

Membrane Proteins

 Course	 Essential Protein Structure and Function
 Confidence	Confident
 Next Review	@April 28, 2024
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Importance of membrane proteins

- 30% of proteins in the human genome are membrane proteins
- 70% of drugs act through membrane proteins - thus great pharmaceutical industrial importance

Properties of membranes

- Lipid bilayer
 - Each phospholipid consists of a polar head group and 2 hydrophobic tails
 - Membrane lipids are relatively small molecules with hydrophilic and hydrophobic moieties - amiphilic
 - These allow spontaneous formation of closed bilayer sheets in aqueous media
 - No covalent forces are involved in membrane formation
 - Vs. detergent molecules
 - One lipid tail enables micelles to form but two tails result in a bilayer
1. Sheet-like structures about 6-10 nm thick
 - 6-bacterial; 10-mammalian
 2. Block the free flow of polar molecules
 3. Contain mostly lipids and membrane proteins with carbohydrate attached to both.
 - Protein:lipid ratio varies from 1:4 (myelin - mostly lipid for insulation) to 4:1 (mitochondrial internal membrane - for ATP production)

4. The lipid acts as a solvent for the membrane proteins
5. The two sides of a membrane are asymmetric, with different properties
6. Membranes are fluid.

a. Diffusion on one face of a membrane by either a lipid molecule or by a protein is usually rapid (lateral diffusion)

i. On the same face of the layer

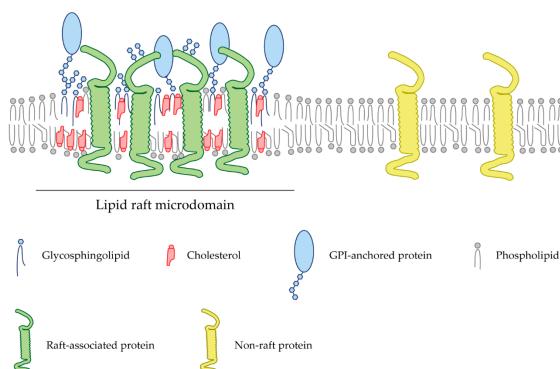
b. Diffusion NEVER occurs across a membrane bilayer or is very slow indeed (transverse diffusion)

i. From one layer to the opposite layer

7. Lipid rafts

a. Lateral separation of lipids to produce areas that are relatively organised in an otherwise highly fluid, disorganised bilayer

i. Lipid rafts are high in sphingolipids & cholesterol, show detergent resistance, and increase the thickness of bilayers. Concentration of proteins in these.



Functions of Lipid Rafts

1. **Signal Transduction:** Lipid rafts serve as platforms for the assembly of signaling molecules, facilitating efficient signal transduction. By concentrating receptors, enzymes, and other signaling proteins, lipid rafts enhance the specificity and speed of cellular responses to external stimuli.
2. **Protein Sorting and Trafficking:** These domains are involved in sorting and trafficking membrane proteins and lipids to specific destinations within the cell or for exocytosis. This sorting is crucial for maintaining the distinct identities of cellular membranes and for the targeted delivery of cellular components.
3. **Endocytosis and Pathogen Entry:** Lipid rafts can be involved in endocytosis, the process by which cells internalize substances from their environment. Certain pathogens, including viruses and bacteria, exploit lipid rafts as entry points to invade host cells.
4. **Cell Membrane Organization:** By segregating specific proteins and lipids into microdomains, lipid rafts contribute to the organization and compartmentalization of the cell membrane. This organization is critical for maintaining the membrane's structural integrity and functional capabilities.

Globular and Fibrous proteins

Functions of membrane proteins

The existence of

- Water-insoluble fibrous proteins
 - alpha-keratin, elastin, collagen
- Water-soluble globular proteins
 - Myoglobin, hemoglobin, immunoglobulin G, enzymes hormones, etc.

is complemented by the existence of

- Membrane-bound fibrous proteins
 - Spectrin, actin, etc.
- Membrane-bound globular proteins
 - Bacteriorhodopsin, porin, etc.

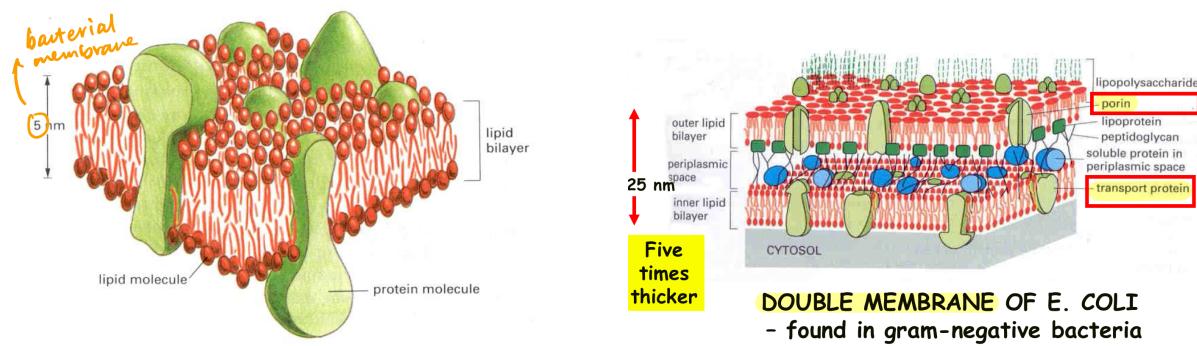
Water-soluble vs. Membrane-bound globular proteins

Water-soluble globular proteins

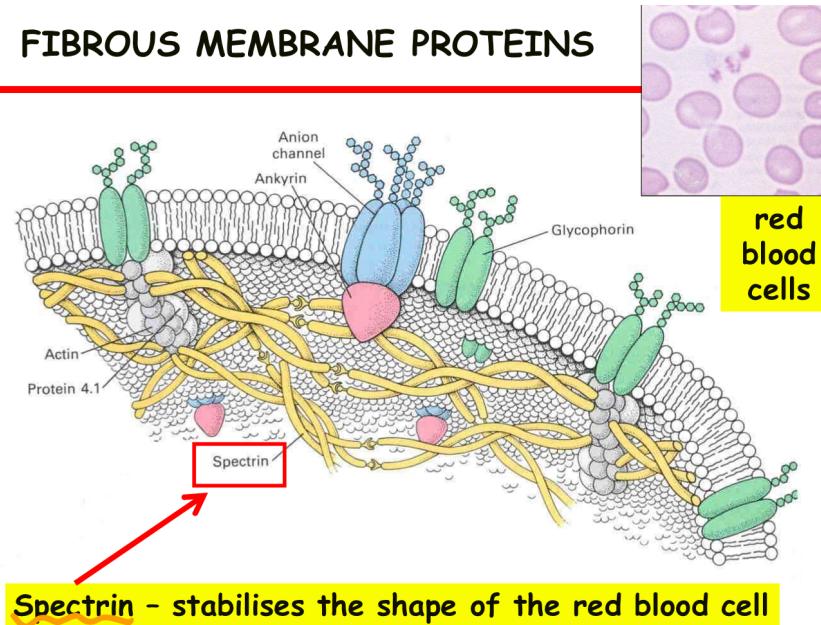
- Diffuses in three-dimensional space in water
- Core of buried hydrophobic residues with alpha-helix and beta-sheet. Surface of hydrophilic residues - protein loops and bends - hydrogen-bonded to water.

Globular membrane proteins: adapt to a lipid environment

- 30-90% freely diffuse in two-dimensional space in membrane, i.e. lateral diffusion
 - But not from one side to the other, only one specific orientation relative to the membrane is permitted, i.e. transverse diffusion
- There are large or small areas of surface hydrophobic residues. Some membrane proteins have hydrophilic centres and hydrophobic surface (ion channels?).
 - That would be a water-soluble protein inside-out



Fibrous membrane proteins



Specific functions of membrane proteins

Fibrous

- **Structural roles**
 - Cytoskeletal proteins such as spectrin in RBCs and dystrophin in muscle

Globular

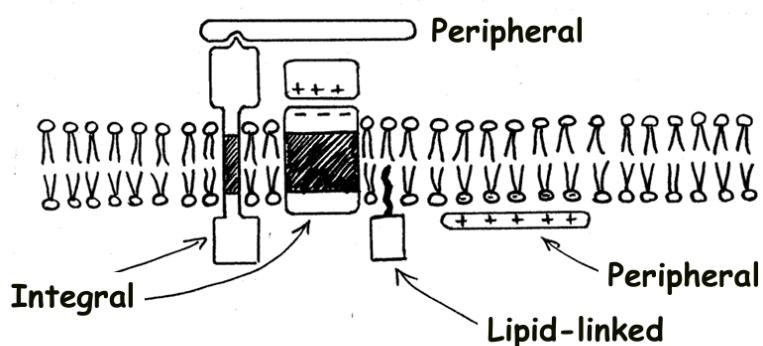
- **Transport** of nutrients and wastes in and out of cells
 - Sugar and lactate transporters

- **Electrical activity** in excitable cells - a gate or a pump - regulates intracellular medium
 - Na, K, Ca channel proteins
- **Signal transduction** - control of information in and out of cells
 - Growth factor and hormone receptors
- **Energy conversion**
 - Photosynthesis: light to chemical energy
 - Oxidative phosphorylation: mitochondria
- **Adhesion between cells**
 - Extracellular matrix

Types of membrane proteins

Four types:

1. Integral membrane proteins
2. Peripheral membrane proteins
 - a. May connect to a integral protein and has no contact with lipids
 - b. May be lipid linked, i.e., lipid tailed globular protein
3. Lipid-linked membrane proteins
4. Water soluble but inserts into membranes



(1) Integral; (2) Peripheral; (3) Lipid-linked

Peripheral membrane proteins

- Bound to lipid bilayer either to the lipid headgroups or to the integral protein
 - by electrostatic and/or hydrogen bond interactions
- Soluble in water. Elute these by chromatography by the use of salt, pH changes or chelators.

Lipid-linked membrane proteins

- Bound to lipid bilayer by means of one of 3 lipid tails.
 - Glycosylphosphatidylinositol (GPI) anchor, or prenylated (isoprenes), or fatty acid acylated (myristic/palmistic)

Integral membrane proteins

- Bound to lipid bilayer by hydrophobic interactions with protein
- Amphipathic with two main regions
 - Surface regions outside the membrane coexists with water, so these are hydrophilic with polar (neutral and charged) sidechains. If carbohydrate present will only be on the outside surface.
 - Surface regions inside the bilayer face a hydrophobic environment with no hydrogen bonding capacity. Thus, these have hydrophobic sidechains (Val, Ile, Leu)
- Insoluble in water in absence of detergent. To purify these by chromatography requires destroying the lipid bilayer with organic solvents or detergents.

Solubilisation of integral membrane proteins

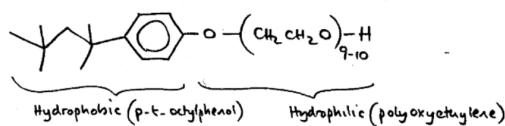
- Use gentle non-ionic detergents
- Hydrophobic segment for interacting with the hydrophobic protein surface
- Hydrophilic segment to render the complex soluble in water
- The critical micelle concentration (CMC) is the concentration above which the detergent can form micelles

SODIUM DODECYL SULPHATE, SDS, charged.

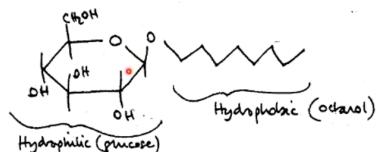
SDS-PAGE

$\text{SO}_4^{2-} \cdot (\text{CH}_2)_{11} \cdot \text{CH}_3$ - note this denatures the protein - useless!

TRITON X-100, neutral, with a low cmc of 0.3 mM

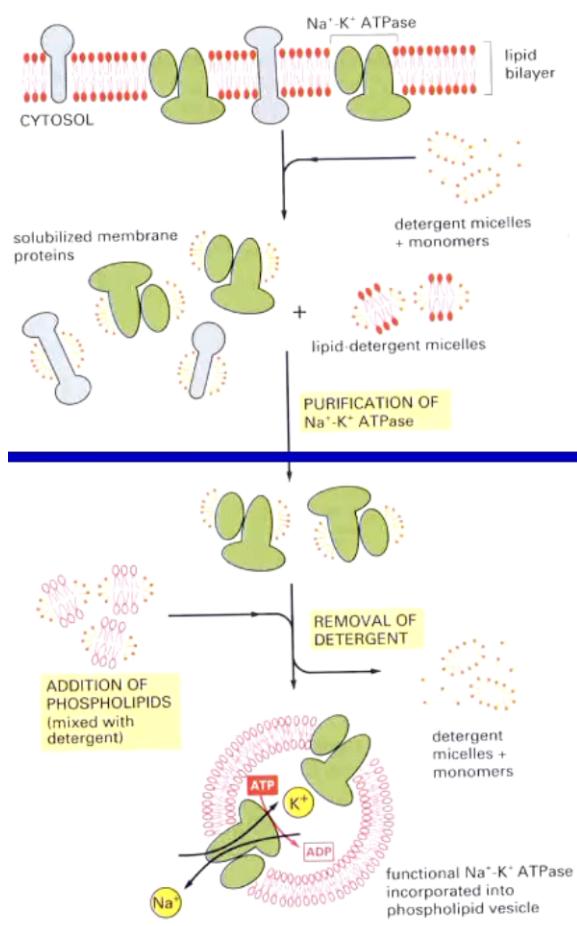


OCTYL- β -D-GLUCOSIDE, neutral, with a high cmc of 23 mM, which is more effective for solubilization.



- Hydrophilic part of SDS is too strong that it will denature the protein
- Triton is better with a neutral property, however, low cmc
 - The lower the CMC, the more stable the micelle and the more slowly molecules are incorporated into or removed from the micelle.
 - CMC values are a guide to detergent hydrophobic binding strengths.
 - The higher the CMC, the weaker the binding and the easier the removal of the detergent, such as by dialysis.
- Octyl-beta-glucoside with high cmc is more effective for solubilisation

Purification



- Detergents have a single hydrocarbon chain, which favors micelle formation, unlike the two chains in lipid molecules which favors bilayers
- Up: Use detergent to break up the bilayer
 - Detergent micelles surround (solubilise) both the membrane proteins and the lipid (lipid-detergent micelles)
- Bottom: Use of phospholipids to reconstitute into vesicles
 - Addition of phospholipids mixed with detergent to remove detergent
 - Dialysis of detergent - high cmc - easier removal of detergent
 - Functional protein incorporated into phospholipid vesicles

Secondary structures

Important

- Hydrophobic sidechains within bilayers
- No free hydrogen bonds are permitted - the alpha-helices and beta-strands must span the bilayer

Alpha-helix

- A helical arrangement of the protein mainchain that repeats

Beta-sheet

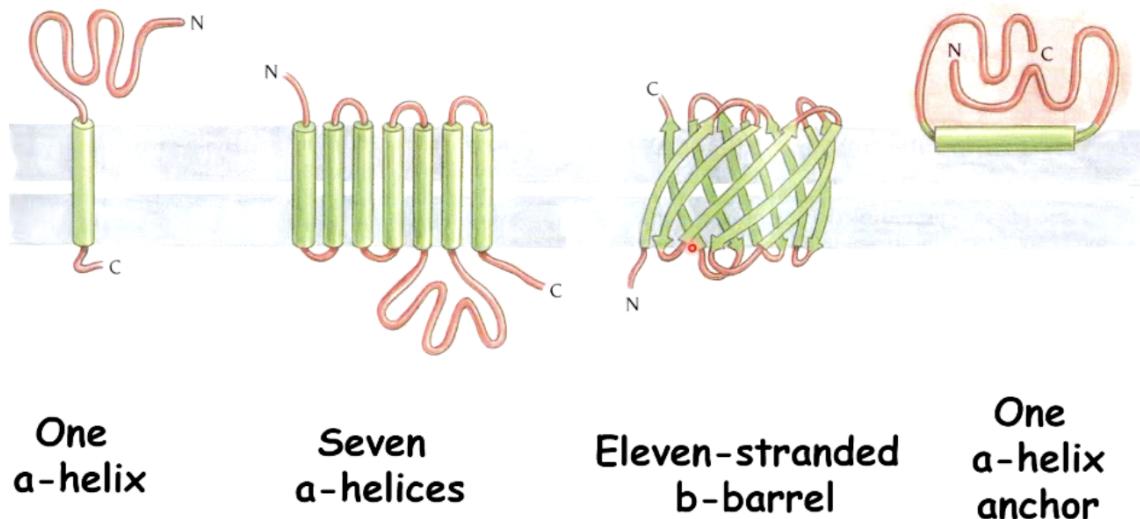
1. Mainchain is extended; the R groups (sidechains) alternate up and down

itself approximately every 4 residue

1. Rod-like with the R groups (sidechains) outside
2. All the C=O and N-H mainchain groups are H-bonded
3. All H-bonds are parallel to the helix axis
 - H-bonds are permitted in bilayers

2. All the C=O and N-H mainchain groups at centre are H-bonded
3. All H-bonds are between separate mainchains and perpendicular to the direction of main chain
 - H-bonds not permeable in bilayers

1. Satisfy H-bonding capacity of the peptide bonds by containing these within α -helices and β -sheets.
 - a. Single or multiple passes of an α -helix are permitted but not β -sheets
 - b. The hydrophobic helix containing about 20-25 amino acids is common in membrane proteins
 - c. Bilayer spanning hydrophobic α -helices are the common structural feature/motif of most membrane proteins
2. A β -barrel formed by β -strands will satisfy all the mainchain H-bonds - giving a β -strand membrane protein.
 - a. Single β -strand will not have its mainchain H-bonds satisfied - this is energetically very unfavorable - not observed.
 - b. A multistranded β -sheet will not have its edge mainchain H-bonds satisfied - again not observed.
3. No-free N-terminal or C-terminal ends, nor loops or turns can exist within the hydrophobic core of the bilayer. The peptide chains must span the bilayer completely.



Note that N and C terminals are all outside of the bilayers.

Prediction of secondary structures

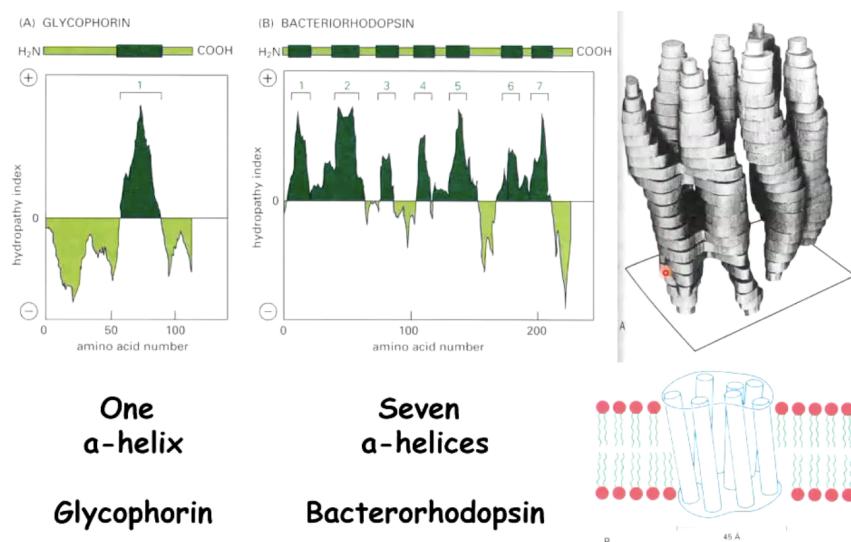
Hydrophobicity plots

1. Secondary structure predictions based on methods for water-soluble globular proteins do not work for membrane proteins
2. From the sequence, it can be accurately predicted whether a protein will be water-soluble or membrane-bound using a different method: hydrophobicity plots
3. A numerical scale of hydrophobicities for the 20 amino acids is needed. These are based on:
 - a. Free energy for the transfer of amino acid sidechains from water to a hydrophobic phase
 - b. Observed distribution of sidechains between the surface and the interior in proteins of known structures. Typical values are as follow:

Hydrophobic	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">Phe</td><td style="text-align: center;">Ile</td><td style="text-align: center;">Val</td><td style="text-align: center;">Thr</td><td style="text-align: center;">Asn</td><td style="text-align: center;">Glu</td><td style="text-align: center;">Arg</td></tr> <tr> <td style="text-align: center;">3.7</td><td style="text-align: center;">3.1</td><td style="text-align: center;">2.6</td><td style="text-align: center;">1.2</td><td style="text-align: center;">-4.8</td><td style="text-align: center;">-8.2</td><td style="text-align: center;">-12.3</td></tr> </table>	Phe	Ile	Val	Thr	Asn	Glu	Arg	3.7	3.1	2.6	1.2	-4.8	-8.2	-12.3	Hydrophilic
Phe	Ile	Val	Thr	Asn	Glu	Arg										
3.7	3.1	2.6	1.2	-4.8	-8.2	-12.3										

4. To create a profile, assign a hydrophobicity score to each amino acid residue in the sequence
5. Slide a 21-residue “window” along the protein sequence.

- a. Calculate the average hydrophobicity inside the window for residues 1-21, 2-22, 3-23.
 - b. Plot this average vs. the position of the window in the sequence.
6. Positive peaks indicate predicted membrane-spanning helices. Negative valleys indicate hydrophilic regions.
7. This method assumes that membrane spanning α -helices are hydrophobic, yet porin (a membrane protein with a β -sheet structure) has no long hydrophobic sequences.
- a. The method only picks up α -helix.



8. Once the helices are identified - the "inside-outside" topology needs to be predicted. If the inter-helix loop is short (>60 residues), the "positive-inside" rule applies.
- a. Whichever side has the most positive charges (residues) in the inner side of the membrane protein
9. Many Web-based tools to predict transmembrane helices
- a. PHDhtm
 - b. HMMTOP
 - c. TMPRED
 - d. SOSUI

Summary

1. Lipid bilayers form a mobile phase in which membrane proteins are embeded.
2. The hydrophobicity of membrane proteins is very different from water-soluble proteins.
3. There are three (four) ways in which a membrane protein can become associated with a lipid bilayer.
4. Membrane proteins secondary structures must not have unsatisfied H-bonds within the bilayer.
5. We can predict a-helical secondary structures, but not b-sheets for membrane proteins.

Survey of known membrane protein structures

Membrane protein structures at high resolution

- In 2006, 100 are known, compared to 40,000 others in PDB. About 2/3 are all-alpha and 1/3 are all beta.
- In 2019, 860 unique ones are known, compared to over 148,000 in the PDB
- Technologically difficult to crystalise membrnae proteins than water-soluble proteins
- Three groups of structures
 - Monotopic membrane proteins
 - Membrane proteins which are attached to only one side of the membrane and do not span the whole way across
 - Transmembrane: alpha helical: 3199/3525
 - Transmembrane: beta barrel: 389/3525

Globular membrane proteins

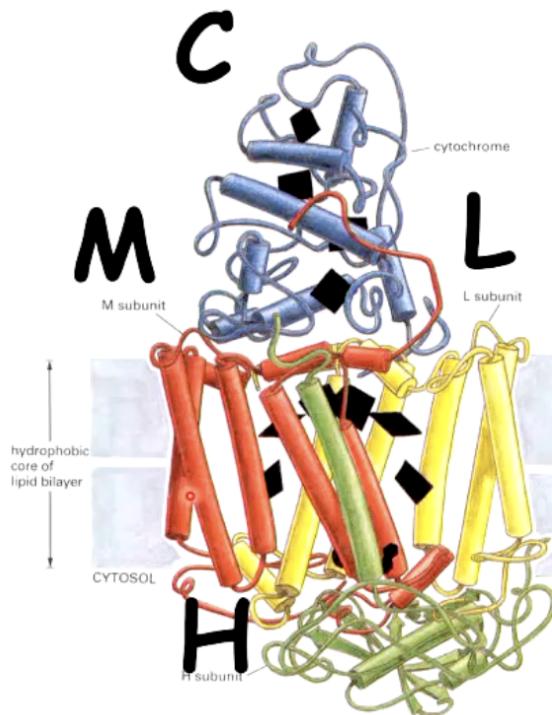
GPCR - alpha helix proteins

- 2012 Nobel price to Lefkowitz and Kobilka for GPCR studies
- Most physiological process depend on GPCRs
 - Include receptors for adrenaline, dopamine, serotonin, light, flavor and odor

- Around half of all medications act through these receptors, among them beta blockers, anti-histamines and various kinds of psychiatric medications
- How does the information that a hormone has bound to a receptor from one side of a lipid bilayer get transmitted to proteins on the other side of the bilayer?
 - Small structural change around the ligand propagates into a much larger structural transition on the inside than previously anticipated
- Mechanisms: A large **displacement of helix 6** and an **opening of a deep hydrophobic cleft** on the intracellular side of the receptor
 1. A hormone binds to the receptor, at the middle of α-helices
 2. The receptor alters shape. Intracellularly, the G protein binds and is activated.
 3. Activated G-protein breaks apart the free α-subunit will trigger a chain of reactions that alters the cell's metabolism
 4. A new G protein binds. The receptor can activate hundreds of G proteins before the hormone on the outside detaches.

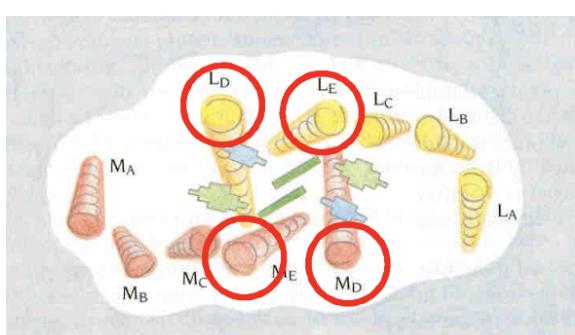
Photosynthetic reaction centre - an α-helix protein

- The photosynthetic reaction centre of the purple bacterium is a complex of 4 membrane proteins that converts energy captured from sunlight into electrical and chemical energy
 - One of the very first example of large membrane proteins



- C-type cytochrome: peripheral protein
- L-subunit: 5 membrane helices
- M-subunit: 5 membrane helices
- H-subunit: 1 membrane helix

- The prosthetic groups (chlorophylls and pheophytin) are contained within a region encased by the helices of the L and M subunits. Note that the helices tilt.
- Absorbed photons produce the energy needed to donate electrons to an acceptor.
 - These electrons are transmitted from the chlorophylls to other groups (quinones).
 - The quinone is then released for further reaction.



- The chlorophylls are central (green). There is a special pair in

- This was crystallised from detergent used to cover hydrophobic regions. Small amphiphiles are present to replace large detergent molecules which can disturb the crystalline order.
- This structure provided an excellent insight into the mechanism of photosynthesis.

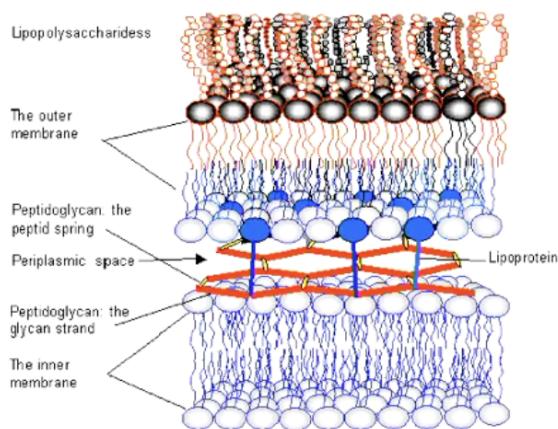
the L and M subunits. Helices D/E form a four-helix bundle.

- Four-helix bundle: common structural motif in proteins, characterized by the arrangement of four α -helices in a compact, globular cluster. This motif is stabilized by hydrophobic interactions among the side chains of amino acids

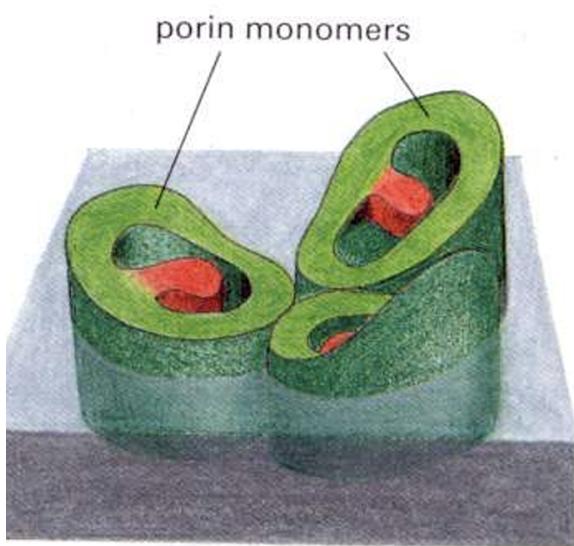
But there is little that can be learnt from this for other α -helical membrane proteins.

- The crystal structure clarified the mechanism of photosynthesis, which convert light energy into electrical and chemical energy.

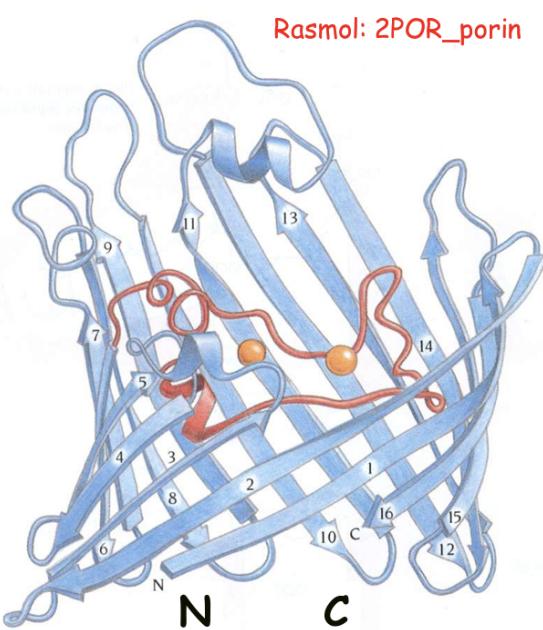
Porins - a beta-sheet protein



- Permits uptake and disposal of small molecule nutrients and waste of molecular weight <600.
 - Abundant in bacterial outer membrane - Gram negative.
 - Found in chloroplasts and outer mitochondrial membranes
 - No predicted long stretches of hydrophobic residues - hence no α -helix were predicted to be in this protein.



- Showed by an X-ray diffraction:
 - A **trimeric** structure
 - Beta structure
 - A long loop (red) which protudes into the pore to narrow this - thus only small molecules allowed through.



- Each monomer is a **16-stranded beta-barrel**, in which the strands are all **antiparallel** and **7-16** residues long.
- Hydrophobic and hydrophilic residues alternate along the beta-strands, giving rise to a **hydrophobic outer surface** and a **hydrophilic inner surface** surrounding the aqueous pore.
 - I.e., No long stretches of hydrophobic residues were detected in the sequence of porin.

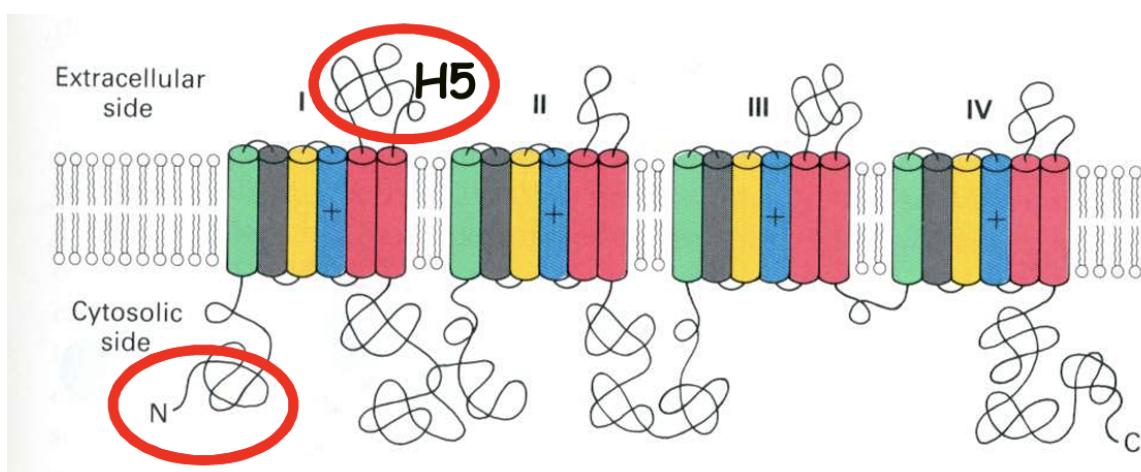
- The crystal structure clarified the mechanism of transport of small molecules across the outer membrane of bacteria.
- Long molecules cannot enter because of the size of the water-filled channel in the centre - about 9 Å long and 8 Å diameter. Size of channel determined by the "red loop"
- The channel also has one side lined with positive charges (His, Lys, Arg) and other side lined with negative charges (Glu, Asp).
 - Affects what passes through.

- Treat meningitis by targeting the porins.

Na/K transporter proteins - four core alpha-helices

- Three types of transporters
 - Active pumps (use ATP) - $10E1-10E3$ ions/s
 - Channel proteins (very rapid ion movements) - $10E7 - E8$ ions/s
 - Transporter (down a concentration gradient - or coupled) - $10E2 - E4$ ions/s

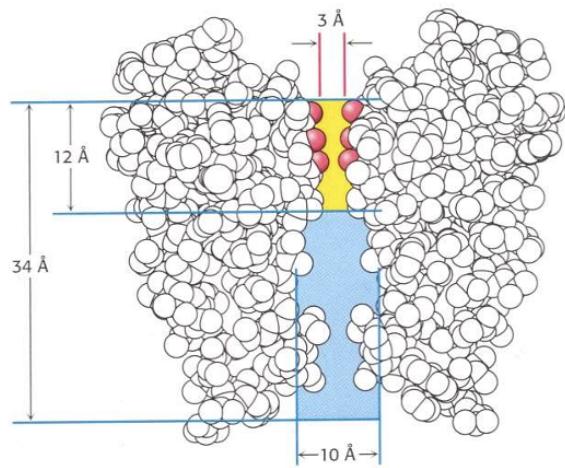
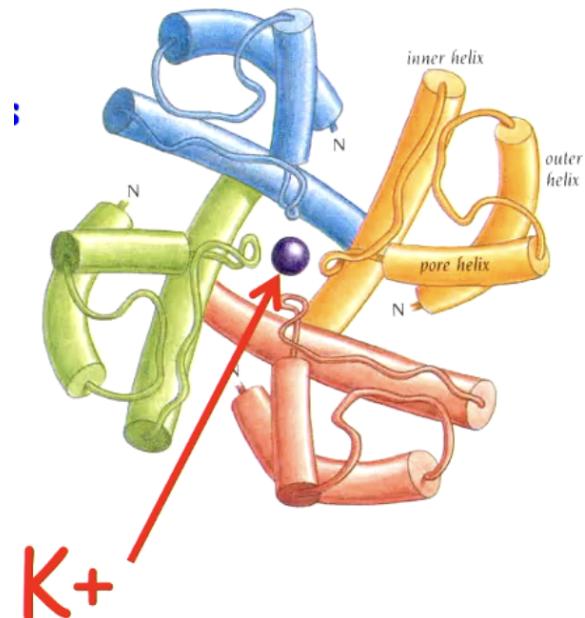
Human Na and K ion channel proteins



- Ion channels mediate very rapid rates of ion transfer
- Four subunits in all these proteins, packed into a tetramer.
- Six α-helices S1-S6, a H5 loop between S5 and S6, and a N-terminal segment that activates/inactivates the open channel
- S4 directs the channel to open, having a positive charge to sense the voltage

Bacterial K ion channel protein

- The bacterial membrane protein has three **a-helices** of each subunit instead of six
 - Two transmembrane helices, one pore helix
- Four **inner helices** in the tetramer forms a pore down the centre, with carbonyl C=O oxygens lining the pore at a fixed separation that permits only K⁺ to pass through
- The crystal structures clarified the mechanism of transport of K⁺ across membranes
- Mainchain C=O groups that point into the pore (diameter 3 Å) constitute binding sites for three K ions.
- K ions are dehydrated and this hydration is compensated by the C=O groups
 - When a potassium ion binds to the selectivity filter (SF), eight water molecules are released from a hydrated potassium ion.
 - The ion is then bound to eight carbonyl oxygens at each of the four sites in the SF.
 - At the last site, the ion binds to four of the carbonyl oxygens as well as four water molecules, keeping the eightfold coordination number.



This simple reaction is favorable because of low energy cost to lose the water molecules and bind the to the selectivity filter.

- Once the ion has completely diffused through the channel, the ion becomes rehydrated once again in the extracellular matrix.

Selection between Na and K in ion channels

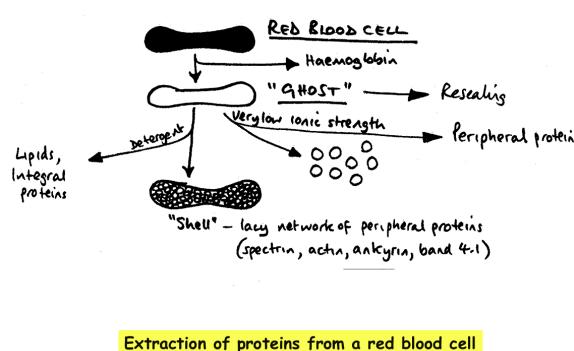
- The sidechains at this selectivity pore (or filter) are rigid, so the mainchain C=O cannot move
 - Potassium ions are normally surrounded by eight water molecules, which are stripped before entering the filter.
 - The filter is designed to have similar dimensions that mimic the shell of water that encloses potassium by lining the pore with eight oxygen atoms, as stated above.
 - These are optimally placed to allow for the interaction with potassium ions. This makes the transfer of energy from the central cavity to the selectivity filter extremely low because the environment is not drastically changing.
- The “core helices” point towards the K ions to neutralise their charges via the helix dipole effect
- Repulsion between successive K ions leads to movement
- This channel protein is 100-fold more specific for K and not Na because of different ion sizes and hydration levels.
 - Na ions are much smaller, and have a higher solvation energy.
 - Smaller ions, which have a higher charge density, interact more strongly with the dipole moments of the solvent molecules, resulting in a higher solvation energy.
 - Sodium ions have a smaller ion radius resulting in the inability to coordinate with all eight oxygens at the first site. This results in sodium ions favoring the shell of water.

Fibrous membrane proteins

RBC has to be very robust in order to survive 120 days of squeezing through capillaries. This is achieved with the help of many contacts made with

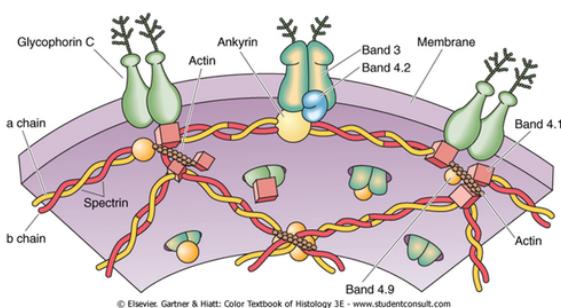
cytoskeleton (peripheral membrane proteins), unlike other cells.

Spectrin



- Puncture RBC and hemoglobin leaks out - ghost
- Detergent to remove lipids and integral proteins
- Left with "shell" with network of peripheral proteins

- Isolated from low-ionic strength extracts of ghosts formed by lysis of RBC
 - Band 1 protein - $M_r = 260,000$ - a chain
 - Band 2 protein - $M_r = 225,000$ - b chain
- In the membrane this exists as the tetramer (1,2)2 or (a,b)2
 - Two of each chain



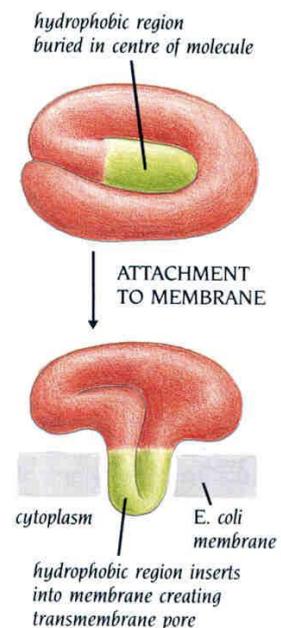
- Spectrin connects with ankyrin which anchors in the bilayer
- With the help of band 4.1 protein connects also to short actin filaments
- A polygonal network is created to form the cytoskeleton of the RBC
- It has a repeat of 106 aa's that forms a triple-stranded a-helix, with 18 or 20 repeats in total

Inserted membrane proteins

Colicin A

- Pore-forming colicins are a family of three-domain toxins (600 amino acids) produced by *E. coli* and related bacteria.

- They form ion channels in the inner membrane of target cells.
- Bactericidal activity against closely related bacterial strains
- Colicin has three domains: A, B, and I that forms a Y shaped structure
 - B - receptor binding to the external membrane
 - A - translocation to the periplasmic space
 - I - ion channel formation
- The channel domain I is well conserved across all colicin sequences
 - Half of this contains 10 α -helices - with 8 amphipathic and 2 hydrophobic (H8 and H9)
 - A repacking of the α -helices occurs on bilayer insertion.
 - Hydrophobic region usually buried in the centre of molecule.
 - So that hydrophobic region inserts into membrane creating transmembrane pore.



Summary

1. GPCR: Integral, α -helical. Hormone signaling to G-proteins inside cells
2. Photosynthetic reaction centre. Integral and peripheral; α -helical. Converts light energy into metabolic energy
3. Porin. Integral; beta-barrel. Enables small molecules to pass into E. coli periplasmic space
4. Na/K channels. Integral, α -helical. Pores are selective for Na or K
5. Spectrin. Peripheral. Mediates structure of the RBC
6. Colicin. Insertion. Able to insert itself into lipid bilayers to disturb these.