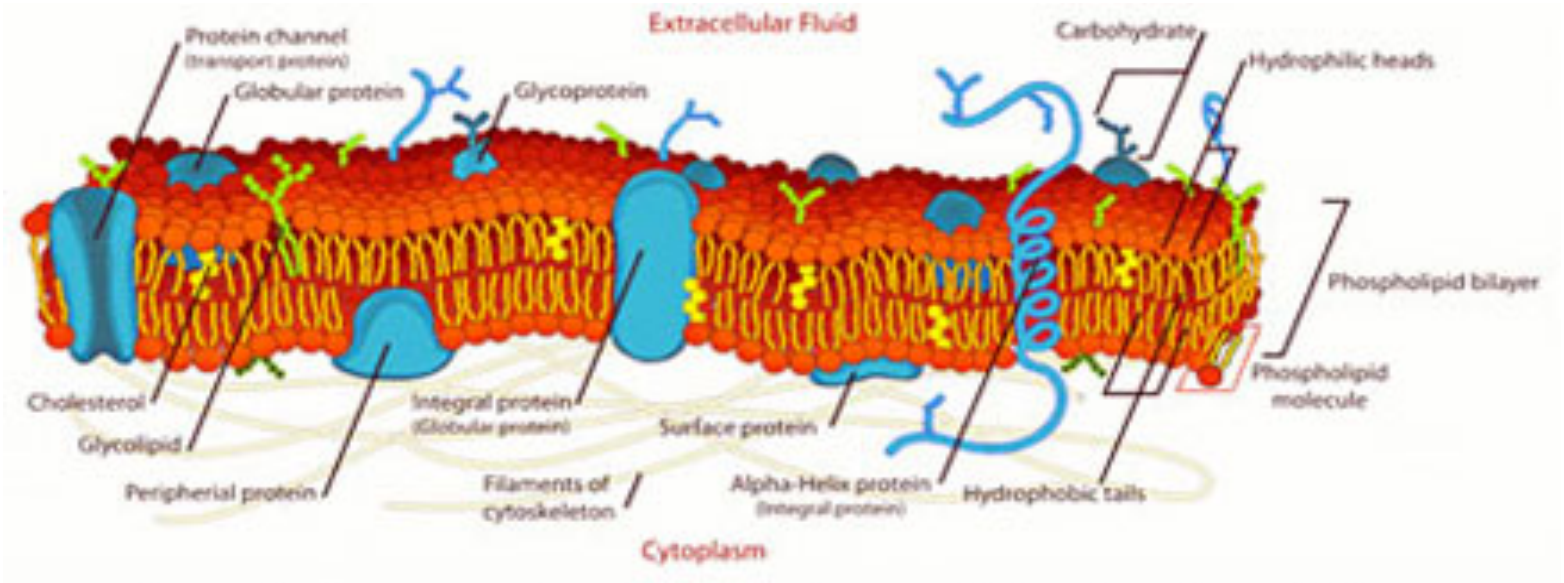
5. Membrane Proteins

Membrane Proteins

- Proteins that interact with biological membranes.
- Targets of over 50% of all modern medicinal drugs.
- Approximately 20–30% of all genes in most genomes encode membrane proteins.

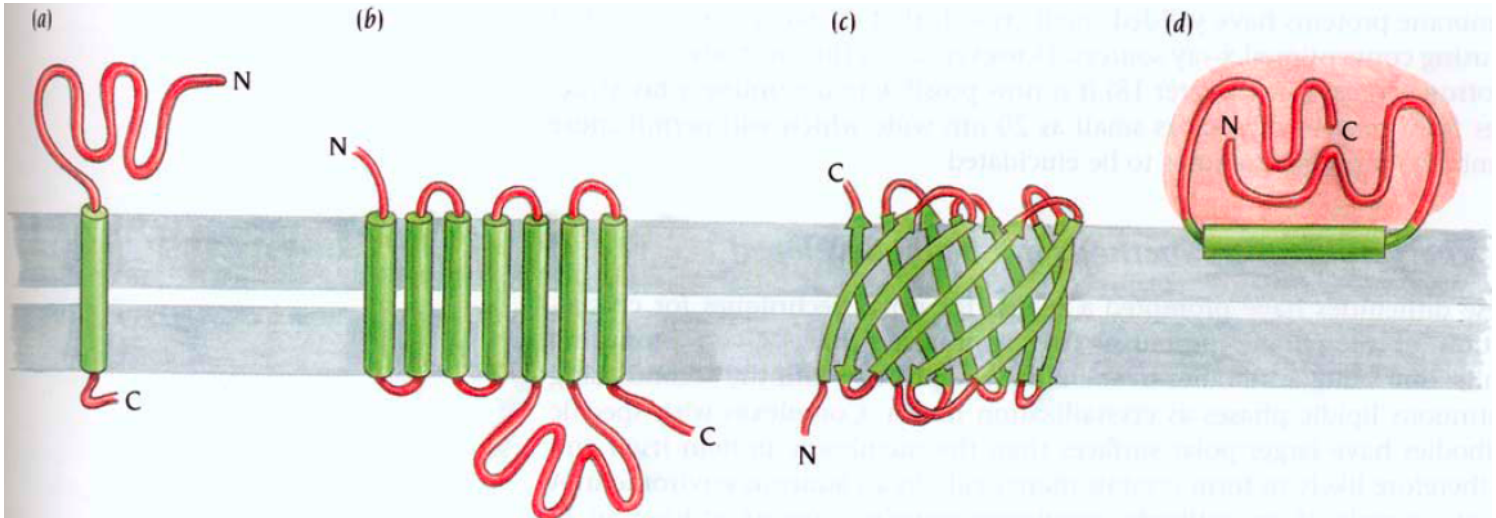


Schematic Representation of Cell Membrane with Proteins shown in Blue

Overington JP, Al-Lazikani B, Hopkins AL (December 2006); Krogh, A.; Larsson, B. R.; Von Heijne, G.; Sonnhammer, E. L. L. (2001); http://drugdiscoveryopinion.com/2009/02/membrane-proteins-anchored/

Definitions

- Cells and organelles are compartmentalized 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

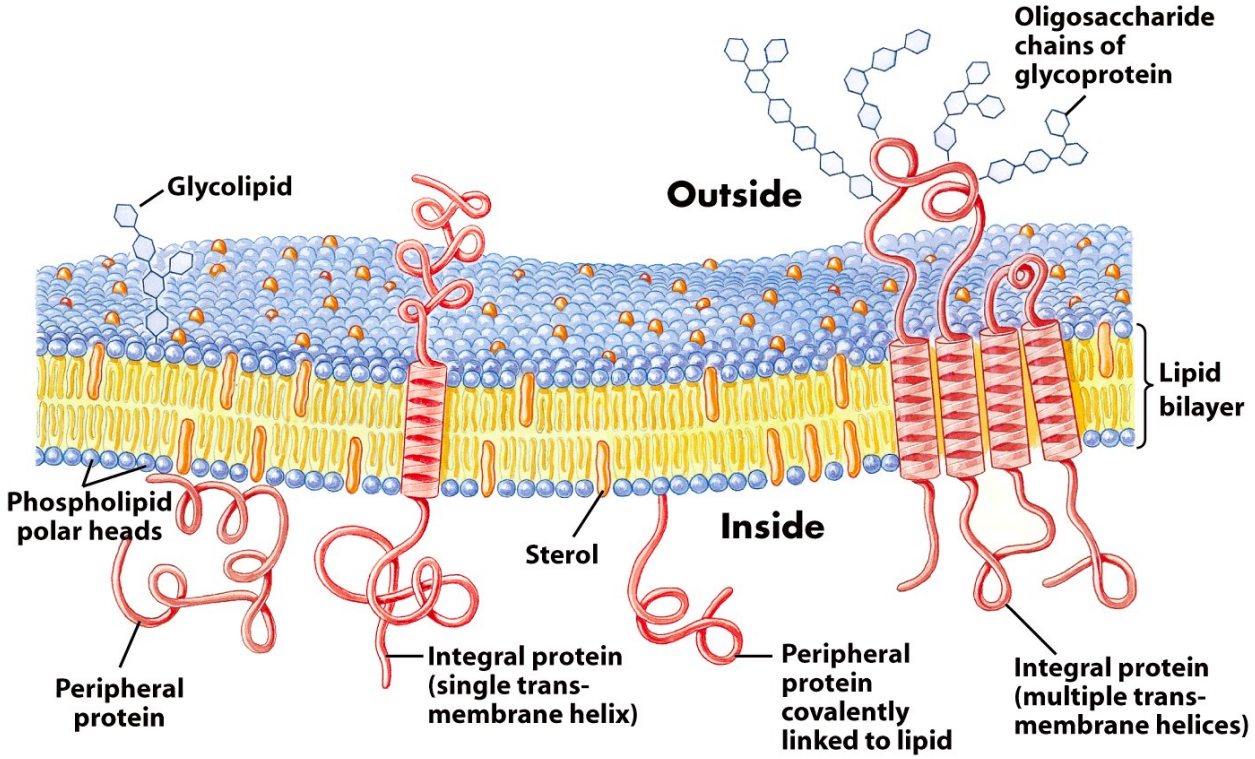


Roles of membrane proteins

- 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

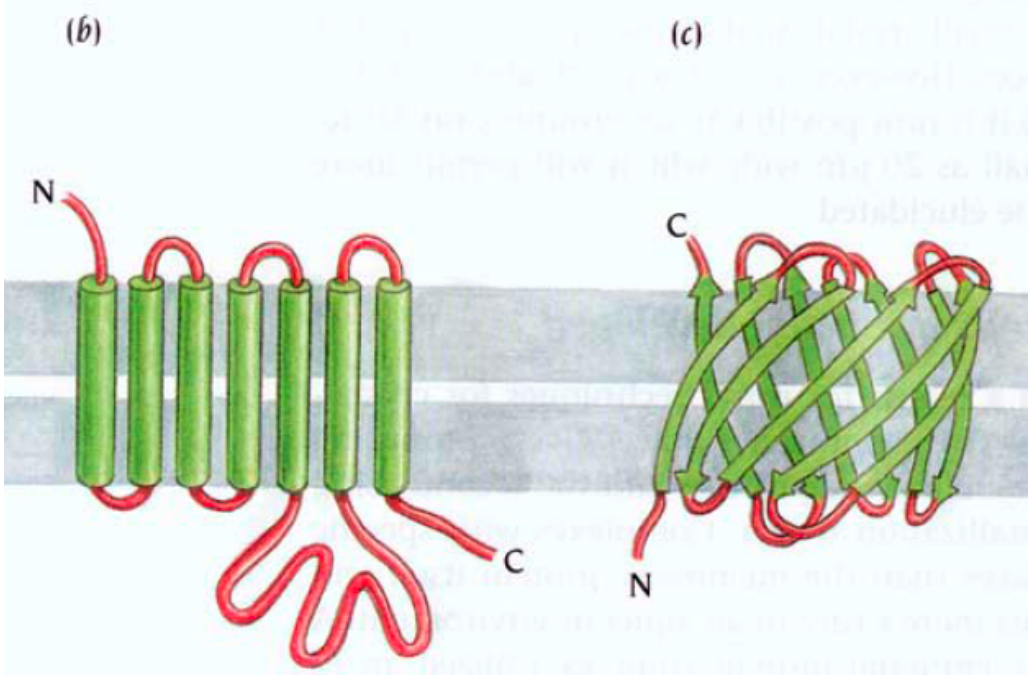
Membrane Protein Classifications

- Integral membrane proteins
  - These are permanently attached to membrane
- Peripheral membrane proteins
  - These are temporarily attached to membrane or to integral membrane proteins



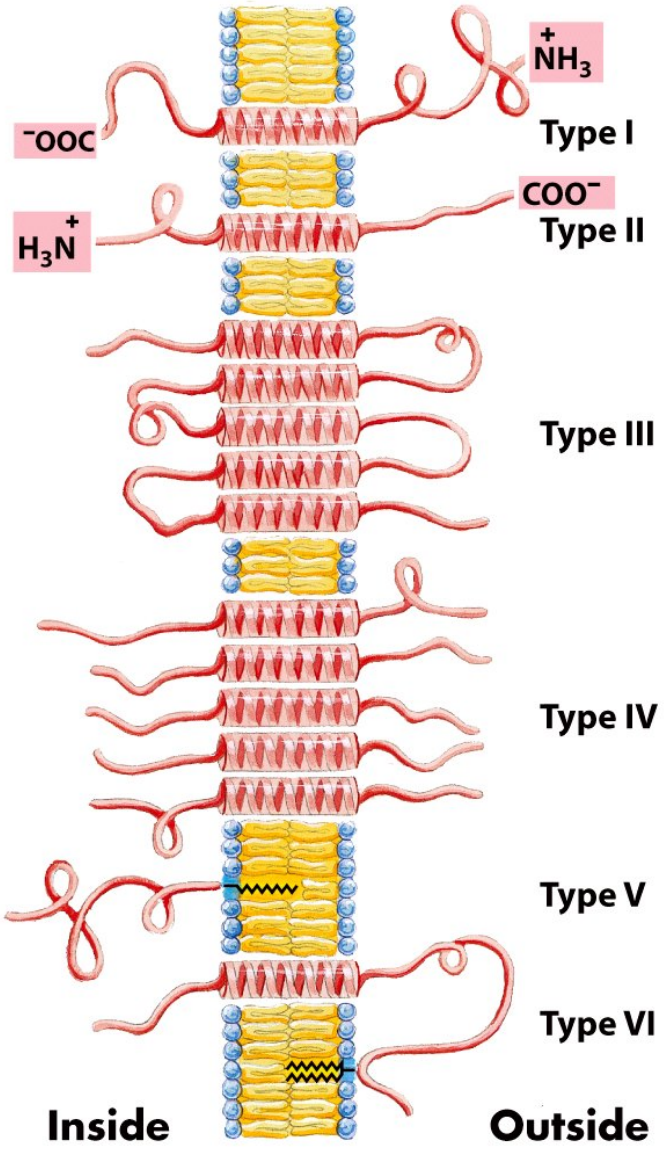
Classification of membrane proteins

- **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



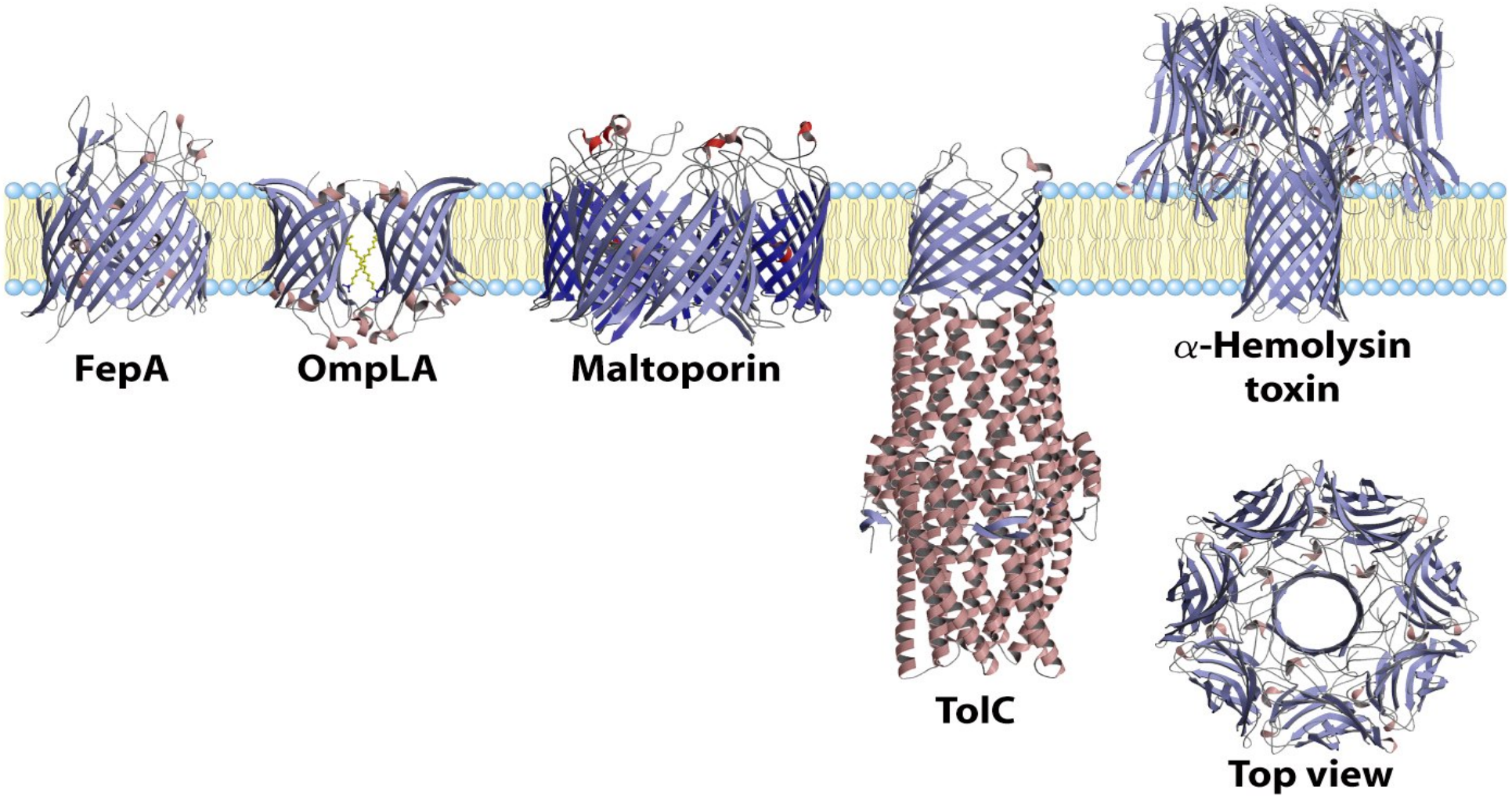
Integral Membrane Proteins

- **Functions**
  - Transfer information
  - Transport material
  - Energy conversion
- **Types**
  - **Type I**: Single transmembrane span, N-terminus in ectodomain, C-terminus in cytosol
  - **Type II**: Single span, C-terminus in the ectodomain, N-terminus in the cytosol
  - **Type III**: Multiple spans
  - **Type IV**: Several different polypeptides assembled to form a channel
  - **Type V**: Lipid-linked protein
  - **Type VI**: Proteins with both transdomain component and lipid anchor



Nelson, D. L., & Cox, M. M. (2008). Principles of Biochemistry (5th ed., p. 377).

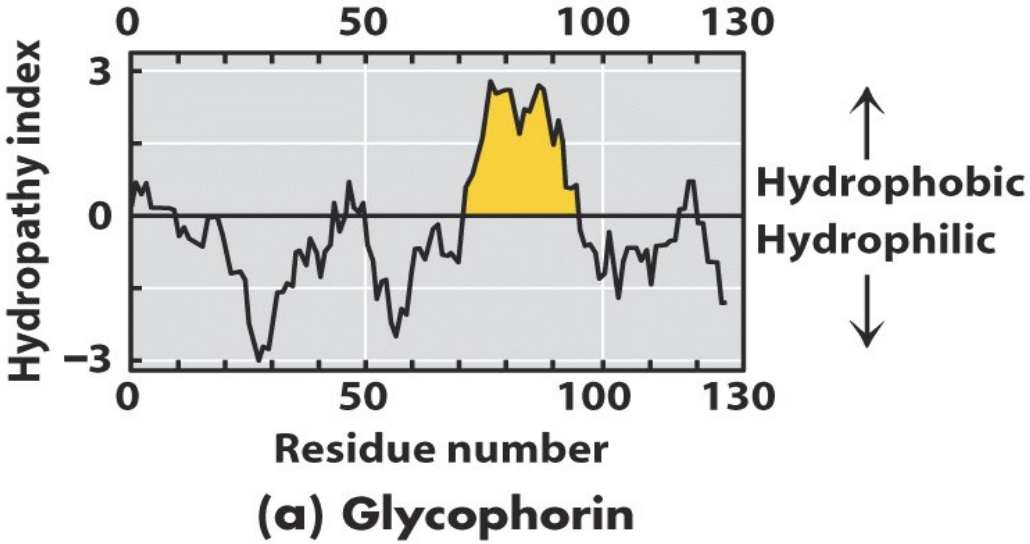
Membrane proteins with β-barrel structure



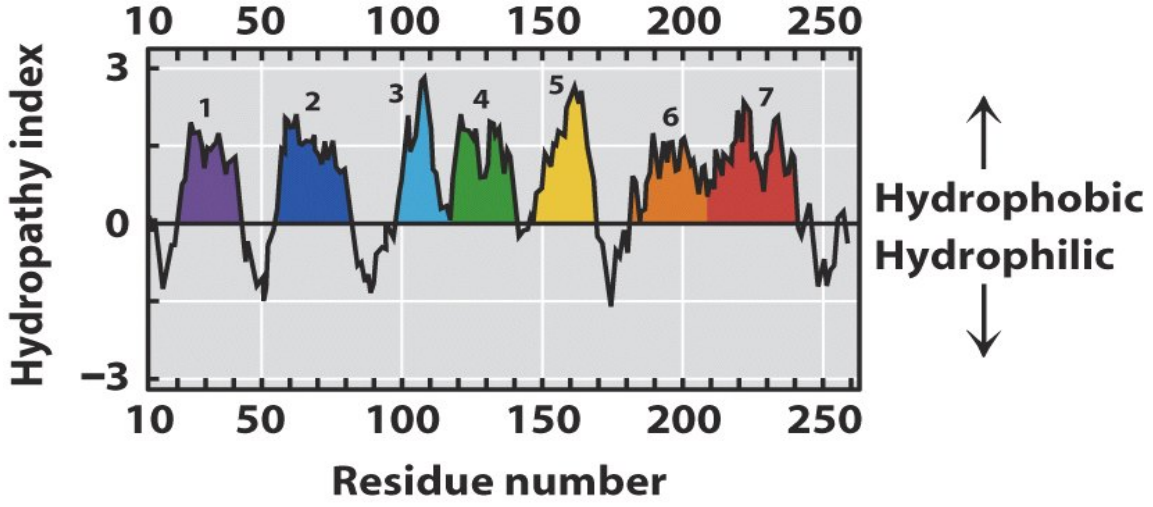
- The Topology of an Integral Membrane Protein Can Be Predicted from Its Sequence
- Hydrophobic plots

- Unbroken sequences of more than 20 hydrophobic residues

  10-20% of all proteins: integral membrane proteins

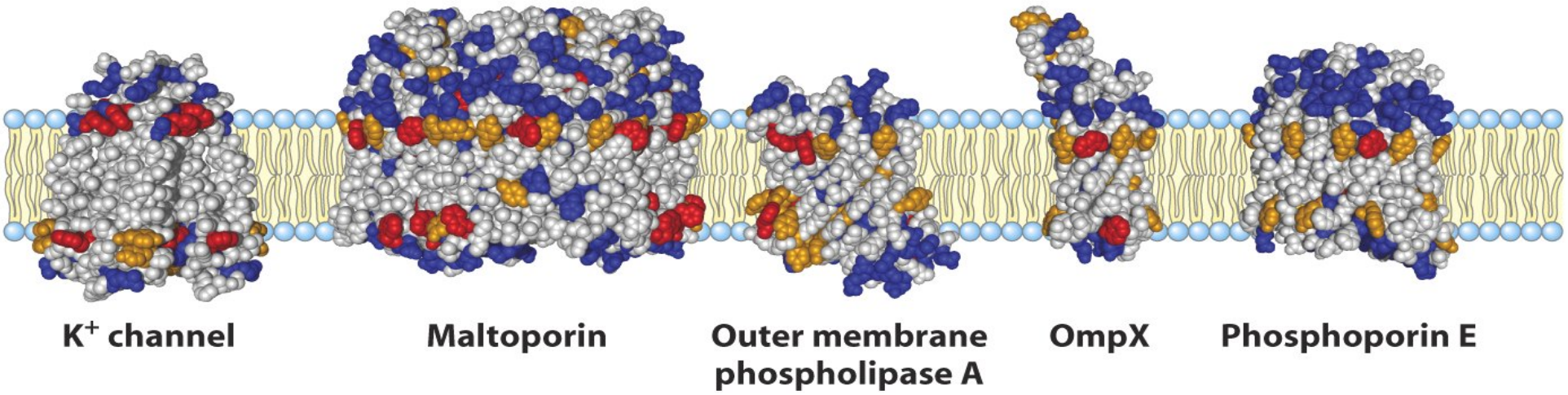


(a) Glycophorin



(b) Bacteriorhodopsin

Tyr and Trp residues of membrane proteins clustering at the water-lipid interface

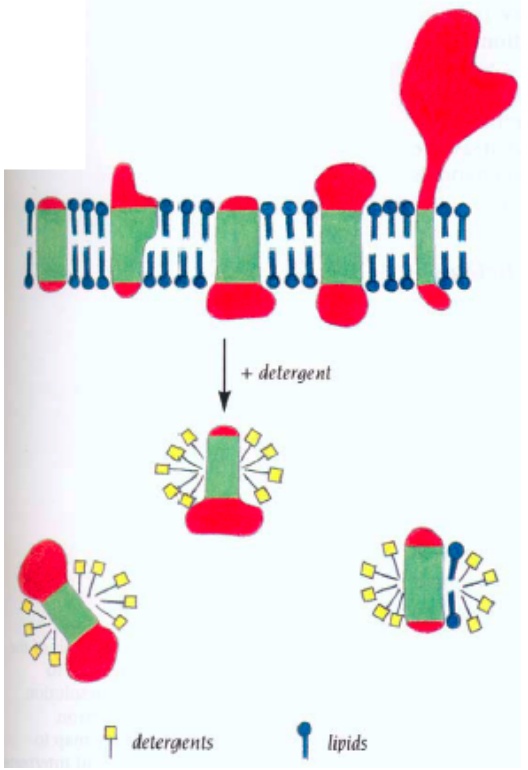


Tyr: orange

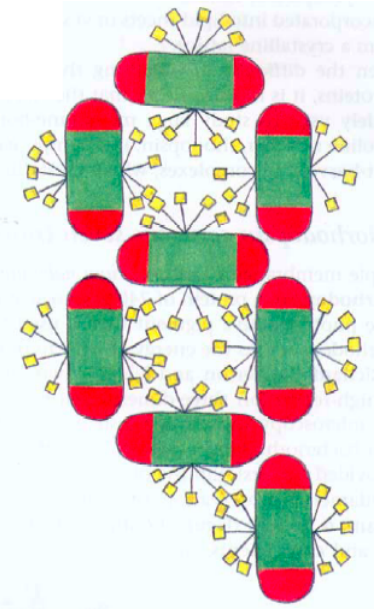
Trp: red

Charged residues (Lys, Arg, Glu, Asp): blue

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

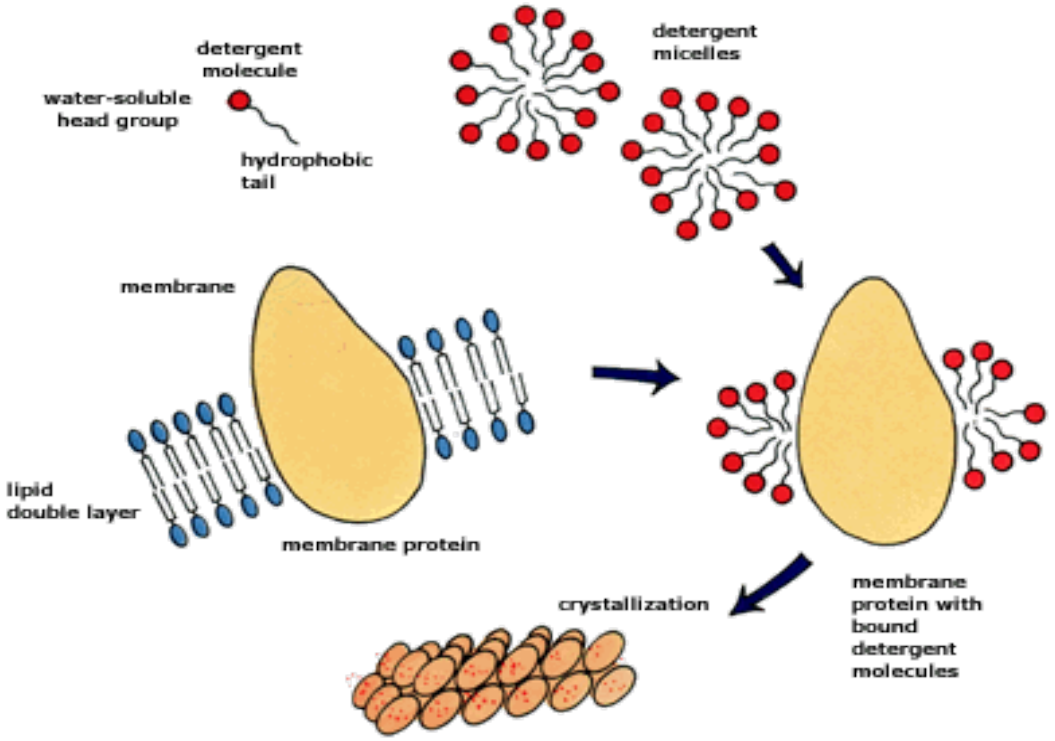


Crystallization of Membrane Proteins

- Difficult to crystallize because:
  - Insoluble in aq. Buffers (Due to hydrophobic surface regions)
  - Tend to denature in organic solvents
- Can be solubilized using aqueous detergents, then purified in native state.

How this works:

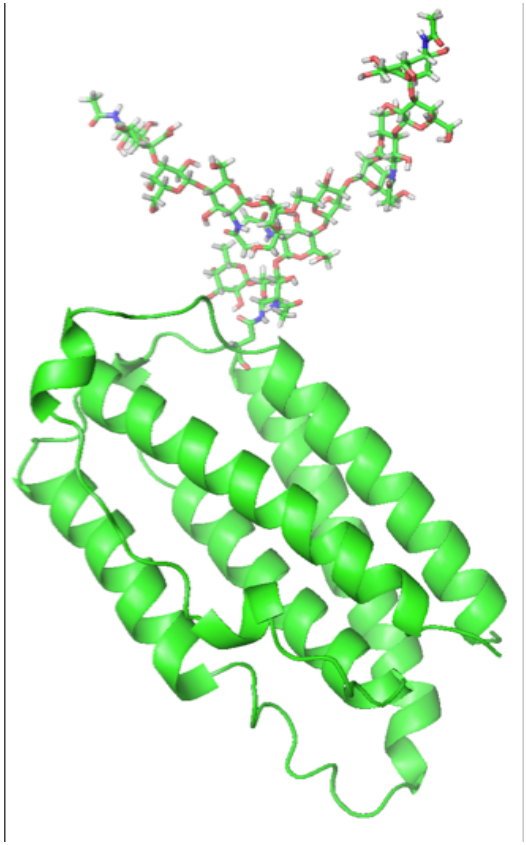
Amphipathic detergent adheres to hydrophobic surface of protein, so protein-detergent complex becomes hydrophilic. Can be crystallized in this state, but frequently produces low resolution crystals

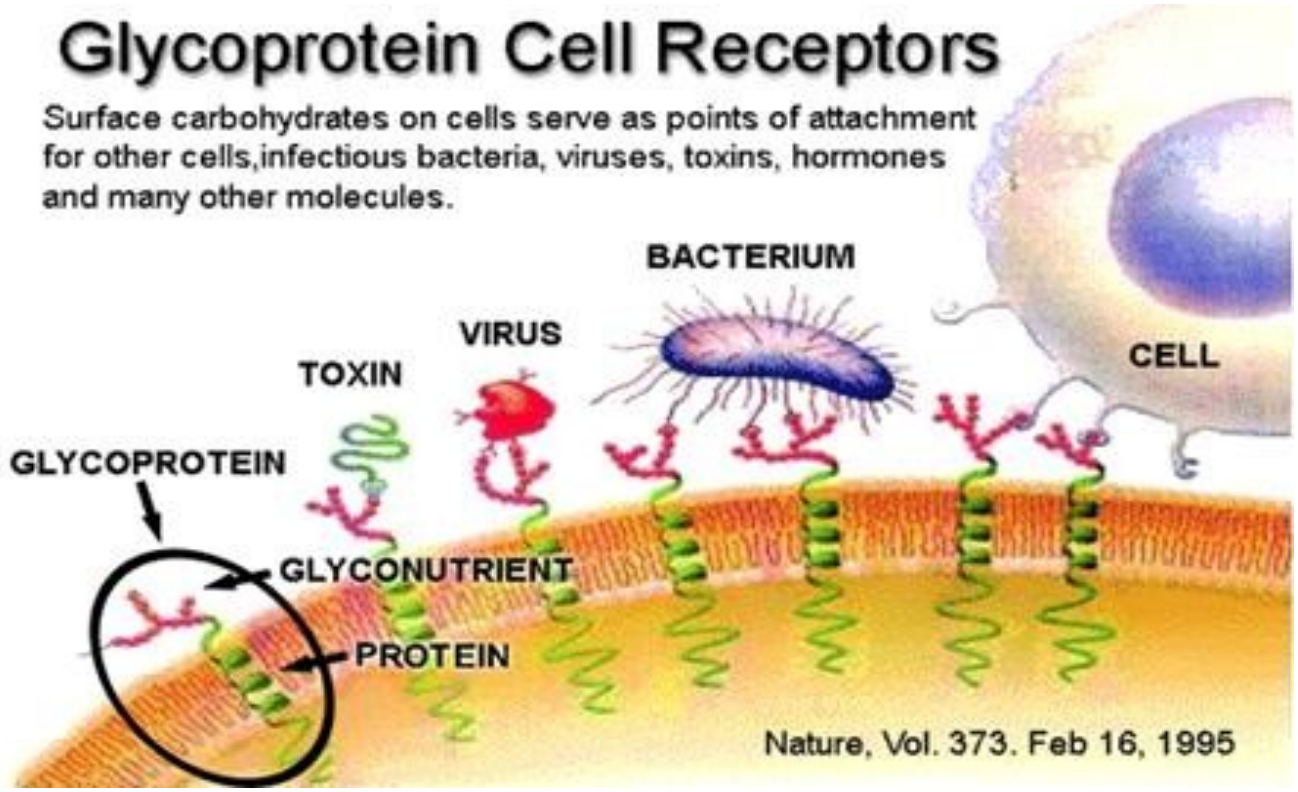


http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1988/illpres/crystals.html

Glycoproteins

- Proteins that contain oligosaccharide chains (glycans) covalently attached to AA side chains as co- or post-translational (N- or O-) glycosylation.
- Function as receptors.

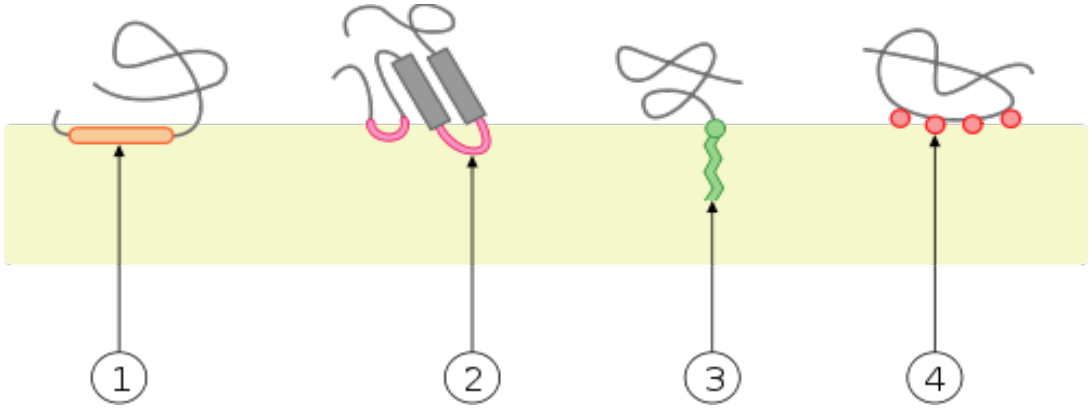




Peripheral membrane proteins

- Adhere only temporarily to the biological membrane with which they are associated (reversible binding).
- Attach to integral membrane proteins, or penetrate the peripheral regions of the lipid bilayer.
  - May be regulatory protein subunits of many ion channels and transmembrane receptors.
- Tend to collect in the water-soluble component, or fraction, of all the proteins extracted during a protein purification procedure.
- Reversible binding of these proteins is associated with regulation of cell signaling and other cellular events.
- These proteins may interact with membrane in several ways:

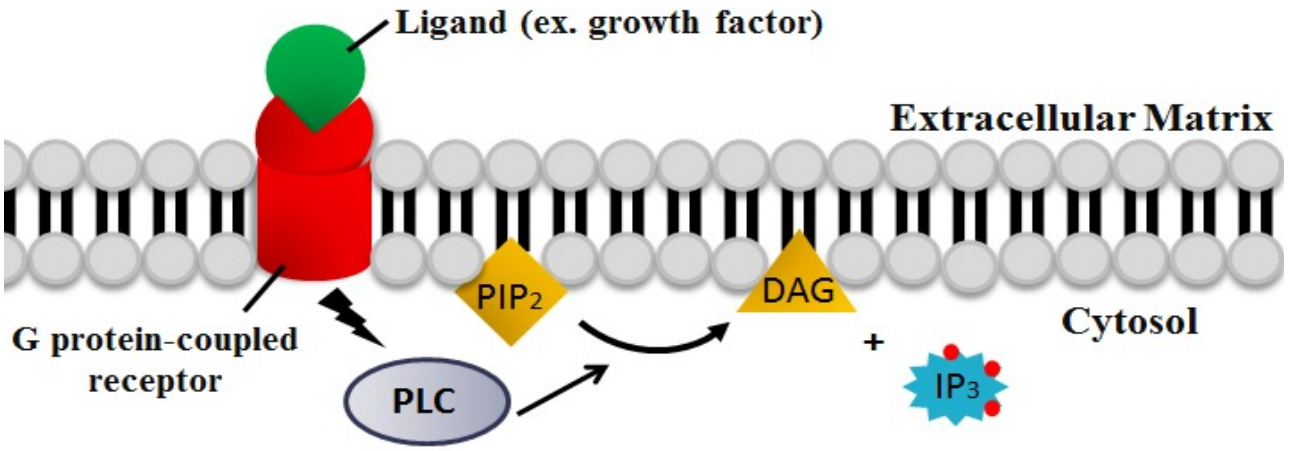
1. By amphipathic α-helix parallel to membrane
2. By a hydrophobic loop
3. By a covalently bound membrane lipid (*lipidation*)
4. By electrostatic or ionic interactions with membrane lipids (*e.g.* through a calcium ion)



Categories of peripheral membrane proteins

- Enzymes
  - Phospholipase C: Hydrolyzes PIP2 into IP3 and diacylglycerol
- Membrane-targeting domains (lipid clamps)
  - C2 domains: Bind phosphatidylserine or phosphatidylcholine
- Structural domains
  - Annexins: Calcium-dependent intracellular membrane/phospholipid binding. Involved in membrane fusion and ion channel formation.
- Transporters of small hydrophobic molecules
  - Glycolipid transfer protein
- Electron Carriers
  - Cytochrome C: Transfers electrons between Complex III (CoQ-Cyt C reductase) and Complex IV (Cyt C oxidase) in the electron transport chain.
- Polypeptide hormones, toxins and antimicrobial peptides
  - Venom toxins (e.g., snake venom)

Phospholipase C

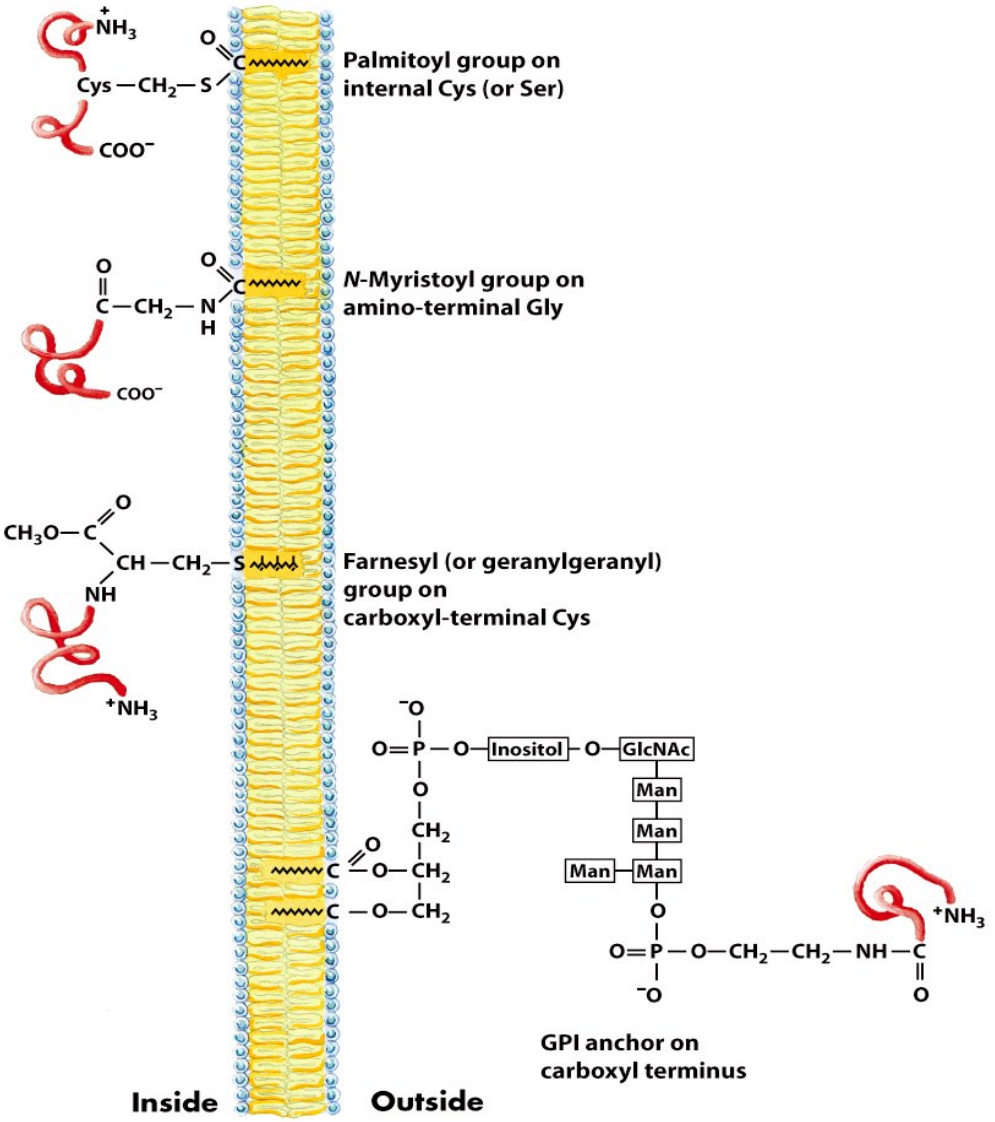


- Enzymes that cleave phospholipids just before the phosphate group.
- PLC cleaves the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) into diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP3).
- DAG remains bound to the membrane.
- IP3 is released as a soluble structure into the cytosol, where it binds to IP3 receptors in the smooth endoplasmic reticulum (ER), releasing calcium into cytosol, causing a cascade of intracellular changes.
- Calcium and DAG also work together to activate protein kinase C, which goes on to phosphorylate other molecules, leading to altered cellular activity.

"PLC role in IP3-DAG pathway" by RaihaT - Own work. Licensed under CC BY-SA 3.0 via Wikimedia Commons - http://commons.wikimedia.org/wiki/File:PLC_role_in_IP3-DAG_pathway.tif#/media/File:PLC_role_in_IP3-DAG_pathway.tif

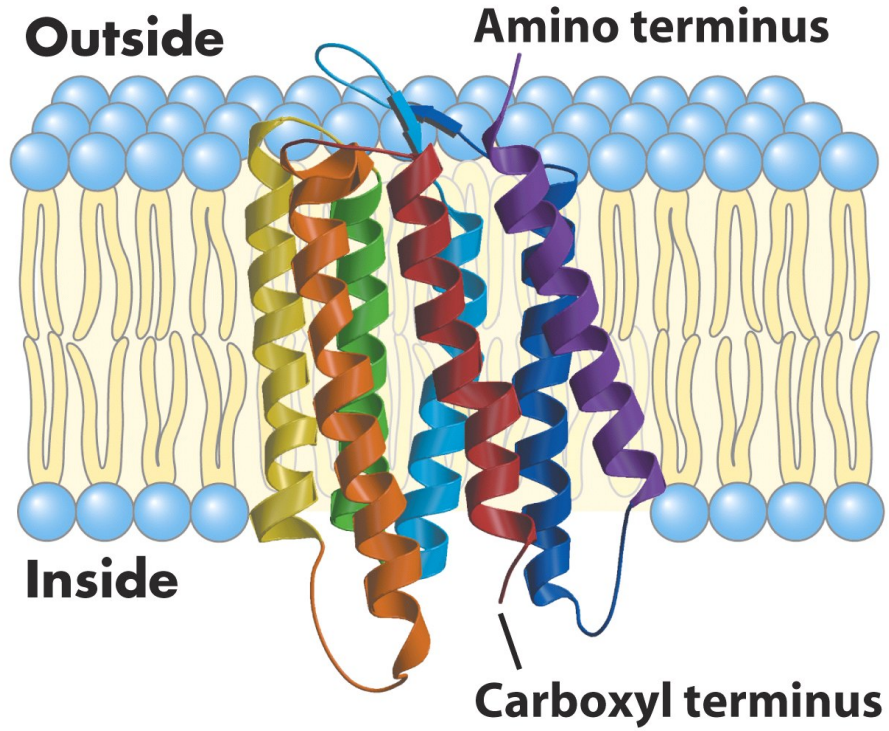
Lipid-linked proteins

- Covalently Attached Lipids Anchor Some Membrane Proteins
- Lipid-linked membrane proteins

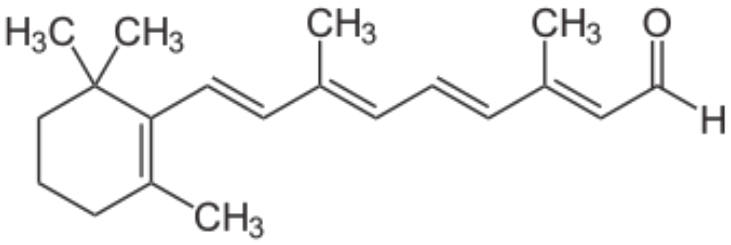


Bacteriorhodopsin

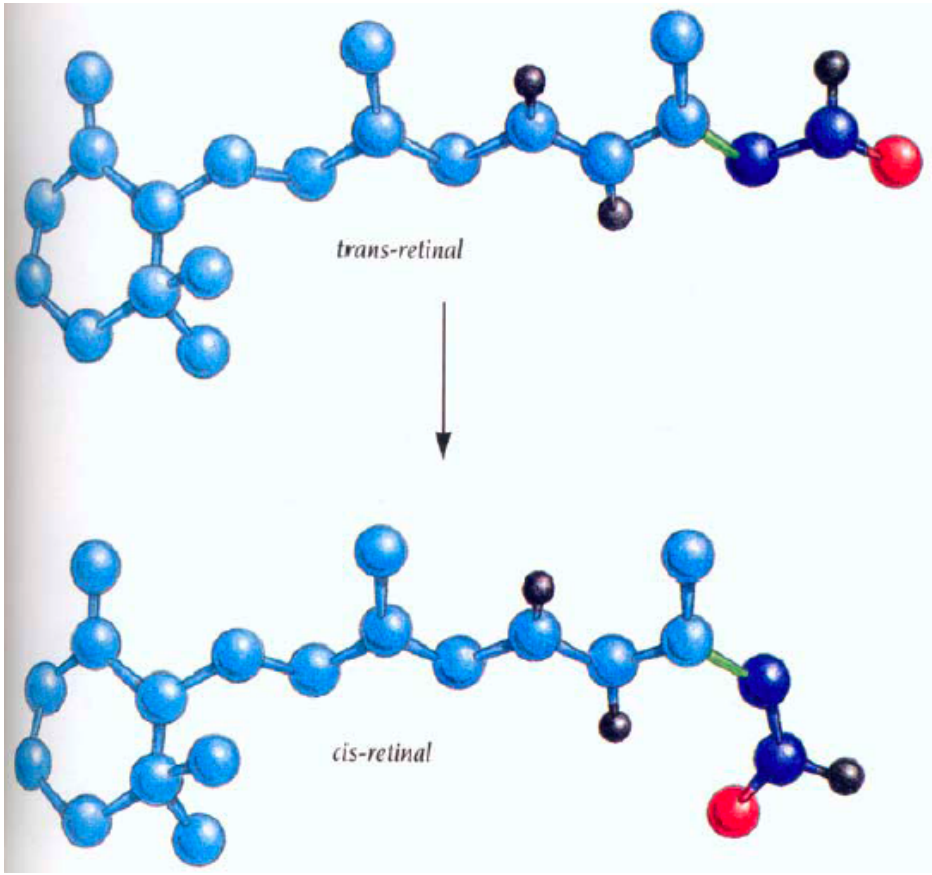
- Membrane protein of 248 aa that binds rethinal, the same pigment that captures light in our eyes
- Made of 7 transmembrane helices, tilted by 20• with respect of the plane of the membrane
- In 1975 thanks to EM a first model of bacteriorhodopsin was obtained at 7Å resolution.
- The model was improved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



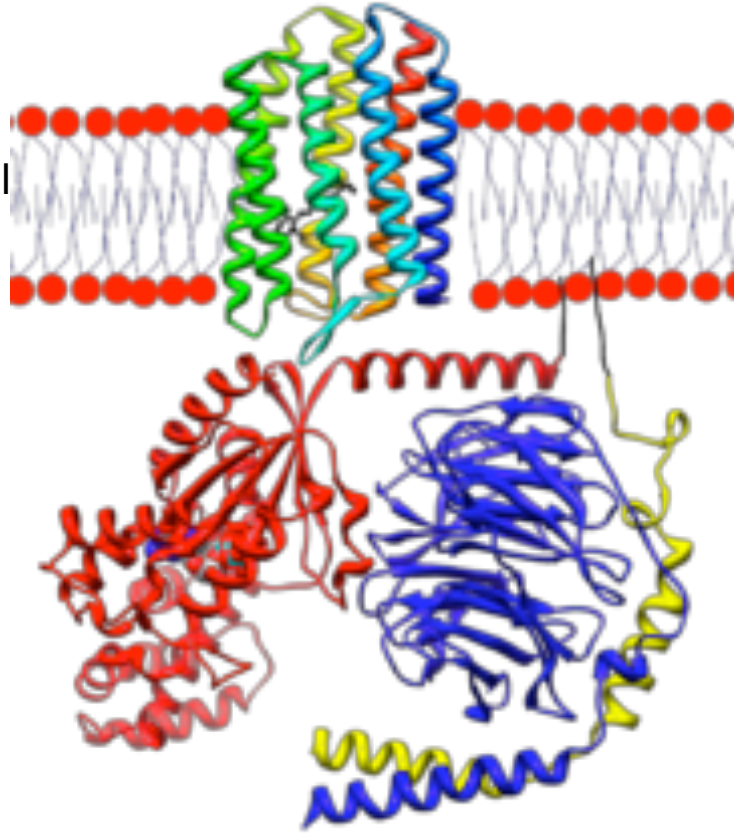
Bacteriorodhopsin function



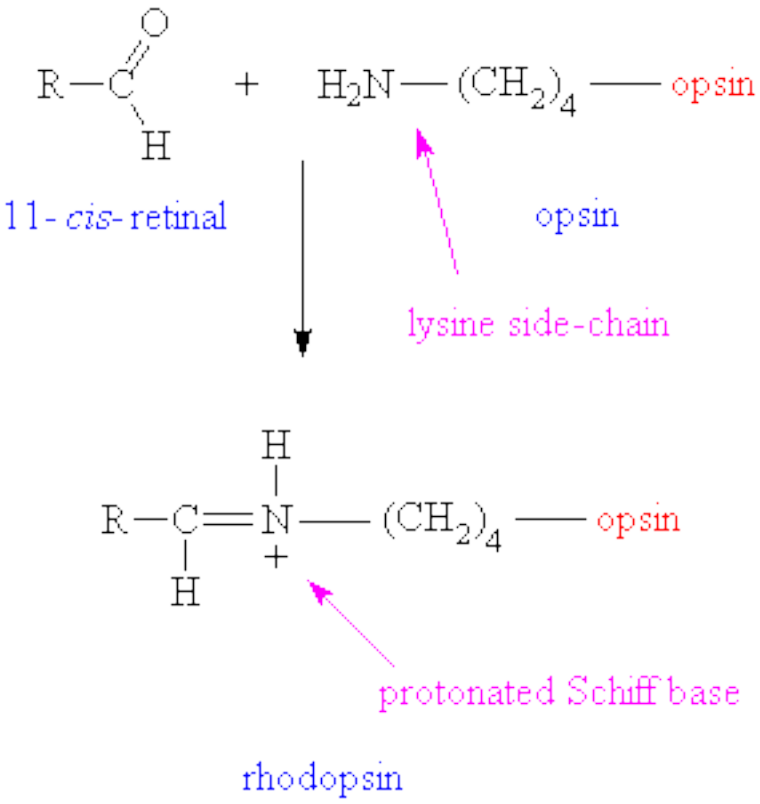
- The archaea halobacteria have a simplified biological system for the conversion of light into chemical energy.
- Under intense illumination bacteria express high quantities of bacteriorhodopsin
- When retinal is bound to bacteriorhodopsin and it absorbs a photon it undergoes an isomerisation *trans*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

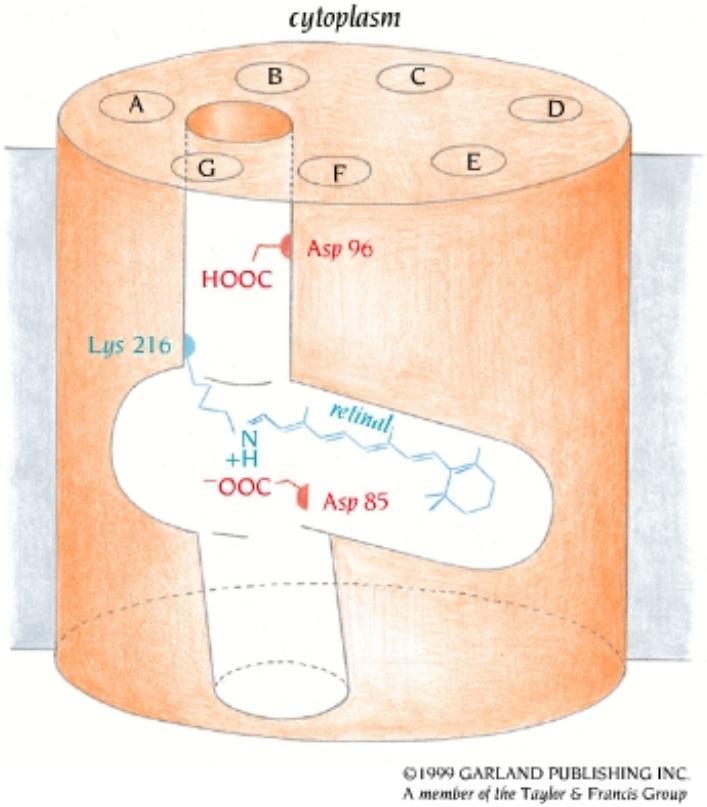


- The retinal is covalently linked to Lys216 in the chromophore by Schiff base action.
- After photoisomerization of the retinal molecule, Asp85 becomes a proton acceptor of the donor proton from the retinal molecule.
- This releases a proton from a "holding site" into the extracellular side (EC) of 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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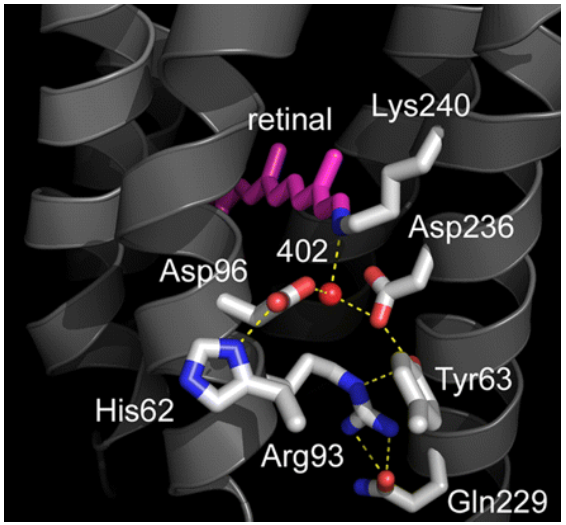
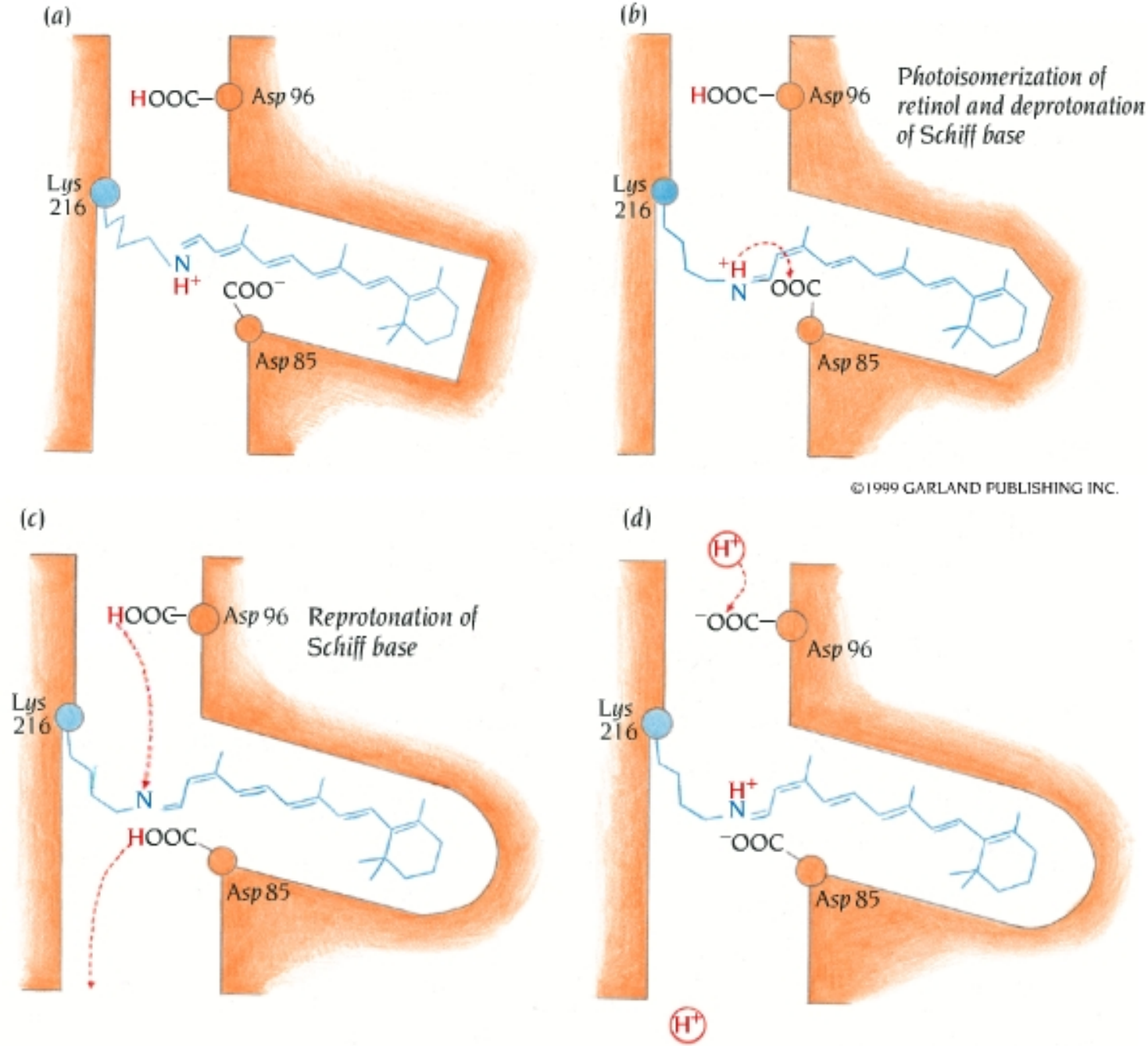

retinal
Lys240
Asp236
402
Asp96
Tyr63
His62
Arg93
Gln229


(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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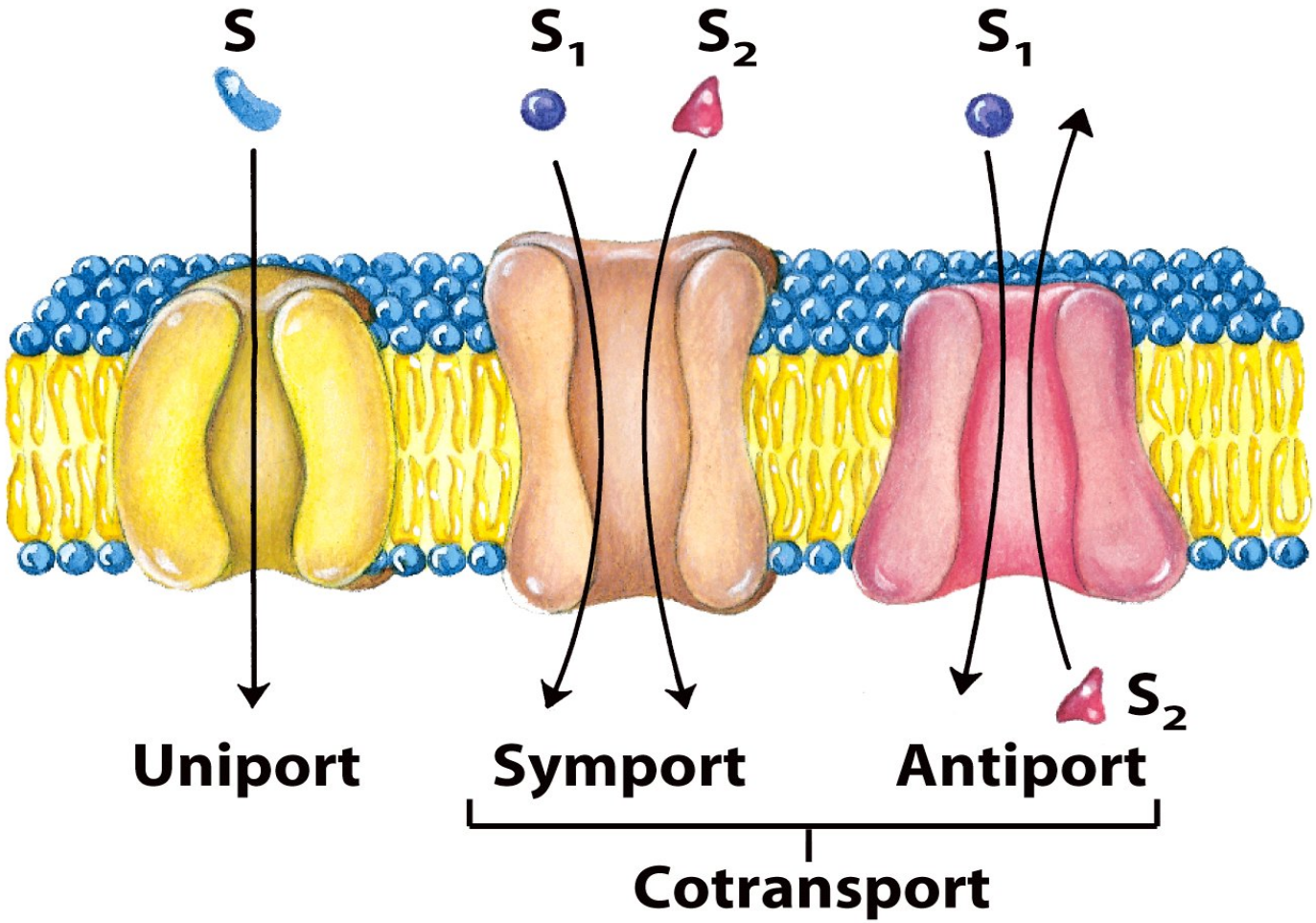
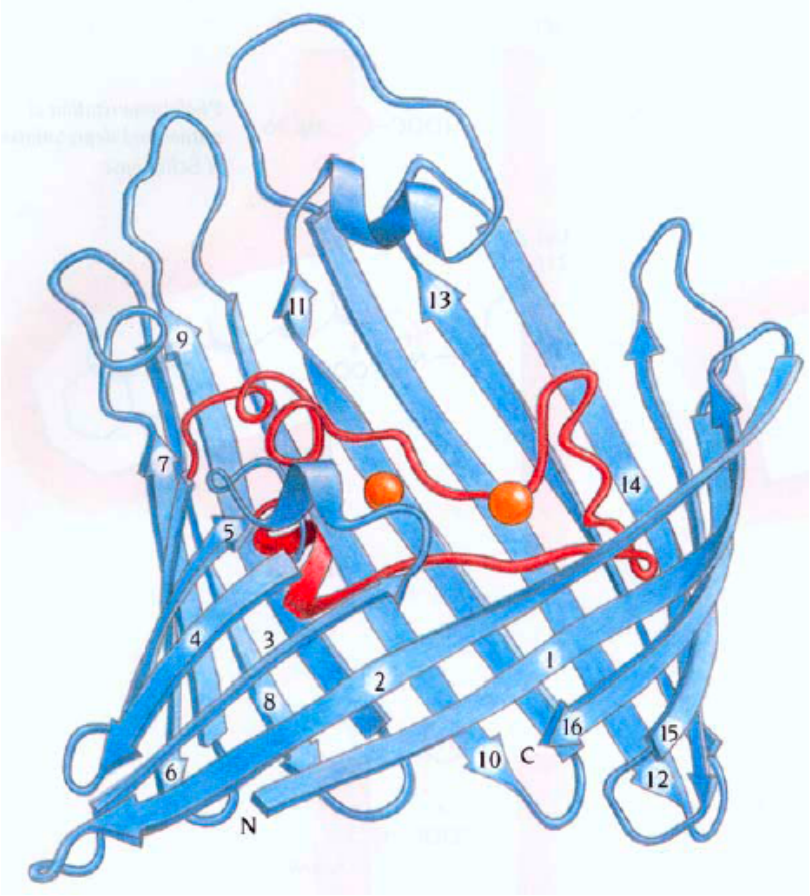
Transporters

Three general classes of transport systems



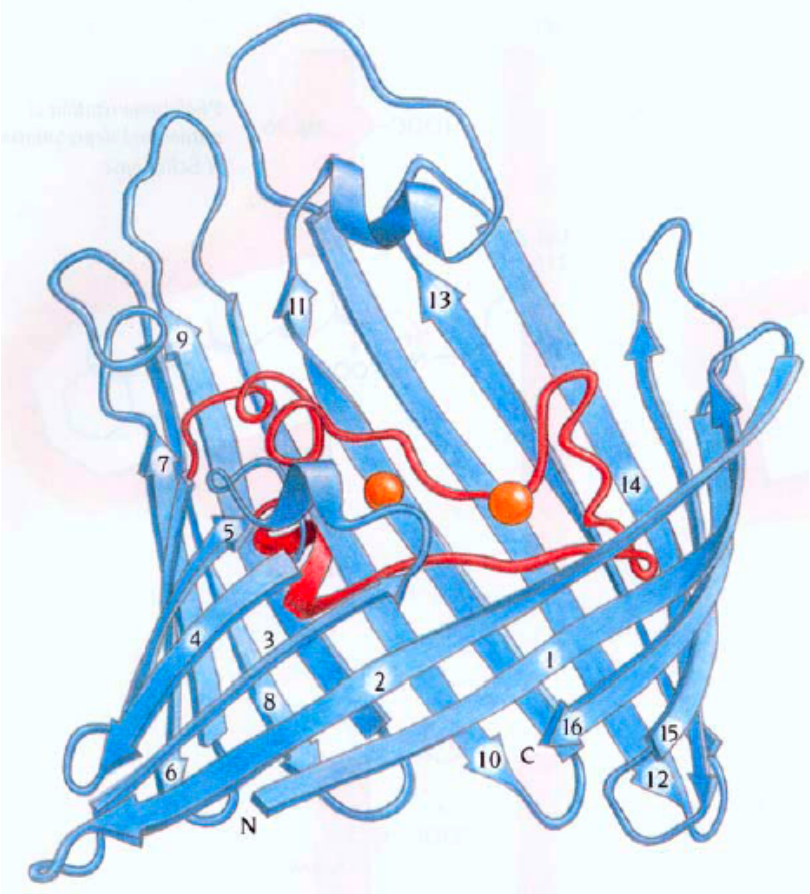
Porins

- 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 topology 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.



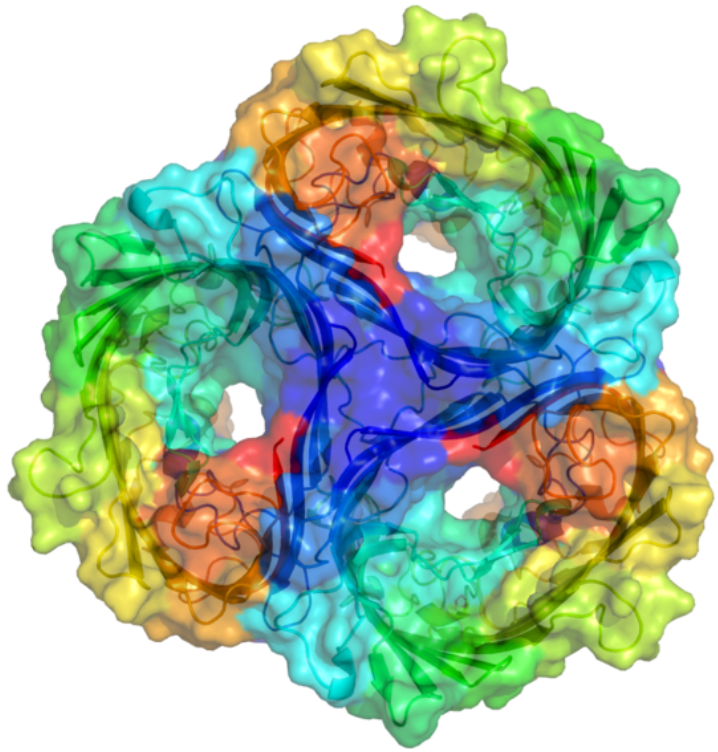
Porins

- 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



Porins

- 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

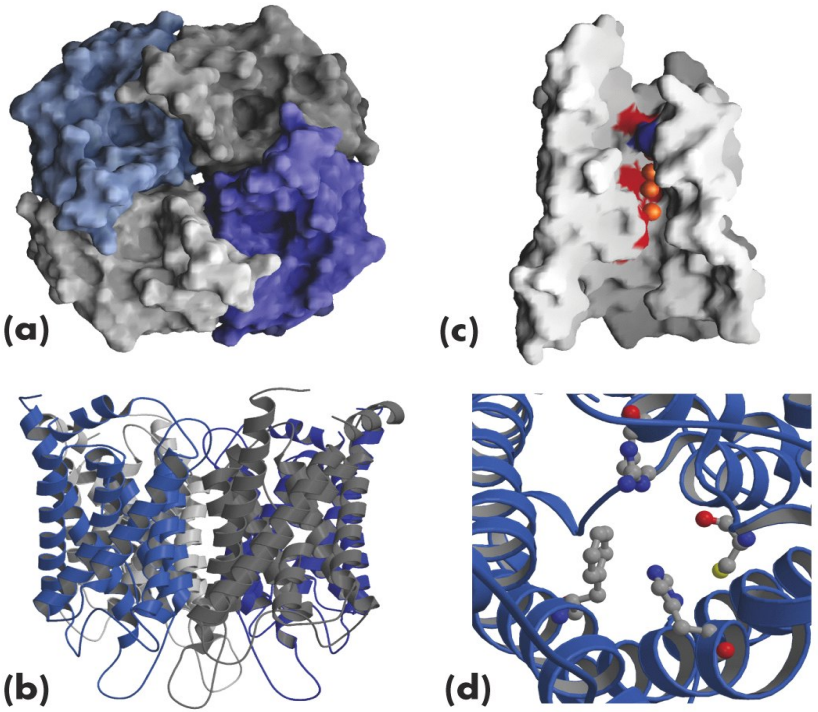


Aquaporins

- Aquaporins form hydrophilic transmembrane channels for the passage of water structure of an aquaporin, aqp-1

TABLE 11–6 Aquaporins

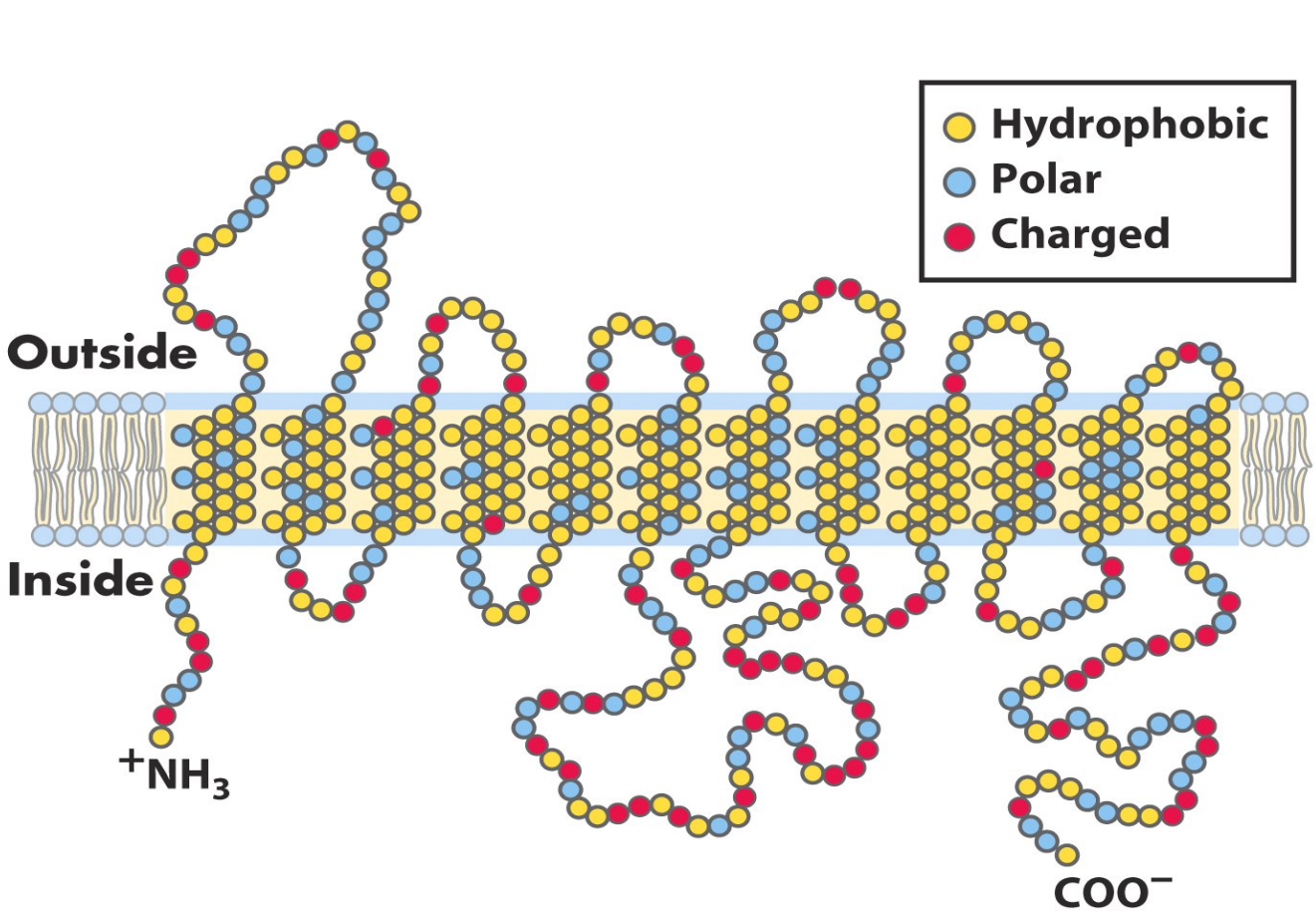
Aquaporin	*Roles and/or location*
AQP-1	Fluid reabsorption in proximal renal tubule; secretion of aqueous humor in eye and cerebrospinal fluid in central nervous system; water homeostasis in lung
AQP-2	Water permeability in renal collecting duct (mutations produce nephrogenic diabetes insipidus)
AQP-3	Water retention in renal collecting duct
AQP-4	Cerebrospinal fluid reabsorption in central nervous system; regulation of brain edema
AQP-5	Fluid secretion in salivary glands, lachrymal glands, and alveolar epithelium of lung
AQP-6	Kidney
AQP-7	Renal proximal tubule, intestine
AQP-8	Liver, pancreas, colon, placenta
AQP-9	Liver, leukocytes
TIP	Regulation of turgor pressure in plant tonoplast
PIP	Plant plasma membrane
AQY	Yeast plasma membrane

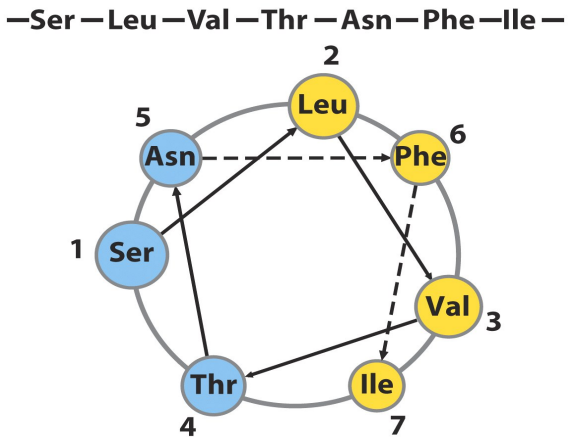


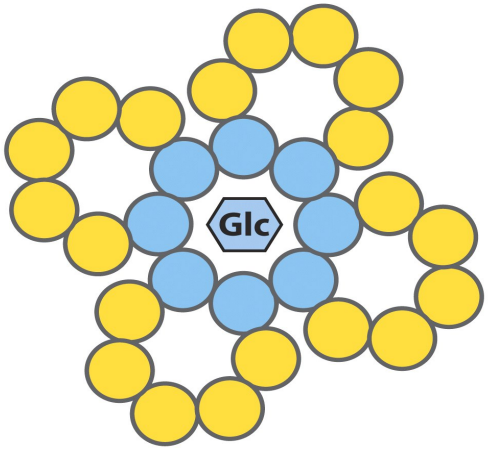
Structure of an aquaporin, AQP-1

GLUCOSE TRANSPORTER GLUT1

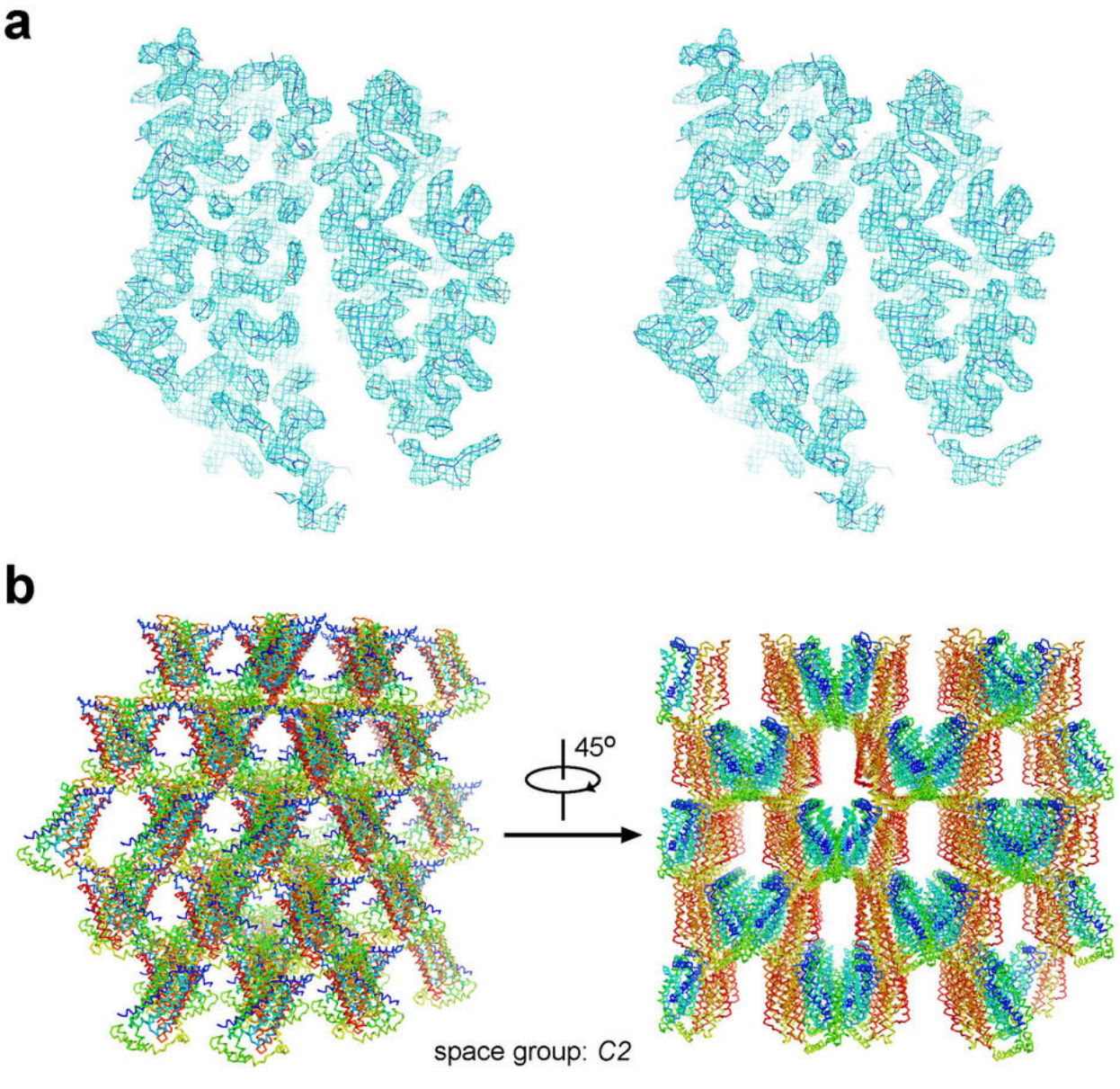
- GLUT1 belongs to the sugar porter subfamily of the major facilitator superfamily (MFS), one of the largest and most ubiquitous secondary transporter superfamilies
- The glucose transporter of erythrocytes mediates passive transport







Crystal structure of the human glucose transporter GLUT1.



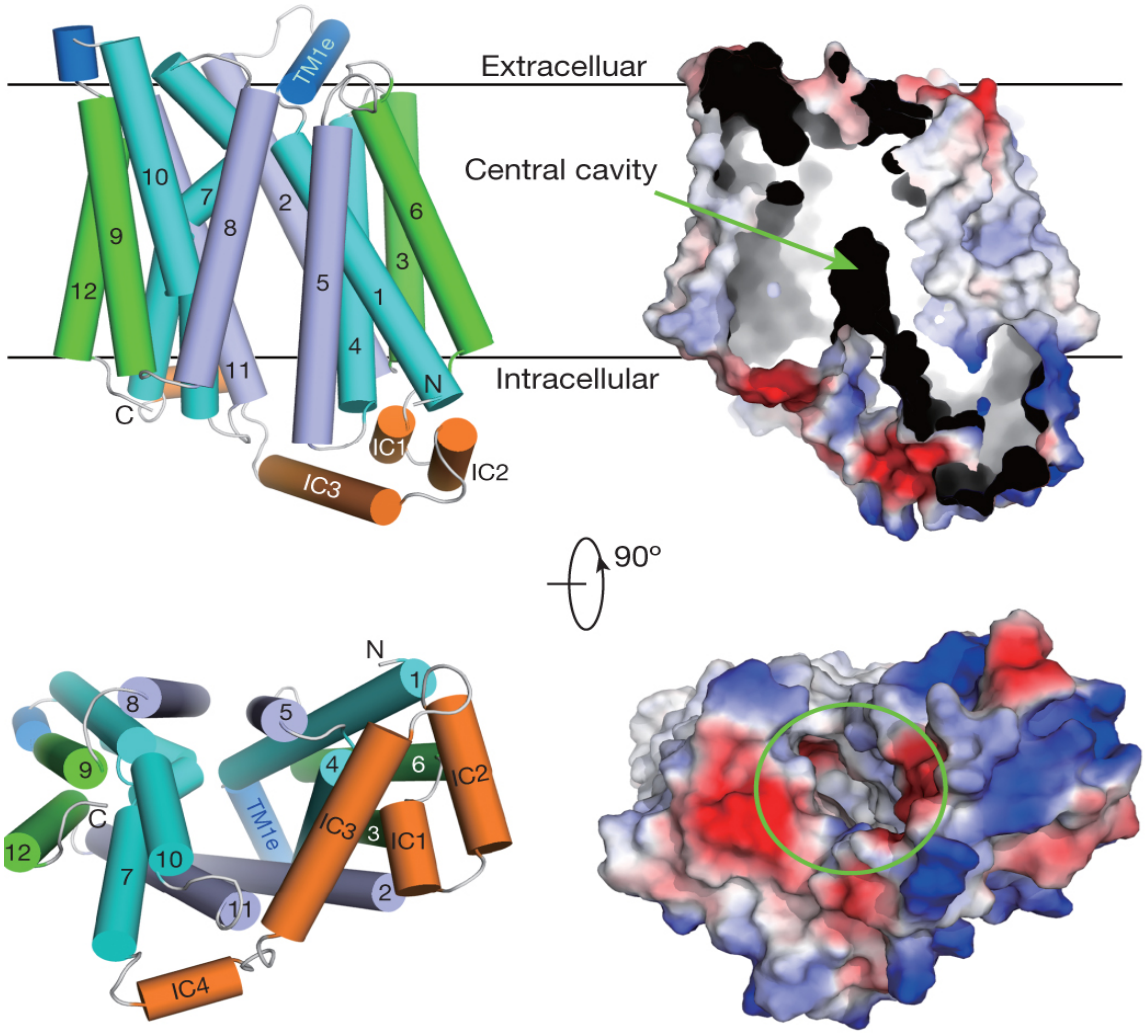
3.2 Å resolution

nature

D Deng *et al. Nature* **000**, 1-5 (2014) doi:10.1038/nature13306

Overall structure of the human glucose transporter GLUT1.

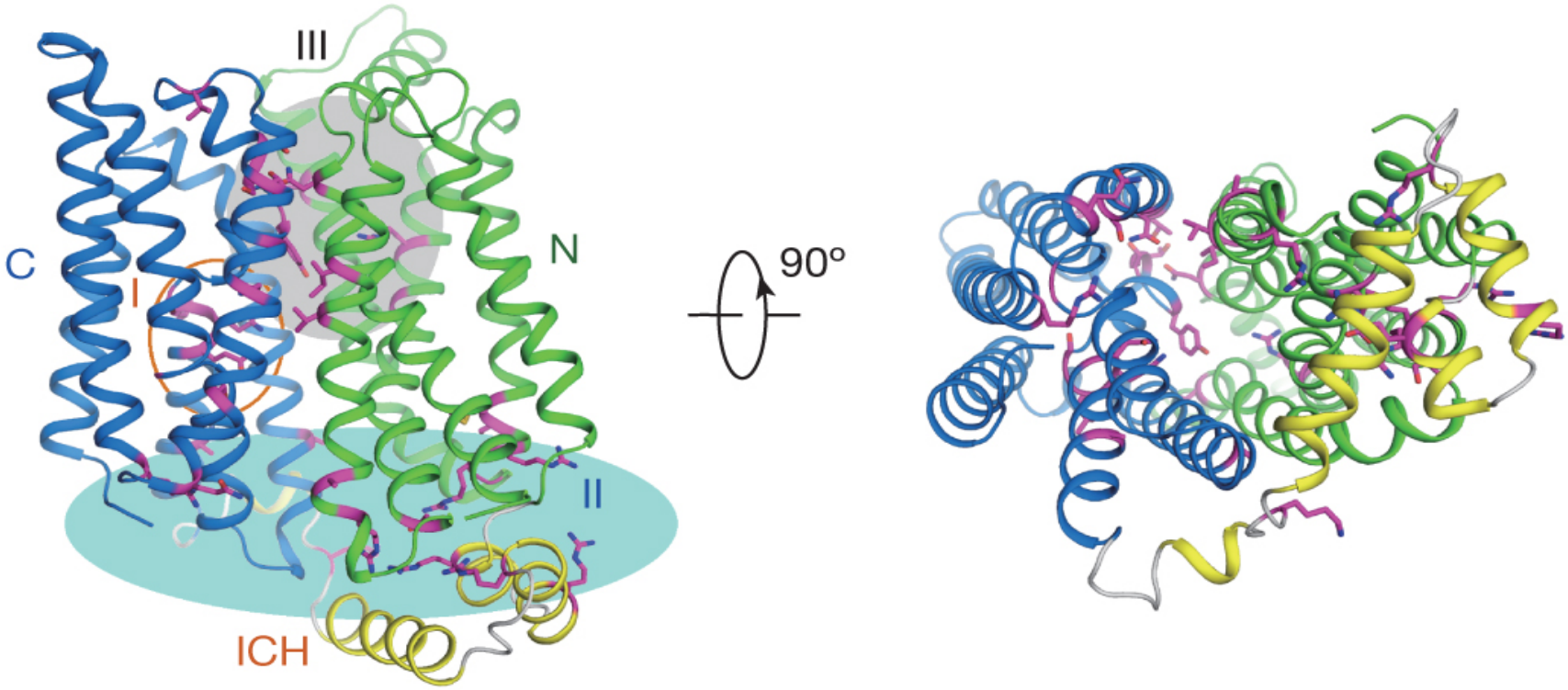
- MFS transporters share a conserved core fold that comprises 12 transmembrane segments organized into two discretely folded domains, namely the amino- and carboxy-terminal domains
- Mounting experimental evidence indicates that the three-helix bundle may represent the basic structural and functional unit.
- The structure of full-length human GLUT1 containing two point mutations (N45T, E329Q) was determined in an inward-open conformation.
- The extracellular and intracellular helices are coloured blue and orange, respectively. IC indicates intracellular helix.



nature

D Deng *et al. Nature* **000**, 1-5 (2014) doi:10.1038/nature13306

Structural mapping of disease-derived mutations in GLUT1.

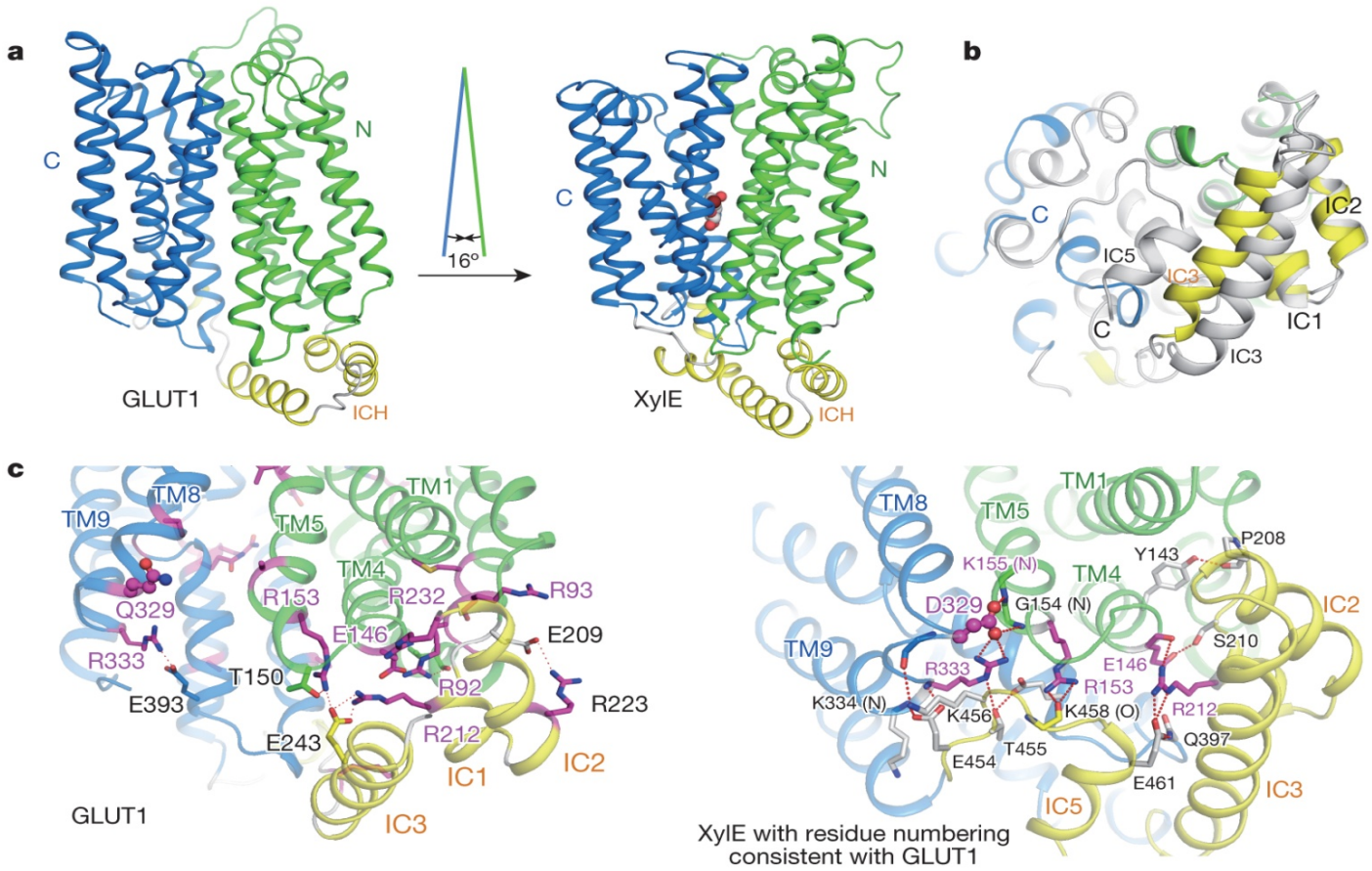


The residues for which mutations have been identified in patients with GLUT1 deficiency syndrome and other symptoms are coloured magenta. The orange circle and the grey and cyan shades indicate the three major clusters of residues for which mutations are associated with diseases. The N, C and ICH domains are coloured green, blue and yellow, respectively.

nature

D Deng *et al. Nature* **000**, 1-5 (2014) doi:10.1038/nature13306

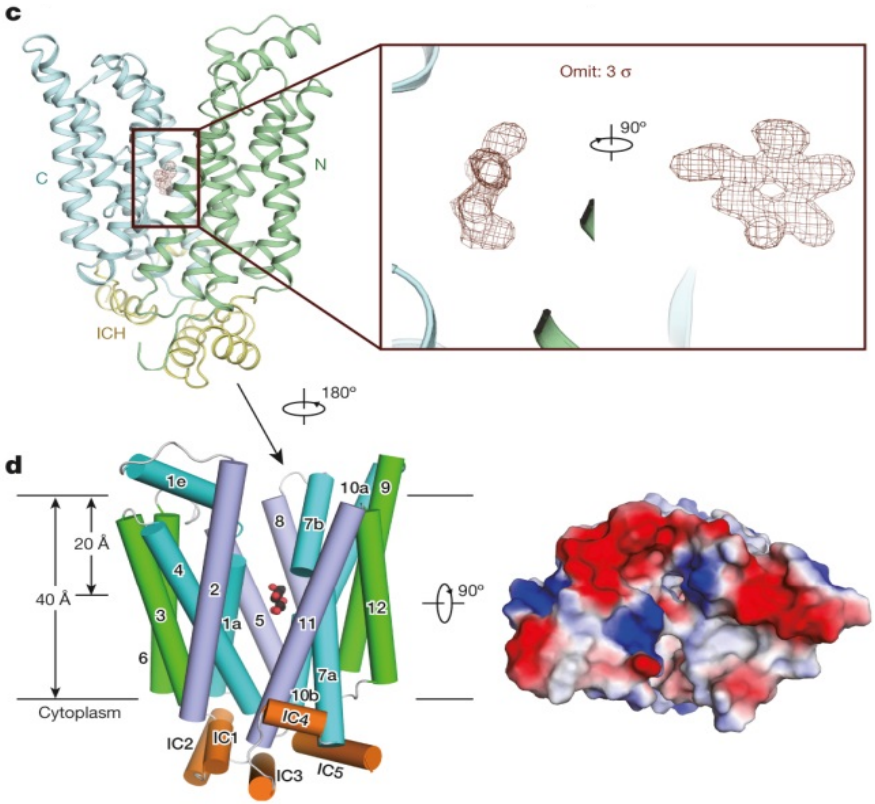
The ICH domain serves as a latch that tightens the intracellular gate.



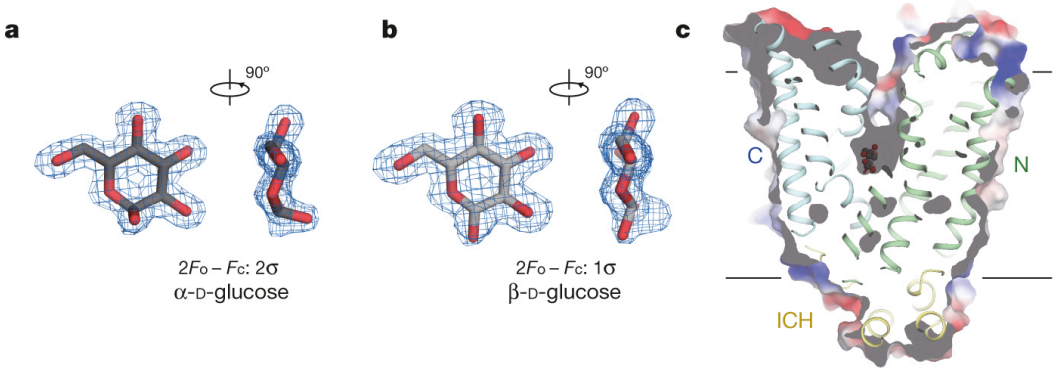
a, Structural comparison of the inward-open GLUT1 to its *E. coli* homologue XylE.
b, Structural overlay of GLUT1 and XylE using their respective N domains. For visual clarity, GLUT1 is displayed in three colours and XylE is coloured grey.
c, Analysis of the disease-related GLUT1 residues that mediate the inter-domain interactions on the intracellular side. Left: positioning and interactions of the disease-related residues on the intracellular side of the inward-open GLUT1. Right: the interaction network of the corresponding residues in the outward-facing XylE.

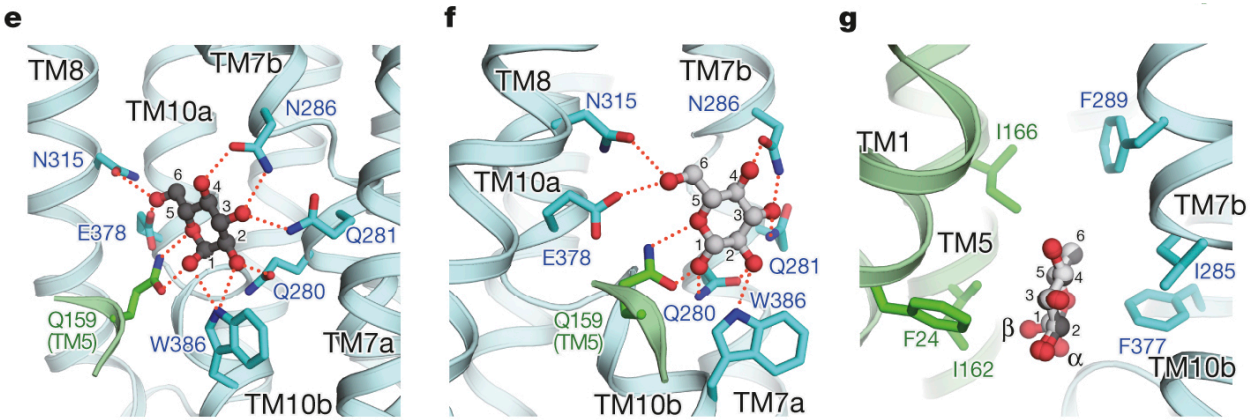
D Deng *et al. Nature*, 1-5 (2014) doi:10.1038/nature13306

Structure of human GLUT3(N43T) bound to D-glucose.



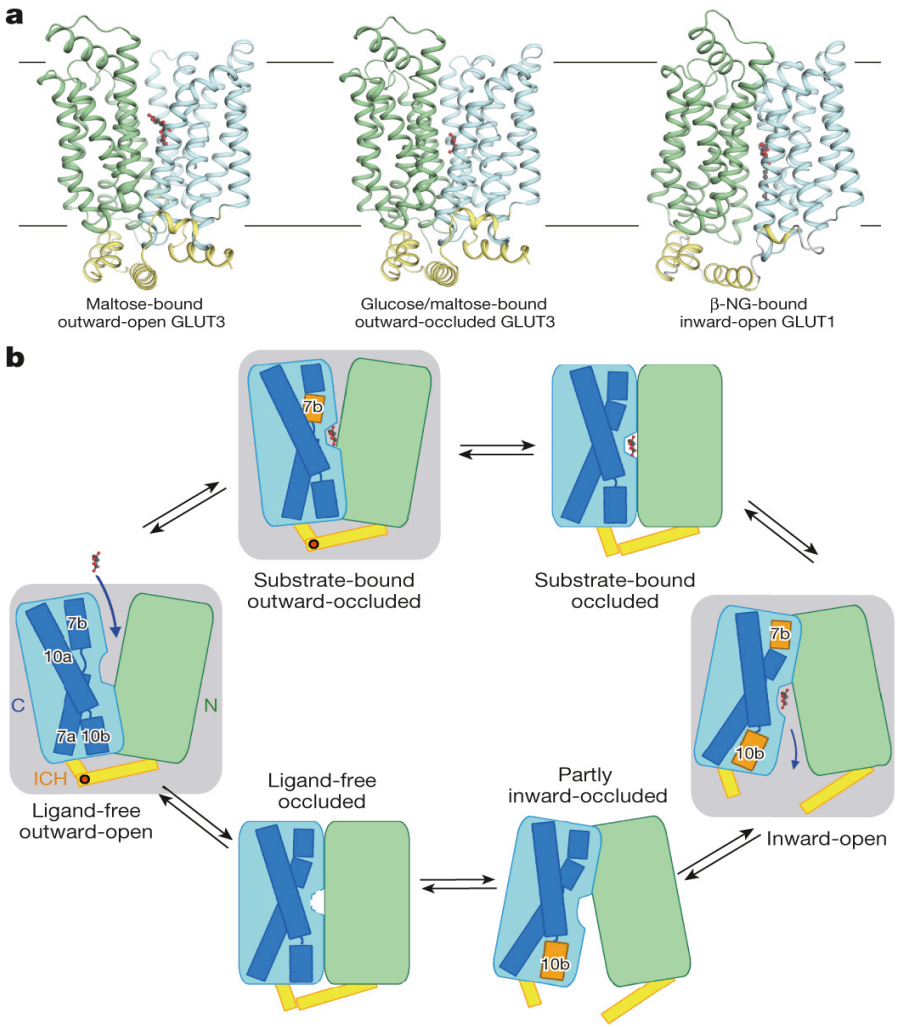
- The 'omit' electron density of the bound ligand is shown as brown mesh.
- The structure of glucose-bound GLUT3 exhibits an outward-occluded conformation. The ligand is shown as black spheres.
- The bound glucose is predominantly coordinated by the C-terminal domain.





D Deng *et al. Nature*, 1-6 (2015) doi:10.1038/nature14655

Updated working model of GLUTs.



a, An overview of the resolved structures of human GLUT1 and GLUT3.
b, Schematic illustration of the alternating access cycle of GLUTs. The rearrangements of the substrate-binding site and the local structural shifts of TM7b and TM10b during the transport cycle are highlighted. The shaded states refer to the structures shown in panel a. The N-terminal, C-terminal and ICH domains are coloured green, cyan and yellow, respectively. The structural elements that undergo prominent local shifts during state transition are highlighted in orange.

https://images.nature.com/full/nature-assets/nature/journal/v526/n7573/extref/nature14655-sv3.mov

D Deng *et al. Nature* **000**, 1-6 (2015) doi:10.1038/nature14655

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**

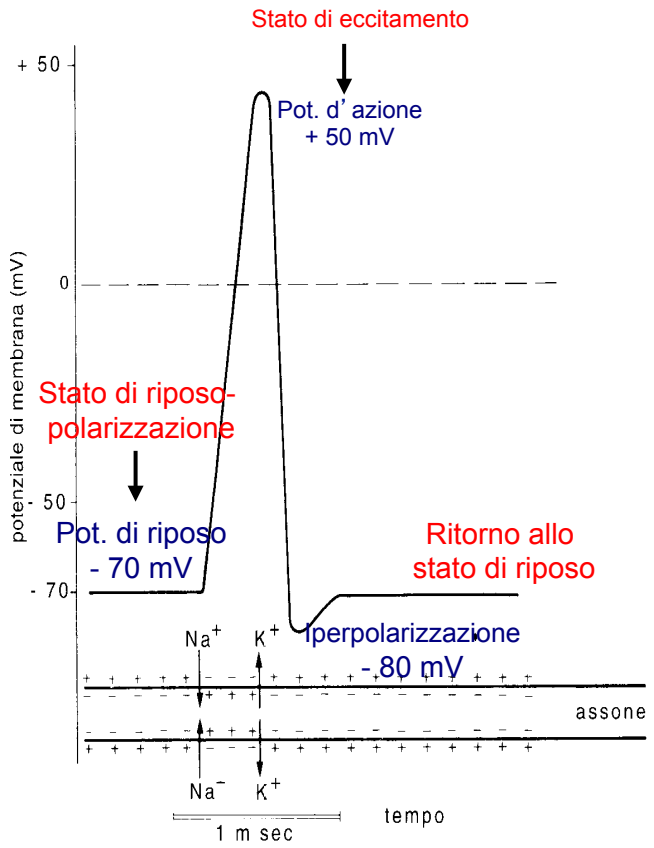
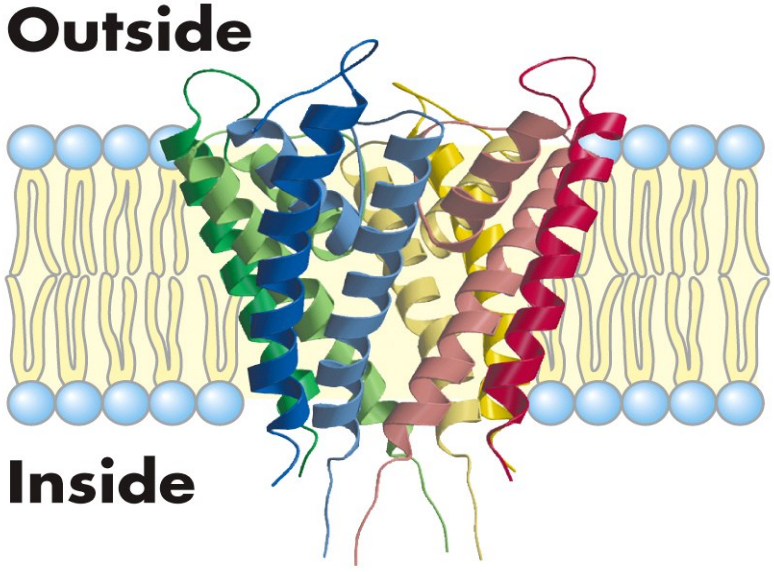


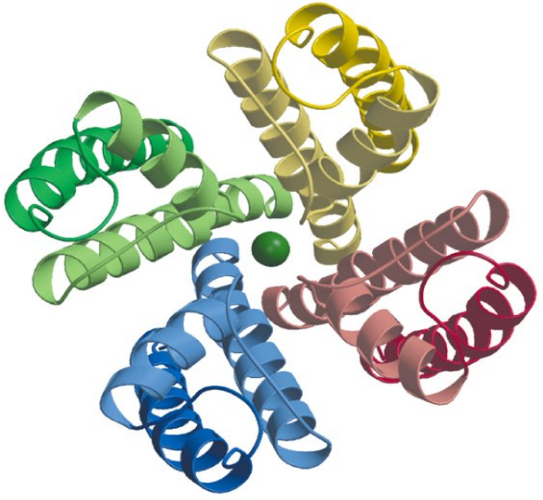
Fig 21.2 *Propagazione dell'impulso nervoso lungo l'assone come onda di depolarizzazione.* In corrispondenza dell'impulso depolarizzante i Na^+ che entrano attraverso la membrana assonale sono in numero maggiore rispetto ai K^+ che escono. Da qui la inversione temporanea della polarizzazione.

Ion channels

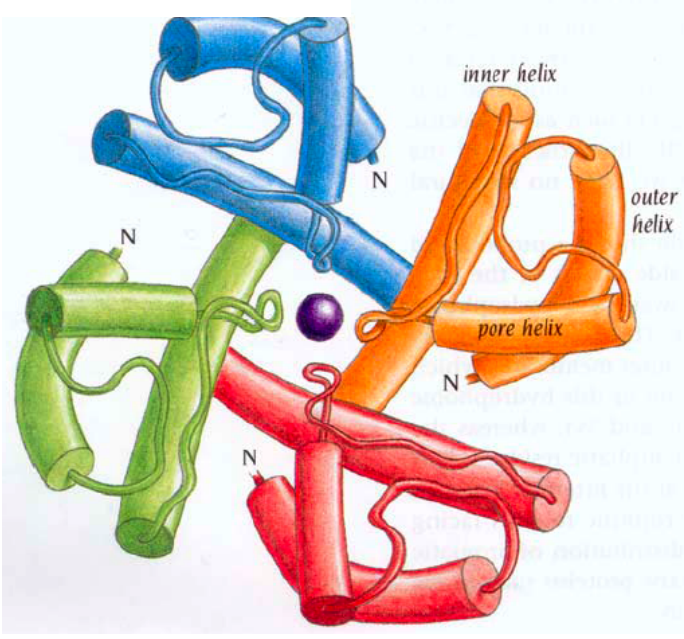
- An ion channel has 2 diverse conformational states:
  - Open: this is when the ions can cross the channel according to the electrochemical gradient that exists on the two sides of the membrane
  - Closed: this is when the channel is impermeable to the ions
- The ion channels must be
  - Highly selective for a particular ion
  - Keep high rate of transport
  - Regulate the flux of ion: this is called *gating*

The K^+ channel





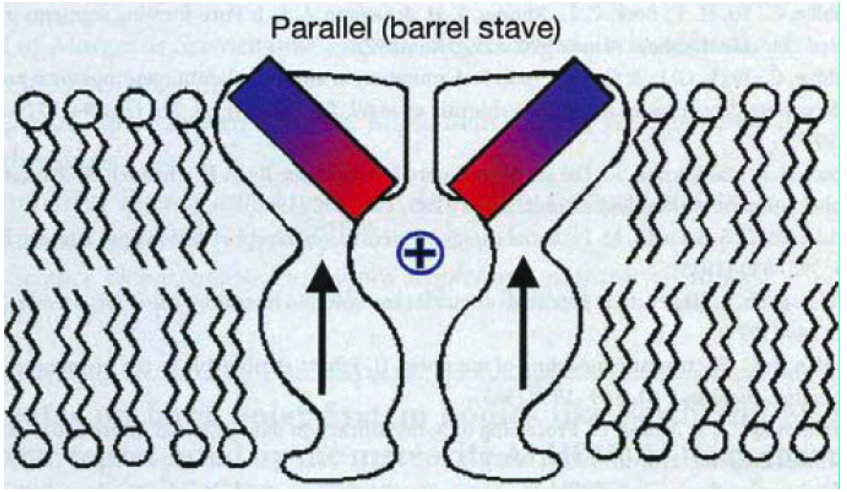
- The structure of the open and closed K^+ channel has been solved at 1.9 and 3.6 Å respectively
- The K^+ channel is a tetramer made of 4 subunits that come together to form the channel.
- Each subunit is made of 3 main trans-membrane helices called external helix, internal helix and pore helix. The 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

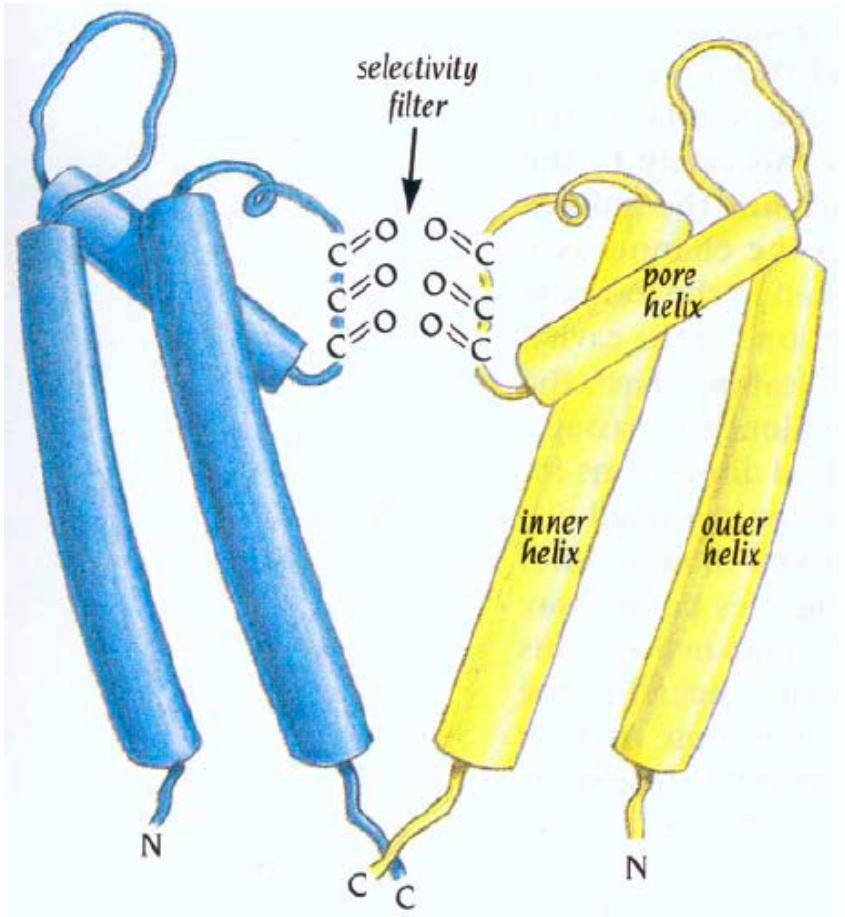


Open and Closed K^+ channels

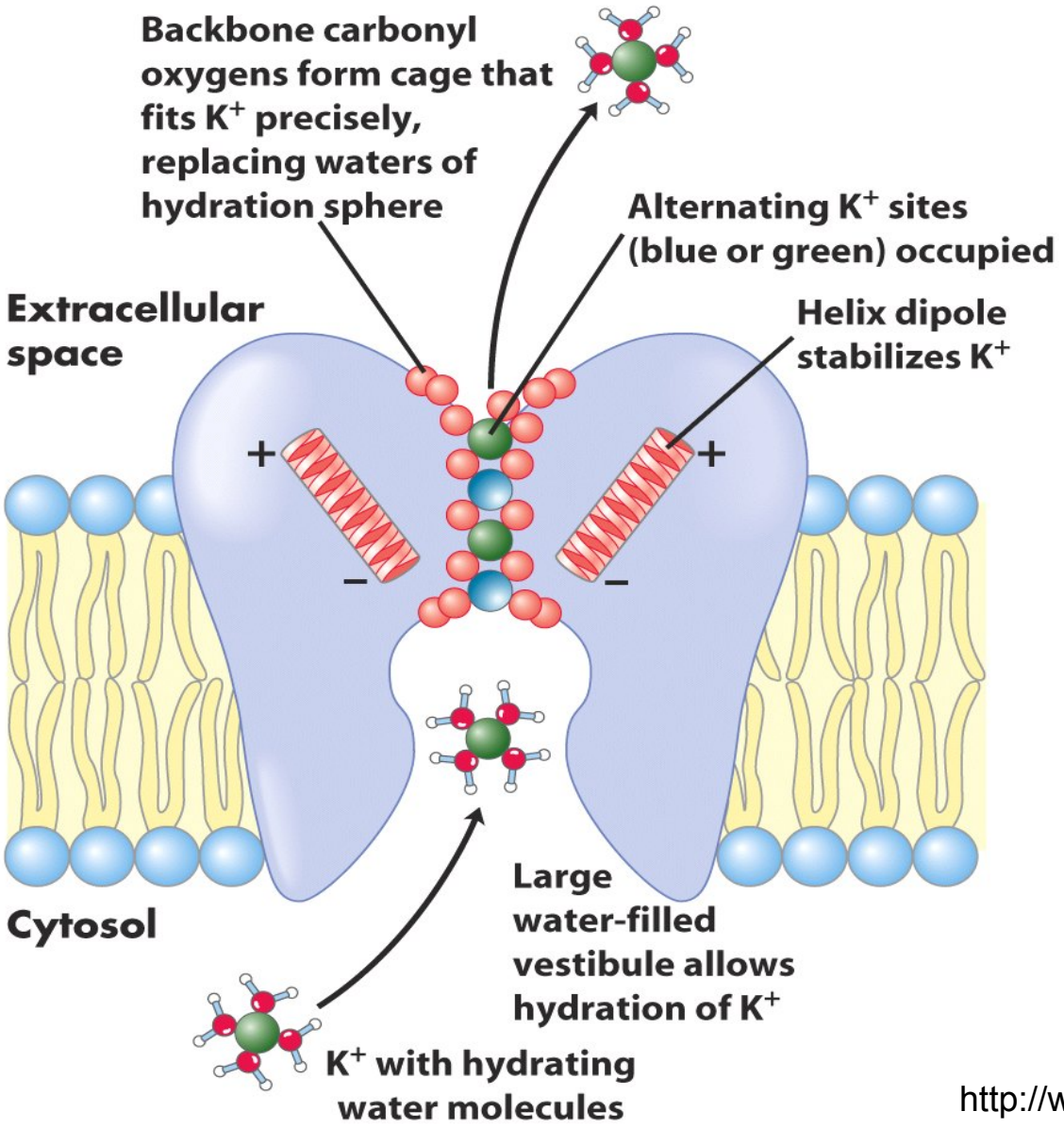
- **Closed K^+ channel:**
  - Here the flux of ions is blocked by the side chains of hydrophobic aa
  - The pore helices are not aligned – the stabilisation of the K^+ is achieved with the helix dipoles that are pointing their C-ter towards the centre of the cavity
  - The volume of the central cavity 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^+

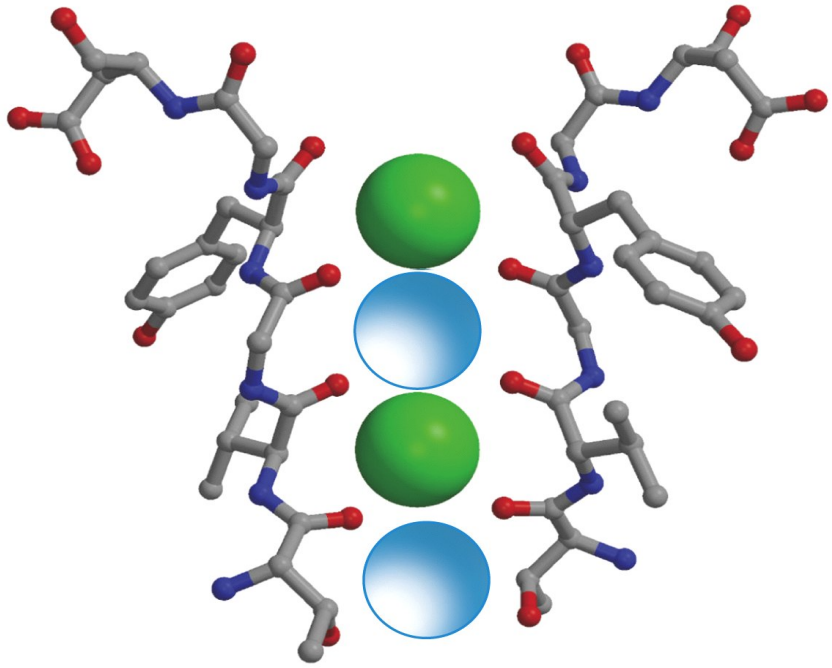
http://www.youtube.com/watch?v=Z1M8s9aLe4Q





The K^+ channel

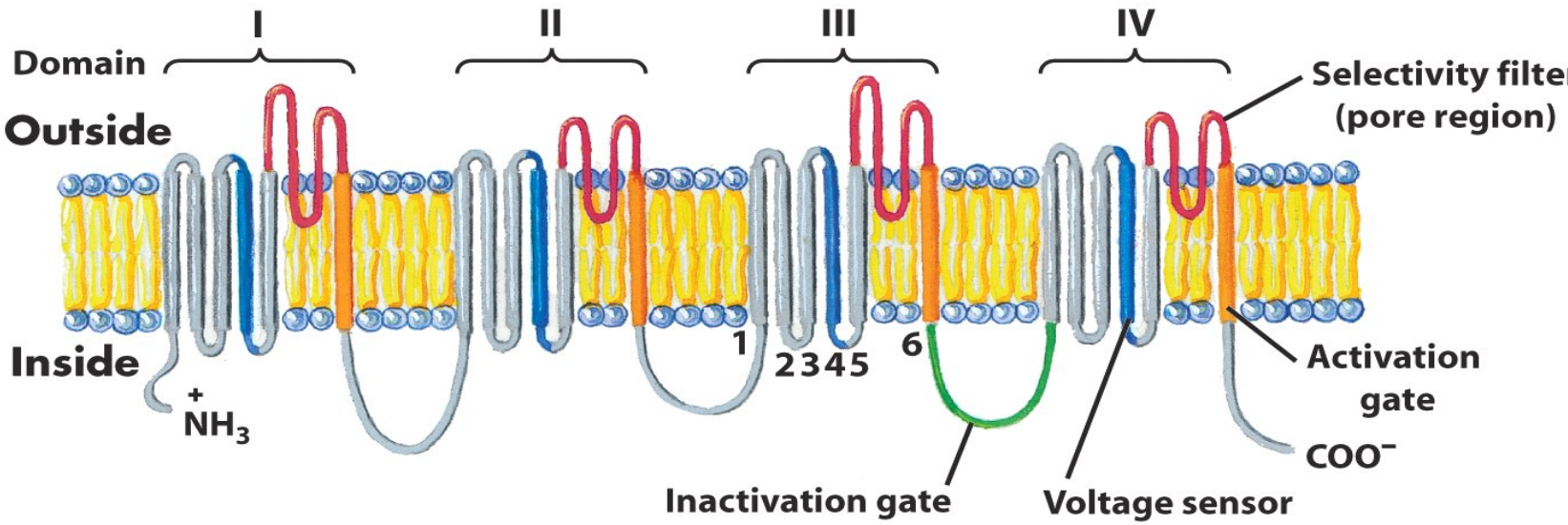




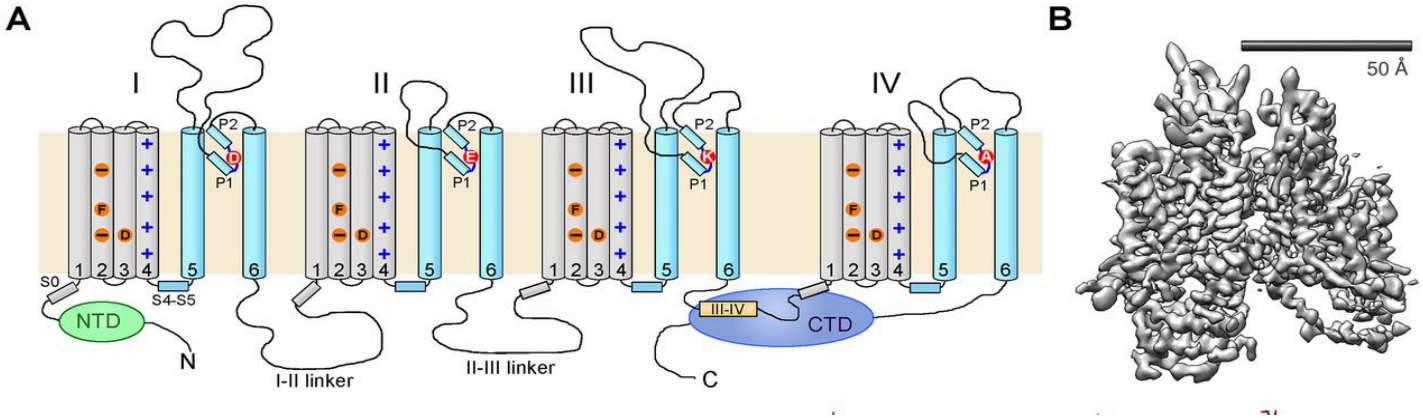
http://www.youtube.com/watch?v=Z1M8s9aLe4Q

Voltage gated ion channels

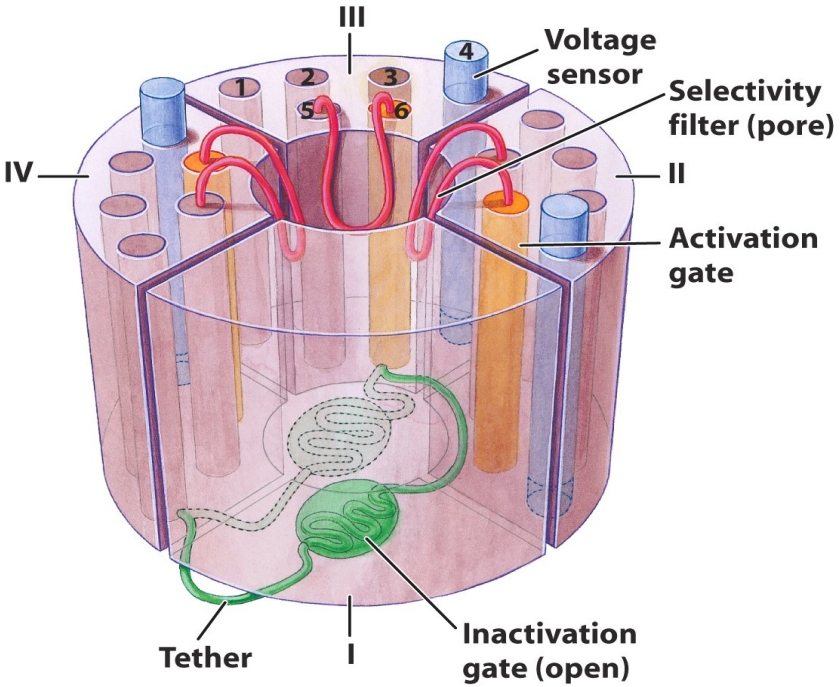
- The neuronal Na^+ channel is a voltage-gated ion channel
- Voltage-gated sodium (Nav) channels open and close ion-selective pores in response to changes in membrane potential, and this gating underlies the generation of action potentials.
- Nav channels are large membrane proteins that contain four peripheral voltage-sensor domains (VSD1–4) that influence the functional state of the central ion-conducting pore.
- There are 9 isoforms of Nav α subunits in human, designated Nav1.1-Nav1.9.
- Mutations within the nine human Nav channel isoforms are associated with migraine (Nav1.1), epilepsy (Nav1.1–Nav1.3, Nav1.6), pain (Nav1.7–Nav1.9), cardiac (Nav1.5), and muscle paralysis (Nav1.4) syndromes.
- Accordingly, Nav channel blockers are used for the treatment of many neurological and cardiovascular disorders.



Voltage gated ion channels

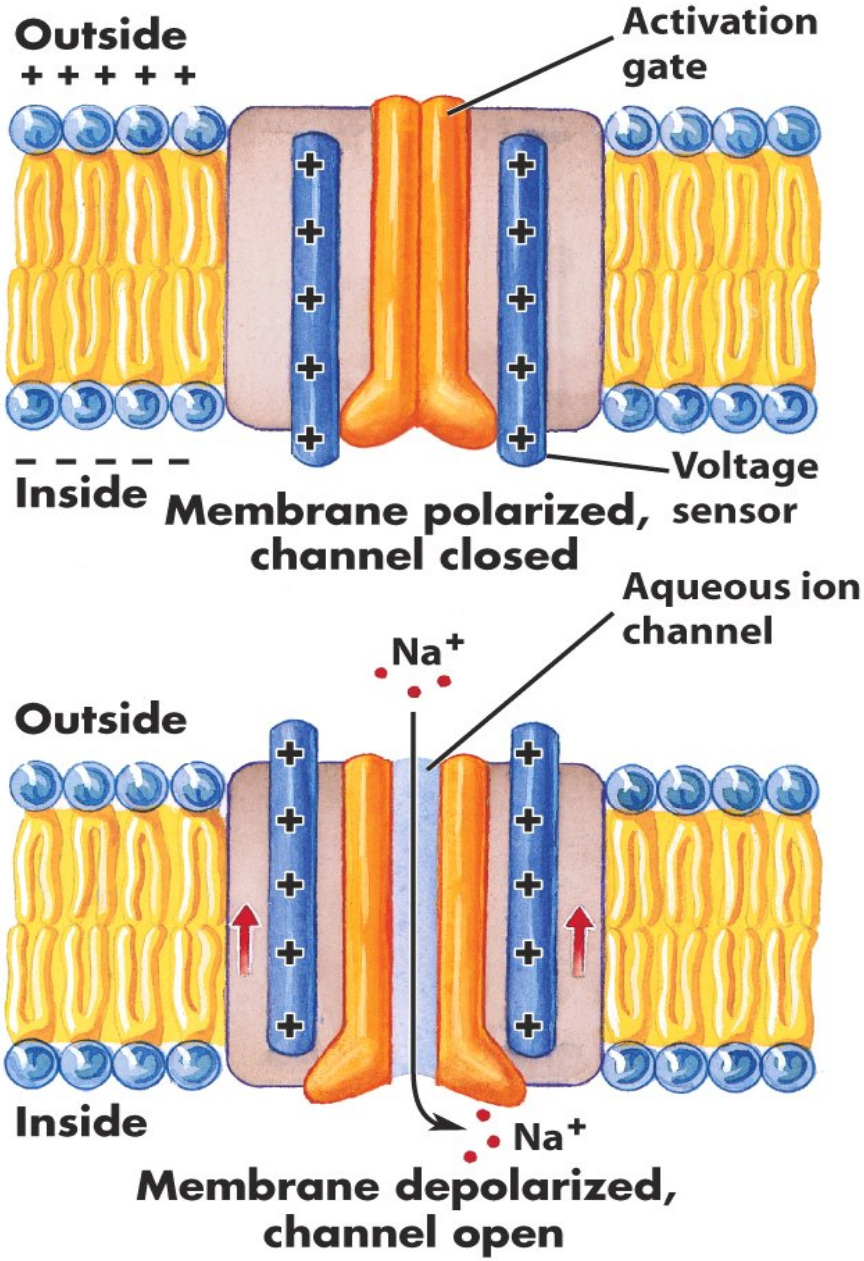


- The eukaryotic Nav channels consist of a pore-forming α subunit and auxiliary β subunits.
- The α subunit is sufficient for voltage-dependent ion conductance, while the β subunits facilitate membrane localization and modulate channel properties.
- The α subunit, similar to the closely related Cav channels, is comprised of a single polypeptide chain that folds to four homologous repeats (domains I-IV) each containing six transmembrane segments S1-S6.
- The four sets of S5 and S6 segments and their intervening sequences enclose the ion permeation pore domain, while the S1-S4 segments in each repeat form a voltage sensing domain (VSD).
- The ion selectivity of a voltage gated ion channel is determined by the selectivity filter (SF), a molecular sieve enclosed by the partial membrane penetration loops between S5 and S6 segments



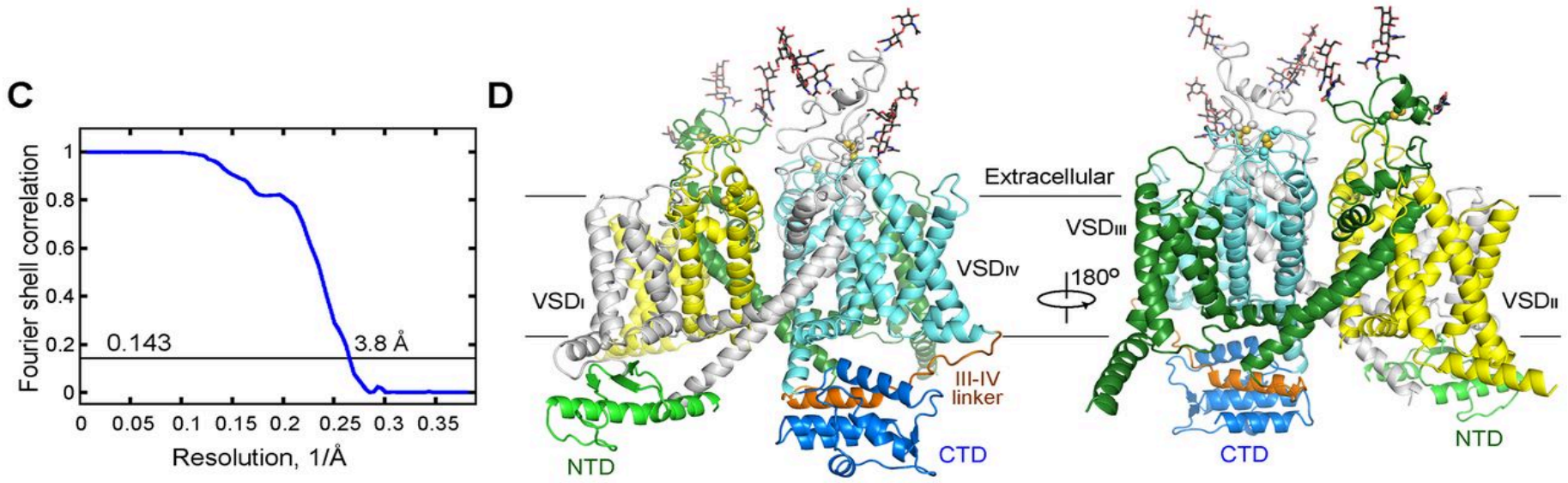
Voltage gated ion channels

- Essential for voltage gating, VSDs contain the “gating charges”, which are a set of highly conserved positively charged residues occurring at every third place along S4 segment.
- Upon depolarization, according to measurements in Kv channels, approximately 12 gating charges per channel are transferred across the membrane from the intracellular side to the extracellular side. During this process, the gating charges interact sequentially with conserved acidic or polar residues on S2 and S3 segments.



Voltage gated ion channels

The overall structure of Na_vPaS. The structure is domain colored. The glycosyl moieties and disulfide bonds are shown as sticks and spheres, respectively.



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