BCHS 6229
Protein Structure and Function

Lecture 10 (Nov 10, 2011)

Special Topics (I)

Membrane proteins

Growth and excitement in membrane protein structural biology

Global collaborative initiatives that work on membrane proteins: the **NIH Roadmap and Protein Structure Initiative**, EU-funded projects such as EDICT, SPINE, BioXhit, the SGC and the Riken Protein-3000 project in Japan.

…behind the obvious technological advances in membrane protein biochemistry and structure determination, there are also other less tangible factors that are equally important: the importance of learning everything there is to know about a specific membrane protein and really getting to know the subtle chemical and biological nuances that exist. This requirement is closely coupled to the overwhelming feeling in the whole field today that anything is now possible.

Key Concepts

- Membrane functions : selective permeability barriers, information processing, organization of reaction sequences, energy conversion

- Lipids (lipid bilayer) responsible for permeability barrier

- Proteins perform essentially **all other membrane functions**, including modulation of permeability barrier by allowing or assisting some solutes to cross membrane (transport processes)

Membrane Proteins

- Membrane protein functions:
  - "pumps" (active transport)
  - "gates" ("passive" transport, facilitated diffusion)
  - receptors
  - signal transduction
  - enzymes
  - energy transduction
- Membrane protein distribution:
  - Both amount of protein in general and which specific proteins are present varies with function of membrane, i.e., with type of membrane and with cell type.
  - Examples:

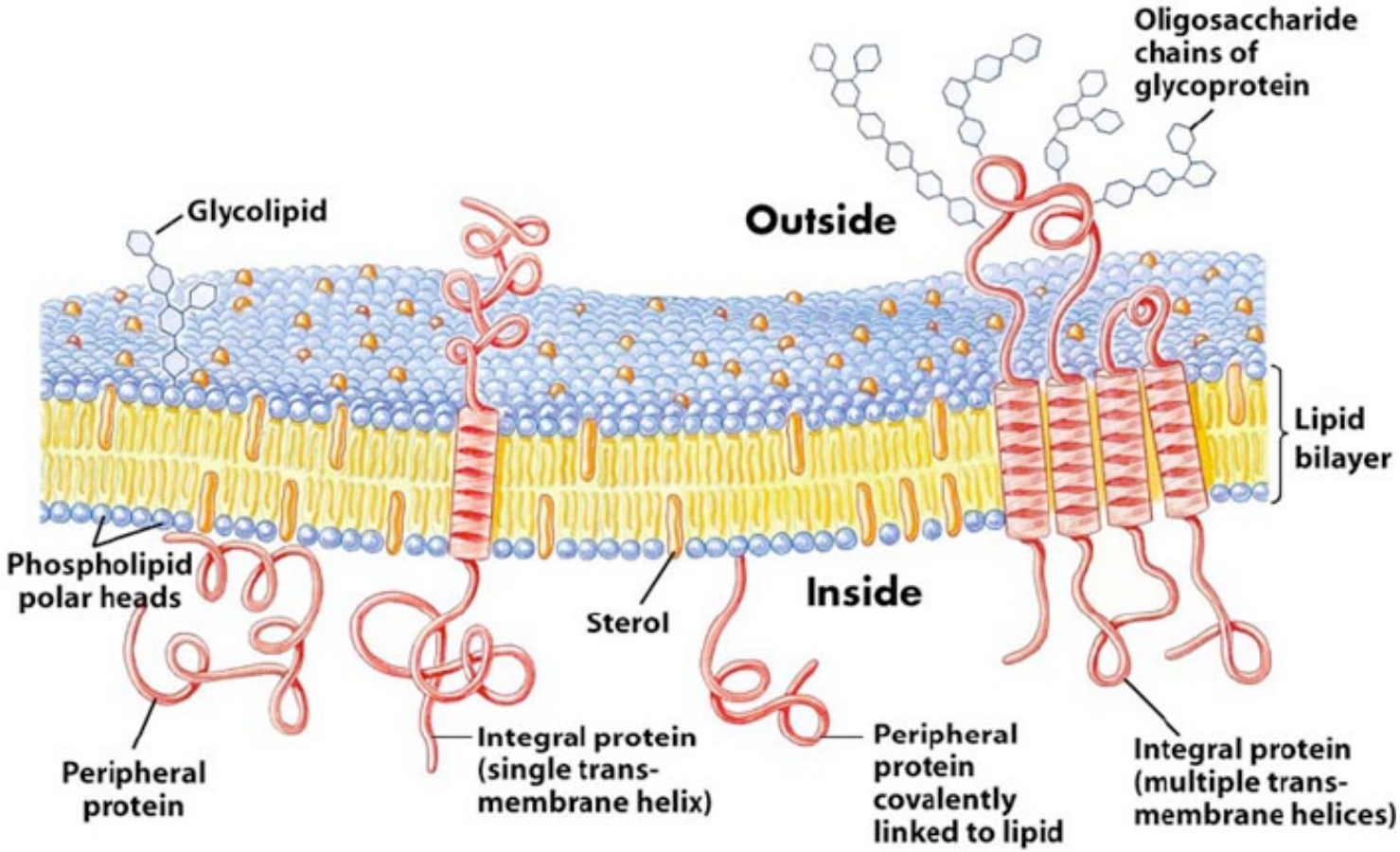
* myelin (membrane around myelinated nerve fibers, function=electrical insulation) mostly lipid (only ~18% protein)
* plasma membrane: enzymes, receptors, etc. (~50% protein)
* mitochondrial inner membrane and chloroplast thylakoid membrane: electron transport, energy transduction (ATP synthesis) (~75% protein)

Membrane Proteins

- Terminology (as applied to membrane proteins): Peripheral, integral, lipid-anchored (operational definition -how can they be extracted from membrane?); α-helical transmembrane proteins, β-barrel transmembrane proteins (structural definition)

- Explain in structural terms how an integral membrane protein can deal with its polar backbone groups in spanning the hydrophobic core of a lipid bilayer.

- Discuss the structural properties of the examples of membrane proteins: the type(s) of secondary structure and types of R groups found in the transmembrane (membrane-spanning) structural components of these membrane proteins.



Nelson & Cox, *Lehninger Principles of Biochemistry*, 4th ed., Fig. 11-3

Membrane proteins

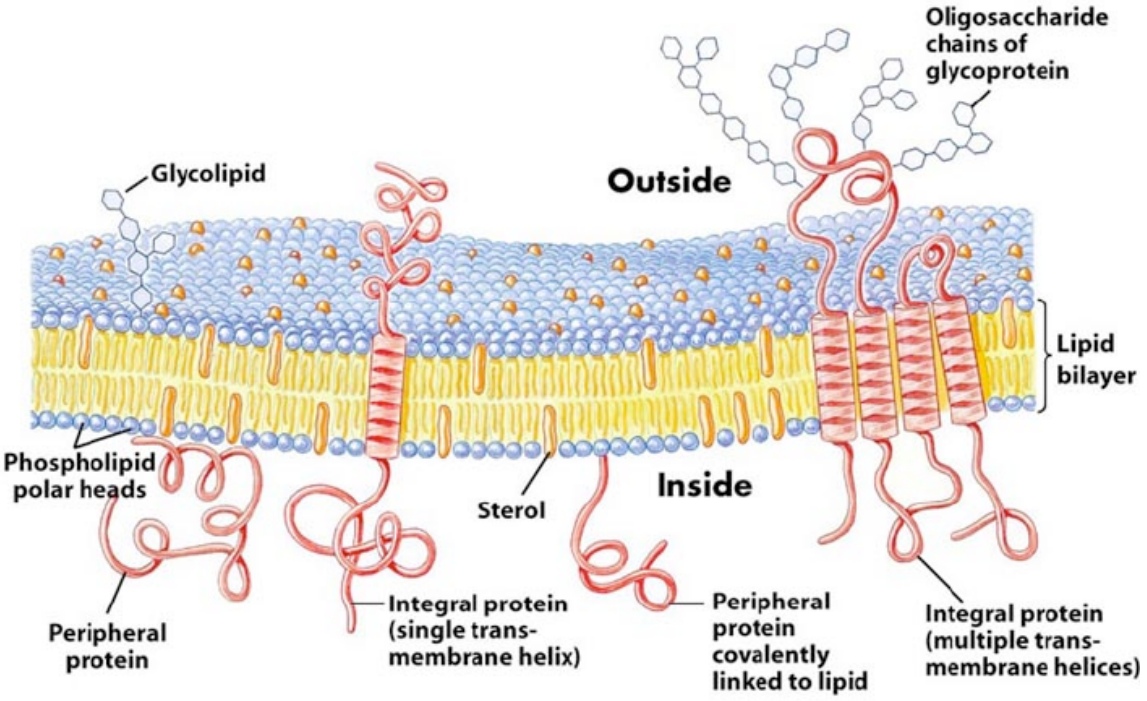
- **3 types based on association with membrane:**
  1. **Peripheral**
  2. **Integral**
  3. **Lipid-anchored**

1. Peripheral membrane proteins

- **weakly associated** with membrane at ***surface***
- bind to polar lipid heads and/or to integral membrane proteins
  - electrostatic interactions predominate (ionic bonds and/or hydrogen bonds)
  - easily extractable from membranes by high salt concentrations (disrupting electrostatic interactions), or by EDTA (chelates Ca^{2+} and Mg^{2+})
- usually water-soluble (globular)
- There can also be fibrous proteins attached to membrane surface (cytoskeletal proteins).
- Some peripheral "membrane" proteins come and go from membrane, e.g., using reversibly attached lipid anchor.
  - Covalently attached C14 fatty acyl chain (myristoyl group) slips into lipid bilayer, holding protein to surface of membrane.

2. Integral membrane proteins

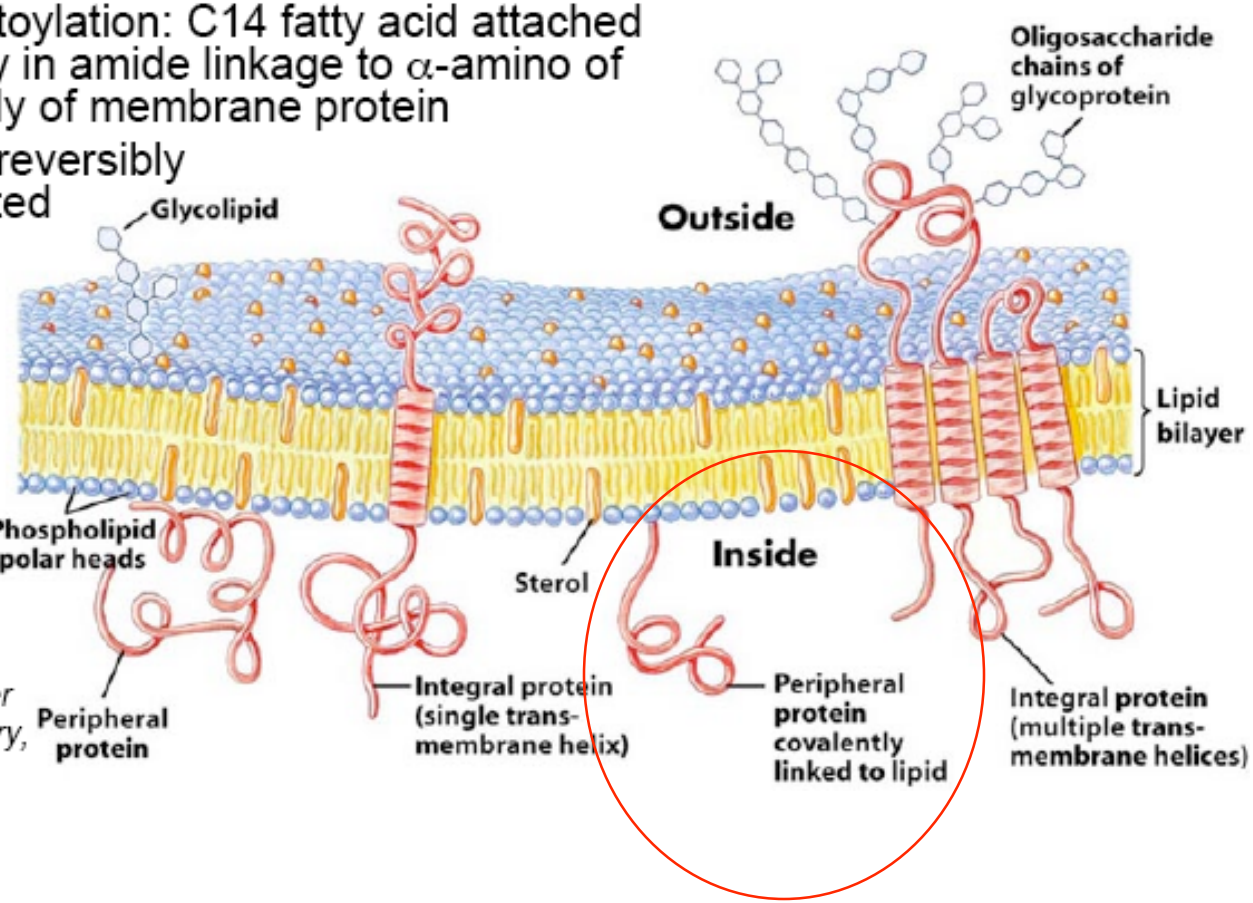
- **tightly bound** to membrane - interact with interior (membrane core, lipid tails) (**hydrophobic interactions**)
- require detergents (or organic solvents) for extraction from membranes
- water-insoluble
- Some proteins have both peripheral and integral domains.
- Some completely span membrane ("transmembrane proteins").
- ***Glycoproteins*** always have carbohydrates on **extracellular side**.



Nelson & Cox, *Lehninger Principles of Biochemistry*, 4th ed., Fig. 11-3

3. Lipid-anchored membrane proteins

- Proteins that are **covalently linked to lipids**, a requirement for their **association with the membrane** (lipid inserts into membrane, associating protein with membrane)
- Some lipid anchors can be **reversibly attached to/detached from proteins**.
  - **"switching device" to *alter affinity of protein for membrane***
- role in **signal transduction** pathways in eukaryotic cells
  - e.g., N-myristoylation: C14 fatty acid attached enzymatically in amide linkage to α-amino of N-terminal Gly of membrane protein
  - examples of reversibly N-myristoylated proteins:
  - **PKA** (cAMP-dependent protein kinase)
  - **α subunit of G proteins**



Nelson & Cox, *Lehninger Principles of Biochemistry*, 4th ed., Fig. 11-3

Monotopic membrane proteins

Cyclooxygenases
Squalene-Hopene Cyclases
Peptidoglycan Glycosyltransferases
ADP-Ribosylation Factors
Etc.

β-barrel transmembrane proteins

- β-barrel membrane proteins: multimeric (porins and relatives)
- β-barrel membrane proteins: monomeric/dimeric (multimeric)
- β-barrel membrane proteins: mitochondrial Outer Membrane
- Omp85-TpsB Outer Membrane Transporter Superfamily
- Adventitious Membrane Proteins: Beta-sheet Pore-forming Toxins

α-helical transmembrane proteins

- Adventitious Membrane Proteins: Beta-sheet Pore-forming Toxins
- Outer Membrane Proteins
- G Protein-Coupled Receptors (GPCRs)
- Bacterial and Algal Rhodopsins
- SNARE Protein Family
- Integrin Adhesion Receptors
- Channels: K+ and Na+ Ion-Selective
- Channels: other ion channels
- Gap junctions
- Sec proteins (Membrane Proteins Involved with Protein Insertion and Secretion)
- Oligosaccharyltransferases (OST) paper#5
- Multi-Drug Efflux Transporters

and many many more …

Transmembrane (TM) Proteins

How does an integral membrane protein accommodate its polar backbone (peptide N-H and C=O groups) in a stable way across hydrophobic core of lipid bilayer????

• By H-bonding as many as possible of polar backbone groups (really ALL of them), *i.e.*, by forming secondary structures, either α-helical or β barrel motifs for membrane-spanning parts of transmembrane protein.

Examples of TM Proteins demonstrating TM secondary structural motifs:

1. glycophorin A (erythrocyte membranes)
2. bacteriorhodopsin (purple membrane of Halobacterium halobium, a salt loving bacterium)
3. prostaglandin H2 synthase (COX, enzyme involved in biosynthesis of prostaglandins/inflammatory response)
4. porins (channel-forming proteins -- outer membranes of gram-negative

Well, basically all…

Glycophorin A found in red blood cells, a TM protein.

has a single transmembrane α-helix

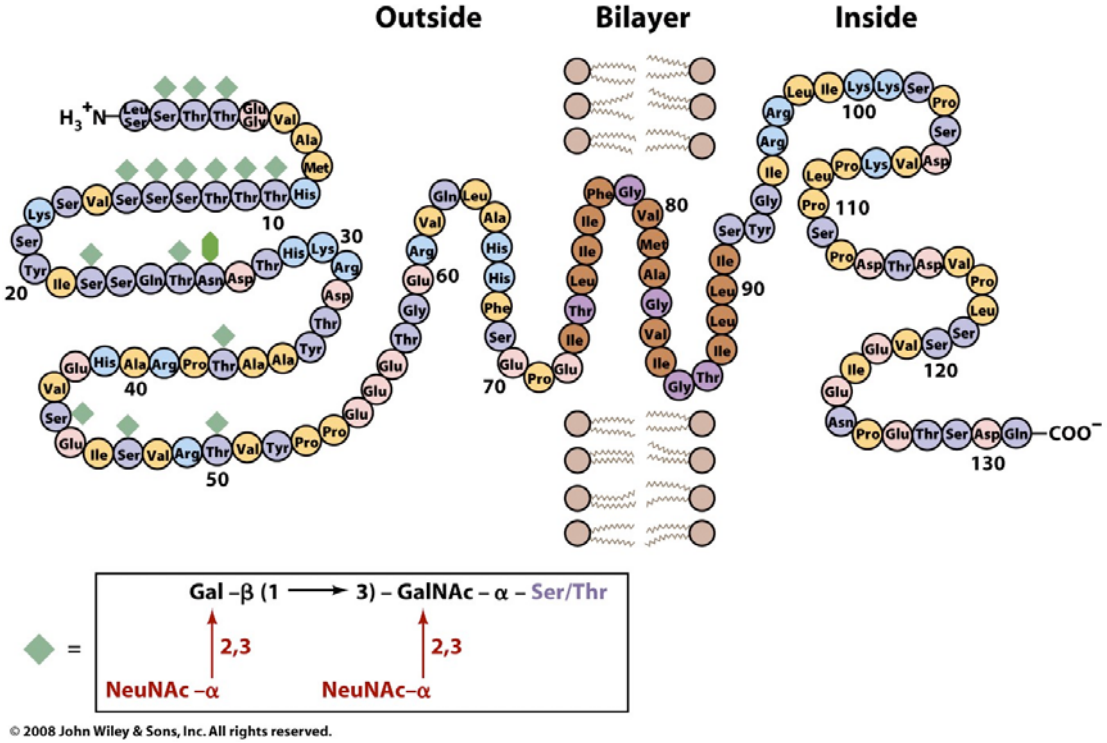
• integral membrane protein in erythrocytes

• total MW ~31,000 but only 131 aa residues

• Remember, average "residue mass" of an amino acid residue in a protein is about 110, so glycophorin A would have MW about 14,400.

• Where does additional mass come from?

• Glycophorin A is a glycoprotein by mass ~60% carbohydrate, ~40% protein.



• Most of protein (N-terminal portion) on outside of cell, exposed to water; mainly hydrophilic residues, heavily glycosylated (lots of carbohydrates in glycosidic bonds to specific Ser, Thr, and Asn residues)

• Carbohydrates include ABO and MN blood group antigen-determining structures.

• Extracellular part of protein also receptor for influenza virus binding to cells

• C-terminal portion on cytosolic side of membrane, interacts with cytoskeletal proteins

• One 19-AA residue hydrophobic segment is exactly right length to span membrane if it's coiled into an α-helix -- with hydrophobic R groups oriented outward, toward "solvent" (hydrophobic core of lipid bilayer).

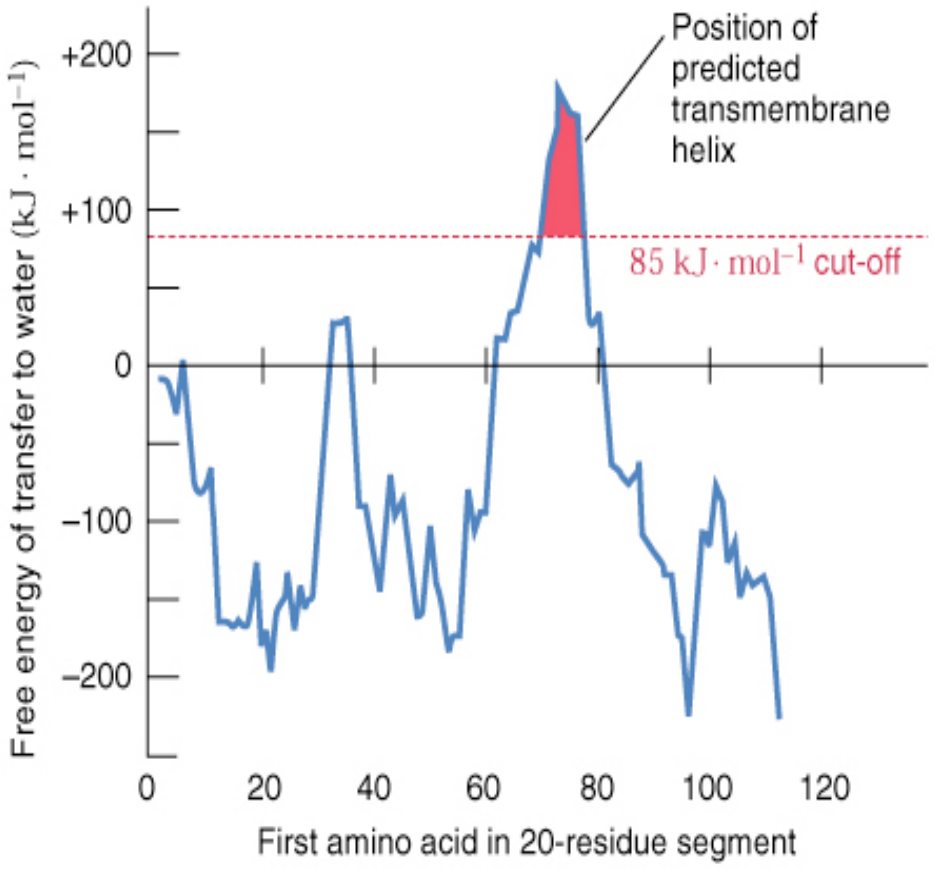
Hydrophobic 19-20 amino acid sequences are a very common way for proteins to span biological membranes, in α-helical conformation.

Polar peptide backbone groups (carbonyl oxygens and amide N-H groups) fully H-bonded

– Hydrogen-bonding "neutralizes" these polar groups, and

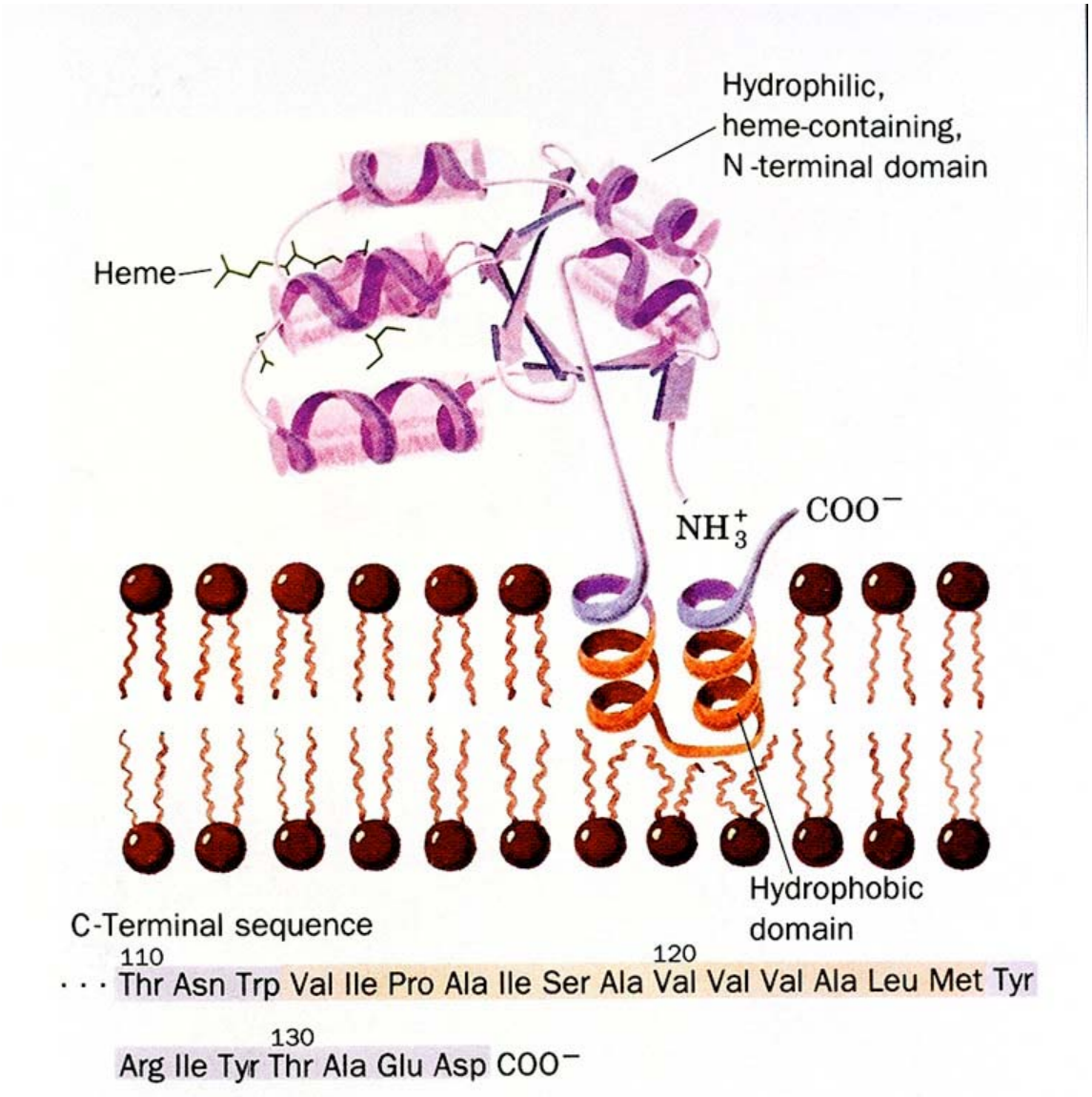
– "screens" them from contact with lipid core by R groups on outside of helix.

• hydrophobic core of membrane (2X length of tails) is ~30 Å across, so 20-residue α-helix (20 residues x 1.5 Å "rise" per residue) is right length to reach across hydrophobic core.

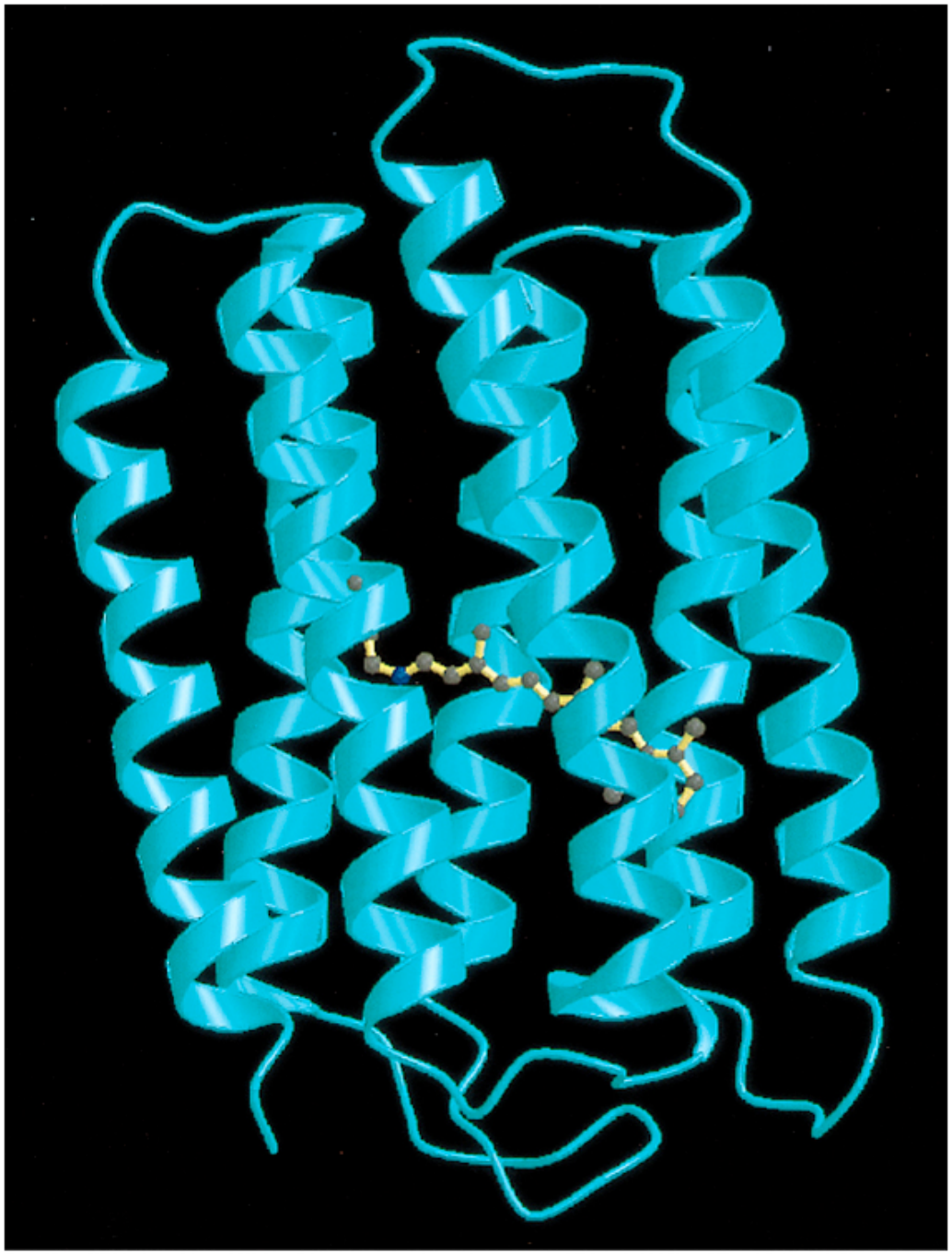


After Engleman, D.M., Steitz, T.A., and Goldman, A., *Annu. Rev. Biophys. Biophys. Chem.* 15, 343 (1986).

A transmembrane helix is characterized by a hydrophobic stretch of 19 amino acids. The hydrophobicity plot is a sliding sum of free energy of transfer for seven amino acids to an aqueous media. The large positive peak indicates the position of the transmembrane helix.



Cytochrome b5 is anchored to the membrane an is a small electron transfer protein involved with supplying electrons to fatty acid desaturase.

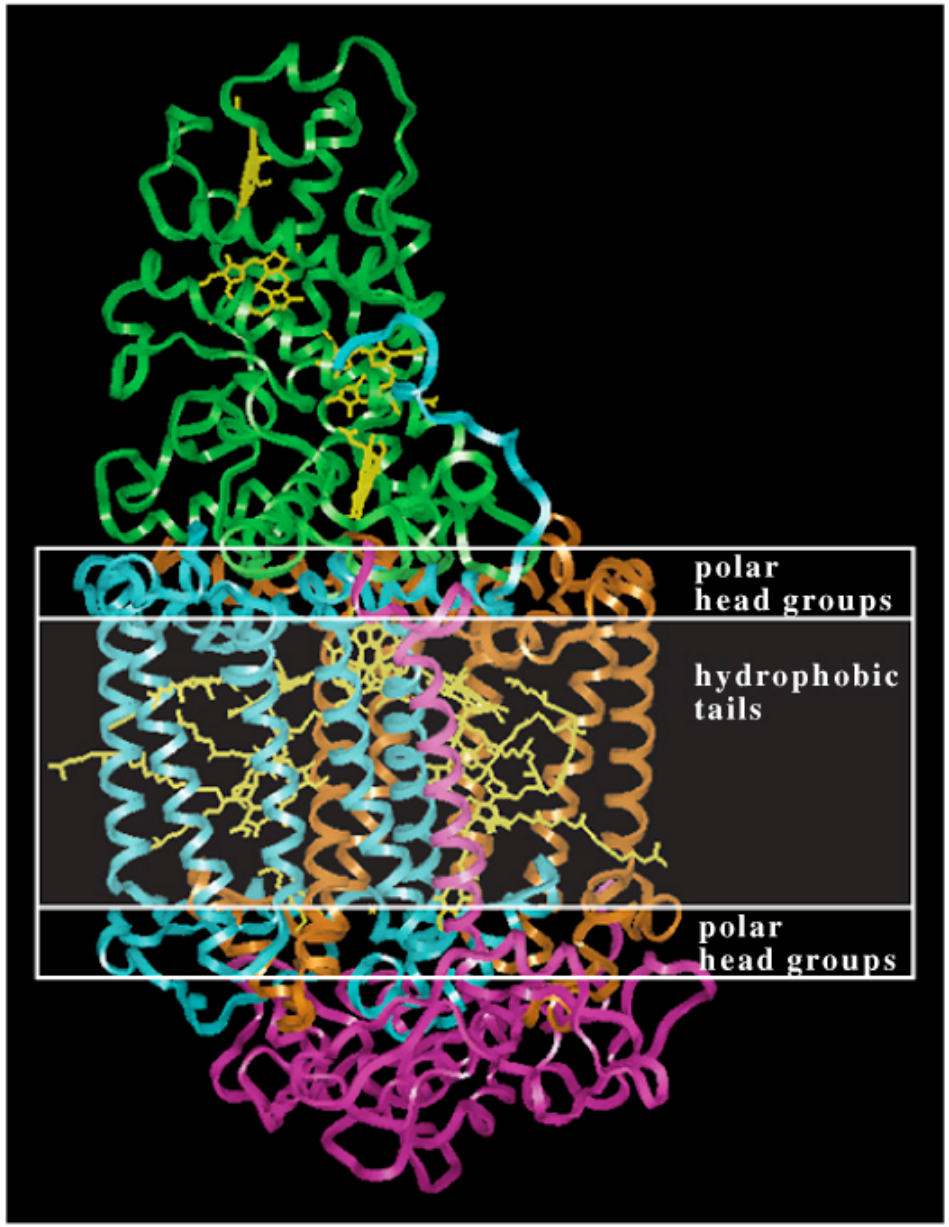


Courtesy of N. Grigorieff and Richard Henderson, MRC Laboratory of Molecular Biology, Cambridge, England.

Bacteriorhodopsin, contains many transmembrane helicies. Its function is to absorb light energy and use it to form a pH gradient by pumping protons into the cytoplasm of the bacteria *Halobacterium holobium*. The same function is used to detect light impulses in our eyes, but instead of proton pumping the signal is converted to nerve impulses. The opsin cofactor is shown in yellow.

Many membrane proteins form large complexes that have very complicated chemistry.

Bacterial photosynthetic reaction center or *Rhodobacter viridus*. It consists of four proteins, the transmembrane proteins, H, M, and L have collectively 11 TM segments which six different chlorophyll like molecules, and Fe and two bound quinones.
The green subunit contains four c-type cytochromes.



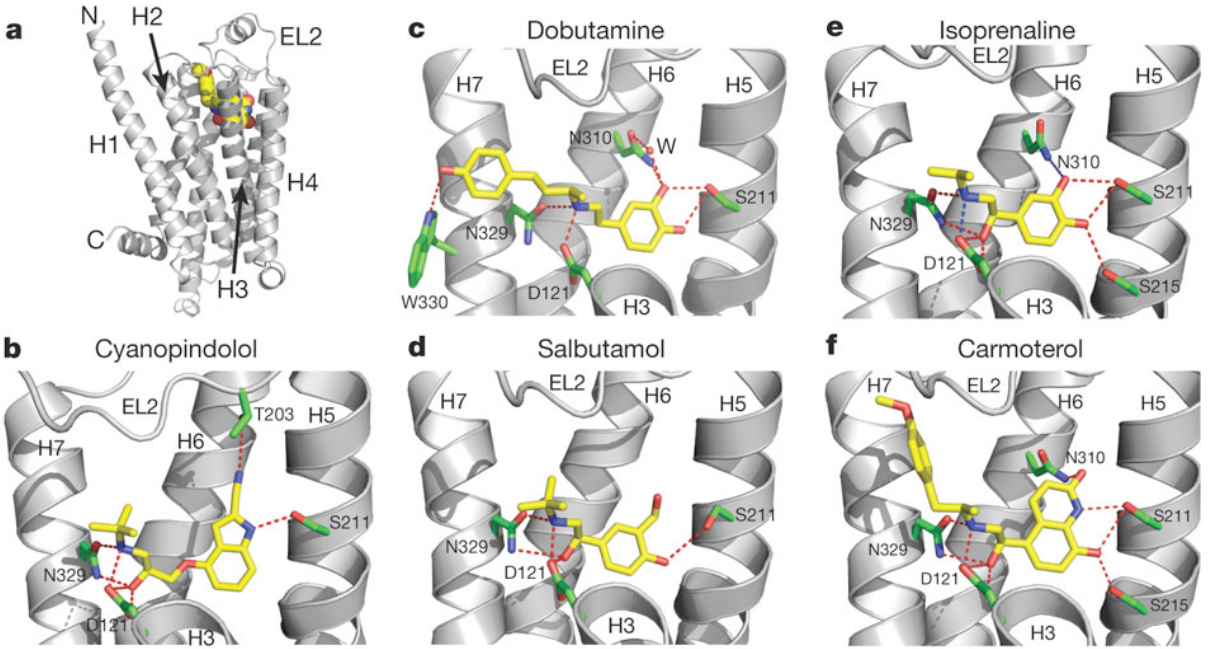
(a)

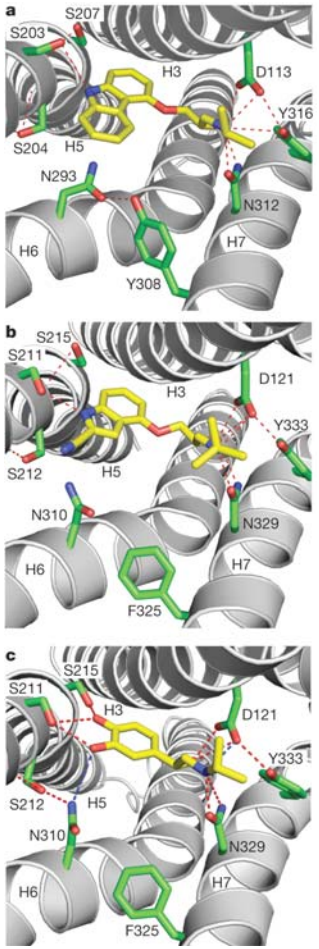
Figure 10-5a. X-Ray structure of the photosynthetic reaction center of *Rhodopseudomonas viridis*. [Based on an X-ray structure by Johann Deisenhofer, Robert Huber, and Hartmut Michel, Max-Planck-Institut für Biochemie, Germany.]

G Protein-Coupled Receptors (GPCRs)

Structure of the β_1-adrenergic receptor bound to agonists.

Comparison of the ligand-binding pockets of the β_1 and β_2 adrenergic receptors.



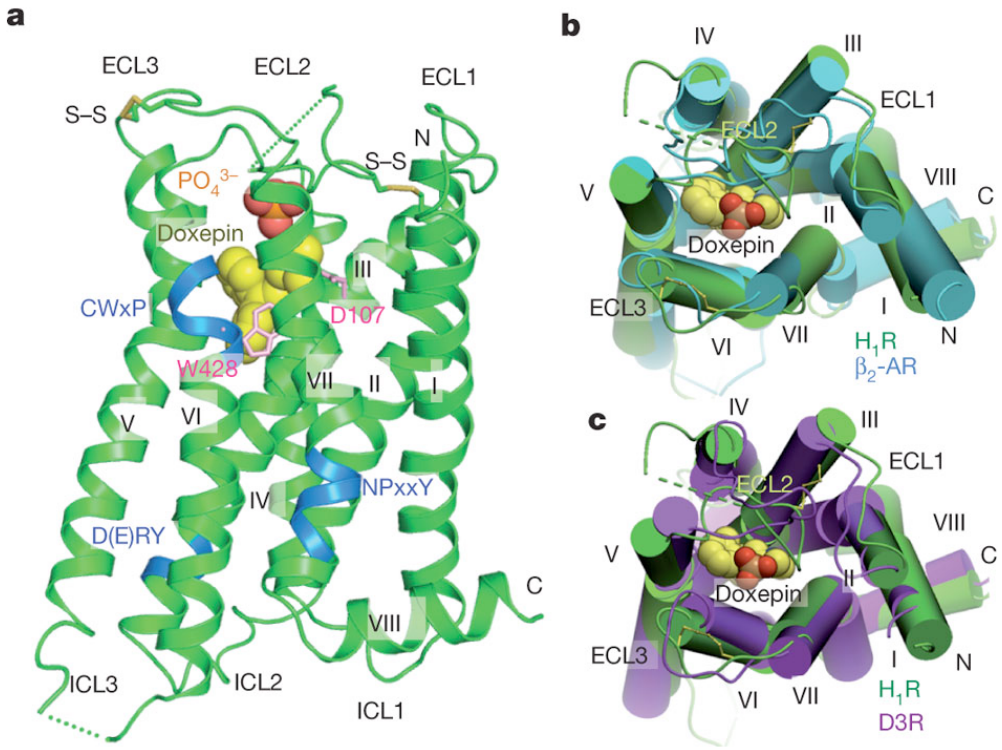


T Warne *et al. Nature* **469**, 241-244 (2011) doi:10.1038/nature09746

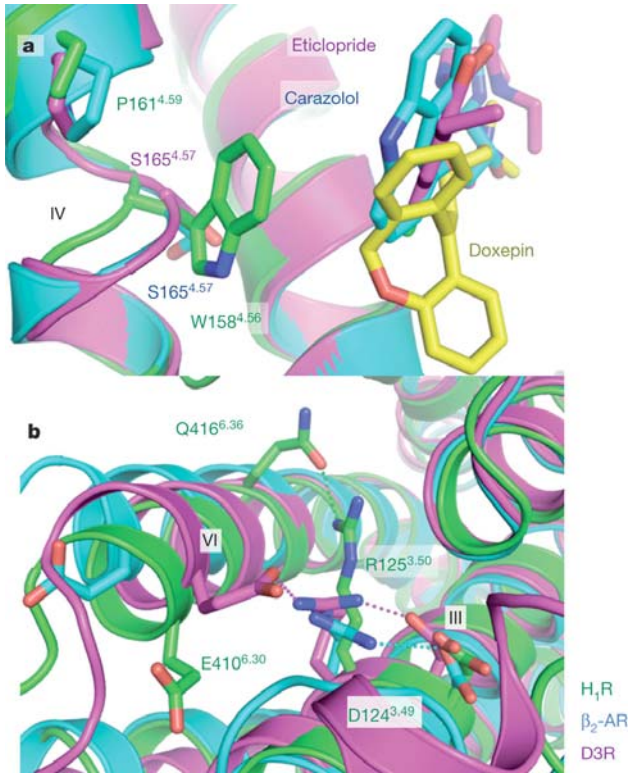
nature

G Protein-Coupled Receptors (GPCRs)

Structure of H_1R complex with doxepin.

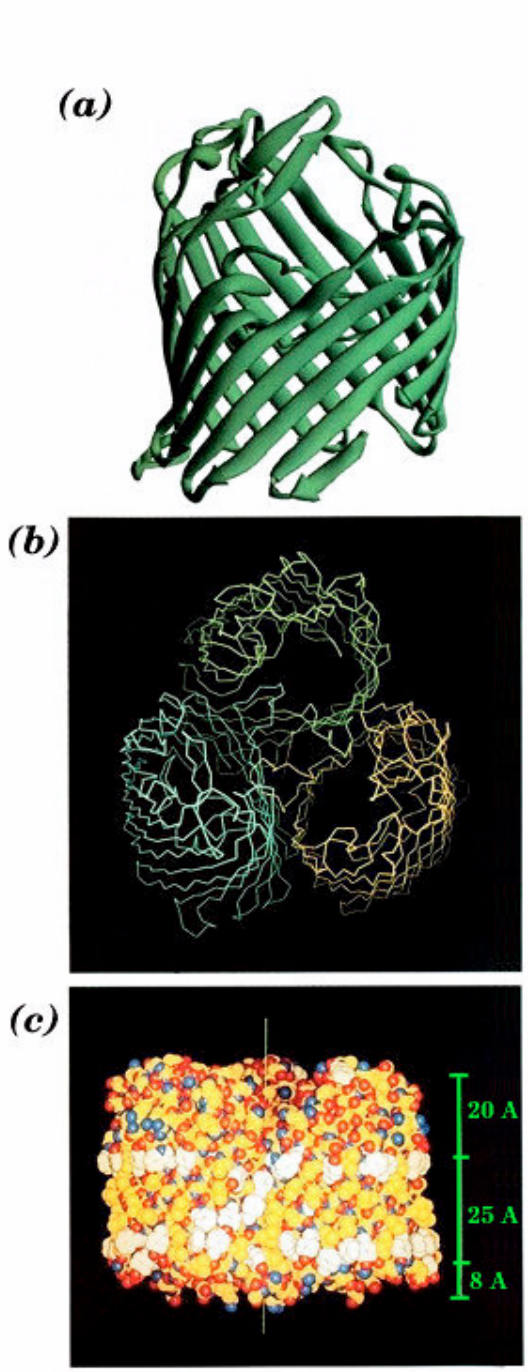


Comparison of the structures of H_1R, β_2-AR and D3R.



T Shimamura *et al. Nature* (2011)

nature



Porins

Pores and channels have functions to regulate flow of small molecules across the membrane. Here is a picture of OmpF, Outer membrane protein. The protein consists of β-pleated sheets across the membrane in a barrel-like structure. Small molecules flow through the center pore. Porins are trimers that have some control over the rate of flux across the membrane and a small loop inside the barrel controls substrate specificity.

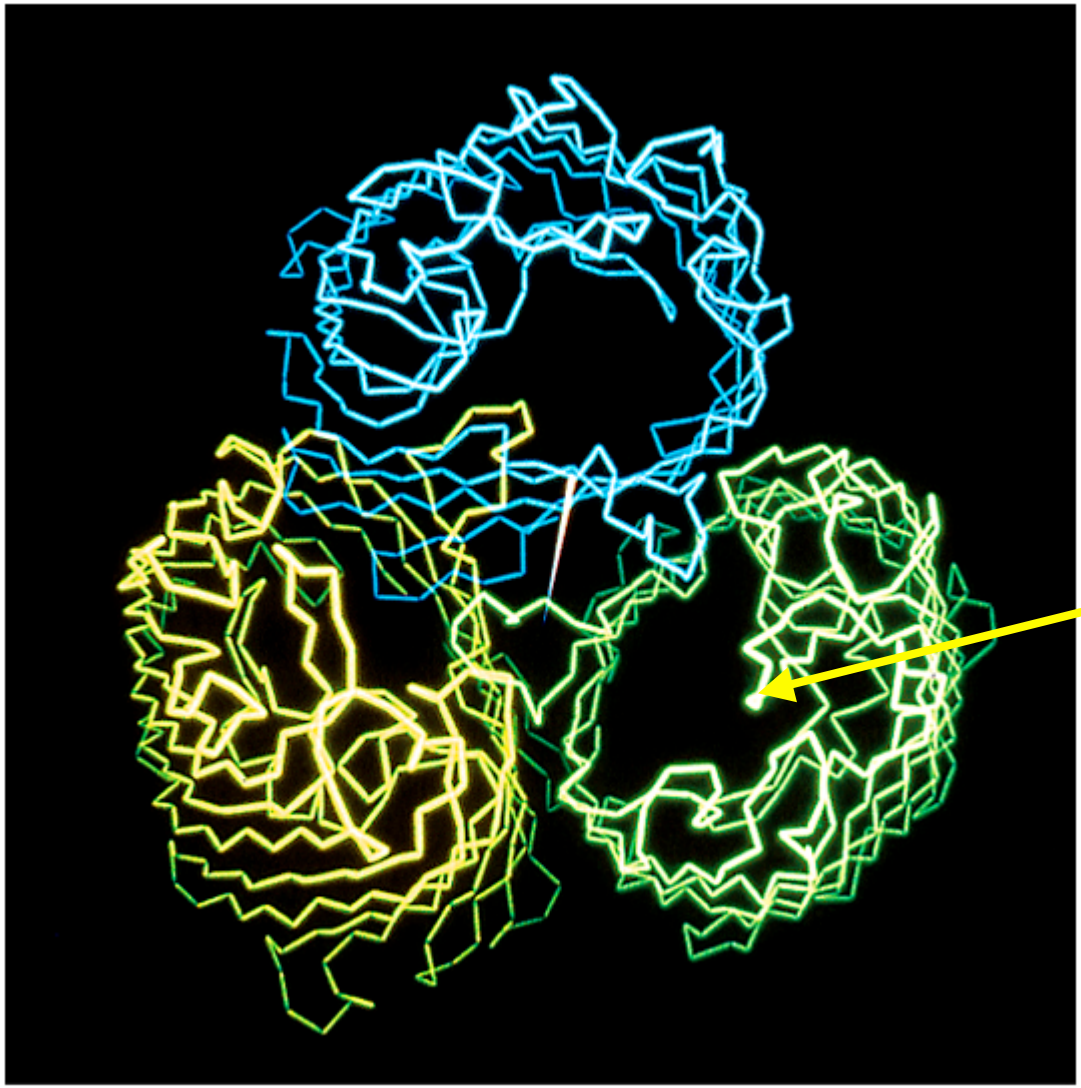


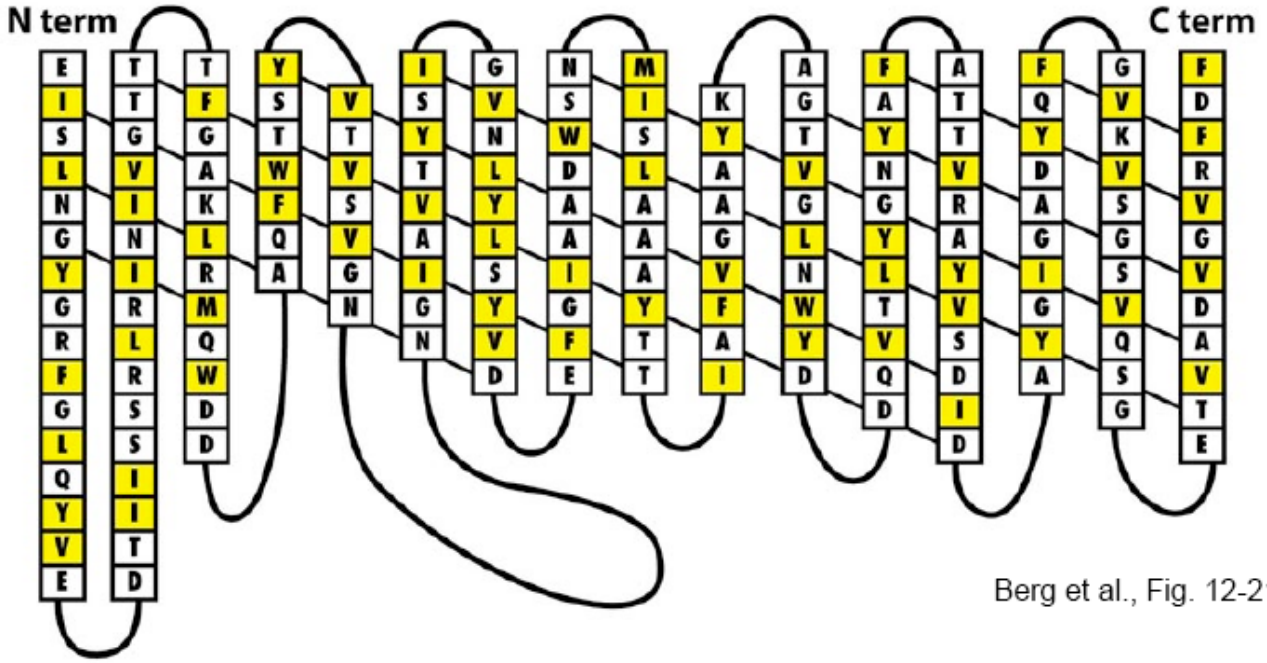
Figure 10-6b. X-Ray structure of the *E. coli* OmpF porin. [Courtesy of Tilman Schirmer and Johan Jansonius, University of Basel, Switzerland.]

The arrow shows the loop that controls the type of small molecules transported.

Amino acid sequence of a porin

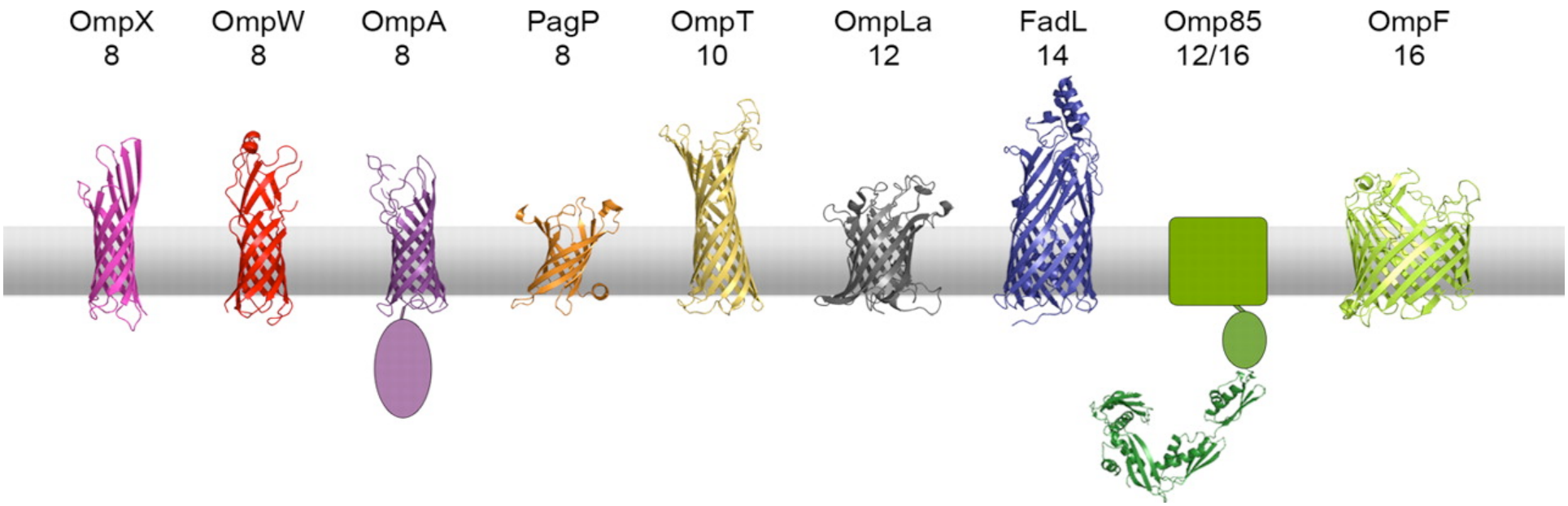
• β strands are indicated, with diagonal lines indicating direction of hydrogen bonding along the β sheet

• hydrophobic residues (F, I, L, M, V, W and Y) shown in yellow



Berg et al., Fig. 12-21

Note the more or less alternating hydrophobic and hydrophilic residues in the β strands (adjacent R groups project out from sheet on opposite sides).



Burgess N K et al. J. Biol. Chem. 2008;283:26748-26758

Current challenges

Emsley et al. *Nature* **381**, 90-2 (1996)

Otto et al. *JBC* **280**, 17339-45 (2005)

Gangwer et al. *PNAS* **140**, 16293-8 (2007)

Yeo et al. *EMBO J* **23**, 1245-56 (2004)

Nummelin et al. *EMBO J* **23**, 701-11 (2004)

Clantin et al. *PNAS* **101**, 6194-9 (2004)

Yeo et al. *JBC* **282**, 31076-84 (2007)

Pertactin

Hbp

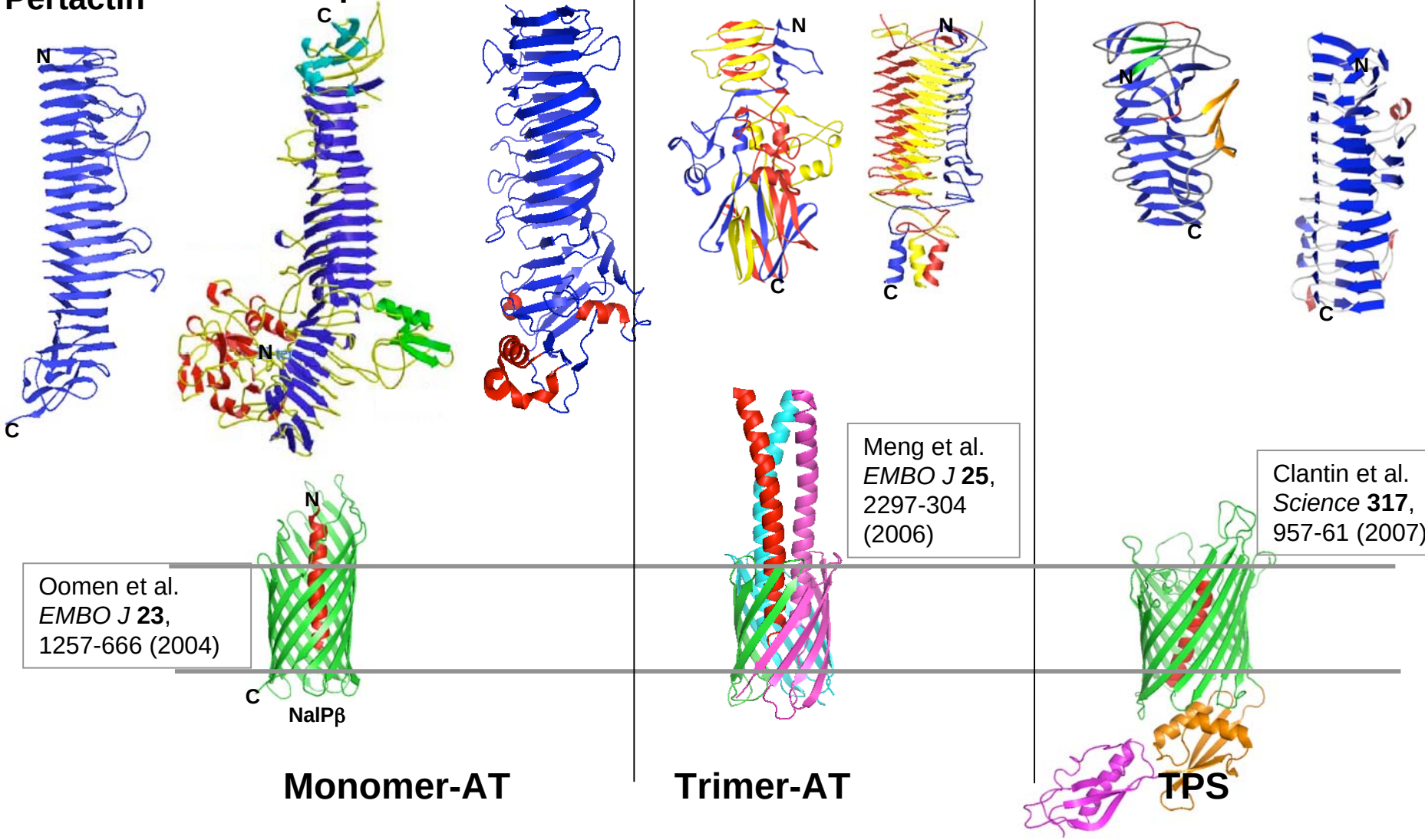
VacAp55

HiaBD

YadA-CBD

Fha30

HMW1-PP



Oomen et al. *EMBO J* **23**, 1257-666 (2004)

Meng et al. *EMBO J* **25**, 2297-304 (2006)

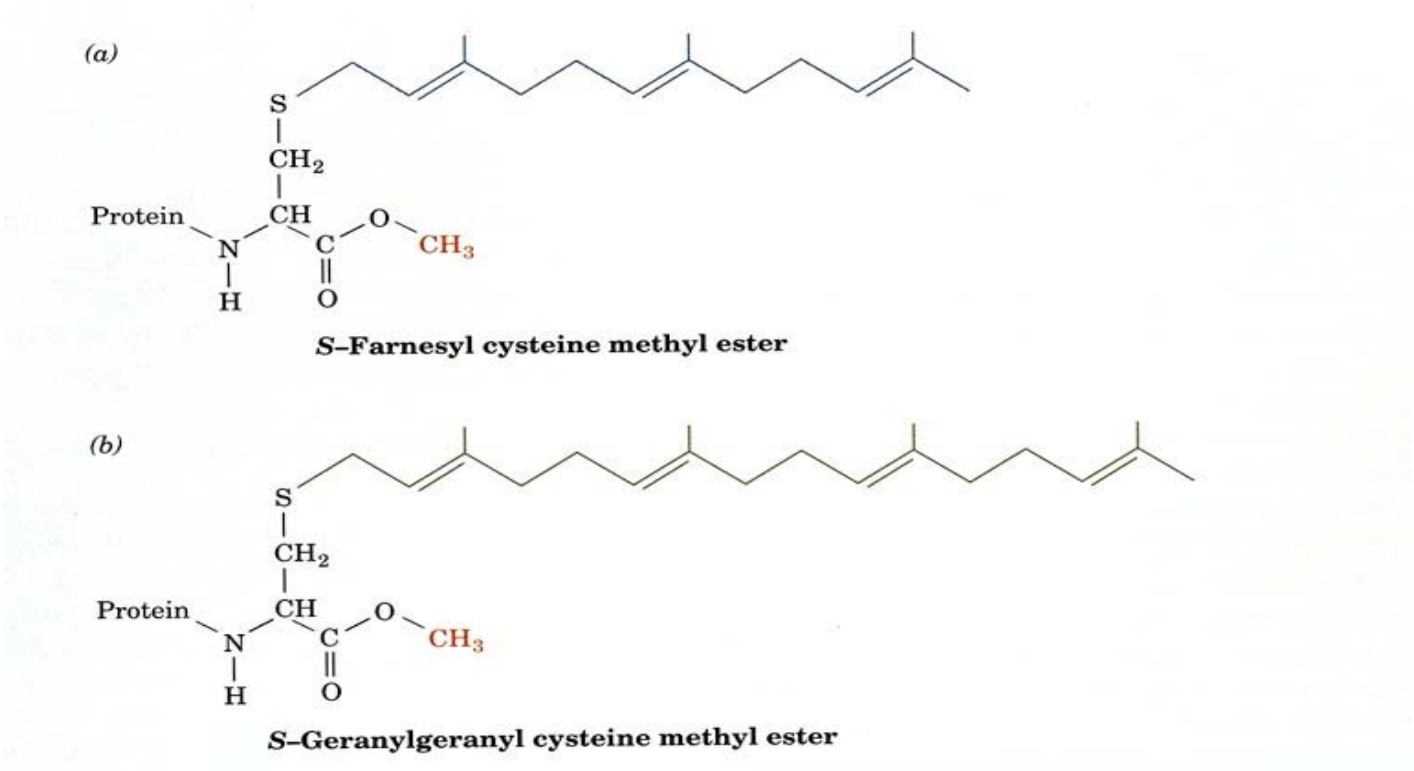
Clantin et al. *Science* **317**, 957-61 (2007)

Monomer-AT

Trimer-AT

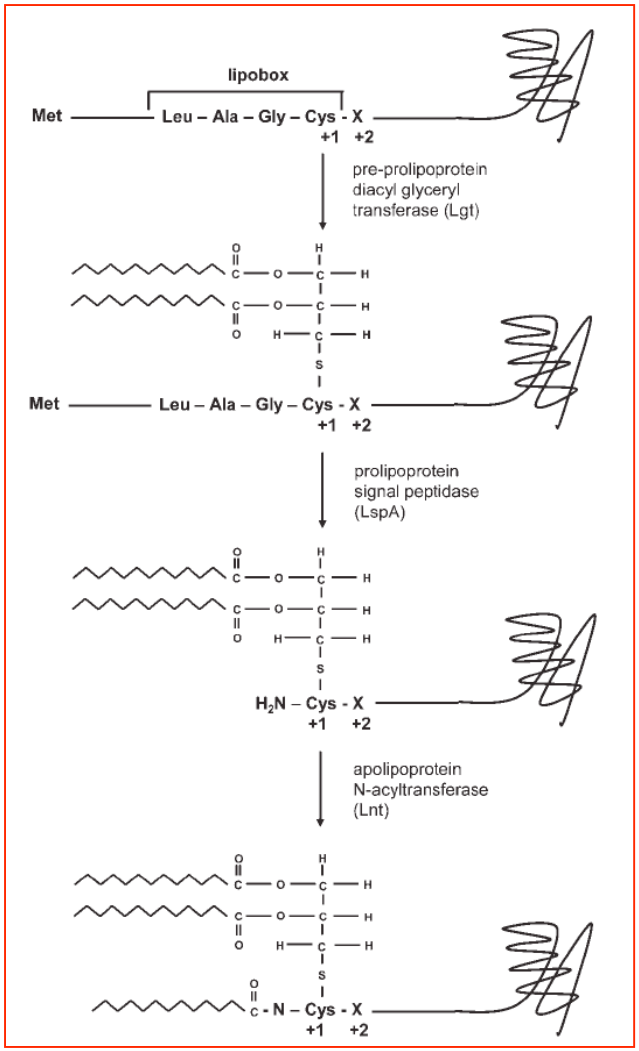
TPS

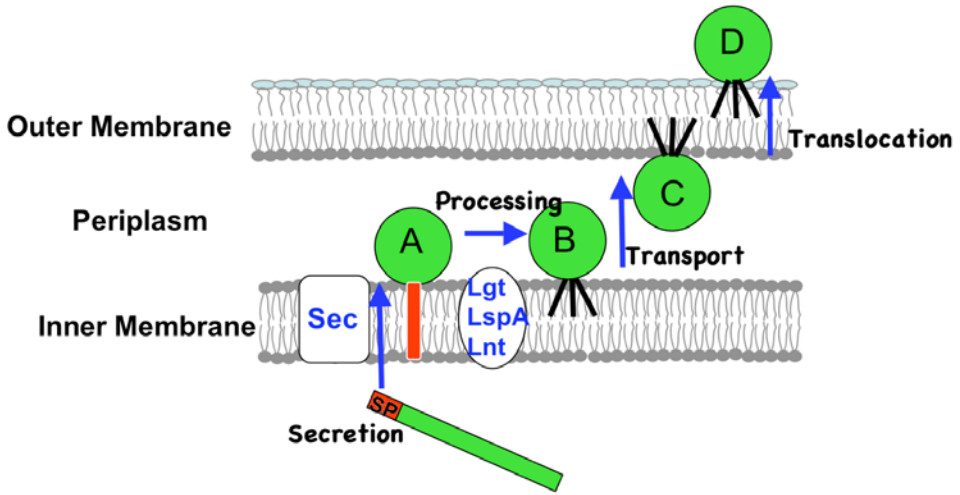
Lipid linked proteins

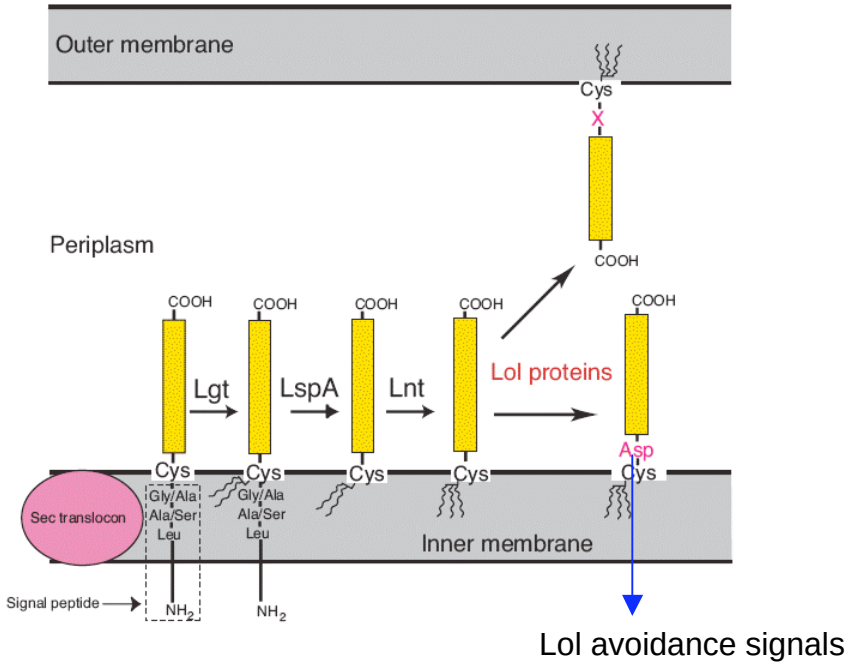


Prenylated proteins: C-X-X-Y linkage site. XZ are aliphatic residues and Y A,M,S for geranly and Leu for farnesyl. Linked to the cys group and the protein cleaved after Y and methylated. Prenylation mediates protein-protein association.

Bacterial lipoproteins







Lol avoidance signals

Rezwan M *et al* (2007) Microbiology 153, 652-58