Membrane Proteins

BC530

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Membrane proteins carry out a wide variety of functions.

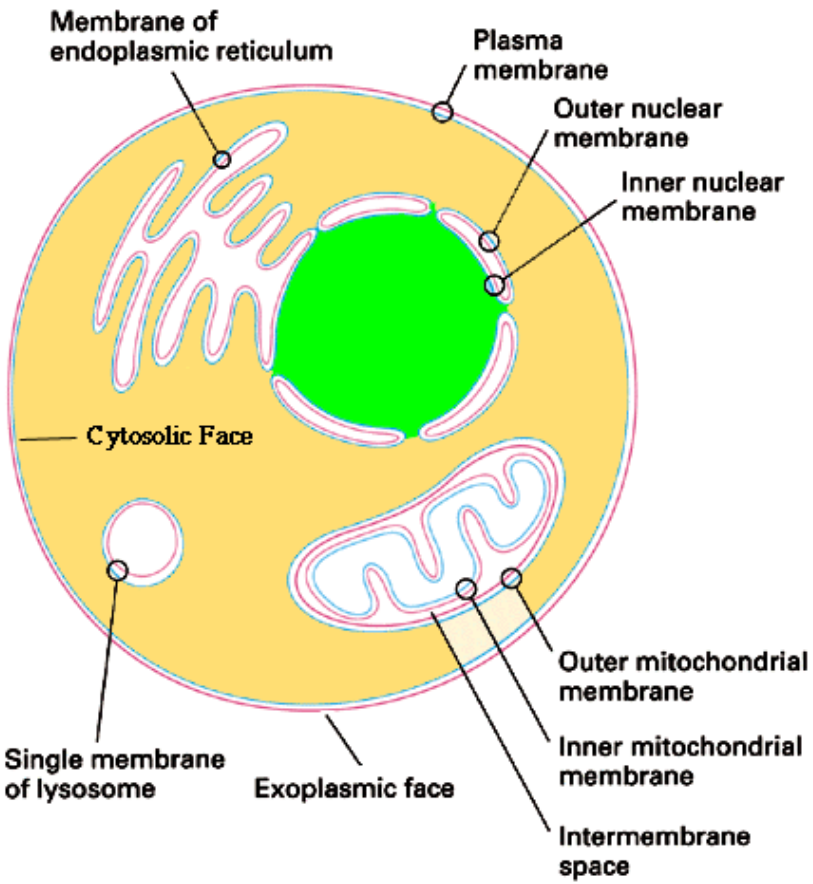
Such as:

- **Controlling the traffic** of metabolites across the membrane
- **Capturing light** and converting this precious energy into an electric and chemical potential across the membrane
- **Converting this electrochemical potential** into chemical energy stored in molecules such as ATP
- **Sending signals** from events occurring outside the cell across the membrane into the cell so that proper action can be taken such as e.g. start or stop of cell division
- **Function as a "vacuum cleaner"** removing unwanted "polluting" hydrophobic molecules from the bilayer
- **Pumping undesired molecules, such as drugs, out of the cell**
- **Allowing DNA to enter cells**
- **Transporting entire proteins across cell membranes:**

  The "substrate" proteins are often unfolded, but sometimes remain largely folded as is the case of the fascinating gigantic "nuclear pore complex".

Membranes in the eukaryotic cell

In Plants there are obviously also "chloroplasts"



In most cells there are also "peroxisomes", with relatives like glycosomes, hydrogenosomes and others, in cells from various species.

In malaria parasite there are very special organelles like "apicoplasts", "rhoptries", "micronemes" and "dense bodies". The latter three of these are loaded with special proteins involved in host cell invasion

Many proteins are membrane proteins

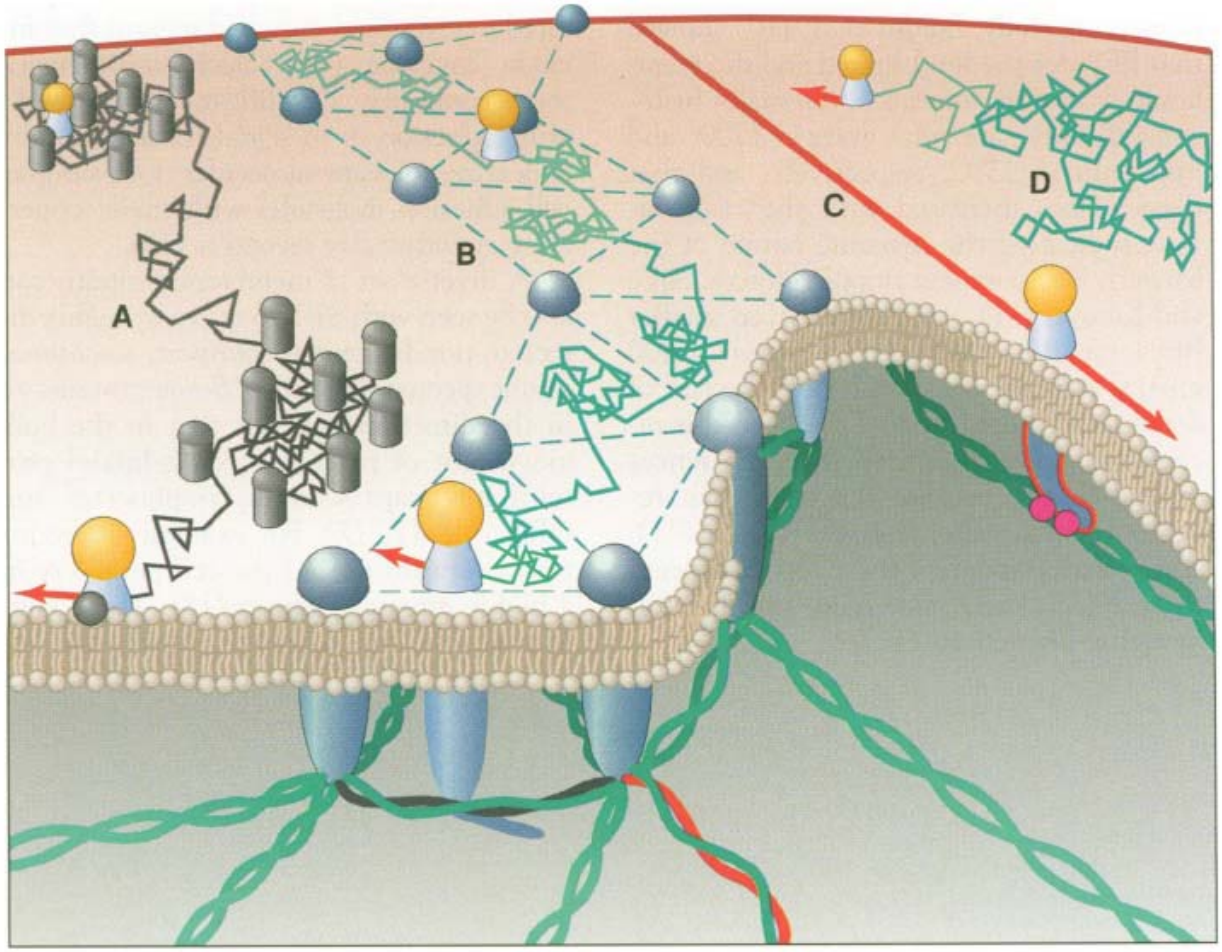
- Bacterial genomes ~20%
  - *E. Coli* ~21%
  - *H. Influenzae* ~19%
  - *M. pneumonia* ~17%
  - Thermophiles ~23%,
  - Mesophiles ~24% (20 genomes studied)
- Yeast
  - *S. cerevisae*: ~33%
- *H. sapiens* ~18.5%

http://dodo.cpmc.columbia.edu/cubic/genomes/human/human_htm.html

http://www.biophys.mpg.de/michel/public/memprotstruct.html

There is plenty organization in biomembranes

Guiding, constricting and preventing protein motion



(A) Transient confinement by obstacle clusters
(B) or confinement by the cytoskeleton. (C) directed motion, and (D) free random diffusion

From : “Revisiting the Fluid Mosaic Model of Membranes”, Jacobson et al., Science 268, 1441 (1995)

Photosynthetic Reaction Centre

The first membrane protein with known structure.

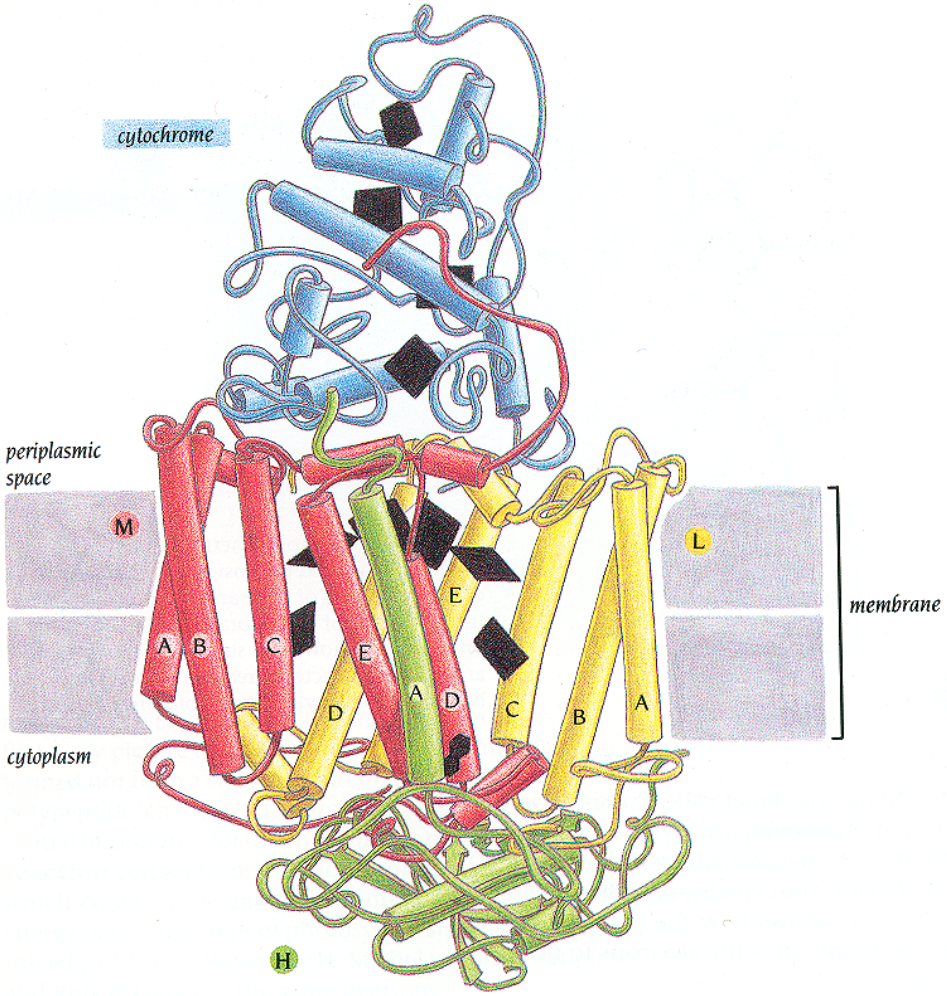
Loaded with complex co-factors.

Showing numerous “trans-membrane helices”.

A bacterial photosynthetic reaction centre

The first atomic structure of a membrane protein.

Captures light and generates a transmembrane proton potential – the mechanism is fascinating



A multiprotein complex consisting of: H, L, M chains and an associated cytochrome.

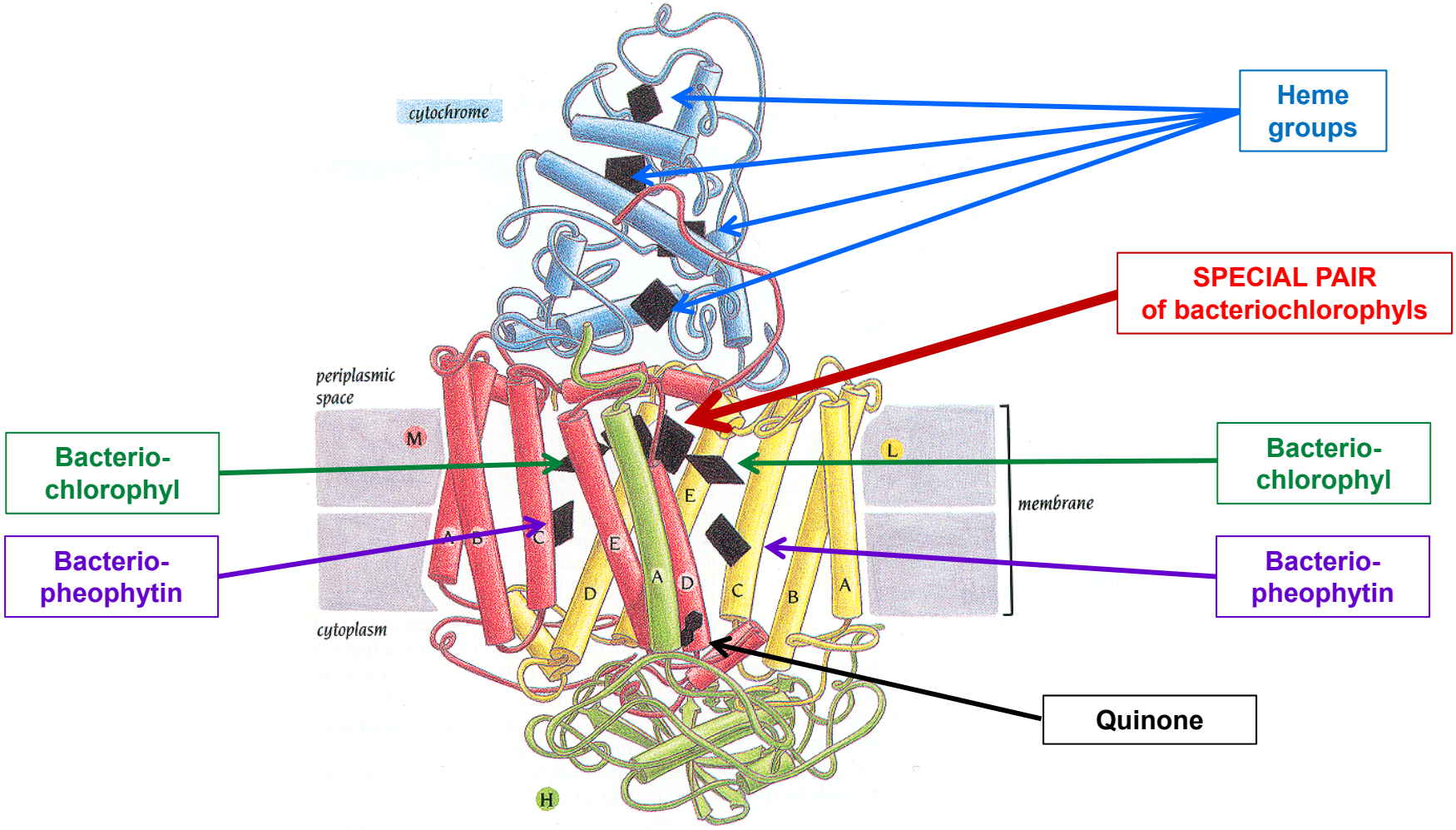
Letters A, B, etc refer to equivalent helices in the L and M chains, and to the anchor helix in the H chain.

Figure from: Brandén and Tooze. Structure solved by Hartmut Michel, Johan Deisenhofer, Robert Huber

A bacterial photosynthetic reaction centre

The first atomic structure of a membrane protein.

Captures light and generates a transmembrane proton potential – the mechanism is fascinating



A multiprotein complex consisting of: H, L, M chains and an associated cytochrome.

Plus bacteriochloryphylls, bacteropheophytins, heme groups, and one or more quinones.

Figure from: Brandén and Tooze. Structure solved by Hartmut Michel, Johan Deisenhofer, Robert Huber

Rhodopsin

The first structure of a member of the G-Protein Coupled Receptors (GPCR's)

Captures light and initiates a signal transduction process

Bovine Rhodopsin

- **Member of the G-Protein Coupled Receptor (GPCR) family**
- **Consists of the protein Opsin (40 kDa) covalently linked to 11-*cis*-retinal through Lys-296**
- **Absorption of a photon by the 11-*cis*-retinal causes an extremely complex series of events:**

  - isomerization leads to a conformational change of the protein

  - the all-*trans* retinal is hydrolyzed

  - the retinal dissociates form the opsin

  - the conformational change of the protein triggers signal events in the cell including a change in ion channel activation

  - rhodopsin is regenerated from newly synthesized 11-*cis* retinal and opsin

- **The structure of rhodopsin reveals seven trans-membrane helices**
- **The chromophore is situated at 2/3 from the cytoplasmic side of the membrane**

  - The chromophore interacts with numerous residues, mainly from helices II, V, VI and VII

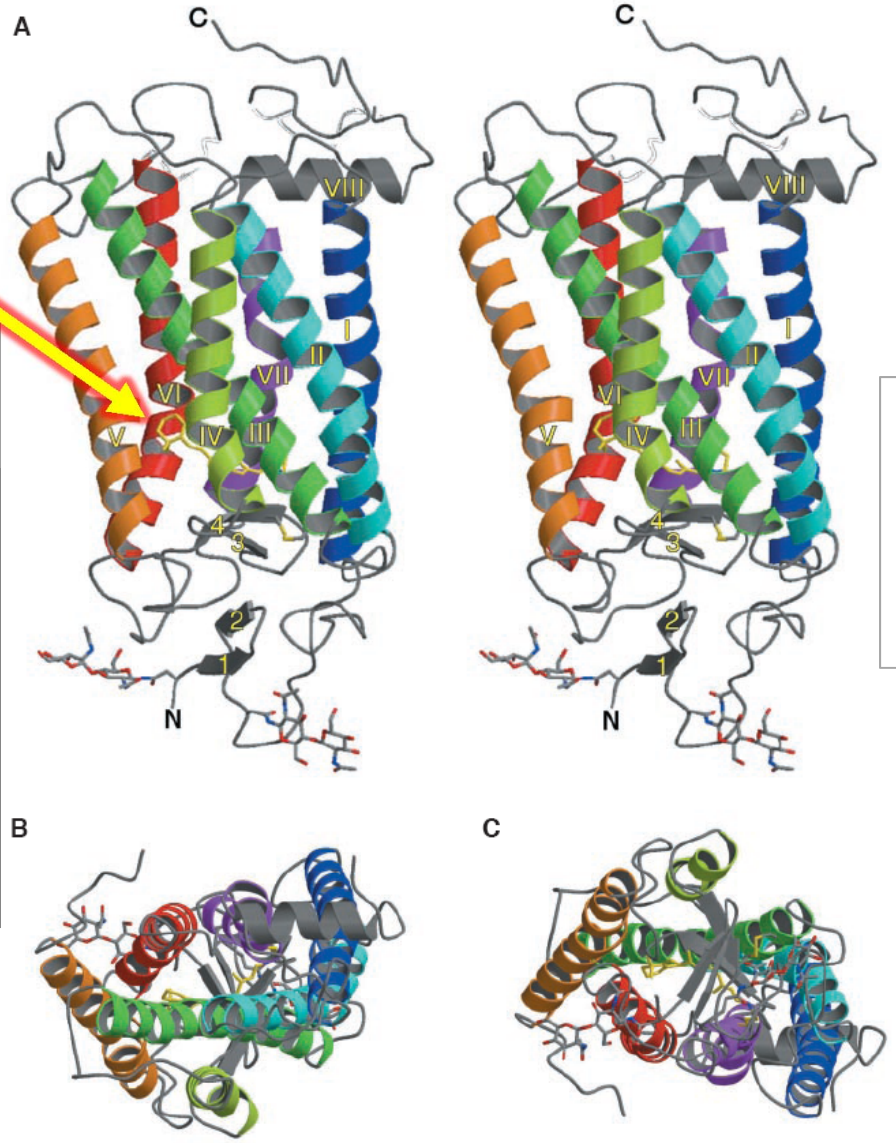
Rhodopsin

Seven transmembrane helices surrounding the essential retinal

RETINAL

Upon light capture:

1. **the 11-cis-retinal isomerizes into all-trans-retinal**
2. **which alters the conformation of rhodopsin**
3. **and the retinal even dissociates from rhodopsin.**



a) **Stereo view parallel to the membrane.**

b) **View from cytoplasm**

c) **Intradiscal view.**

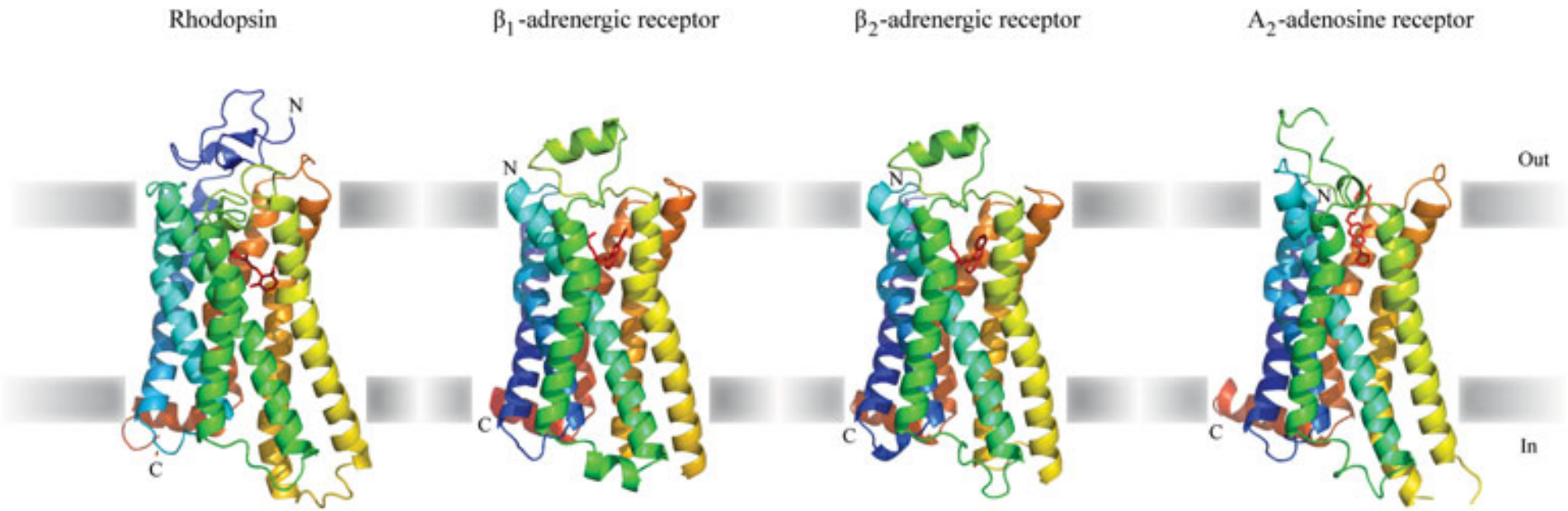
Palczewski, K.,et al & Stenkamp, R. E., Yamamoto, M. & Miyano, M. (2000). *Science* **289**, 739-745. "Crystal structure of rhodopsin: A G protein-coupled receptor. "

G-protein-coupled receptor structures

Seven transmembrane helices and critically important loops

Recognizes a ligand and initiate a signal transduction process

Essential for motion and emotion



A gallery of G-protein-coupled receptors with known structure.

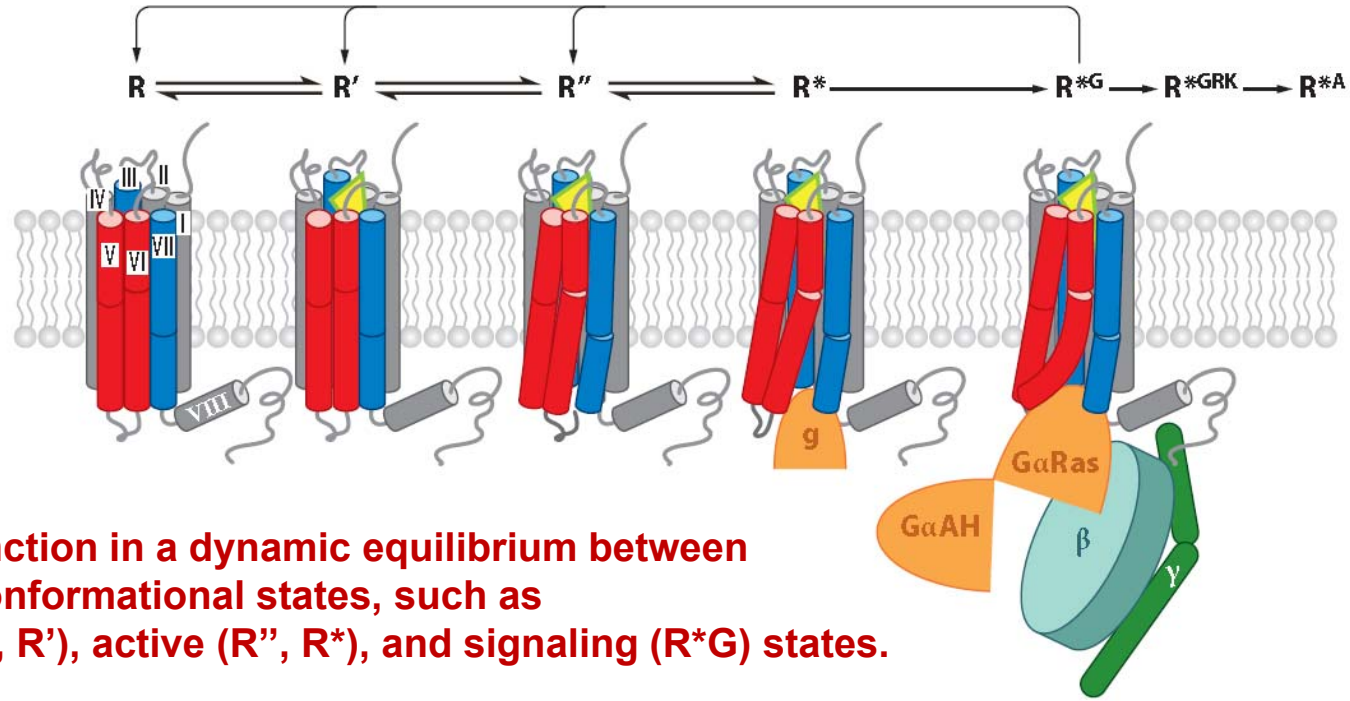
(a) bovine rhodopsin, (b) β_1-adrenergic receptor, (c) β_2-adrenergic receptor, (d) adenosine A_{2a} receptor.

The three structures on the right represent inactive, antagonist-bound, conformations of different G-protein-coupled receptors. Upon binding small molecules on the outside of the cell, structural changes occur in the GPCRs which is triggers numerous complex signaling events inside the cell.

The structures are related as they all have a homologous seven-helix TM bundle, but differ in the details of their binding sites and structures at both cytoplasmic and extracellular surfaces.

Vinothkumar and Henderson, Structures of membrane proteins, Quart. Rev. Biophysics 43: 65–158 (2010)

Key intermediates in the GPCR activation mechanism



GPCRs function in a dynamic equilibrium between multiple conformational states, such as inactive (R, R'), active (R'', R*), and signaling (R*G) states.

In case you like more details:

- R represents inactive (ground) states, which can be stabilized by binding of inverse agonists or antagonists.
- R' represents inactive low-affinity agonist-bound states, which differ from R states by only small local changes in the receptor binding pocket.
- R'' represents activated state(s), characterized by substantial global rearrangement of helices and side-chain microswitches on the intracellular side that exposes, at least partially, the G protein binding crevice.
- R* represents activated substates with initial insertion of the G protein C-terminal α-helix (or its surrogate mimic g) into the intracellular crevice.
- Finally, R*G is a distinct G protein signaling conformation of a receptor, which can be achieved upon full engagement and activation of the GPCR-Gαβγcomplex.

Katritch, Cherezov & Stevens, Structure-function of the G protein-coupled receptor superfamily. Annu. Rev. Pharmacol. Toxicol. 53, 531–556 (2013)

BACTERIAL PORINS

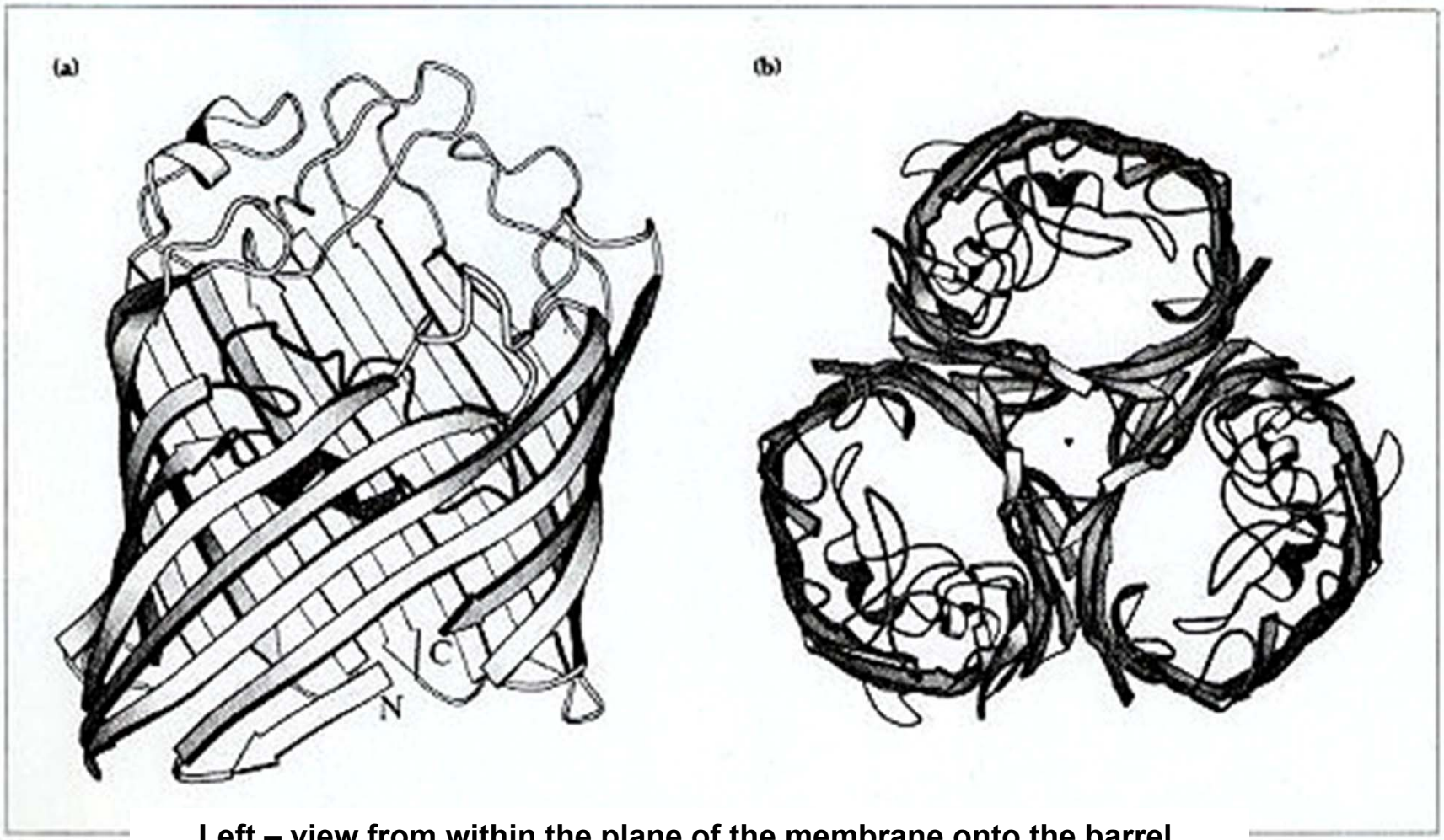
All-β structure membrane proteins.

Using "β-barrels" to insert themselves into the bilayer.

A porin allows transport of molecules through a biomembrane

(Often the outer membrane of Gram-negative bacteria)

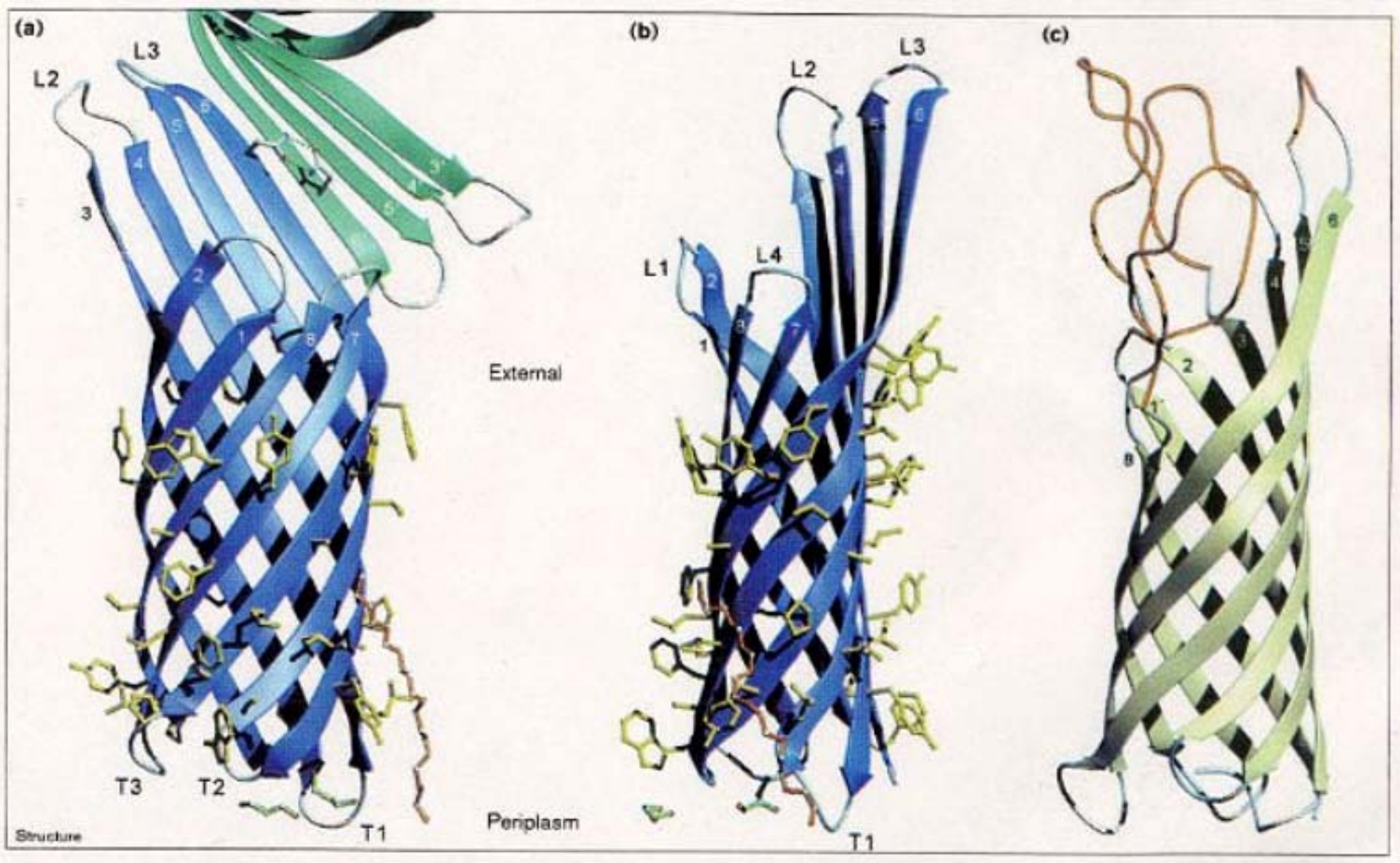
Ribbon Diagram of porin "OmpE" from *E. coli*



Left – view from within the plane of the membrane onto the barrel.

Right – view along the three-fold axis of the trimer looking approximately along the pore inside each porin subunit.

Porins “OmpX” and “OmpA” from *E. coli*



Ribbon plots of **(a,b) OmpX** (blue) and **(c) OmpA** (yellow).
(a,b) Two perpendicular views of OmpX with the residues of the aromatic girdle and the nonpolar ribbon in yellow. Two glycerol (green) and one C8E4 detergent molecule (orange) could be located.

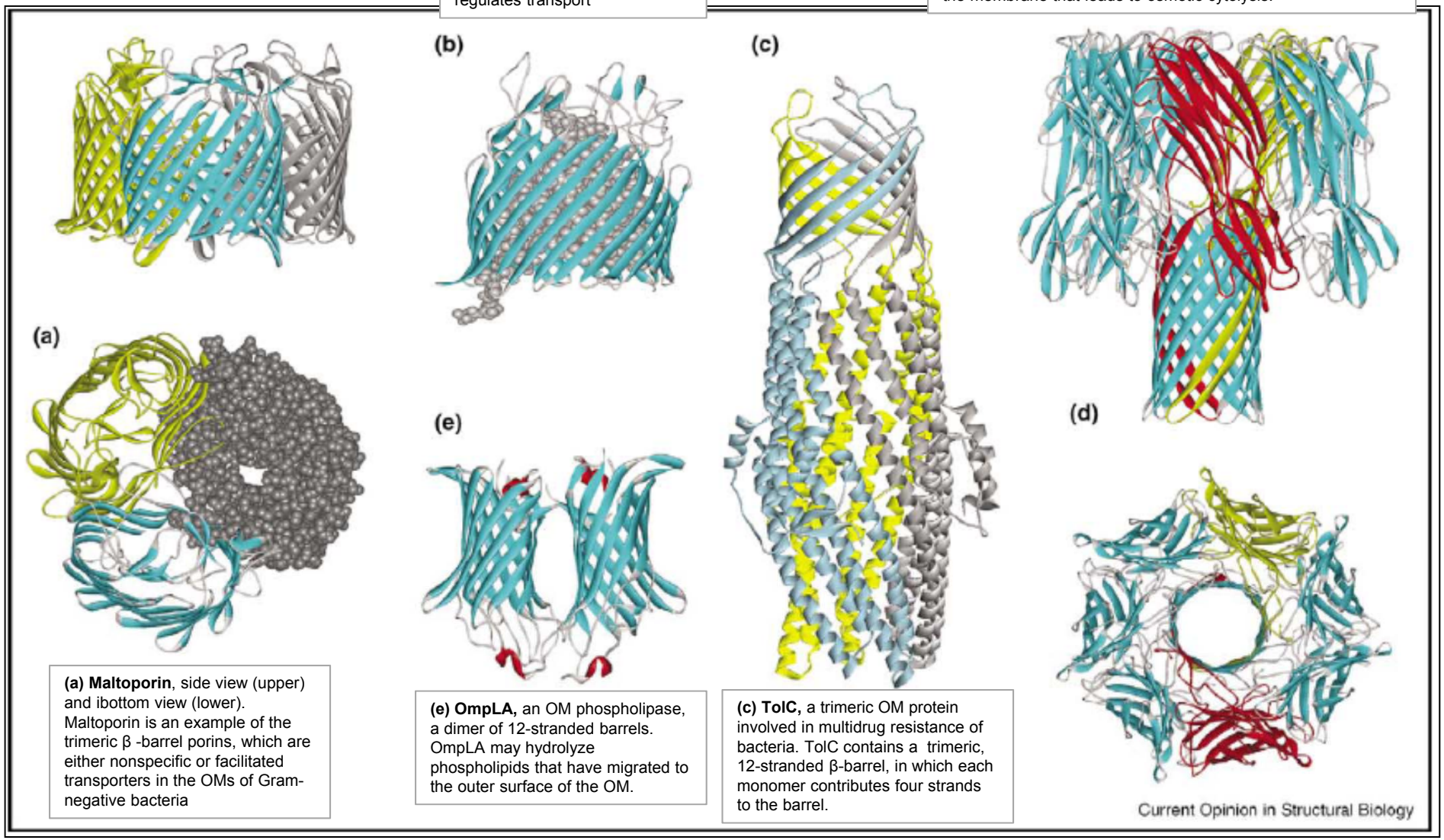
(c) The **OmpA** membrane domain.

Vogt & Schulz, Structure 7:1301-1309 (2000).

A gallery of β-barrel membrane protein structures.

(b) FepA, a TonB-dependent Fe-siderophore transporter with 22 membrane-spanning β-strands. A 'hatch'domain, shown in gray, plugs the barrel and regulates transport

(d) The protein toxin α-hemolysin from *S. aureus*. This toxin is secreted as a monomeric protein that subsequently assembles into a heptameric 'prepore' on the membrane, followed by the cooperative insertion of the β-barrel across the membrane. Each monomer contributes one β-hairpin to the 14-stranded barrel. The central pore is an ungated path through the membrane that leads to osmotic cytolysis.



(a) Maltoporin, side view (upper) and ibottom view (lower). Maltoporin is an example of the trimeric β -barrel porins, which are either nonspecific or facilitated transporters in the OMs of Gram-negative bacteria

(e) OmpLA, an OM phospholipase, a dimer of 12-stranded barrels. OmpLA may hydrolyze phospholipids that have migrated to the outer surface of the OM.

(c) TolC, a trimeric OM protein involved in multidrug resistance of bacteria. TolC contains a trimeric, 12-stranded β-barrel, in which each monomer contributes four strands to the barrel.

Wimley, "The versatile beta-barrel membrane protein", COSB 13, 404 (2003)

β-Barrel construction principles

Quite a number of transmembrane β-barrels from outer bacterial membranes follow 10 construction rules:

1. **The number of β strands is even, and the N and C termini are at the periplasmic barrel end.**
2. **The β-strand tilt is always around 45° and corresponds to the common β-sheet twist. Only one of the two possible tilt directions is assumed, the other one is an energetically disfavored mirror image.**
3. **The shear number of an n-stranded barrel is positive and around n+2, in agreement with the observed tilt.**
4. **All β strands are antiparallel and connected locally to their next neighbors along the chain, resulting in a maximum neighborhood correlation.**
5. **The strand connections at the periplasmic barrel end are short turns of a couple of residues named T1, T2 and so on.**
6. **At the external barrel end, the strand connections are usually long loops named L1, L2 and so on.**
7. **The β-barrel surface contacting the nonpolar membrane interior consists of aliphatic sidechains forming a nonpolar ribbon with a width of about 22 Å.**
8. **The aliphatic ribbon is lined by two girdles of aromatic sidechains, which have intermediate polarity and contact the two nonpolar-polar interface layers of the membrane.**
9. **The sequence variability of all parts of the β barrel during evolution is high when compared with soluble proteins.**
10. **The external loops show exceptionally high sequence variability and they are usually mobile.**

From: Schulz, G.E. (2000). β-Barrel membrane proteins. *Curr. Opin. Struct. Biol.**10***, 443-447

Aquaporin

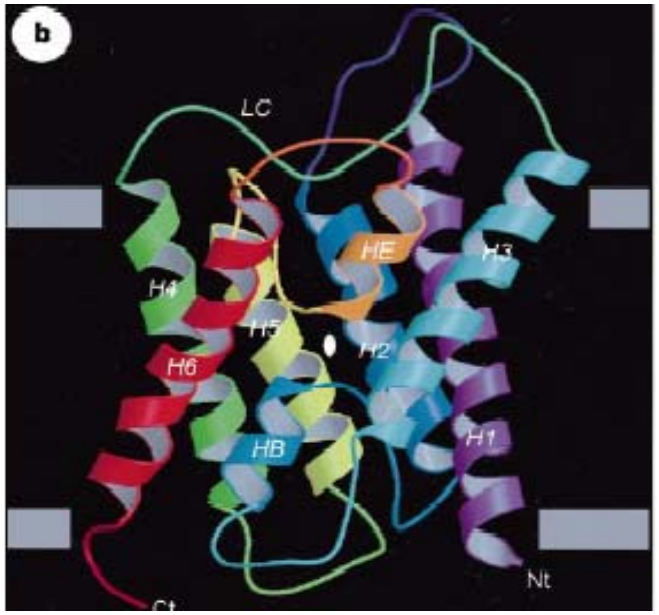
A water channel.

Aquaporins form pores in the membranes of cells and selectively conduct water molecules through the membrane,

while preventing the passage of ions (such as sodium and potassium) and other small molecules.

Structure of Aquaporin 1

Ribbon representations of the AQP1 fold depicting the six membrane-spanning helices, the two pore helices, and the connecting loops in different colours.

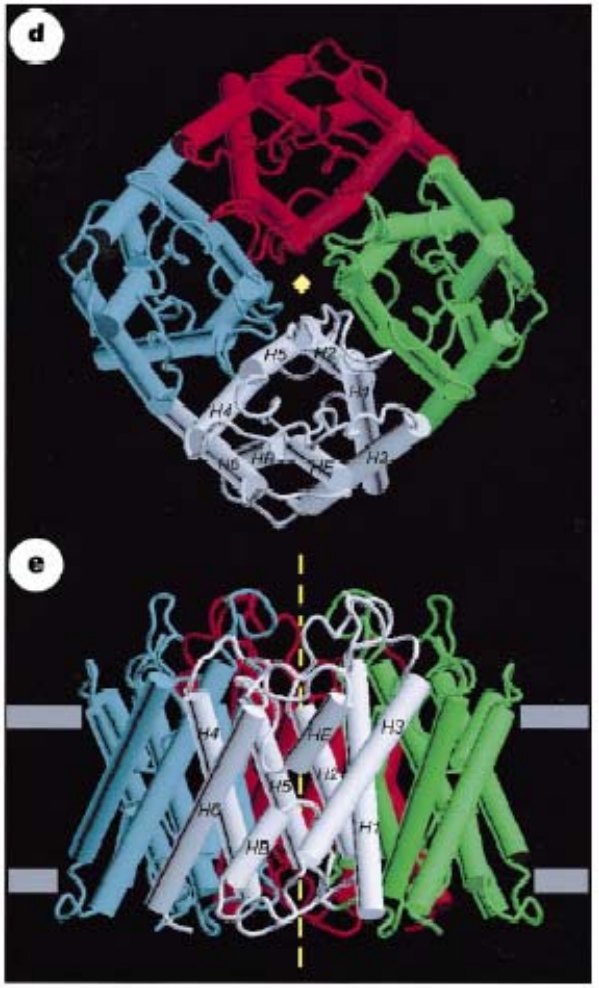


B. Side view of single subunit, viewed along pseudo 2-fold which runs between the two pore helices HB and HE.

D. Cylinder model of the AQP1 tetramer; end-on view from the extracellular surface.

NOTE: the yellow square in the center is NOT the pore! In this case EACH individual subunit has a, crooked, pore!

E. Side view. Yellow diamonds and yellow dotted line indicate the four-fold axis of the AQP1 tetramer. Dotted lines and spindle in white show the pseudo two-fold axis of the AQP1 monomer. Grey bands indicate the surface of the lipid bilayer.



Murata K et al. “Structural determinants of water permeation through aquaporin-1.” Nature 407:599-605 (2000). Figure 2

Key Aquaporin questions:

Q1. Why no conduction of positive ions?

A1. Unfavorable interaction with N-termini of two helix dipoles at point of restriction.

A2. Dehydration in channel.

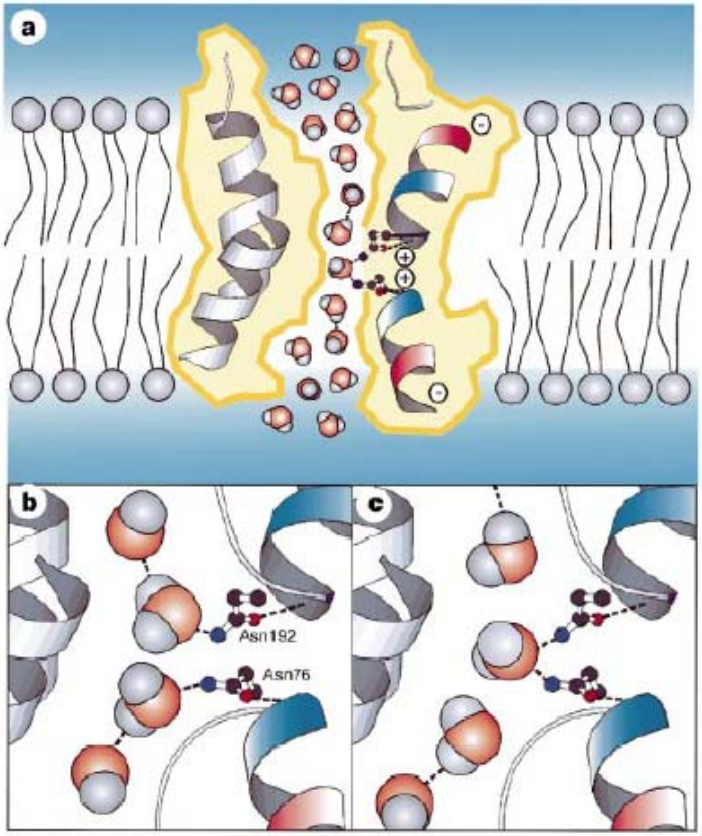
Q2. Why no conduction of negative ions?

A. Full or partial dehydration along channel very unfavorable.

Q3. Why no conduction of hydrogen ions?

A. No suitable chain of water molecules seen in experiments – nor in calculations – in channel: The "hydrogen bond isolation mechanism" aka the "proton exclusion mechanism".

Water permeation through aquaporin-1



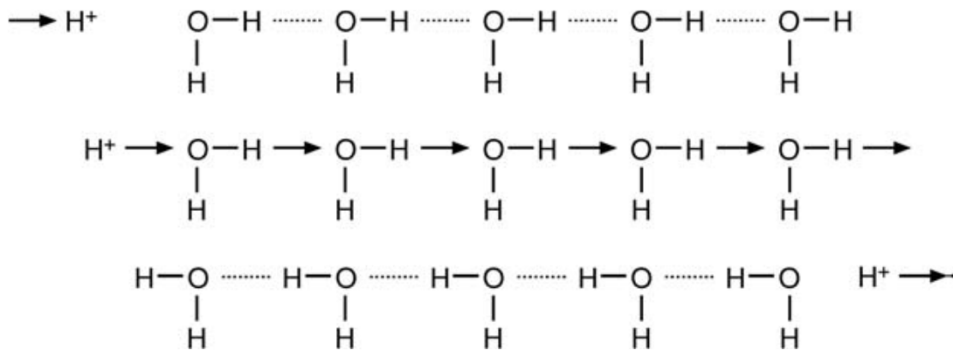
Schematic representations explaining the mechanism for blocking proton permeation of AQP1.
a. Diagram illustrating how partial charges from the helix dipoles restrict the orientation of the water molecules passing through the constriction of the pore.

b and c. Diagram illustrating hydrogen bonding of a water molecule with Asn 76 and/or Asn 192, which extend their amido groups into the constriction of the pore.

(The electrostatic effect of the helix dipole can be approximated by ½+ unit charge at the N-terminus and ½ – unit charge at C-terminus of an α-helix. See: Hol, Van Duynen, Berendsen, Nature (1978))

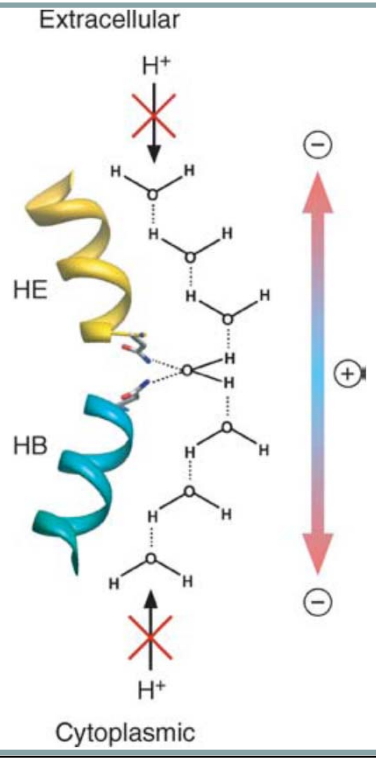
Murata K et al."Structural determinants of water permeation through aquaporin-1." Nature. 2000; 407:599-605.

Water permeation through aquaporin-1 (Ctd)



The "Grothuss" mechanism of proton transport along a "proton wire".

The proton exclusion mechanism.



The two Asn residues of the NPA motifs (Asn76 and Asn192 in AQP1) are held tightly in position and extend their side-chains into the pore. In this way the positive ends of the dipoles (which can be approximated by ½ + and ½ – unit charges) of the two pore helices are focused onto the amido groups of the NPA's Asn residues in the center of the pore.

Based on this observation the 'hydrogen bond isolation mechanism' was proposed (Murata et al. 2000):

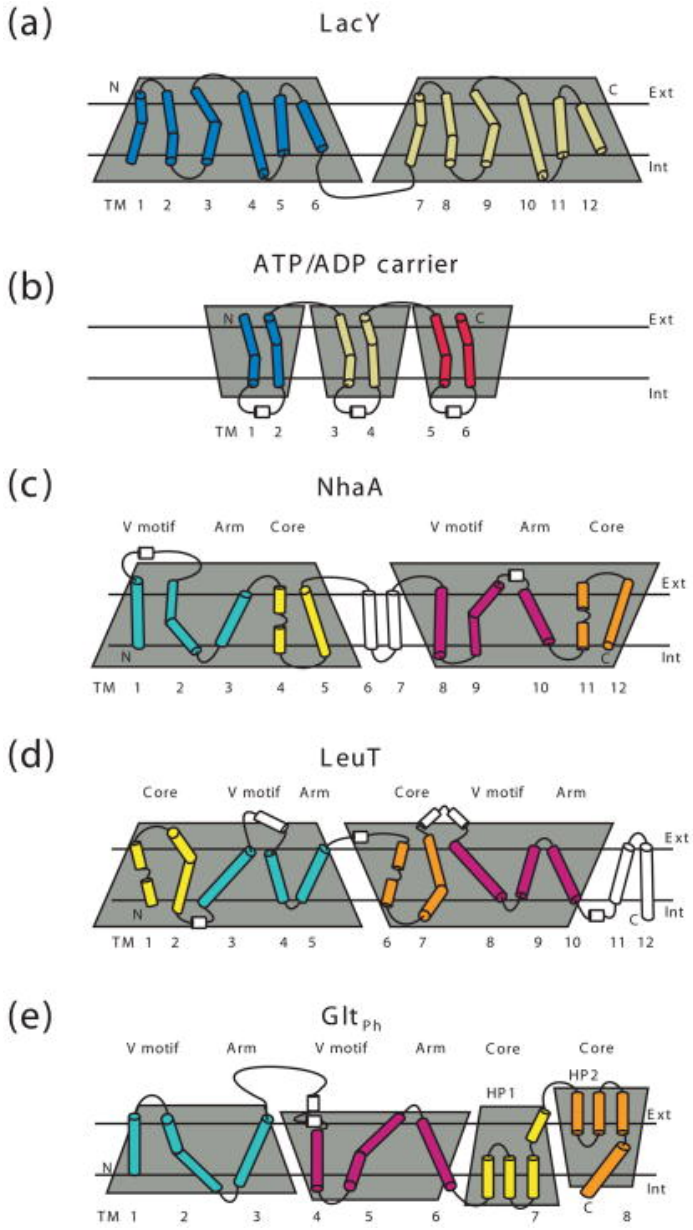
- **A water molecule approaching the center of the pore would orient itself so that its oxygen atom can form hydrogen bonds with the side chains of the two Asn residues.**
- **The two hydrogen atoms of the water molecule would be oriented perpendicular to the pore axis, preventing the formation of hydrogen bonds with neighboring water molecules in the pore.**
- **The isolation of this central water molecule in the pore would break the continuous line of hydrogen bonds and thus be a very efficient means to prevent protons from crossing the pore.**

Gonen & Walz, "The structure of aquaporins, Quarterly Reviews of Biophysics 39: 361–396 (2006)

Secondary Transporters

Enable the crossing of secondary metabolites across membranes

Secondary Transporters



Membrane topology of transporters with parallel and inverted structural repeats.

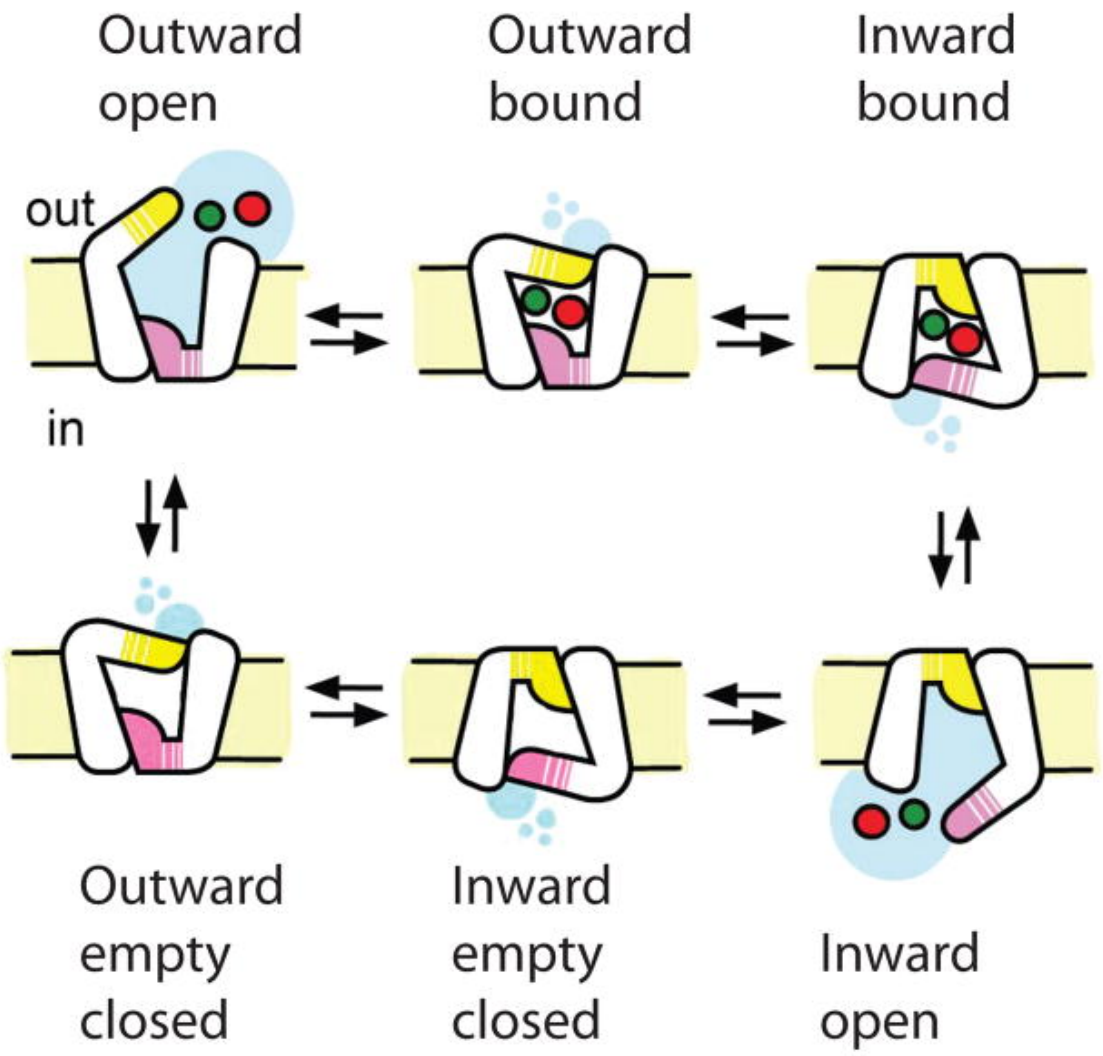
Secondary active transporters catalyze concentrative transport of substrates across lipid membranes by harnessing the energy of electrochemical ion gradients.

These transporters bind their ligands on one side of the membrane, and undergo a global conformational change to release them on the other side of the membrane.

Boudker and Verdon,Trends Pharmacol Sci. (2010) 31:418–426. "Structural perspectives on secondary active transporters"

Secondary Transporters

Schematic of transport across a membrane without leaking (too many) protons and metabolites



A remarkably "lock-like" mode of action
– to prevent leakage of other metabolites, ions and protons

Boudker and Verdon,Trends Pharmacol Sci. (2010) 31:418–426. "Structural perspectives on secondary active transporters"

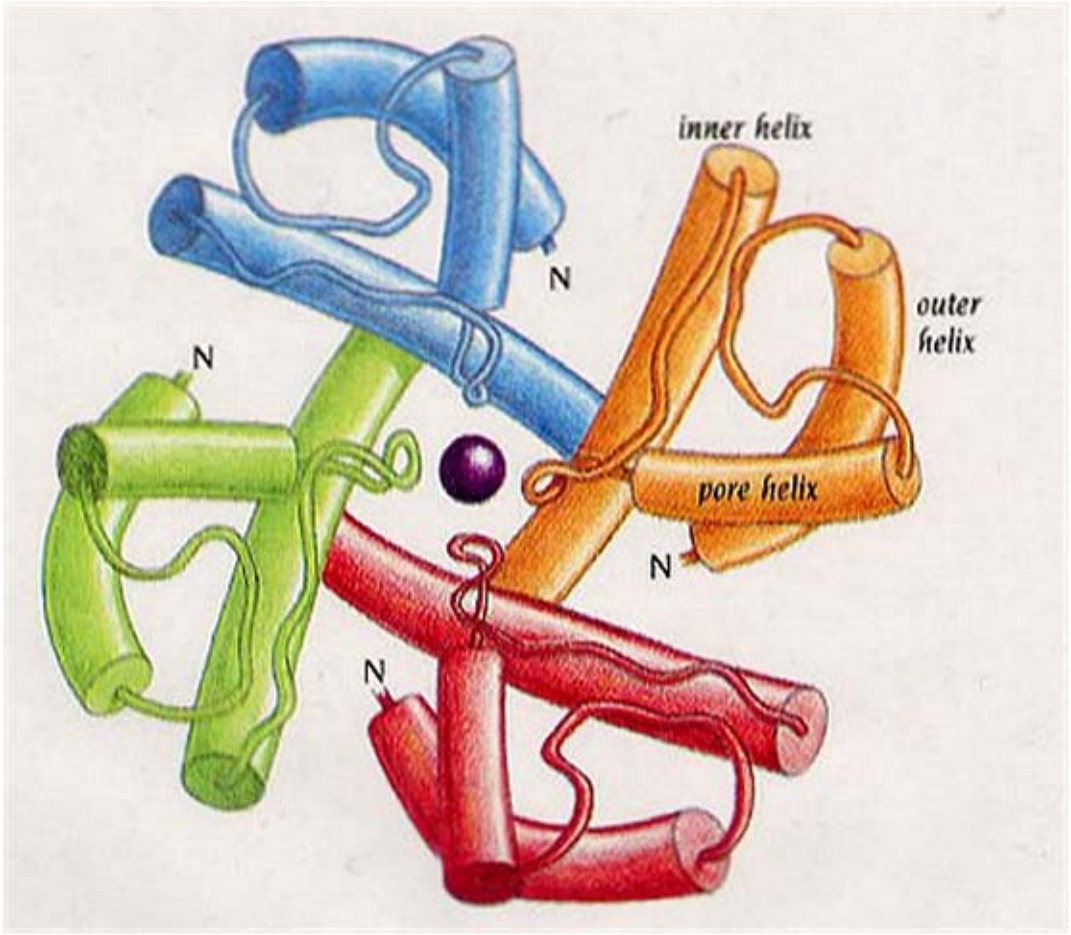
Potassium Channel

Regulates the crossing of potassium ions across the membrane of nerve cells

The K^+ channel is a tetrameric molecule with one ion pore in the interface between the four subunits

- The polypeptide chain of the bacterial K^+ channel subunit comprises 158 residues folded into two transmembrane helices, a pore helix and a cytoplasmic tail of 33 residues.
- Four subunits arranged around a central fourfold symmetry axis form the K^+ channel molecule.
- The subunits pack together in such a way that there is a hole in the center which forms the ion pore through the membrane.
- The C-terminal transmembrane helix, the inner helix, faces the central pore while the N-terminal helix, the outer helix, faces the lipid membrane.
- The four inner helices of the molecule are tilted and kinked so that the subunits open like petals of a flower towards the outside of the cell.
- The open petals house the region of the polypeptide chain between the two transmembrane helices. This segment of about 30 residues contains an additional helix, the pore helix, and loop regions which form the outer part of the ion channel.
- One of these loop regions with its counterparts from the three other subunits forms the narrow selectivity filter that is responsible for ion selectivity. The central and inner parts of the ion channel are lined by residues from the four inner helices.

Potassium Channel



Schematic diagram of the structure of a potassium channel perpendicular to of the membrane.

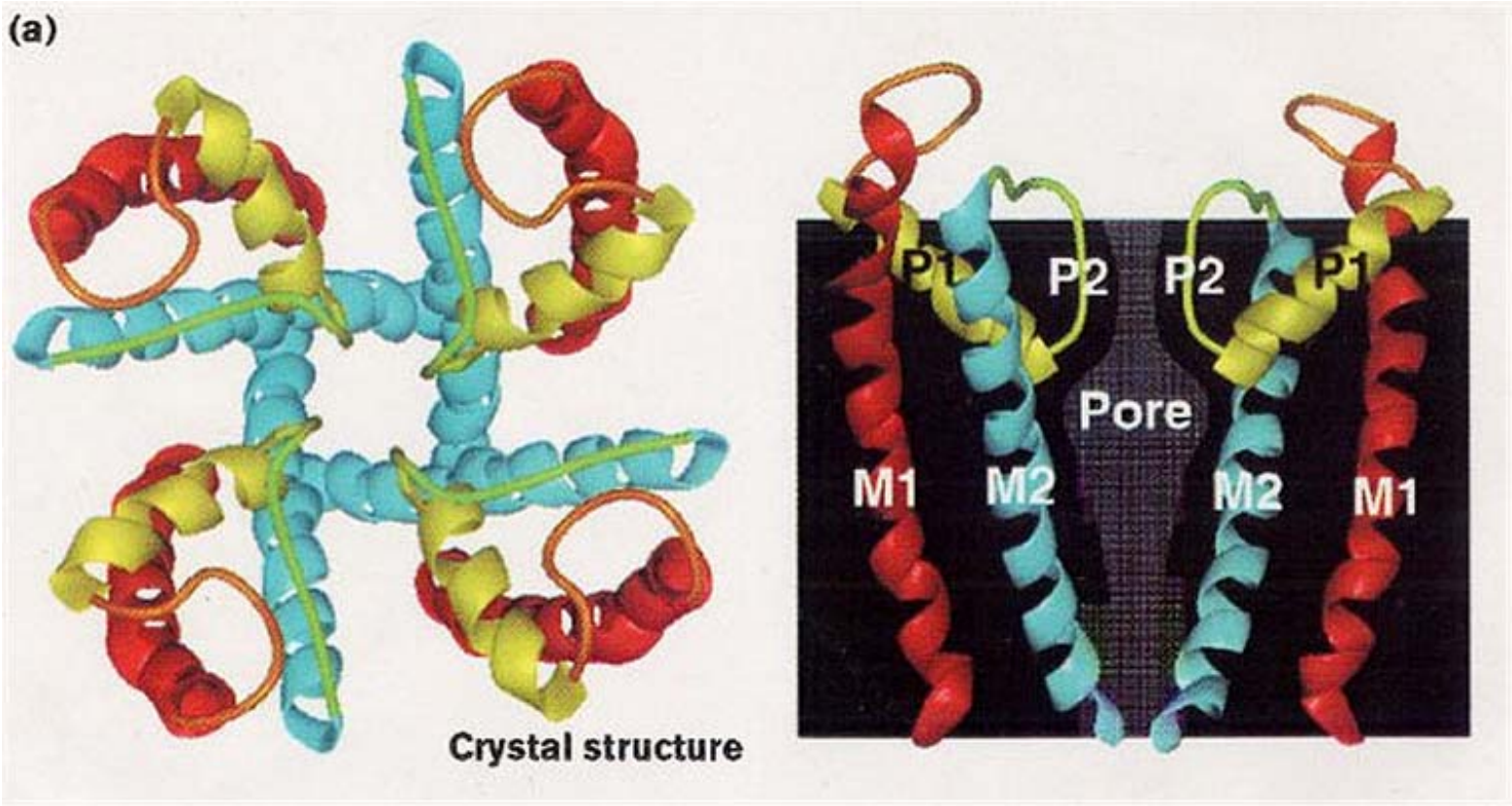
The molecule is tetrameric with a hole in the middle that forms the ion pore (purple).

Each subunit forms two transmembrane helices, the inner and the outer helix.

The pore helix and loop regions build up the ion pore in combination with the inner helix

Branden, C. & Tooze, J. (1999) *Introduction to Protein Structure*. 2nd edit.

Potassium Channel (Ctd.)



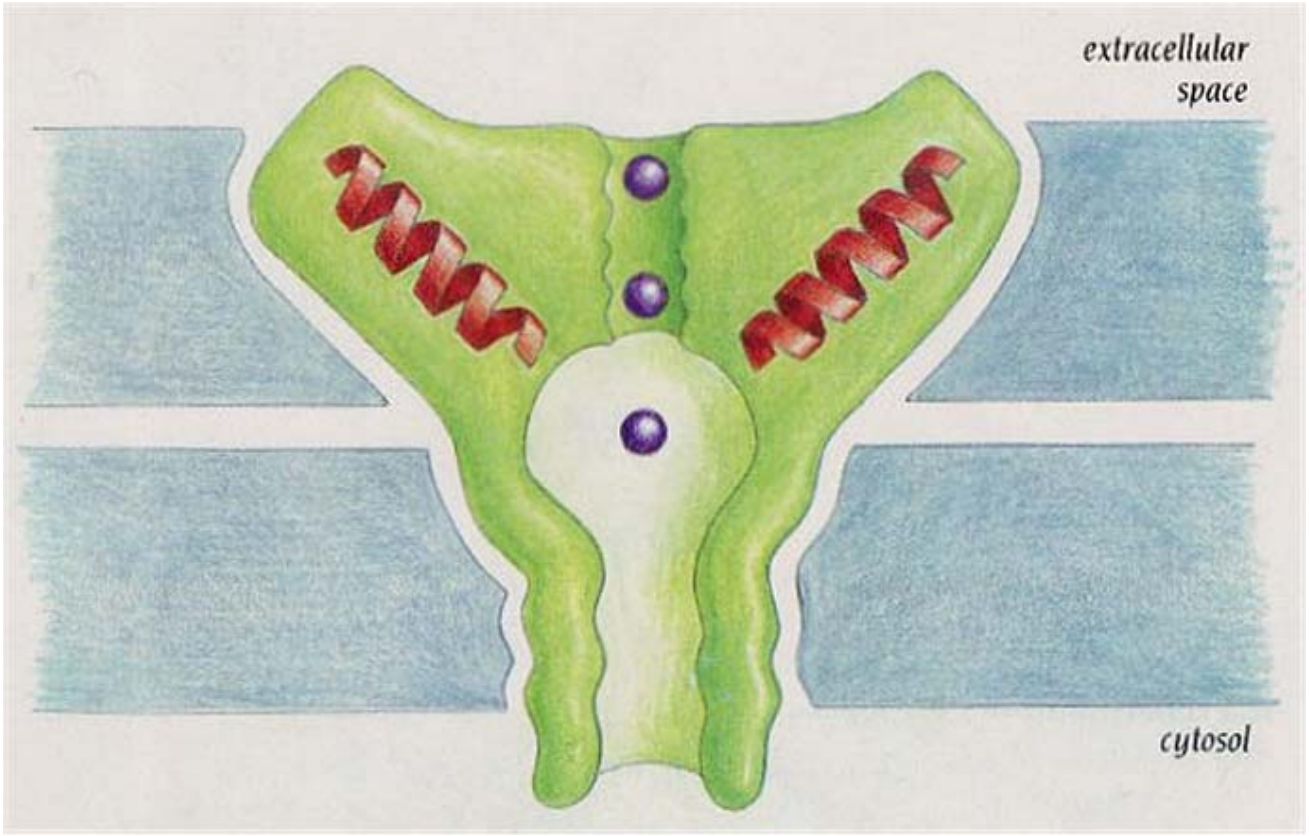
Ribbon diagram of the KcsA structure compared to a proposed model.

LEFT: a diagram of the crystal structure of the KscA tetramer as viewed from the extracellular surface.

RIGHT: a side view of two monomers of KcsA showing an outline of the internal pore and cavity.

Moczydlowski, E. (1998) Chemical basis for alkali cation selectivity in potassium-channel proteins. *Chem. Biol.* 5, R291-R301.

Potassium Channel (Ctd.)



Schematic diagram of the ion pore of the K^+ channel.

From the cytosolic side the pore begins as a water-filled channel that opens up into a water-filled cavity near the middle of the membrane. A narrow passage, the selectivity filter, links this cavity to the external solution.

Three potassium ions (purple spheres) bind in the pore.

The pore helices (red) are oriented such that their carboxyl end (with a negative dipole moment) is oriented towards the center of the cavity to provide a compensating dipole charge to the K+ ions
(Adapted from D.A. Doyle et al., *Science* 280: 69-77, 1998)

Branden, C. & Tooze, J. (1999) *Introduction to Protein Structure*. 2nd edit.

Potassium Channel (Ctd.)

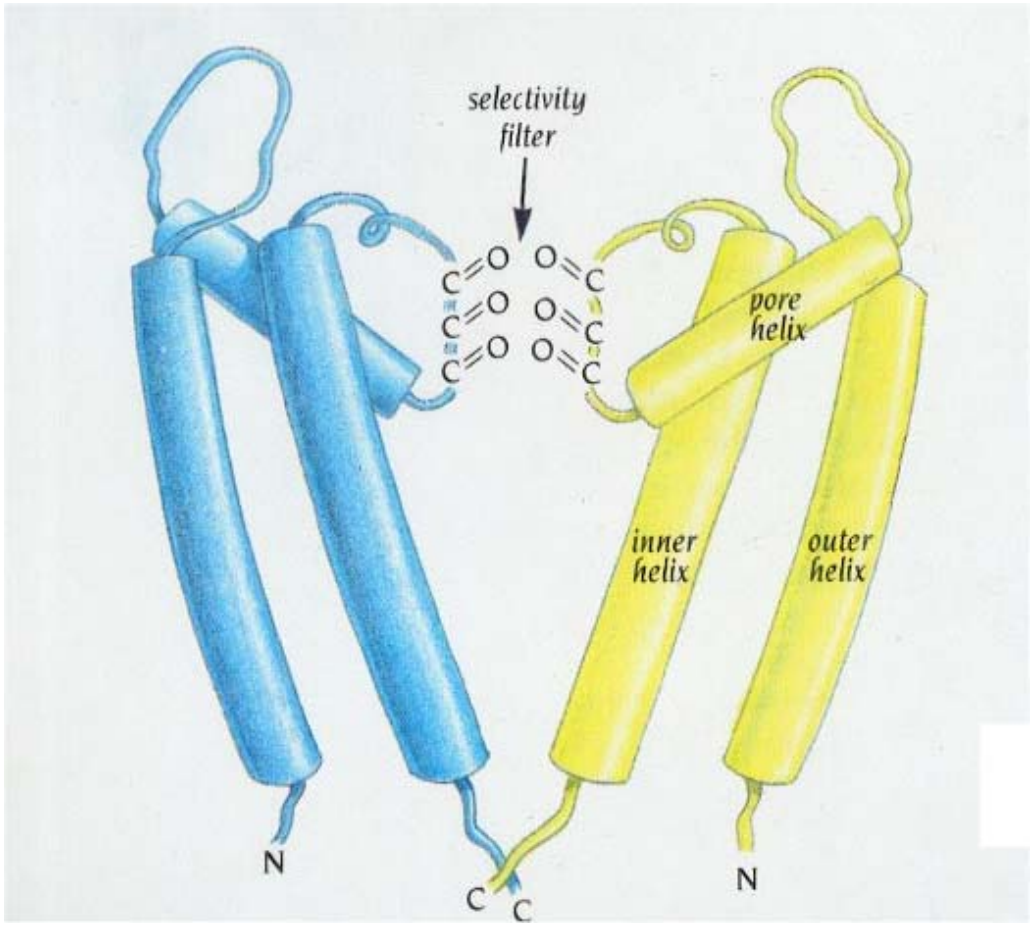


Diagram showing two subunits of the K+ cannel, illustrating the way the selectivity filter is formed.

Main-chain atoms line the walls of this narrow passage with carbonyl oxygen atoms pointing into the pore, forming binding sites for K+ ions.

Branden, C. & Tooze, J. (1999) *Introduction to Protein Structure*. 2nd edit.

The ion pore has a narrow ion selectivity filter

- The overall length of the ion pore is 45 Å and its diameter varies along its length.
- As expected for a K^+ channel, there is a surplus of negative charges at both ends of the pore, which attract positively charged ions.
- From the cytosolic side, the pore begins as a channel 18 Å long, which opens into a wider cavity of about 10 Å diameter near the middle of the membrane.
- A narrow passage, the selectivity filter, links this cavity to the external solution.
- Main-chain atoms from all four subunits line the walls of this passage with carbonyl oxygen atoms pointing into the channel.

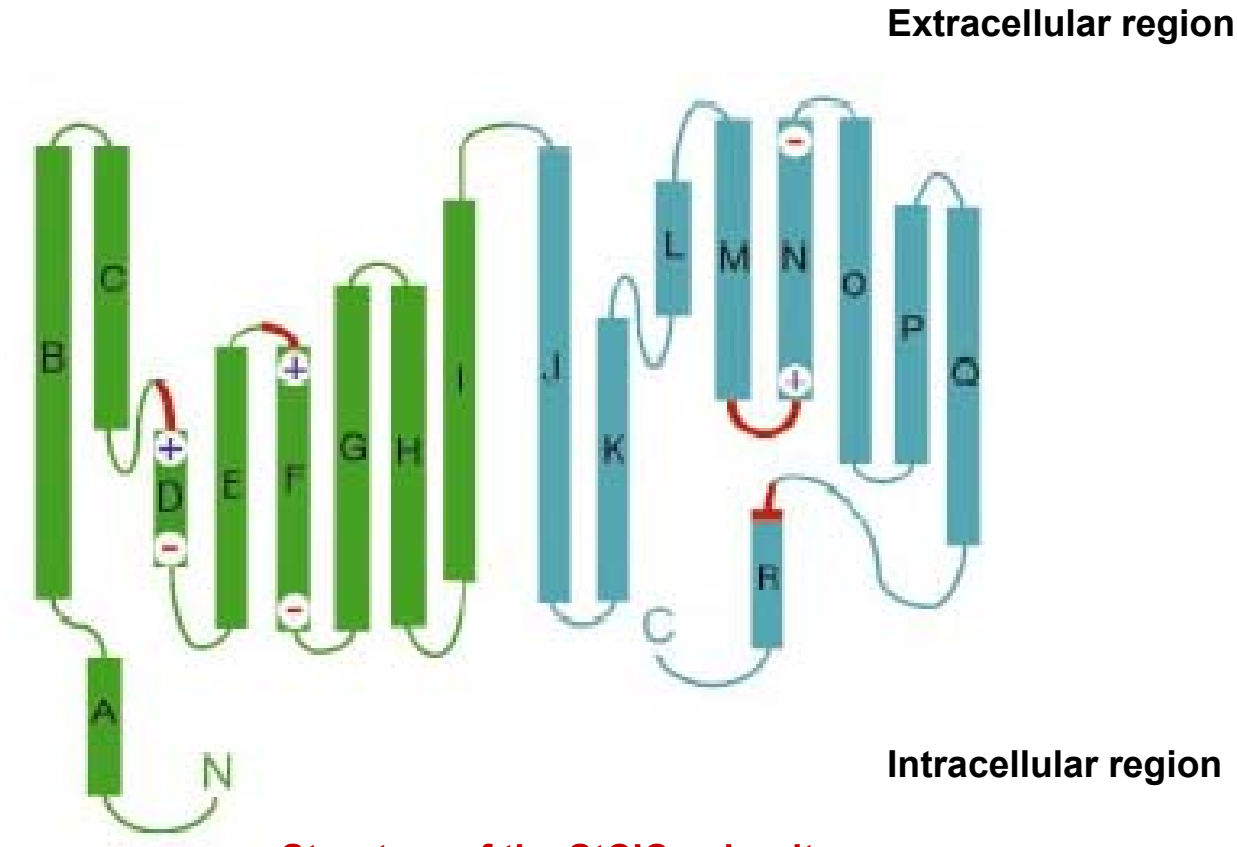
MacKinnon has suggested a plausible mechanism for the ion selectivity and conductivity of the channel:

- When an ion, which in solution has a water hydration shell, enters the selectivity filter it dehydrates.
- Binding to the carbonyl oxygen atoms in the filter compensates the energetic cost of dehydration.
- The dimensions of the binding sites are such that a K^+ ion fits in the filter precisely so that the energetic costs and gains are well balanced, but the firm packing of the side chains prevents the carbonyl oxygen atoms from approaching close enough to compensate for the cost of dehydration of a Na^+ ion.

Chloride Channel

Chloride channels display a variety of important physiological and cellular roles that include regulation of pH, volume homeostasis, organic solute transport, cell migration, cell proliferation and differentiation.

Chloride Channel

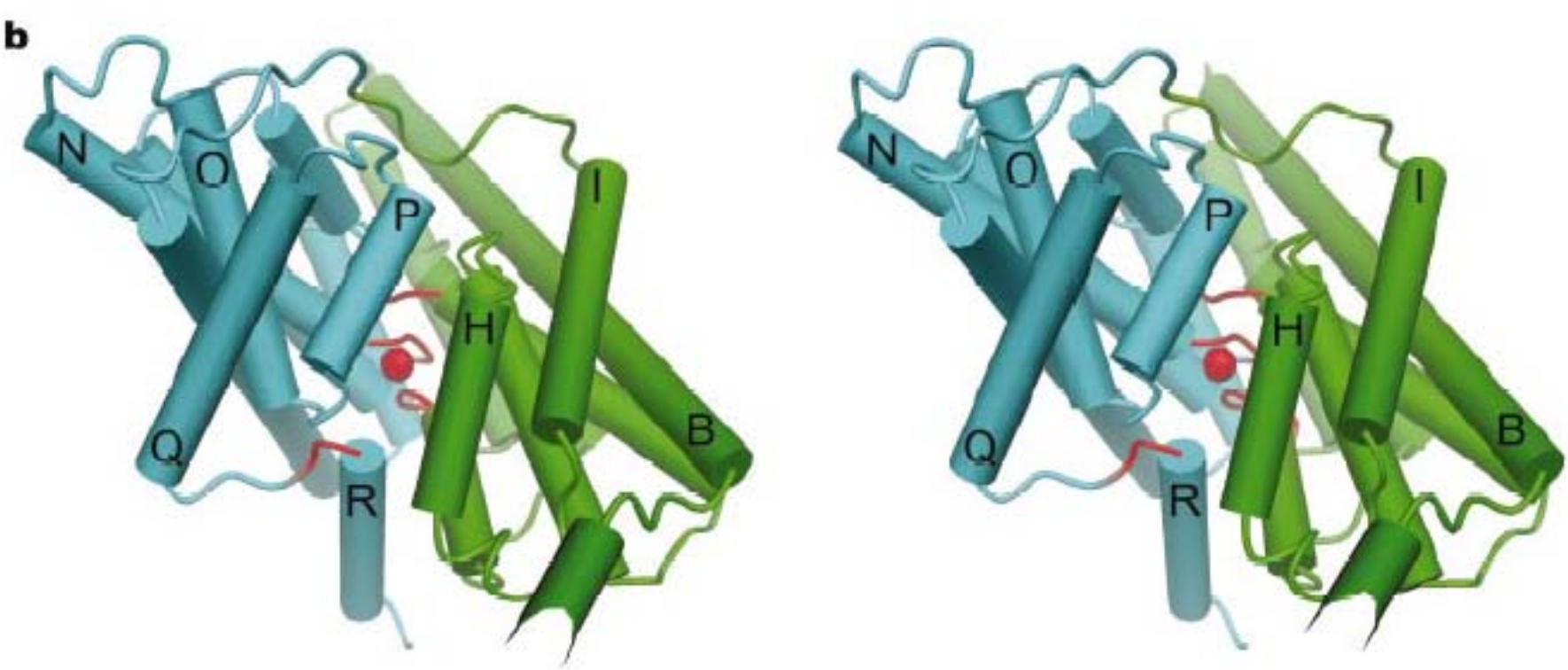


Structure of the StClC subunit

- **Two similar domains (green and cyan) are related by a pseudo-twofold axis.**
- **The α-helices (A-R) shown as cylinders.**
- **Regions forming the Cl^- selectivity filter in red.**
- **Partial charges at the end of helices involved in Cl^- binding are indicated by + and – (end charges) to indicate the sense of the helix dipole.**
- **(Should have been ½+ unit charge at N-termini and ½ – unit charge at C-termini of the helices)**

"X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity." Dutzler et al & McKinnon, Nature. 2002 ,415:287-94.

Chloride Channel

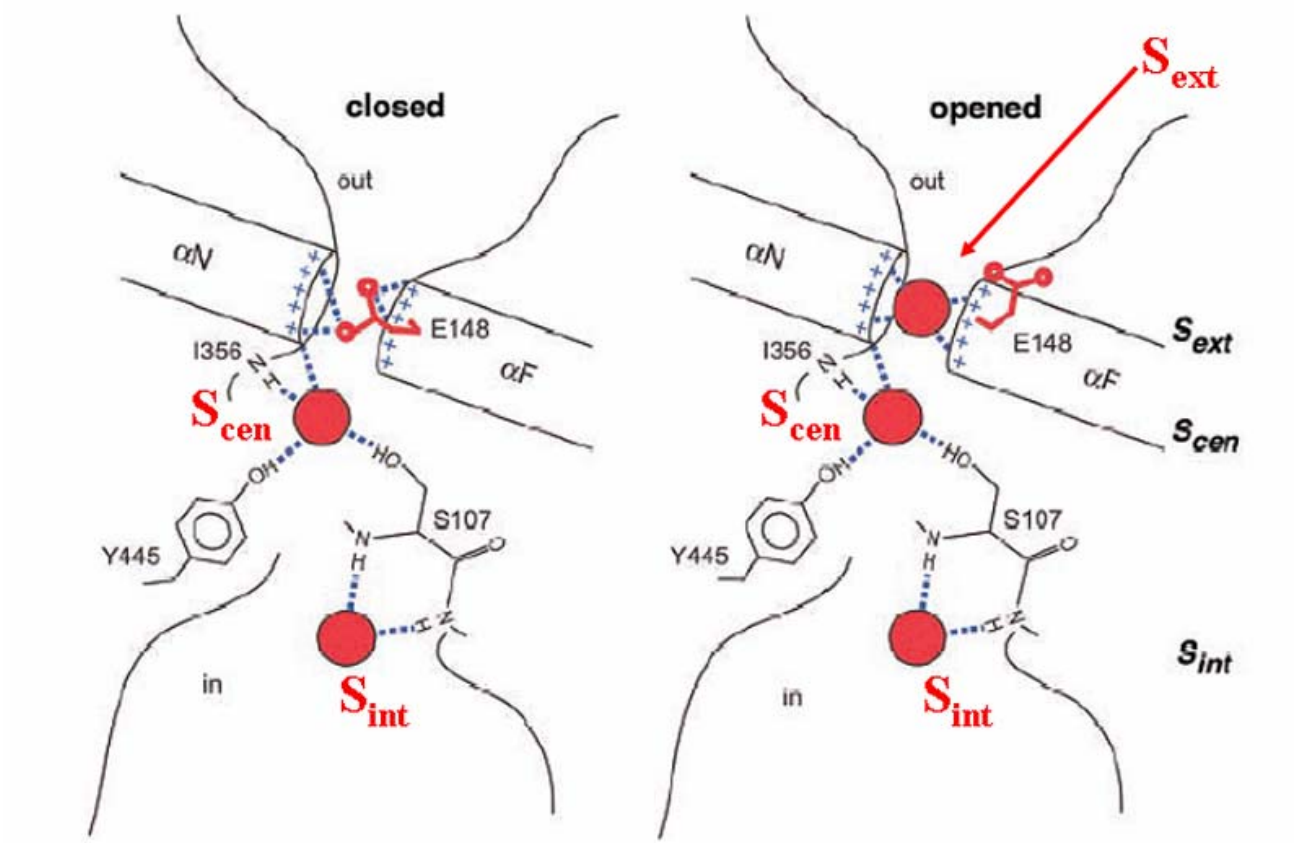


Stereo view of the StClC subunit viewed from within the plane of the membrane from the dimer interface with the extracellular solution above.

The α-helices are drawn as cylinders, loop regions as cords (with the selectivity filter red), and the Cl^- ion as a red sphere.

"X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity." Dutzler et al & McKinnon, Nature. 2002 ,415:287-94.

Chloride Channel



Schematic drawing of the closed and opened conformation of a ClC chloride channel.

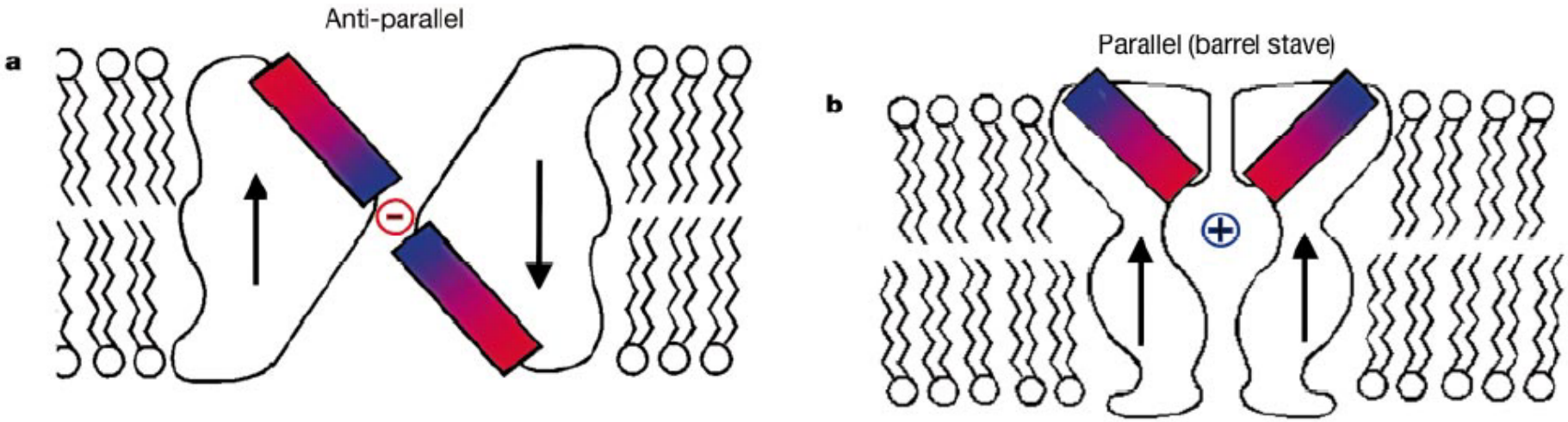
In the closed conformation, the ion-binding sites S_{int} and S_{cen} are occupied by chloride ions, and the ion-binding site S_{ext} is occupied by the side chain of Glu^{148}.

In the opened conformation, the side chain of Glu^{148} has moved out of binding site S_{ext} into the extracellular vestibule. S_{ext} is occupied by a third chloride ion.

Chloride ions are shown as red spheres, the Glu^{148} side chain is colored red, and hydrogen bonds are drawn as dashed lines.

"X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity." Dutzler et al & McKinnon, Nature. 2002 ,415:287-94.

Two architectures of ion-channel proteins



a) The antiparallel architecture of ClC Cl^- channels contains structurally similar halves with opposite orientations in the membrane (arrows). This architecture permits like ends (same dipole sense) of a α-helices to point at the membrane centre from opposite sides of the membrane (180° separation).

b) The parallel or barrel stave architecture of K^+ channels contains structurally similar or identical subunits with the same membrane orientation (arrows). Helices point at the membrane centre from the same side of the membrane. Helices are depicted as dipoles with blue (positive) and red (negative) ends.

(The electrostatic effect of the helix dipole can be approximated by ½+ unit charge at the N-terminus and ½ – unit charge at C-terminus of an α-helix. See: Hol, Van Duynen, Berendsen, Nature (1978))

"X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity." Dutzler et al & McKinnon, Nature. 2002 ,415:287-94.

MULTI-DRUG EFFLUX

- Several types of systems have evolved in gram-negative bacteria to pump deleterious molecules out of the cytosol.

- Several of these systems consist of three partners:

  (i) an inner membrane transporter,

  (ii) a periplasmic membrane "fusion protein",

  (iii) an outer membrane channel.

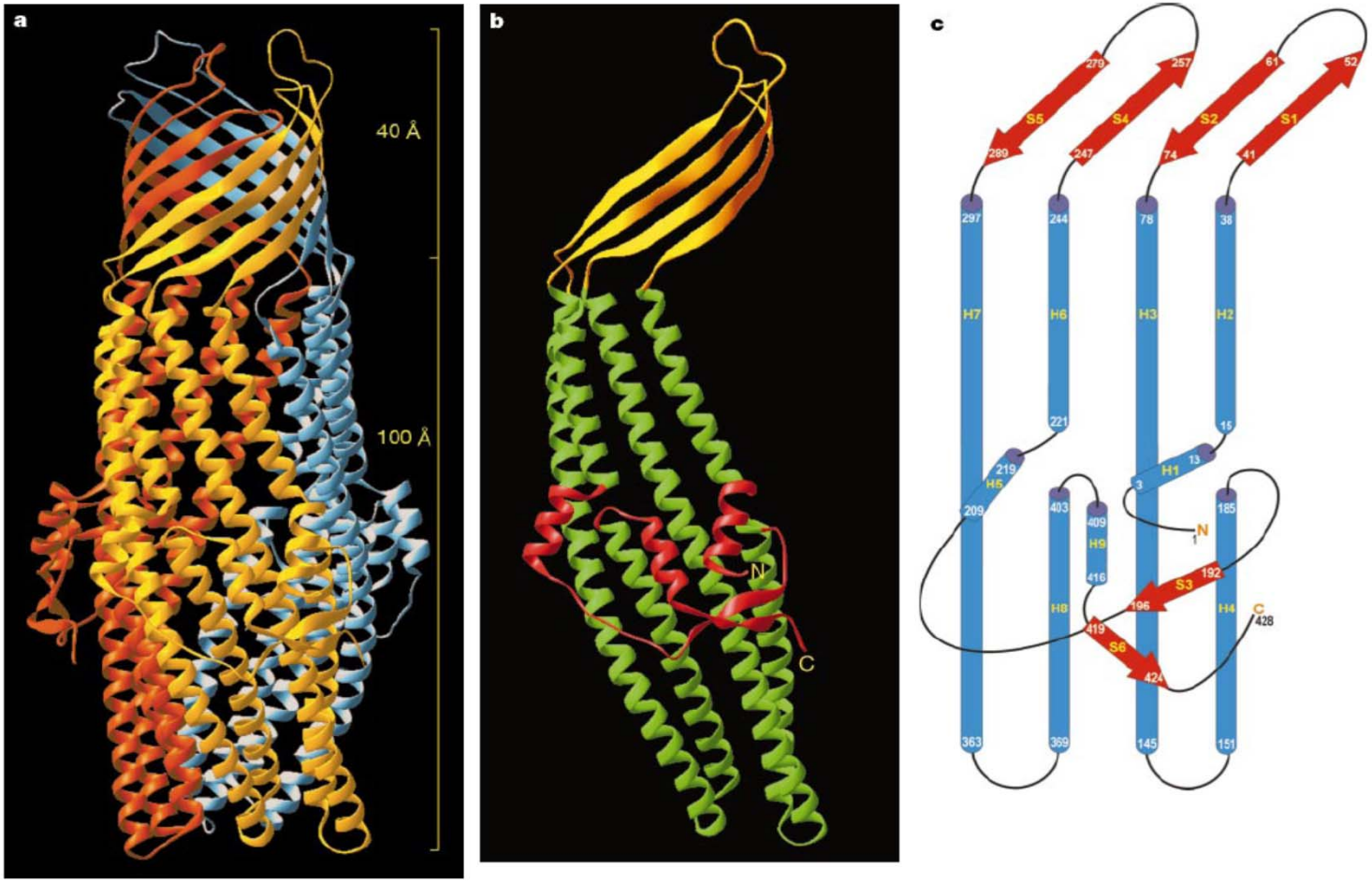
- An example is: AcrB, AcrA and TolC in *E. coli*
- Another example: MexB+MexA+OprM in *P. aeruginosa*

Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export

- Diverse molecules, from small antibacterial drugs to large protein toxins, are exported directly across both cell membranes of Gram-negative bacteria.
- This export is brought about by the reversible interaction of substrate-specific inner-membrane proteins with an outer-membrane protein of the TolC family, thus bypassing the intervening periplasm.
- Three TolC protomers assemble to form a continuous, solvent-accessible conduit—a ‘channel-tunnel’ over 140 Å long that spans both the outer membrane and periplasmic space. The periplasmic or proximal end of the tunnel is sealed by sets of coiled helices.
- We suggest these could be untwisted by an allosteric mechanism
- The structure provides an explanation of how the cell cytosol is connected to the external environment during export, and suggests a general mechanism for the action of bacterial efflux pumps.

Koronakis et al.,Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export.
Nature 405:914-919.(2000)

The overall architecture of TolC



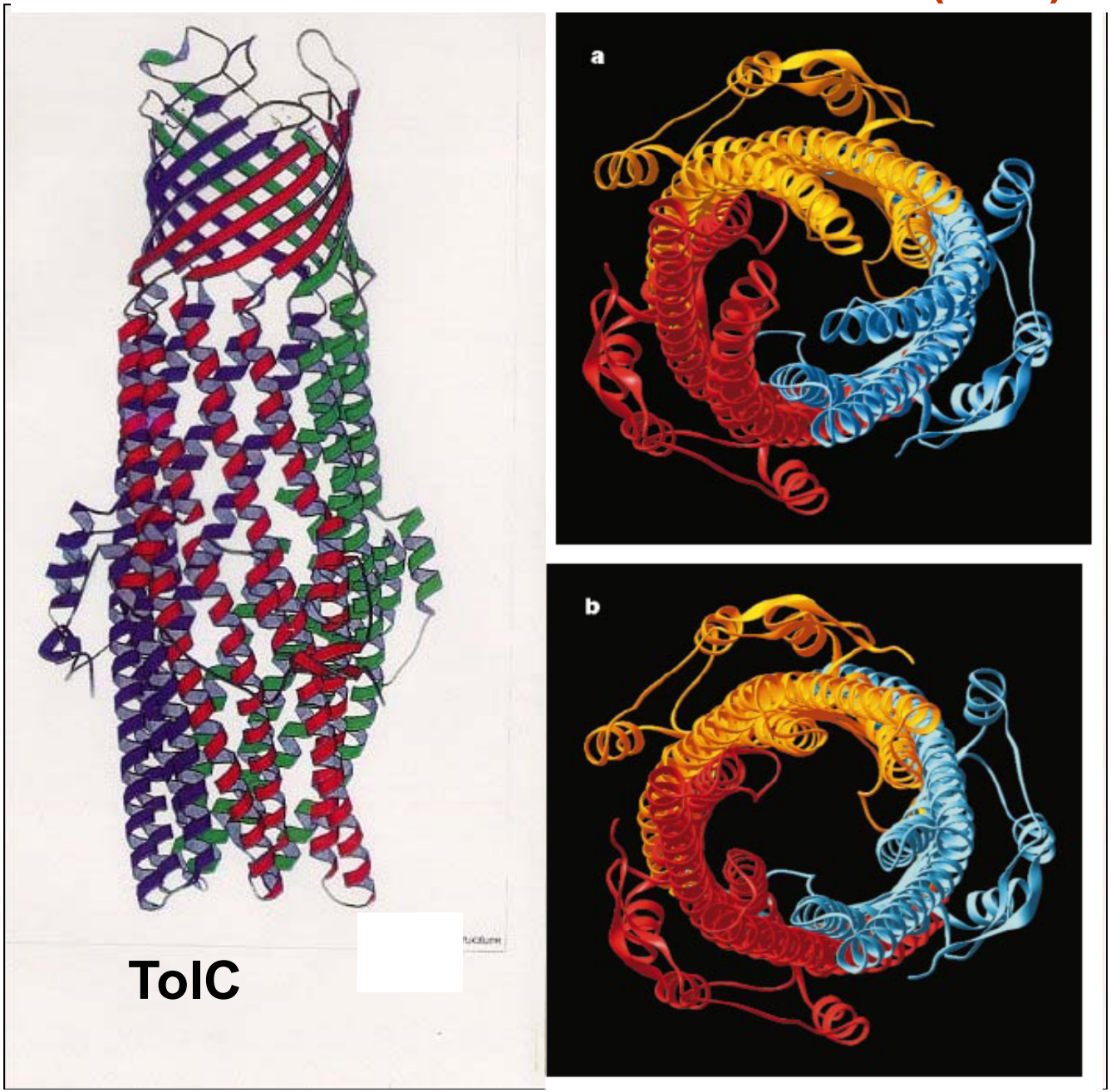
THE OVERALL ARCHITECTURE OF TOLC.

a. **Cα trace of TolC. The protomers are individually coloured. The molecular threefold axis is aligned vertically, normal to the plane of the outer membrane. The β-barrel is at the top (distal) end, and the α-helical domain is at the bottom (proximal) end.**
b. **Cα trace of a single protomer. The β-barrel domain is yellow, the α-helical barrel domain is green and the equatorial domain is red.**
c. **Topology diagram of the protomer. Secondary-structure elements are indicated: helices (H) in blue, strands (S) in red. The structural repeat comprises the sets (H1, H2, S1, S2, H3, H4) and (H5, H6, S4, S5, H7, H8).**

The overall architecture of TolC (Ctd.)

Beta barrel in OM

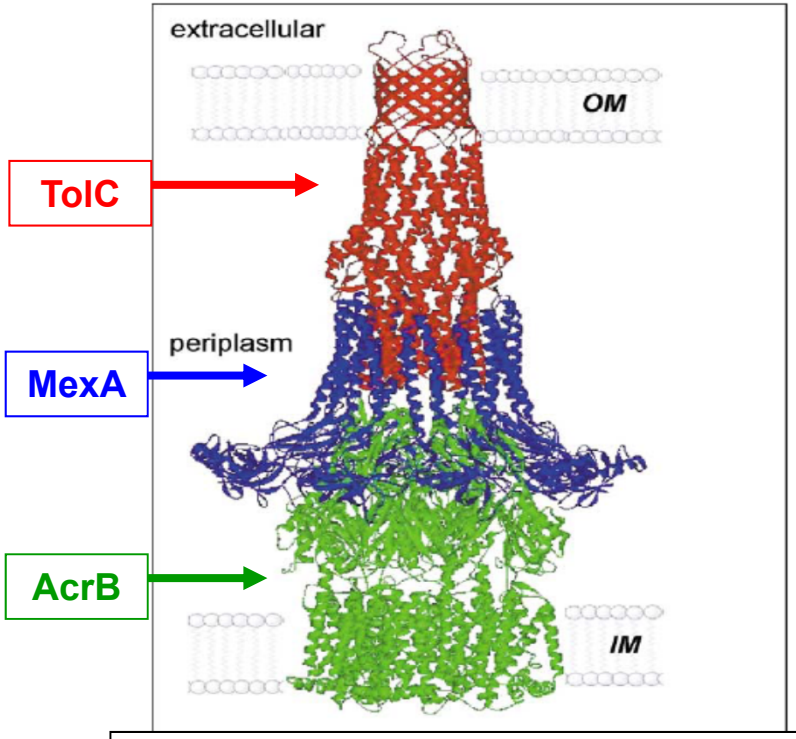
Helical Funnel in Periplasm



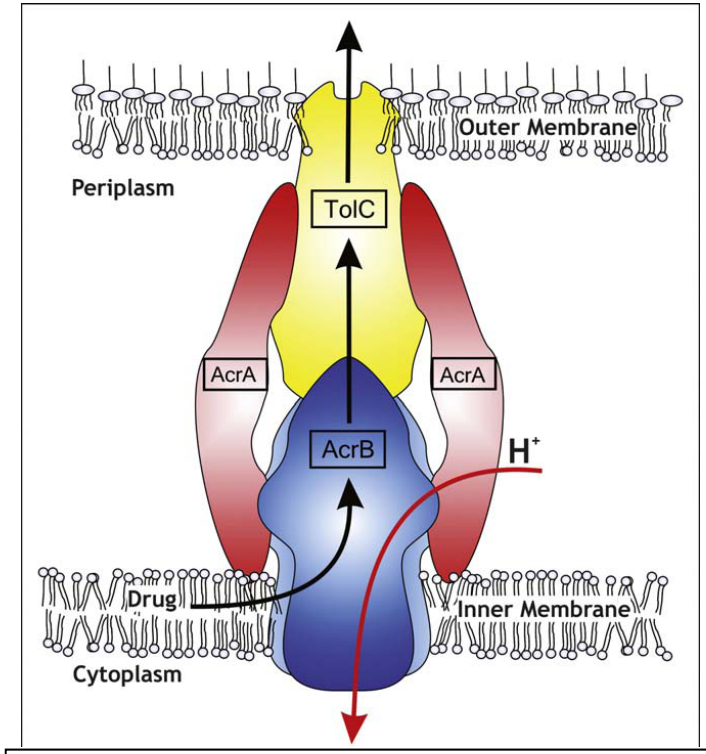
"Diafragm" closed (observed)

"Diafragm" open (model)

Model of one complete Drug efflux Pump



Model of an assembled tripartite drug efflux pump
AcrB+AcrA+TolC in *E. coli* ≈ MexB+MexA+OprM in *P. aeruginosa*



Schematic Model of an assembled tripartite drug efflux pump
Blair & Piddock – CurrOpMicrobiol 12:512 (2009)

Model of an assembled tripartite drug efflux pump.

- This model of an RND class drug efflux pump is based on the open-state model of TolC (red) forming a minimal contact interface with the six hairpins at the apex of AcrB (green).
- A ring of nine MexA molecules (blue) is modelled to form a sheath around AcrB and the α helix barrel of TolC (MexA is a close homologue of AcrA, the natural partner of AcrB/TolC).

For recent, low resolution, cryo-EM reconstruction see: Du, Structure of the AcrAB–TolC multidrug efflux pump, Nature 509:512 (2014)
Eswaran, Curr Opin Struct Biol 14: 741-747 (2004); Blair & Piddock, Curr Opin Microbiol 12:512 (2009)

AcrB≈MexB:

the inner-membrane component of a Multi-Drug Efflux Pump

***E coli* AcrB is :**

- **Located in the inner-membrane**
- **A transporter that is energized by a proton-motive force**
- **Shows the widest substrate specificity among all multi-drug pumps, including:**
  - **Antibiotics**
  - **Disinfectants**
  - **Dyes**
  - **Detergents**

Yu et al & Koshland, Science 300, 976 (2003)

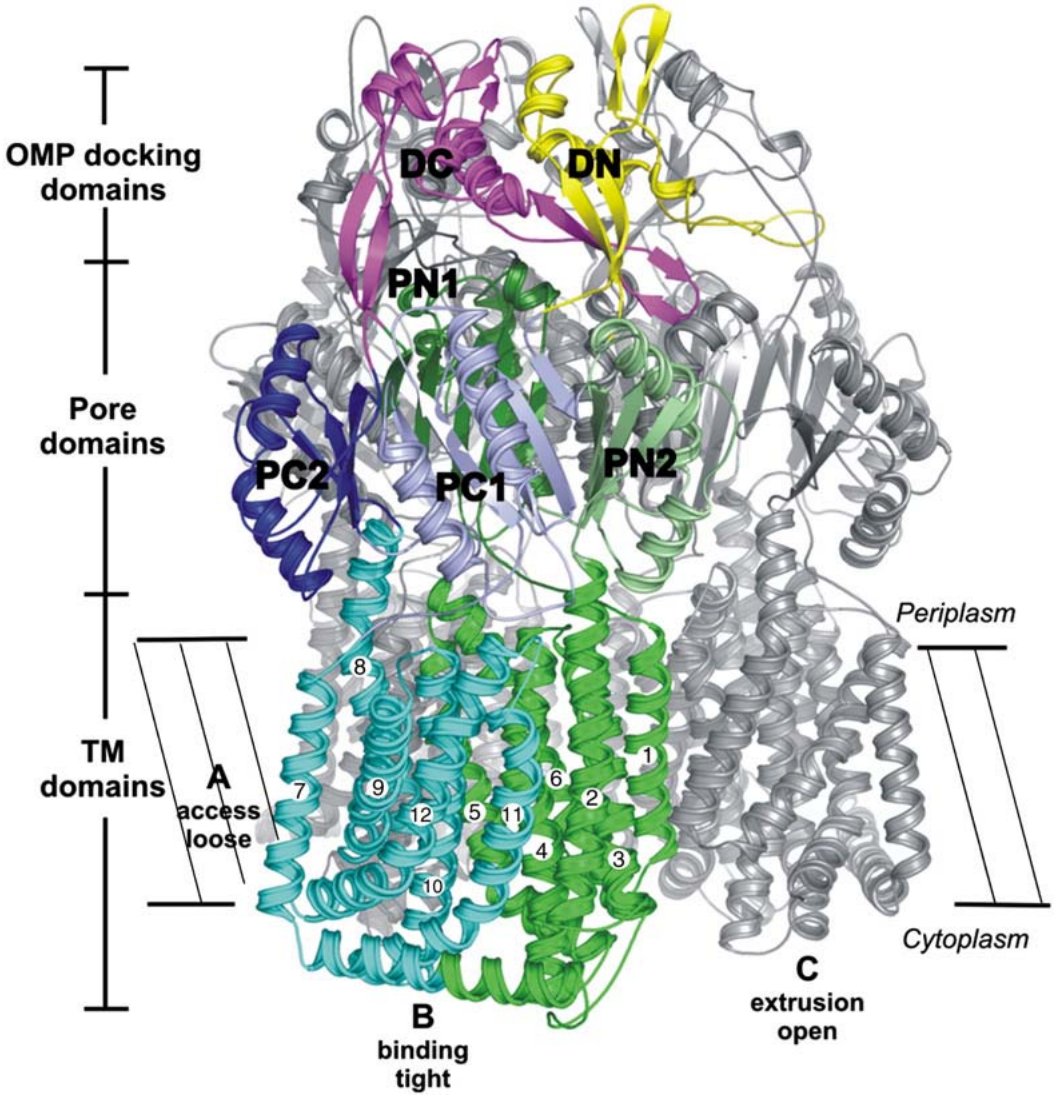
Crystal Structure of AcrB≈MexB

- A homotrimer containing 36 transmembrane α-helices, and a "headpiece" protruding by 70 Å into the periplasm.
- The top of the headpiece, the "TolC docking domain," opens as a 30 Å wide funnel (similar to the diameter of the TolC base). The bottom of the funnel connects to the central pore domain.
- A 35 Å long hairpin structure from each protomer completely penetrates an adjacent protomer, forming an interlocking weave near the interface of the 2 headpiece domains.
- The transmembrane region is loosely packed – it is believed the pore between protomers is filled with phospholipids.

Murakami, . Crystal structure of bacterial multidrug efflux transporter AcrB. Nature 2002, 419:587-593

Crystal Structure of MexB≈AcrB

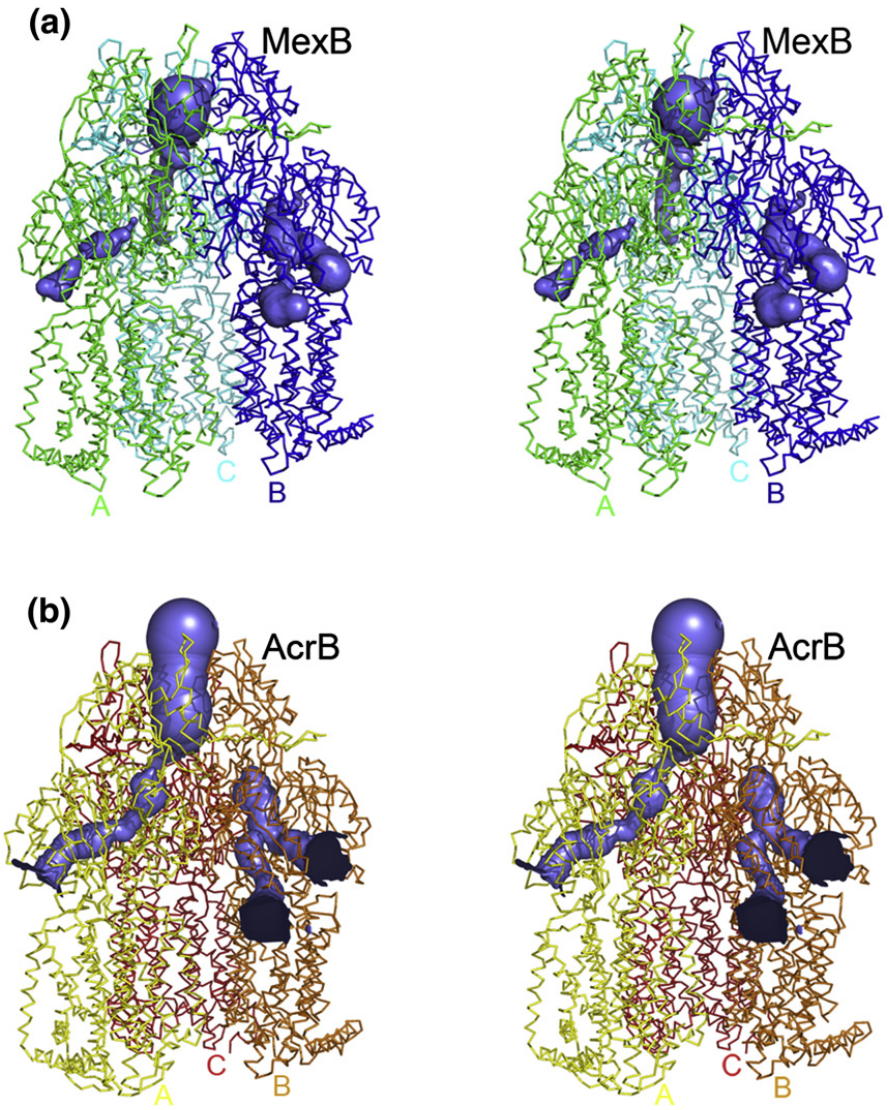
Side View – parallel to the membrane



Sennhauser, Crystal Structure of the Multidrug Exporter MexB from Pseudomonas aeruginosa JMB 389:134-145 (2009)

O2. Drug Efflux

Increase drug efflux



Stereo views of the channels in the MexB≈AcrB trimers

leading to the drug-binding cavity.

View parallel to the membrane

(a) The channels detected in the inner membrane MexB trimer are shown as blue surfaces.

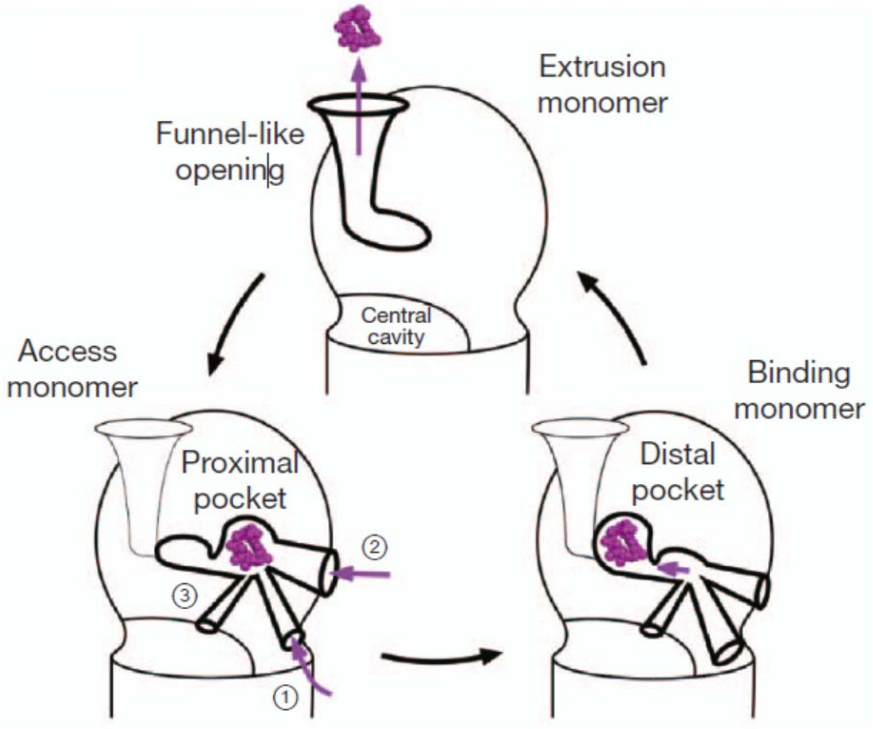
(b) Channels detected in the inner membrane AcrB trimer.

The channels were calculated using the program CAVER [http://loschmidt.chemi.muni.cz/caver].

Sennhauser, Crystal Structure of the Multidrug Exporter MexB from Pseudomonas aeruginosa, JMB 389:134-145 (2009)

Increase drug efflux

Peristaltic drug transport mechanism by the AcrB trimer



The surfaces of the proximal and distal binding pockets are very different so that very different substrates can be exported.

- The three subunits have three different conformations: Access monomer. Binding monomer, Extrusion monomer.
- Each subunit has several channels, which differ in each conformation.
- The Access monomer has a proximal binding pocket where large substrates initially bind.
- The Binding monomer has a distal binding pocket where small substrates bind immediately.
- Large drugs are transported from the proximal pocket to the distal pocket by a peristaltic motion due to a conformational change from the Access state to the Binding state.
- Drugs move next through a channel at the top to the outer-membrane channel formed by TolC.

Nakashima, Structures of the multidrug exporter AcrB reveal a proximal multisite drug-binding pocket, Nature 480: 565 (2011).

Membrane Proteins - General

- So far the slides have mainly shown rather simple membrane proteins.
- However, many membrane proteins are parts of incredibly complex assemblies.
- A very famous complex is the ATPase in bacterial cell walls, mitochondria and chloroplasts.
- Quite impressive systems are also Protein Secretion Systems, called Type 1 to Type 7 secretion systems (T1SS – T7SS) which export unfolded or even folded proteins across one or two membranes of Gram negative bacteria.
- All components of the electron transport chain are super-sophisticated membrane proteins, in particular Systems I, III and IV in mitochondria.
- There are also sophisticated membrane proteins machineries which put membrane proteins into… membranes (yes, yes, nice chicken and egg problem)
- Etc, etc, etc

Membrane Proteins - Literature

Good web site:

http://blanco.biomol.uci.edu/

General Reference:

Vinothkumar and Henderson, Structures of membrane proteins, Quart. Rev. Biophysics 43:65–158 (2010)

Annual Reviews:

Membrane Protein Section of: “Current Opinion in Structural Biology”

Journals with many membrane protein structures:

Top journals, like Nature, Science, Cell, etc.