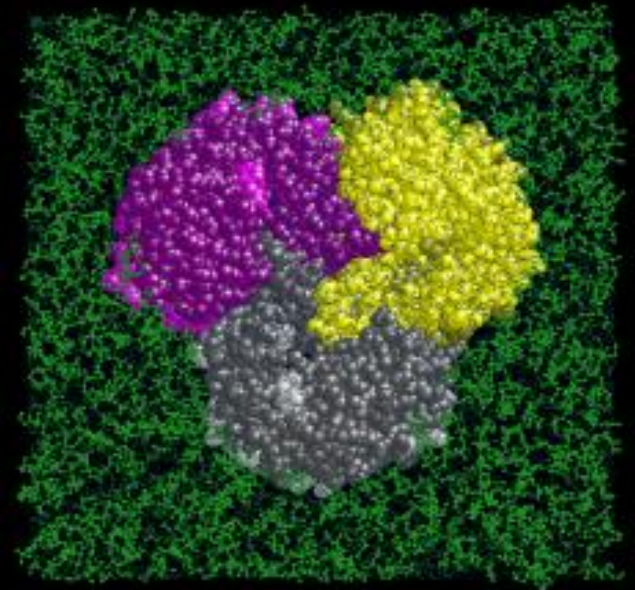
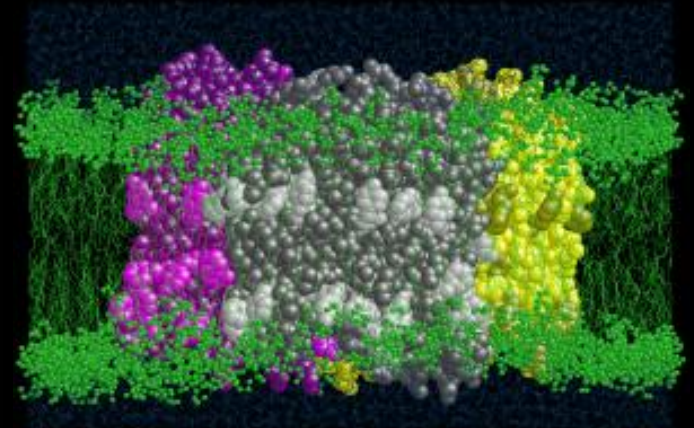
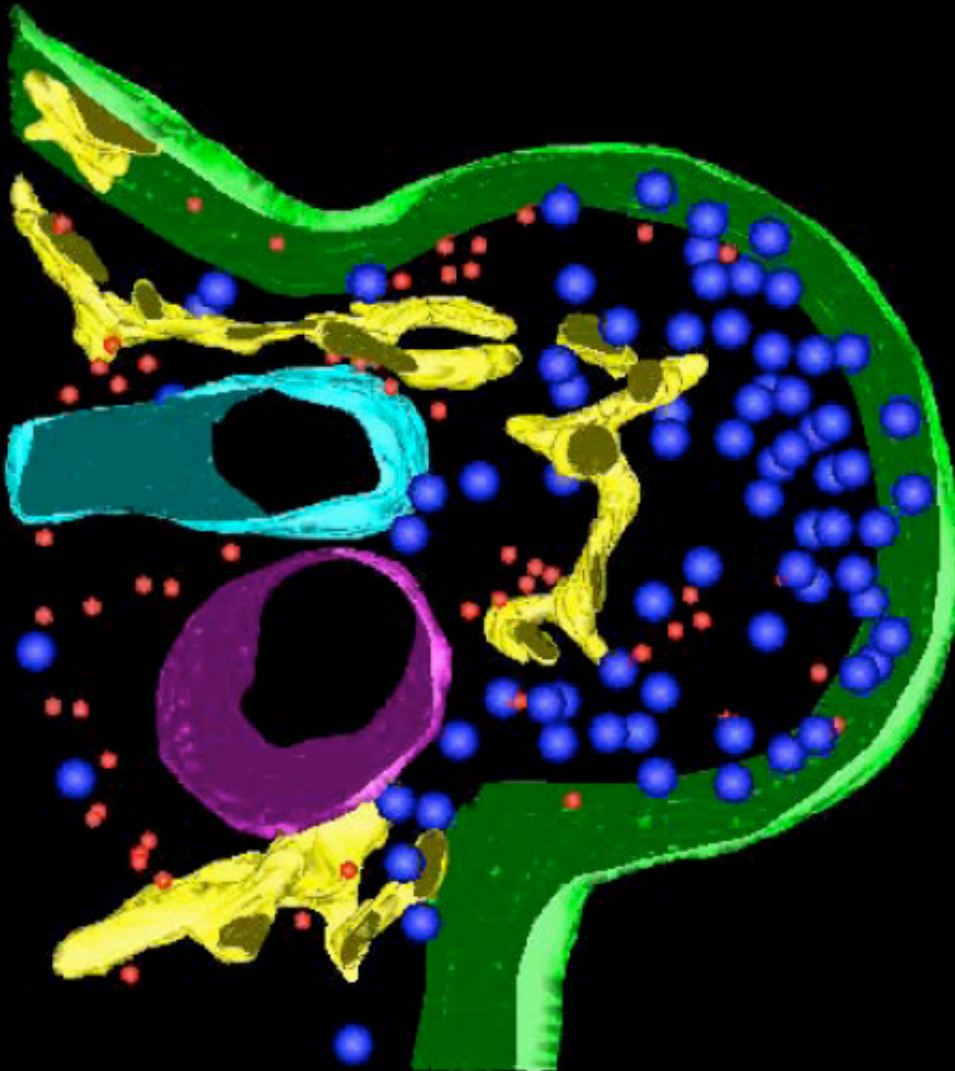
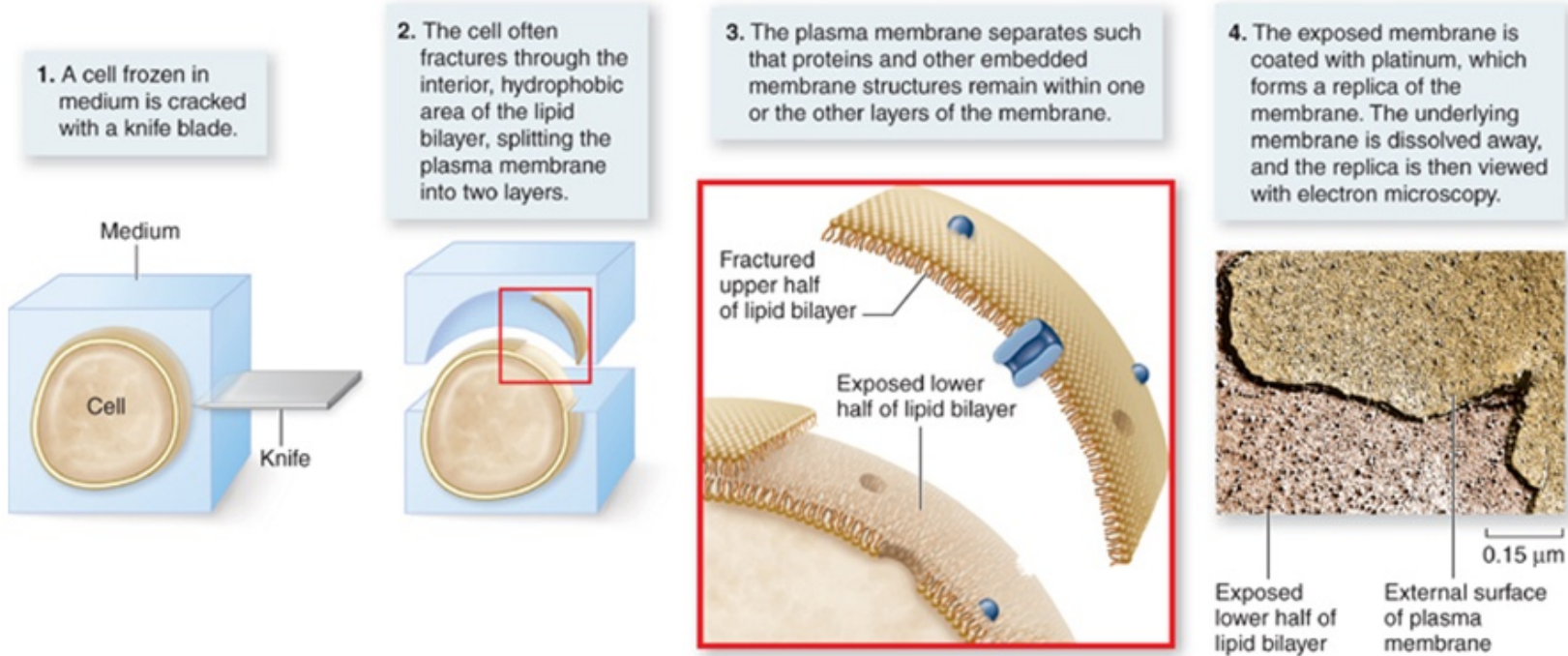


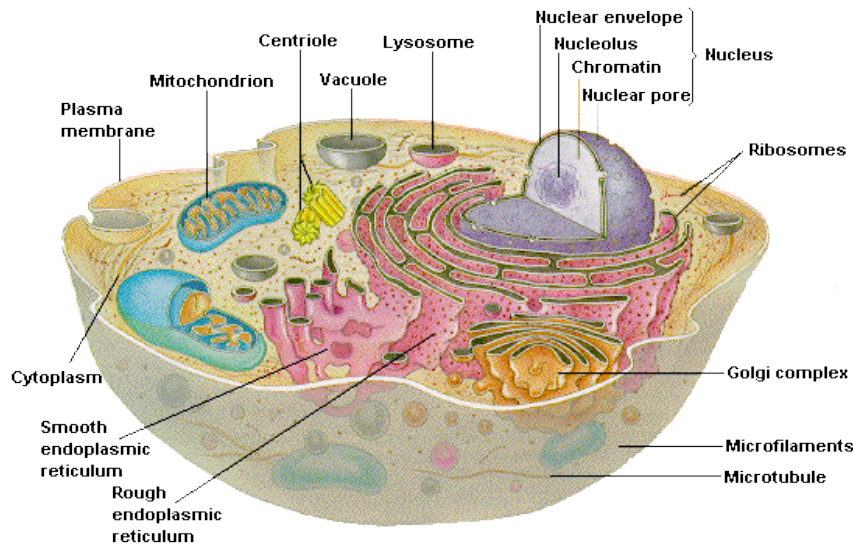
*Electron  
tomographic  
reconstructions*

# Biomembranes - Yeast Cell



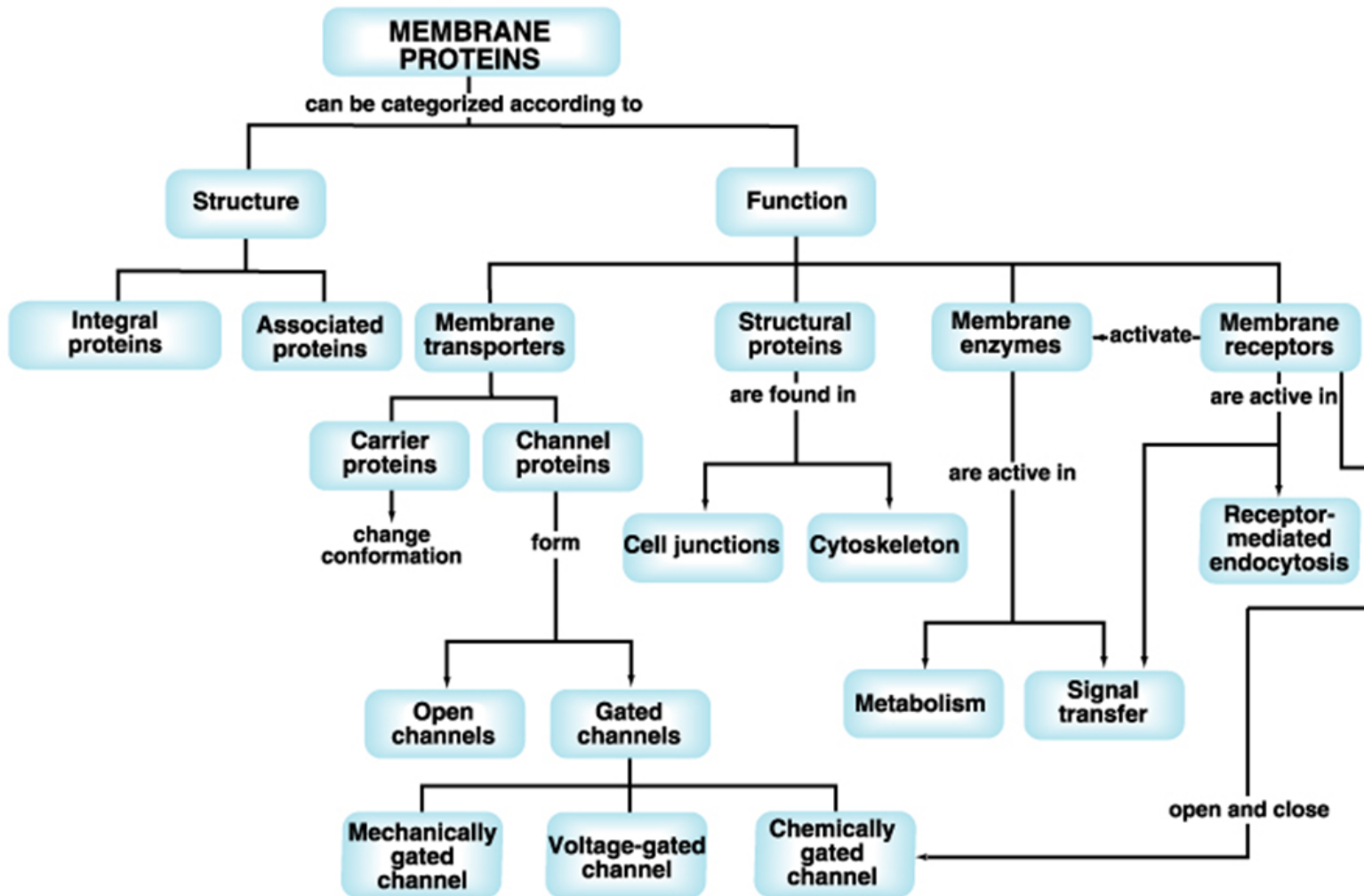
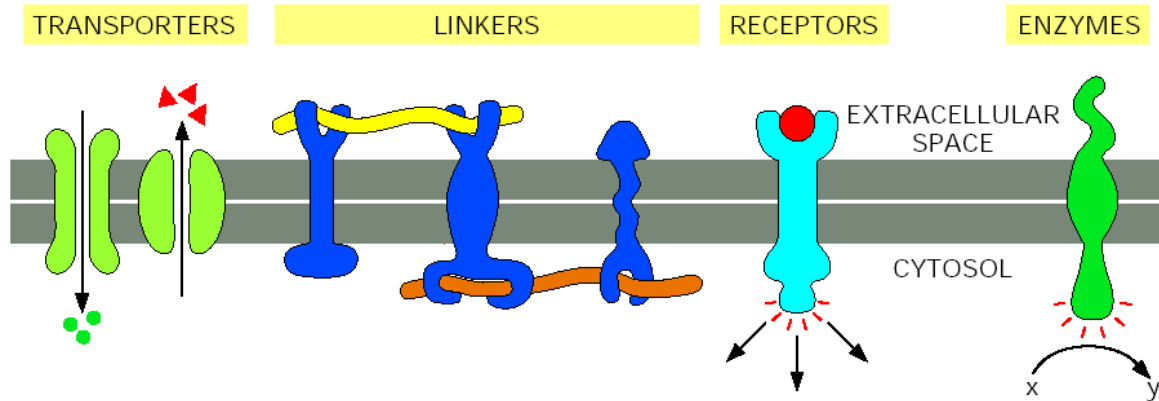


The *fluid mosaic model* of membrane structure proposed by S. J. Singer and G. L. Nicolson. *Membrane is ~ 5nm thick.*



*Membrane proteins  
account for ~30%  
of all proteins.*

# Membrane Proteins





# ***$\alpha$ -helical transmembrane proteins***

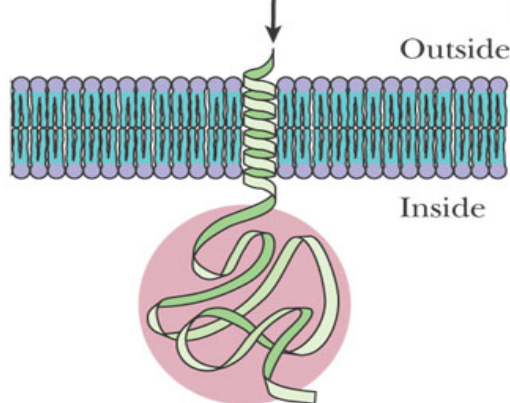
*Sugars are covalently attached to certain amino acid side chains in regions that face outside the cell.*

*The major  
histocompatibility  
(MHC) antigen  
HLA-A2*

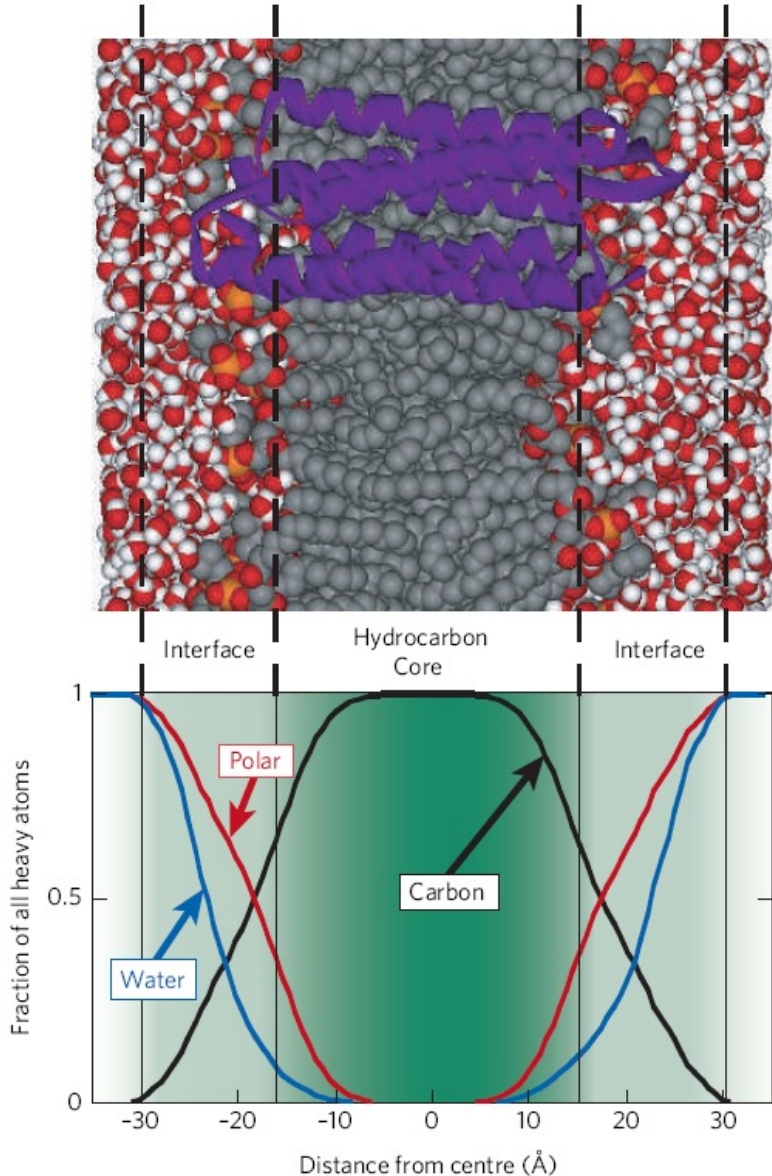


***Polypeptide chain folds  
independently in these  
three regions***

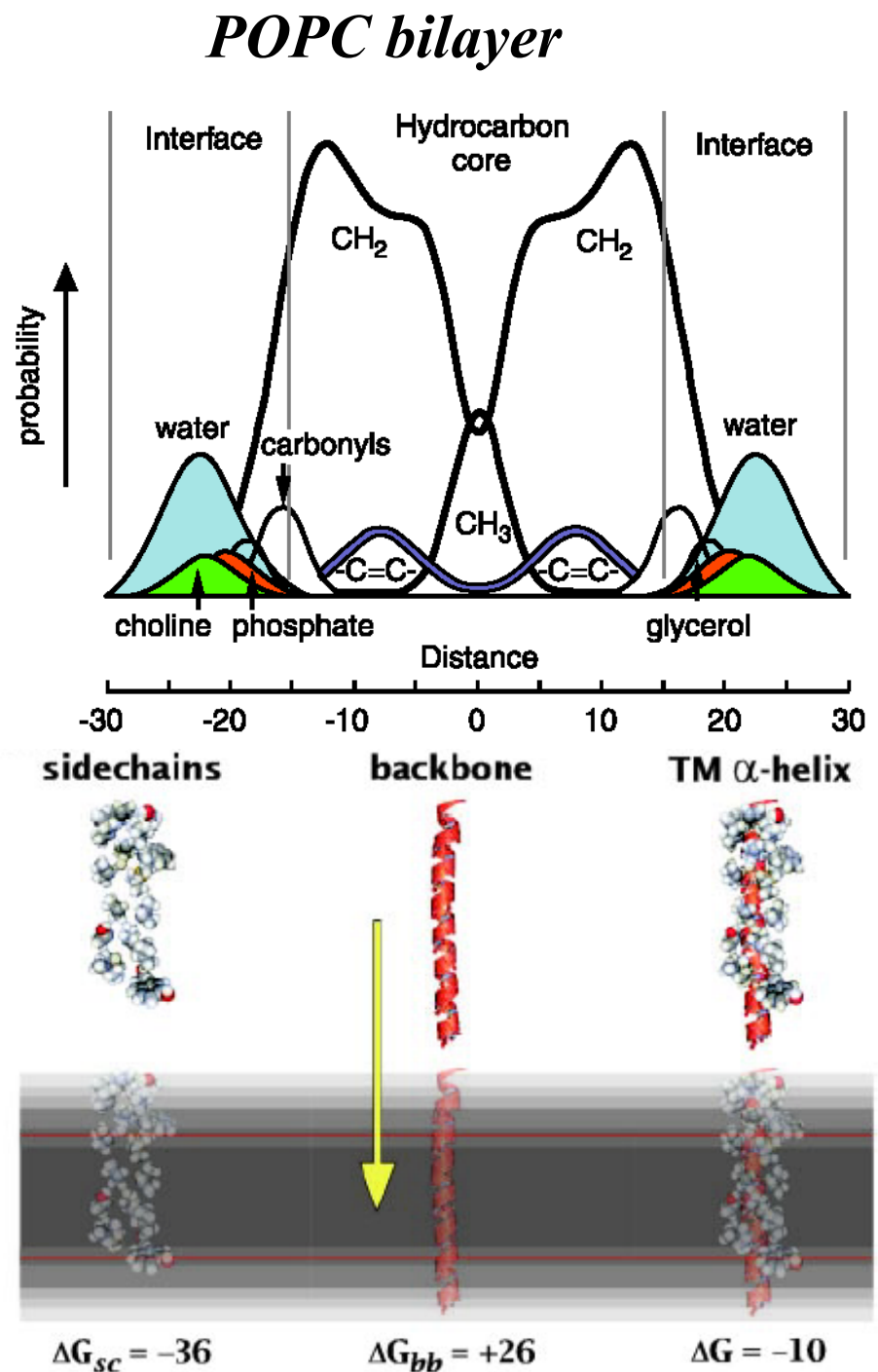
*SH groups facing outside the cell  
are oxidized to S-S.*



*SH groups facing the cytoplasm  
are reduced (by glutathione in  
eucariotes and thioredoxin in  
bacteria).*



*The hydrogen bonds within the protein backbone and amino acids polarity are the determining factor in the intramembrane protein segment.*



# Each amino acid has different hydrophobicity

$$\Delta G_{o \rightarrow w} = -1.36 \log K_p$$

**Table 12.1** Hydrophobicity scales

Amino acid	Phe	Met	Ile	Leu	Val	Cys	Trp	Ala	Thr	Gly	Ser	Pro	Tyr	His	Gln	Asn	Glu	Lys	Asp	Arg
A	2.8	1.9	4.5	3.8	4.2	2.5	-0.9	1.8	-0.7	-0.4	-0.8	-1.6	-1.3	-3.2	-3.5	-3.5	-3.5	-3.9	-3.5	-4.5
B	3.7	3.4	3.1	2.8	2.6	2.0	1.9	1.6	1.2	1.0	0.6	-0.2	-0.7	-3.0	-4.1	-4.8	-8.2	-8.8	-9.2	-12.3

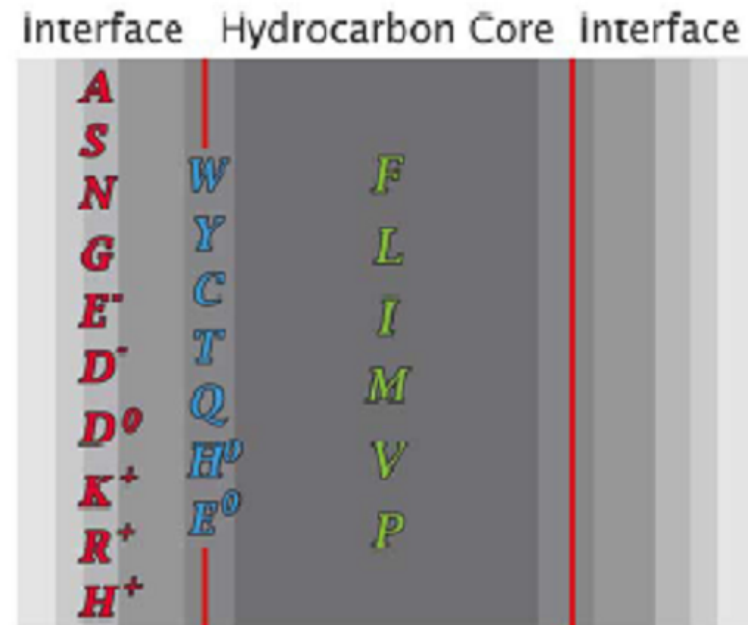
Row A is from J.Kyte and R.F. Doolittle; row B, from D.A. Engelman, T.A. Steitz, and A. Goldman.

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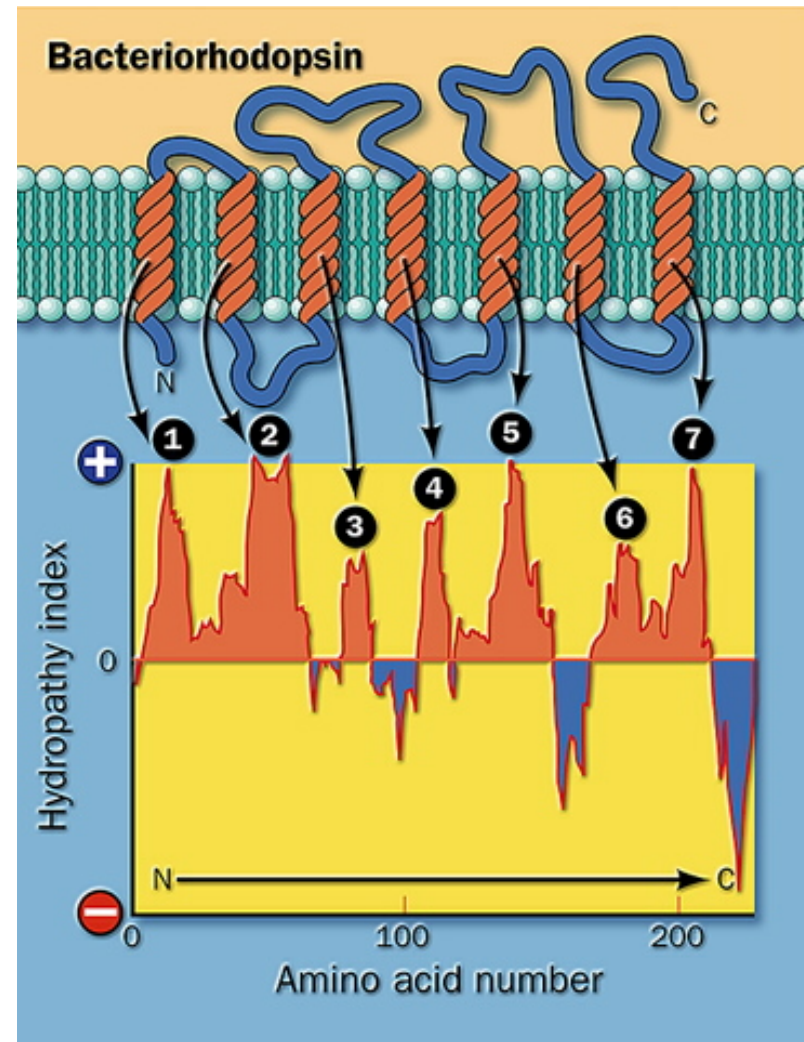
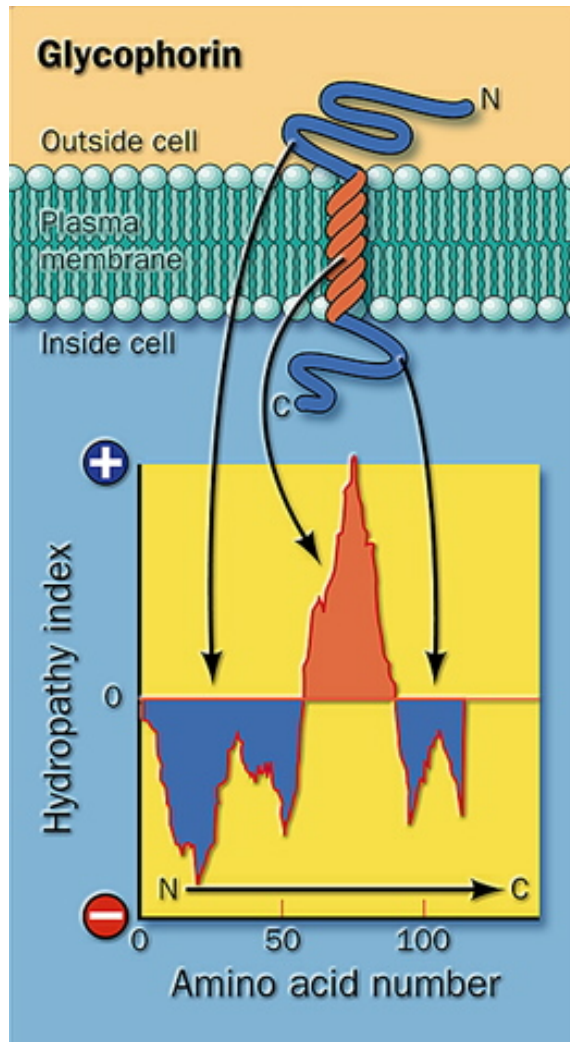
Hydrophobicity index is high for nonpolar side chains

$K_p < 1 \longrightarrow$  Hydrophobic compound

$K_p > 1 \longrightarrow$  Hydrophilic compound



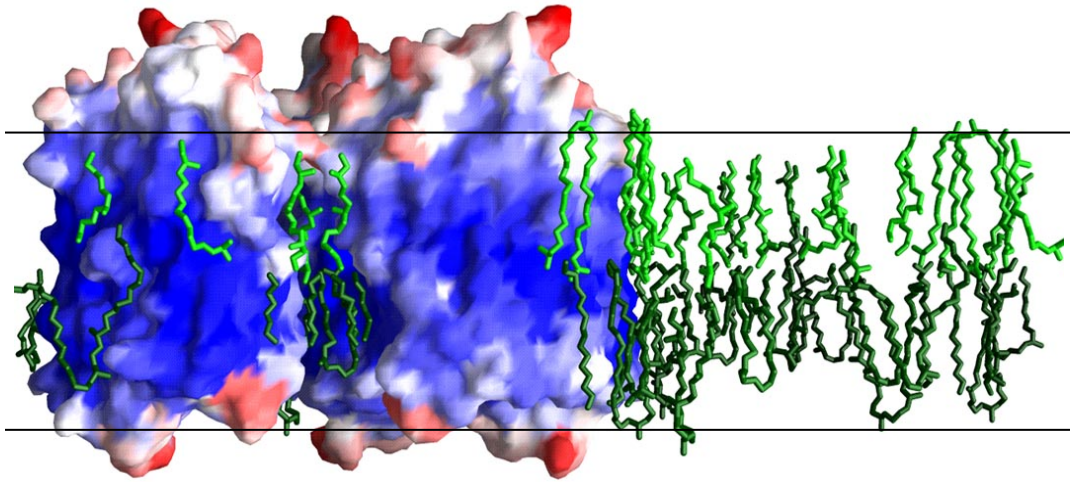
*Hydrophobicity indices plotted against residue number gives a curve that is called hydropathy plot*



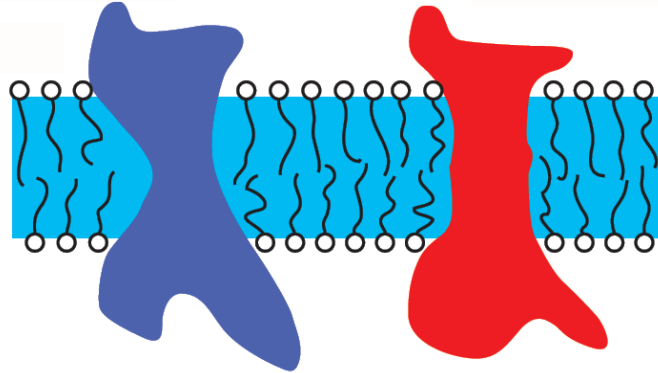
But the reality is not so simple !!!



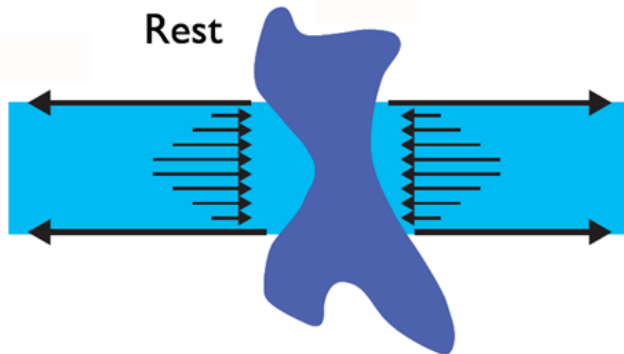
# *The effect of lipid stress on transmembrane proteins*



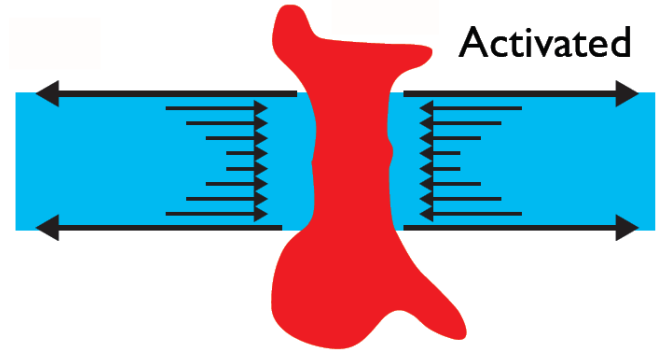
Rest  $\rightleftharpoons$  Activated



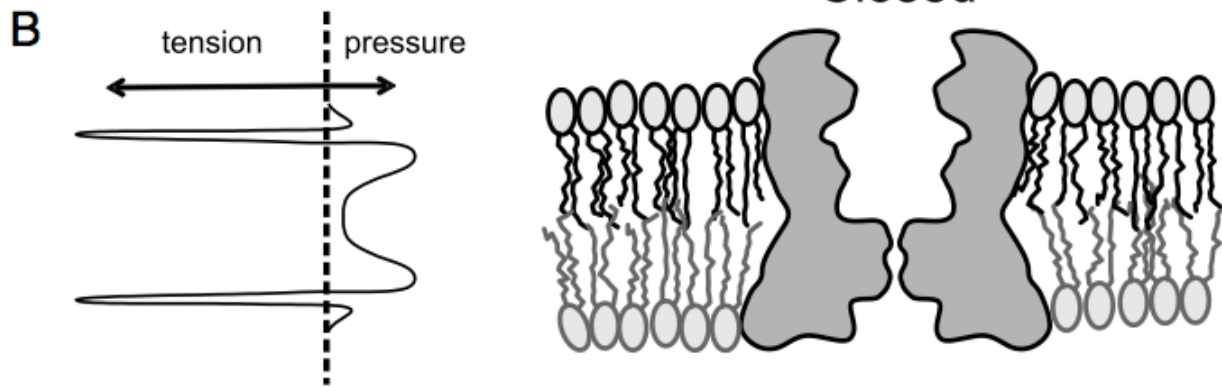
Rest



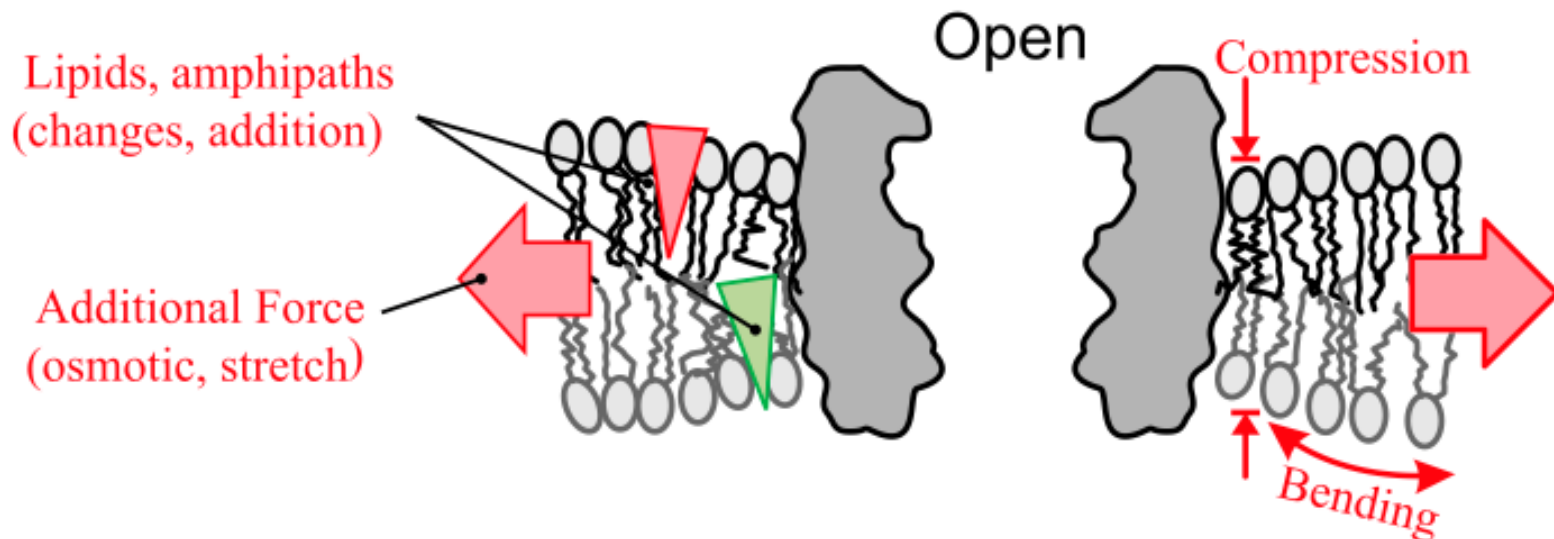
Activated



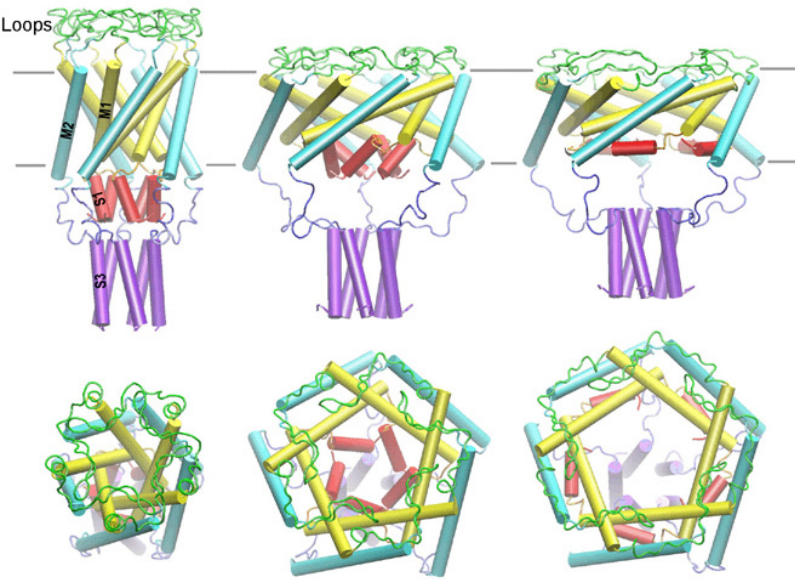




External stretch force and/or amphipaths with positive or negative spontaneous curvature asymmetrically added to one leaflet can thin and bend the bilayer at the protein–lipid interface, changing the force vectors and therefore prefer a better matched protein conformation, say, the open state.



# Mechanosensitive Channels as Osmotic Pressure Relief Valves

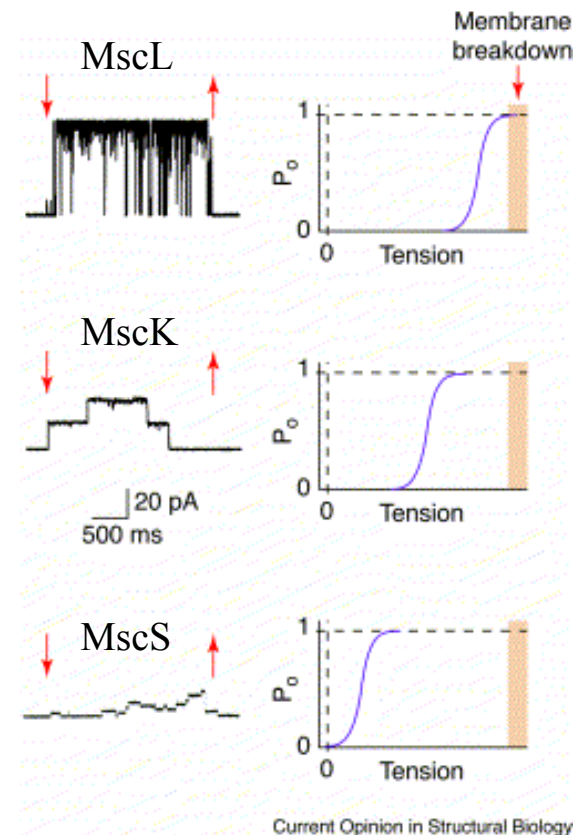
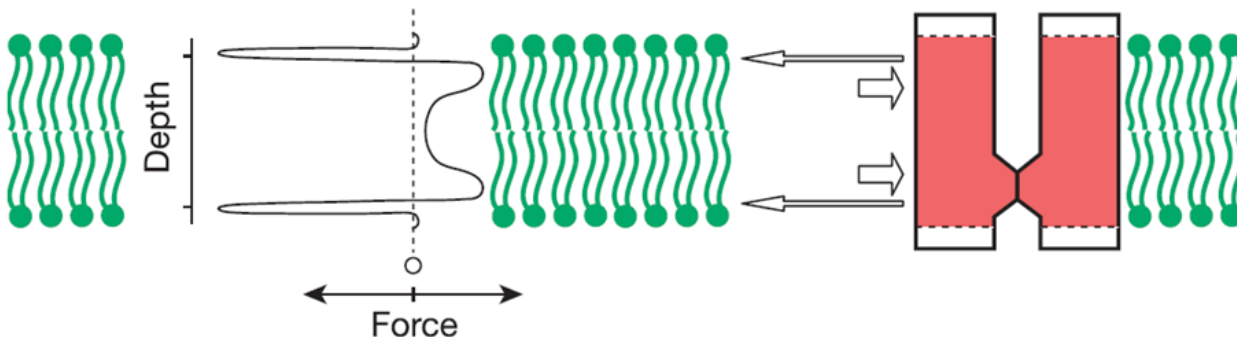


(Sukharev *et al.*)

*Hierarchy of mechanically-gated channels.*

*Gating tension of MscL serves to avoid membrane rupture.*

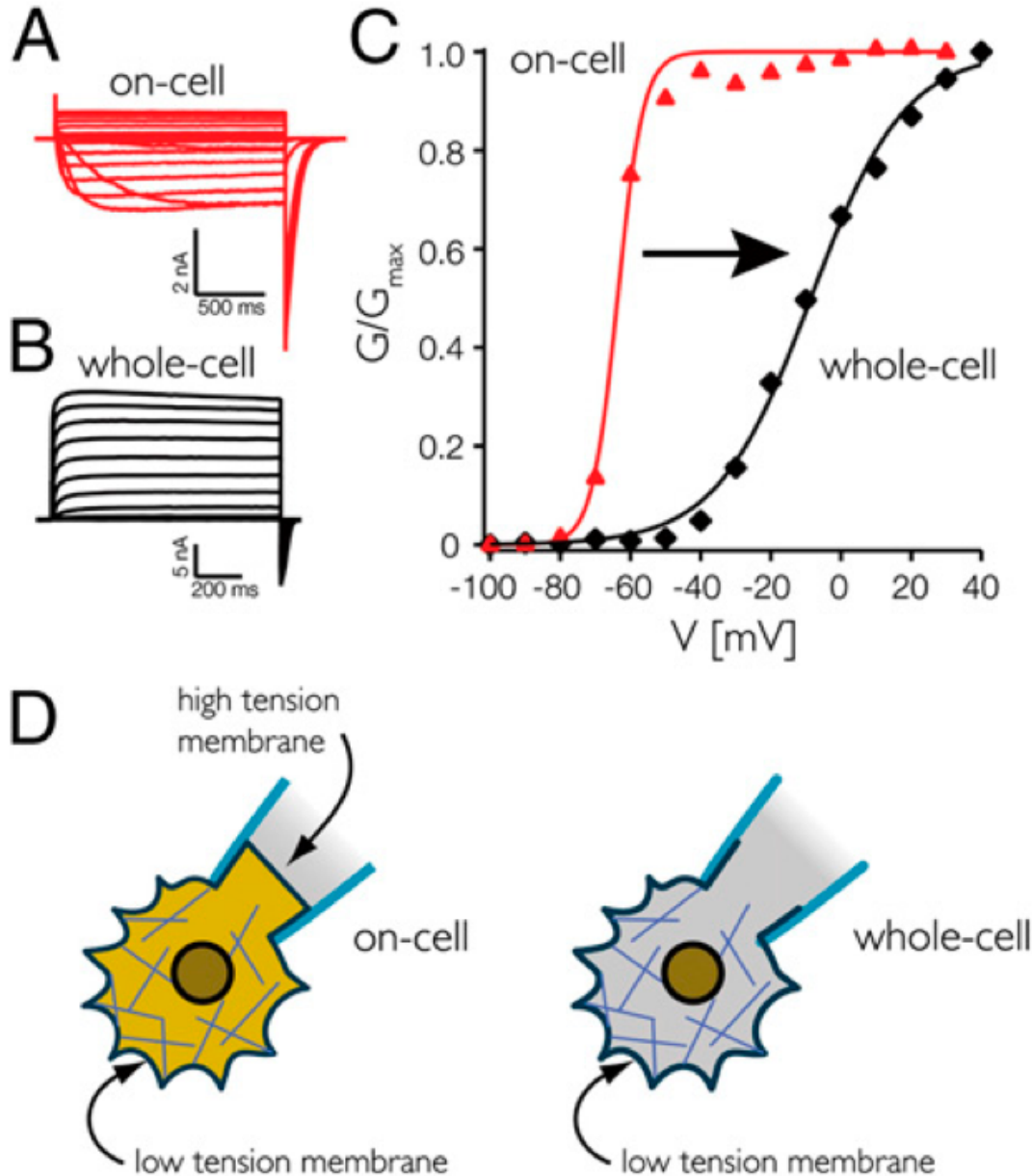
**Key Question: How does mechanical tension couple to the conformational change ?**



Current Opinion in Structural Biology

(Perozo and Rees)

# *The mechano-sensitivity of Kv*



*The Kv1.2 expressed in an Sf-9 cell responds to voltage steps ranging from  $-100$  to  $40$  mV. The responses in on-cell mode (A) are drastically different from those in the whole-cell mode (B).*

*The normalized conductances are graphically compared in C.*



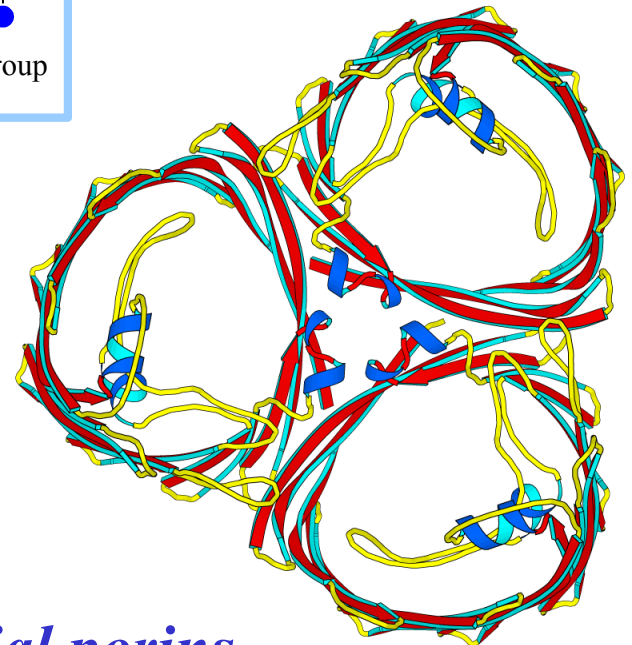
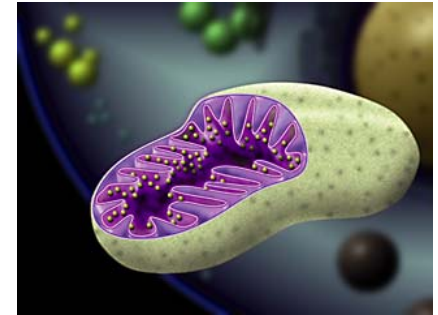
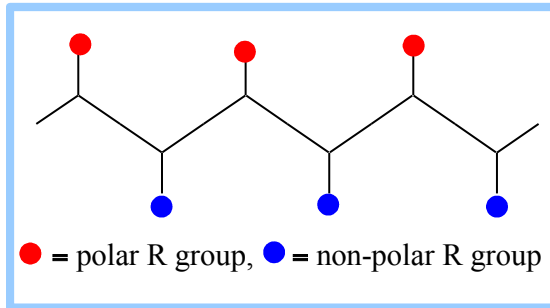
# Porins

✚ The outer membrane of gram negative bacteria, or in the outer membranes of mitochondria in eukaryotes.

✚ Diameter 40 Å; 30 – 50 Å high; mass 30-50 kD.

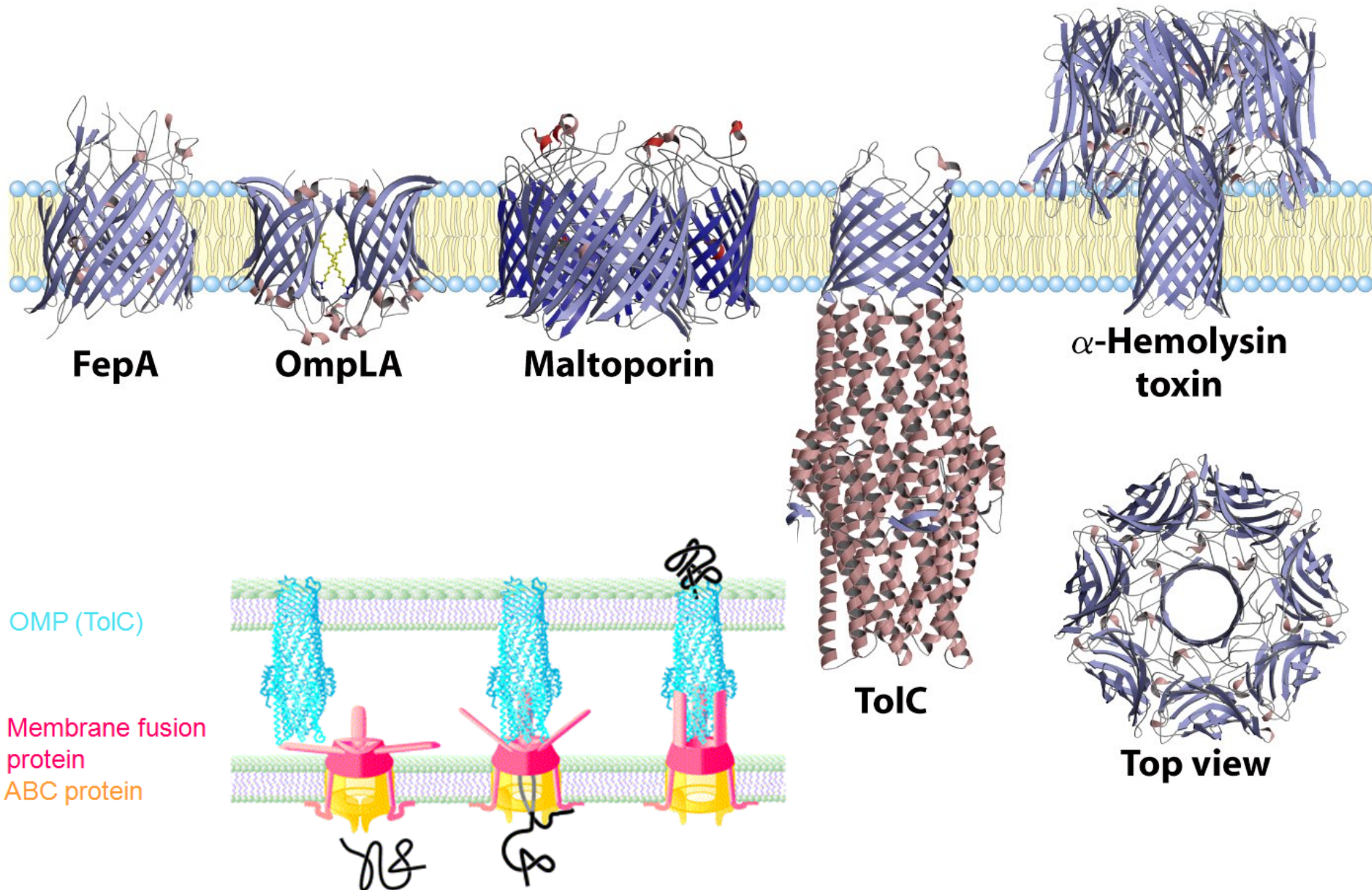
✚ The hydrophobic, lipid-exposed surface of a  $\beta$ -barrel is about 27 Å thick.

✚ Membranes are spanned by  $\beta$ -strands of 9–11 residues with a tilt of 20–45° out of the TM axis.



*Quaternary structure of bacterial porins*

# *Membrane Proteins with $\beta$ -Barrel Structure*

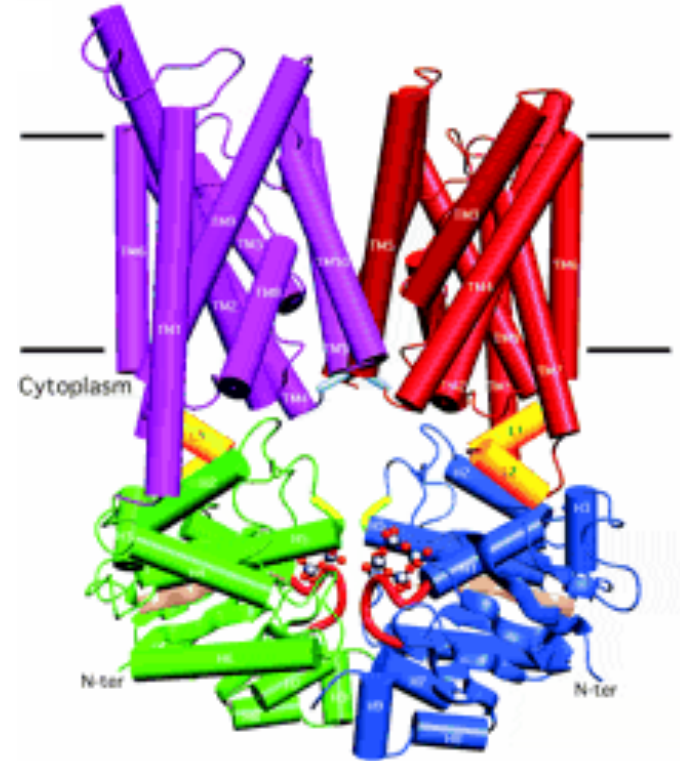


# *ABC transporters*

Largest class of membrane proteins known to pump various substrates ranging from chloride ions to vitamin B<sub>12</sub> into or outside of cell.

## *Vitamin B<sub>12</sub> Transporter – BtuCD – a tetrameric protein*

- ❑ *Cystic fibrosis* (CFTR) – cystic fibrosis transmembrane regulator  
-defect in ABC genes.
- ❑ *Cancer* - multidrug resistance proteins (e.g. MDR1 and MDRP1-over expression in tumor cells causes resistance to chemotherapeutic agents).
- ❑ *Bacterial multidrug resistance* - involved in export of harmful substances out of cell.

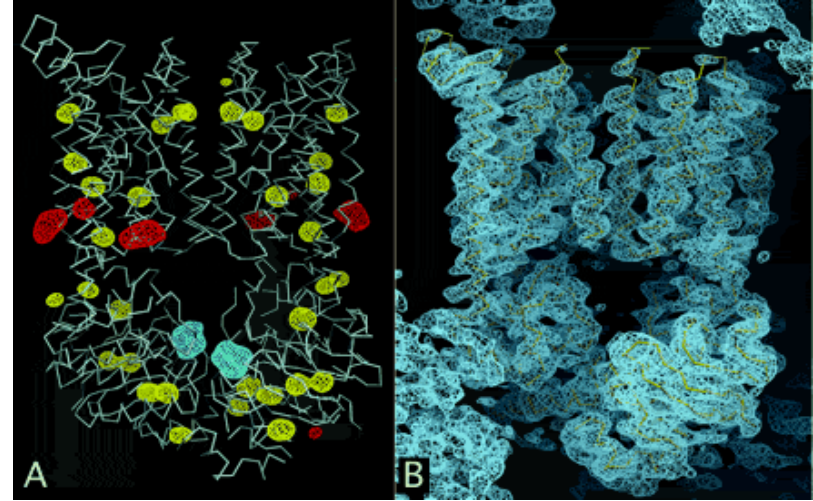


*The E. coli BtuCD structure: a framework for ABC transporter architecture and mechanism. Locher KP, Lee AT, Rees DC, Science 2002 May 10;296(5570):1091-8*



# Structure

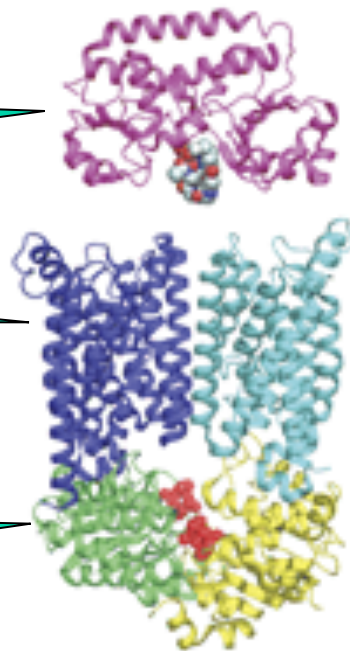
- Tetrameric protein
- 2 membrane spanning domains (BtuC)
- 2 ATP binding cassettes (BtuD)



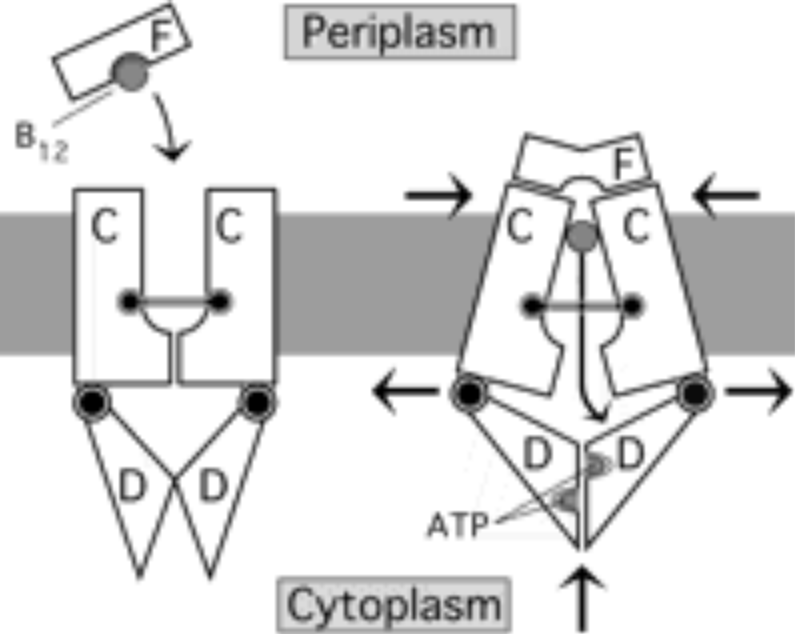
*BtuF periplasmic binding protein delivers  $B_{12}$  to the mouth of BtuC*

*BtuC (membrane spanning domain)*

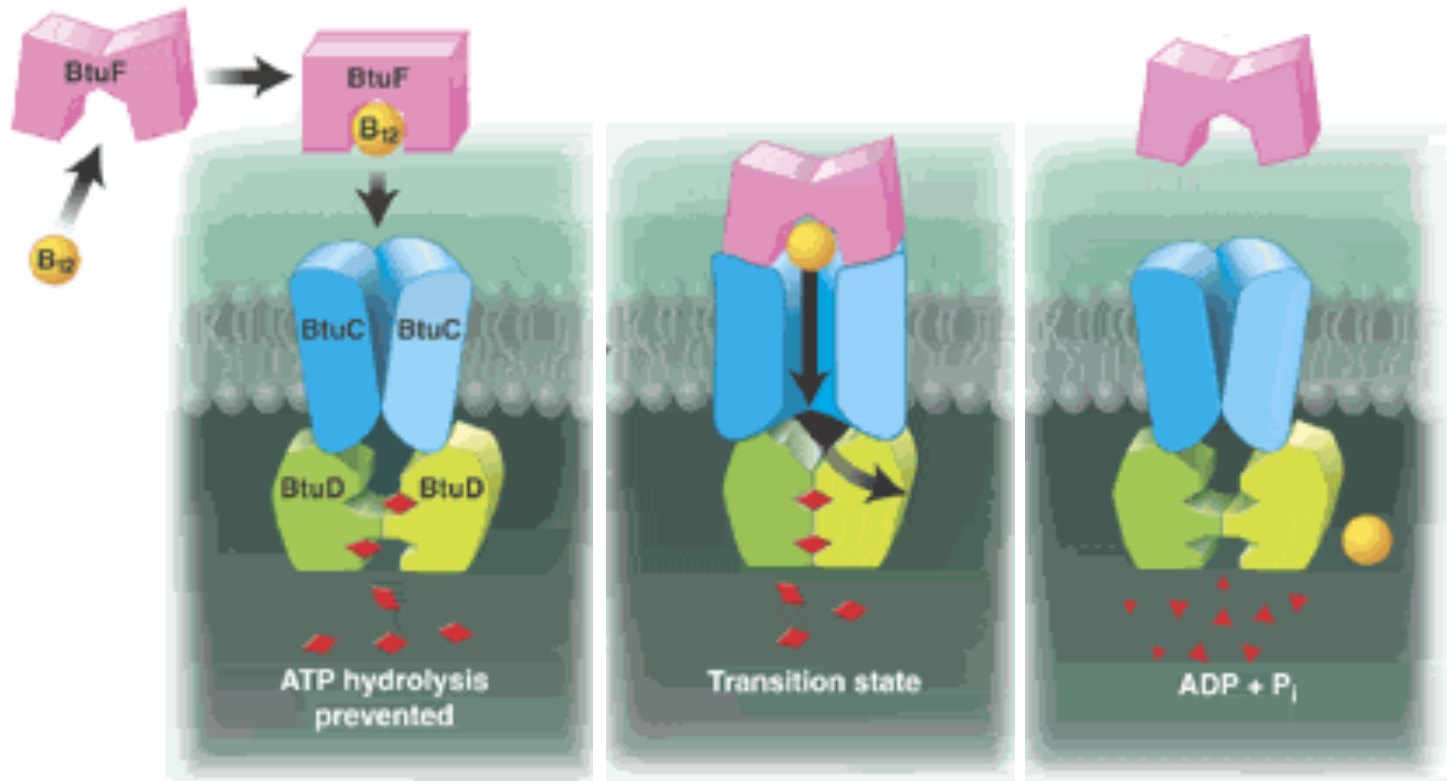
*BtuD(ABC Cassettes) located just below the membrane surface*



This molecular assembly is  $\sim 90$  Å tall,  $\sim 60$  Å wide and 30Å thick.



*The transport mechanism for Vitamin  $B_{12}$*



# *Non-constitutive membrane proteins*

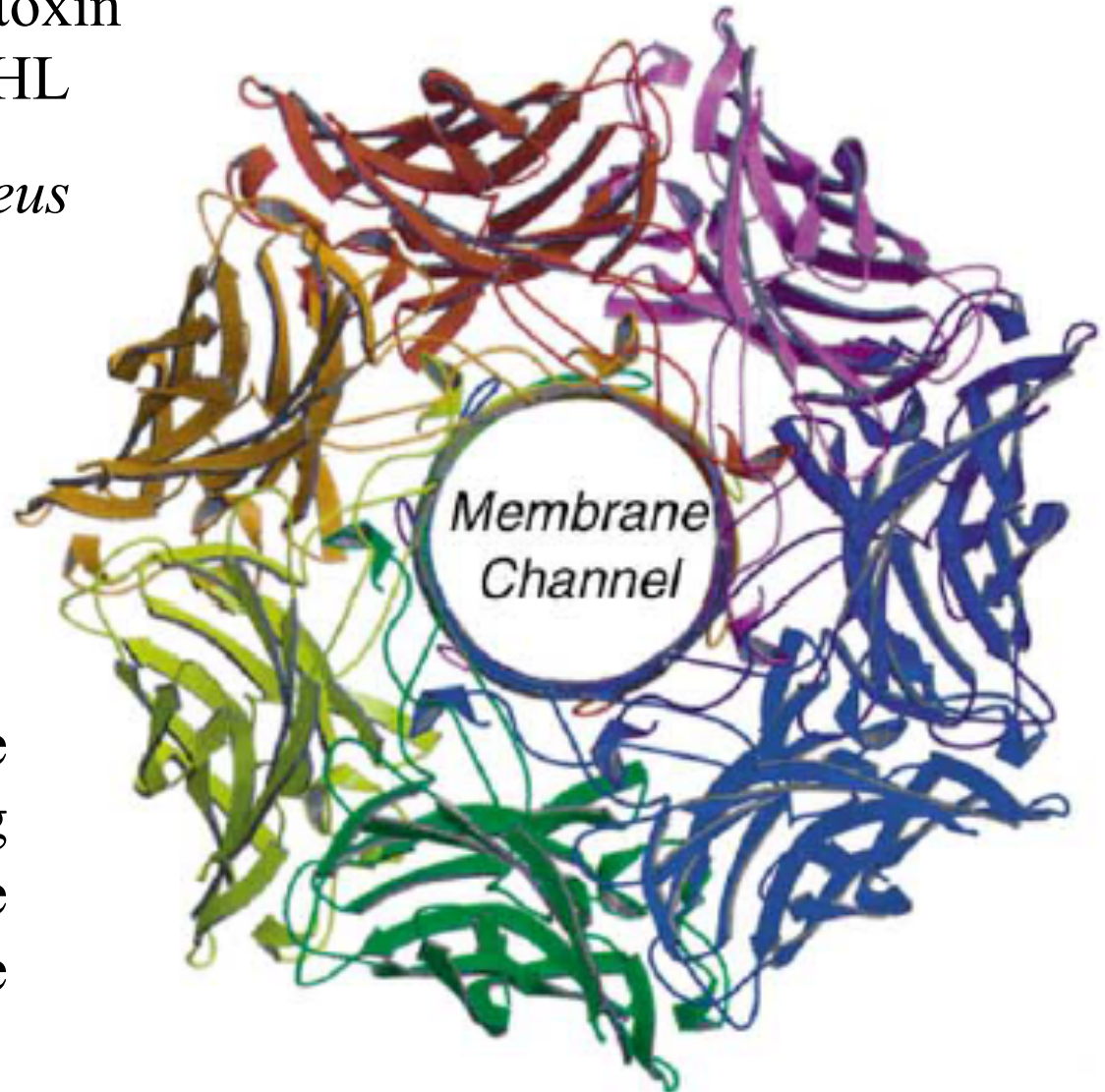
Protein pore-forming toxin

Alphahemolysin  $\alpha$ -HL

*Staphylococcus aureus*

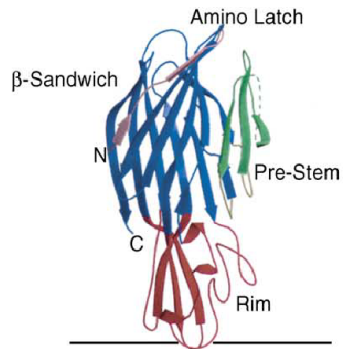
✚ The heptamer is 100 Å tall and 100 Å at its widest point.

✚  $\alpha$ -HL can self-assemble into a lipid bilayer creating an aqueous nanopore (inside diameter ~2 nm) across the lipid membrane.





The water-soluble form of the toxin (LukF)



LukF

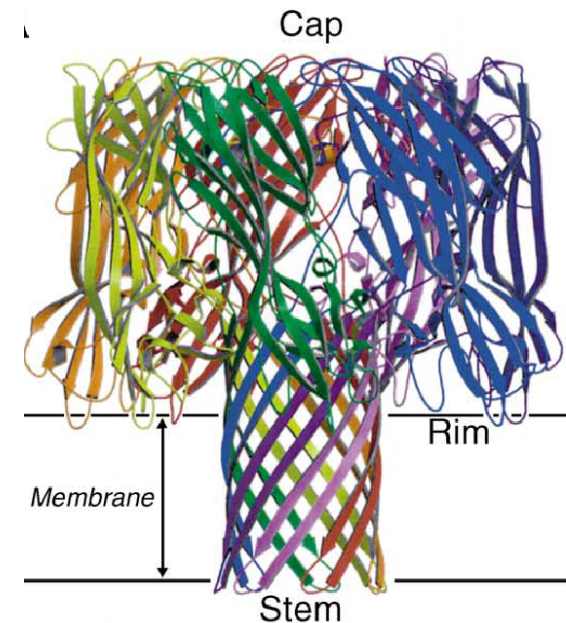
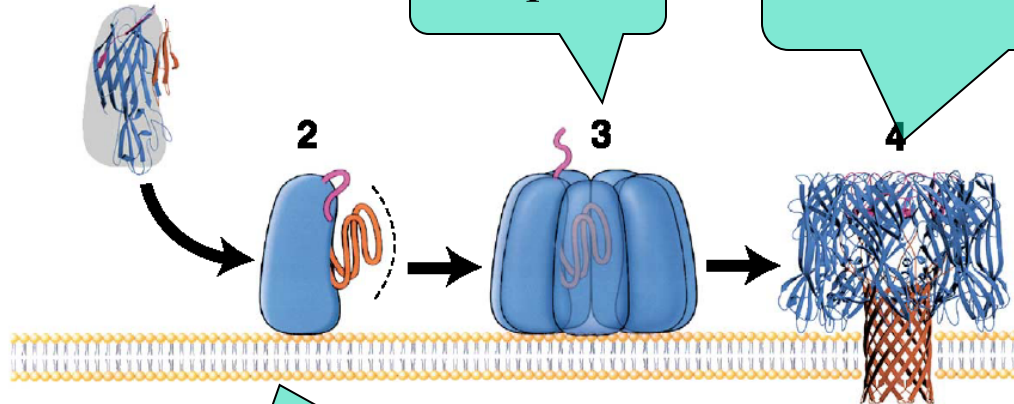
## $\alpha$ HL assembly

Prepore

The assembled heptamer

The membrane-bound monomer

*$\alpha$ HL buries  $\approx 154$  residues in the membrane, the total effect on the decrease of free energy is so great that pore formation is essentially irreversible.*



$\alpha$ HL