Membrane Proteins

A **membrane protein** is a protein molecule that is attached to, or associated with the membrane of a cell or an organelle. More than half of all proteins interact with membranes.
Membrane Proteins - Function

Biological membranes consist of a phospholipid bilayer and a variety of proteins that accomplish vital biological functions.

* Structural proteins are attached to microfilaments in the cytoskeleton which ensures stability of the cell.

* Cell adhesion molecules allow cells to identify each other and interact. Such proteins are involved in immune response.

* Membrane enzymes produce a variety of substances essential for cell function.

* Membrane receptor proteins serve as connection between the cell's internal and external environments.

* Transport proteins play an important role in the maintenance of concentrations of ions. These transport proteins come in two forms: carrier proteins and channel proteins.
Membrane Proteins - Main categories

* Integral membrane proteins which are permanently bound to the lipid bilayer

* Peripheral membrane proteins that are temporarily associated with lipid bilayer or with integral membrane proteins

* Lipid-anchored proteins bound to lipid bilayer bound through lipidated amino acid residues
Integral membrane proteins
Integral membrane proteins are permanently attached to the membrane.

They can be defined as those proteins which require a detergent (such as SDS or Triton X-100) or some other apolar solvent to be displaced.

They can be classified according to their relationship with the bilayer:

**Integral polytopic proteins**, also known as *transmembrane proteins*, are proteins that are permanently attached to the lipid membrane and span across the membrane (at least once). The transmembrane regions of the proteins are either beta-barrels or alpha-helical. The alpha-helical domains are present in all types of biological membranes including outer membranes. The beta-barrels were found only in outer membranes of Gram-negative bacteria, lipid-rich cell walls of a few Gram-positive bacteria, and outer membranes of mitochondria and chloroplasts.

**Integral monotopic proteins** are proteins that are permanently attached to the lipid membrane from only one side and do not span across the membrane.
Peripheral membrane proteins

Peripheral membrane proteins are temporarily attached either to the lipid bilayer or to integral proteins by a combination of hydrophobic, electrostatic, and other non-covalent interactions. Peripheral proteins dissociate following treatment with a polar reagent, such as a solution with an elevated pH or high salt concentrations.

Integral and peripheral proteins may be post-translationally modified, with added fatty acid or prenyl chains which may be anchored in the lipid bilayer.
Membrane Proteins

Cells and organelles within them are bounded by membranes, which are extremely thin (4.5 nm) films of lipids and protein molecules. The lipids form a bilayered sheet structure that is hydrophilic on its two outer surfaces and hydrophobic in between. Protein molecules are embedded in this layer, and in the simplest case they are arranged with 3 distinct regions: 1 hydrophobic transmembrane segment and 2 hydrophilic regions (one on each side of membrane).
A biological membrane functions basically as a permeability barrier that establishes discrete compartments and prevents the random mixing of the contents of one compartment with those of another. However, biological membranes are more than passive containers. The embedded proteins serve as highly active mediators between the cell and its environment or the interior of an organelle and the cytosol. They catalyze specific transport of metabolites and ions across the membrane barriers. They convert the energy of sunlight into chemical and electrical energy, and they couple the flow of electrons to the synthesis of ATP. They act as signal receptors and transduce signals across the membrane. The signals can be neurotransmitters, growth factors, hormones, light or chemotactic stimuli. The transmembrane proteins of the plasma membrane are also involved in cell-cell recognition.
Membrane proteins are difficult to crystallize

Membrane proteins, which have both hydrophobic and hydrophilic regions on their surfaces, are not soluble in aqueous buffer solutions and denature in organic solvents. However, if detergents, such as octylglucoside, are added to an aqueous solution, these proteins can be solubilized and purified in their native conformation. The hydrophobic part of the detergent molecules binds to the protein’s hydrophobic surfaces, while the detergents’ polar head-groups face the solution. In this way the protein-detergent complex acquires an essentially hydrophilic surface with the hydrophobic parts buried inside the complex.

Such solubilized protein-detergent complexes are the starting material for purification and crystallization. For some proteins, the addition of small amphipathic molecules to the detergent-solubilized protein promotes crystallization, probably by facilitating proper packing interactions between the molecules in all 3 dimensions in a crystal. Therefore, many different amphipathic molecules are added in separate crystallization experiments until, by trial and error, the correct one is found.
Not many known → around a dozen but it’s being worked on → smaller crystals and synchrotron will be better.

Turns out you can get 2D crystals much earlier than 3D crystals (the membrane makes the 3\textsuperscript{rd} dimensions) and this allows electron microscopes to be the best structural investigative tool so far.

Examples...
An **integral membrane protein (IMP)** is a protein molecule (or assembly of proteins) that is permanently attached to the biological membrane. Proteins that cross the membrane are surrounded by "annular" lipids, which are defined as lipids that are in direct contact with a membrane protein. Such proteins can be separated from the biological membranes only using detergents, nonpolar solvents, or sometimes denaturing agents. IMPs comprise a very significant fraction of the proteins encoded in an organism's genome.
Rhodopsin

Rhodopsin

Transducin
Bacterial rhodopsins

**Bacterial rhodopsins** are a family of bacterial opsins. They are retinal-binding proteins that provide light-dependent ion transport and sensory functions to a family of halophilic and other bacteria. They are integral membrane proteins with seven transmembrane helices, the last of which contains the attachment point for retinal (a conserved lysine).

Bacteriorhodopsin = trimer of 7 helices.
Bacteriorhodopsin contains 7 transmembrane α-helices

The purple membrane of Halobacterium halobium contains ordered sheets of bacteriorhodopsin, a protein of 248 amino acids residues which binds retinal, the same photosensitive pigment that is used to capture light in our eyes. Bacteriorhodopsin uses the energy of light to pump protons across the membrane.
Bacteriorhodopsin has 7 transmembrane $\alpha$-helices hooked together by loops

The helices are tilted $\sim 20^\circ$ to the plane of the membrane
What does it do?

It’s a light-driven protein pump.

Trans-retinal gets into the helices – binds and absorbs a photon and changes to cis-retinal – as a consequence → protons are pumped from the cystol to the extracellular space, setting up a proton gradient.

This gradient is used to generate ATP and to transport ions and molecules across the membrane ➔ PROTON PUMP
Trans-retinal enters

Light changes cis to trans by placing proton close to NH + group
Porin

Porins are beta barrel proteins that cross a cellular membrane and act as a pore through which molecules can diffuse. Unlike other membrane transport proteins, porins are large enough to allow passive diffusion, i.e., they act as channels that are specific to different types of molecules. They are present in the outer membrane of Gram-negative bacteria and some Gram-positive bacteria.

Porins typically control the diffusion of small metabolites like sugars, ions, and amino acids.

In gram-negative bacteria, the inner membrane is the major permeability barrier, whereas the outer membrane contains porins, which render it largely permeable to molecules less than about 1500 daltons.
Porin channels are made up and down β barrels

A 16 strand up and down β barrel all hydrogen bonded to neighbors

Porin barrels have a large central channel which is partially blocked by a long loop called the eyelet. Causes a narrowing in the middle of the channel – defines the size of the solute that can pass through (acts as a filter)

Calciums offset lots of negative charge in the middle.
The amino acid composition of the porin beta sheets is unique in that polar and nonpolar residues alternate along them. This means that the nonpolar residues face outward so as to interact with the nonpolar lipid membrane, whereas the polar residues face inwards into the center of the beta barrel to interact with the aqueous channel.

For antibiotics to be effective against a bacterium, they must pass through the outer membrane, using a porin.

Bacteria can develop resistance to the antibiotic by mutating the gene that encodes the porin—the antibiotic is then excluded from passing through the outer membrane.
The complete porin arrangement has 3 channels – it assembles as a trimer.

The outer surface of each interacts with the others – through hydrophobics and polar interactions with loops.

Large molecules get filtered out at the top of the trimer funnel.

Next – further screening at the entrance of each barrel.

Final screening by the restriction down the barrel by the loop on top.

3 stages of filtering: large molecules stooped and small one allowed through.
Ion channels

Ion selectivity and high ion conductance

The function of such ion channels is to allow specific inorganic ions, mainly $K^+$, $Na^+$, $Ca^{2+}$, and $Cl^-$ to diffuse rapidly across the lipid bilayer and balance differences in electric charge between the 2 sides of the membrane— the membrane potential

The membrane potential has two basic functions. First, it allows a cell to function as a battery, providing power to operate a variety of "molecular devices" embedded in the membrane.

Second, in electrically excitable cells such as neurons, it is used for transmitting signals between different parts of a cell. Opening or closing of ion channels at one point in the membrane produces a local change in the membrane potential, which causes electric current to flow rapidly to other points in the membrane.
Ion channels

The membrane potential of resting cells is largely determined by $K^+$ which can move freely in or out through $K^+$ leak channels.

These channels are selective for $K^+$ ions by a factor of 10,000 over $Na^+$.

This is quite interesting........Both $K^+$ ions and $Na^+$ ions are spheres and featureless. $K^+ > Na^+$ sizewise so how can selectivity be so great? Why doesn’t the $Na^+$ just fall through? The rate of selection is amazing...~$10^8$ ions per second.

Selectivity implies strong interactions between $K^+$ and the pore.
The $K^+$ channel is a tetramer molecule with one ion pore in the interface between the 4 subunits.

The polypeptide chain of the bacterial $K^+$ channel comprises 158 residues folded into 2 transmembrane helices, a pore helix and a cytoplasmic tail of 33 residues that was removed before crystallization.

Perpendicular to the plane of the membranes

Fits $K^+$ perfectly not $Na^+$

How does it do this?
2 subunits of the K\(^+\) channel. More chain atoms line the walls of the narrow passage with carbonyl oxygens pointing into the pore, forming binding sites for K\(^+\).

Helices point C=O into channel – for binding site for ion (4 at a time)
Same with potassium ion.........
The ion must lose its waters to fit in the pore.

It will only do this if the energy gain from the ion binding in the pore outweighs the energy loss of losing the water molecules.

$K^+$ has the perfect size to form fit nicely in the pore and pick up energy. It outweighs the desolvation energy – SO IT WILL DO THIS.

$Na^+$ does NOT have the perfect size to form fit nicely in the pore. It’s too small. Doesn’t make good contacts with the C=O’s in the pore. So it’s not energetically favorable to desolvate. So it won’t.
Negative charges at both end – attract the ions

K\(^+\) enters hydrated so must lose H\(_2\)O before gets in because knows will have more energy when bound with carbonyl’s

Na\(^+\) is too small to bind in the C=O O=C channel therefore will not be worth it’s time dehydrating.