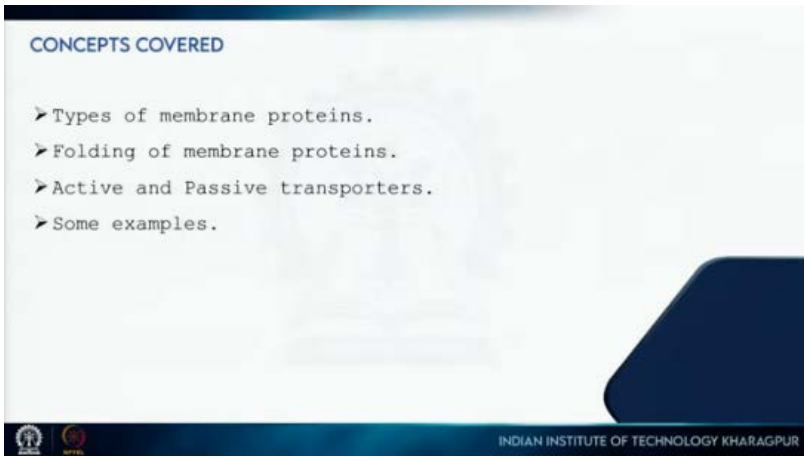
Introduction to Complex Biological Systems
Professor Dibyendu Samanta and Professor Soumya De
Department of Bioscience and Biotechnology
Indian Institute of Technology, Kharagpur
Lecture 15
Membrane proteins

Welcome back. So, in this week's lecture, we have looked at proteins and how these proteins can perform all these different functions in different living systems. So, today I am going to talk about a special type of protein called membrane proteins. So, in membrane proteins, the topics or the concepts that I will cover are types of membrane proteins, folding of membrane proteins, so we will see that membrane proteins fold in a slightly different manner compared to water-soluble cytoplasmic proteins.

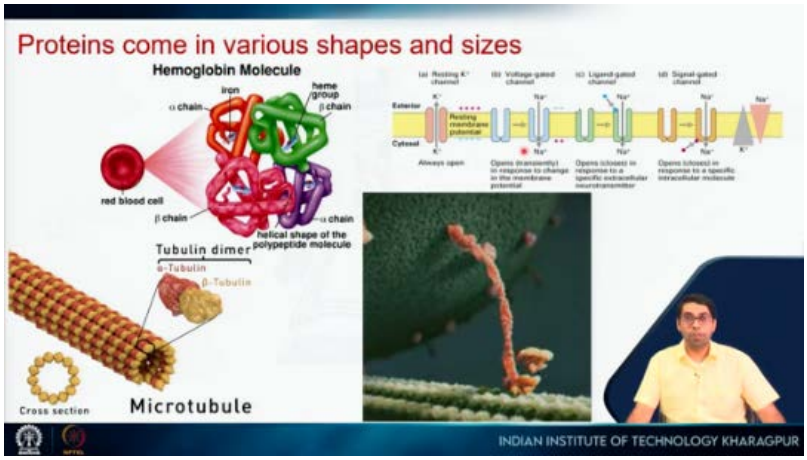


Then, we will look at two different types of transporters, the active and passive transporters, and I will give you a few examples. So, this is something that I have already shown you that proteins come in various shapes and sizes, and they perform all sorts of different functions. We have already seen hemoglobin, which is present in our red blood cells, and how it can carry oxygen. We are going to see microtubules and these other proteins like dynein or kinesin, which carry huge cargo from one end of the cell to the other. Today, we are going to focus on proteins that look like this.

So, these two lines indicate a lipid bilayer. So, I will discuss that in detail in the next few slides, and these proteins are embedded in this bilayer and perform various tasks. So, some examples are shown here, for example, potassium and sodium channels. So, these are ion channels that are very specific to these particular ions, and they can let these ions either

along the gradient or away from the gradient, depending on the type of channel. And you can see that these are so specific that they can distinguish between potassium and sodium ions. So, if you think about the size of a potassium and sodium ion, their diameter differs by only 0.7 angstrom. So, if you think about a benzene ring, the distance between two carbons, the bond between two carbons, is 1.5 angstrom.

So, here the diameter is even smaller than that; the difference in diameter is even smaller than that or half of that, which is 0.7 angstrom, and these ion channels can differentiate even that small change in size between potassium and sodium ions. So, we will see how these ion channels work in some examples. So, membrane proteins not only transport ions, but they also have all sorts of other functions. So, we will see channels, transporters, and pumps. So, channels are membrane proteins which allow sodium or potassium ions, like sodium and potassium.



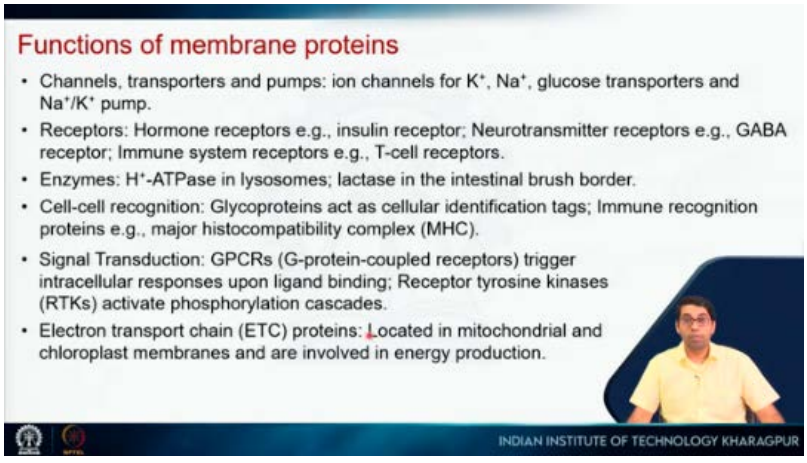
They can also allow calcium to pass across the membrane, and that happens along the gradient. There are pumps where sodium and potassium are pumped. In the reverse direction, they hydrolyze ATP; they use that energy to pump out protons or pump in sodium, so they will establish that gradient. These are called pumps. Then there are transporters which transport small molecules. We will see one example today where we will see maltose, which is a glucose molecule, a sugar molecule, and a maltose transporter. Apart from these, there are receptors, for example, hormone receptors like the insulin receptor. So, insulin comes and binds to the insulin receptor and results in the uptake of blood glucose.

There are neurotransmitter receptors, and in the immune system, there are T cell receptors. So, we will see all these receptors in more detail in the coming lectures. There are also enzymes which are embedded on the membrane, for example, in vesicles like lysosomes, where various protein molecules are degraded. The internal pH of lysosomes is acidic. To maintain that acidic pH, there are enzymes which will pump in protons. These are proton pump ATPases, so they will hydrolyze ATP to pump in protons. We will see one such example today. In the case of bacteria. Then there is lactase on our intestinal brush border.

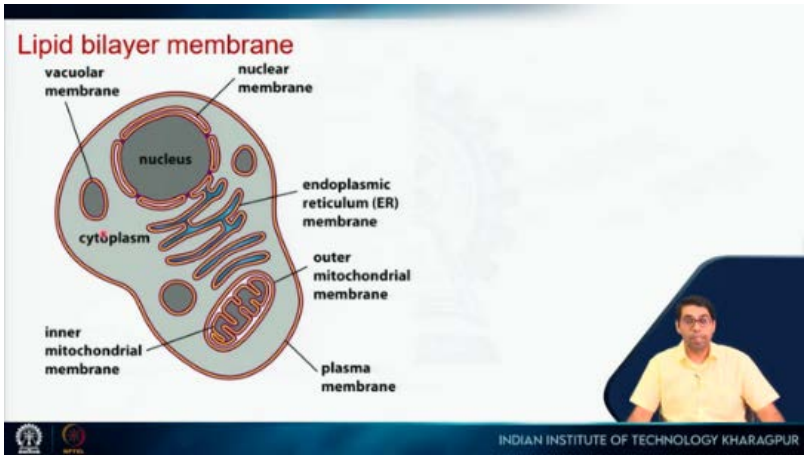
So, in the small intestine, these enzymes break lactose into glucose and galactose, and they help us to digest all dairy products. So, if you are drinking milk or if you are taking any milk product, they will have a lot of lactose, and this enzyme helps us to digest those. There are proteins which help in cell-cell recognition. So, there is a lot of cell-cell communication that happens. Two cells can talk to each other through membrane proteins.

So, there are examples of, we will see examples of that. We will see one particular example in great detail when we talk about our immune system, and that is the major histocompatibility complex. Membrane proteins also help in signal transduction. GPCRs, or G protein-coupled receptors, are very important in that. We will see receptor tyrosine kinases when we talk about the cell cycle.

So, we will see that tyrosines are phosphorylated by these enzymes. We have already seen one such example in a previous lecture. Then, there are electron transport chains. So, we will talk about this in more detail when we talk about photosynthesis and respiration. So, this is the general architecture of a eukaryotic cell.

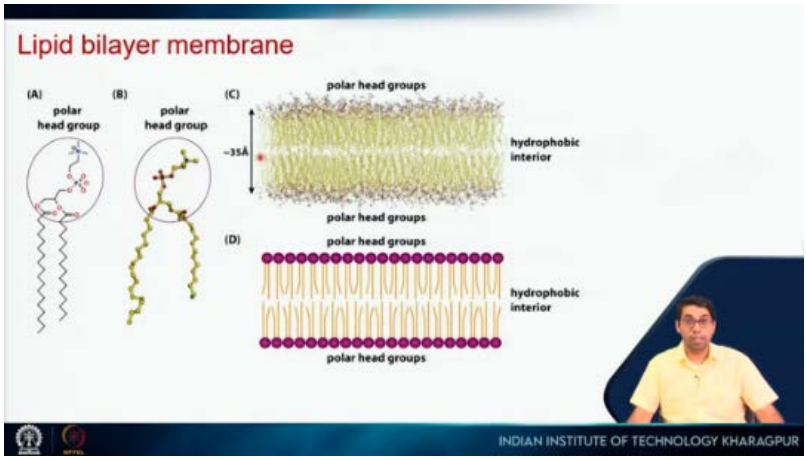


I will talk about cells in more detail in next week's lecture. But briefly here you see these two lines. This is the plasma membrane. And then there are also these internal organs, which are also surrounded by membranes. These membranes are formed by lipid bilayers.

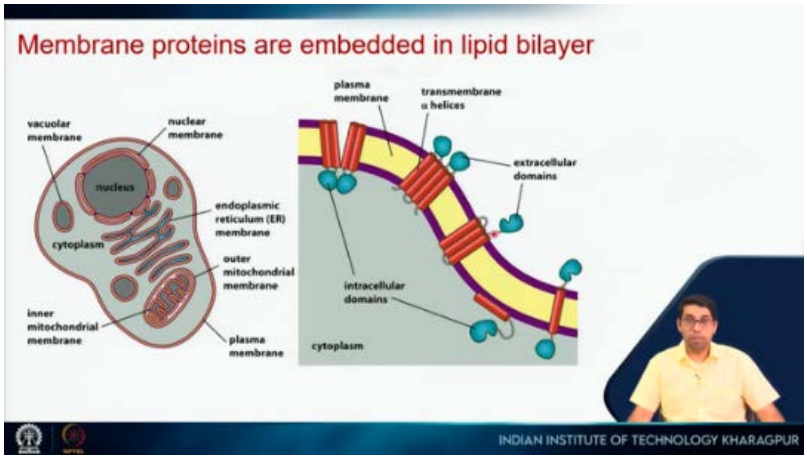


So, what are these lipid bilayers? So, these are lipids. So, these have this particular type of structure. So, you have a hydrophobic tail and a polar head group, and these get arranged in this manner. So, you have this polar head group and the hydrophobic tail on the inside, the polar head group on the outside, and that is why it is called a bilayer because this is one layer and this is the other layer.

So, one is the inner leaflet, and the other one will be the outer leaflet. And if you put the actual structures, not the schematic, then it will look something like this. And it turns out that this layer is very thin. It is only 35 angstroms or 3.5 nanometers in thickness. And on a cell, when we have this lipid bilayer, there are proteins that are embedded in the lipid bilayer like this.



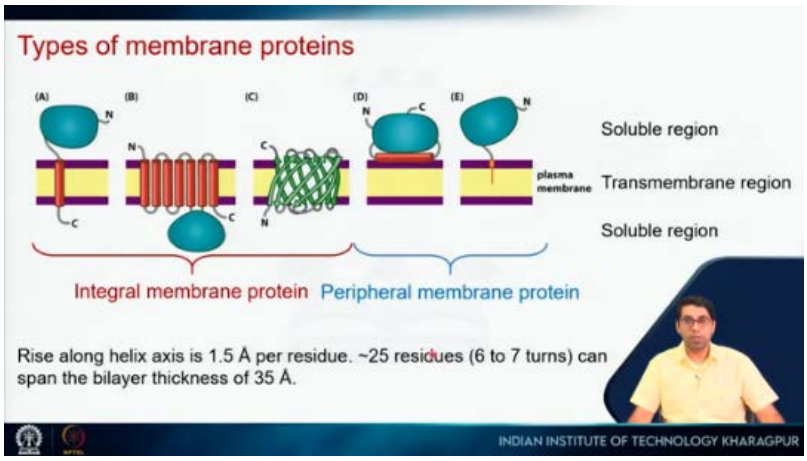
So, these two purple lines show the two head groups. So, that is the lipid bilayer, and there are these membrane proteins, which we will see the types of membrane proteins in the next slide. They are embedded in this bilayer, and they perform different functions. So, these proteins are not only present in the plasma membrane, but they are also present in these internal organelles that are present inside a cell. So, depending on how the proteins are anchored to the membrane, we can have these five different types of membrane proteins.



So, this is the membrane, and anything that passes through it will be called the transmembrane region of the protein. Anything that is outside or inside will be soluble. So, these are very similar to our soluble proteins, but these transmembrane regions have different characteristics compared to what we have seen for soluble proteins. Now, this transmembrane region can be a single helix where the helix is what is anchoring the protein. So, the protein can be outside or it can be inside.

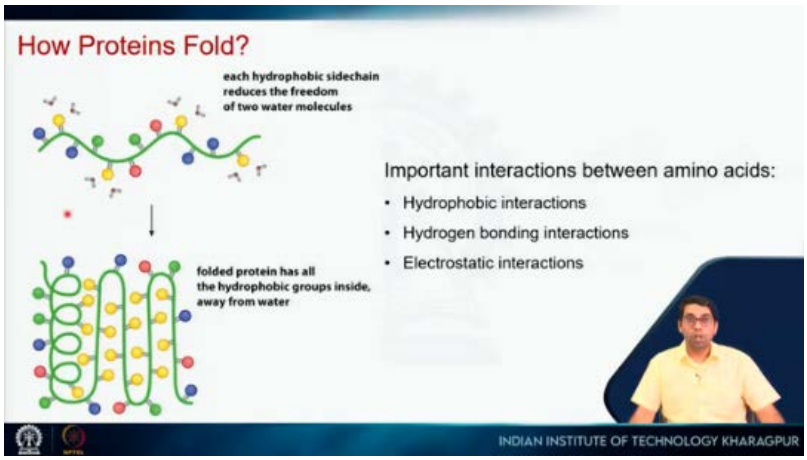
There can be multiple helices. Again, the protein can be inside or outside. Instead of helices, we can also have an arrangement of beta strands. So, these three, where the protein or the secondary structure is nicely embedded in the lipid bilayer. These types of proteins are called integral membrane proteins.

Then there are two more types where the helix is not inserted into the membrane but interacts with one or the other side of the membrane like this. Or the protein might be anchored to the membrane by forming a covalent bond with one of the lipids. So this will be a special lipid that has formed a covalent linkage with one of the amino acid side chains of the protein. So these types of proteins are called peripheral membrane proteins. Now if I turn my attention to this helix, you will see that this is a helix. It is a single helix. Normally for soluble proteins, we do not see such an isolated helix, but such an isolated helix embedded in a membrane is quite stable. We have already seen that the rise along the helix of a helical axis is 1.5 angstrom per residue, right? And we know that we have just seen that this distance is around 35 angstrom. So, if we divide that by 1.5, we get roughly 25 residues. So, 25 residues will form this transmembrane helix, and that will mean that it will have around 6 to 7 turns, right? So, these 6 to 7 turns or 25 residues can form a helix that will span the bilayer thickness of 35 angstrom. Let's look at the characteristics of this helix.

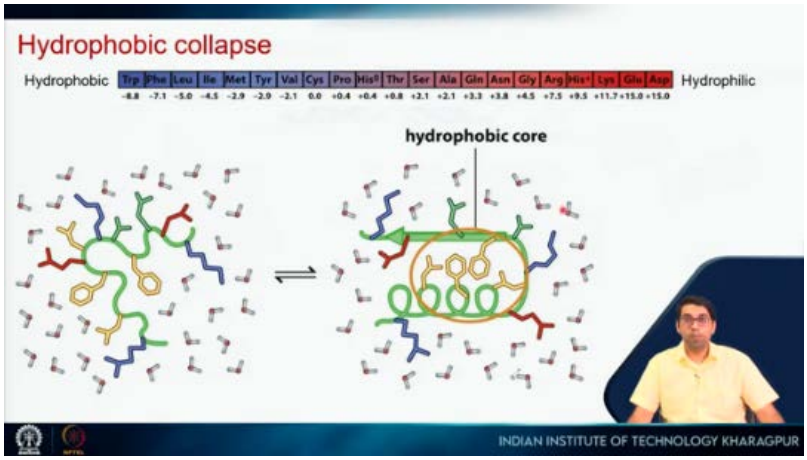


Now, this is just a recapitulation of what I have shown you before, and this is true for soluble proteins. This is a polypeptide chain, and it folds into a structure like this. One of the important driving forces of this is called hydrophobic collapse, where all the hydrophobic groups, which are shown by these yellow circles, get inside, and all the polar

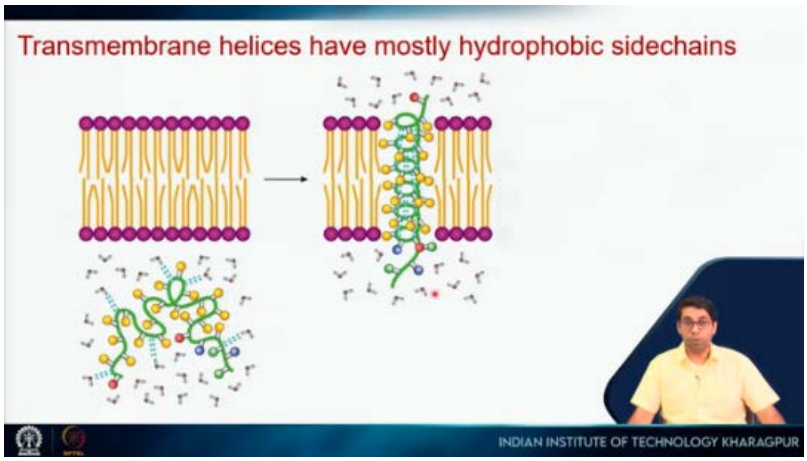
groups stay on the outside. So, when this hydrophobic collapse happens, we have all the hydrophobic groups inside and all the polar groups outside. Now, amino acids can be categorized on a hydrophobicity scale, so you can imagine that charged residues will be highly hydrophilic. And residues which have aromatic groups like tryptophan, phenylalanine, and also methyl side chains will be highly hydrophobic.



So, anything that you see here, negative numbers mean highly hydrophobic, and positive numbers mean highly hydrophilic, meaning water-loving. So, for a normal water-soluble protein, these residues or hydrophobic residues will be inside, and these residues will be on the outside. But when it comes to a transmembrane region where a helix will get embedded, what we see is that all the side chains will not interact with water. They are going to interact with these very hydrophobic lipid chains, okay? So, in this case, the helix will be entirely formed of or mostly formed of amino acids which are highly hydrophobic. So, these amino acids will be hydrophobic so that they can interact with this hydrophobic part of the lipid bilayer, and towards the terminus, we will have hydrophilic or charged amino acids.



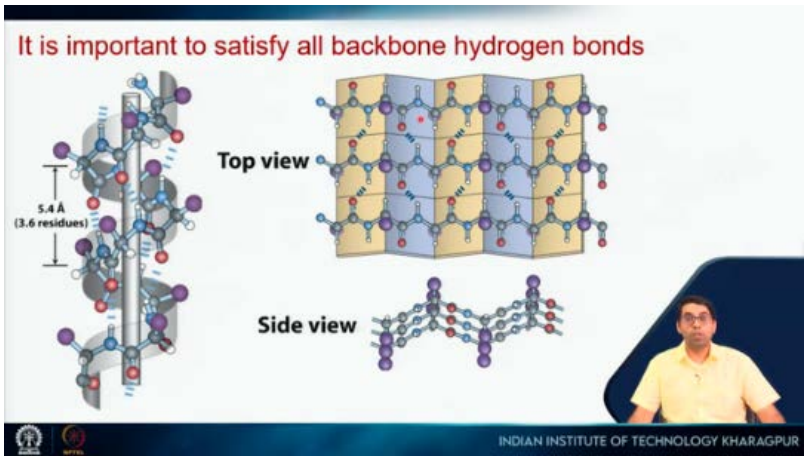
So, this is a very characteristic feature of helices that are embedded in lipid bilayers. Okay, so that is for the helix. What about beta strands? Can we have beta strands inside a lipid bilayer? So, one important thing that you should consider is that in the case of helices, all the hydrogen bonds are internal.



So, all the amino acids form hydrogen bonds. So, this, and I'm talking about the backbone. So, the backbone amide *NH* and *CO* form hydrogen bonds in this pattern of *i*, *i+4*, which means that the first three amino acids and the last three amino acids will have unsatisfied hydrogen bonds, but if my helix is long enough so that this is just outside the membrane or it is interacting with the polar head group and this is also interacting with the polar head group, All these residues which are inside will have their hydrogen bonds satisfied, and we have seen that the side chains are already non-polar, so there are no hydrogen bonds for the side chain, so helix is something that can very nicely satisfy this criteria of forming all the hydrogen bonds for the backbone residues.

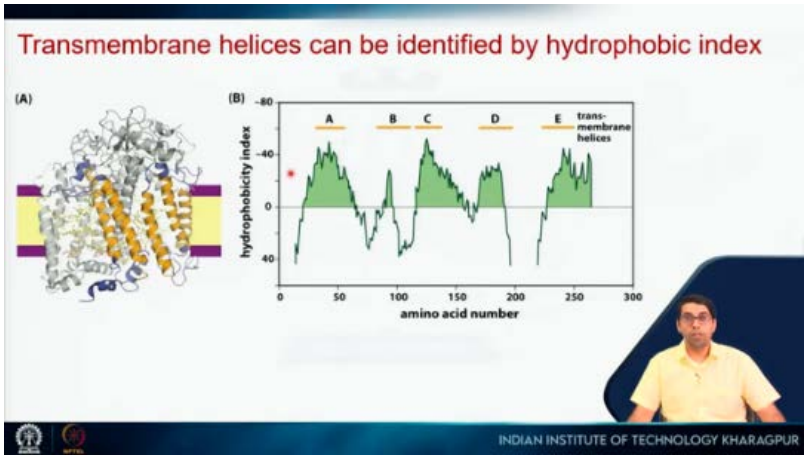
In the case of beta strands, we can have that because hydrogen bonds are formed between two beta strands. However, if we look at a beta sheet like this, you see there are three beta strands. This one has these amide and *CO* groups which will not have their hydrogen bonds satisfied. Similarly, this strand will have the amide and the *CO* groups which will not have their hydrogen bonds satisfied, which means that in the center, everything is fine, but what about the edges?

One way to solve this problem is if we take this sheet and roll it into a cylinder, which means that this will form a circle so that this *CO* will form a hydrogen bond with this *NH* and this *CO* will form a hydrogen bond with this *NH*. So, if we can roll it into a cylinder, we can convert this into a transmembrane region, and that is exactly what nature has done. So, we see this type of beta barrel structures where the beta sheet is rolled into a barrel or cylinder-like structure where all the hydrogen bonds inside the transmembrane region are satisfied. In the case of helices, you can have a single-pass helix because all the backbone hydrogen bonds will be satisfied, or you can have multiple helices, and we will see examples of both. So, in the case of helices, since these helices will be mostly hydrophobic, one can identify this transmembrane region.



In a membrane protein, if we have a membrane protein, then what we have to do is plot the hydrophobicity for all the amino acids. Here we see that this stretch of amino acids has high hydrophobicity, and this stretch of amino acids also has high hydrophobicity. So we can tell that these residues will most likely form a helix. If the length is somewhere close to 25 amino acids. We can say with good certainty that this will form a transmembrane

helix, right? So, this way we can actually identify transmembrane helices for a membrane protein just by looking at its amino acid sequence and calculating the hydrophobicity index. So, let's look at some examples. Here we are looking at a beta barrel protein called a porin.

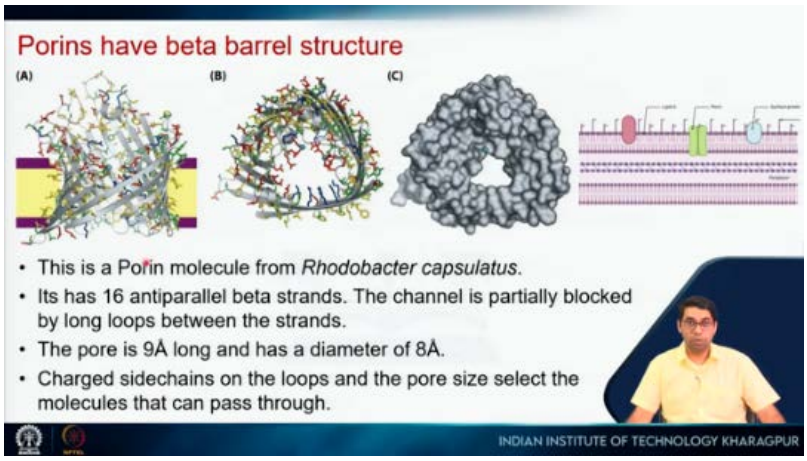


These proteins, or porins, are present in the outer membrane of gram-negative bacteria. I will discuss gram-negative and gram-positive bacteria in more detail in next week's lecture. But in this case, I will just briefly tell you that gram-negative bacteria have two membranes. So, there is an inner membrane and an outer membrane. On the outer membrane, these proteins called porins are embedded. This is a porin embedded in the outer membrane.

These are very broad; you can see that the channel is quite big. These are open, water-filled channels, and they allow passive diffusion of nutrients and waste products through these channels. E. coli is an example of a gram-negative bacterium, and each E. coli cell contains 100,000 copies of porin molecules in its outer membrane. These are very important molecules because they allow the diffusion of nutrients into the cell and also allow waste products to go out of the cell. The structure that we are seeing here is a porin molecule, not from E. coli, but from a particular bacterium called Rhodobacter capsulatus.

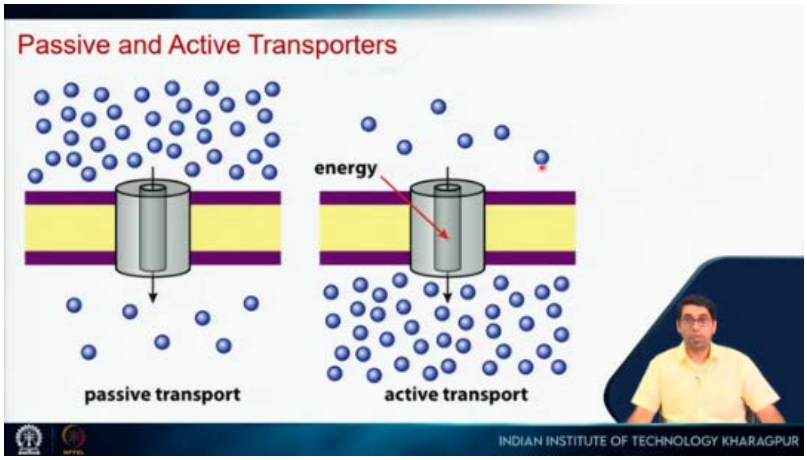
If you look at this structure, there are 16 antiparallel beta strands, which means that one strand goes like this, there will be a loop, then it comes back, there will be a loop, then it goes up, there will be a loop like that. These are all antiparallel, and they are connected by long loops between the strands. The pore that we see here has a length of 9 angstroms. So, you can see it is much smaller. This is 35 angstroms, but the pore size is not much.

So, this is 9 angstroms. And it has a diameter of 8 angstroms. There are charged residues on the loops. So, these charged residues combined with the pore size together determine which type of molecules can pass through this channel. Through this pore, when we talk about transporters, a pore is a transporter. We can think of two types of transporters. One that is a passive transporter, which results in passive transport, and the other one is active transporters.



In a passive transporter, just like in the case of a pore, molecules are transported across the channel via diffusion. So, they will follow the gradient or the concentration gradient of the molecule. So, if there are more molecules here and fewer molecules there, you will see transport in this direction. However, in the case of active transport, they can transfer molecules from low concentration to higher concentration, and to do that, they use energy. In many cases, this energy is the hydrolysis of ATP. In this case, in the next example, we will see that the energy is provided by light.

So, light energy is used to transport hydrogen ions from one side to the other side of the membrane. So, light energy will create this gradient of hydrogen ions, which will be used by the bacteria to perform certain other tasks. So, light energy is converted to this energy, which is like a capacitor where you have more hydrogen ions here and fewer hydrogen ions there. Then, that energy stored across the membrane will be used by the bacteria to perform other tasks. So, that is done by this particular protein called bacteriorhodopsin.

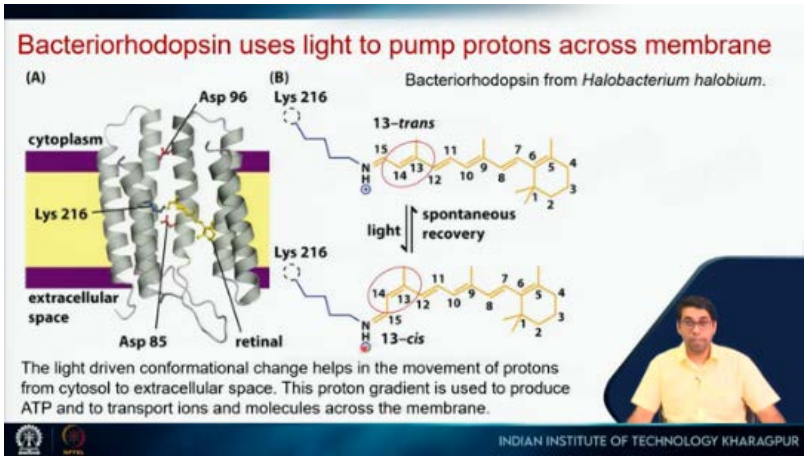


So, bacteriorhodopsin is found in Halobacterium halobium. So, this is the bacteria that loves salt. In this case, what it does is it uses light. So, light-driven conformational change helps in the movement of protons from the cytosol to the extracellular space, and this proton gradient is used to produce ATP and to transport ions and molecules across the membrane. So, it uses light energy to establish this proton gradient and then uses this energy to perform other tasks like the production of ATP. So, this is the membrane protein, a transmembrane protein. It has seven helices.

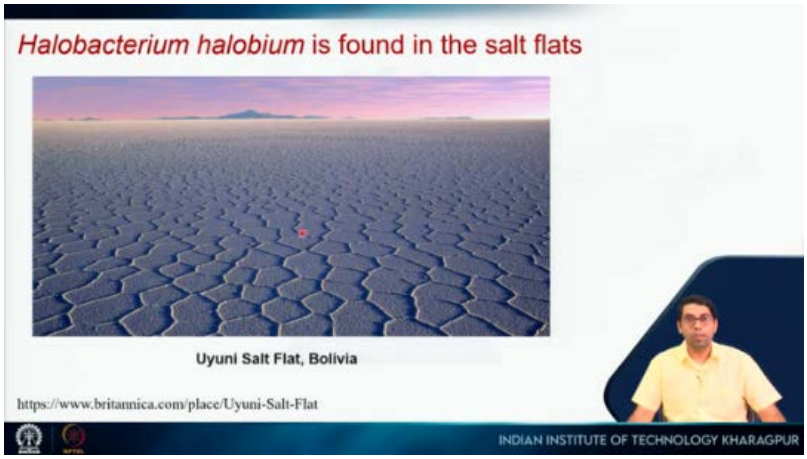
So, you can see there are 1, 2, 3, 4, 5, 6. The seventh helix is here, and it is not shown. So, it has been removed so that we can see this molecule, which is right at the center of the transmembrane region. And this molecule is called retinol. So, retinal, as it is written here, is basically a pigment. It is very similar to the pigment that is present in our eye, the light-sensitive pigment that we have in our eye.

So, the retinal has this particular structure, and you can see that there are these alternating double and single bonds. All these carbons across the double bond are arranged in the trans conformation. So, we are going to look at this particular double bond. You can say this is trans because across this double bond, the two carbons, which are carbon number 12 and carbon number 15, are on the opposite side of this double bond. When this molecule absorbs light, a photon of light flips this into the cis conformation. So now you see 12 and 15 are on the same side. This molecule, the retinal, is covalently linked to this lysine 216 side chain. So, the lysine 216 side chain is an amine group, NH_3, so it forms a Schiff base with this side chain. It is covalently attached to this side chain, and you can see that there

is a positive charge on this nitrogen. And this will become important in the next few slides. So, this type of bacteria is found in salt flats, if you have not seen salt flats.

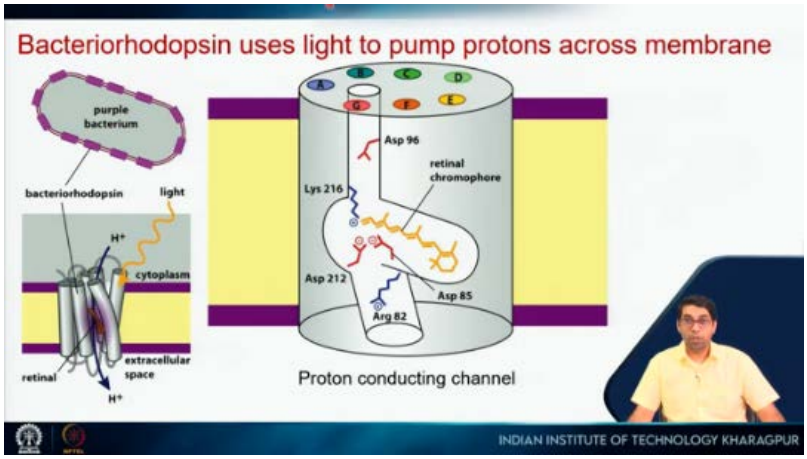


So, this is an example of a salt flat. It is the Uyuni salt flat in Bolivia. So, these bacteria love salt, and they are found in this type of salt flat. So, this is the bacteria. It is a purple bacterium, and the color comes from these proteins. The membrane protein is here, the bacterial rhodopsin. It absorbs light and results in the transfer of a proton from the outside, from the cytosol to the extracellular space, thereby establishing this proton gradient. Schematically, the protein will look like this. So, these are the seven helices, and there is this proton-conducting channel. You can see that this molecule, the retinal, is almost at the center, and this proton-conducting channel is lined with charged residues.



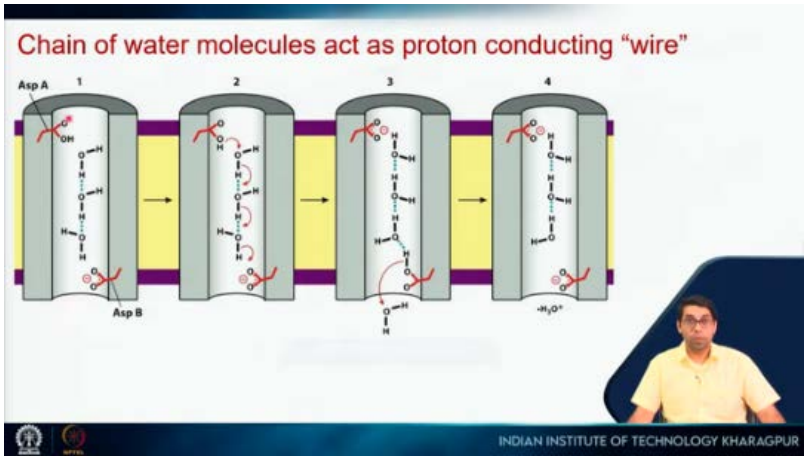
So, there are aspartic acid, lysine, and arginine, right? So, they are lined with these charged groups. We will see that this aspartic acid 96 and this one 85, are particularly important. They play a very critical role in the transfer of the proton through this channel and also

ensure that the proton transfer happens only in one direction. So, it happens in this direction and not in the reverse direction. So, to understand that, let us look at this hypothetical protein where there are two aspartic acids, aspartic acid A and aspartic acid B, and there is this channel. In this channel, there are water molecules.



So, it turns out that these water molecules, this chain of water molecules, act as a proton-conducting wire. So what happens is this aspartic acid will donate its proton to this water molecule. It will take the proton here; this proton will go to the next water molecule, this proton will go to the next water molecule, and this proton will be taken up by the aspartic acid. And finally, this aspartic acid will donate the proton to the water molecule which is on the other side of the membrane. Thereby, a proton from this side gets transferred to this side. Right now, there is a problem here because a proton can follow this path and go in this direction, or it can follow this path and go in the other direction. So, there is no directionality in this proton channel, and this directionality is provided by the pigment retinal.

So, it results in or allows proton transfer only in one direction and not in the other direction. So, that is shown in this particular schematic diagram. So, here you see this is retinol, and, as I mentioned earlier, there are two aspartic acids which are important. Those are aspartic acid 96 and aspartic acid 85. So, this is the resting state, and you can see that here the bond is in a trans conformation. Light comes then it absorbs a photon of light and gets converted to cis.

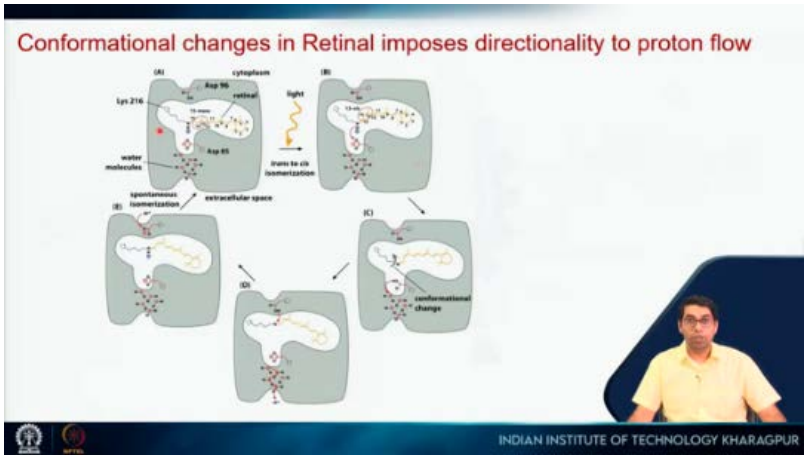


So now you can see that it is in cis conformation. So, 12 and 15 are on the same side of the double bond. In this condition, what happens is this NH group from the Schiff base gets very close to this aspartic acid 85 and donates its proton to this aspartic acid. Once that happens, this becomes neutral. Once this becomes neutral, that results in a conformational change in the protein. So, all the helices, the seven helices that are there, readjust their packing, and that moves this Schiff base away from this 85 and close to this 96. So now it has moved close to 96 and away from 85. Aspartic acid 85 has the proton that readily transfers this proton to the water molecule in this extracellular space. So here we have the water chain. So it transfers it to the water molecules, and finally, the water is, the protein is released in the extracellular space here.

So, at this stage this bond is still cis. We have transferred the proton here. This is close to this aspartic acid. Now, this schiff base will abstract a proton from this aspartic acid, which has taken the proton from the cytoplasmic side, from a water molecule in the cytoplasmic side. So, this proton will be transferred to the Schiff base. It will again become positively charged, and this structure will relax back to the trans state. So, it is cis that will relax back to the trans state because energetically trans is more favorable than cis. So, it will relax back to the trans state, and that is where this proton channel is regenerated. So, it can absorb another photon of light, and it will go through the same cycle.

So, in each cycle, it will transfer a proton from the cytoplasmic side to the extracellular space. This is another example where a sugar molecule, so this is maltose, is transferred into the bacterium. So, again, this is the outer membrane, and this is the molecule that we

have already seen; this is porin. So, porin will allow all sorts of ions and molecules inside the periplasmic space, right? So, this is the periplasm; maltose will just get in through passive diffusion, and in this region, there is a particular protein which is called the maltose-binding protein.

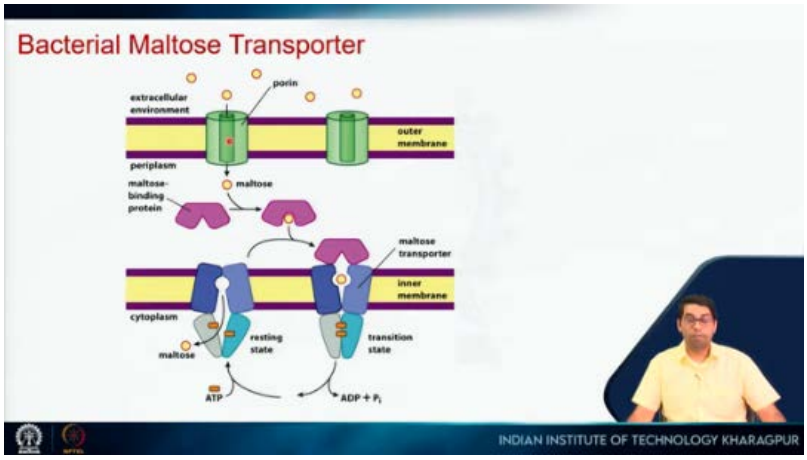


So, this maltose-binding protein will bind maltose very tightly, but now it has to transfer this maltose into the cytoplasmic side of the bacterium, and that is done by this maltose transporter. So, this maltose transporter, this is the transmembrane part, and this is the cytosolic. This is the one which is the actual transporter, and this one binds ATP. So, these two molecules that we see are ATP, and when they come close, it will hydrolyze ATP. So, this transporter has two conformations. One is the resting state where it is closed towards the periplasmic side and open towards the cytoplasmic side.

The other one is the tension state, where it opens towards the periplasmic side and closes towards the cytoplasmic side. So this is the resting state of this transporter. When the maltose-binding protein binds maltose, it changes its conformation and can bind this. Maltose transporter, when it binds this maltose transporter, drives a change in the conformation from this closed state to this open state. So now maltose gets into this, and that also triggers a change in this ATP-binding domain so that these two are close to each other, and in this state, these domains will hydrolyze ATP into ADP, so ADP is released and an inorganic phosphate. This hydrolysis will now trigger a second conformational change so that this periplasmic side is now closed, and the cytoplasmic side is open. Once

this happens, maltose, which is trapped here, cannot go back. It will diffuse into the cytoplasm.

So, in essence, maltose diffuses through the porin into the periplasmic site and is transported from the periplasm to the cytoplasm via this maltose transporter, and to do this transport, the energy is supplied by the hydrolysis of ATP. So, we have seen an example of passive diffusion or passive transport by porin, and we have seen examples of two active transports. In one case, the energy is supplied by light, and in the other case, energy is supplied by the chemical hydrolysis of ATP.



So, you can follow these books to study the membrane proteins, especially this first and the second book. Thank you.