect being charged and altering ion concentration such as calcium
3-Into myelin, they might influence the insulation effect
4-Receptors
5-Interactions

Glycocalyx or Cell Coat

binds to LECTINS (proteins binding sugars)

It is a cell outer layer rich of carbohydrates belonging to:

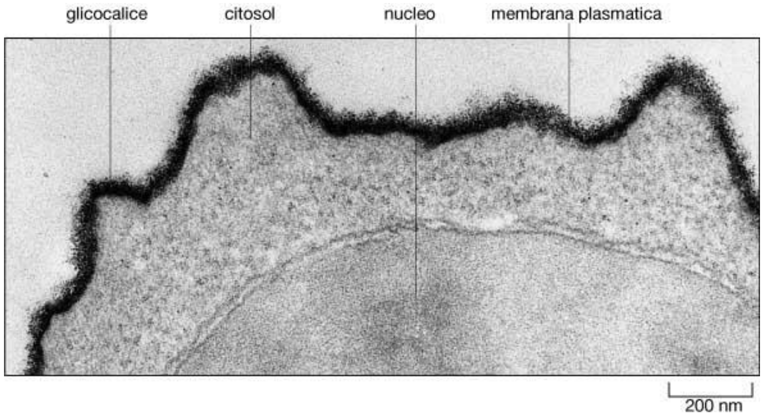
- PM Glycolipids

- PM Glycoproteins

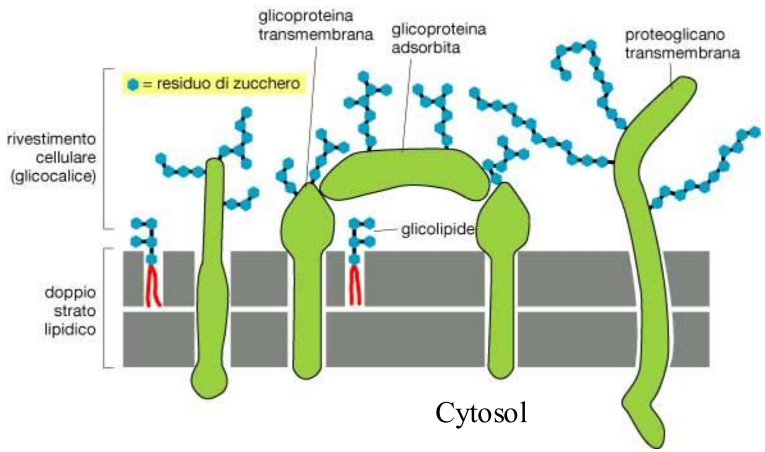
- Secreted Glycoproteins remaining in proximity

-Secreted or not Proteoglycans (Syndecans)

-Functions: protection from mechanical or chemical damage, cell-cell interaction or repulsion.



Extracellular space



Cytosol

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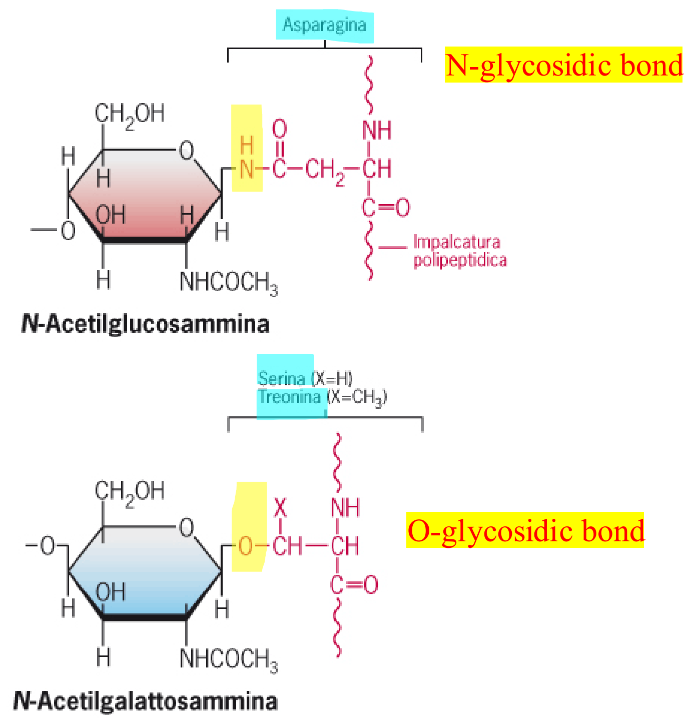
2 types of bonds in glycoproteins



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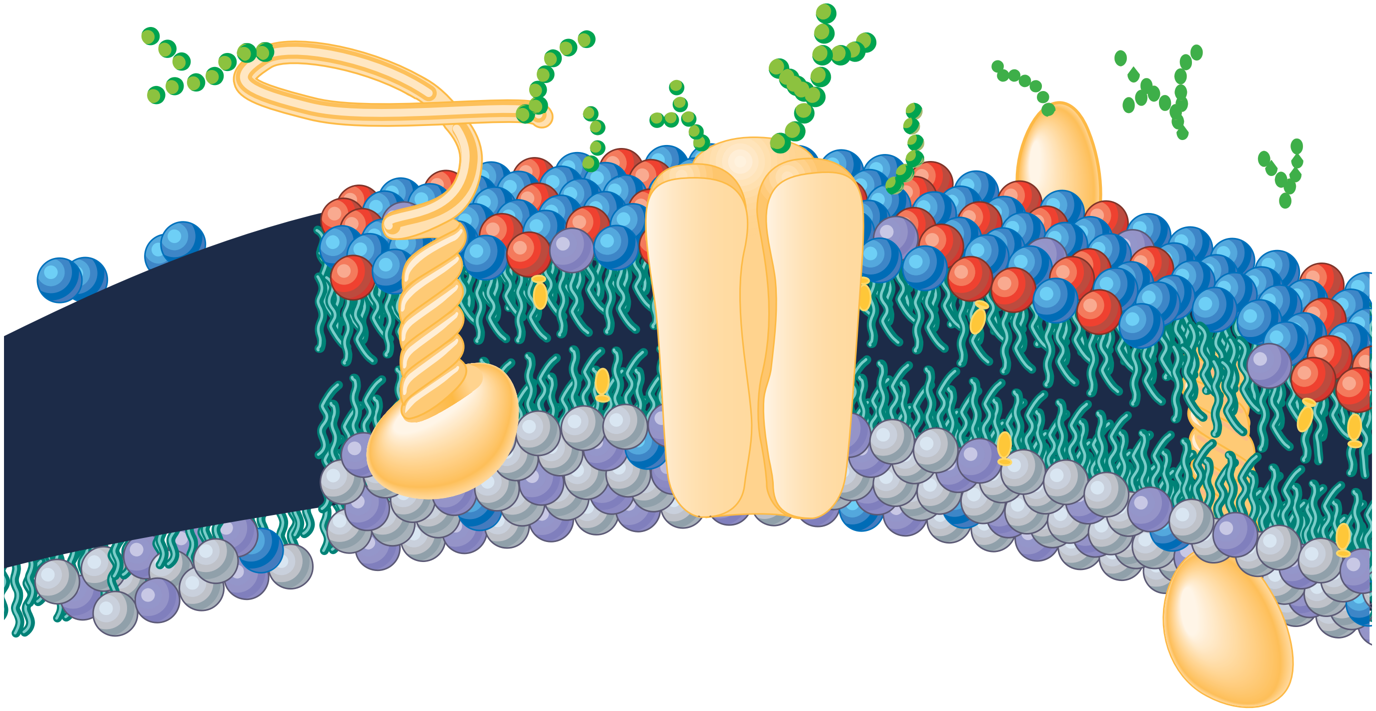
MEMBRANE PROTEINS

Receptors, channels and carriers, performing electron transfer in photosynthesis and respiration, structural role, enzymes



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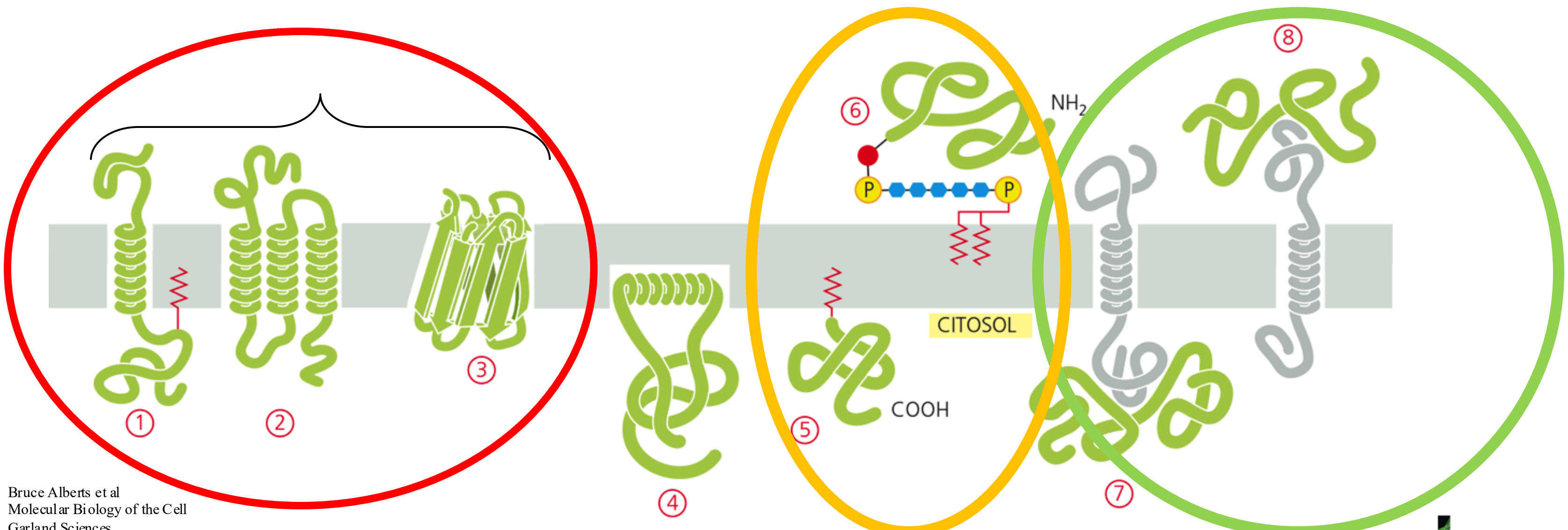
The proteins of membranes
Responsible of DISCONTINUITYand ASYMMETRY in the membranes

3 classes of membrane PROTEINS

1. Transmembrane/Integral (20-30% of all proteins) { 1 time SINGLE-PASS / More times MULTIPASS / Barrel }

2. Covalently bound to lipids (phosphatydilinositol, prenyl group or a fatty acid)

3. Peripheral (non covalent/elettrostatic weak bonds association to the lipid hydrophilic head or to other proteins, on either side).



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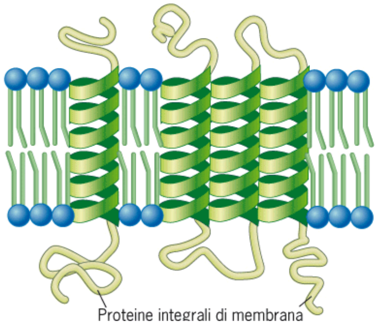
1. Transmembrane/Integral proteins

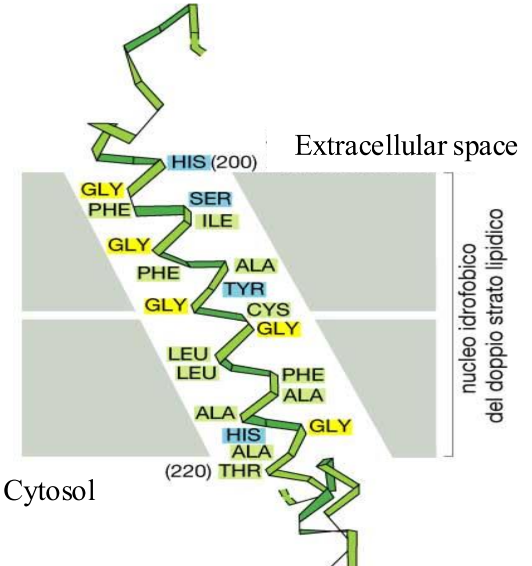
Amphipatic

The transmembrane domains are generally hydrophobic and associated (VdW forces) to lipid tails to assure the impermeability of the membrane.
Often α-helices



Those portions of a transmembrane protein that cross the lipid bilayer usually cross as α helices composed largely of amino acids with nonpolar side chains.
The polypeptide backbone, which is hydrophilic, is hydrogen-bonded to itself in the α helix and shielded from the hydrophobic lipid environment of the membrane by its protruding nonpolar side chains

Hydrophobic/
Apolar
20 aa



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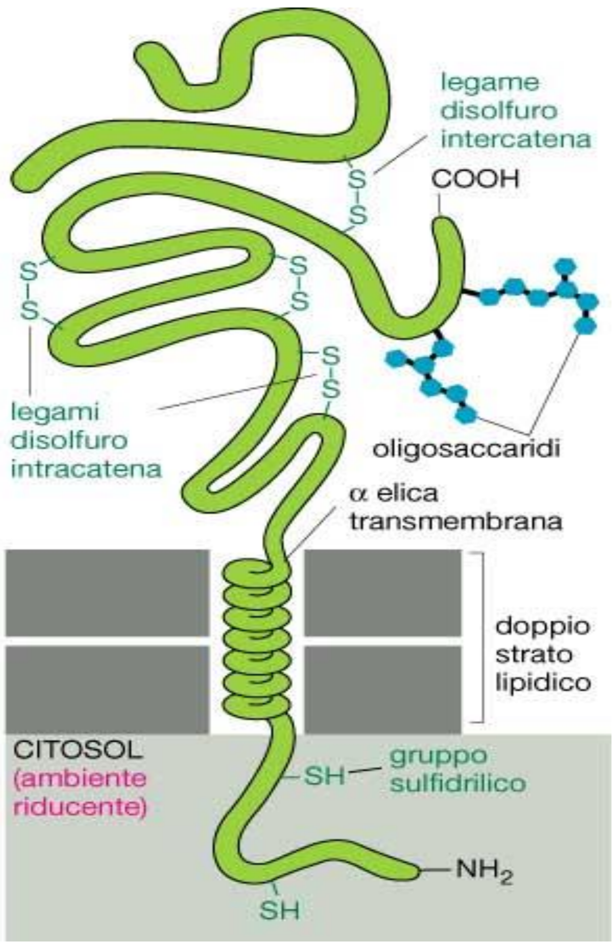
1. Transmembrane/Integral proteins

Mostly GLYCOSILATED

Hydrophilic



Hydrophobic
/Apolar

Often disulfide S-S bonds for 3° and 4° structures

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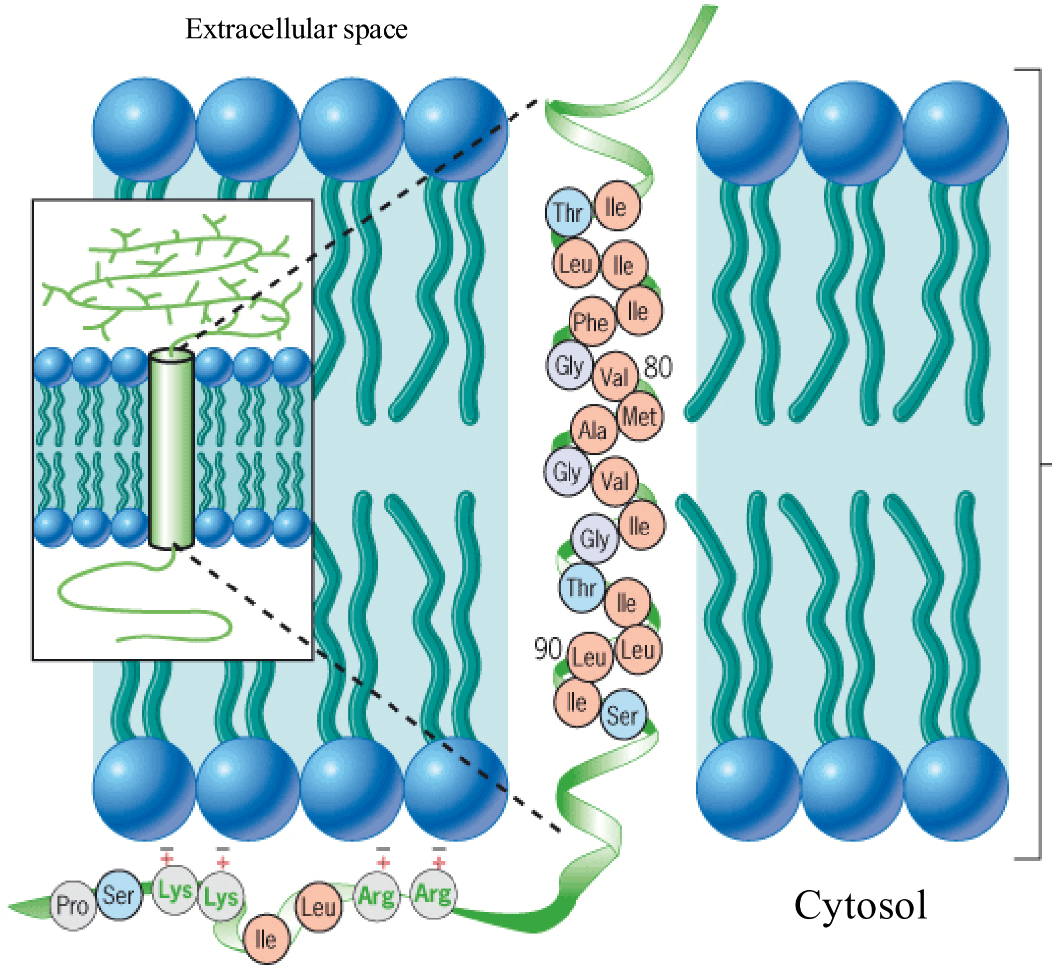

Polar aa interact with lipid heads

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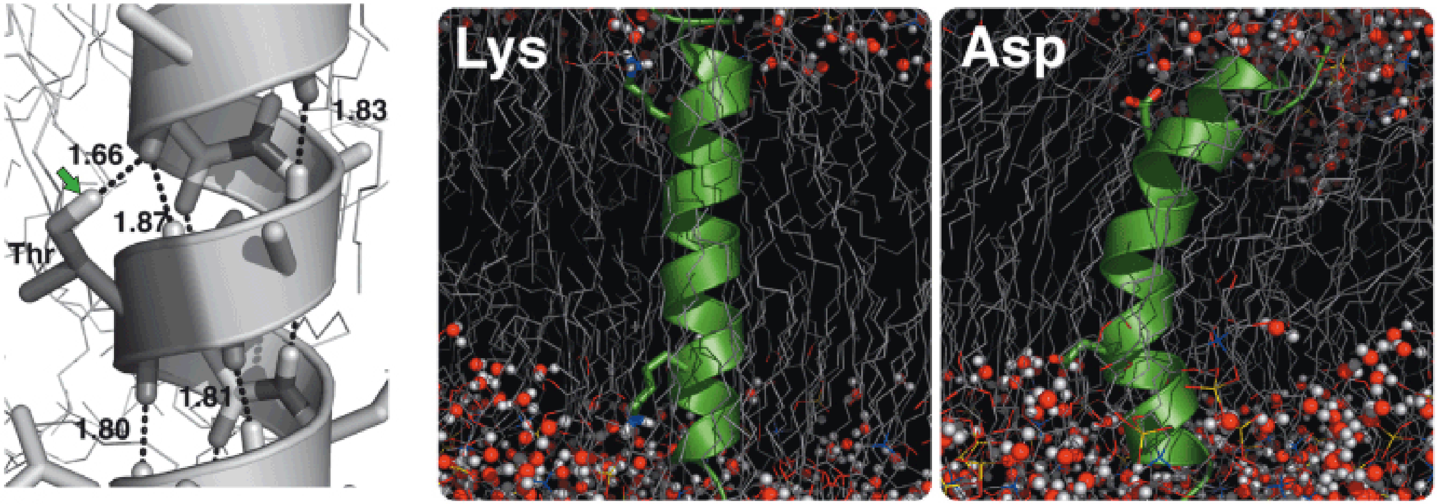

(a) (b) (c)

The aa sequence of a protein can give information on its 3D structure. For example the HYDROPHOBICITY GRAPH, (e.g. 20-30 hydrophobic aa) might suggest/predict the presence of an α-helix.

Transmembrane proteins are difficult to crystallize.

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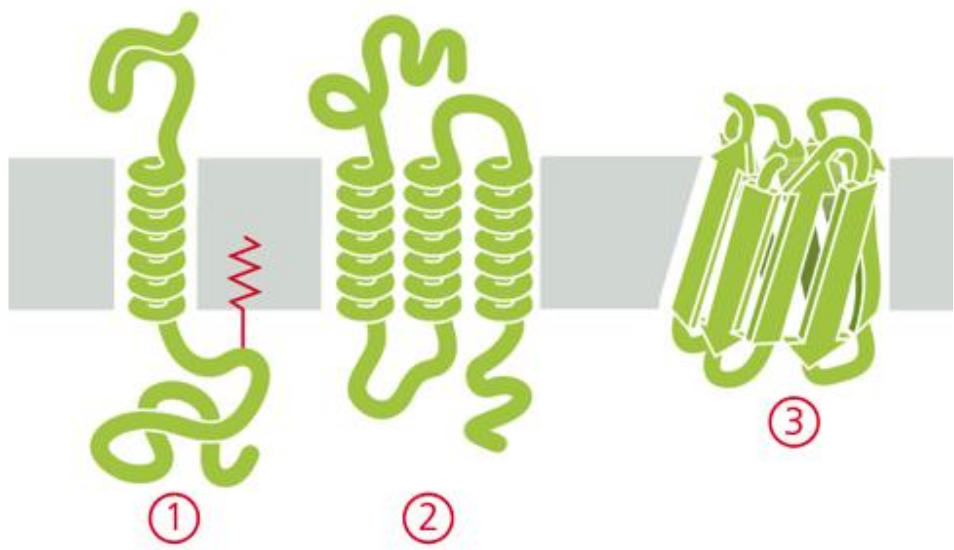
1. Transmembrane/Integral
(20-30% of all proteins)
They can laterally move in the bilayer (MOBILITY)

1 time
More times
β-Barrel

(receptors, channels and carriers, and perform electron transfer in photosynthesis and respiration)



β-Barrels can form channels for the passage of hydrophilic molecules like ions through the PM.

The inner channel is surround by hydrophilic lateral chains.

In bacteria (porins), chloroplasts and mitochondria but not into PM of eukaryotic cells

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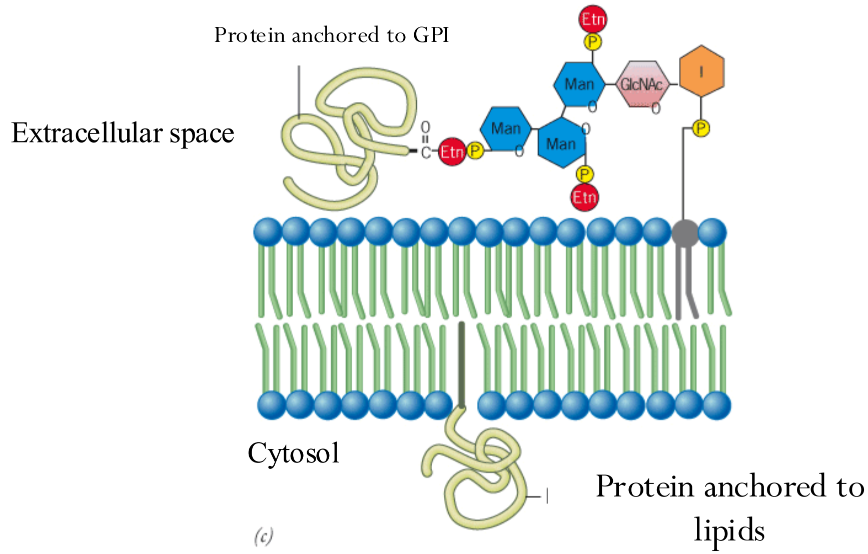
2. Covalently bound to lipids
Receptors, enzymes, adesion proteins

- Proteins bound to the GPI (glicosyl-phosphatydilinositol/PI), a short oligosaccharide bound to a PI

- Proteins bound to a prenyl group inseted between fatty acids (on the cytosolic side) (e.g. Ras, intracellular signaling)

- Proteins bound to a fatty acid



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3. Peripheral

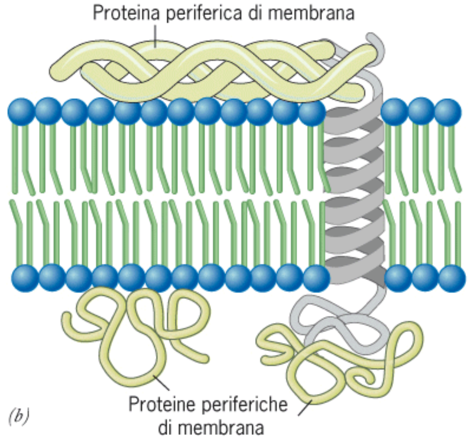
(non covalent/elettrostatic weak bonds association to the lipid hydrophilic head or to other proteins, on either side).

Enzymes, or mediating cell-to-cell comunication or structural role forming a skeleton below the inner cytosolic lipidc layer.

Not always associated but the association might be modulated.

Extracted or solubilized by gentle procedures such as high or low ionic strenght or extreme pH. In this case the bilayer remains intact.

For the integral ones, we need detergents.



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How to look at cells

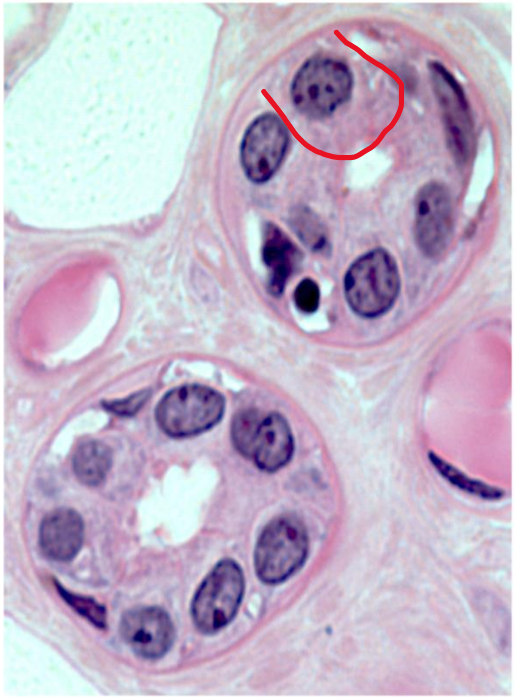
1
Histological dye (Acids and Bases)

2
Immunohistochemistry or Immunocytochemistry (BASED ON ANTIGEN-ANTIBODY REACTION).

- Enzymatic (light microscope)
- Immunofluorescence

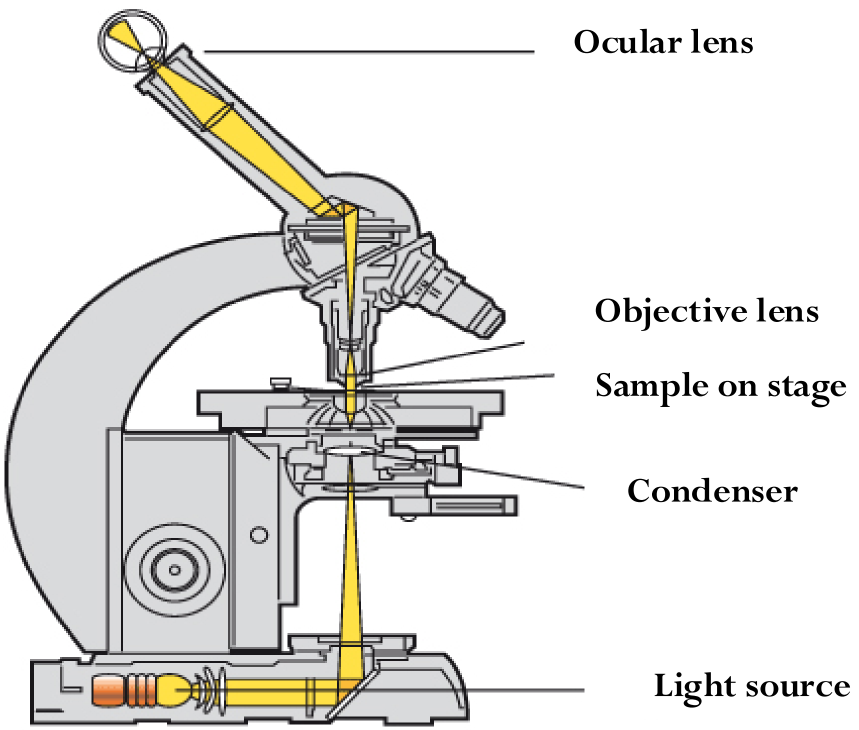
Histological dye (Acid and Bases)

Observed by a light microscope



Hematoxylin
Eosin

Light Microscope



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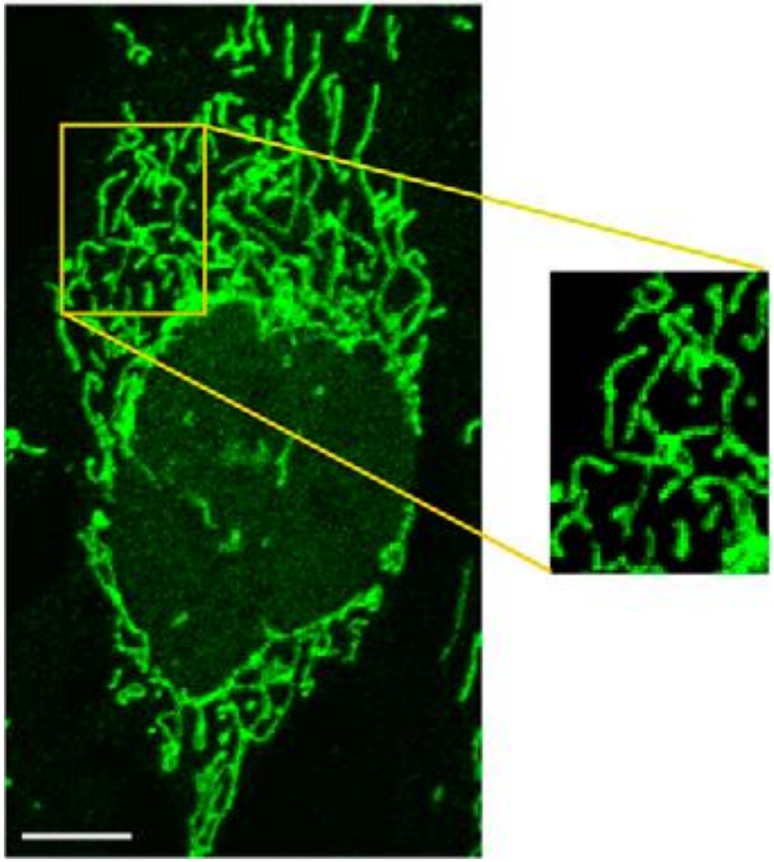
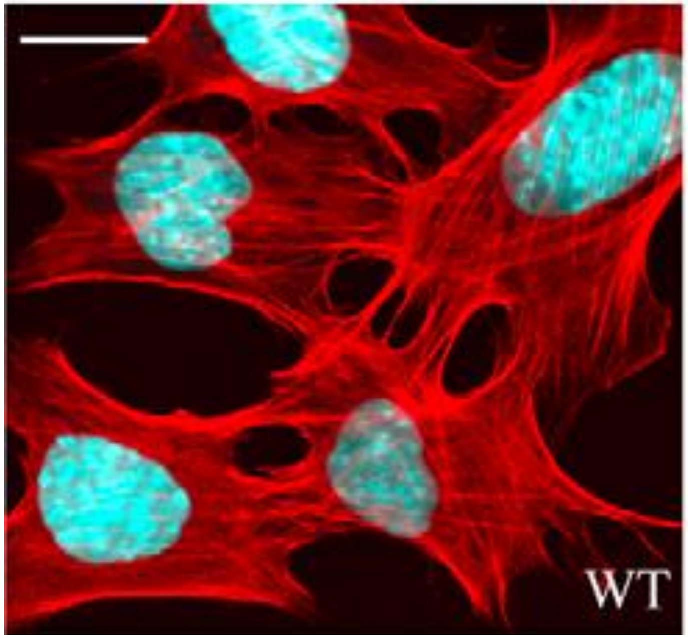
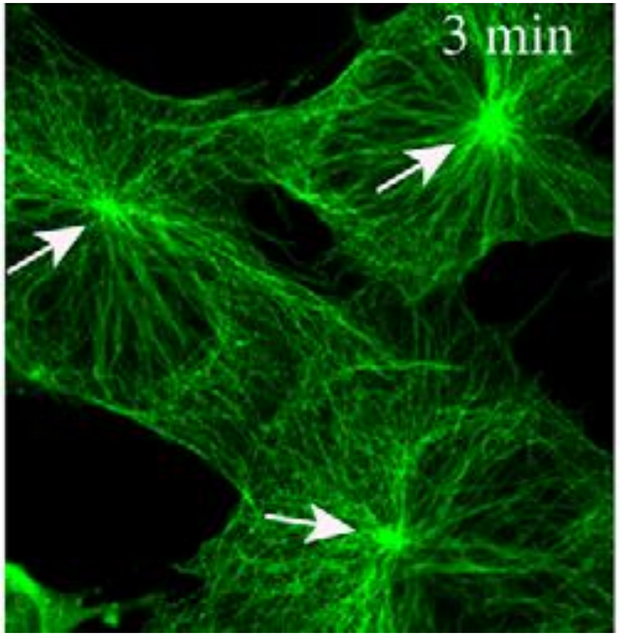
Mitochondria net



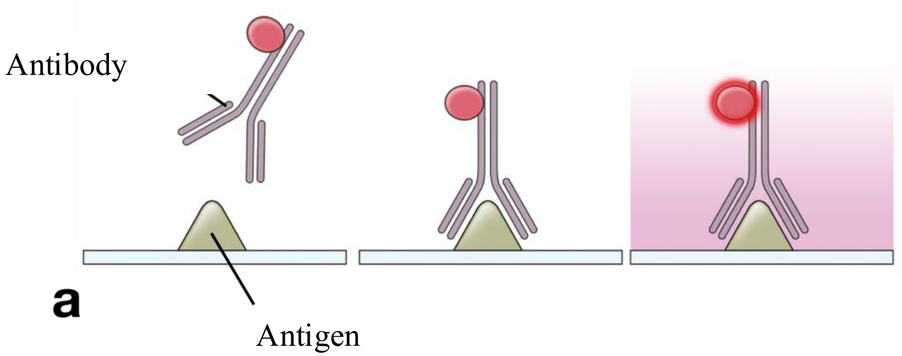
Cytoskeleton





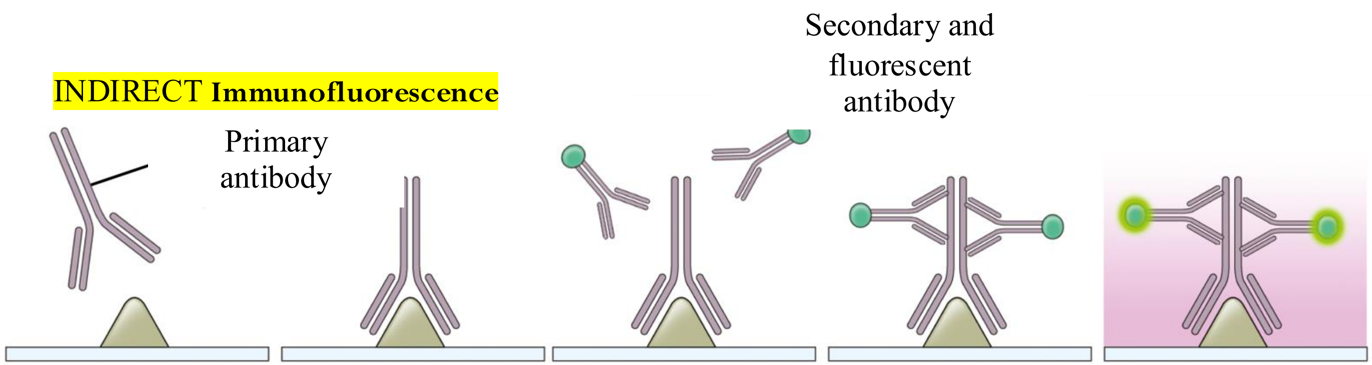
2
Immunofluorescence

DIRECT **Immunofluorescence**

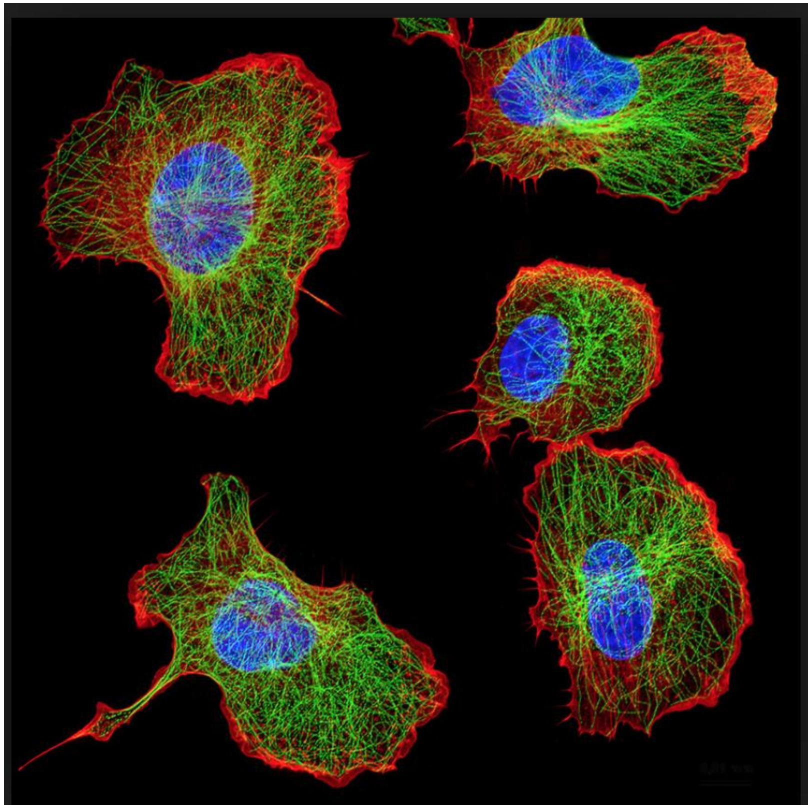


a

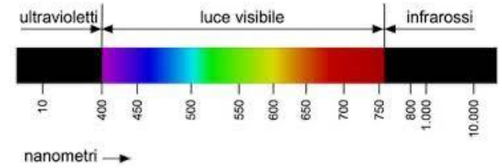
INDIRECT **Immunofluorescence**

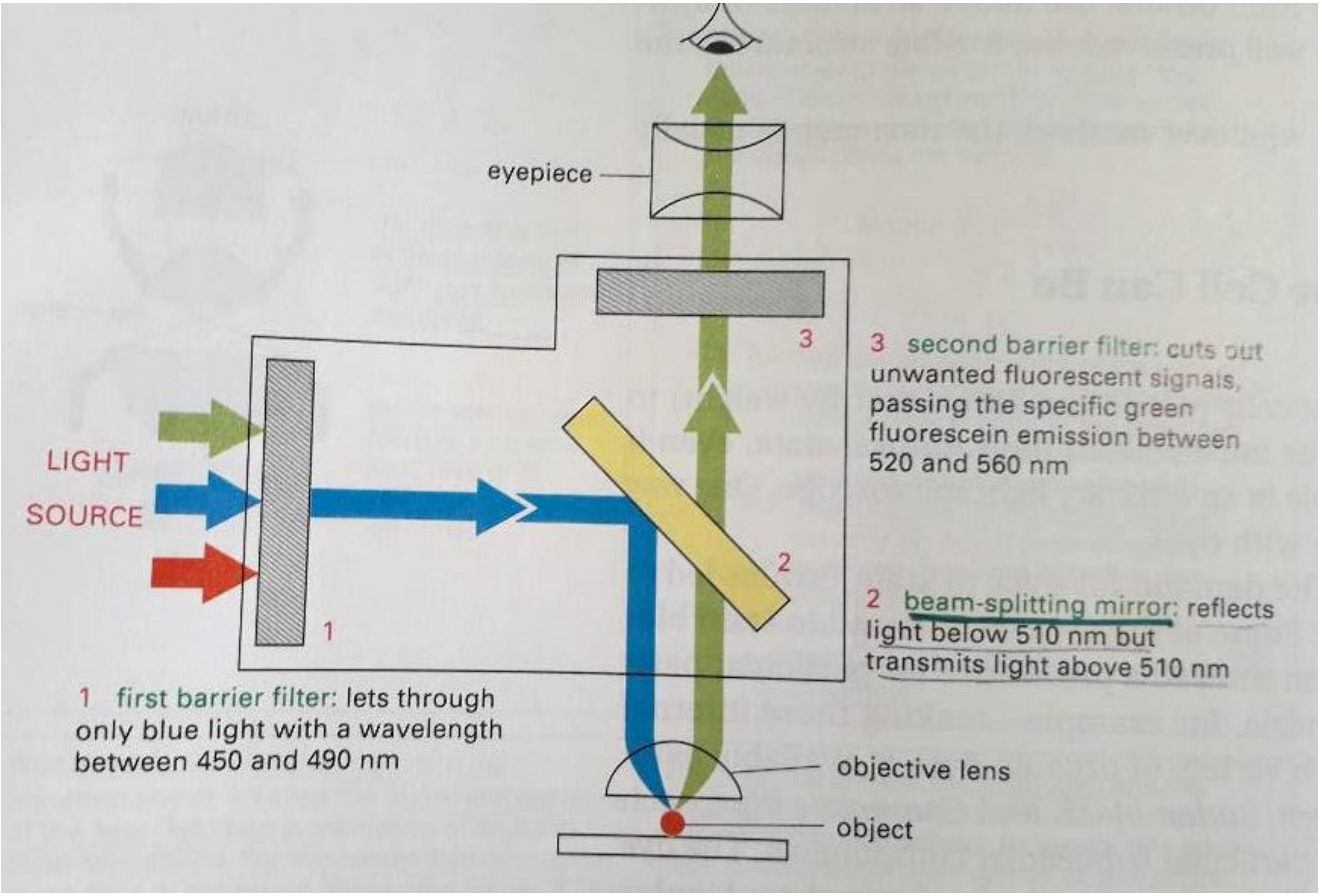


b



Fluorescence Microscope



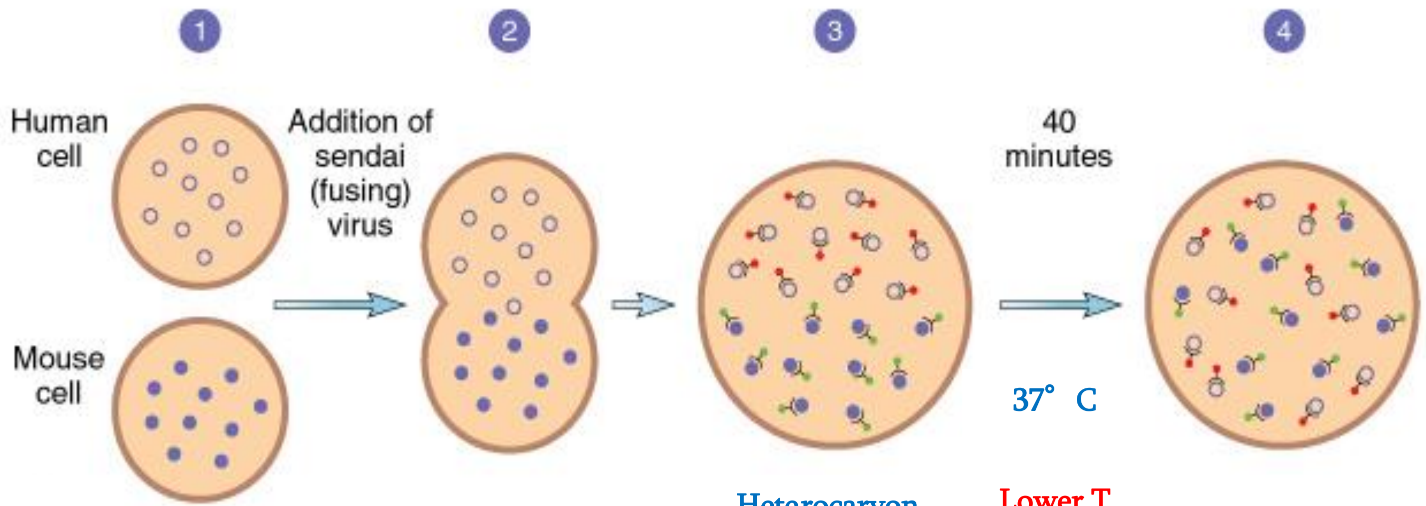


Both lipids and proteins

can **move** in the bilayer

MOBILITY of membrane proteins

1-FUSION of membrane and HETEROCARYON formation



37° C

Heterocaryon immediately after fusion

Lower T higher viscosity

Even distribution of both fluorescencs

Antibodies (conjugated with a fluorescent group) against mouse and human surface proteins

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MOBILITY of membrane proteins

2-Fluorescence Recovery After Photobleaching (FRAP) by laser

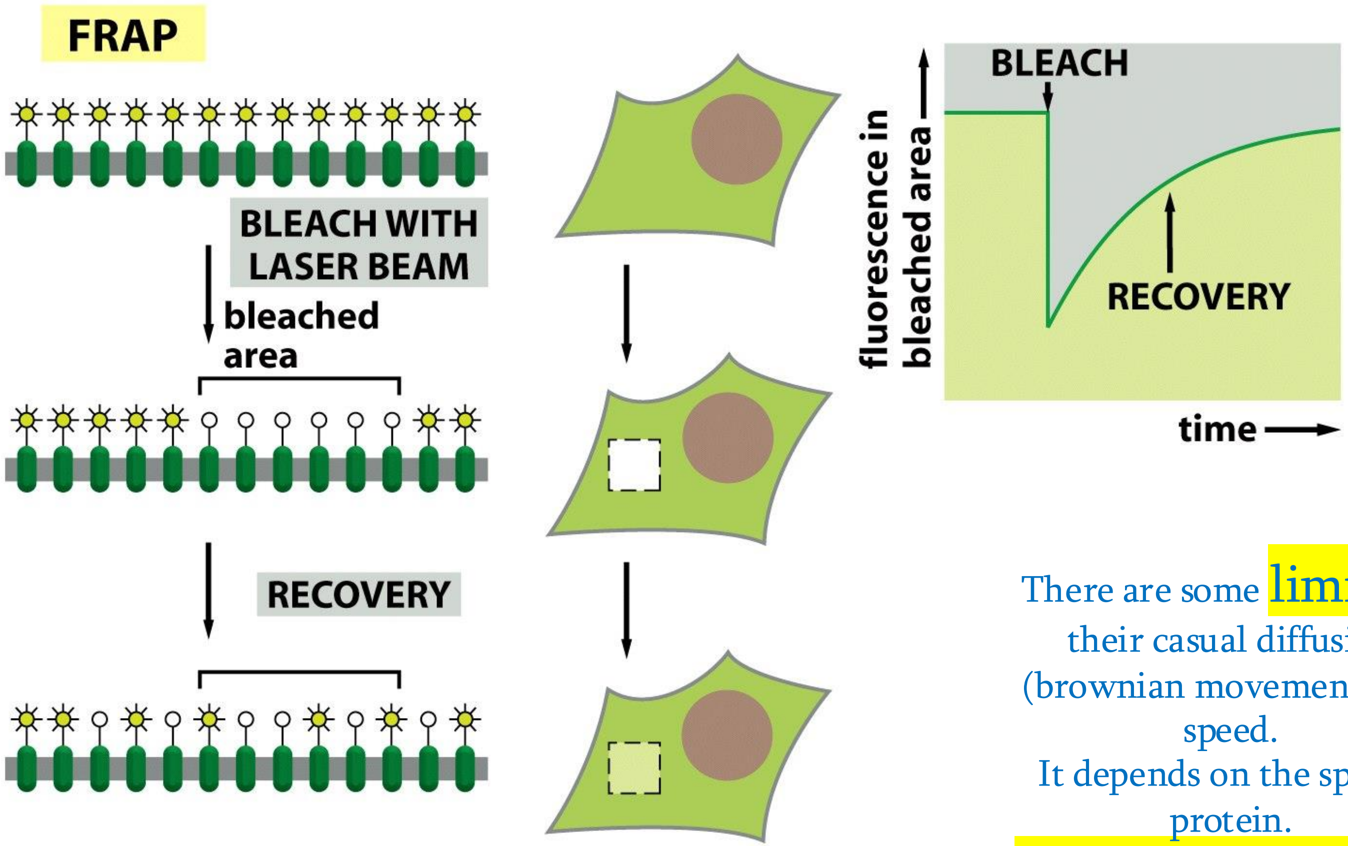


Figure 10-36a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Bruce Alberts et al
Molecular Biology of the Cell
Garland Sciences

There are some limits in their casual diffusion (brownian movement) and speed.
It depends on the specifc protein.
Some barriers, cytoskeleton on the cytosolic leaflet

1972

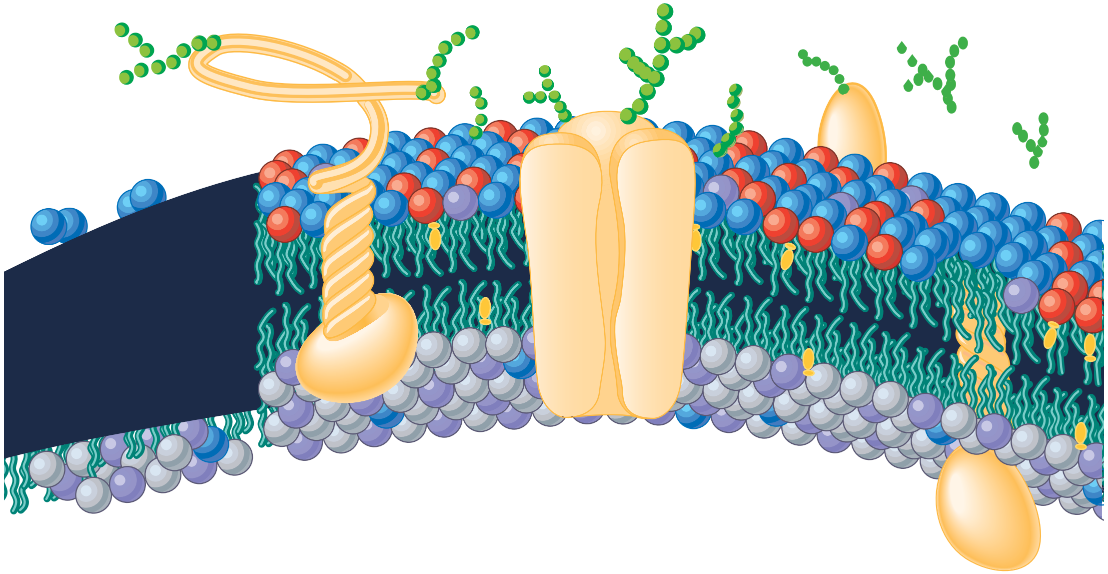
Singer and Nicholson

San Diego

FLUID MOSAIC

The state of membrane is fluid and lipids can move.
Proteins are localized in a mosaic, in a discontinuous way.

Membranes are DYNAMIC and ASYMMETRIC



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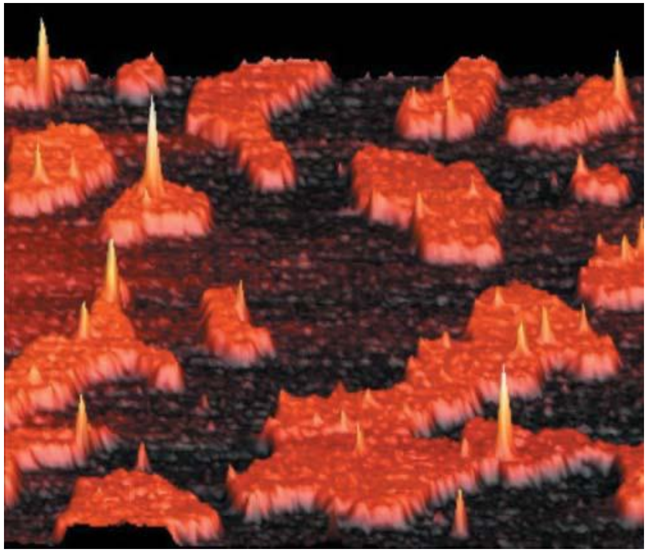
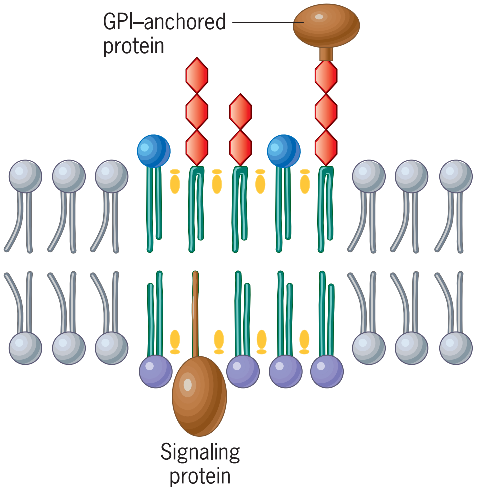
LIPID RAFTs
(an artifact?)

More gelified and ordered areas FLOATING
on a more fluid region (phospholipids).





Cholesterol, Sphingolipids and long satured lipids

Some proteins prefer the rafts (GPI binding proteins).

They might be functional areas for cell signaling

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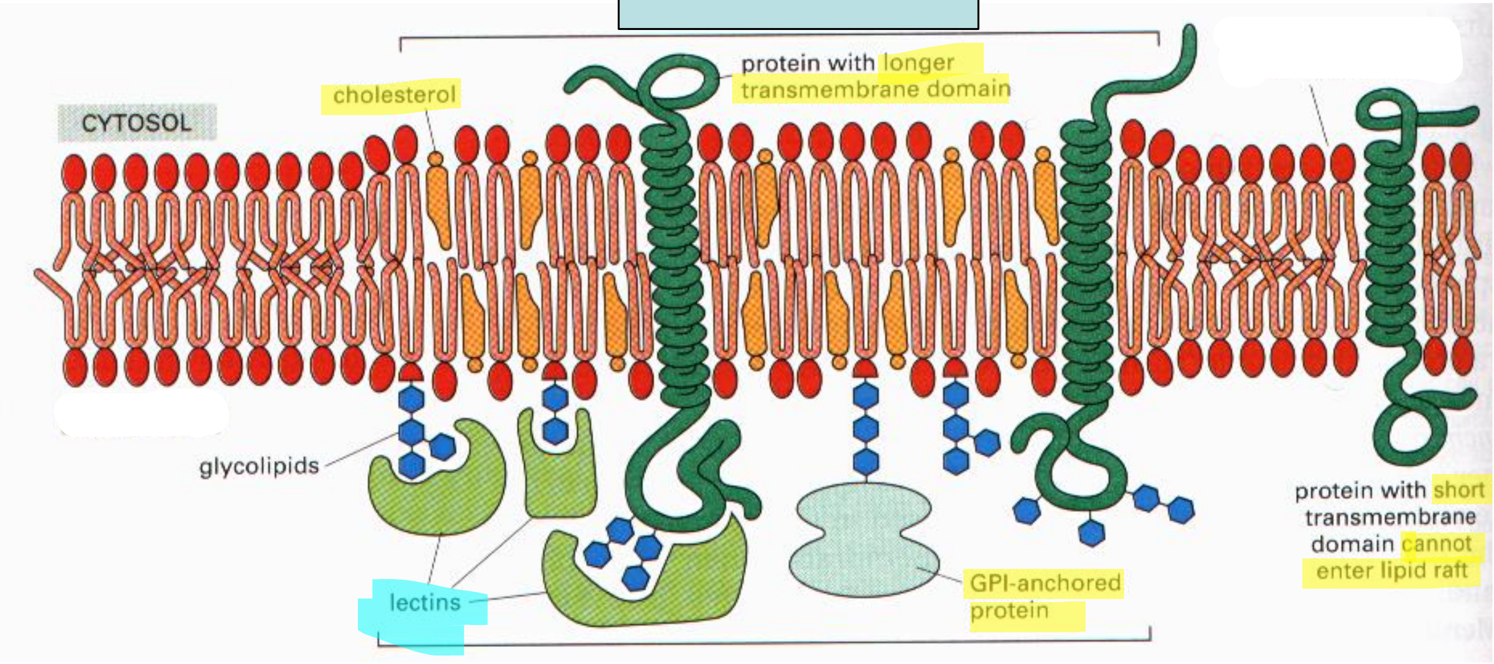
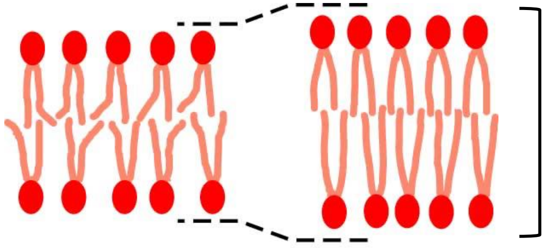
LIPID RAFT





Sphingolipids have more satured tails and tend to be longer and to get together closer

Bruce Alberts et al
Molecular Biology of the Cell
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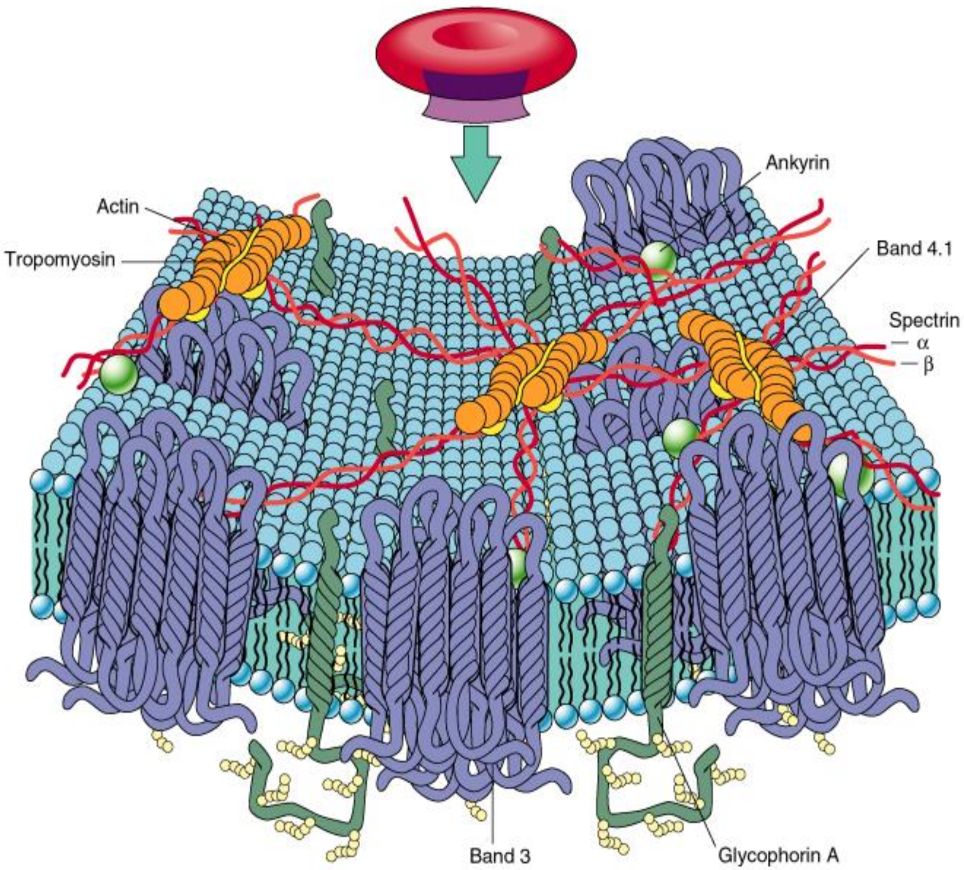
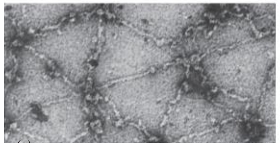
Example:

Plasma membrane of red blood cells

PM of red blood cells





A scanning electron micrograph of human red blood cells. the cells have a biconcave shape and lack a nucleus and other organelles

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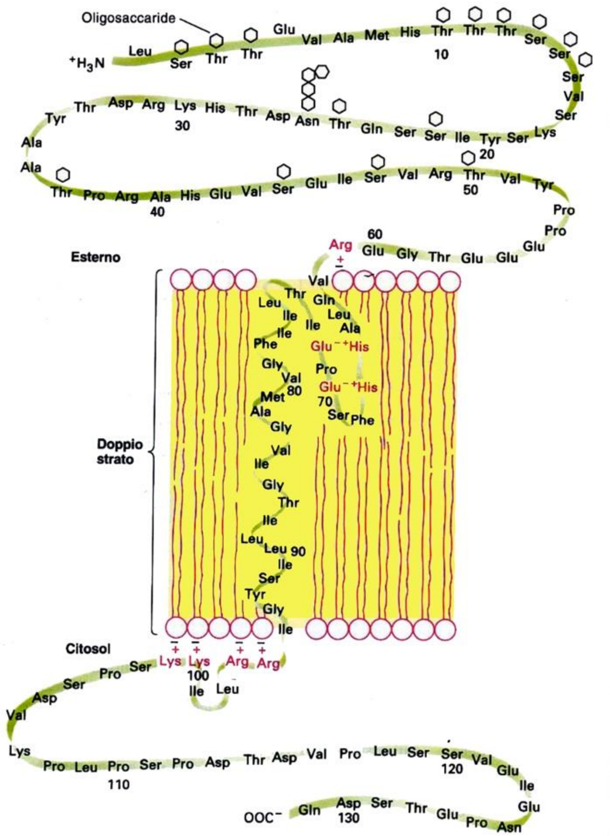
Glycophorin A



Sialic acid-Repulsion-Pushing away erythrocytes.

Also this protein allows the cytoskeleton to associate to the PM

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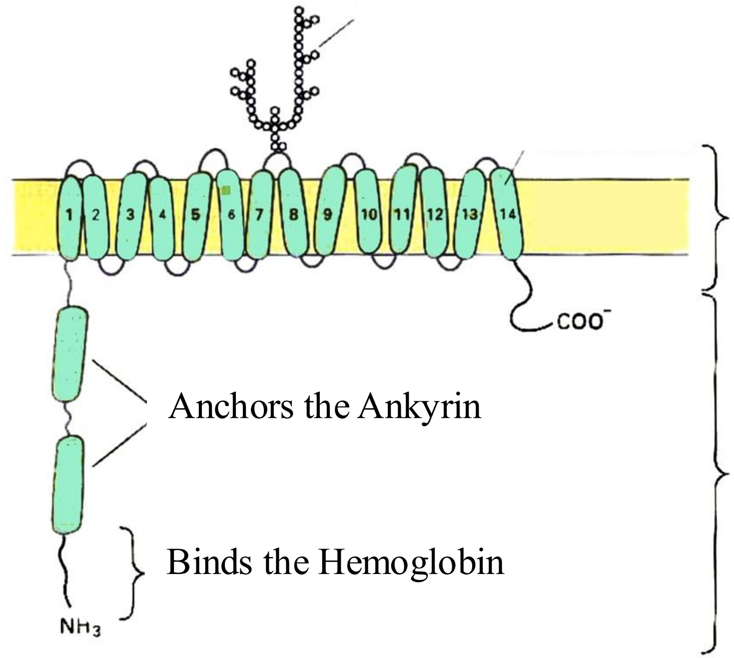
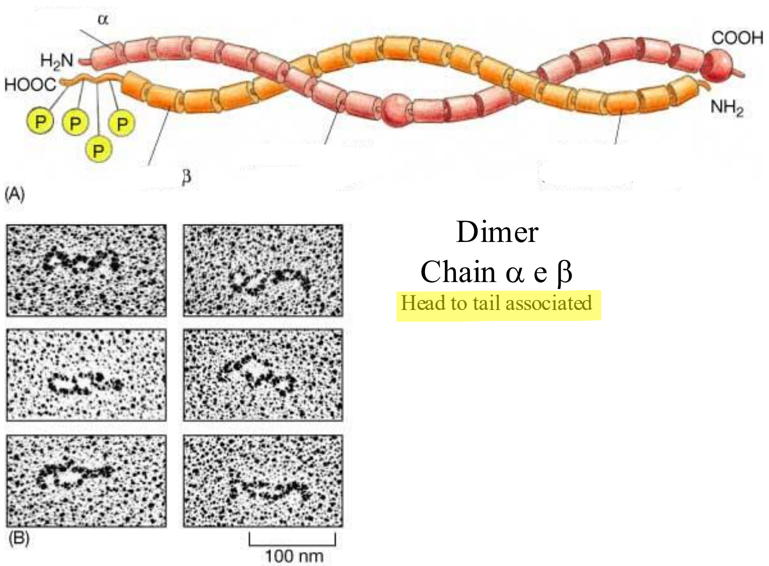
Band 3 protein



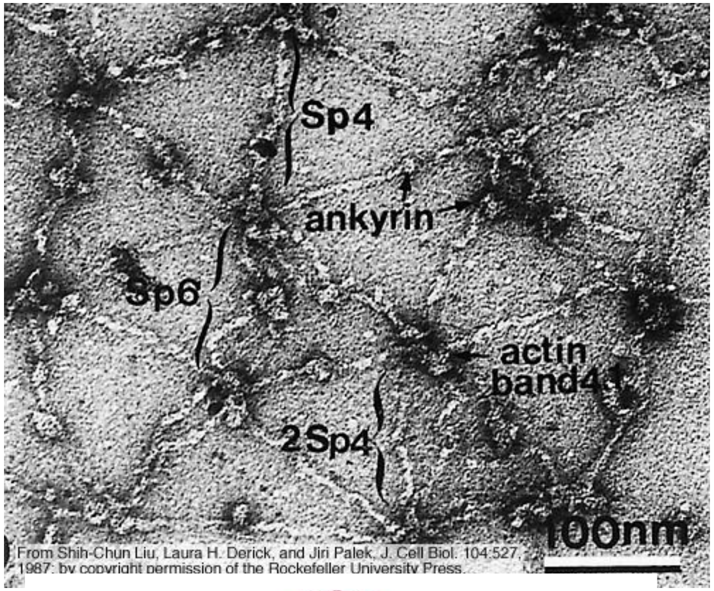
- Dimer
- Channel for anion exchange (PASSIVE facilitated TRANSPORT)
- Also this protein allows the cytoskeleton to associate to the PM

CORTEX
Cytoskeleton

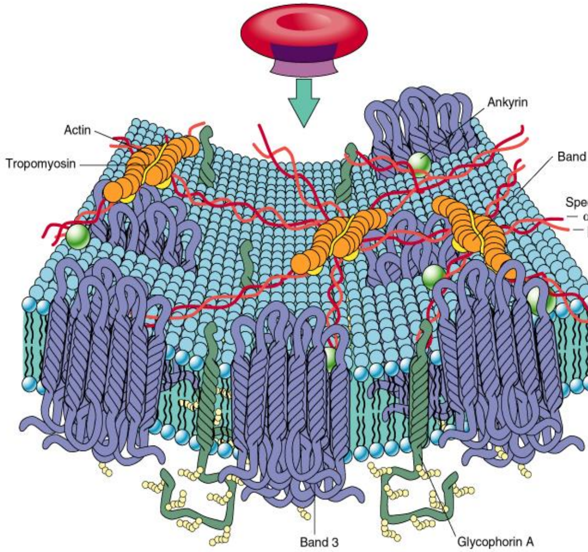
Spectrin



Dimer
Chain α e β
Head to tail associated



From Shih-Chun Liu, Laura H. Derick, and Jiri Palek, J. Cell Biol. 104:527, 1987; by copyright permission of the Rockefeller University Press.



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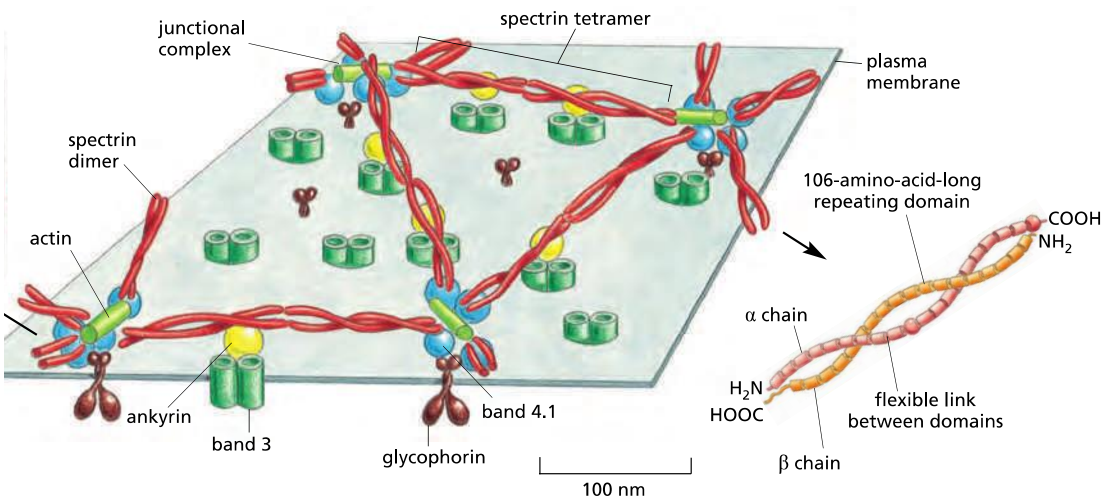
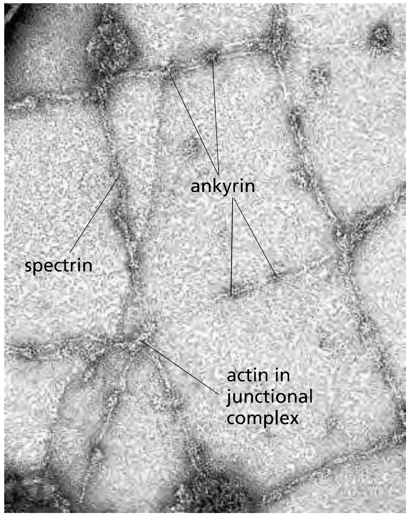
PM of red blood cells





The electron micrograph shows the cytoskeleton on the cytosolic side of a red blood cell membrane. It is like a meshwork.

The cytoskeleton is linked to the membrane through two transmembrane proteins: a multipass protein called *band 3* and a single-pass protein called *glycophorin*. Spectrin binds to some band 3 proteins via *ankyrin* molecules, and to glycophorin and band 3 (not shown) via band 4.1 proteins.

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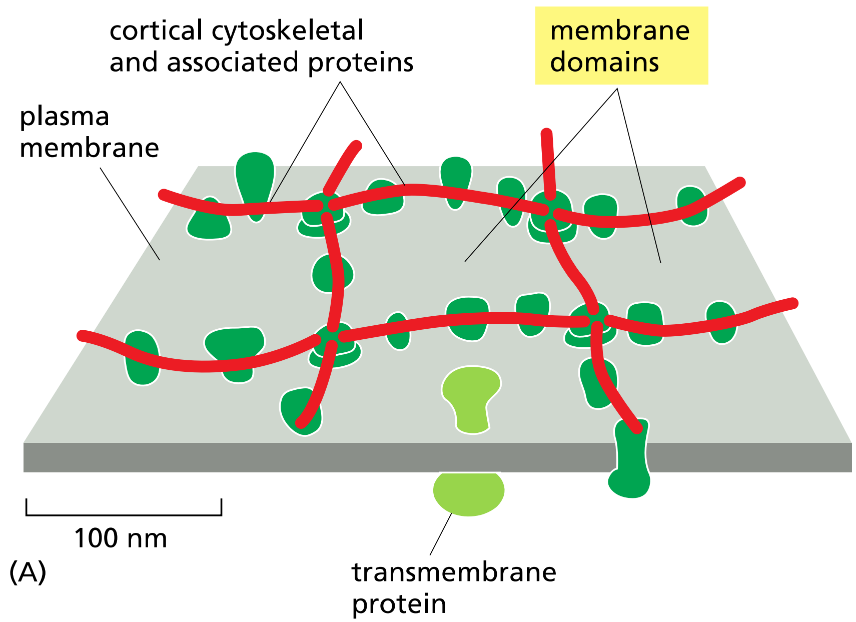
Because the cytoskeletal filaments are often closely apposed to the cytosolic surface of the plasma membrane, they can form mechanical barriers that obstruct the free diffusion of proteins in the membrane. These barriers partition the membrane into small domains, or *corrals*.



- Fibrous proteins (long shape-structural role: collagens, silk, keratin…)

- Globular proteins (compact and more frequent)