

Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

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The Project has been realised with the support of the European Union and has been co-financed by the European Social Fund ***

**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

***A projekt az Európai Unió támogatásával, az Európai Szociális Alap társfinanszírozásával valósul meg.

INTRODUCTION TO BIOPHYSICS

(Bevezetés a biofizikába)

BIOLOGICAL MEMBRANES

(Biológiai membránok)

GYÖRFFY DÁNIEL, ZÁVODSZKY PÉTER

Introduction

- Cells and compartments of cells are surrounded by membranes
- These membranes separate the interior of cells from the extracellular space but they also connect them to each other
- Material, energy and information can get across the membrane
- Biological membranes are mainly built of phospholipids

Phospholipids

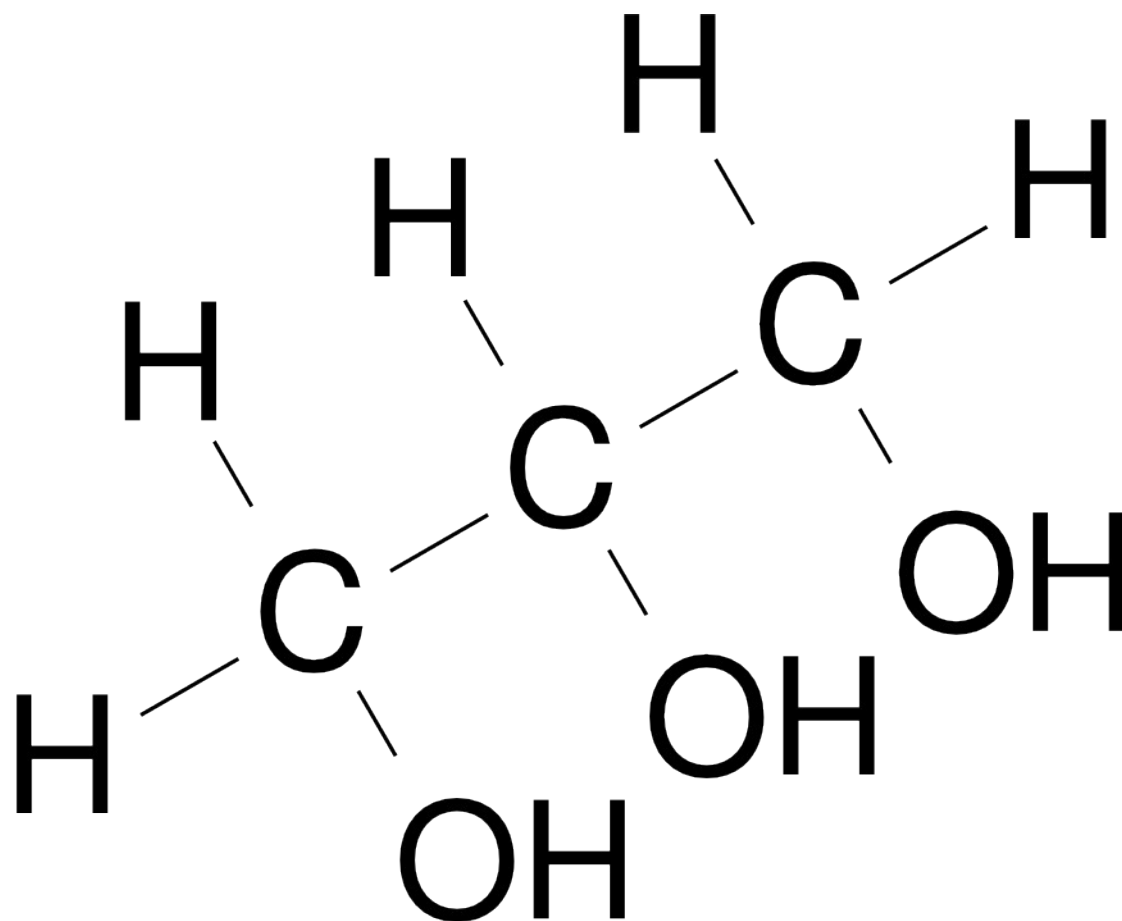
- Lipids are a group of organic compounds
- Their characteristic property is the hydrophobicity of at least a part of the molecule
- Some of them are amphiphilic, that is they contain a polar group in addition to the apolar bulk
- Phospholipids are a class of lipids which contains a phosphate group

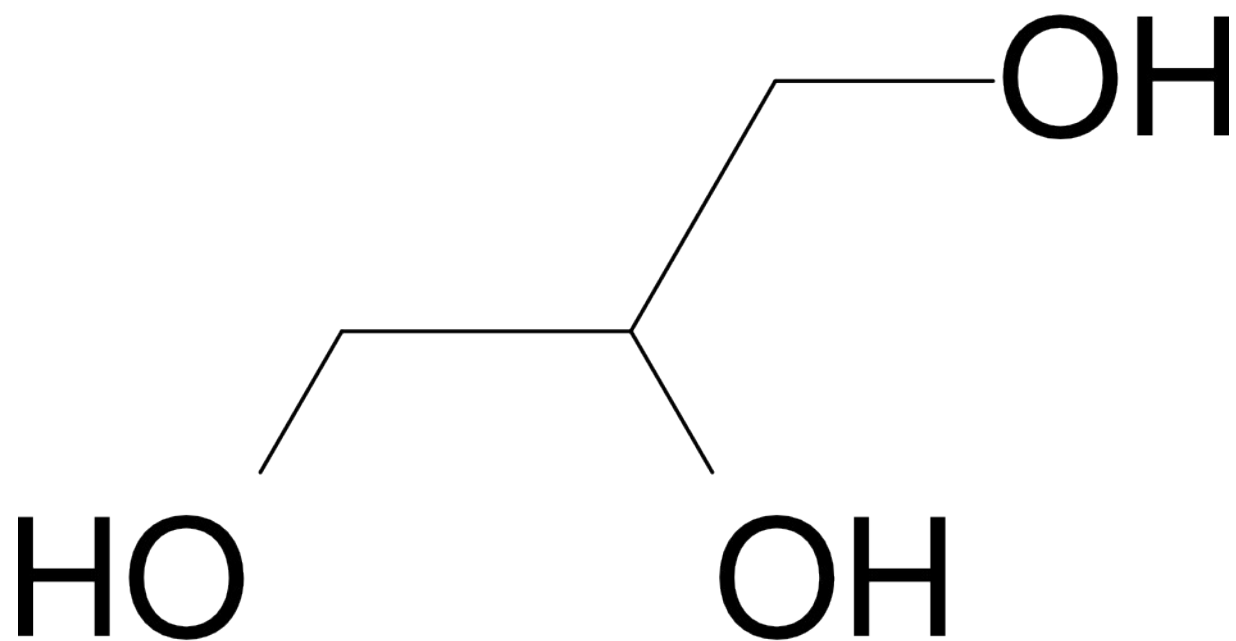
- The fundamental building blocks of phospholipids are:
 - Glycerol
 - Fatty acids
 - Phosphate group
 - Other groups

Glycerol

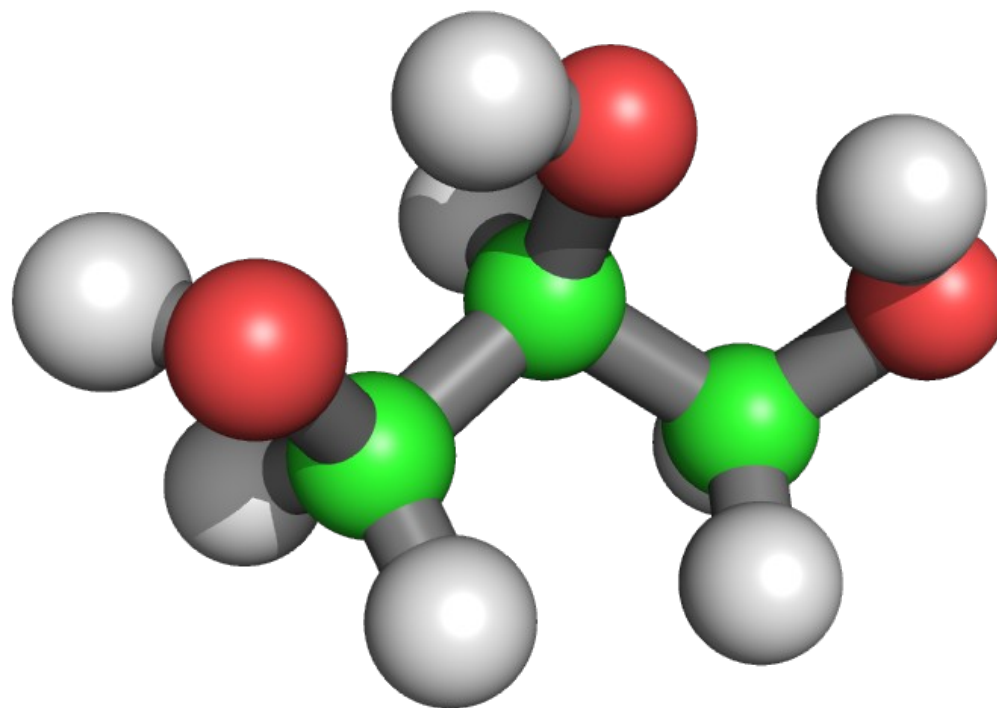
- Glycerol is an alcohol containing three hydroxyl groups
- It can form three ester bonds by these three hydroxyls
- Glycerol has a large solubility in water
- In itself glycerol is toxic for the human organism

2D structure of glycerol





3D structure of glycerol

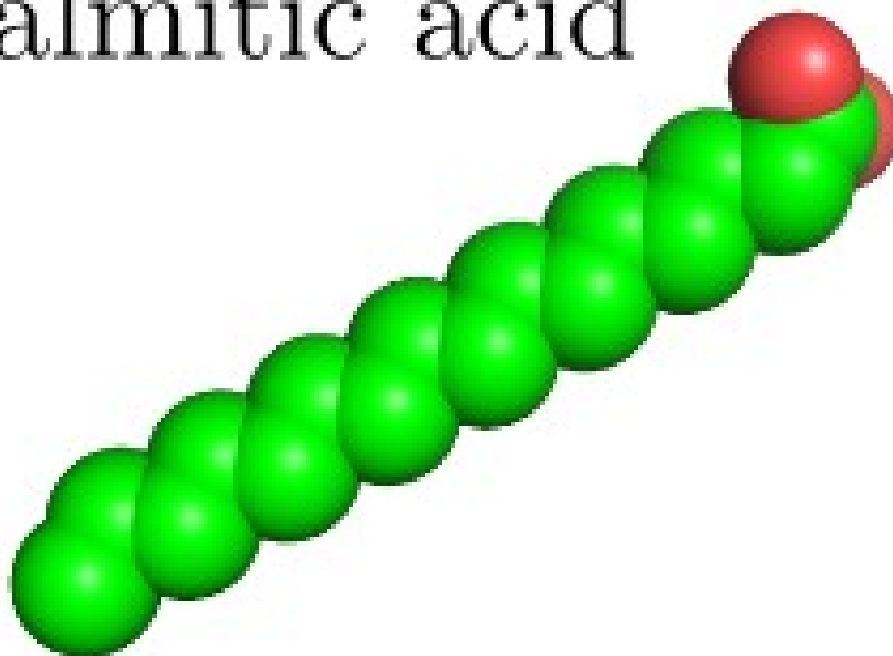


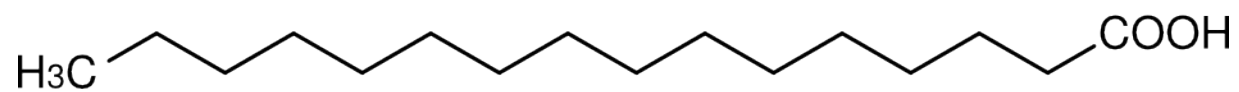
Fatty acids

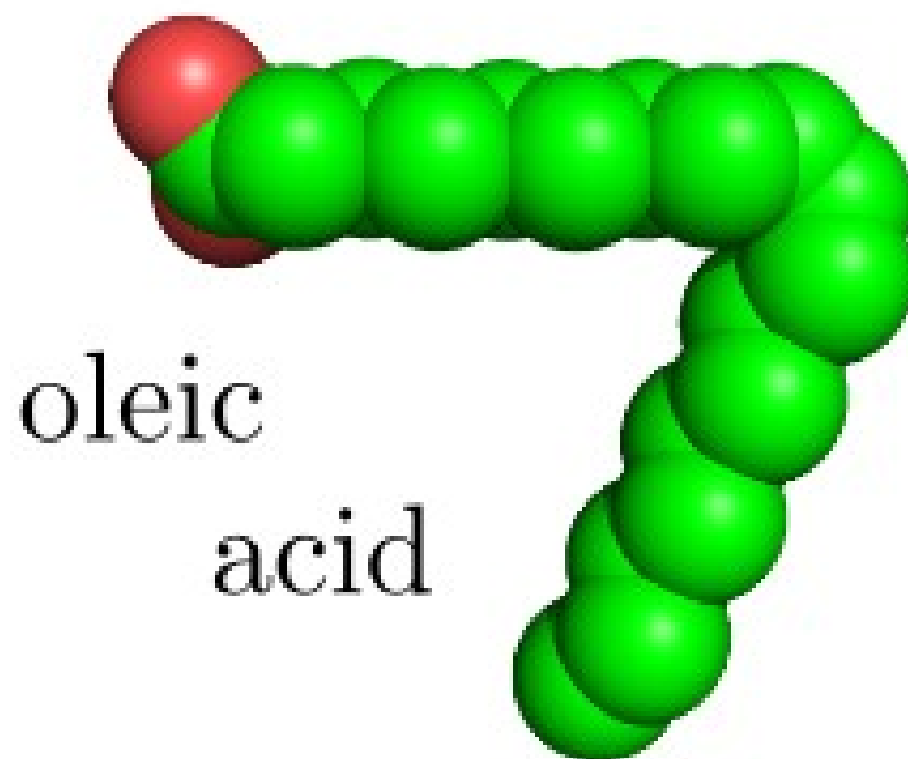
- Fatty acids are carboxylic acids with a long linear aliphatic chain
- They can be unsaturated or saturated according to whether or not they contain double covalent bonds between carbon atoms
- Fatty acids in an organism usually have an even number of carbon atoms

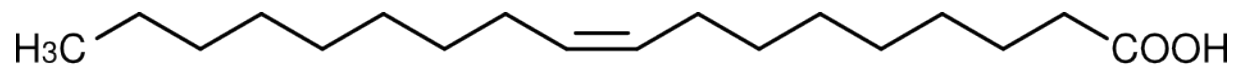
Some characteristic fatty acids

palmitic acid

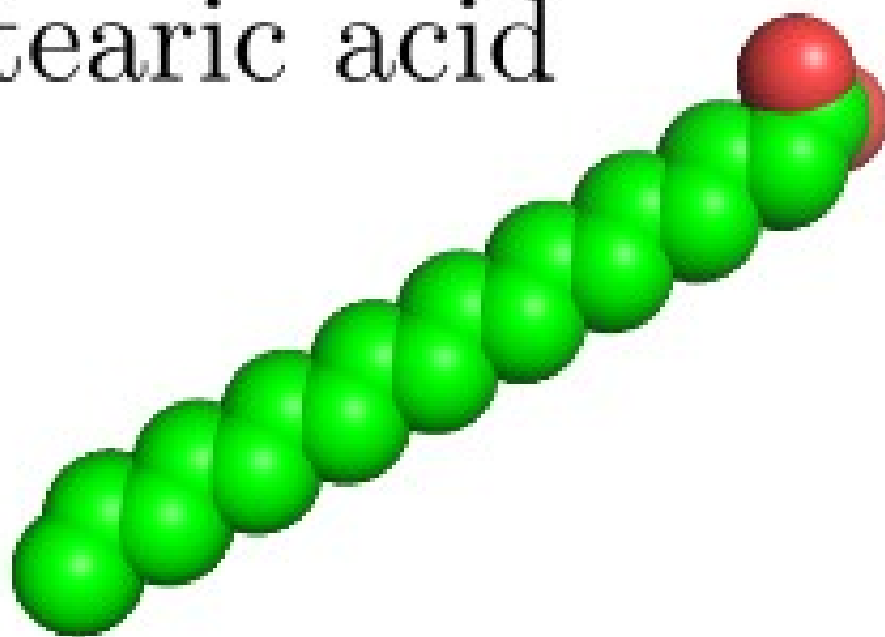


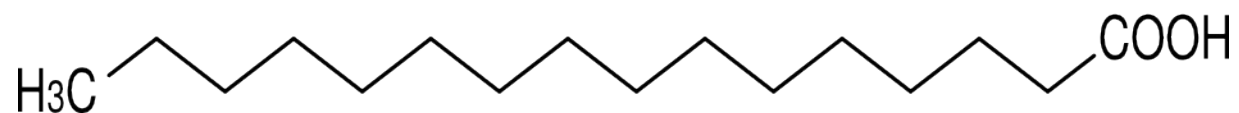


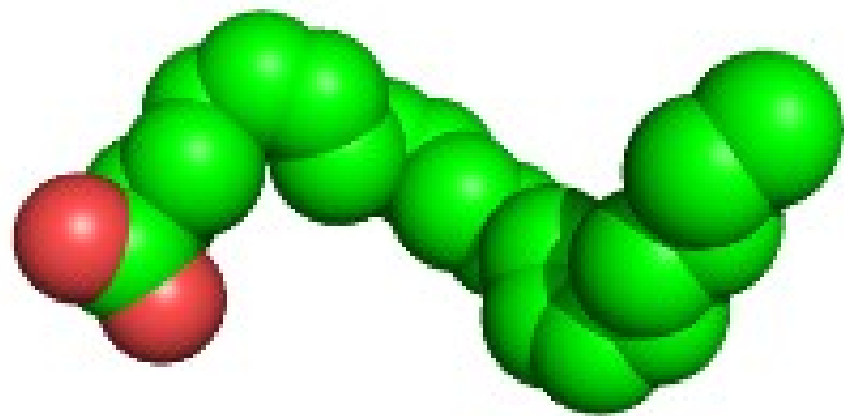




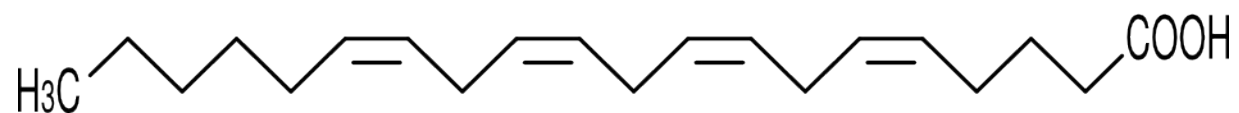
stearic acid





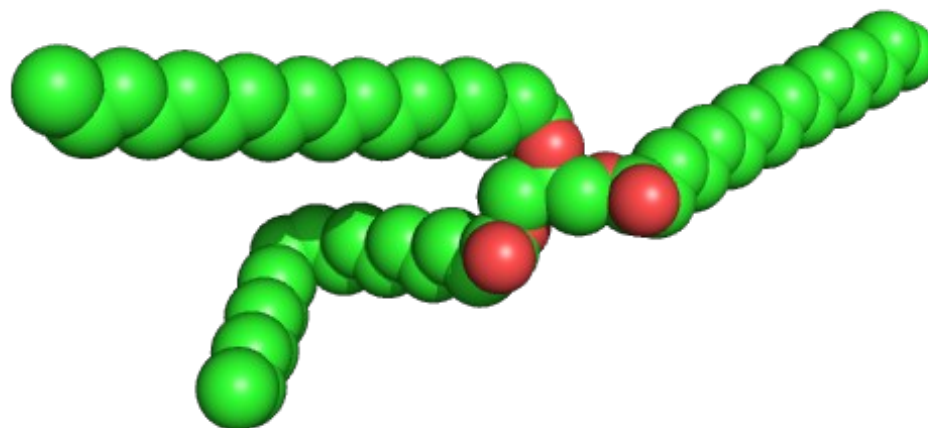


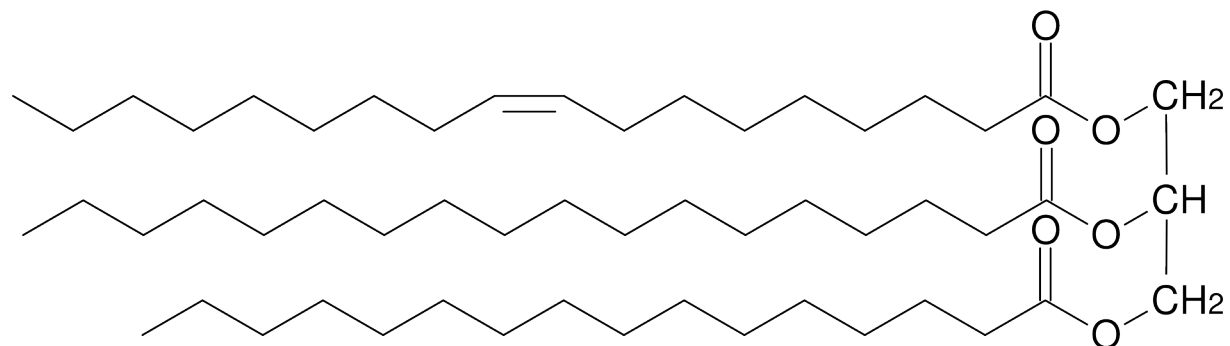
arachidonic acid



- Glycerol and fatty acids can be connected by ester bonds forming glycerides
- Common fats and oils are triglycerides, that is they consist of a triester of glycerol with three fatty acids
- This glyceride structure is the basis of more complex lipids like phospholipids

Triglyceride





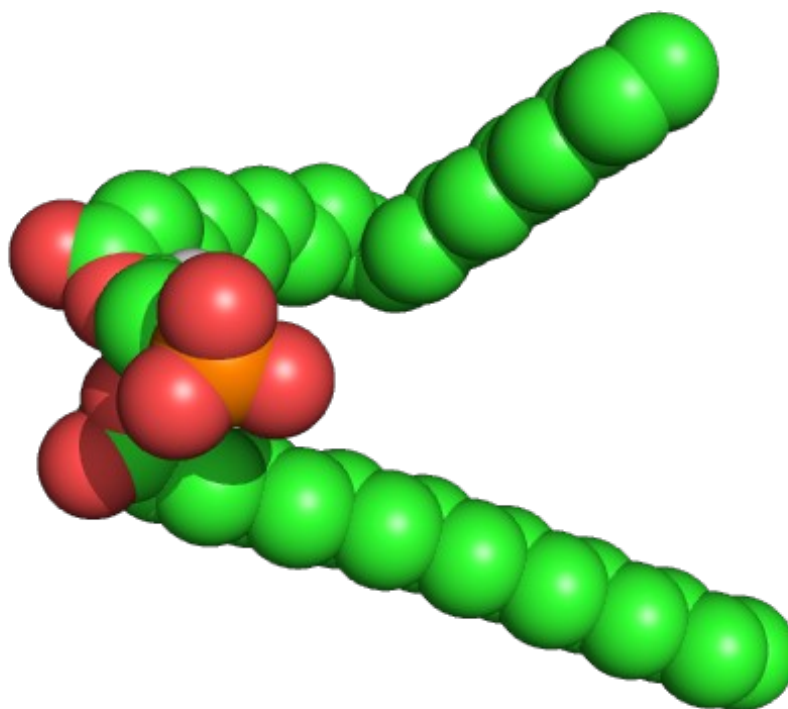
Phospholipids

- Most phospholipids consist of a glycerol (but for example sphingomyelin contains sphingosine) esterized by two fatty acids and a phosphoric acid, and some organic group connected to the phosphate
- Phospholipids are the main lipid building blocks of biological membranes
- The basic compound of phospholipids is phosphatidic acid

Phospholipids

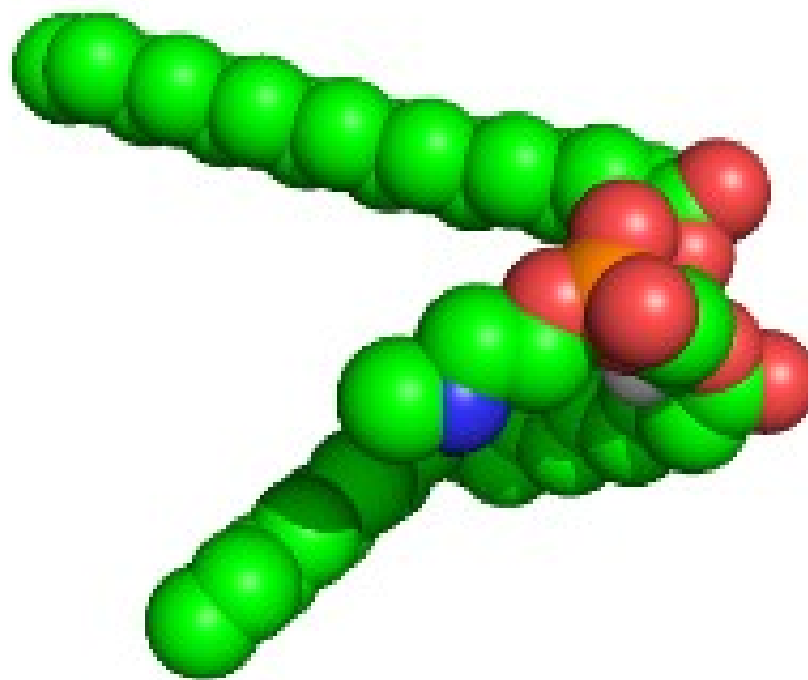
Diacylglyceride phospholipids	Phosphosphingolipids
Phosphatidic acid	Ceramide phosphorylcholine
Phosphatidylethanolamine	Ceramide phosphorylethanolamine
Phosphatidylcholine	Ceramide phosphorylglycerol
Phosphatidylserine	
Phosphoinositides	

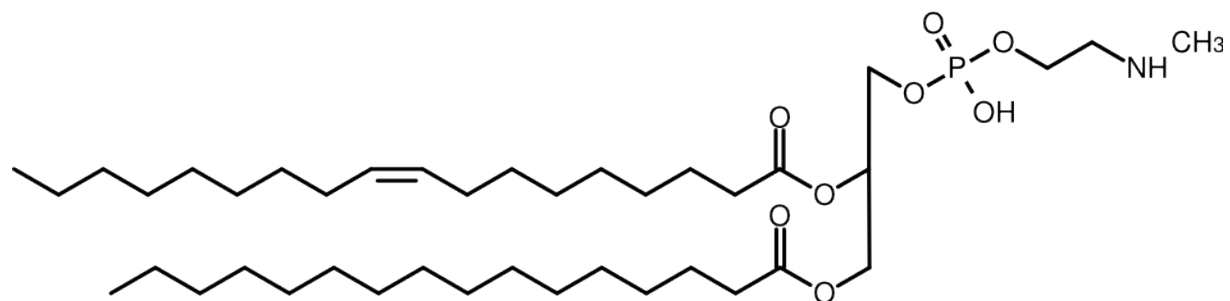
Phosphatidic acid



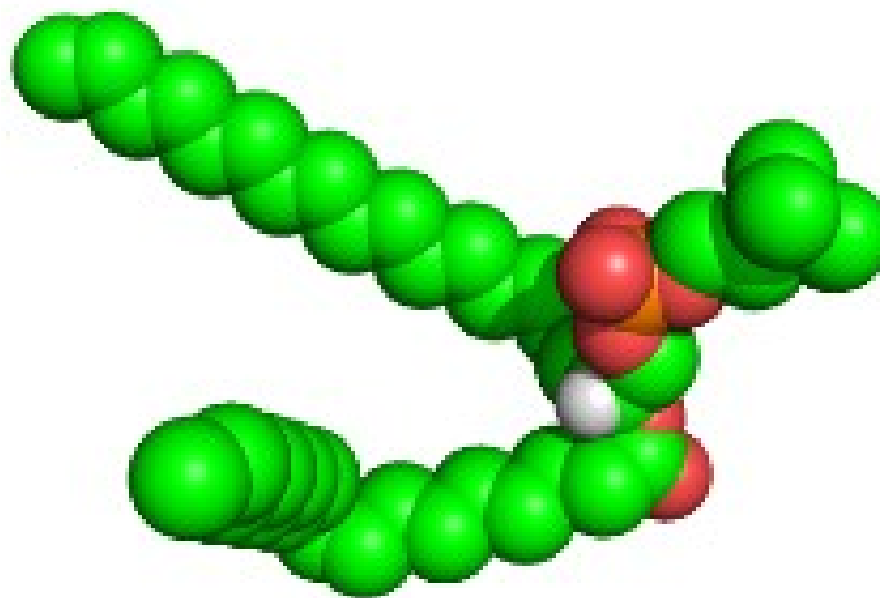
Other phospholipids

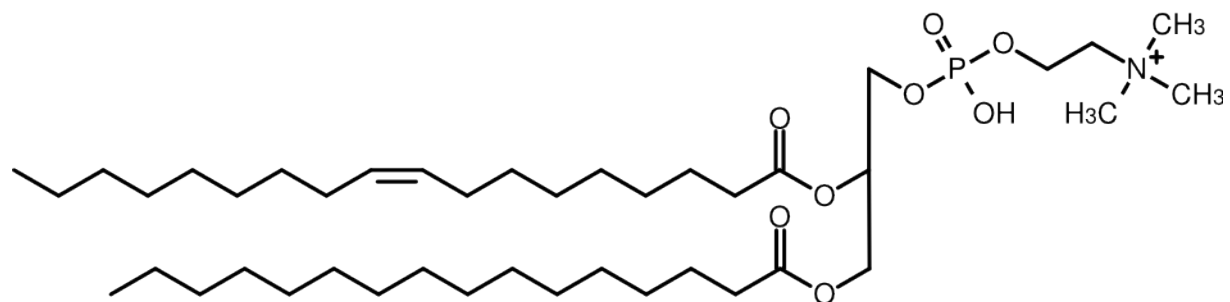
phosphatidylethanolamine



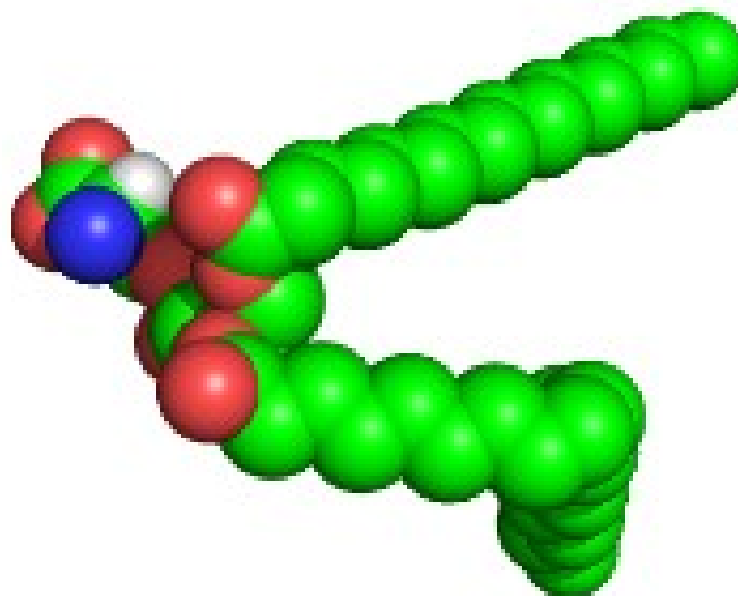


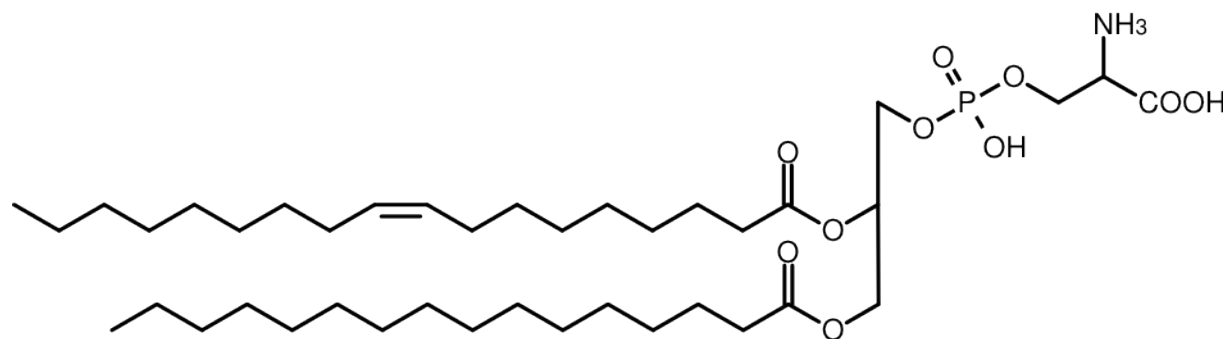
phosphatidylcholine



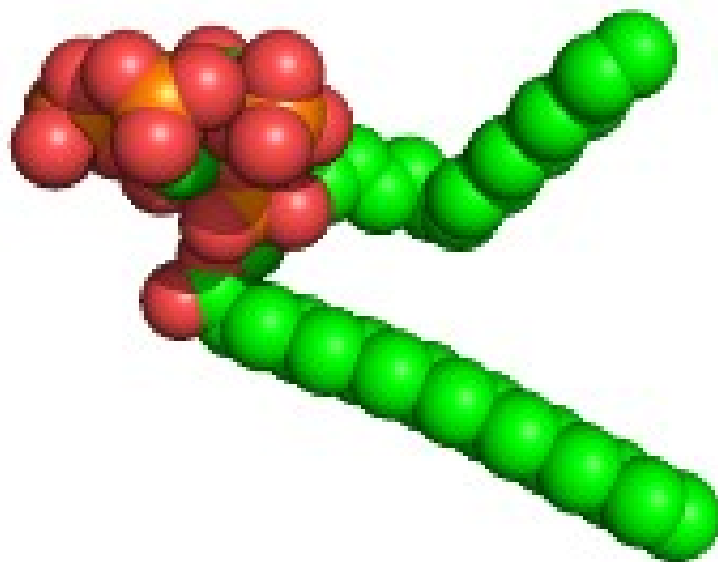


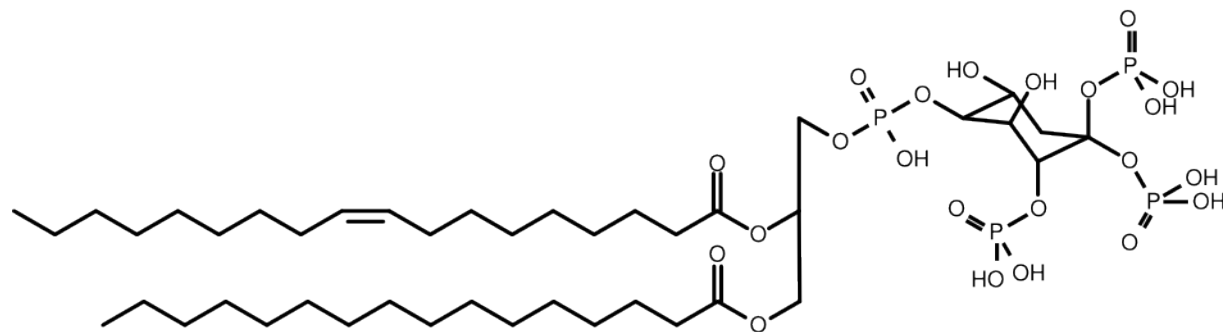
phosphatidylserine



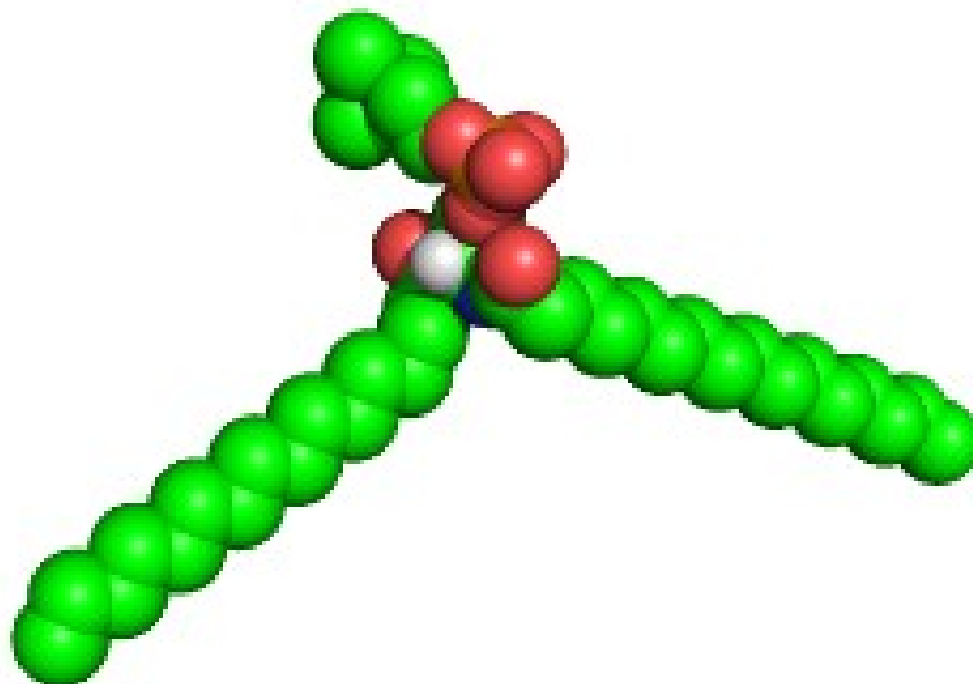


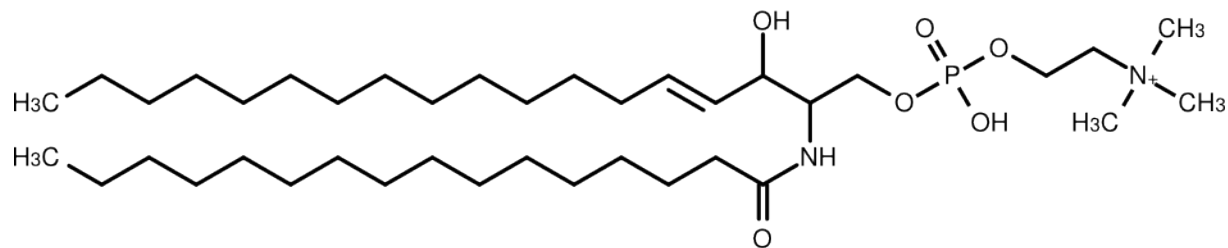
phosphatidylinositol triphosphate





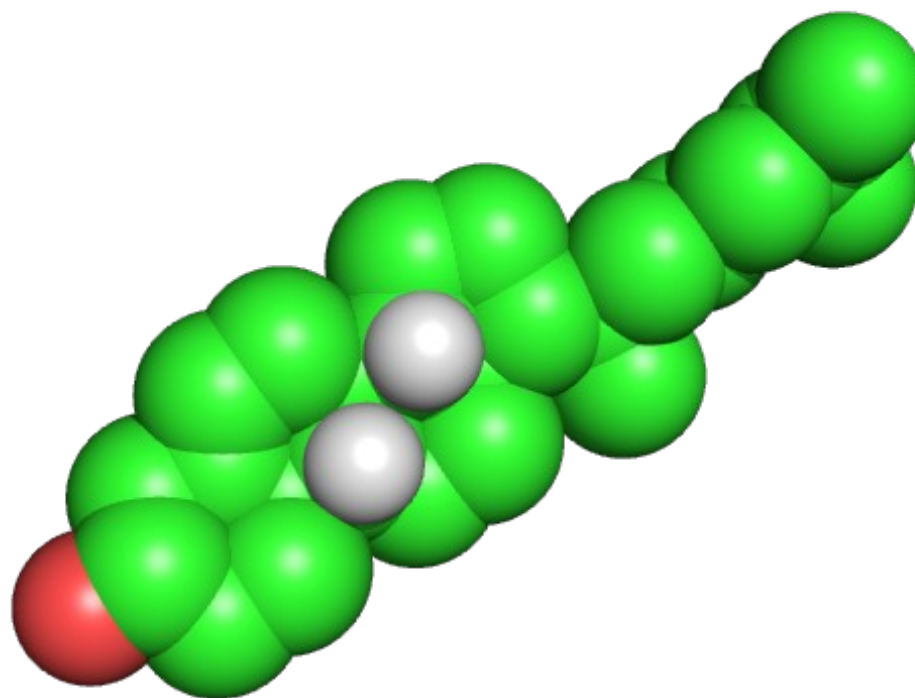
sphingomyelin

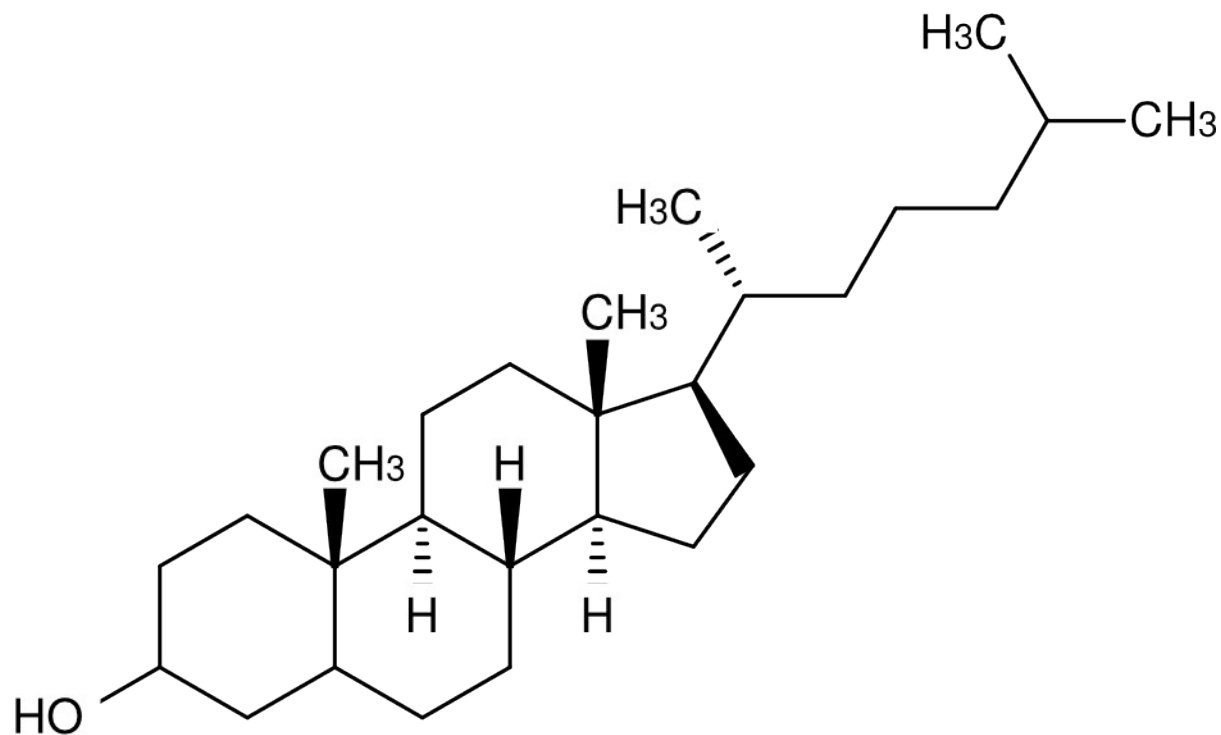




- Membranes also contain other lipids such as steroids, for example cholesterol

Cholesterol

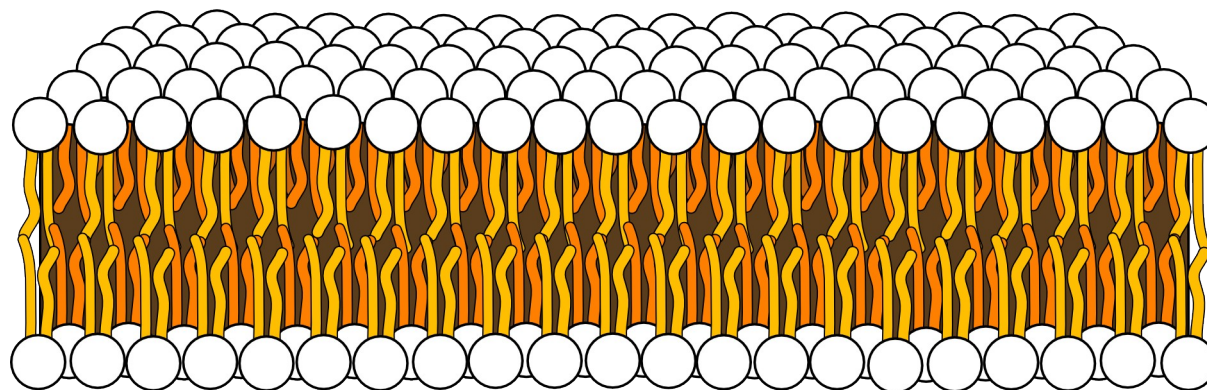




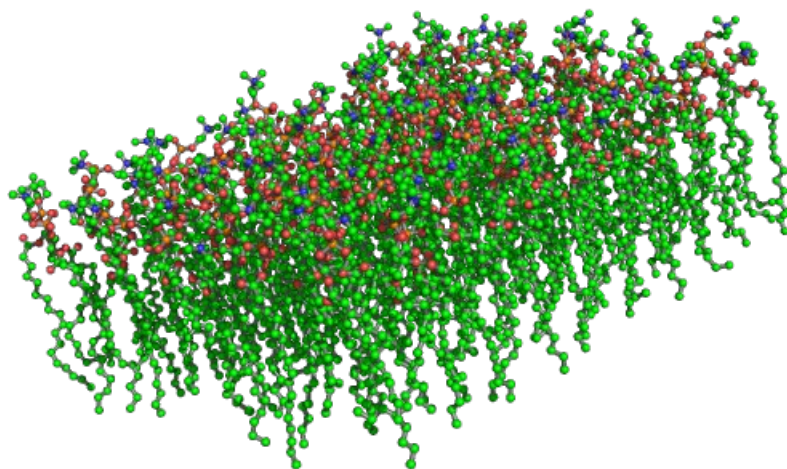
Lipid bilayer

- The lipids building up biological membranes have a polar head and an apolar tail
- In consequence of this property, they are arranged in a bilayer structure such that the apolar tails point toward the centre of the membrane and the polar heads point outside

Schematic lipid bilayer



Atomic picture of one layer of a membrane

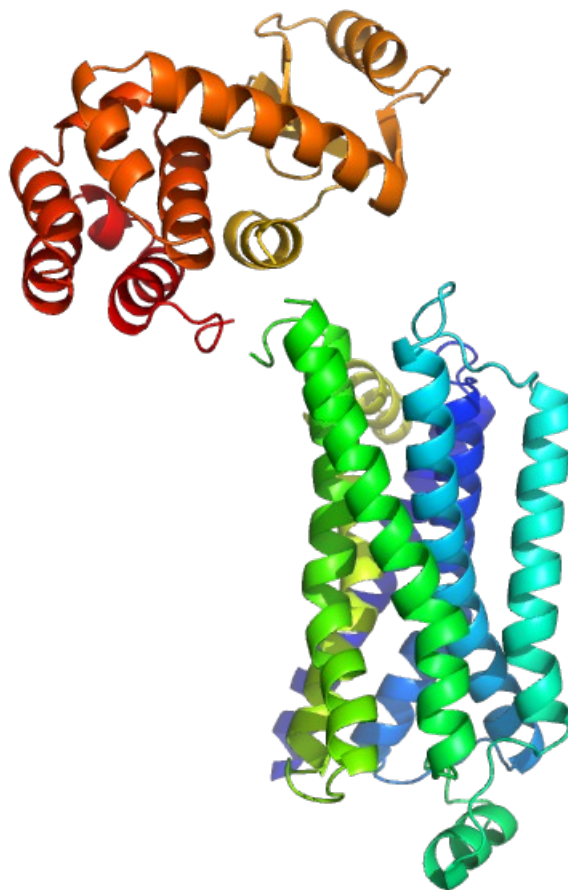


Membrane proteins

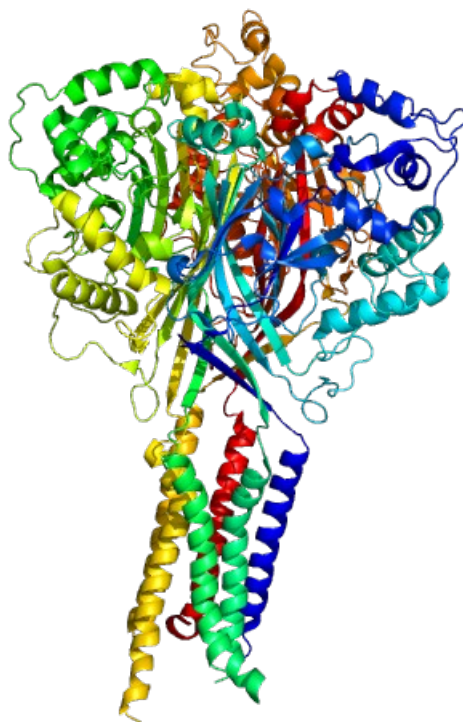
- Biological membranes also contain proteins
- Some of them span across the membrane; these are called *transmembrane proteins*
- Some of them are attached to the membrane permanently but do not necessarily span across the membrane; these are called *integral membrane proteins*

- In contrast, some of them are only attached temporarily to the membrane; these are called *peripheral membrane proteins*
- Such membrane proteins are, for example, the regulatory subunits of receptors and ion channels

A 7TM receptor



A Na⁺ channel



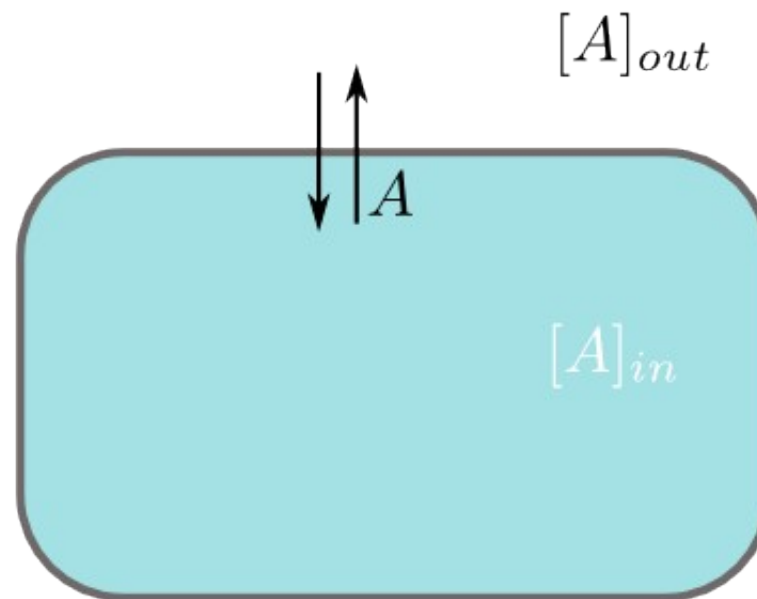
Membrane potential and the Nernst equation

- If the charges on the two sides of a membrane are not equal, an electric potential gradient appears
- For ions that can pass freely across the membrane, an equilibrium develops
- The Nernst equation describes the relationship between the potential gradient and the concentrations of the ion inside and outside the cell

The Nernst equation

- Let us consider a cell bounded by a semi-permeable membrane
- For an ion A which can freely pass across the membrane, let $[A]_{in}$ denote its concentration inside and $[A]_{out}$ outside the cell, respectively

The membrane is permeable for the ion A



- An ion is subject to two opposing forces, one caused by the concentration gradient and the other caused by the Coulomb forces between electric charges
- The chemical potential of a substance gives us the free energy of one mole of it
- For one mole of A the free energy is

$$\mu = \mu^0 + RT \ln [A]$$

where μ^0 is the standard chemical potential and $[A]$ is the molar concentration of A

- The free energy difference between the two sides of the membrane for one mole of A is

$$\Delta\mu = \mu_{inside} - \mu_{outside}$$

- Since the standard chemical potential is independent of the ion concentration and depends only on the properties of the substance, it is the same on both sides of the membrane

$$\mu_{inside}^0 = \mu_{outside}^0$$

- Taking into account the preceding equation, the free energy increase when one mole of A is taken into the cell is

$$\Delta\mu = RT \ln [A]_{inside} - RT \ln [A]_{outside}$$

$$\Delta\mu = RT \ln \frac{[A]_{inside}}{[A]_{outside}}$$

- Now let us consider the effect of electric charges
- The free energy of one mole of ions due to an electric potential is

$$G_{electric} = zF\Phi$$

where z is the valence of the ion, for example +1 in the case of sodium and -1 in the case of chloride, F is the Faraday constant and Φ is the electric potential

- The free energy increase when one mole of ions is moved from outside to inside the cell due to the electrical potential is:

$$\Delta G_{electric} = G_{(electric \text{ in})} - G_{(electric \text{ out})}$$

that is

$$\Delta G_{electric} = zF\Delta\Phi$$

where

$$\Delta\Phi = \Phi_{inside} - \Phi_{outside}$$

is the *membrane potential*

- Based on these equations, the total free energy increase when one mole of A is taken inside the cell is

$$\Delta\mu + \Delta G_{electric} = RT \ln \frac{[A]_{inside}}{[A]_{outside}} + zF\Delta\Phi$$

- At equilibrium, ions moving into the cell neither gain nor lose energy so this free energy increase is zero

$$RT \ln \frac{[A]_{inside}}{[A]_{outside}} + zF\Delta\Phi = 0$$

- After rearrangement, we get for the *membrane potential* that

$$\Delta\Phi = \frac{RT}{zF} \ln \frac{[A]_{outside}}{[A]_{inside}}$$

- This is the *Nernst equation* which is one of the most important relationships in electrochemistry

- When deriving the Nernst equation, we assumed that an equilibrium occurs for the ion species being considered
- This has the consequence that Nernst equation is only valid for ions for which an equilibrium can develop, i.e. ions that can freely pass across the membrane
- Usually, such permeability is made possible by ion channels as we can see next

Walther Nernst (1864-1941)



Problem 1

- In leukocytes, the value of the membrane potential is

$$\Delta\Phi = -90\text{mV}$$

- What is the ratio of the concentrations of potassium ions inside and outside the cell at temperature

$$T = 37^{\circ}\text{C} = 310\text{K}$$

?

- As there are potassium channels in the membrane and thus, an equilibrium develops, the Nernst equation is valid for potassium in this example thus

$$\Delta\Phi = \frac{RT}{zF} \ln \frac{[K^+]_{outside}}{[K^+]_{inside}}$$

- After rearrangement, we obtain that

$$\frac{[K^+]_{outside}}{[K^+]_{inside}} = e^{\Delta\Phi zF / RT} = 0.0345$$

- So the potassium concentration inside the cell is almost thirty times greater than outside

- If in the cytoplasm the potassium concentration is

$$[K^+]_{inside} = 140\text{mM}$$

what is the potassium concentration outside?

$$[K^+]_{outside} = 0.0345 \cdot 140\text{mM} = 4.8\text{mM}$$

Transport across the membrane

- Biological membranes such as the plasma membrane, do not only separate the interior of the cell from the environment but also connects them
- This connection occurs by the transport of substances into or out of the cell
- Different mechanisms exist for different types of substances

- Transport processes can be grouped according to whether they require or not energy to occur
- If for a given substance, a concentration gradient exist between the two sides of a membrane, the transport along the concentration gradient is energetically favorable (*passive transport*), but in the opposite direction, additional energy must be invested (*active transport*)

- Since membranes have a hydrophobic layer which also must be passed, transport processes can be grouped by the chemical characteristic of the substance, namely, according to whether it can pass this hydrophobic layer
- Substances with hydrophobic character and small gas molecules such as O_2 or CO_2 can diffuse across the membrane
- Larger molecules or charged particles can pass only with the help of special proteins

- The transport of multiple substances can be coupled so that one of them moves along the concentration gradient and releases energy which can be used for the transport of the other substance moving against the concentration gradient
- If the two substances move in the same direction we speak about *symport* and in the opposite case we speak about *antiport*
- If only one substance gets transported we say that *uniport*

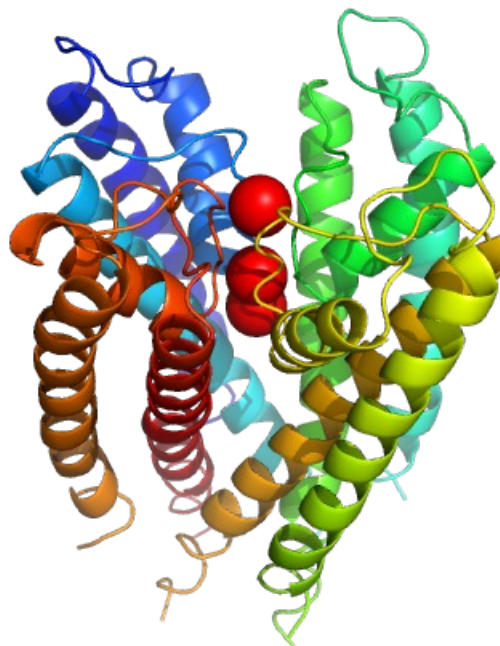
Molecular diffusion

- This is the simplest way to pass a membrane
- Only hydrophobic or small molecules are capable of this type of transport
- For example O_2 , H_2O and CO_2 can be transported by diffusion

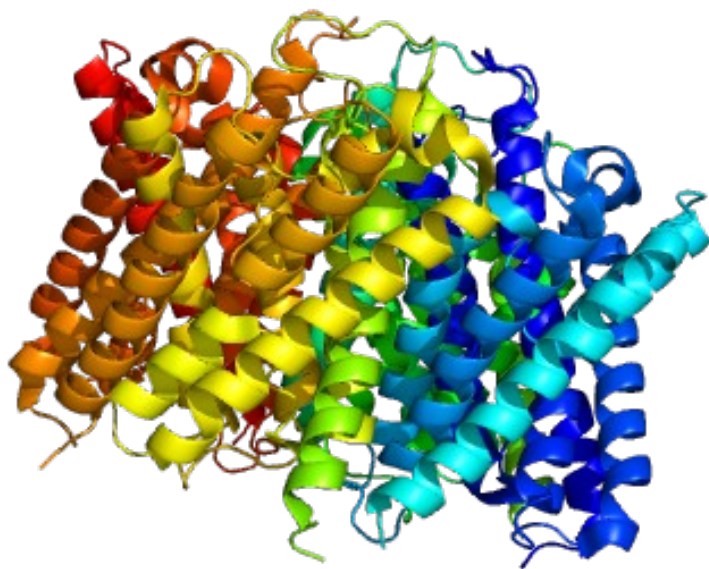
Channels

- Channels are transmembrane proteins which make passive transport possible
- Channels exist for ions and some other substances, e.g. aquaporins transport water
- Some channels are very specific for one type of ion so a sodium ion cannot pass through a potassium channel and vice versa

Potassium channel



Aquaporin



Carriers

- While channels form a permanently open tube across the membrane, carriers are always closed on one end
- In the case of voltage-gated carriers, a given membrane potential causes the transporter to open
- In the case of ligand-gated carriers, the binding of some ligand molecule triggers the opening

Passive transport through carriers

- An example for a carrier through which passive transport occurs is the glucose transporter
- This transporter is open towards one direction
- If a glucose molecule approaches the carrier from that direction it can bind to the protein
- When the protein switches over, i.e. becomes open towards the other direction, the glucose is released from the carrier

- Another possibility is that glucose is released before the carrier switches
- On the side where the concentration of glucose is higher, more glucose molecules will bind to the carrier and fewer will be released before the switch
- Thus, macroscopically, a glucose flow will be observed from the side with higher to that with the lower concentration

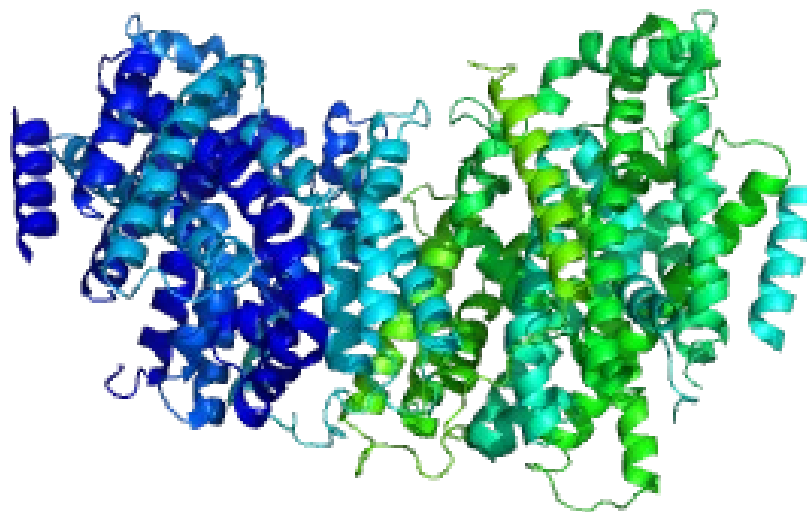
Active transport through carriers

- Active transport processes are often distinguished by whether they utilize directly the energy of ATP hydrolysis (*primary transport*) or utilize the flow of another substance along its concentration gradient (*secondary transport*)

Secondary transport by symporters and antiporters

- An example of a symporter is the glucose-sodium symporter where a glucose molecule is carried into the cell with the simultaneous transport of sodium
- The sodium-calcium exchanger is an antiporter where the inward flow of sodium is utilized to pump calcium out of the cell

Sodium-glucose symporter



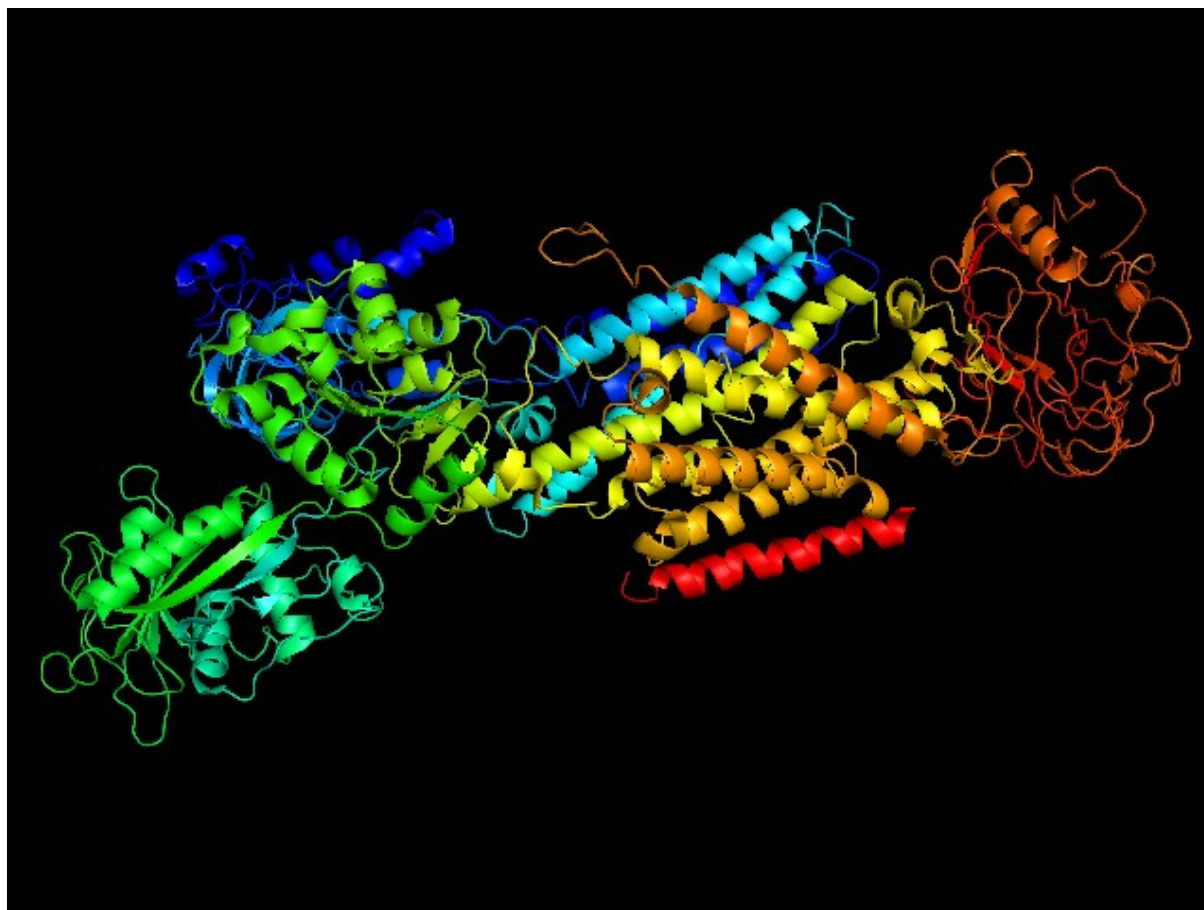
Ca²⁺ binding domain of a sodium-calcium antiporter



Primary transport by pumps

- Through primary transport, the energy of ATP hydrolysis is directly utilized to move ions against an electrochemical gradient
- A well-known and rather important pump is the Na^+/K^+ -ATPase which has a role in the adjustment of the action potential during impulse transmission in the nervous system
- Both sodium and potassium are transported against their electrochemical gradients

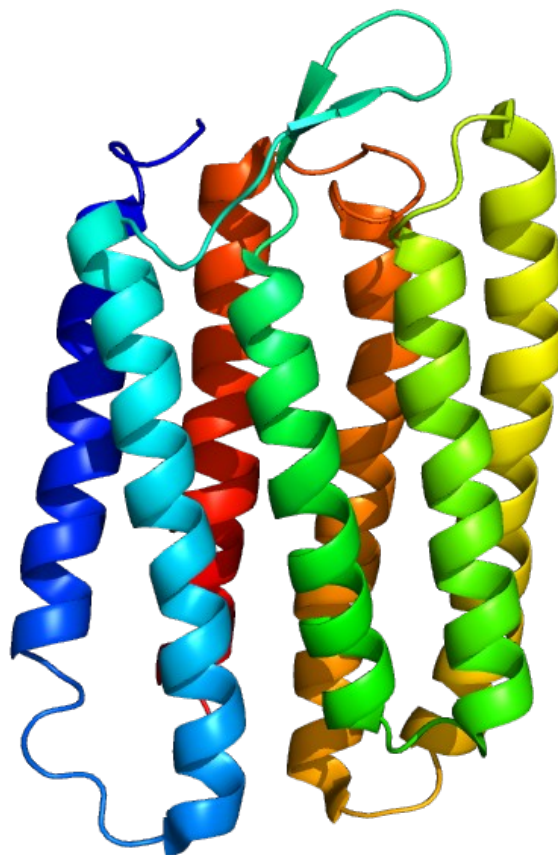
Na⁺/K⁺-ATPase

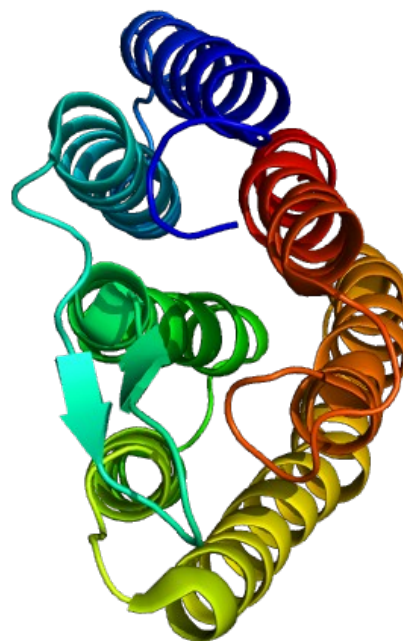


Two membrane machines

- We will present the structure and function of two membrane machines of particular interest
- First of them is *bacteriorhodopsin* which is responsible for capturing the energy of light to produce a proton gradient in some halobacteria
- Bacteriorhodopsin is an *integral membrane protein* complex
- It is found in the *purple membrane*

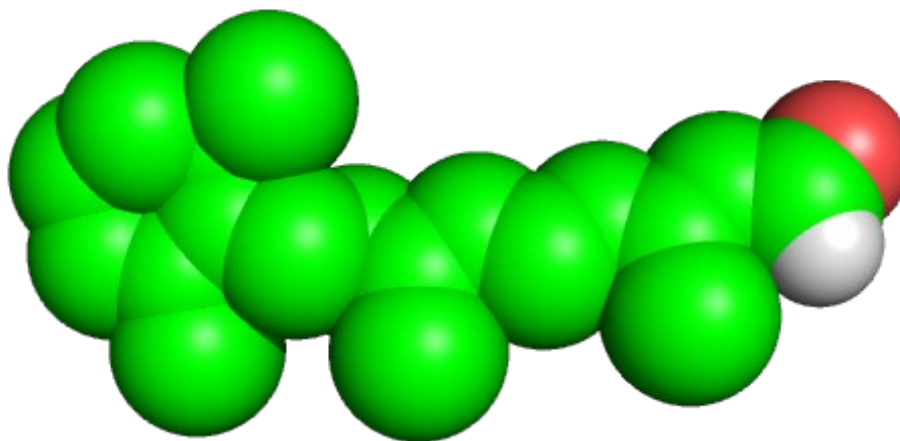
Bacteriorhodopsin

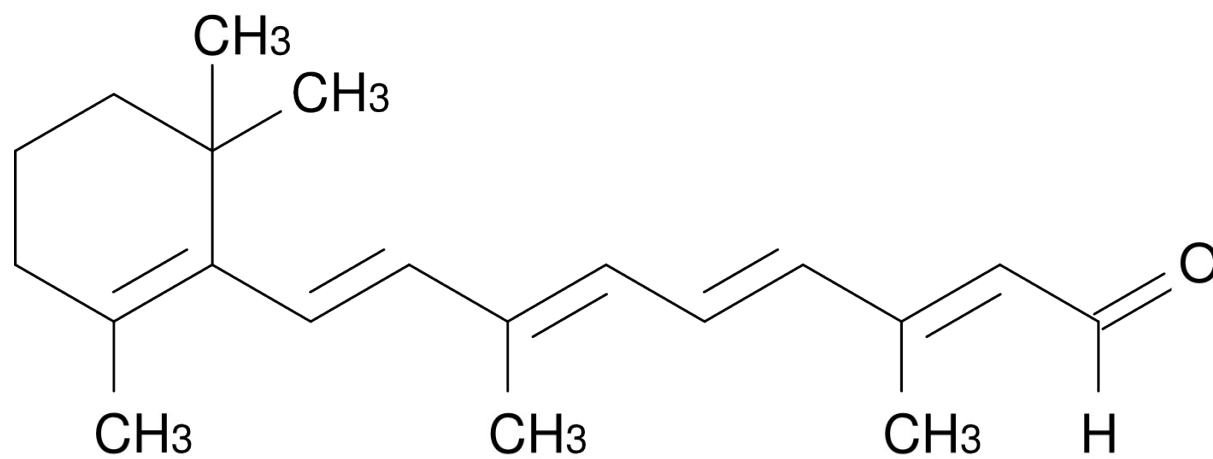




- In addition to the protein itself, bacteriorhodopsin contains one retinal molecule which is a chromophore
- Retinal has a role in the vision of animals

Retinal



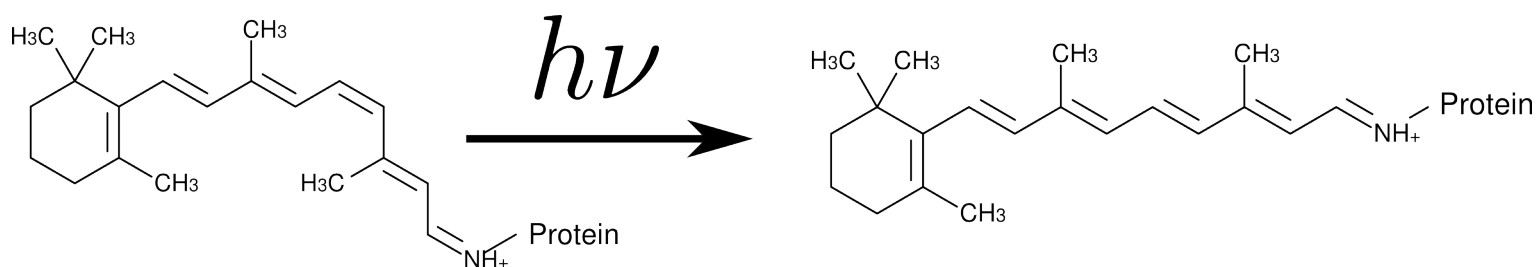


Operation of bacteriorhodopsin

- The retinal changes its conformation upon absorption of light
- This conformational change of retinal causes a change in the conformation of the protein
- This conformational change allows the protein to function as a proton pump and release a proton to the extracellular site

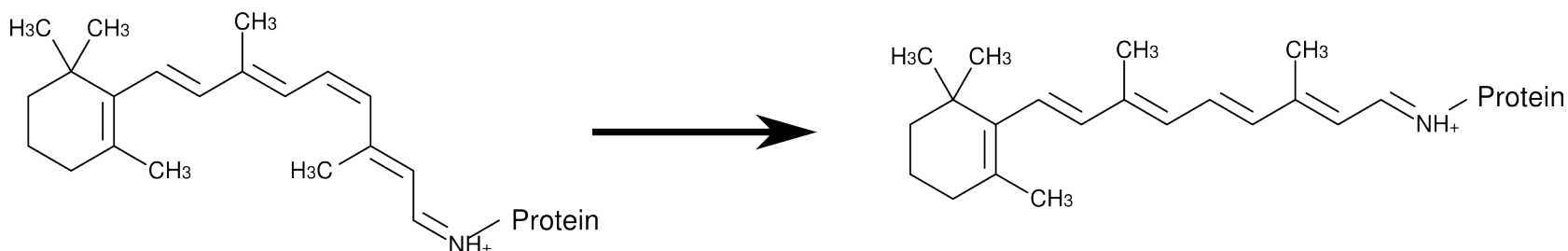
The photoisomerization cycle of retinal

- The cis-retinal binds to the protein as a Schiff-base
- This complex can absorb a photon and the cis-retinal isomerizes to the all-trans form



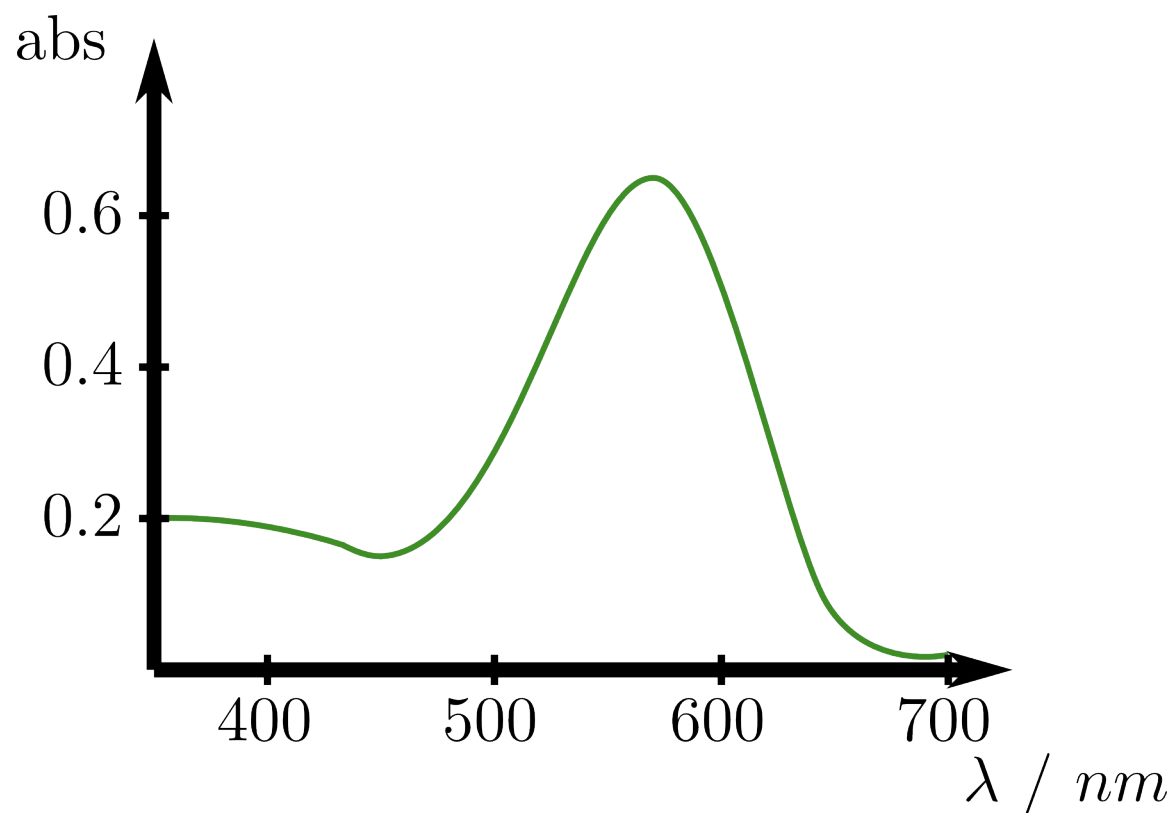
- Following the isomerization of the chromophore, a proton transfer occurs from the Schiff base to the Asp-85 residue of the protein
- Another aspartate residue of the protein, Asp-96, provides a proton to reprotonate the Schiff base
- A reprotonation of Asp-96 occurs from the cytoplasm of the cell

A reverse isomerization of retinal from the cis to the all-trans form while both Asp-85 and Asp-96 are protonated



- Finally, Asp-85 releases the proton outside the cell
- Repeating this cycle many times, a proton gradient is established which can be used for ATP synthesis

Absorption spectrum of bacteriorhodopsin

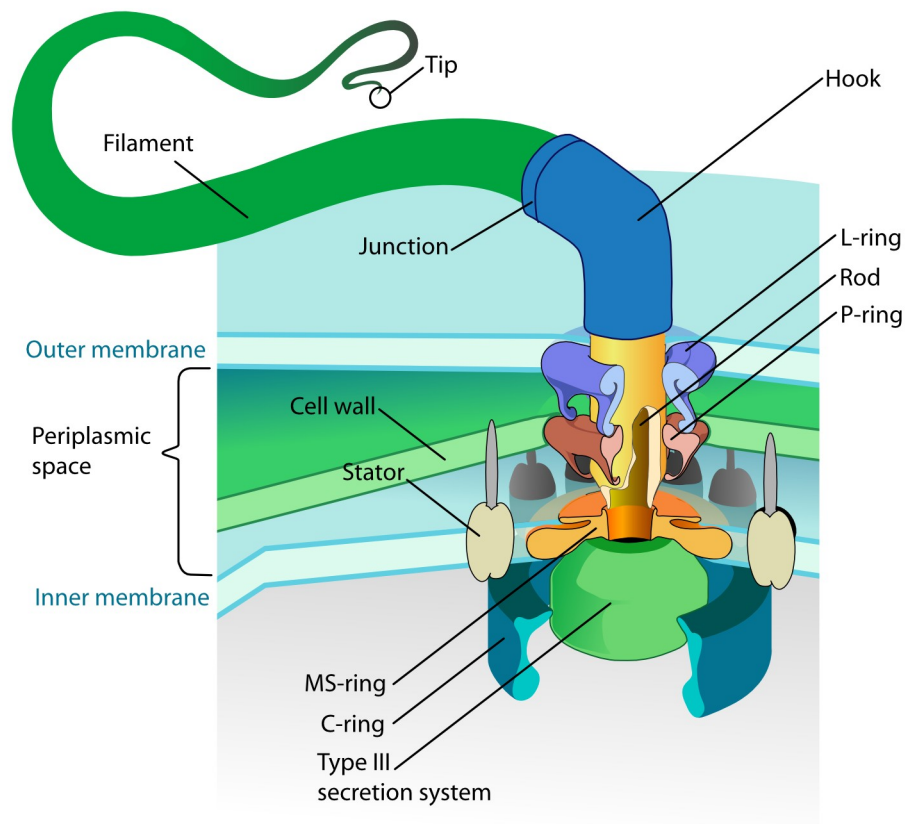


Bacterial flagellum

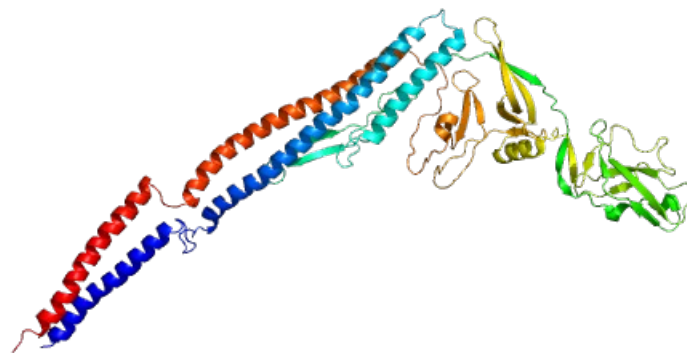
- Some bacteria have a special organ called flagellum which takes part in the motion of the organism
- The flagellum consists of a basal body located in the cell wall of the bacterium and a filament attached to it
- The filament is built of a protein called flagellin
- The filament is attached to the basal body through a protein called hook which ensures a quasi rectangular junction

- Gram-negative bacteria such as *Escherichia coli* have a basal body consisting of four protein rings
 - L ring is located in the outer membrane
 - P ring is located in the peptidoglycan layer
 - M ring is located in the plasma membrane
 - and S ring is attached to the plasma membrane

Flagellum of a Gram-negative bacterium



Flagellin



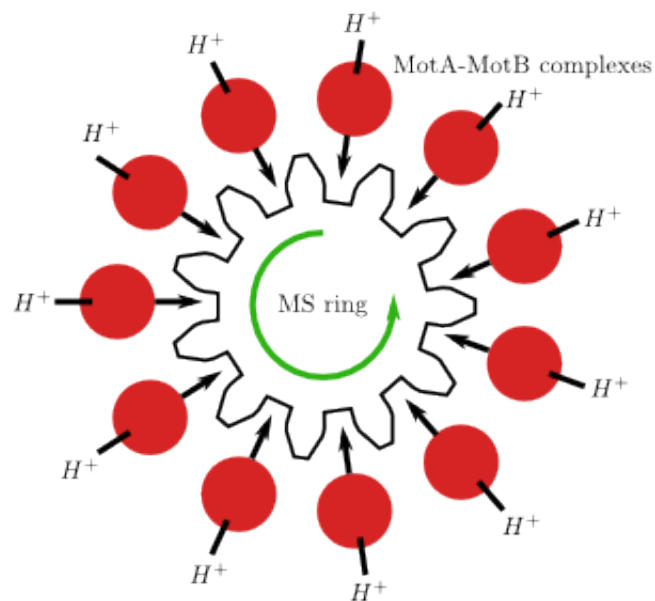
Operation of bacterial flagellum

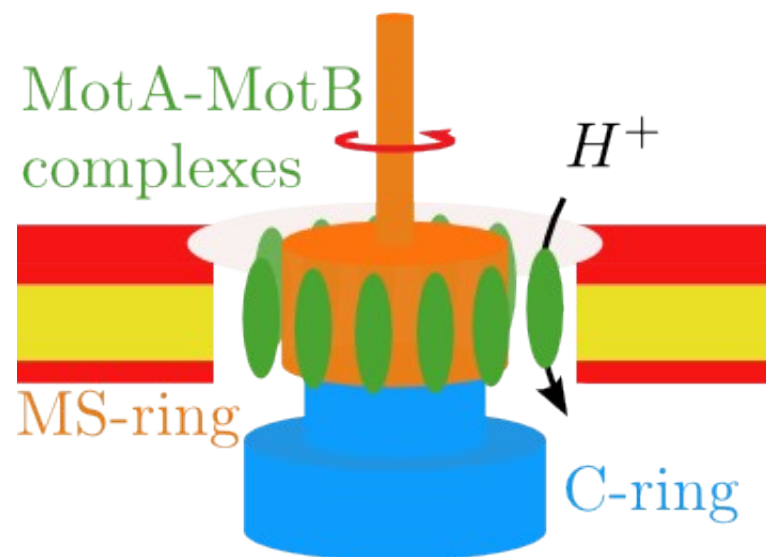
- Several models have been proposed to explain the flagellar rotation
- These models agree that it is not the ATP but an electrochemical potential gradient through the plasma membrane which drives the rotation
- In *E. coli* a flow of protons into the cell generates a rotational motion

Proteins functioning in rotation

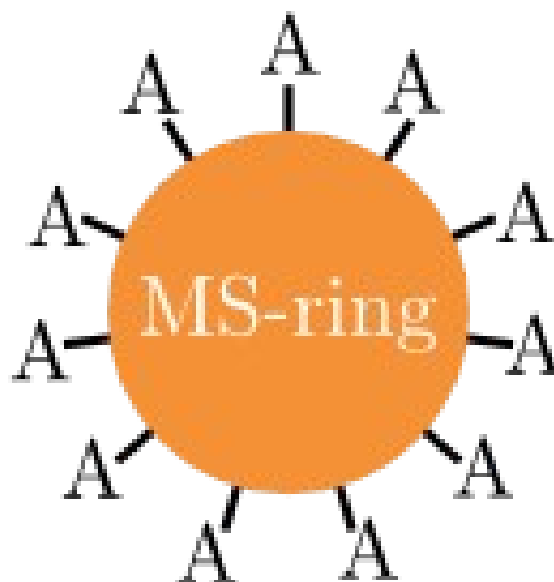
- Several proteins have been shown to participate in the generation of rotary motion
- MotA and MotB are membrane proteins being localized in the plasma membrane that form the stator and serve as channels to conduct ions across the membrane
- FligF, FliG, FliM and FliN proteins form the rotor itself

Schematic structure of the rotary complex





Proton acceptor groups are on the MS-ring equally spaced along the ring



A general scheme for energy conversion

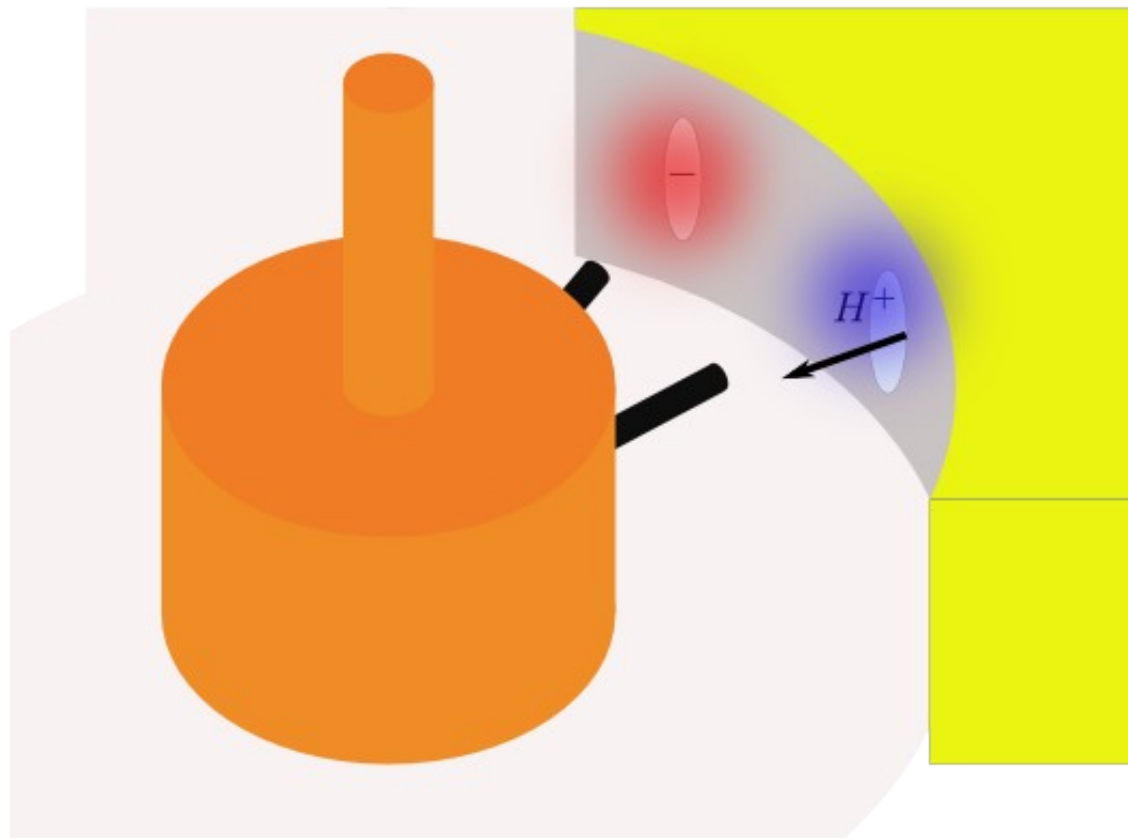
- A general model is shown based on models describing the rotary motion generation process
- There are several disagreements between models with respect to the details of the process

General scheme of rotary motion generation

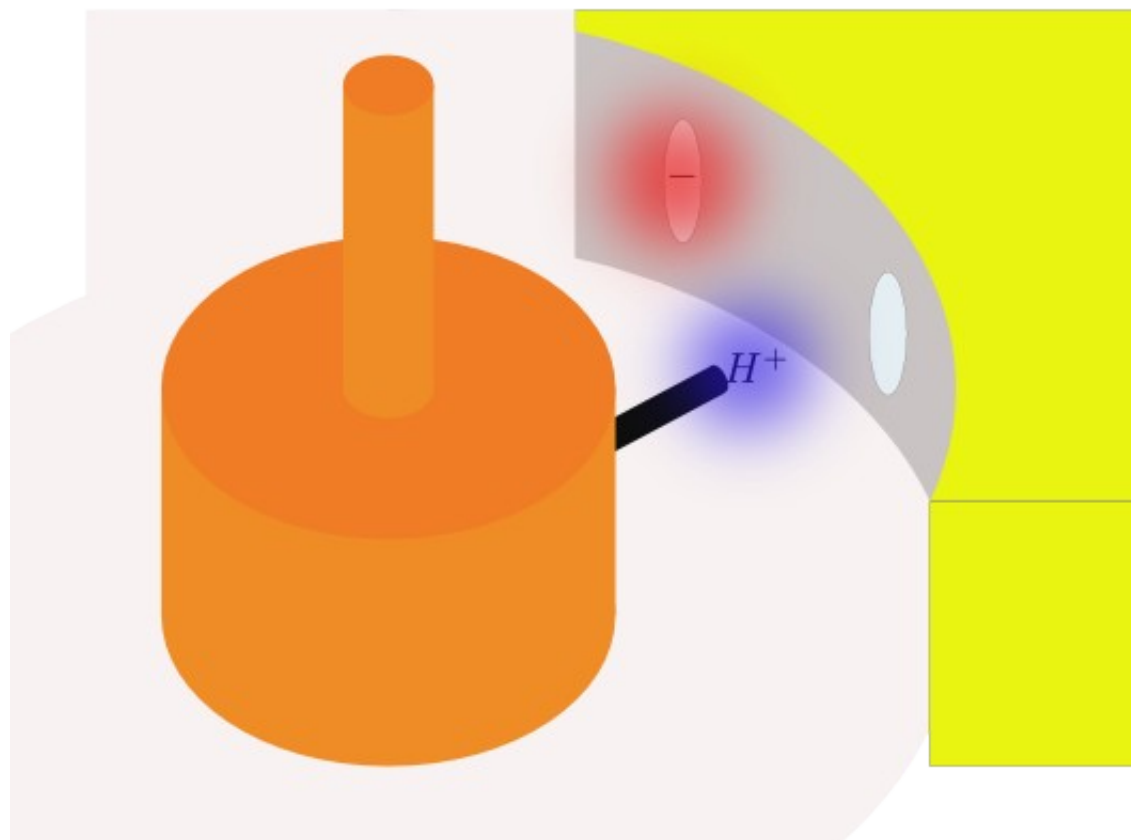
- Around the MS-ring, proton acceptor groups are located equally spaced
- From outside, a proton arrives to the nearest acceptor group through a channel formed by the MotA-MotB complex
- The proton binds to the acceptor group, lending it a positive charge
- There is another channel with a negatively charged group at its entrance

- This negatively charged group attracts the positively charged group, causing the rotation of the whole ring
- Due to this rotation, the next acceptor group turns to the channel through which another proton arrives
- This cycle repeats several times, generating the rotation of the ring and thus the rotation of the flagellar filament as well

A proton arrives from outside through a channel formed by the MotA-MotB complex



The proton binds to the proton acceptor group



MS-ring rotation driven by the attraction of electric charges occurs

