Types of transmembrane proteins

Transmembrane proteins span entire biological membrane. They are also called as integral proteins because they run throughout the biomembrane. The firm attachment of Integral proteins to membranes is actually the result of hydrophobic interaction between membrane lipids and hydrophobic domains of the protein.

There are 2 types of transmembrane proteins

- Alpha Helical: Present in inner membranes of bacterial cells or plasma membranes of eukaryotes. This is the major category of transmembrane proteins
- Beta Barrels: Found only in outer membranes of Gram Negative Bacteria, cell wall of Gram Positive Bacteria, and outer membranes of mitochondria and chloroplast.

Another classification refers to the position of the N- and C-terminal domains.

Types I and II have only one transmembrane helix; the amino-terminal domain is outside the cell in type I proteins and inside in type II.

Type III proteins have multiple transmembrane helices in a single polypeptide.

In type IV proteins, transmembrane domains of several different polypeptides assemble to form a channel through the membrane.

Type V proteins are held to the bilayer primarily by covalently linked lipids

Type VI proteins have both transmembrane helices and lipid (GPI) anchors.

Reference: Lehninger Principles of Biochemistry 4 edition

Single Pass transmembrane protein: These allow only one component to pass through the membrane. This single-pass transmembrane protein has an extracellular N-terminus and cytoplasmic C-terminus for a cell membrane.

Multipass transmembrane protein: Multipass transmembrane protein basically allows more than one ion or molecule to pass through the membrane protein. In this protein the polypeptide crosses the lipid bilayer multiple times. Most of the membrane-spanning segments of polypeptide chains are thought to have an alpha helical conformation. This is because in a lipid environment the hydrogen bonding between peptide bonds would be maximized if the polypeptide chain were to form a regular alpha-helix

Find two examples of each transmembrane protein shown in this picture. Preferably your two choices should come from vastly different species for example (glutamate receptors in plants and mouse maybe (Arabidopsis thalia and Mus musculus)

- Answer:

A single transmembrane alpha-helix:

Human glycophorin A (GpA): Glycophorin is a glycoprotein of the erythrocyte plasma membrane, where 60% of the mass consists of complex oligosaccharide units are covalently attached to specific amino acid residues. Ser, Thr, and Asn residues are the most common points of attachment

Human Growth Hormone Receptors

Other examples include G-protein linked receptors, insulin receptors, Receptor Tyrosine kinase.

A polytopic alpha-helical protein:

Bacteriorhodopsin (PDB ID 2AT9): The single polypeptide chain folds into seven hydrophobic alpha helices, each of which traverses the lipid bilayer roughly perpendicular to the plane of the membrane. The seven transmembrane helices are clustered, and the space around and between them is filled with the acyl chains of membrane lipids. The light-absorbing pigment retinal is buried deep in the membrane in contact with several of the helical segments. It is light driven proton pump densely packed in regular arrays in purple membrane of the bacterium Halobacterium salinarum.

Reference: Lehninger Principles of Biochemistry 4th edition

Prominin: a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells. It is targeted to plasma membrane protrusions of non-epithelialâ€‰cells. It is a membrane associated 15Kda protein. (Reference: Anja Weigmann*, Denis Corbeil*, Andrea Hellwig, and Wieland B. Huttnerâ€ PNAS November 11, 1997 vol. 94 no. 23 12425-12430)

A transmembrane Beta barrel:

a) The Staphylococcus aureus alpha-hemolysin toxin (PDB ID 7AHL) is composed of seven identical subunits, each contributing one hairpin-shaped pair of beta strands to the 14-stranded barrel.

b) E. coli outer membrane protein TolC (PDB ID 1EK9) central to multidrug efflux and protein export. It is a transporter molecule, has three separate subunits, each contributing four beta strands in this 12-stranded barrel.

Other examples are:

Maltoporin (PDB ID 1MAL) & E. Coli outer membrane proteins FepA (PDB ID 1FEP) and OmpLA (PDB ID 1QD5).

Reference: Lehninger, Principles of Biochemistry 4th edition

Please discuss thoroughly the difficulties membrane proteins pose to crystallization and the ramifications of this, and any novel crystallization methods being used to overcome this challenge. It would be great if you can use your knowledge of amino acid side chains and maybe even some sequences from your membrane proteins from part to build a very convincing argument.

Answer: Membrane proteins are difficult to handle. This is because the difficulties reside in the amphipathic nature of their surface. They possess a hydrophobic surface where they are in contact with the alkyl chains of the lipids, and they possess a polar surface where they are in contact with the aqueous phases on both sides of the membrane or with the polar head groups of the lipids. In order to solubilize and to purify membrane proteins one has to add a vast excess of detergents- amphiphilic molecules that form micelles above their critical micellar concentration. The detergent micelles take up the membrane proteins and cover the hydrophobic surface of the membrane protein with their alkyl chains in a belt-like manner. The polar head groups of the detergents face the aqueous environment.

Hydrophobic domains tend to aggregate when taken out of the lipid bilayer – result in sticky precipitant of unfolded proteins.

Solution: Include mild detergents in the crystallization steps. This will mask the hydrophobic regions and will help the protein to solubilize and an amphiphillic microenvironment will be created which will be suitable for the crystallization. At sufficiently high concentrations of detergents i. e. above CMC, detergents form micelles that cover the hydrophobic regions of the membrane protein

In some cases mild detergents such as DDM form large micelles that can make crystallization difficult. A detergent may not be able to fully mask the transmembrane regions of a protein, and these regions may associate in order to sequester their hydrophobic surfaces from the bulk aqueous phase. This results in the aggregation and precipitation of the protein.

Current ways to improve solubilization and crystallization

Design, synthesis, and use of:

- Detergents with novel structures
- Novel detergent/peptide hybrids
- Cubic lipid phases-the in cubo crystallization.
- Increase hydrophilic surface area by using Abs fragments.

Address how ion channels can have such amazing specificity and ion conductance. I would like you to explain in your discussion the following observations “ The K+ leak channels are highly selective for K+ ions by a factor of 10, 000 over Na+ . What is the chemical basis for this selectivity for K+ ions since both ions are featureless spheres? Steric occlusions cannot account for the selectivity since Na+ is a smaller ion than K+(0. 95Å and 1.

35Å respectively). Finally, How can K+ channels be so selective and at the same time exhibit a throughput of 108 ions per second?”

Reference: © 2002 by Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter.

Answer: Ion channels have amazing specificity and ion conductance and this mystery was solved by X- ray crystallography. The channel is formed by 4 transmembrane subunits and these form central pore throughout the membrane. At the cytosolic end amino acids are negatively charged so they would attract the cations and repel anions and therefore makes the channel cation selective. Each subunit contributes two transmembrane helices, which are tilted outward in the membrane and together form a cone, with its wide end facing the outside of the cell where K+ ions exit the channel. The polypeptide chain that connects the two transmembrane helices forms a short a helix (the pore helix) and a crucial loop that protrudes into the wide section of the cone to form the selectivity filter. (Reference: Adapted from D. A. Doyle et al., Science 280: 69 77, 1998.)

The selectivity loops from the four subunits form a short, rigid, narrow pore, which is lined by the carbonyl oxygen atoms of their polypeptide backbones. Now since the selectivity loops of all known K+ channels have similar amino acid sequence so all of them form similar structure. The structure of the selectivity filter explains the exquisite ion selectivity of the channel. For a K+ ion to enter the filter, it must lose almost all of its bound water molecules and interact instead with the carbonyl oxygen’s lining the selectivity filter, which are rigidly spaced at the exact distance to accommodate a K+ ion. A

Na+ ion, in contrast, cannot enter the filter because the carbonyl oxygen’s are too far away from the smaller Na+ ion to compensate for the energy expense associated with the loss of water molecules required for entry. (Reference: Adapted from D. A. Doyle et al., Science 280: 69 77, 1998.)

Structural studies about bacterial K+ channels have shown how these channels open or close. The loops that form the selectivity filter are rigid and do not change conformation when the channel opens or closes. In contrast, the inner and outer transmembrane helices that line the rest of the pore rearrange when the channel closes, causing the pore to constrict like a diaphragm at its cytosolic end.

I would like you guys to do a little study of glutamate receptors. There are three types of human glutamate receptors. I will give you two out of the three (NMDA, AMPA) I need you to tell me

The different types of glutamate receptors in human beings

- What is the overall quaternary, tertiary, and secondary structures for each type of receptor?
- For each type of receptor tell me what are the functions of each different domain
- What are the differences between each receptor on the basis of amino acid sequence? How do these differences account for observed physiological differences between these receptors?

Answer: Different types of glutamate receptors are:

Ionotropic Glutamate Receptors: These receptors directly control the ion channels. These form ion channel pore that activates when glutamate binds to the receptor. These are quicker in relaying the information. These receptors include

a) AMPA (Î±-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor)

b) NMDA (N-methyl D- aspartate receptor)

c) Kainate receptor

Metabrotropic Receptor: These indirectly activate ion-channels on the plasma membrane through a signaling cascade that involves G proteins. These act through second messenger systems. They have prolonged stimulus because different messengers are used to begin the cascade i. e. signal.

The three classes of iGluR (NMDA, AMPA and kainite receptors) exist as macromolecular complexes that combine into numerous receptor assemblies.

- DOMAINS AND THEIR FUNCTIONS:

All iGluR subunits contain three transmembrane domains (Ml, M3 and M4) and a re-entrant membrane loop (MZ) on the cytoplasmic side that lines the inner channel pore and defines the distinct ion selectivity’s of the ion channel.

The large, extracellular amino-terminal domain of each subunit includes a necessary component of the glutamate recognition site (S1)

The M3-M4 loop includes a second required component of the glutamate recognition site (S2) and RNA splice variants that affect receptor desensitization. The intracellular carboxyl terminus is involved in signal transduction, receptor anchoring and contains phosphorylation sites that modulate receptor activity.

Two globular domains (Sl and S2, adjacent to Ml and the M3-M4 loop, respectively) form a bi-lobed glutamate binding pocket that is in dynamic equilibrium between open and closed states.

- Physiological differences between receptors:

ION SELECTIVITY: AMPA receptors have a low permeability ratio of divalent to monovalent ions as a direct consequence of RNA editing on the M2 re-entrant loop of GluR2. The gene-specified glutamine (Q) codon (CAG) is changed to an arginine (R) codon (GIG) for the pore-lining segment of virtually all GluR2 transcripts and, as a consequence, severely reduces the permeability of the AMPA channel for Ca”+ ions.

RECEPTOR DESENSITIZATION: Receptor desensitization modifies the processing of synaptic information and the plasticity of synapses. Desensitization of AMPA and kainate receptors occurs rapidly in the presence of glutamate and is controlled by subunit composition and a flip/flop splice region.

Glycine-independent NMDA receptor desensitization is dependent on two regions that flank the putative agonist binding domain of the amino terminus of the NR2 subunit. These regions are the first 350 amino acids, which

contains the leucine-isoleucine-valine-binding-protein-like (LIVBP-like) region, and a short segment preceding Ml.