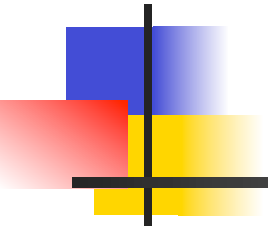
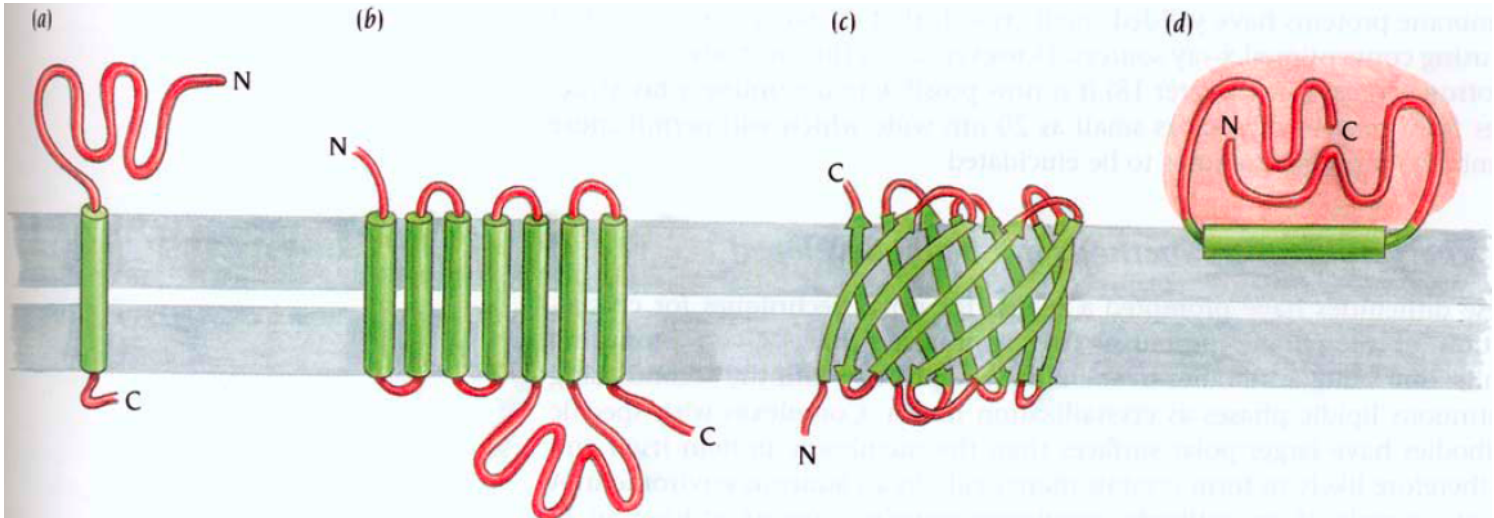
5. Membrane Proteins



Definitions

- Cells and organelles are compartimentalised by biological membranes, a 5 nm thick layer of lipids and proteins
- The lipids form a double layer, hydrophilic on the two solvent exposed sides, hydrophobic in the inside.
- The proteins are inserted in the lipid double layer. We can distinguish 3 zones: 1 hydrophobic zone inside the membrane, 2 hydrophilic zones on either side of the membrane

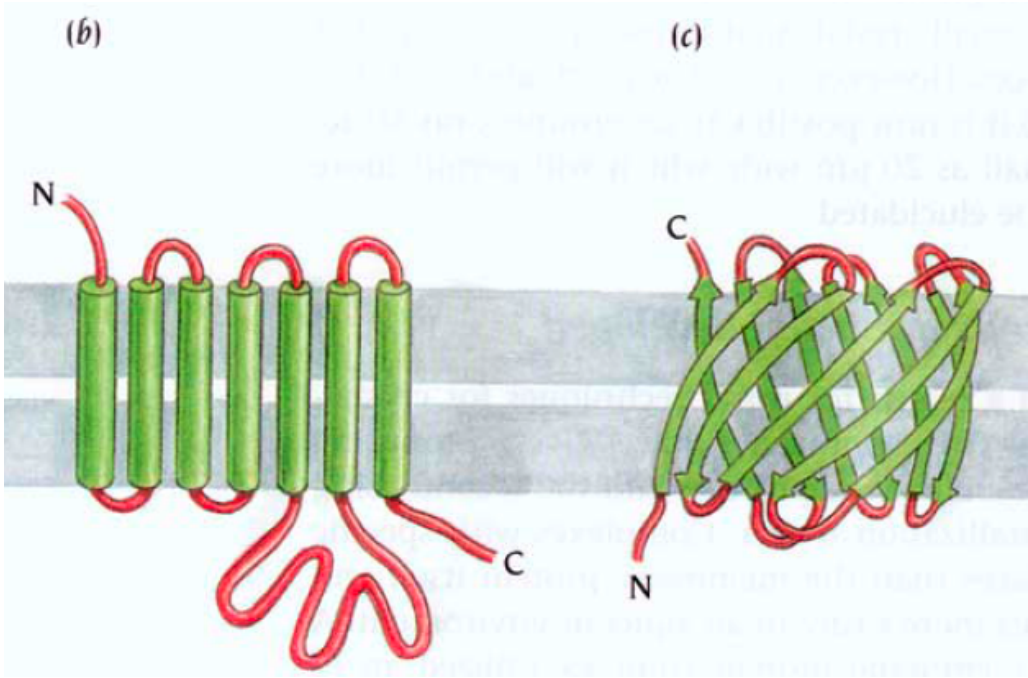


Roles of membrane proteins

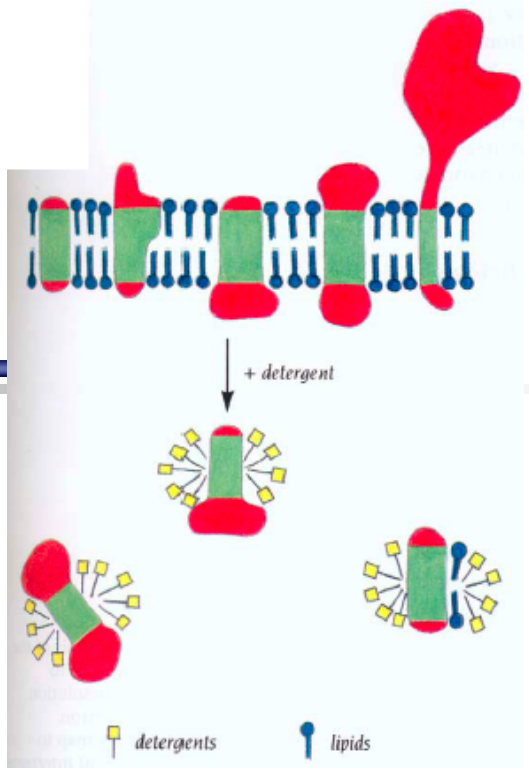
- In their role of compartimentalisation, the biological membranes control the passage or crossing of nutrients, refuges, ions, messangers between the outside environment and the cell/ organelles
- Many biologically key processes occur on the membranes:
  - Electron transport / respiratory chain
  - Photosynthesis
  - Intercellular communication
  - Ligand / receptor interaction
  - Hormon / receptor regulation
  - Nervous impulse transmission

Classification of membrane proteins

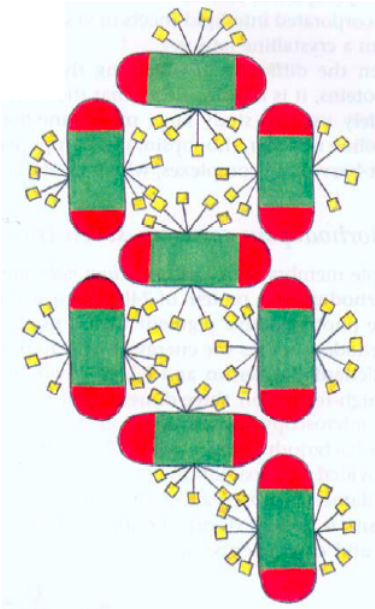
- **Intrinsic or integral membrane proteins**
  - These are proteins tightly bound to the membrane
  - The polypeptide crosses the membrane many folds, usually as alpha-helix but at times also as beta strands,
  - The hydrophilic solvent exposed areas are the C- and N-terminals and the loops
  - These proteins tend to precipitate in acqueous buffer, they require detergents for their extraction and solubilisation



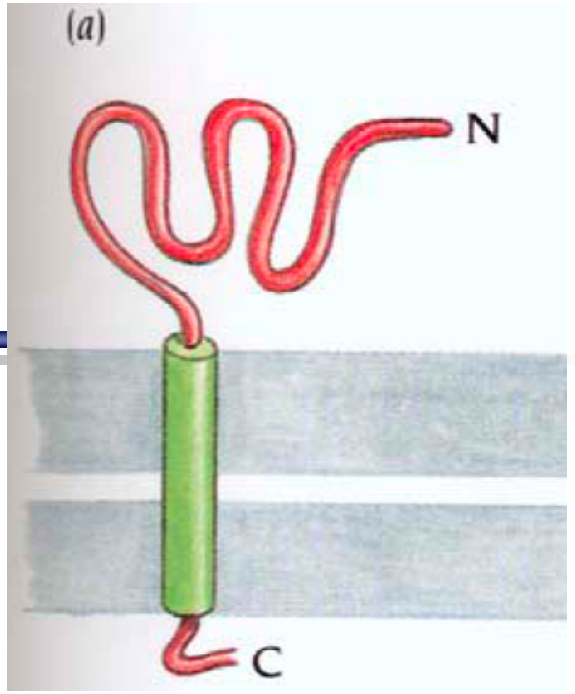
Solubilisation of integral membrane proteins

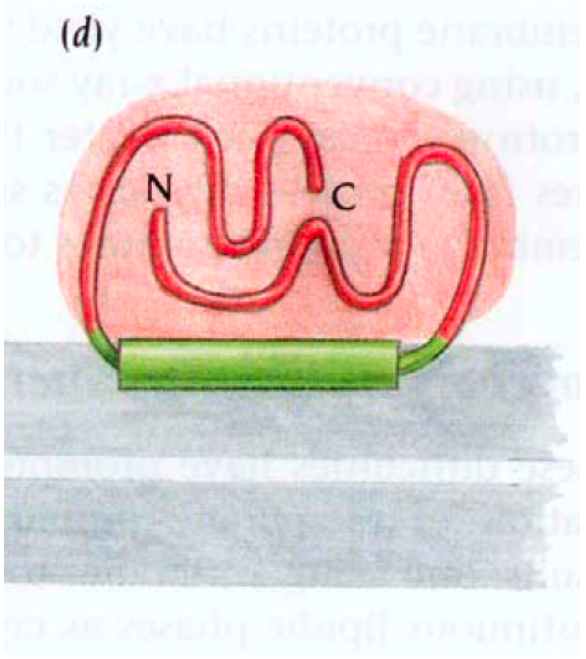


- Solubilisation of integral membrane proteins is achieved by adding **detergents** to the buffer
- Detergents allow to solubilise and purify these proteins in their native state
- The hydrophobic part of the detergent molecule binds to the hydrophobic surface of the protein, while the polar groups of the detergent are oriented towards the solution
- This gives a complex protein-detergent that has an hydrophilic surface and an hydrophobic interior
- Working with integral membrane proteins is much more challenging than working with globular extrinsic or soluble proteins
- The result is that are much fewer structures of membrane proteins, that are normally studied by X-ray crystallography. This is the frontline of structural biology



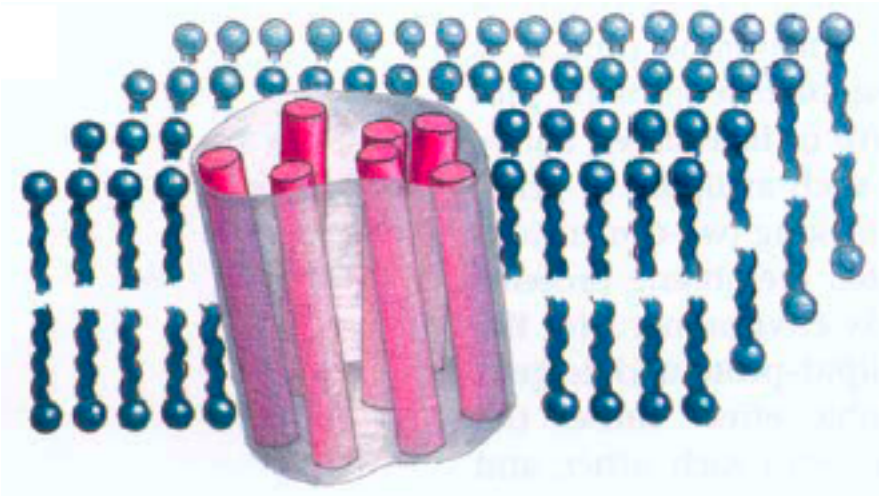
- **Estrinsic or periferic membrane proteins**
  - These proteins have interactions with the surface of the membrane
  - Usually the polypeptide crosses the membrane only once
  - They tend to form globular domains on one side of the membrane that can be digested/cut by proteases
  - Sometimes the proteins do not cross the membrane but are anchored on one side of the membrane by one alpha-helix, or by fatty acids covalently linked to the protein. These fatty acids penetrate into the lipid double layer
  - These proteins are normally soluble in buffer



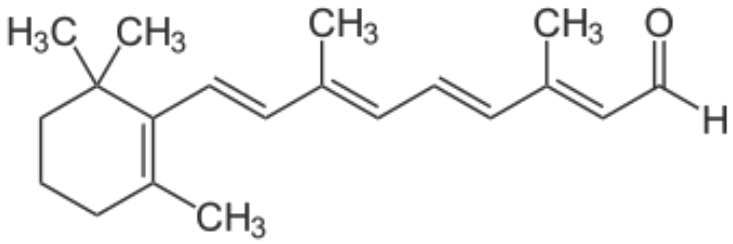


Bacteriorhodopsin

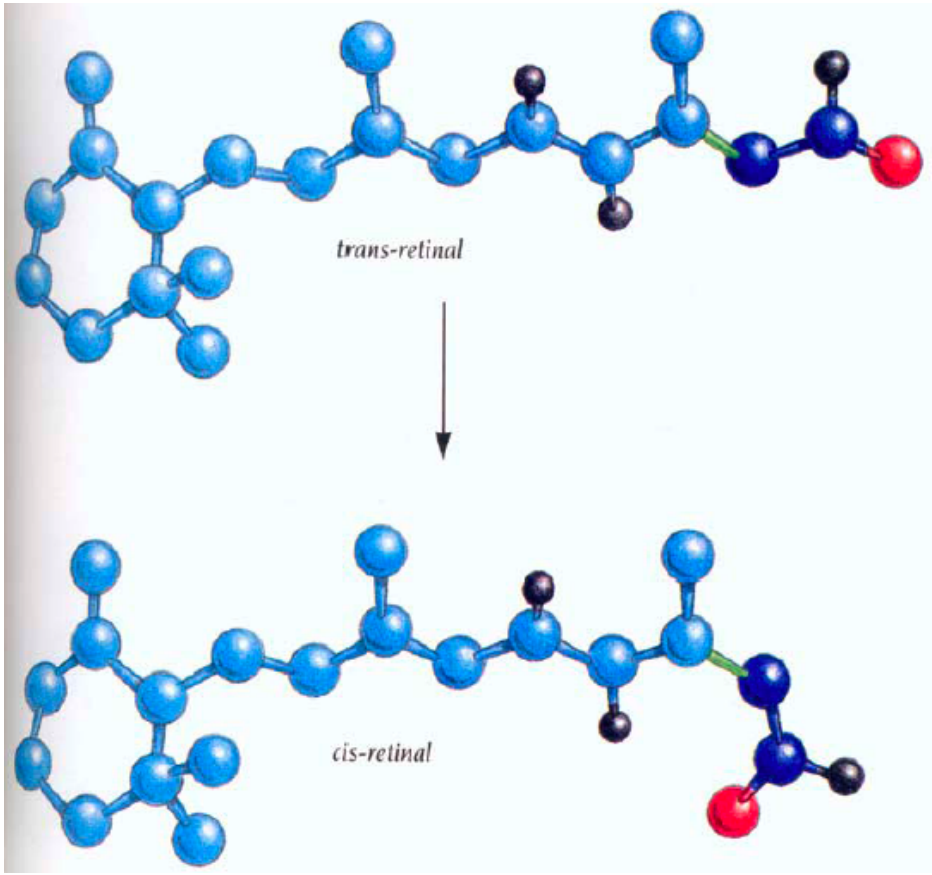
- Membrane protein of 248 aa that binds rethinal, the same pigment that captures light in our eyes
- Made of 7 transmembrane helices, tilted by 20• with respect of the plane of the membrane
- In 1975 thanks to EM a first model of bacteriorhodopsin was obtained at 7Å resolution.
- The model was improved to 2Å in 1990 and the X-ray structure confirmed the model at 3Å resolution



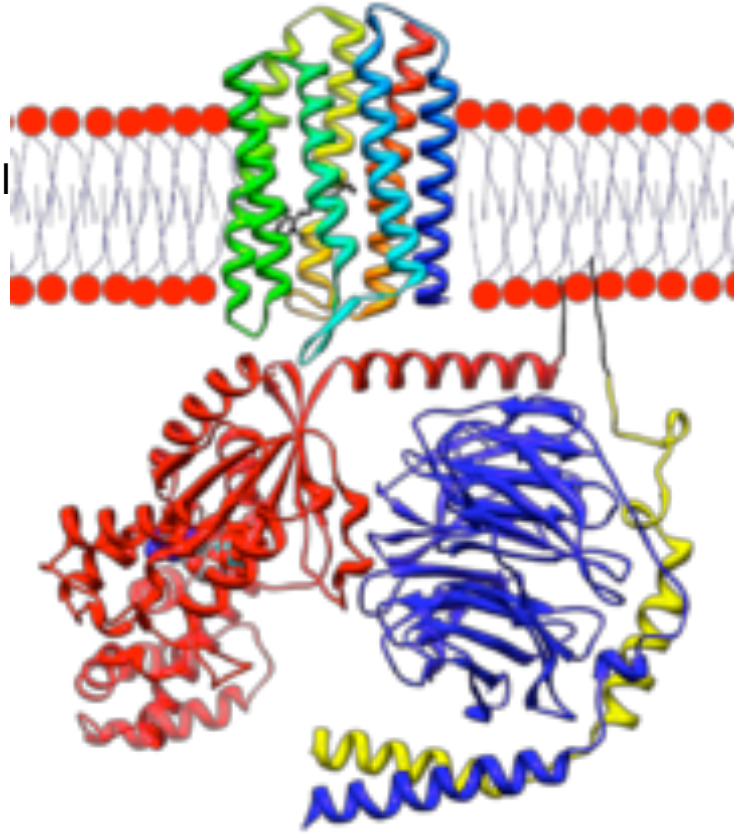
Bacteriorodhopsin function



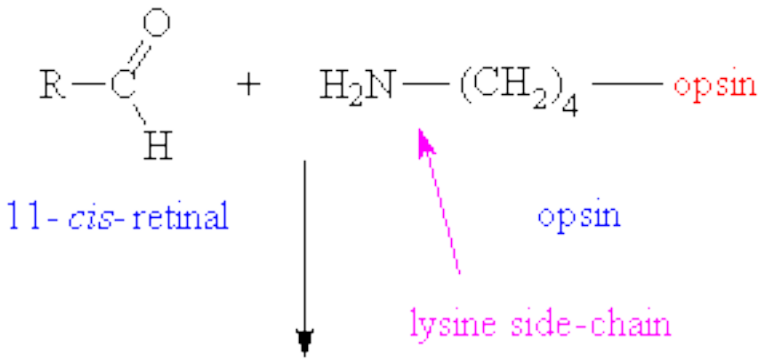
- The archaea halobacteria have a simplified biological system for the conversion of light into chemical energy.
- Under intense illumination bacteria express high quantities of bacteriorhodopsin
- When retinal is bound to bacteriorhodopsin and it absorbs a photon it undergoes an isomerisation *trans* ➔ *cis*
- This causes a conformational change in the active site that causes a proton transfer from the cytosol to the extracellular medium

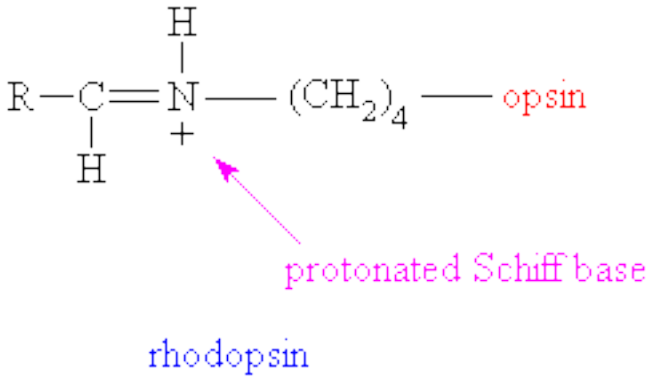


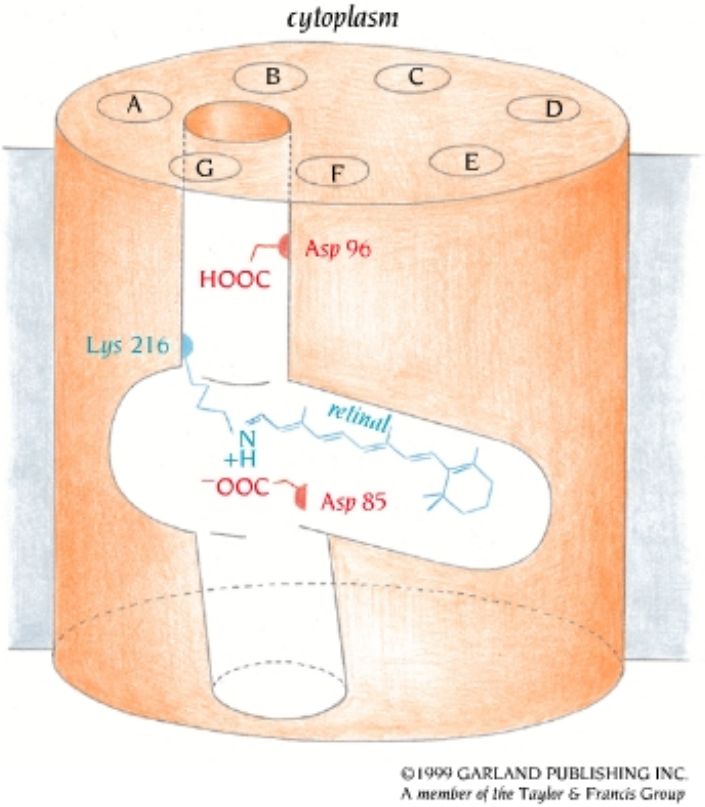
- The retinal is covalently linked to Lys216 in the chromophore by Schiff base action.
- After photoisomerization of the retinal molecule, Asp85 becomes a proton acceptor of the donor proton from the retinal molecule.
- This releases a proton from a "holding site" into the extracellular side (EC) of the membrane.
- Reprotonation of the retinal molecule by Asp96 restores its original isomerized form.
- This results in a second proton being released to the EC side. Asp85 releases its proton into the "holding site" where a new cycle may begin.
- The bacteriorhodopsin molecule is purple and is most efficient at absorbing green light (wavelength 500-650 nm, with the absorption maximum at 568 nm).
- The three-dimensional tertiary structure of bacteriorhodopsin resembles that of vertebrate rhodopsins, the pigments that sense light in the retina.
- Rhodopsins also contain retinal; however, the functions of rhodopsin and bacteriorhodopsin are different and there is only slight homology in their amino acid sequences.
- Both rhodopsin and bacteriorhodopsin belong to the 7TM receptor family of proteins, but rhodopsin is a G protein coupled receptor and bacteriorhodopsin is not.



Type 2 rhodopsin (rainbow colored) embedded in a lipid bilayer (heads red and tails blue) with transducin below it. Gtα is colored red, Gtβ blue, and Gtγ yellow. There is a bound GDP molecule in the Gtα-subunit and a bound retinal (black) in the rhodopsin. The N-terminus terminus of rhodopsin is red and the C-terminus blue. Anchoring of transducin to the membrane has been drawn in black.


R—C(=O)—H
+
H2N—(CH2)4—opsin
11-cis-retinal
opsin
lysine side-chain


R—C(—H)=N+(—H)—(CH2)4—opsin
protonated Schiff base
rhodopsin


cytoplasm
A
B
C
D
E
F
G
Asp 96
HOOC
Lys 216
retinal
N
+H
⁻OOC
Asp 85
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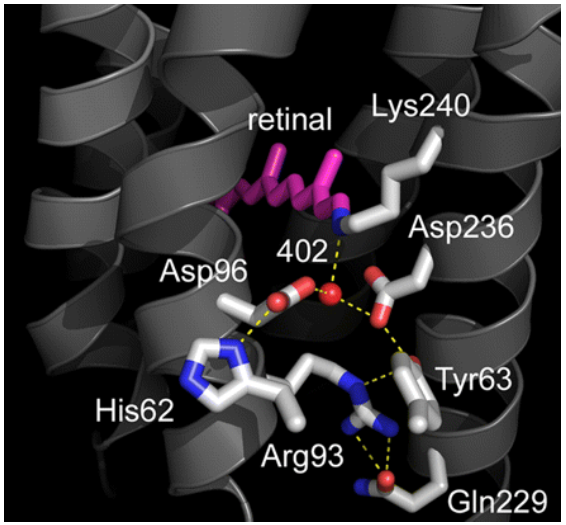
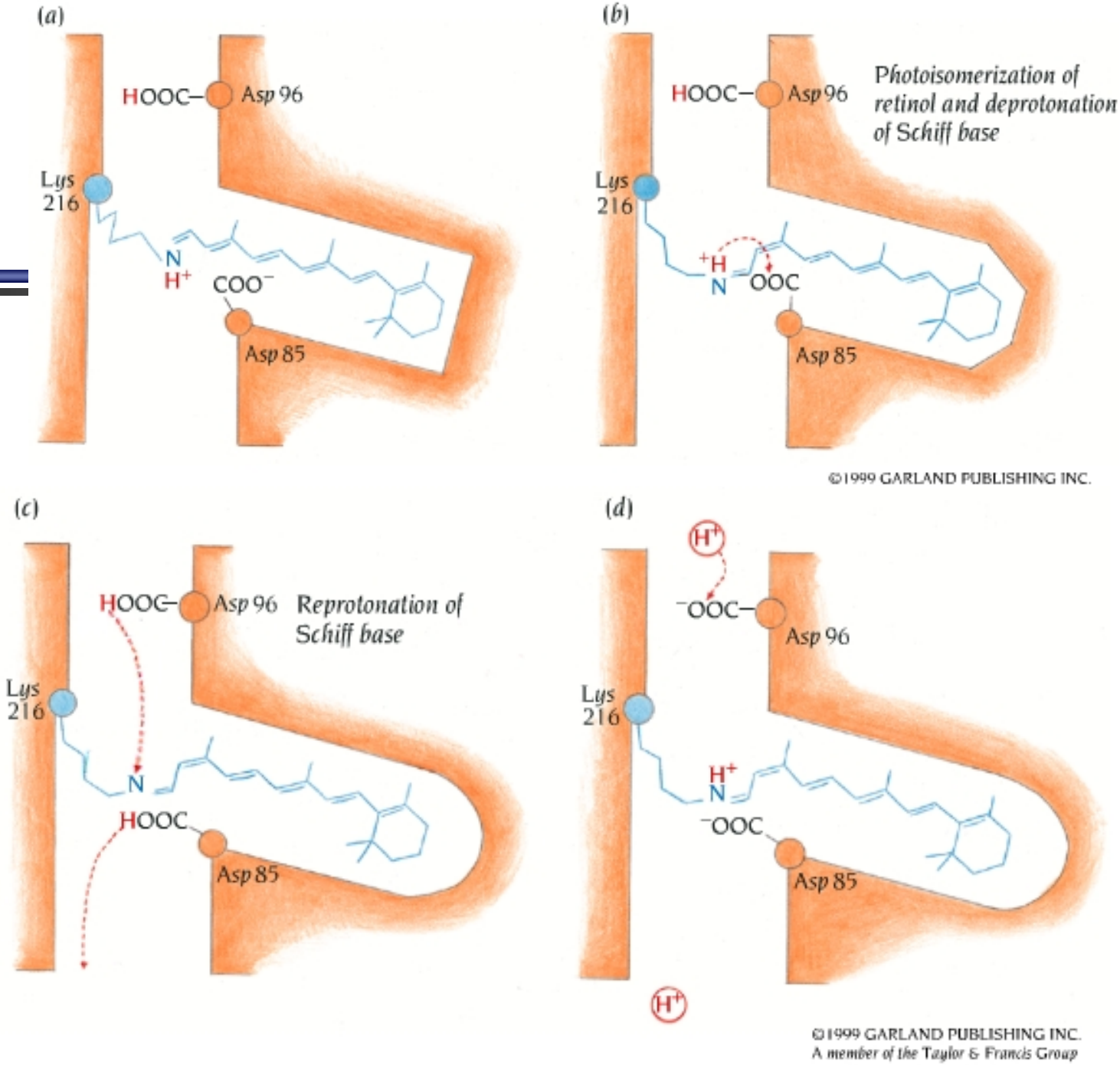

retinal
Lys240
Asp236
402
Asp96
Tyr63
His62
Arg93
Gln229


(a)
HOOC–
Asp 96
Lys 216
N
H+
COO⁻
Asp 85
(b)
HOOC–
Asp 96
Photoisomerization of retinol and deprotonation of Schiff base
Lys 216
+H
N
OOC
Asp 85
©1999 GARLAND PUBLISHING INC.
(c)
HOOC–
Asp 96
Reprotonation of Schiff base
Lys 216
N
HOOC
Asp 85
(d)
H+
⁻OOC–
Asp 96
Lys 216
H+
N
⁻OOC
Asp 85
H+
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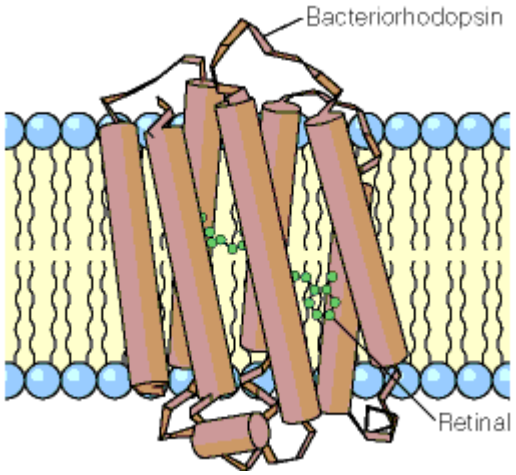
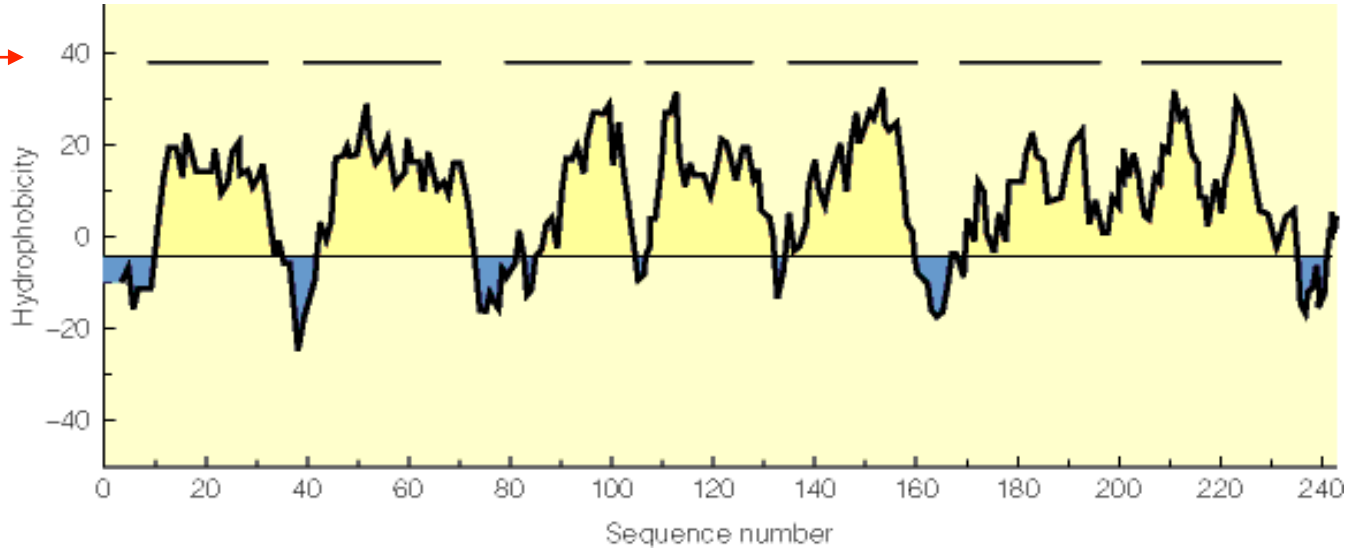
Membrane proteins: structure predictions

- Predictions of the topology of membrane proteins can sometimes be done on the basis of sequences:
  - Transmembrane segments can be inferred from hydrophobicity plots
    - This can be constructed with the Kyte and Doolittle method



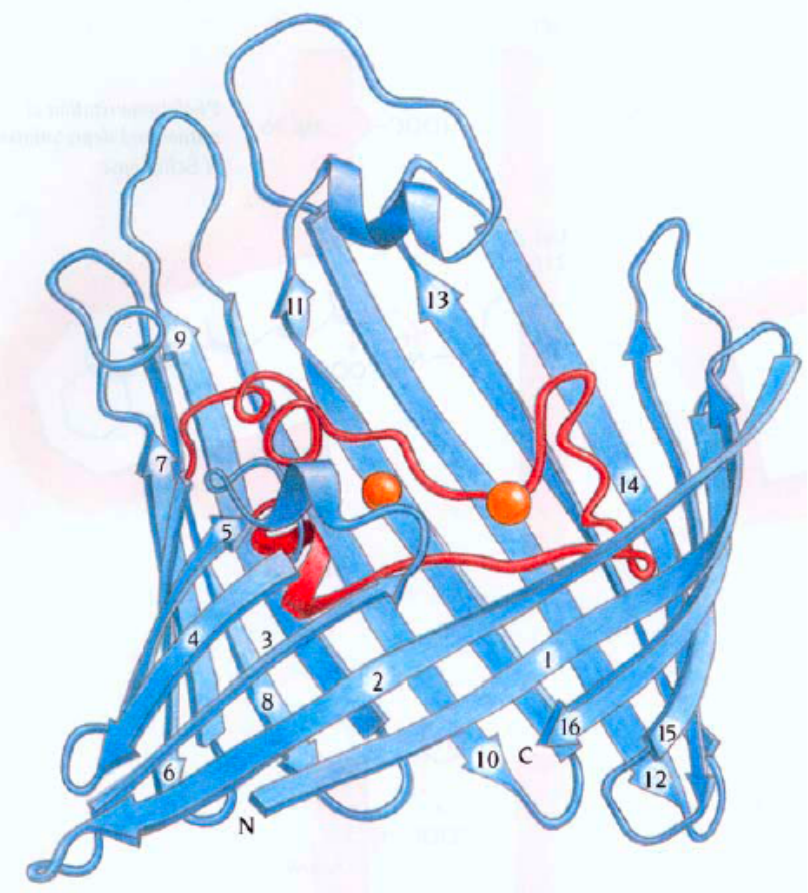
Trans-membrane helices



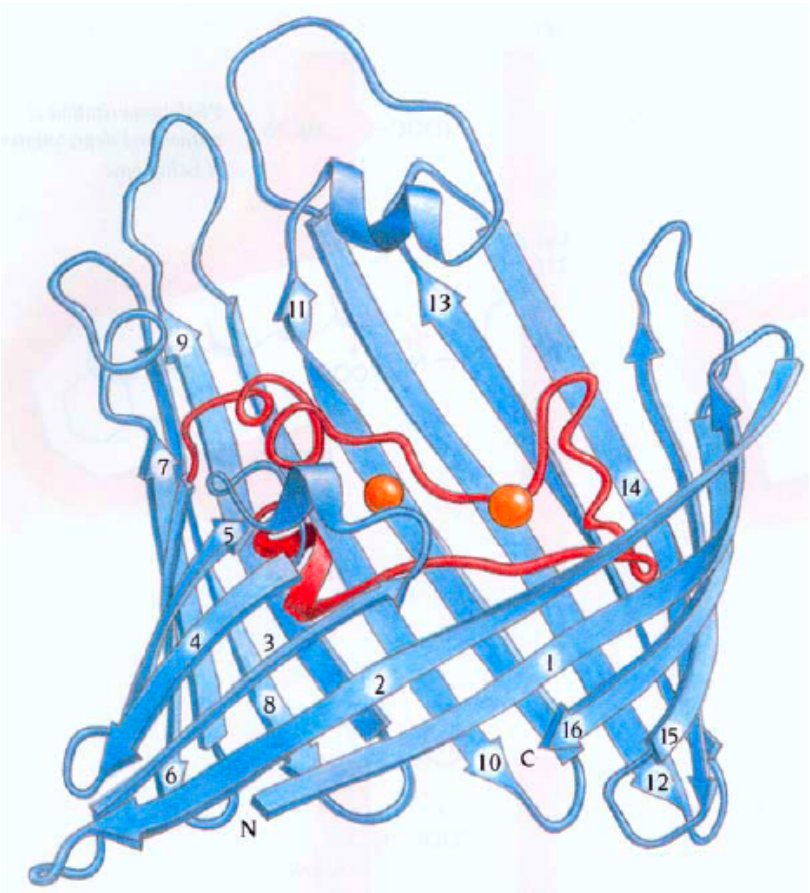
Hydrophobicity plot for Bacteriorhodopsin

Porins

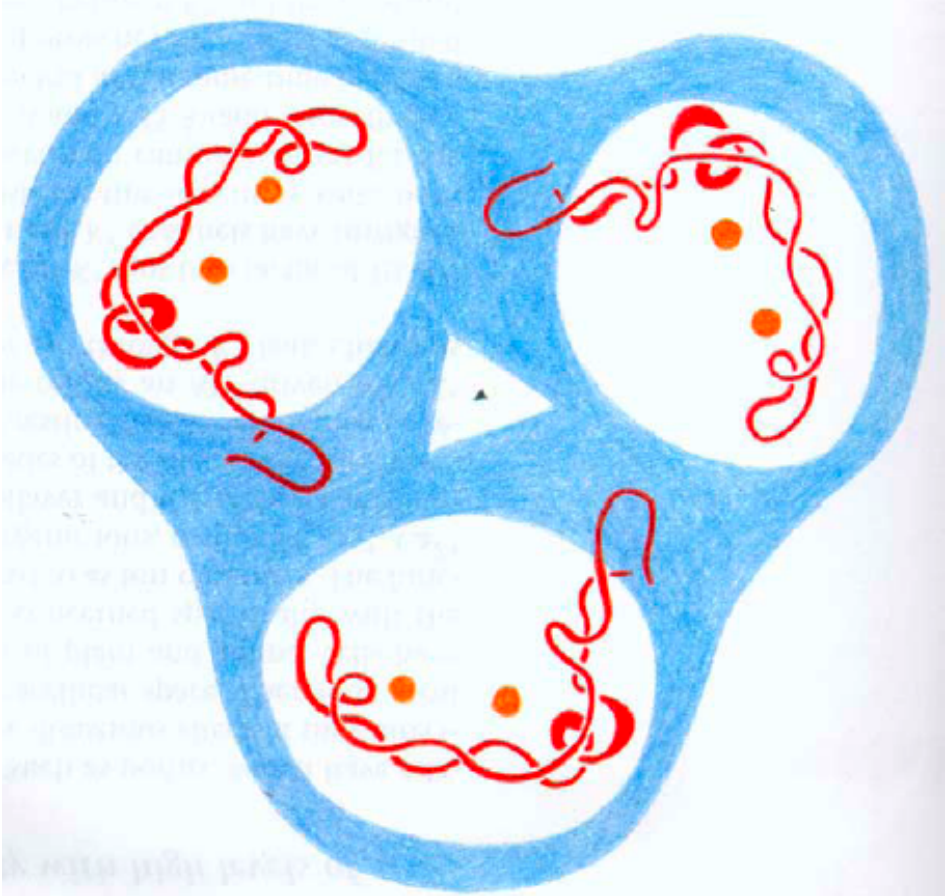
- Gram negative bacteria have 2 membranes: the inner and the outer membranes separated by the periplasmic space
- The porins are transmembrane proteins located on the outer membrane
- They are characterised by beta-strands
- Porins are the most abundant proteins in bacteria: the form an open channel filled with water allowing the passive diffusion of nutrients and waste across the membrane
- The discriminating factor is the diameter of the channel that stops the crossing of large cumbersome molecules that are usually toxic
- The structure of the first porin was solved in 1990
- All known porins are trimers, each formed by a beta-barrel made up by an even number of beta strands, 16 or 18, with topologu up and down, where each strand makes H-bonds with the adjacent strand
- The loops protruding on the extracellular side are long if compared with those on the intracellular side.



- The large numbers of beta-strands that form the barrel describe a large inner channel.
- This is partially blocked by a long loop that is located between strand 5 and 6, extending in the inside of the cavity
- This describes a bottle neck long 9Å and with a diameter of 8Å, that defines the size of the type of molecule that can cross the pore
- The fact that the external part of the barrel is in contact with the membranes and the inside pore is hydrophilic, dictates that the beta strands have an alternating pattern hydrophobic/hydrophilic. An exception are the internal aa in contact with the long loop
- This makes structural predictions difficult to make



- The porins form stable trimers, made of 3 identical subunits, each with a functional pore
- Circa 1/3 of the external surface of each barrel is involved in interactions with other 2 subunit



Ion channels

- The presence of porins in the animal and plant cells would have disastrous effect. These cells have much more selective proteins that descibe very narrow channels involved in the transport of inorganic ions across the membrane: **the ion channels**
- The function of these channels is to allow the rapid passive diffusion of *specific* inorganic ions, such as K^+, Na^+, Ca^{++}, Cl^- across the membrane. This will compensate the difference of charge between the two sides of the membrane, phenomenon that is at the origin of the **action potential**

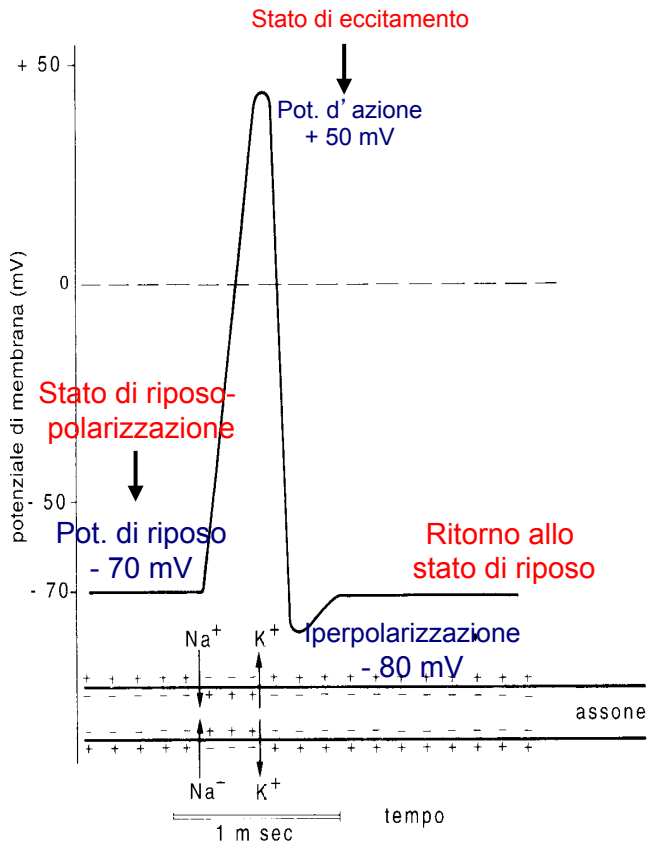
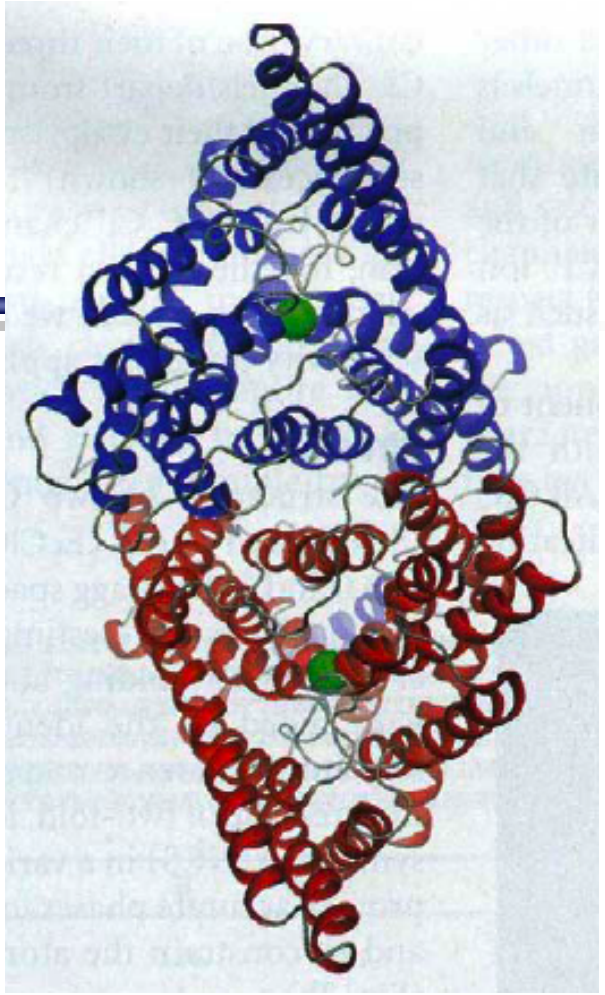


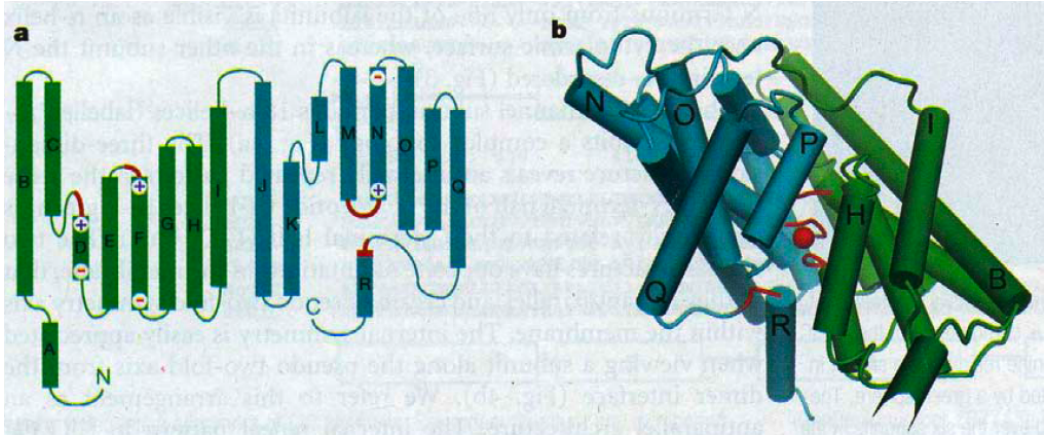
Fig 21.2 *Propagazione dell'impulso nervoso lungo l'assone come onda di depolarizzazione.* In corrispondenza dell'impulso depolarizzante i Na^+ che entrano attraverso la membrana assonale sono in numero maggiore rispetto ai K^+ che escono. Da qui la inversione temporanea della polarizzazione.

- An ion channel has 2 diverse conformational states:
  - Open: this is when the ions can cross the channel according to the electrochemical gradient that exists on the two sides of the membrane
  - Closed: this is when the channel is impermeable to the ions
- The ion channels must be
  - Highly selective for a particular ion
  - Keep high rate of transport
  - Regulate the flux of ion: this is called *gating*
- Examples of channels for which the 3D X-ray structure has been solved are:
  - The Cl^- channel
  - The K^+ channel

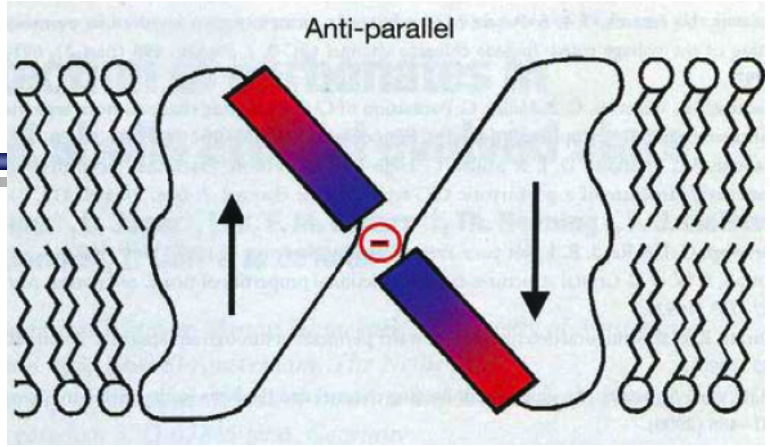
The Cl^- channel

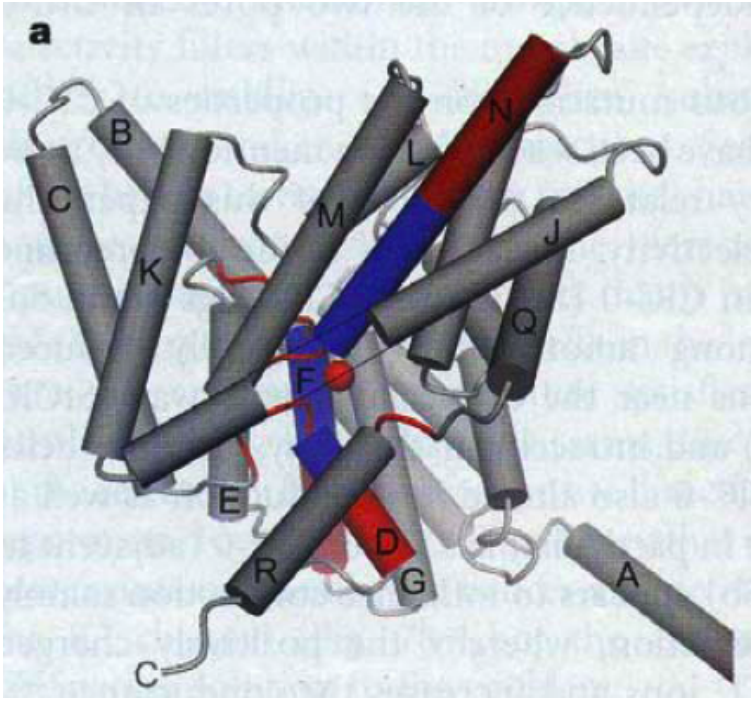
- The structure of the Cl^- channels from *E.coli* and *S.typhimurium* have been solved at 3.5 and 3.0 Å respectively
- They are made of circa 460 aa
- The channel is made of 2 identical subunits, with a high interface contact surface of 2300 $Å^2$ each with a channel and a selectivity filter
- Each subunit is made of 18 alpha helices (A-R)
- The pattern of helices A-I is comparable to that of helices J-R. These 2 groups of helices are positioned in an antiparallel way in respect to one another
- The helices are very tilted with respect to the membrane





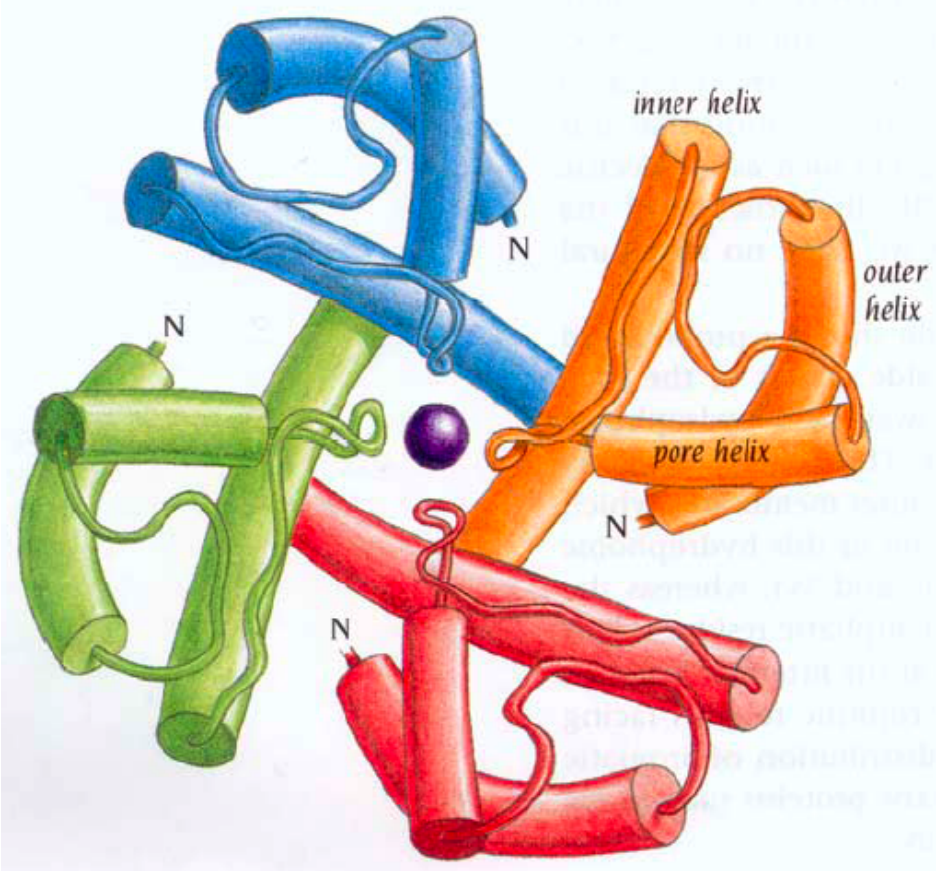
- The channel has the shape of a clepsydra (hourglass): the wider funnel-shaped side has positively charged aa (Arg). These have the task of attracting the Cl^- ions in the channel
- The Cl^-–binding site is made of highly conserved residues belonging to loops that precede helices D, F and N
- The N-ter of these helices have a partial positive charge that are oriented towards the binding site, making this the electrostatically favoured environment for anions (selectivity filter)
- Between the N-ter of the helices F and N there is a Glu highly conserved; oxygen atoms of the side chain makes the binding of the anion not too tight to warrant a rapid diffusion
- This Glu must move to allow Cl^- to enter.



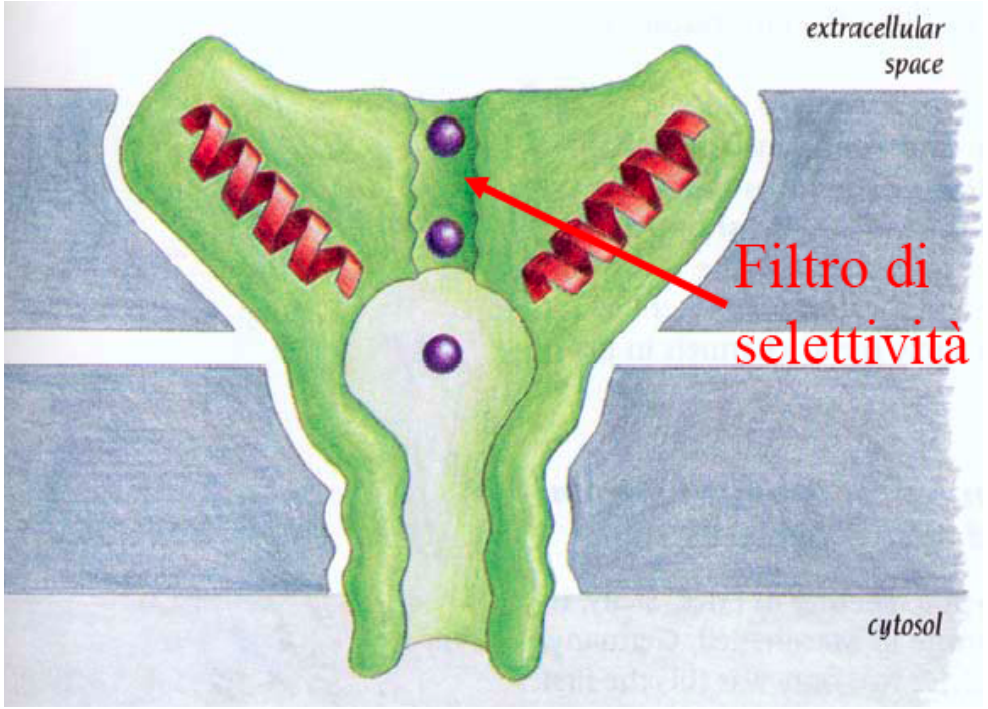


The K^+ channel

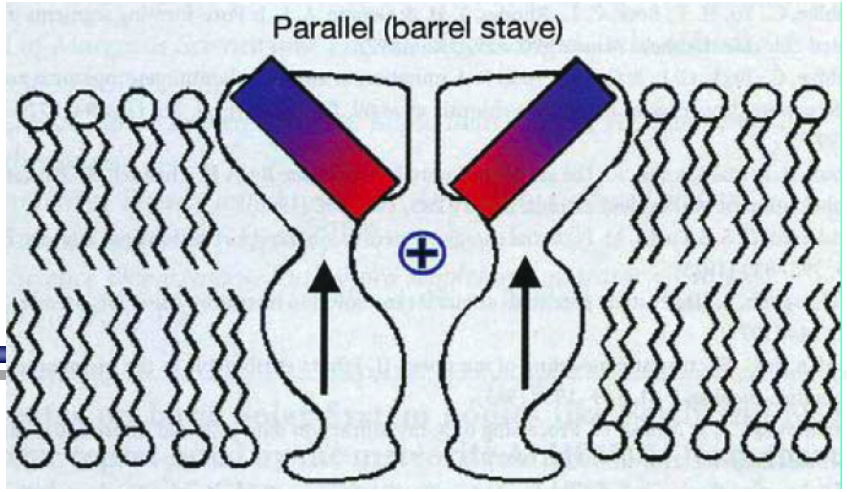
- The structure of the open and closed K^+ channel has been solved at 1.9 and 3.6 Å respectively
- The K^+ channel is a tetramer made of 4 subunits that come together to form the channel.
- Each subunit is made of 3 main trans-membrane helices called external helix, internal helix and pore helix. The internal helix is facing the pore, the external helix is in contact with the membrane lipids. A long internal loop makes the selectivity filter for the ions



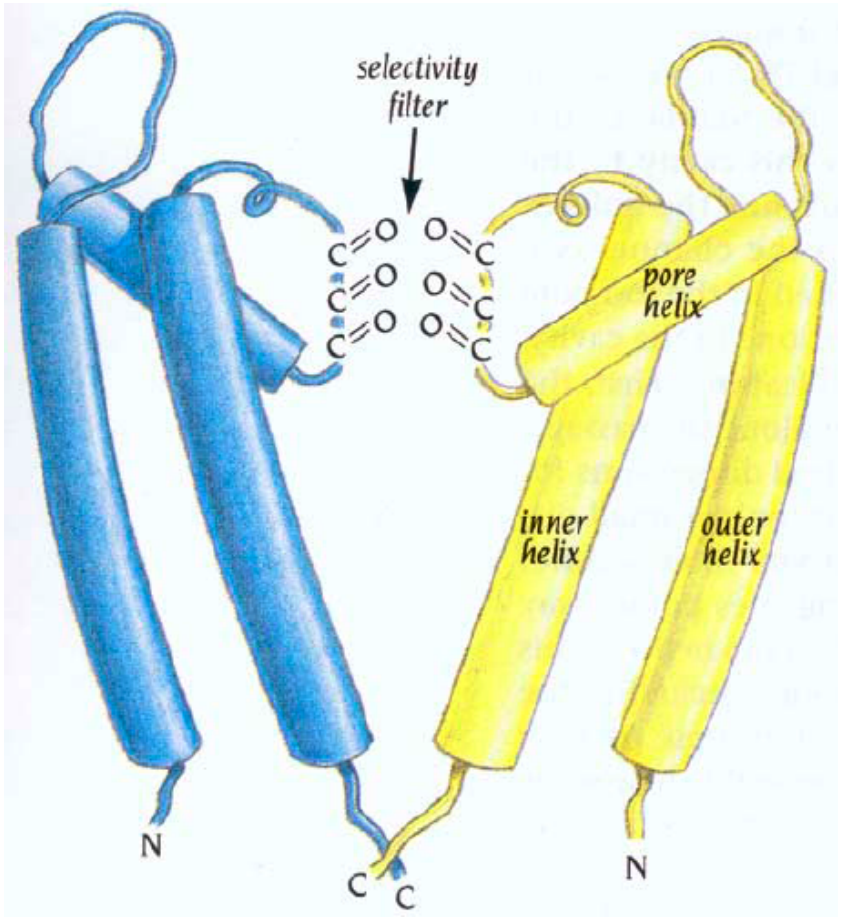
- The length of the channel is 50Å, the diameter varies
- Both ends of the channel contain negative charges to be able to attract the positively charged K^+
- On the cytosol, the channel starts with a segment 18Å long, that opens in a larger cavity that is 10Å in diameter, located half way in the membrane thickness
- This cavity is then connected to the extracellular space by a narrow passage called selectivity filter



Open and Closed K^+ channels



- **Closed K^+ channel:**
  - Here the flux of ions is blocked by the side chains of hydrophobic aa
  - The pore helices are not aligned – the stabilisation of the K^+ is achieved with the helix dipoles that are pointing their C-ter towards the centre of the cavity
  - The volume of the central cavity is diminished
  - The conformation of the selectivity filter is altered
- **Open K^+ channel:**
  - Here the carbonylic oxygens of the peptide bonds are directed towards the selectivity filter to direct the K^+
  - The hydrophobic side chains are oriented towards an hydrophobic ring that surrounds the selectivity filter that speed up the passage of the K^+



http://www.youtube.com/watch?v=Z1M8s9aLe4Q

Enzymes at the border

- Schematic view of the catalytic strategies used by enzymes working with hydrophobic and hydrophilic substrates; lateral diffusion and hydrophilic funneling (top), desorb-and-modify (middle), and working at the interface (bottom).