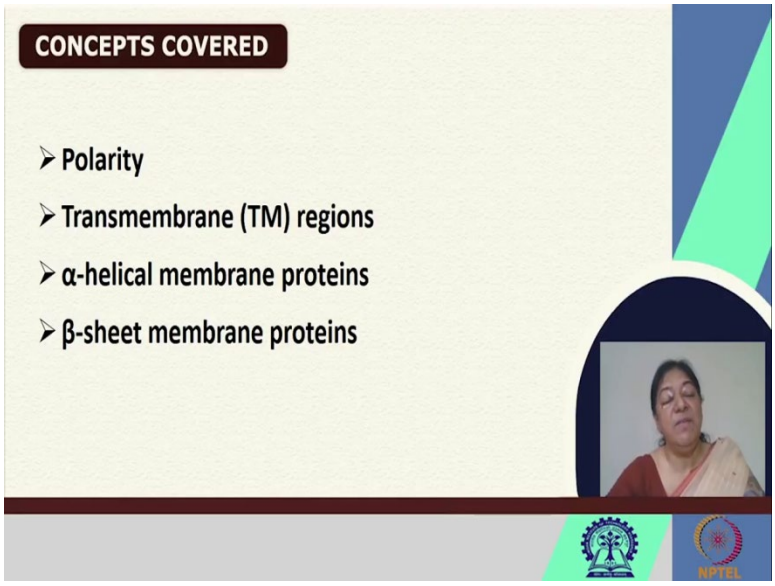
Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 09
Membrane Proteins and Transport
Lecture - 42
Membrane Proteins - II

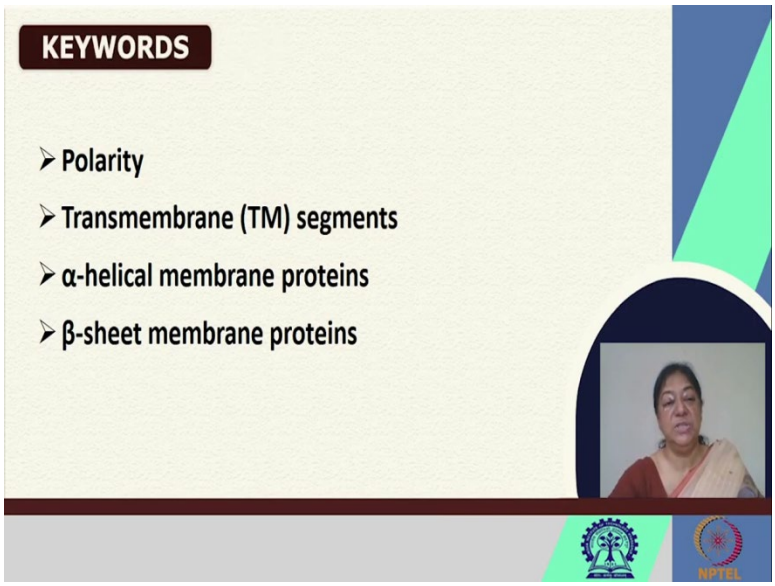
In a continuation of our discussion on membrane proteins and transport, we will be looking at specific aspects of membrane proteins from a structural point of view, considering the types of amino acids that are present in these membrane proteins and more about the transmembrane segments in proteins.

(Refer Slide Time: 00:35)

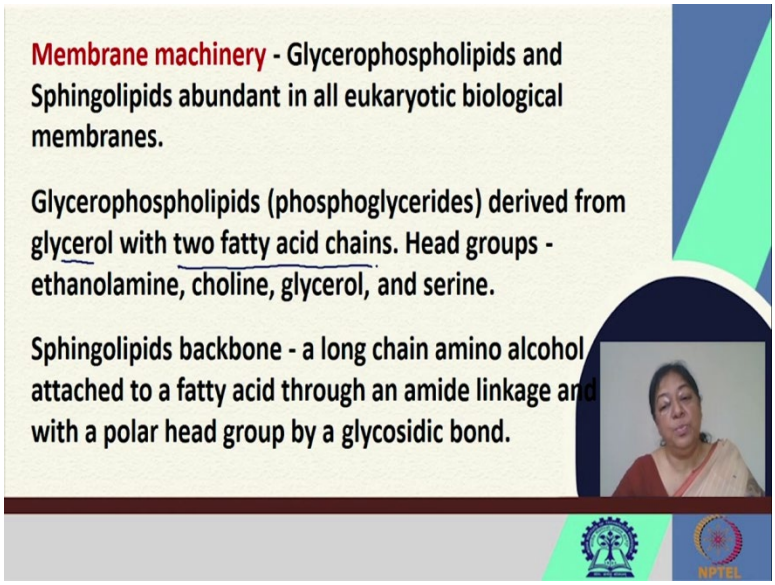


We will be covering the polarity of membranes, the transmembrane regions, α-helical and β-sheet types of proteins.

(Refer Slide Time: 00:42)

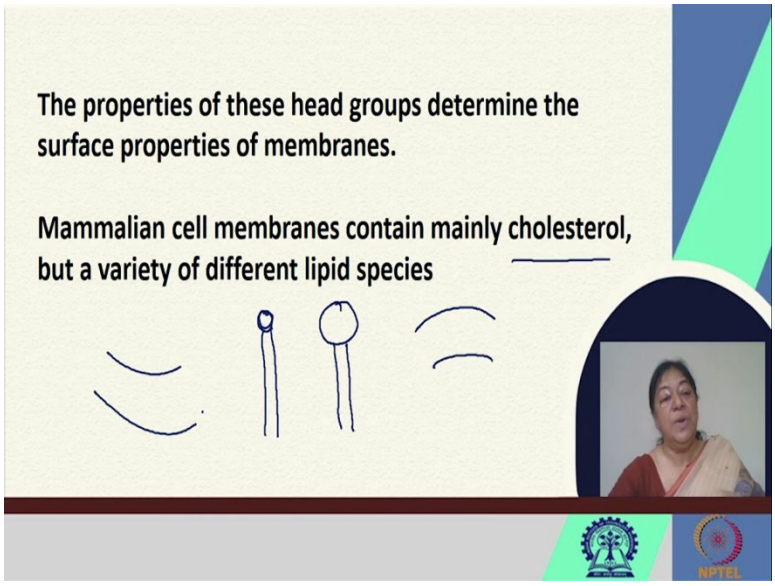


(Refer Slide Time: 00:44)



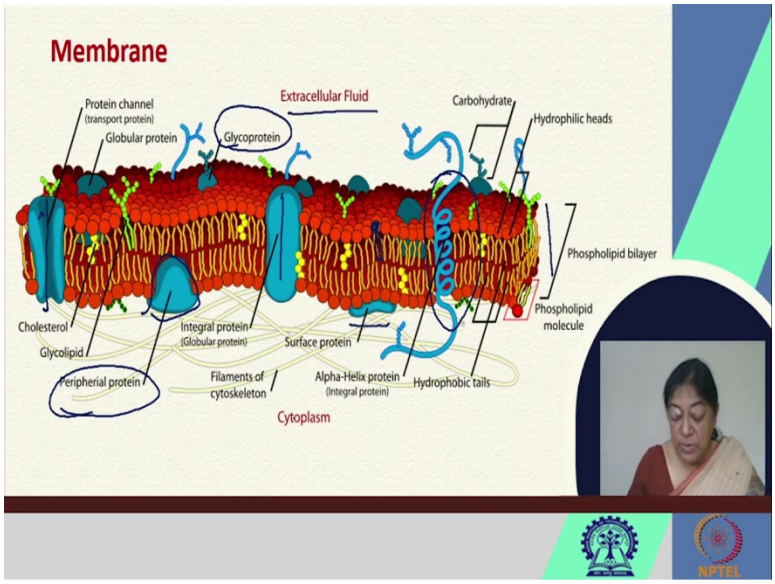
When we look at the membrane machinery, what we learned in the previous lecture was the understanding of the presence of several different types of lipids; the glycerophospholipids the sphingolipids. The glycerophospholipids that were derived from glycerol with two fatty acid chains and several different types of head groups. And then the sphingolipids, that also had a long two fatty acid chains, that then would bring a specific linkage to the lipid.

(Refer Slide Time: 01:18)



The properties of these head groups we found, determine the surface properties of the membranes and also the presence of cholesterol was important in bringing the fluidity to the membrane. So, we understood that when the length of the chain and the size of the head group did have a role to play in the specific curvature of our membrane.

(Refer Slide Time: 01:48)



If we look at what is called the fluid mosaic model of a membrane, we will see the various components that are present in the membrane. So this [refer to slide] is our phospholipid bilayer, to this we can have a glycoprotein, a protein carbohydrate interaction that indicates a protein connected to a carbohydrate, which we will study in the following module.

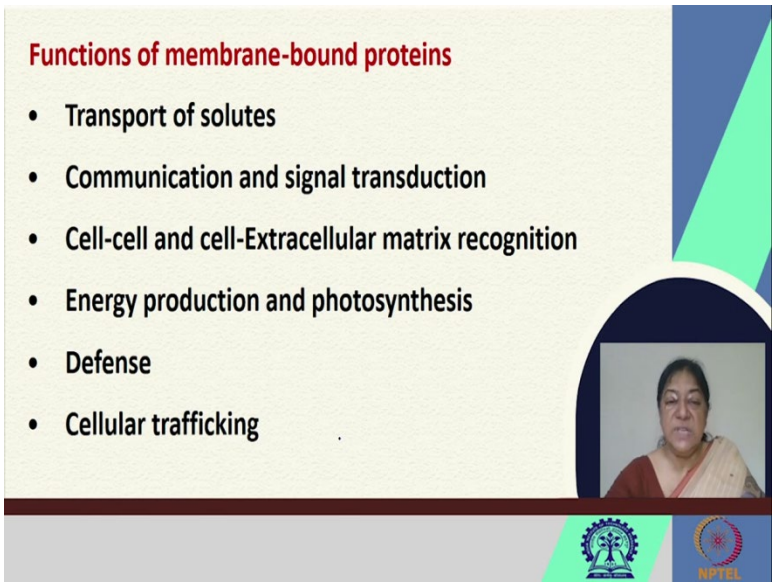
We have a specific channel, a transport protein, that is what membrane proteins are mostly composed of, we have a peripheral protein that is this part of the protein that has specific

activity. Another type of integral protein that would have an α-helical type of structure, that transverses the membrane.

In the lipid structure, we have the head groups, we have the head groups here and we have their chains. And, this forms the lipid bilayer embedded in which we have the carbohydrates, we have the globular proteins, we have glycoprotein's where we have the carbohydrates linked to the proteins, we have protein channels and we have integral proteins, that could be globular proteins, that are part of the membrane.

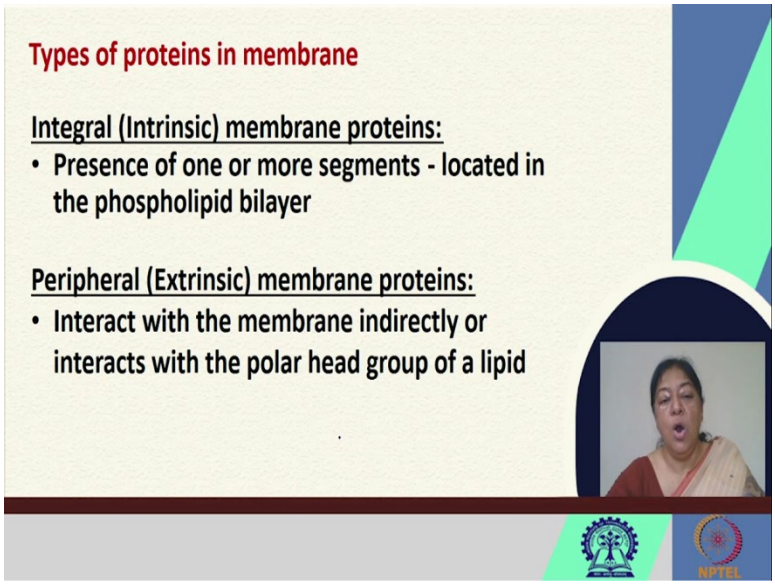
Each of them have a specific function and this specific functionality in terms of membrane proteins is membrane transport. We have 2 lectures devoted to membrane transport, where we will be looking at the active and passive transport of these ions through the membranes, from the extracellular fluid to the cytoplasm and vice versa.

(Refer Slide Time: 03:44)



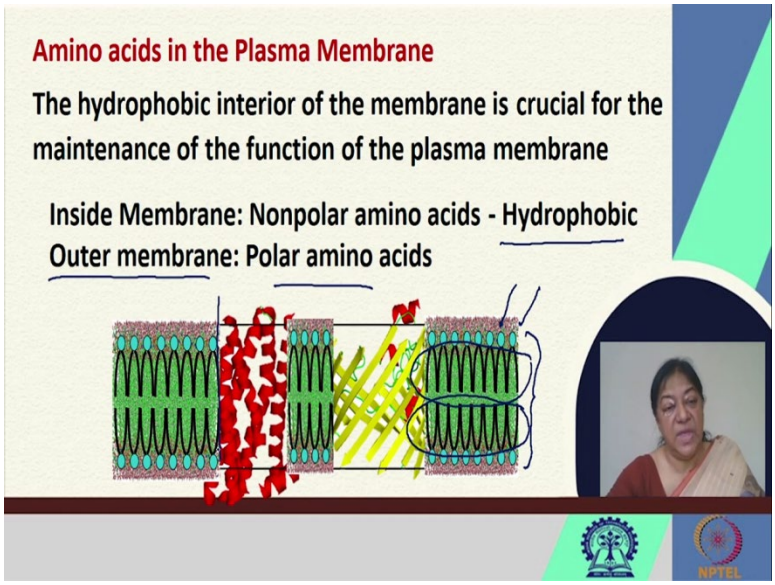
So the functions of membrane bound proteins is, the transport of solutes, communication and signal transduction, cell-cell and cell-extracellular matrix recognition, energy production, photosynthesis, defense and cellular trafficking. This gives us an idea of how we need to understand the structural aspects and associated with it the specific functional aspects.

(Refer Slide Time: 04:15)



The types of proteins we saw that were present in membranes, were the integral type where we had one or more segments that were located in the phospholipid bilayer and the peripheral type that interacted with the membrane indirectly or with the polar head group of a lipid.

(Refer Slide Time: 04:34)

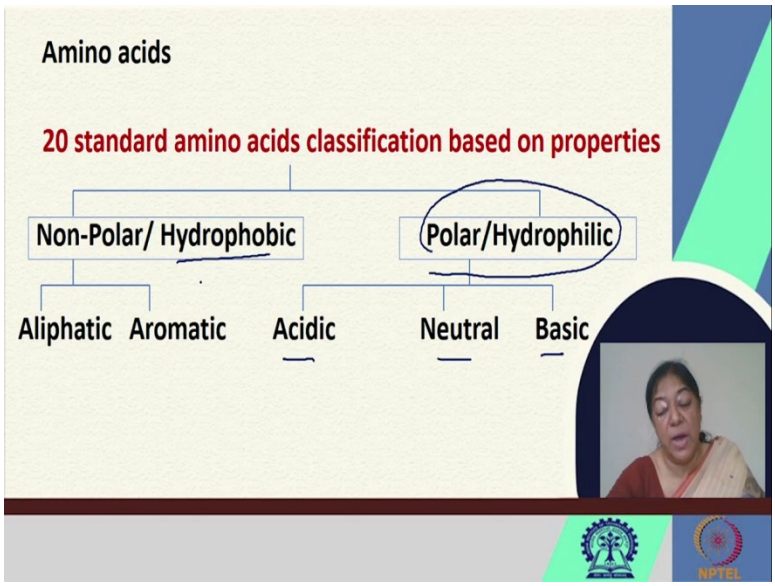


Now to look at the amino acids in the plasma membrane. We understand that there is a hydrophobic interior of the membrane that is crucial for the maintenance of the function of the plasma membrane. So inside the membrane these nonpolar amino acid residues, hydrophobic type amino acid residues, is what we would expect to interact.

At the outer membrane, the polar head being present, we would have polar amino acid residues. If we look [refer to slide] at our lipid bilayer structure, we have the lipid content and these are the polar head groups. Then what we would expect for a certain amino acid or a certain structural

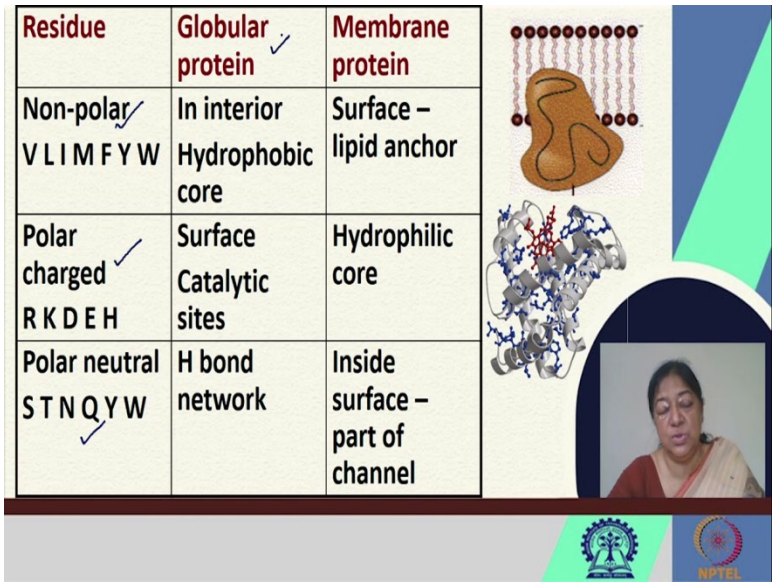
aspect of the protein, any part that would interact with the lipid bilayer, would have nonpolar amino acids present there.

(Refer Slide Time: 05:33)



If we look at the 20 standard amino acids that are classified based on their properties, we know we have the polar hydrophilic type, that could be acidic neutral or basic and we have the hydrophobic type, that could be aliphatic or aromatic in nature.

(Refer Slide Time: 05:48)

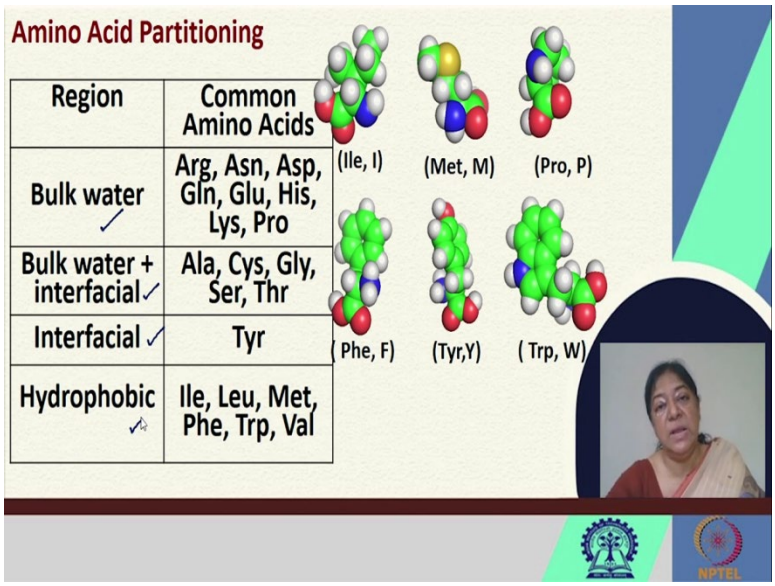


Based on this there is a distribution of where these amino acids can lie. So, we can have nonpolar types, polar charge types and polar neutral types. Usually when we are looking at a globular protein, we have seen that we expect the non polar to form the hydrophobic core of the protein,

the interior of the protein. And the polar charged amino acids or the polar neutral amino acid would be on the surface of the protein and the others would be involved in a hydrogen bonding network.

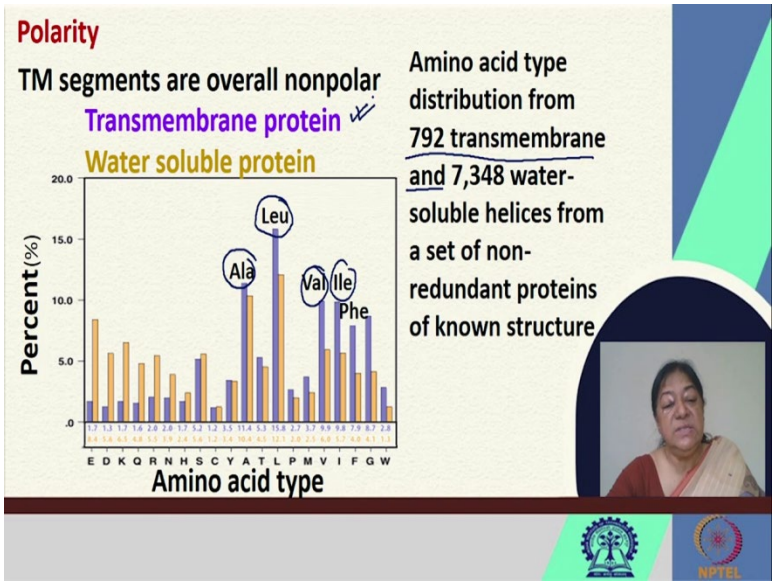
However for the membrane proteins we need a lipid anchor and this lipid anchor is brought about by the presence of the non-polar proteins, the core is hydrophilic because there has to be the transport of ions which would prefer an ionic environment or an aqueous environment for this transfer. We have the inside surface of this pore that forms part of the channels and we will be looking at how they transport ions in the next 2 lectures.

(Refer Slide Time: 07:03)



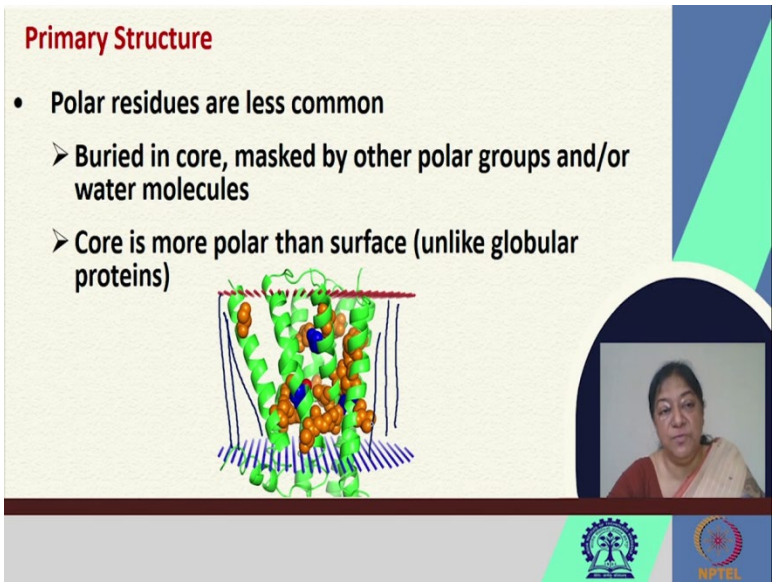
If we look at amino acid partitioning, we have the region which will be called bulk water, bulk water and interfacial, interfacial and hydrophobic. Now what we mean by these is, we would have the small amino acid residues, the hydrophobic amino acid residues, the aromatic type of amino acid residues; each having their own role to play in their interaction with the membrane lipid bilayer.

(Refer Slide Time: 07:33)



If we look at the polarity, these transmembrane segments are overall nonpolar. However, in a study where several transmembrane segments and water soluble helices were looked at [refer to slide], the transmembrane protein is marked here in purple and the water soluble protein marked here in yellow; we see a larger number of hydrophobic types of amino acid residues present in the transmembrane proteins.

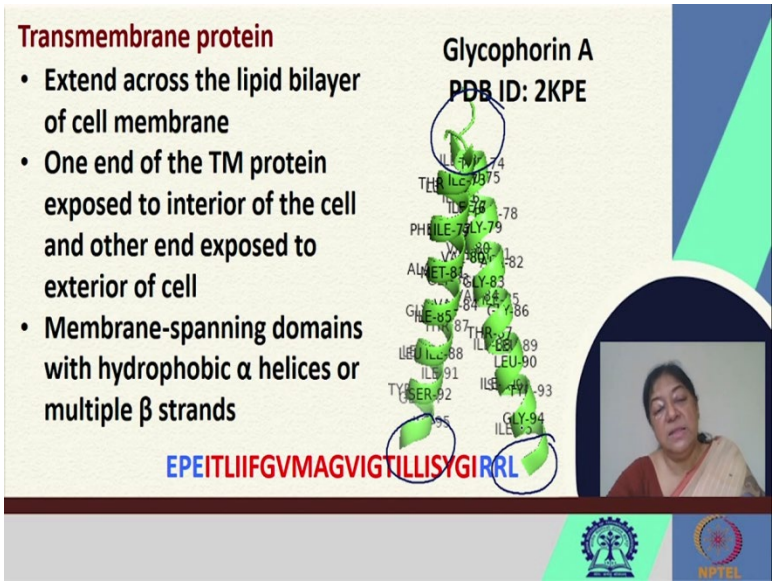
(Refer Slide Time: 08:11)



Now this gives us an idea of trying to understand, what is present in the primary structure, in consideration of what types of amino acids. So, the polar residues are buried in the core. They are masked by other polar groups and/or water molecules and the core has to be more polar than the surface, unlike globular proteins because the interaction type of the surface is with the lipid bilayer, that is the hydrophobic fatty acids.

If we look at the way the membrane is embedded in the protein, we would have the lipid bilayered at these positions and we would expect the interaction of the surface of these to be interacting with the lipid bilayer. The amino acids present there would have to be hydrophobic in nature.

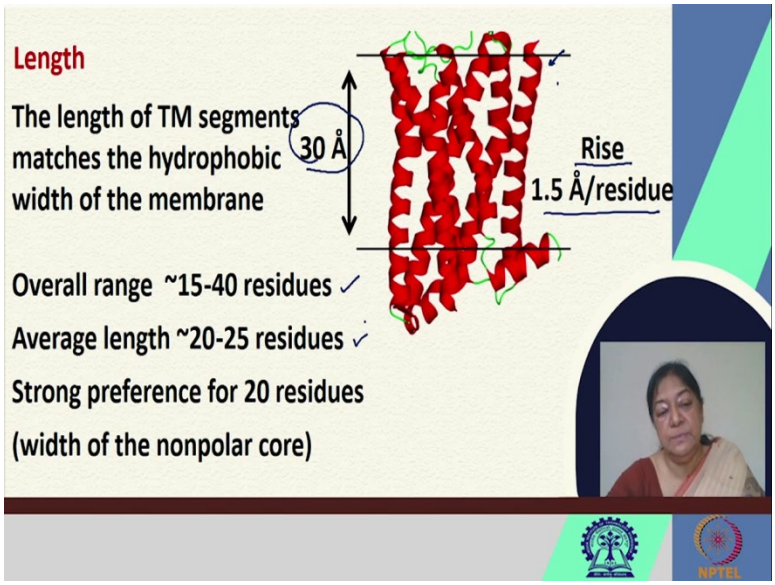
(Refer Slide Time: 09:00)



The transmembrane proteins, they extend across the lipid bilayer of the cell membrane and one end of the transmembrane protein is exposed to the interior of the cell and the other is exposed to the exterior of the cell.

So at the terminal regions, we would expect the amino acids present there to be of a polar nature, because they would have to interact with the matrix; either the cytoplasm or the external media of the cell. The membrane spanning domains now will have hydrophobic α-helices or could have multiple β-strands and the presence of the specific types of amino acids is important in bringing about their presence in the membrane.

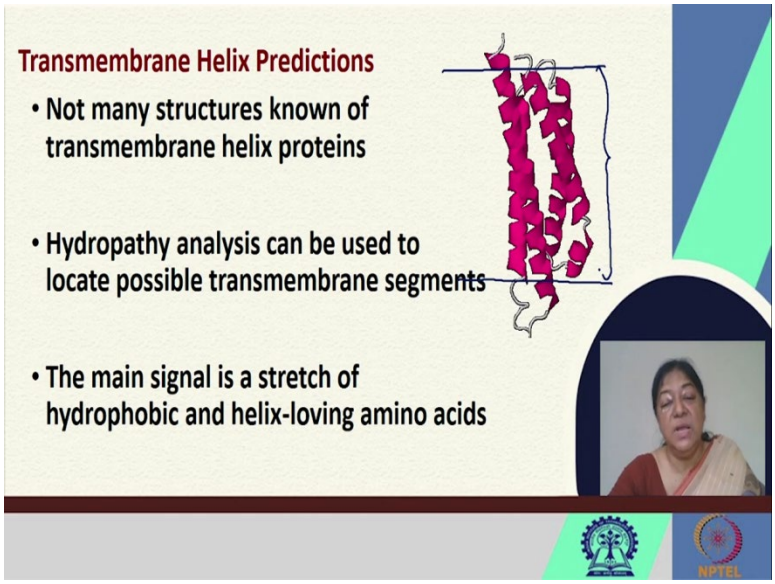
(Refer Slide Time: 09:50)



If we look at the length of a typical membrane, that is 30 Å and we try and identify how many residues need to span the membrane or be part of this transmembrane segment. We learned from module 1, where we studied amino acids and the structures of proteins in modules 1 and 2, we looked at the rise per residue for a helix which is 1.5 Å.

If we want to identify or determine the length of the transmembrane segments, we have to see the match with the hydrophobic width of the membrane. The overall range is around 15 to 40 residues and the average length is around 20 to 25 residues. Considering that the span of the membrane is around 30 Å, there is a strong preference for 20 residues for the width of the nonpolar core.

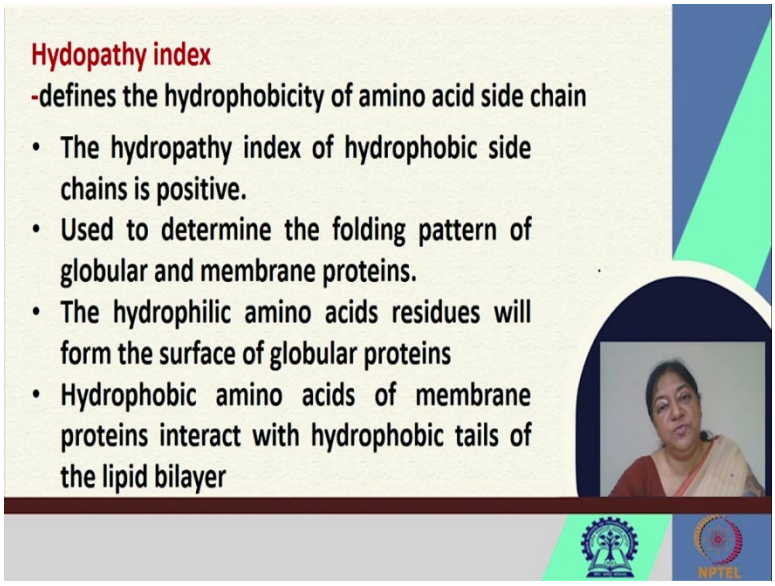
(Refer Slide Time: 10:56)



When we look at transmembrane helix predictions, we know that there are not many structures known of transmembrane helix proteins, because there are solubility issues for their determination. A hydropathy analysis is done to locate possible transmembrane segments.

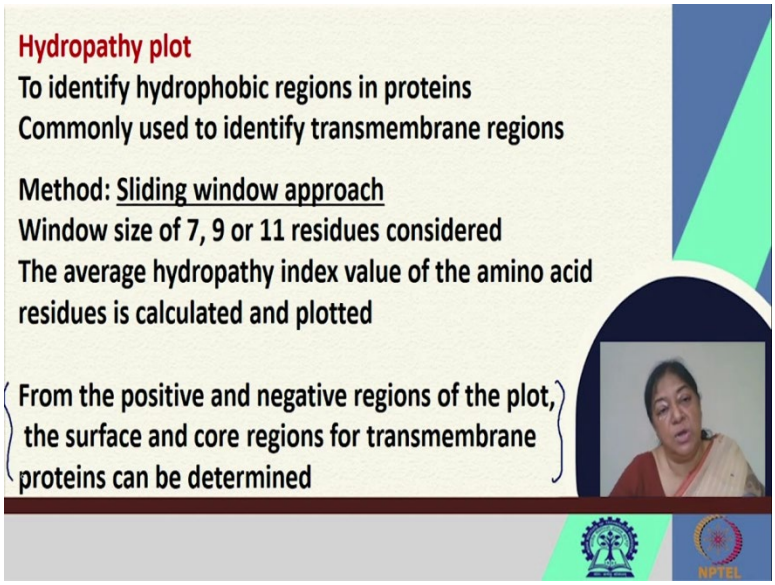
So, a knowledge of the types of residues that are present in this is understandable because of their interaction with the lipid bilayer. But to try and identify which residues are likely to form the transmembrane helix or the transmembrane segment of the protein, we need the hydropathy index.

(Refer Slide Time: 11:42)



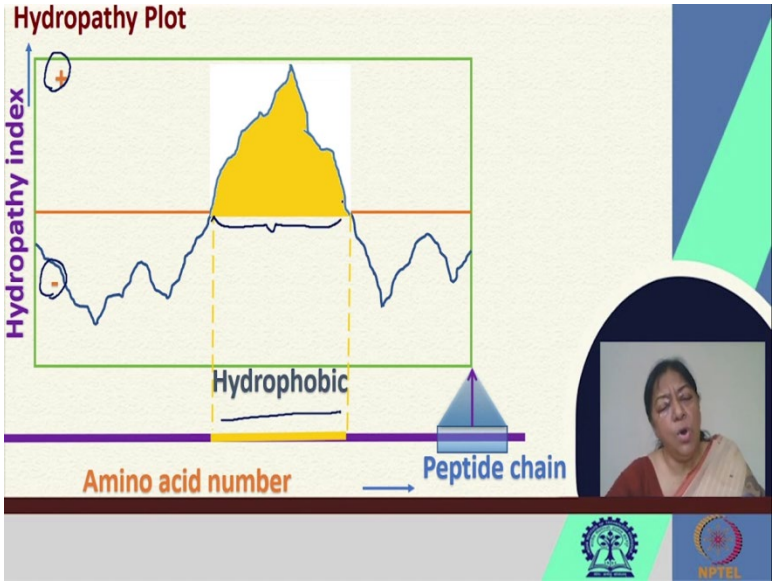
This hydropathy index defines the hydrophobicity of an amino acid side chain and for hydrophobic side chains, it is positive and it can be used to determine the folding pattern of globular and membrane proteins. It is widely used to identify transmembrane segments in proteins. The hydrophilic amino acid residues will form the surface of the globular proteins as we already know and the hydrophobic amino acids of the membrane proteins, will interact with the hydrophobic tails of the lipid bilayer.

(Refer Slide Time: 12:24)



If we want to identify these hydrophobic regions in the proteins that are used to determine the transmembrane regions, we use what is called a sliding window approach. Without going into the details of the method that has been described earlier, we will try and see what we can get or what knowledge we can gain, from a hydropathy plot of the typical membrane proteins. So from the positive and negative regions of the plot, the surface and the core regions for the transmembrane proteins can be determined.

(Refer Slide Time: 13:03)

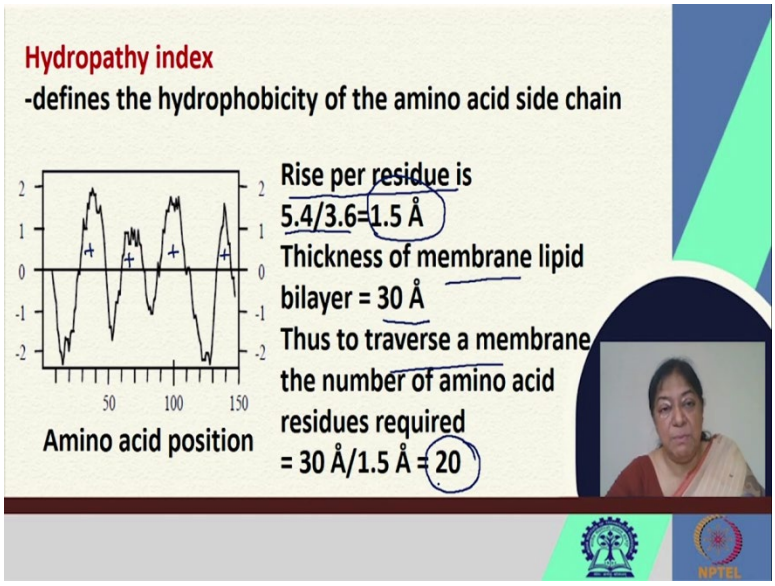


If we look at a general plot we would have the hydropathy index plotted on the y axis. Anything positive would be hydrophobic in nature, anything negative would be hydrophilic in nature. Then

if we look [refer to slide] at the positive region, this would be hydrophobic and when we look at the negative region, this is hydrophilic in nature.

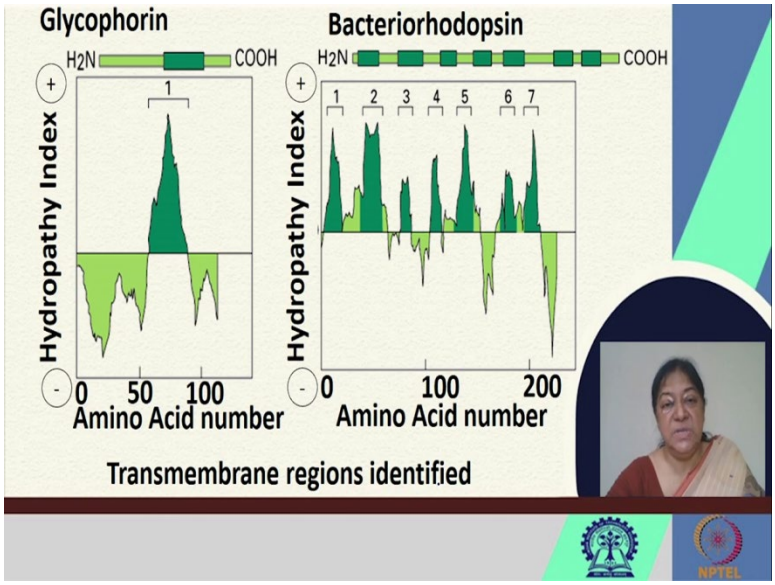
So now if we go along the residue, have this sliding window approach, we know how to look at the average and we plot the average hydropathic index of a sliding window size of 79 or 11. What we find is we find a positive region here and this positive region indicates, that this is the hydrophobic region. If this now spans around 20 amino acids or is more than 20 amino acids long, we can say that it is a transmembrane helix.

(Refer Slide Time: 13:59)



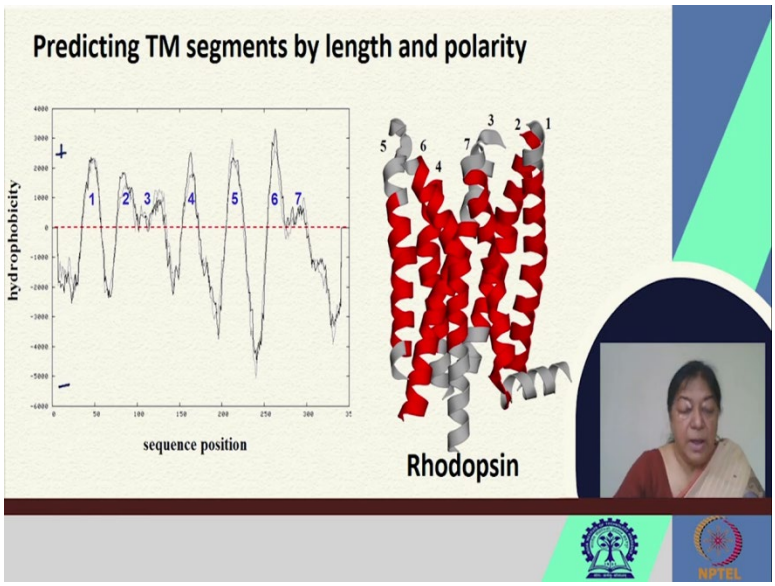
As we looked at the rise per residue where we had a 1.5 Å rise per residue, considering that the thickness of the membrane lipid bilayer is around 30 Å, thus to traverse the membrane the number of amino acid residues required would be 20. So if we look at our hydropathy plot, that has a positive region and spans around 20 amino acid residues, we can consider this to be a transmembrane helix following a transmembrane pattern.

(Refer Slide Time: 14:36)



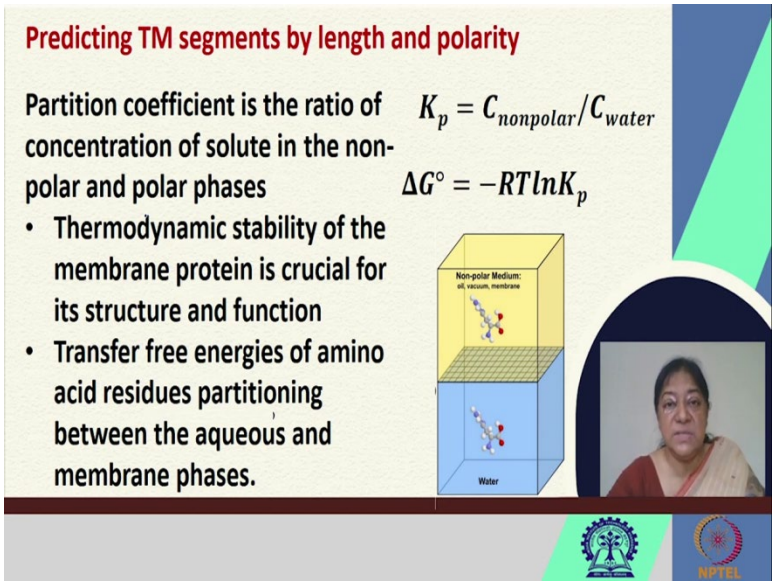
This is a typical transmembrane region identification in two types of proteins, where we have a single helix or we can have multiple helices like in bacterialrhodopsin.

(Refer Slide Time: 14:49)



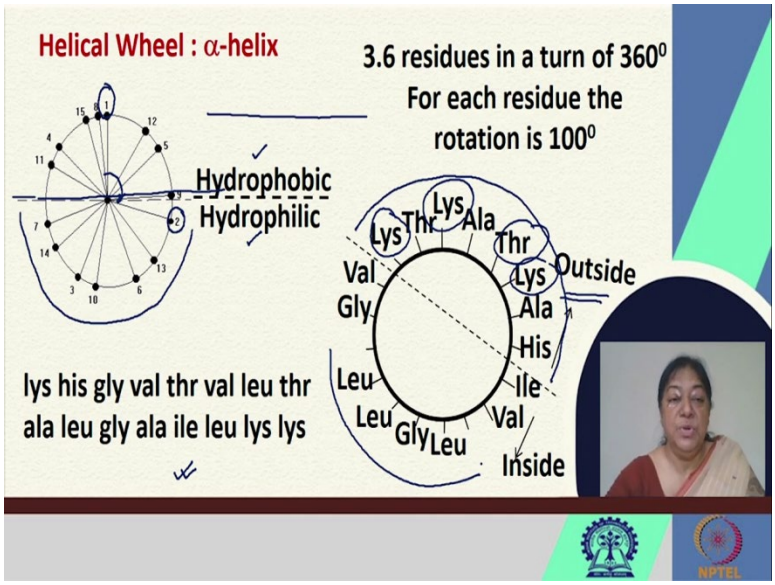
So, when we predict these TM segments by length and polarity, this is what we can look at [refer to slide] in terms of the hydrophobicity; that tell us from the hydropathy index, from the hydropathy plot, (which plots the average of the hydropathy indices values for the specific sliding window), our number of helices.

(Refer Slide Time: 15:15)



Now, we can look at the polarity. The polarity is measured by a partition coefficient, that is the ratio of the concentration of the solute in the nonpolar and polar faces. In this case our solute is our membrane protein and we can look at the specific types of amino acids to understand their relative polarity. And the thermodynamic stability of the membrane protein is crucial for its structure and function, to maintain its fluidity and to allow for the proteins to be embedded in the lipid membrane.

(Refer Slide Time: 15:53)



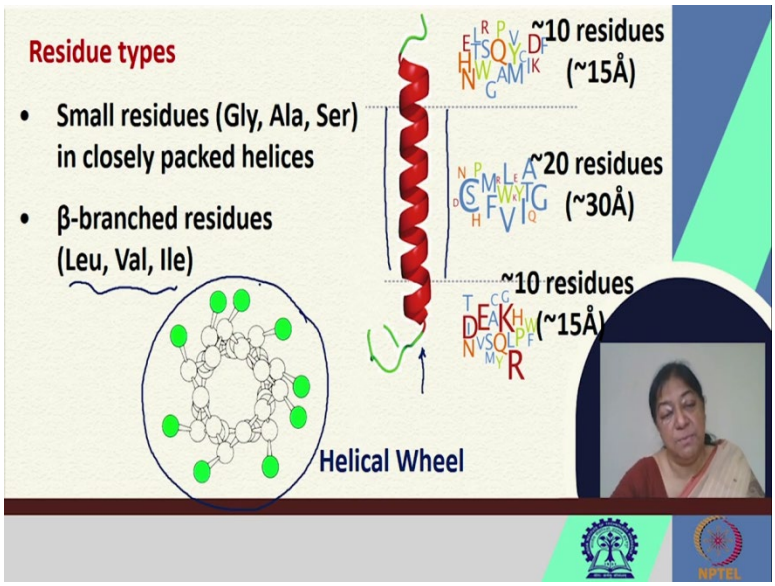
We also looked at a helical wheel in our understanding of proteins and protein structure, for what an α-helix would be in the identification of the types of residues involved and whether we can predict an α-helix being on the surface of a protein or not. Knowing that there are 3.6 residues in a turn of 360°; it meant that for every rotation the residue would rotate by 100°. So you could go from 1 to 2 in an angle of 100°.

So this would give us an indication, if we had the location or we had a sequence of residues that would fall in an amphipathic type of set, where we would have a hydrophobic component and a hydrophilic component; we can safely say that this [refer to slide] portion faces the exterior in a globular protein. However, if we do the same exercise for a membrane protein, this is not what we would expect.

So in this case, if we construct a helical wheel for the sequence given here [refer to slide], we would get a sequence that looks like this or the helical wheel that looks like this; which would indicate with all these types of residues present here, that this would preferably face the external portion of the protein for the globular protein.

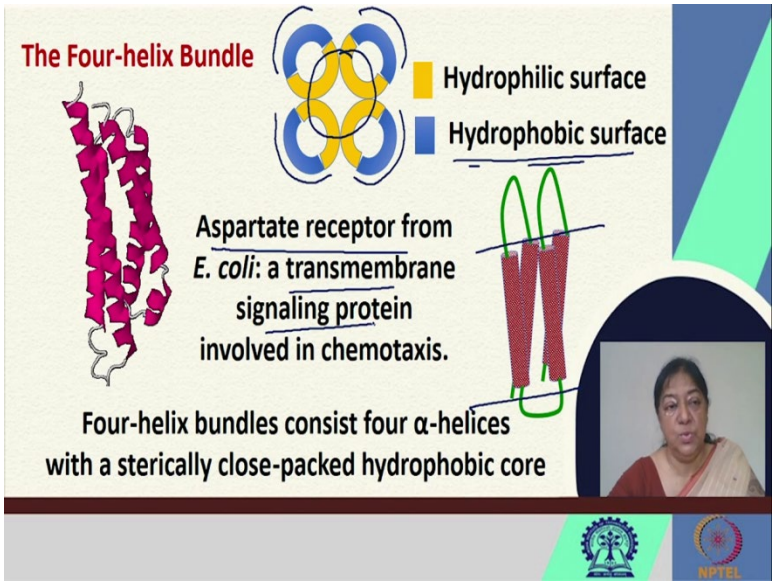
However, the presence of the hydrophobic amino acid residues would mean that this faces the inside of the protein.

(Refer Slide Time: 17:39)



However, when we look at our residue types for the transmembrane helices, we know that there are small residues that can actually be in closely packed helices. We also have β branched residues that are hydrophobic in nature, that can assist in the compact formation that has to be embedded in the membrane lipid bilayer. So, if we look at a helical wheel constructed of this particular helix that is embedded in the membrane, we would expect all of these residues to be hydrophobic in nature because this is a transmembrane helix.

(Refer Slide Time: 18:22)

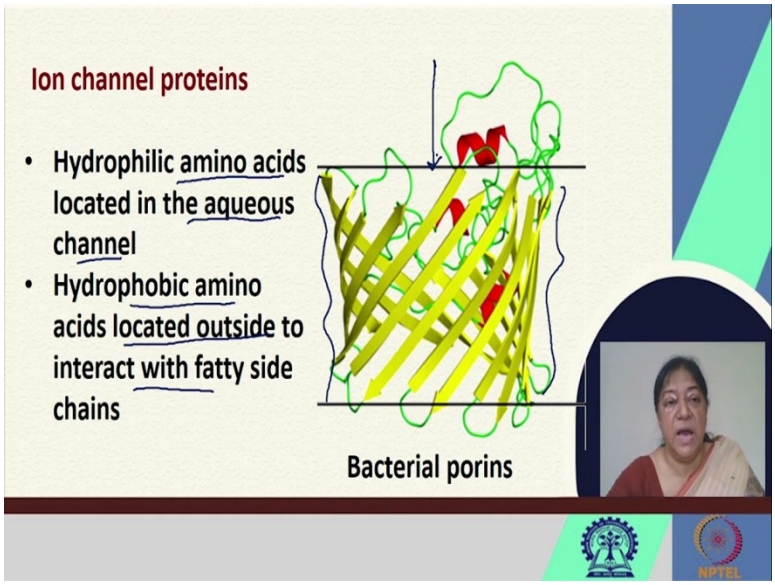


If we now look at a four-helix bundle that is a common structural motif in membrane proteins, this [refer to slide] is what the structure would look like, where this would be the transmembrane region and we would have in this case a four-helix bundle that consists of 4 α-helices with a sterically close packed hydrophobic core.

When we look [refer to slide] down a helical wheel construct, we would have these regions that would be hydrophobic in nature because those are the regions that would be interacting with the lipid bilayer. However, the internal portion that is away from the membrane would be hydrophilic in nature because that has a very important role to play in the transport of ions.

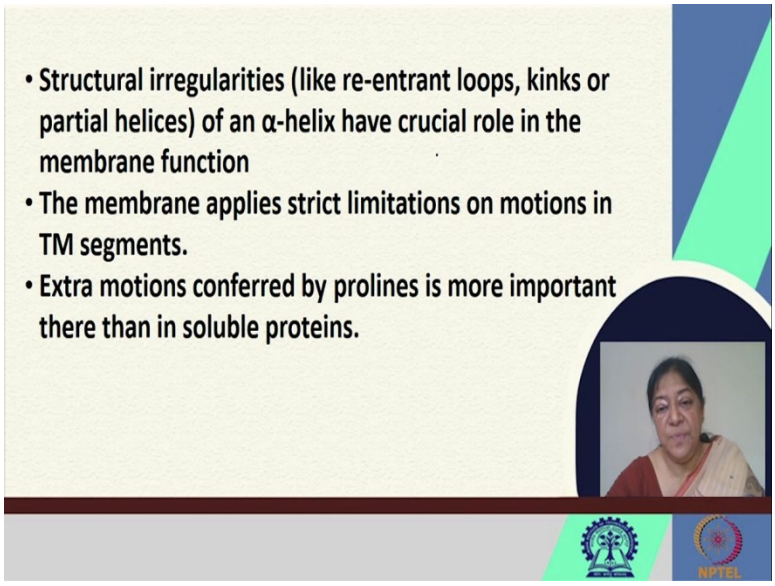
An aspartate receptor is an example of a four-helix bundle protein, that is a transmembrane signaling protein involved in chemotaxis. We will be looking at the transport mechanism, the specific free energy and the membrane potential involved in the next 2 lectures.

(Refer Slide Time: 19:40)



We have the ion channel proteins with their function, we have hydrophilic amino acid residues located in the aqueous channel and hydrophobic amino acid residues located outside. So, these [refer to slide] would be the outside portions that would again interact with the lipid bilayer, but we would have hydrophilic amino acids located in the internal region for the transport of the ions.

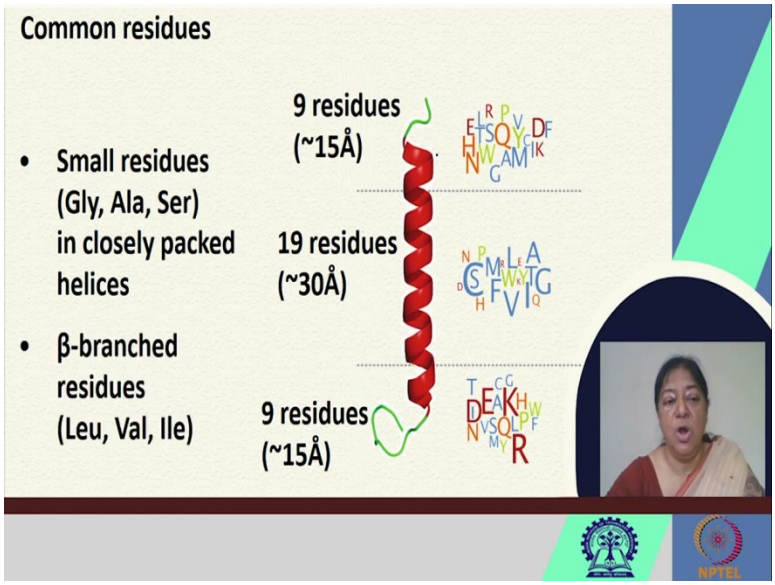
(Refer Slide Time: 20:14)



When we look at structural irregularities like re-entrant loops, kinks or partial helices, they have a very crucial role in membrane function. The membrane applies strict limitations on the motions of the transmembrane segments because they are compacted due to the presence of the lipid bilayer and the membrane cannot be too porous, to allow the transport or the diffusion of anything from the extracellular region to inside the cell.

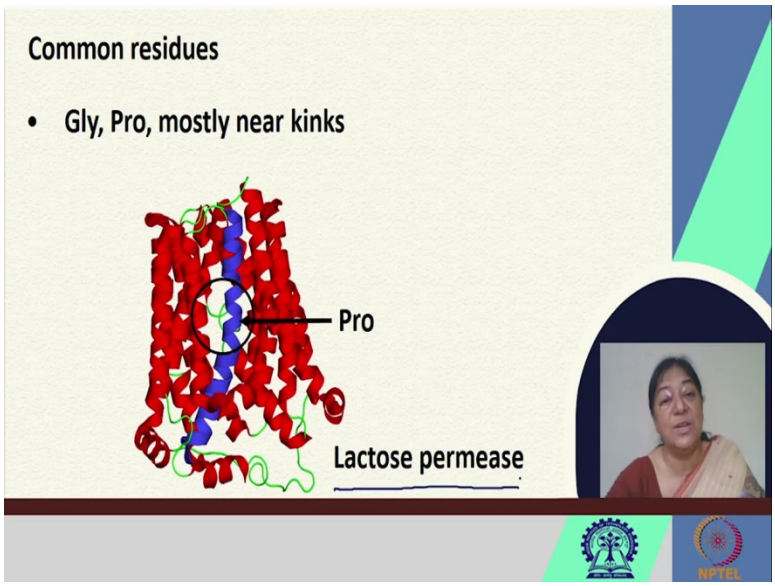
This control is brought about by the specific proteins that are present in the lipid bilayer. There are extra motions that are conferred by specific amino acids such as prolines, which are more important.

(Refer Slide Time: 21:04)



If we look at the closely packed residues, they have a very important role to play in bringing about the compact structure of these types of helices that are present in the membrane protein.

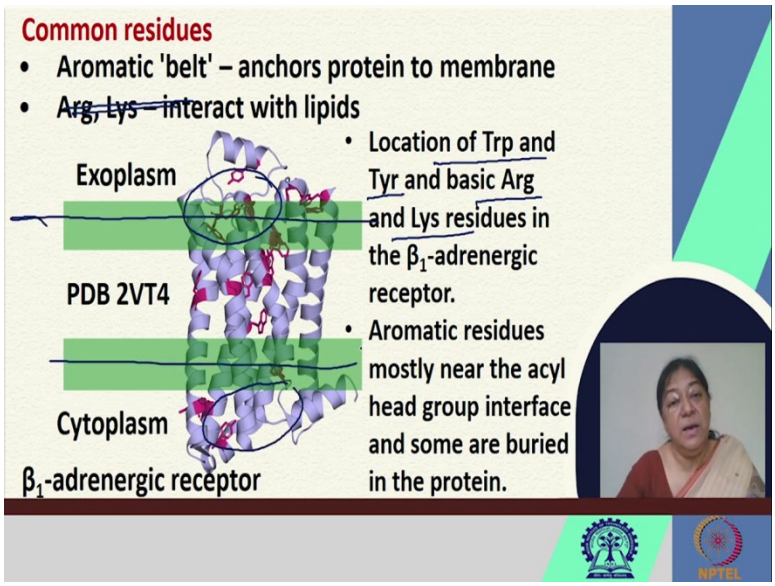
(Refer Slide Time: 21:19)



We have proline here [refer to slide] and this glycine and proline are helix breakers, in a sense that with the transmembrane helix they could bring about a break in the structure. But this

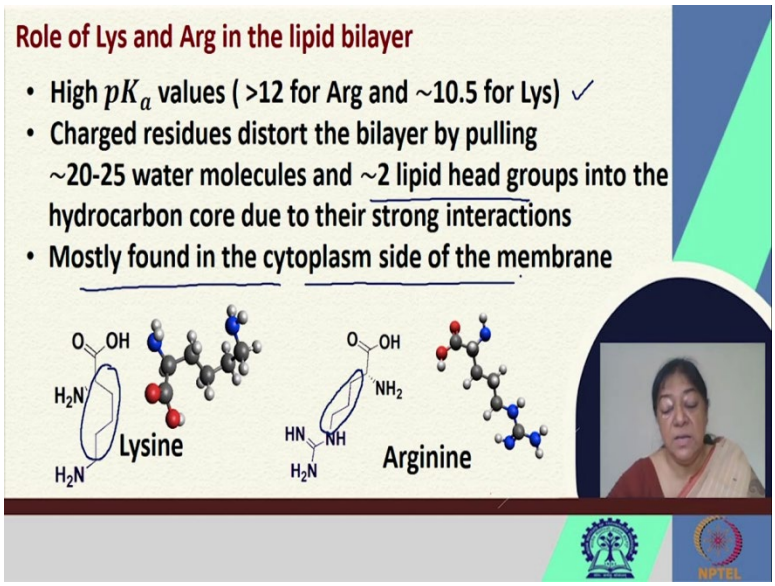
structure is sometimes required to bring about the fluidity that is important for example, in a protein like lactose permease.

(Refer Slide Time: 21:44)



The other important aspect is the aromatic belt, that is present to anchor the protein to the membrane. So, there is a specific location for the aromatic amino acids for example, tryptophan and tyrosine and basic arginine and lysine residues. In this case what happens, the arginine in the lysine can interact with the lipids and we have the locations of the aromatic amino acid residues, in this [refer to slide] aromatic belt to anchor the protein to the membrane.

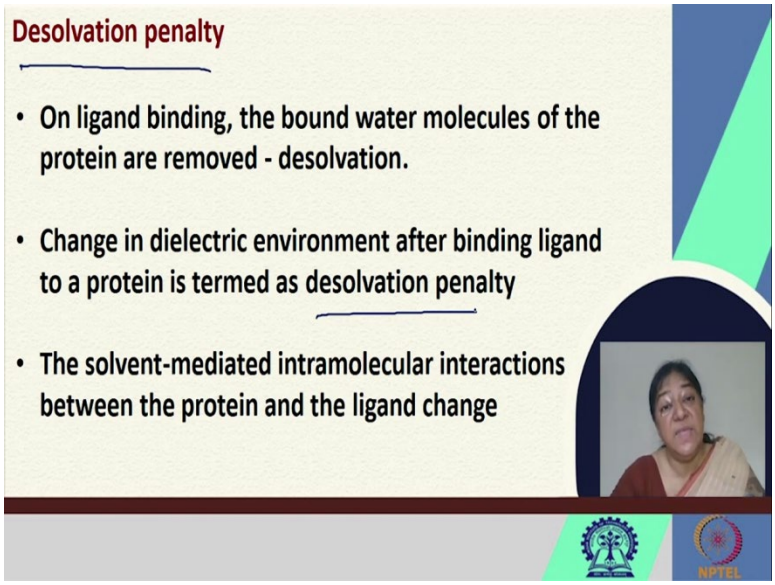
(Refer Slide Time: 22:24)



Now if we look at the role of lysine and arginine in the lipid bilayer, they have a very high pK_a value because of they being basic amino acid residues and the charge residues can distort the bilayer by attracting water molecules to them. There are approximately 2 lipid head groups that can be pulled into the hydrocarbon core due to their strong interactions.

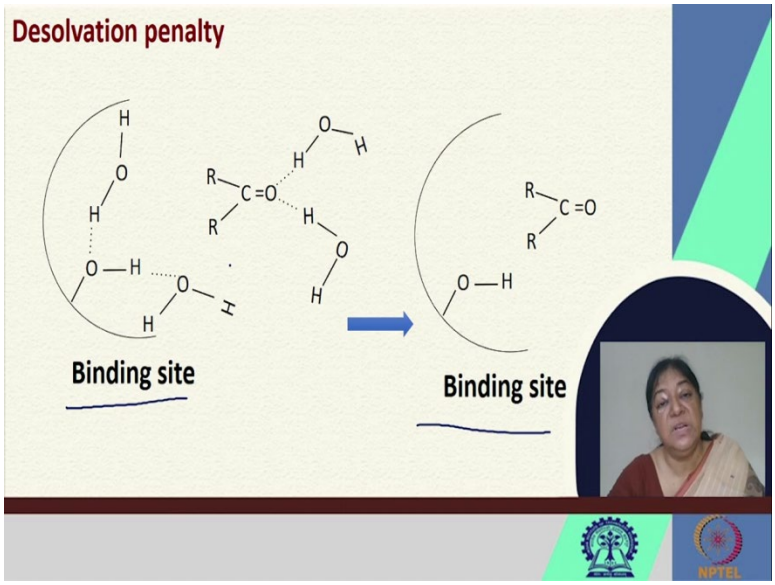
The presence of these long chains, allow them to interact with the lipid bilayer fatty acid chains, but they are found mostly in the cytoplasmic side of the membrane. There are many reasons for their presence in the membrane.

(Refer Slide Time: 23:14)



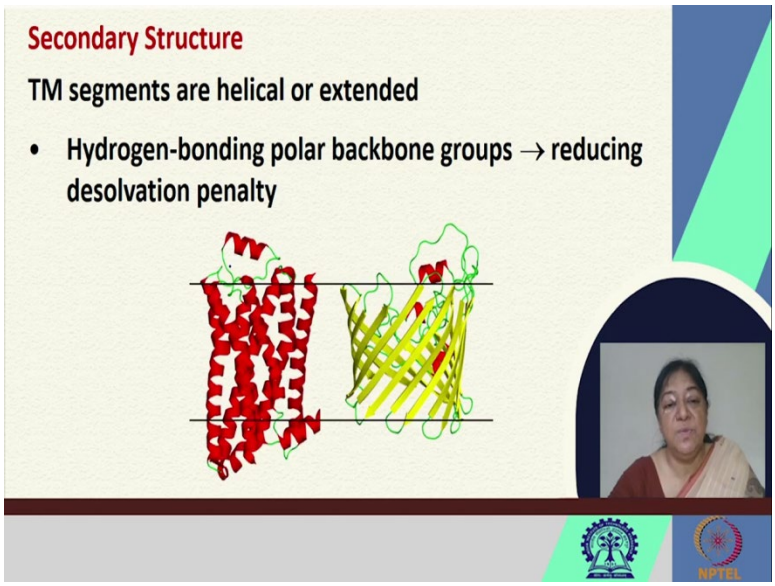
Now on ligand binding there is a property called the desolvation penalty. On ligand binding the bound water molecules of the protein are removed which results in a change in the dielectric environment after binding, that is called the desolvation penalty and the solvent mediated intramolecular interactions between the protein and the ligand, then change.

(Refer Slide Time: 23:43)



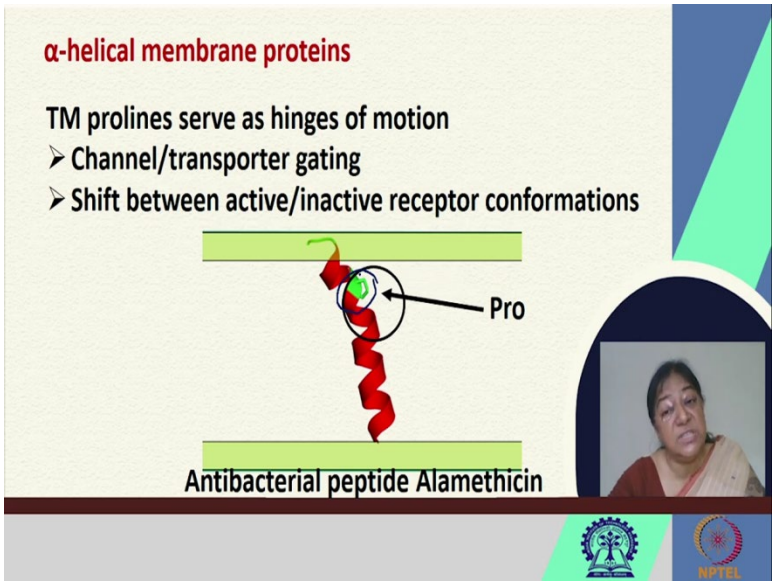
As a result, there is a variation in the binding site due to the presence of the water molecules or their absence, when they are pulled away due to the other interactions.

(Refer Slide Time: 24:02)



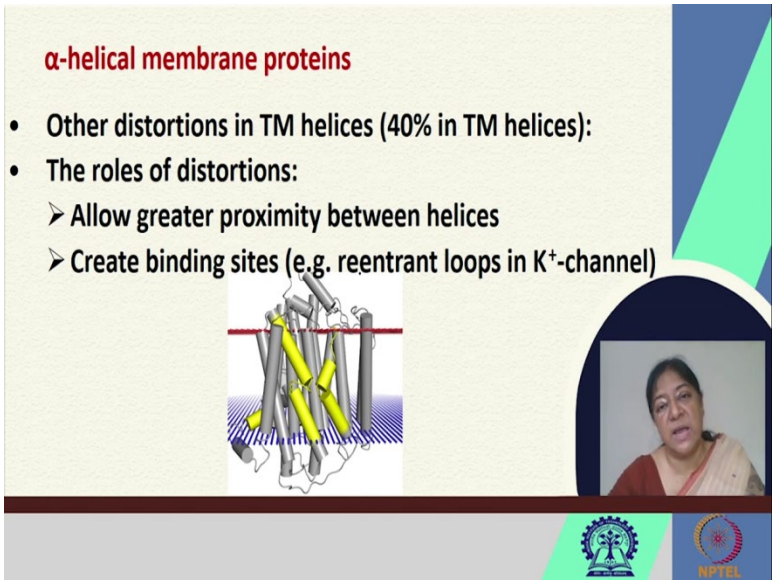
For example if the lysine and arginine pull away the water molecules, the secondary structure of this, where we have the hydrogen bonding of the polar backbone, can reduce this desolvation penalty by interacting or forming hydrogen bonding network, that has been lost due to the loss of the water.

(Refer Slide Time: 24:21)



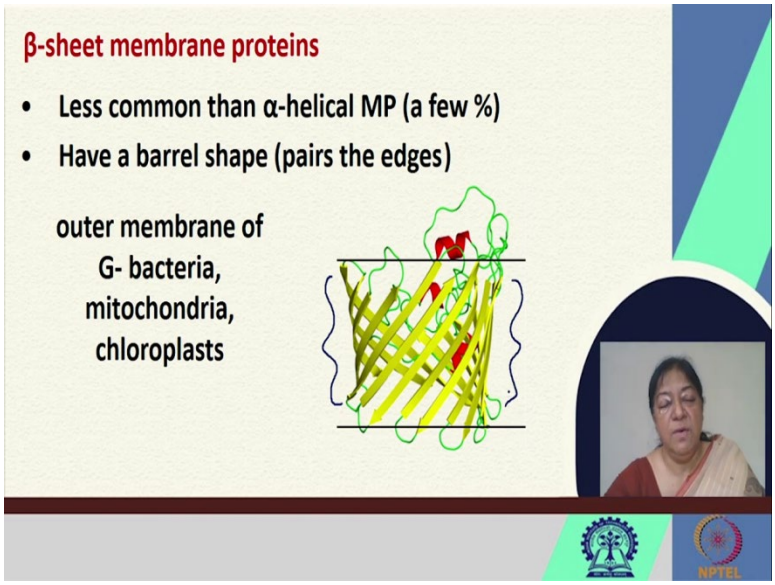
We have α-helical membrane proteins the presence of the proline will bring about hinges in the motion. So, we realize that this hinge is of a utility here in the sense, that there is motion possible and it is useful for channel and transport gating and there is a shift between the active and inactive receptor conformations possible.

(Refer Slide Time: 24:46)



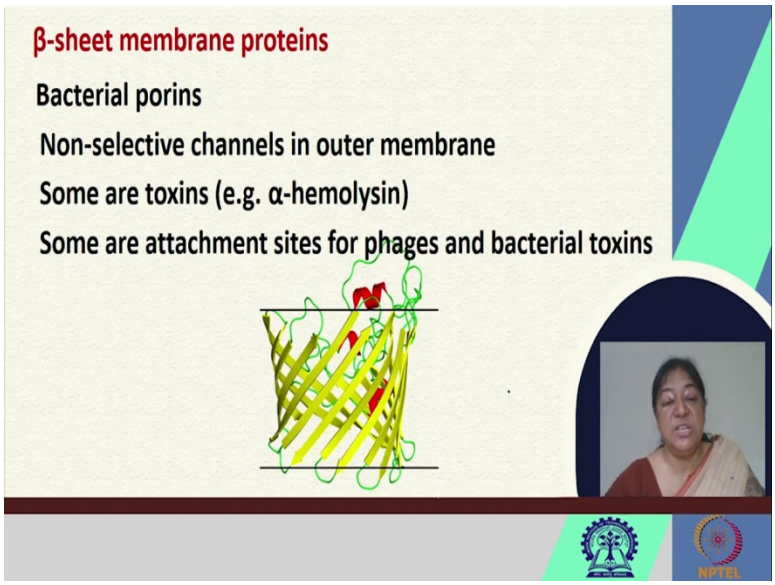
The other distortions in the transmembrane helices, which is useful for bringing about some structural variation for the allowance of the ion channels or for the porosity of the membrane, they create specific binding site in the proximity, in the way they are constructed to interact with the transmembrane region.

(Refer Slide Time: 25:14)

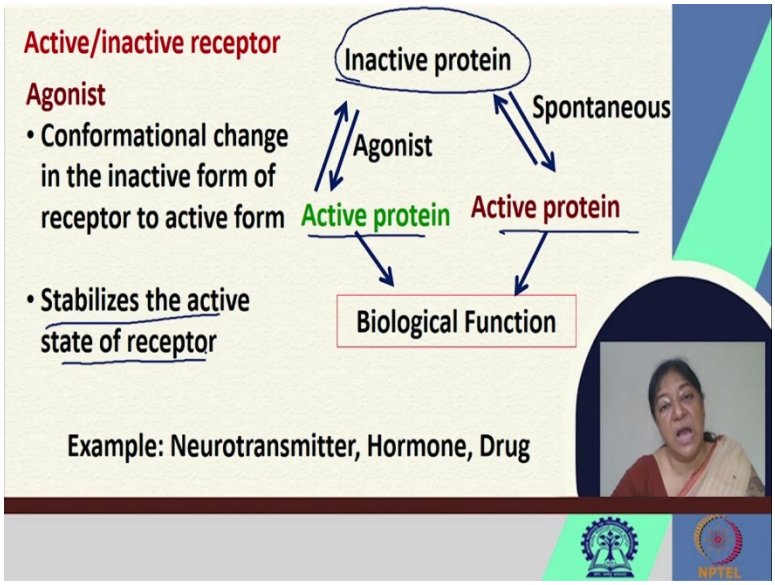


So, the β-sheet membrane proteins are less common than the α-helical membrane proteins, as they have a barrel shape and their interactions with the hydrophobic lipid bilayer are found in specific porins for a nonselective channel to the outer membrane and some of them are even toxins, some are attachment sites for fudges and bacterial toxins.

(Refer Slide Time: 25:32)

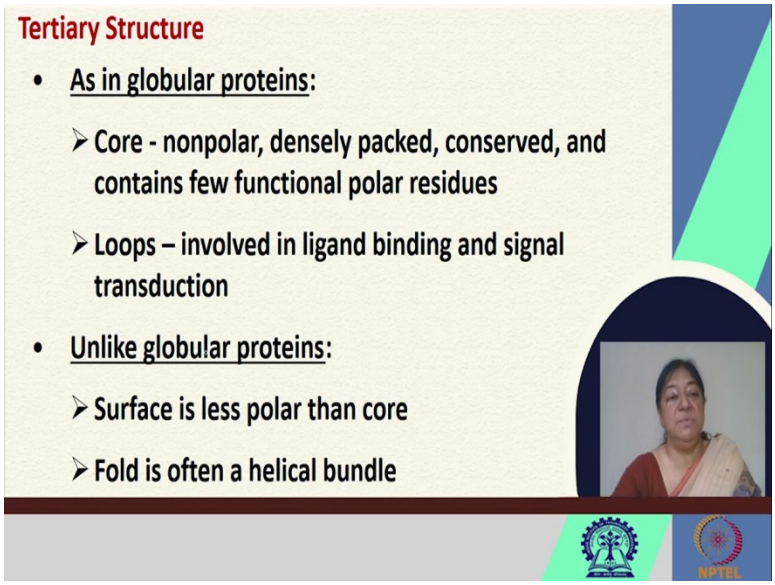


(Refer Slide Time: 25:42)



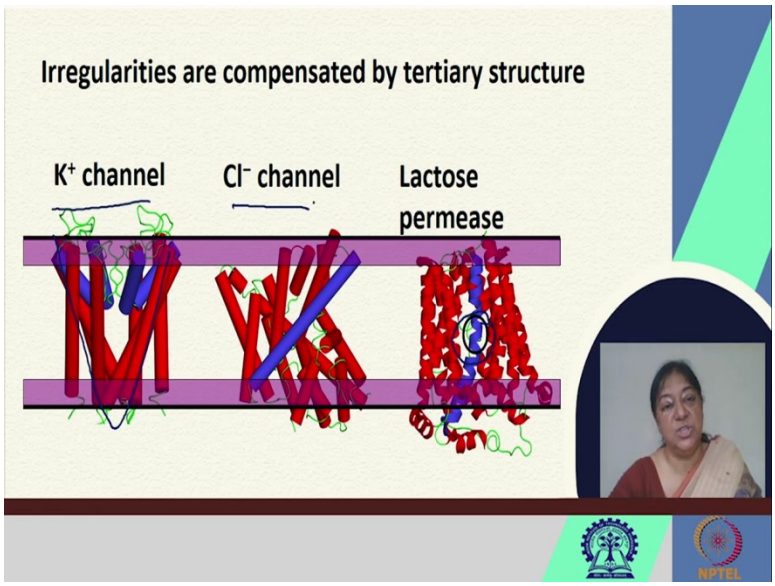
What we have here now is an inactive protein, that can be activated by the conformational change in the inactive form of the receptor to an active form. This can happen spontaneously or it can happen in the presence of an agonist, where we can have stabilization of the active state of the receptor. Now, this is useful for the transport under specific conditions, where we would have the signal transduction occur or any material transfer, ion transfer from the outside to the inside of the cell.

(Refer Slide Time: 26:20)



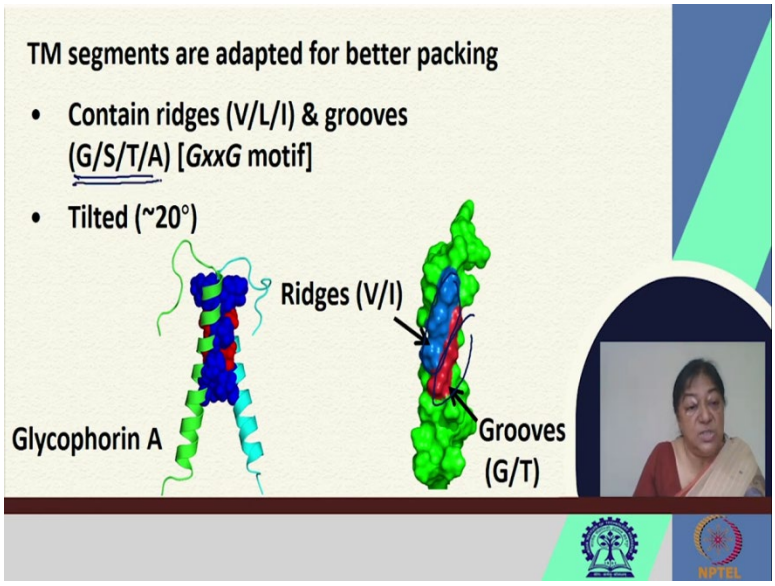
So we have the tertiary structure as in globular proteins. The core is nonpolar, dense packed structure in globular proteins and the loops that are involved in ligand binding. However, unlike globular proteins, we have the surface that is less polar than the core and we have the fold, as often a helical abundant or a β-sheet type, that gives us the bacterial poring structure.

(Refer Slide Time: 26:49)



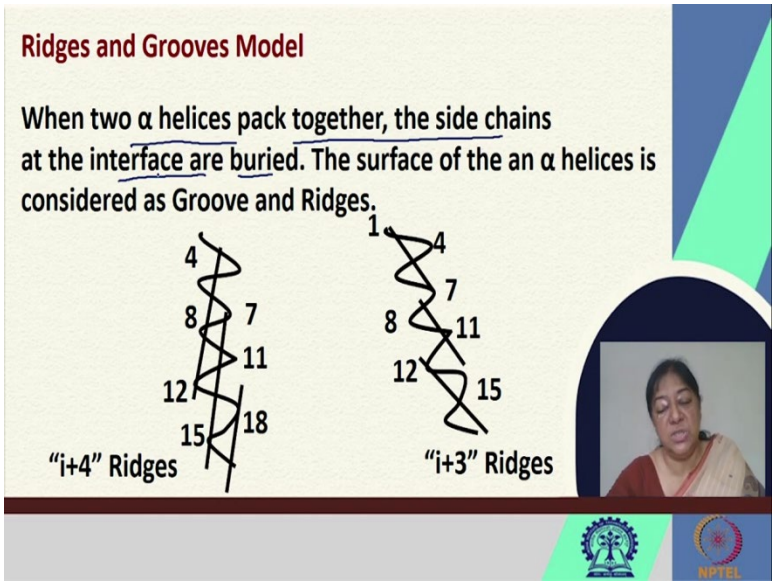
The irregularities are compensated by the tertiary structure, where we can see [refer to slide] variations due to the typical amino acids presence and the shape of these specific types of channel receptors is what we will be looking at in detail in the next lecture and how they can actually work as transport proteins, that being their most important job.

(Refer Slide Time: 27:17)



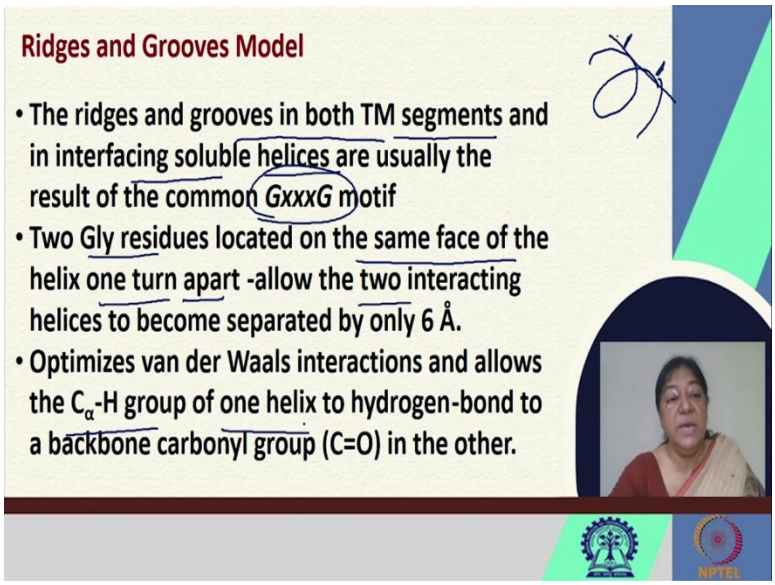
The TM segments now can be adapted for better packing and there are specific ridges on the grooves on the protein, that allow for this.

(Refer Slide Time: 27:29)



So when two α-helices pack together, the side chains of the interface are buried and the surface of the α-helices have grooves and ridges. What happens is, these can come together if there are small amino acid residues present and form the compact structure that is required for a decrease in the porosity of the membrane. So we need this compact structure.

(Refer Slide Time: 28:05)

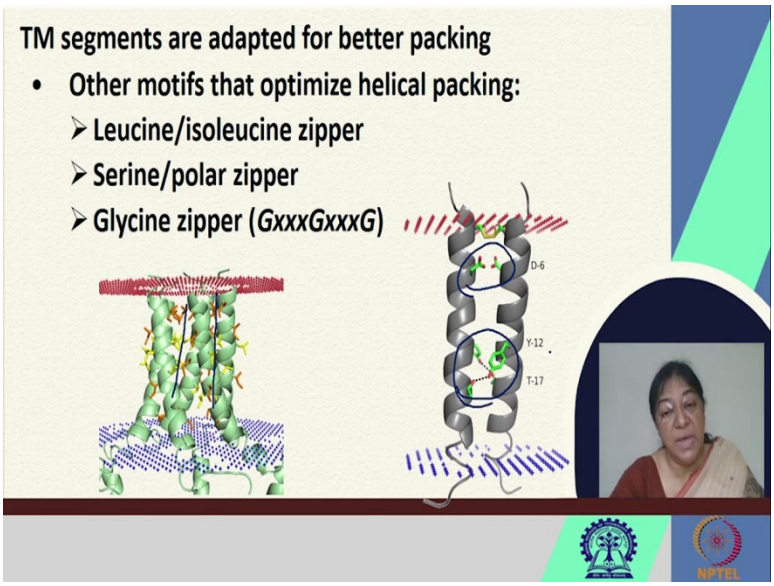


The ridges and the grooves in both the TM segments and the interfacing soluble helices are the result of sometimes what is called a GxxxG motif. What happens is the two glycine residues are on the same side and they can assist in this cap packing.

What happens is, they are located on the same face of the helix one turn apart. They allow the interacting helices to be separated by only a small distance and this can optimize the van der

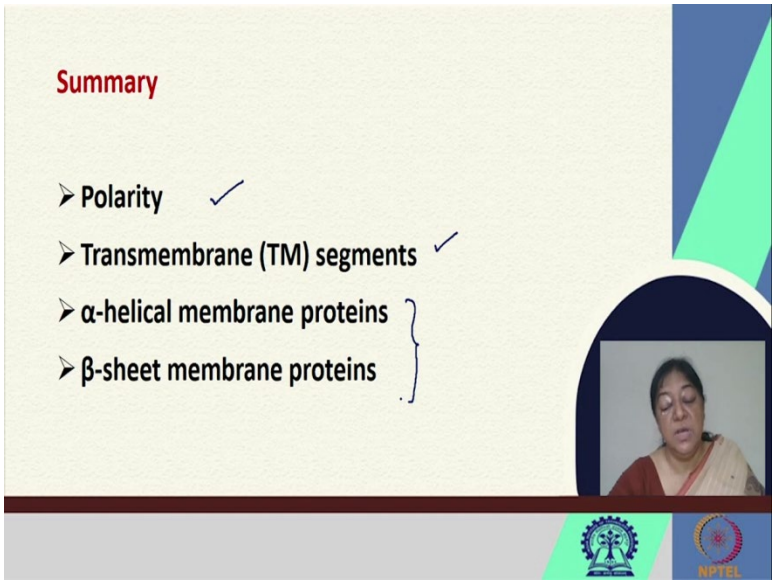
Waals interactions. It allows the C α H group say of one helix to hydrogen-bond even with another helix, bringing about this compact structure.

(Refer Slide Time: 28:55)



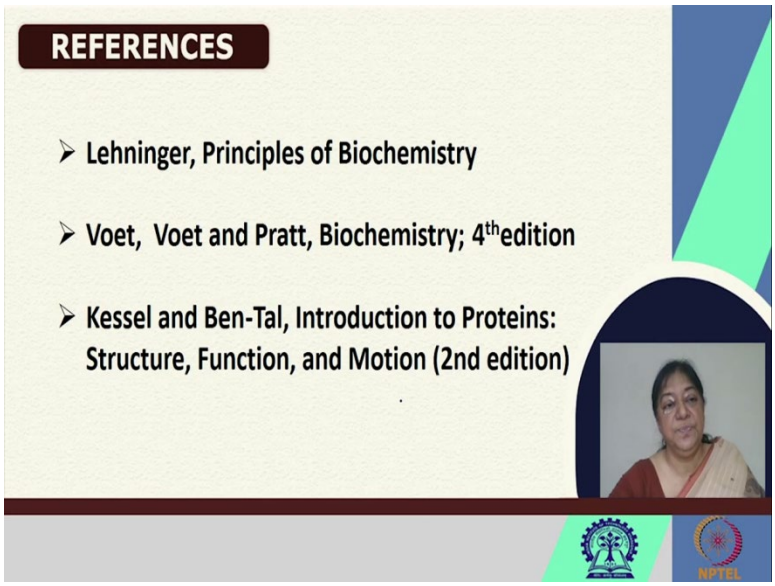
So, we see how they are adapted for a better compactness and there are other zipper molecules which actually look at hydrophobic zipping, in the sense that we have hydrophobic components on the edges of the helices. The side chains that stick out would have different types of interactions that bring about this compact structure for better packing. That gives us the zipper types of molecules or the zipper type of helices; the motifs that allow the transmembrane helix to remain in the membrane for better packing.

(Refer Slide Time: 29:36)



So, we looked at the polarity, the transmembrane segments and the types of proteins that would be involved and the stability in terms of the protein amino acids present.

(Refer Slide Time: 29:50)



These [refer to slide] are the references.

Thank you.