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

Module - 02
Protein Architecture
Lecture - 08
Tertiary and Quaternary Structure

In our next lecture of module 2 on protein architecture, we are going to talk about the tertiary and the quaternary structure of proteins.

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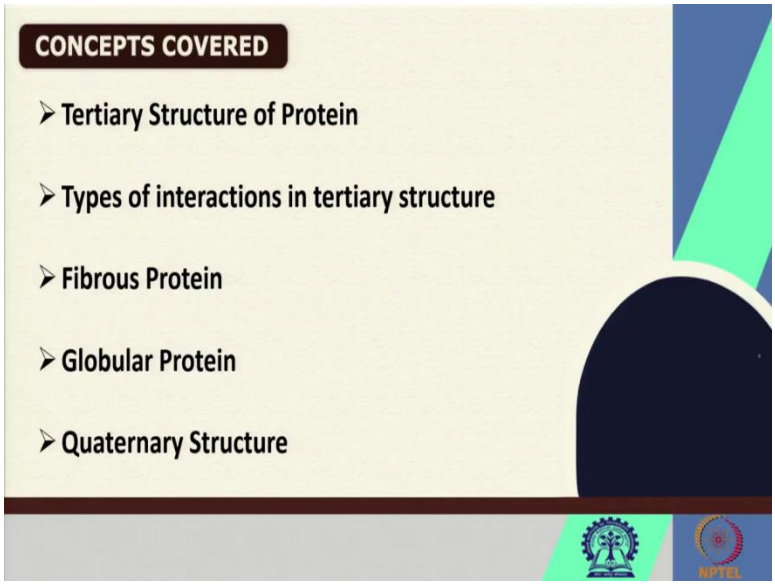

The concepts that will be covered here are the tertiary structure of proteins, the types of interactions in the tertiary structure which we will look at gradually, what we mean by fibrous and globular proteins.

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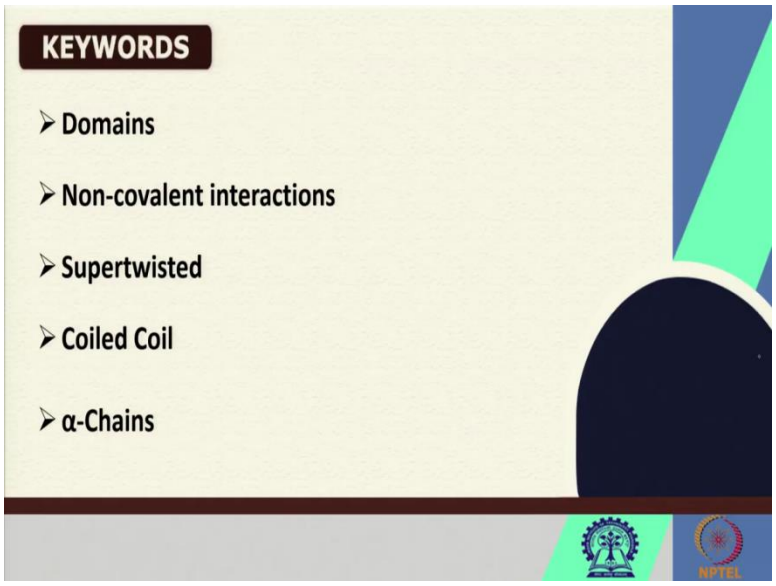

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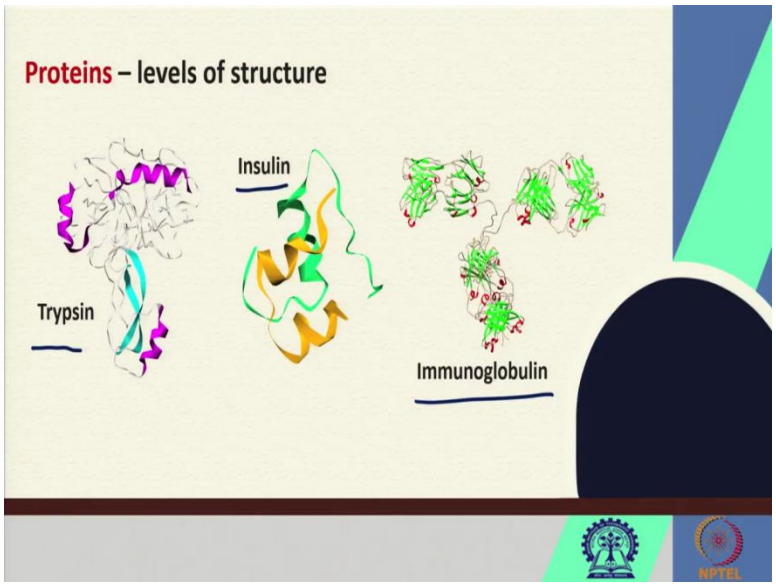

So, in this particular lecture we will look at the levels of structure. Now, we found out that when we look at the different levels of structure they fold into component such as the structure of trypsin, insulin and immunoglobulin.

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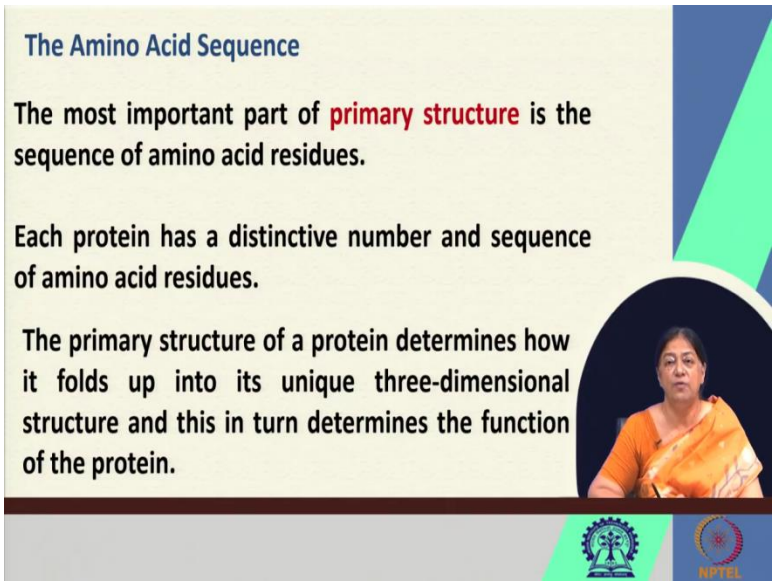

What do we mean by the amino acid sequence? It forms the primary structure of proteins in the linking of the amino acids one after the other. Now, each protein that we talk about or each protein that we mention has a distinctive number and sequence of amino acid residues.

This is very important because later on when we look at the folding of the protein and we understand that it is this sequence that decides the folding of the protein and not only that the folding depends upon where the active site residues are going to be for an enzymatic structure or for a structural protein.

So, it is this major scaffold that determines what the function of the protein is going to be. There is what is called a structure function relationship. The primary structure of the protein determines how it is going to fold up into its unique three dimensional structure and how this in turn determines the function of the protein.

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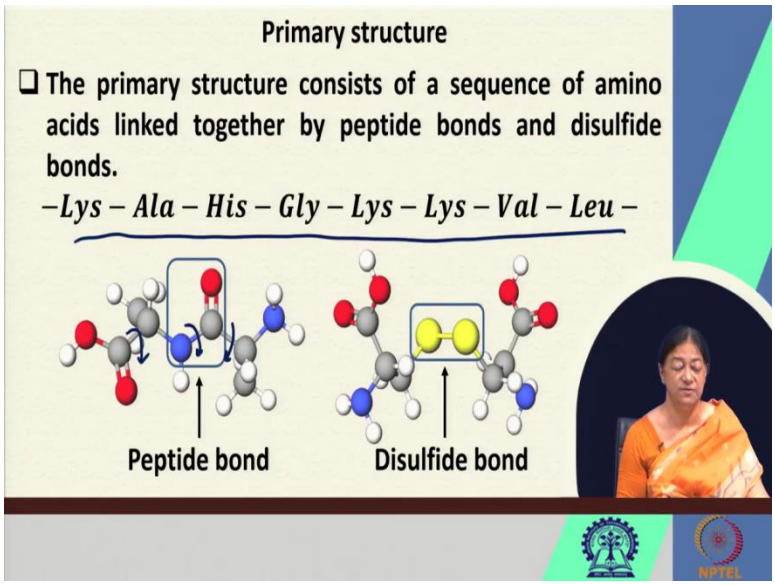

So, the primary structure consists of as we saw in the previous class, the sequence of amino acid linked together by the peptide bond which we found out was a planar structure. We also looked at the possible rotations about the other bonds that are present and we know that along the omega angle we have restricted rotation due to the partial double bond character.

Now when we look at a link of amino acids put together in the formation of the peptide bond, we find that the disulfide bond also gives us a covalent linkage between two cysteine residues forming a cystine disulfide bond.

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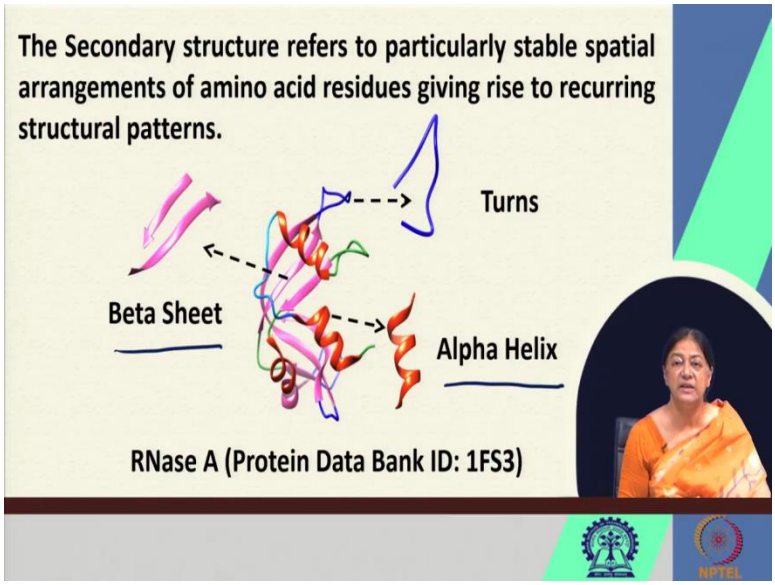

Then next came the secondary structure of proteins. Now the secondary structure gave the primary structure some geometric characteristic. In the sense that the amino acid residues were linked in a particular fashion. They formed what we call the alpha helix and the beta sheet.

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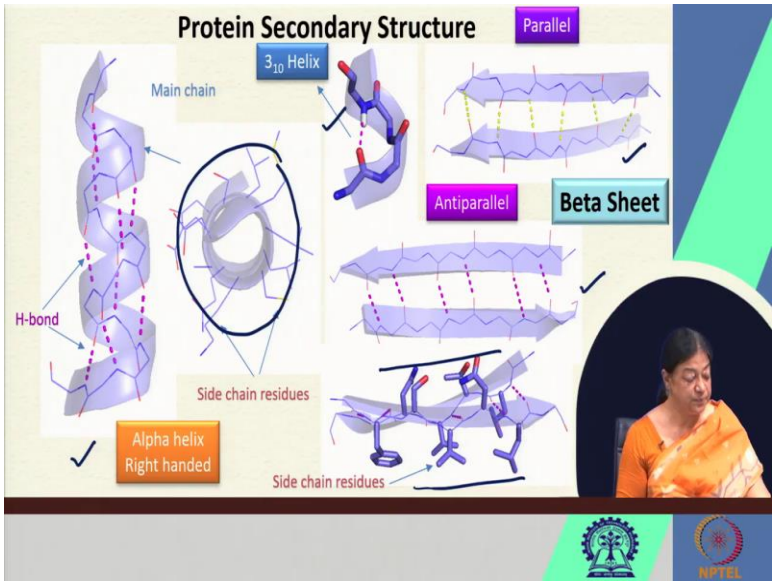

And we looked at specific characteristics of the alpha helix, specific characteristics of the beta sheet and found out that the pattern that emerges when we look at this, is their connectivity, their interaction, in terms of hydrogen bonding patterns. They have distinctive hydrogen bonding patterns for the alpha helix, distinctive hydrogen bonding patterns for the beta sheet and we found out that the pattern also changes when we consider the parallel or the anti parallel type of beta sheet.

For the alpha helices we found out that there was a 3_{10} type of helix, a regular, right handed alpha helix as we know and also a pi helix. The side chain residues disposition for the alpha helix was at the periphery, if we look down the axis and this was important in the construction of the helical wheel. Similarly, we have the side chain residues of the beta sheet oriented in this fashion.

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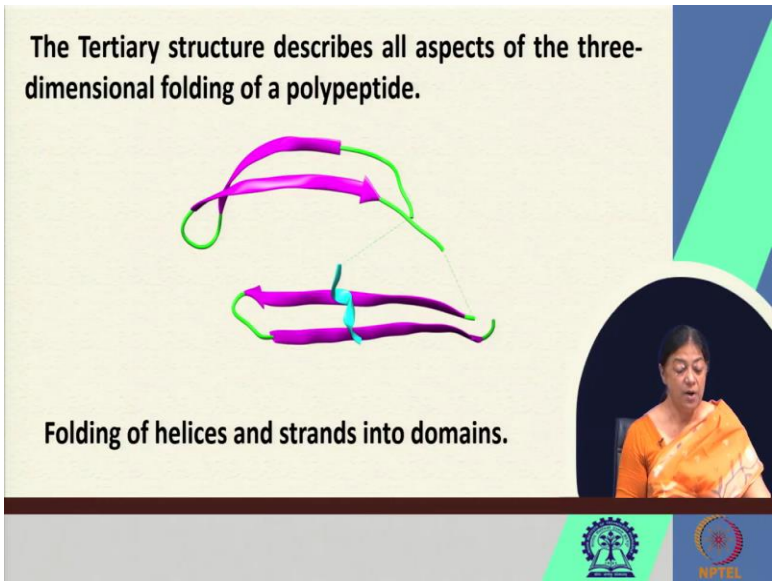

Our next topic now is looking at the tertiary structure. Now, what happens in the tertiary structure is we have a combination of the beta sheets and the alpha helices that are coming together in a matter, folding into what we call domains or super secondary structures, because this is going to then lead us to the compact structure that we know as proteins.

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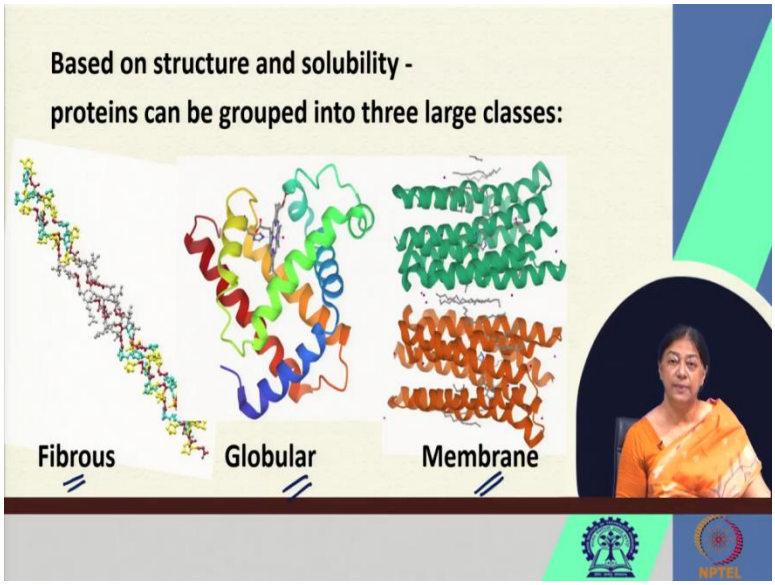

So, if we go into a bit more detail we look at the structure and the solubility. There are different classes of proteins. If we put them into major classes, we have a fibrous class, we have a globular class and we have a membrane class of proteins. Now the importance of these types of proteins is their structure, the amino acids that are present in the protein because we realize that the sequence of the proteins is important and these can be grouped according to their solubility the fibrous, the globular and the membrane.

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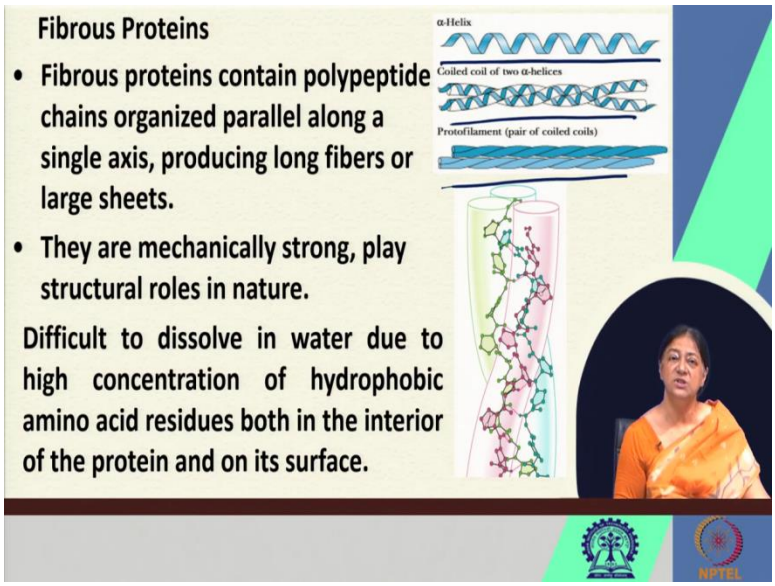

If we look at fibrous proteins, they contain polypeptide chains that are organized parallel along a single axis that produce long fibers or large sheets, as the name implies. What we have is we can have a helix. This is a coiled coil of two alpha helices, which looks like a fiber and because of their fibrous nature these are mechanically very strong and they play structural roles in nature. Now these, as we can understand because of their fibrous nature, are difficult to dissolve in water because of the high concentration of hydrophobic amino acid residues in the interior of the protein and on its surface. So, here again we realize that the type of amino acid is important in deciding how the protein is going to behave.

So, the fact that we have an insoluble type of mechanically strong protein fiber indicates that there are a larger amount of hydrophobic amino acid residues in this particular sequence.

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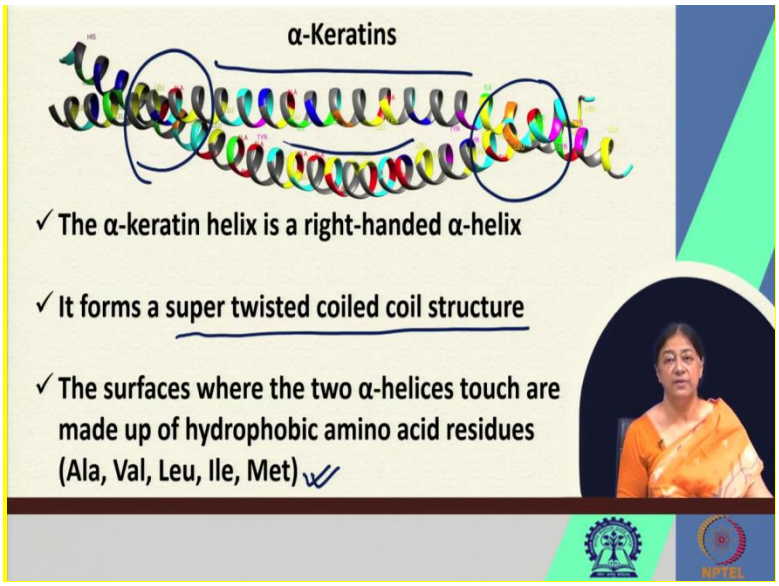

α-keratin is one of such example, where the α-keratin helix is a right handed alpha helix and it forms, as we were talking about, a super twisted coiled coil structure. And even though we may have other residues along this chain, along the helix, the parts where they overlap, where they interact with each other, have mostly hydrophobic amino acid residues.

We will see why that is the case. Hydrophobic amino acid residues prefer to interact with each other rather than the solvent.

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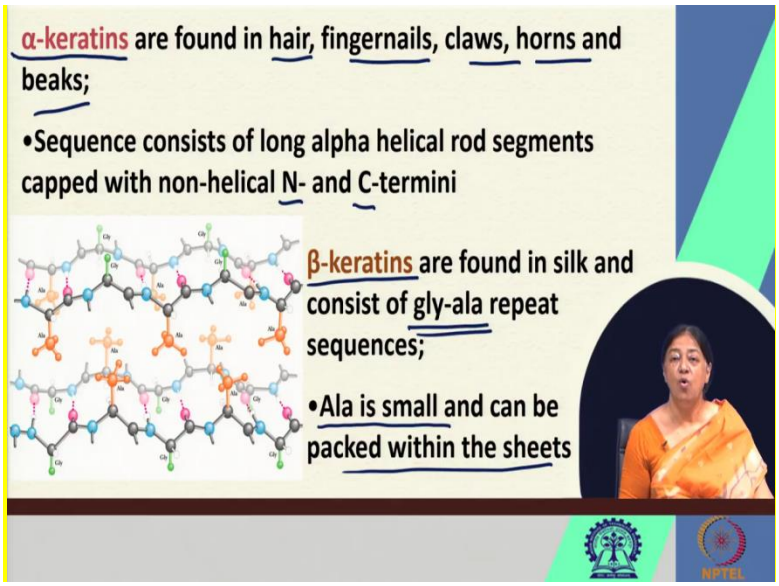

This leads us to where we can find α-keratins. We find α-keratin in hair, in fingernails, claws, horns and beaks. And the sequence as we were talking about, the types of amino acids that are present consists of long alpha helical rod segments and it is capped with non helical N and C-termini. The beta keratins that we talk about are found in silk.

They preferably form sheets and they have a glycine-alanine repeat sequences. Now we know that glycine is the simplest amino acid, the next amino acid that has only CH_3 as the side chain is alanine. So alanine is small in size, as definitely is glycine. And what is happening because of their size is, they can be packed within the sheets that form β-keratin. This is important.

So, we straight away understand the importance of the amino acid sequence, the presence of specific amino acids in their overall structural aspect.

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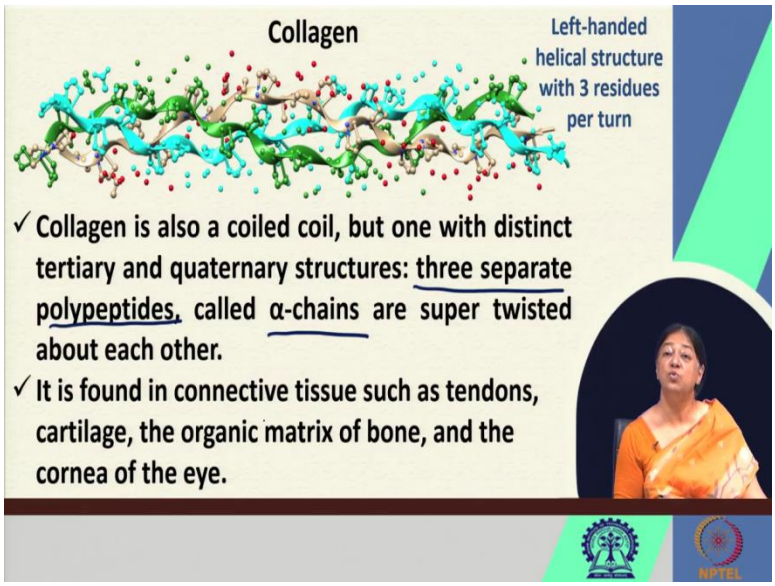

If we look at collagen, another muscle protein, it is a left handed helical structure with 3 residues per turn indicating that it forms a 3_{10} helix. But here also we have a coiled coil structure. And the specific characteristic is that there are three separate polypeptides, each of them called α-chains that are super twisted about each other.

So, the way in which they are formed or the way in which they structurally interact with each other is also important. In this particular case we have a left handed helical structure that has 3 residues per turn, a coiled coil structure and 3 polypeptide α-chains forming a super twisted structure.

Now, this is found in connective tissue such as tendons, cartilage, organic matrix, the muscle. So, this is important in the sense that we are looking at a muscular fiber type protein that has distinctive hydrophobicity issues, in the sense that it would remain insoluble.

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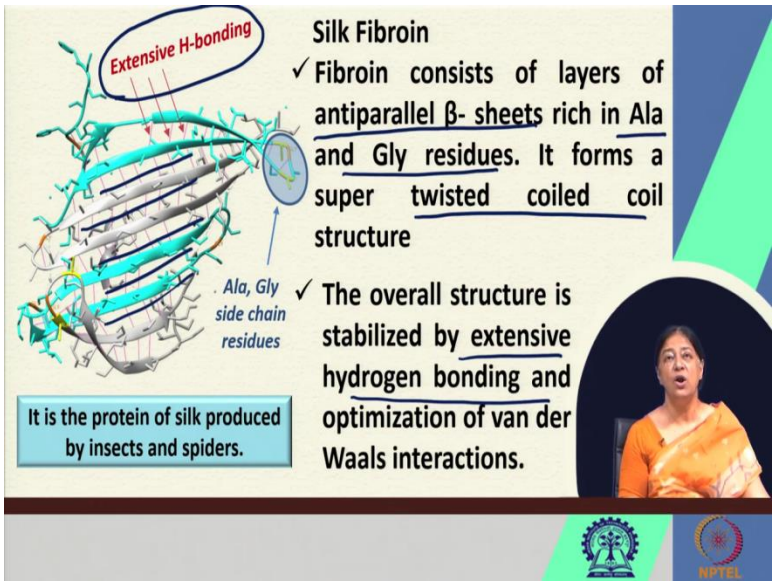

If we look at silk fibroin we see layers of anti parallel beta sheets that are rich in alanine and glycine residues. This particular type forms a twisted coiled coil structure, as is evident from the beta strands that form this twisted structure and this is the protein of silk that is produced by insects and spiders in their web that we know of.

So, the alanine and glycine side chain residues here again play an important role because of their small size and the fact that we have a twisted beta sheet indicates the presence of extensive hydrogen bonding between the strands of this beta sheet. And the overall structure is stabilized by the extensive hydrogen bonding and the optimization of van der Waals interactions, which we will see as we go along in an understanding of what we mean by protein structure.

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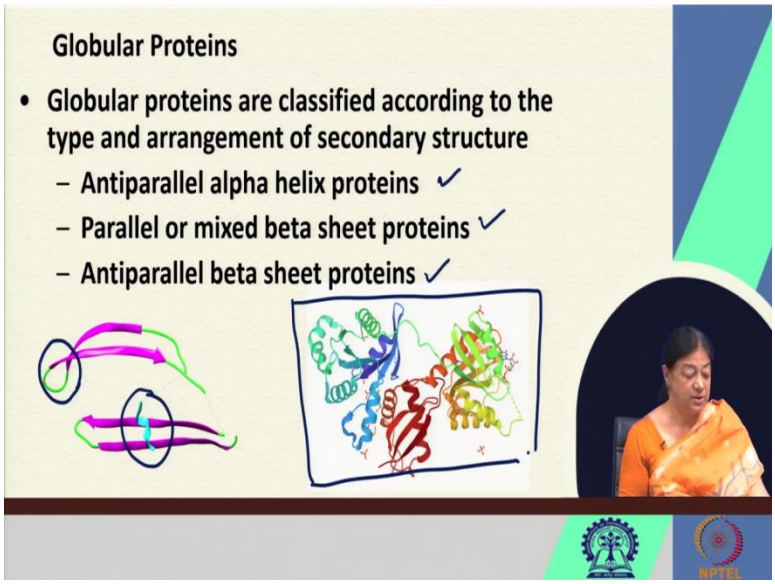

In globular proteins, the proteins are classified according to the type and the arrangement of secondary structures. Interestingly, we can have here anti parallel alpha helix type proteins, parallel or mixed beta sheet proteins and anti parallel beta sheet proteins. There are therefore, proteins that are majorly alpha helix, that have majorly a beta sheet structure or we can have a mixed alpha beta type.

We will look at the linkers later on. So, we can have a structure like this [refer to slide] where we can have the beta sheets, the beta strands linked together or we can have them with an alpha helix. We can have these structures as we can see a mixture now of helices and beta sheets. Now, we know that the beta strands interact with each other to form the beta sheet by hydrogen bonds.

We know that the alpha helix itself has hydrogen bonds between the residues that form the alpha helix, but what is the interaction that is actually forming this type of a structure, bringing the alpha helix and the beta sheet together?

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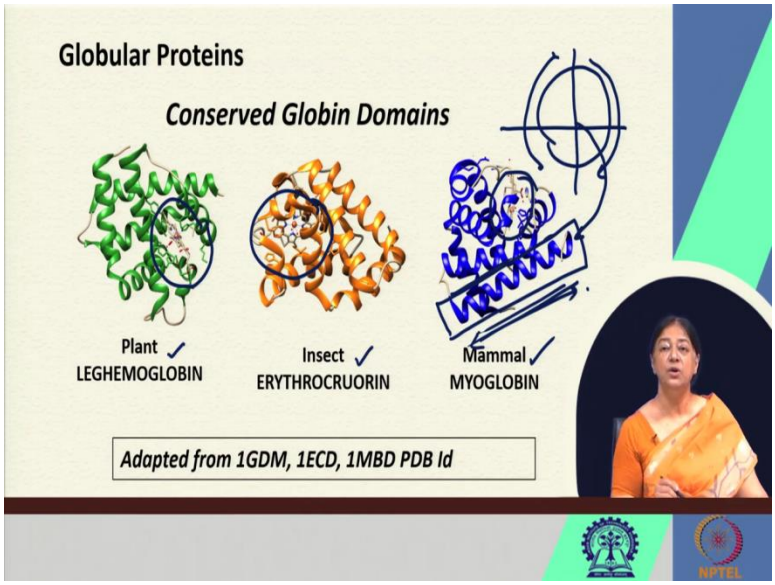

The globular proteins are interesting in the sense that they form what are called conserved domains, many of them. In myoglobin for example, [refer to slide] we have here a picture of a plant leghemoglobin, of an insect erythrocruorin and a mammal. Now, what we see here is if we just study the structures for a moment, we see that they have major alpha helical structures.

But, they do not look very much different, simply because they perform the same function in oxygen binding, where we can see the heme group attached. These globular proteins are water soluble proteins. When we look at water soluble proteins as opposed to the fibrous proteins, what would we expect on the surface? These are the questions that we are going to answer.

If we were to take [refer to slide] this particular sequence of the helix and construct the helical wheel that we looked at in the previous class, then we know that we would have a hydrophilic

surface and a hydrophobic surface in a protein helix such as this, where this face would be facing the solvent.

When we look down the axis here, we have a hydrophilic surface and we have a hydrophobic surface facing the interior of the protein because we know that this is a globular protein, this is going to be soluble in polar solvents. So the surface amino acid residues are preferably going to be polar in nature.

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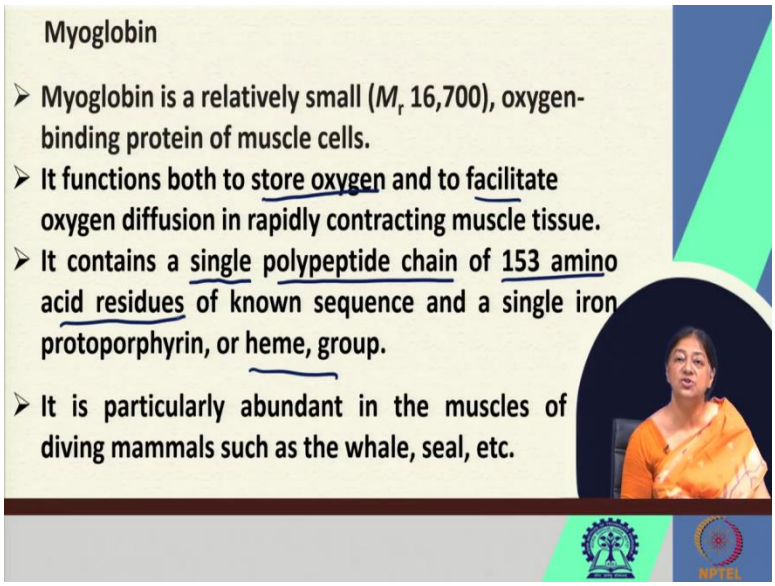

Myoglobin is one such example. It is a small protein, an oxygen binding protein present in muscles. We will look at this in greater detail later on. It functions not only in the storage of oxygen that facilitates oxygen diffusion in rapidly contracted muscle tissue, but is also an extremely important protein.

It contains a single polypeptide chain of 153 amino acid residues of known sequence and a single iron protoporphyrin group or heme group. It is abundant in muscles

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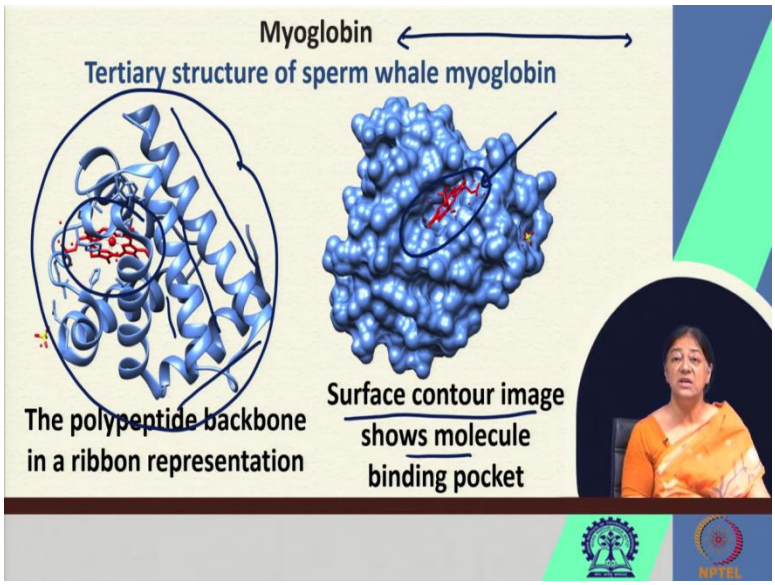

[Refer to slide] this is what the structure of myoglobin is, the tertiary structure. This means as we look at the helices that we have been looking at here, this is where we have the heme group, here we have the oxygen molecule and this is the polypeptide backbone in a ribbon representation. Then we have the surface contour image that also shows the molecular binding pocket.

We will be looking at such instances later on when we look at enzyme inhibitor or enzyme substrate binding, as well as protein ligand binding because this is what is extremely important in understanding the sequence of the protein. So, we have 153 amino acid residues that are linked in the primary structure to form this single polypeptide chain.

Now what has happened. This polypeptide chain has now folded or formed several helices and these helices have now come together in this folded structure of the protein. And we know that this folded structure is giving such a disposition of amino acid residues in three dimensional space that we have the perfect orientation or the perfect scaffold, the perfect structure or cleft to hold the particular molecular binding pocket.

This is extremely important in understanding the structure and the function of the proteins and we will see how it is important as we go along.

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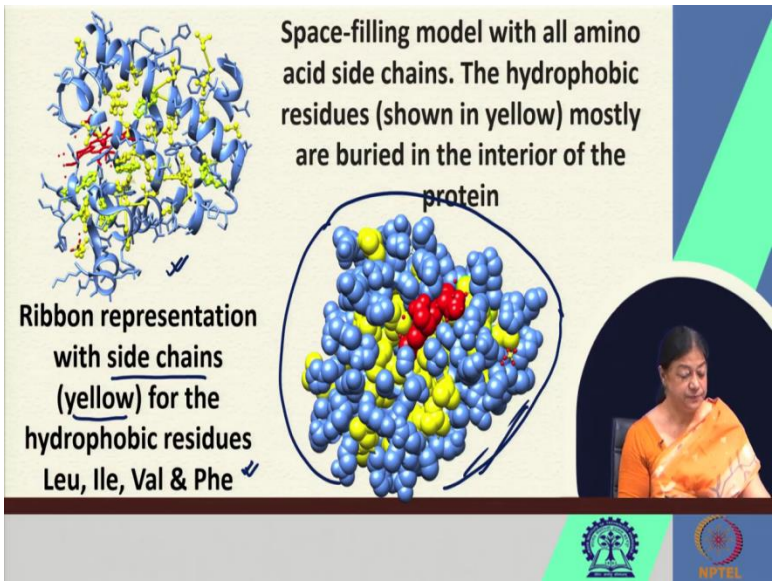

So when we look at the ribbon representations [refer to slide], there are different types of representations where we can look at the structures and the type of amino acid residues. The side chains are there in yellow for the hydrophobic amino acid residues. Now, it is not very apparent in the ribbon representation, what do we mean by hydrophobic amino acid residues?

These are the residues that would prefer to be in the central part of the protein and the hydrophilic ones would be there at the surface of the protein. Now, the question is, is this true for all types of amino acids or rather all types of proteins. The answer is no. For a globular protein because we have a polar solvent around it, we would preferably have hydrophilic type of amino acids on the surface. And what do we see in this space filling model?

We see that the yellow side chains are the hydrophobic side chains. And where are they? They are in the central part of the protein. Why is it so? Because we have a polar solvent and we would prefer to have hydrophilic amino acid residues on the surface.

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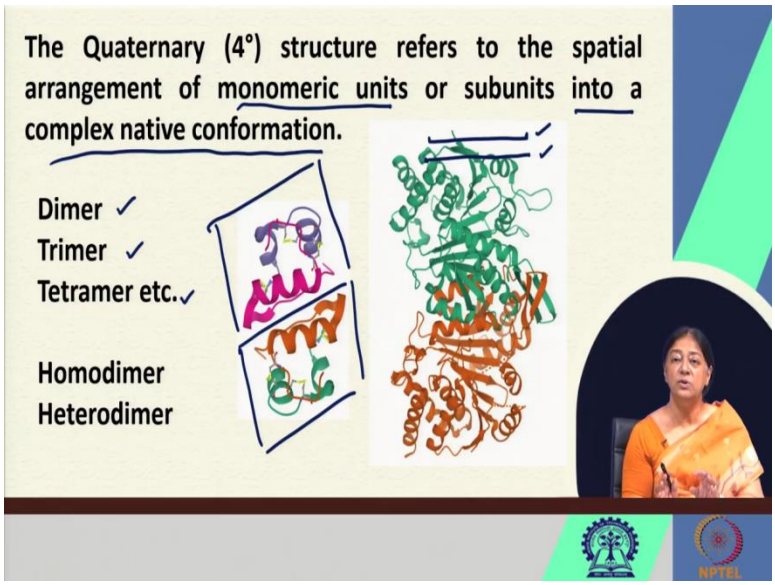

The quaternary structure refers to the spatial arrangement of monomeric units or subunits into a complex native conformation. What do we mean by this? We found out that myoglobin has a single polypeptide chain. Is this true for all proteins? Yes, all proteins will have a single polypeptide chain and they will fold into a native tertiary structure.

But there are some proteins that form quaternary structures. In these quaternary structures the several monomeric units can come together to form the functional unit of the protein, the native protein. We can have dimeric proteins, we can have trimeric proteins, we can have tetrameric proteins.

As the name implies when we have a dimeric protein we would have 2 monomeric units or rather 2 polypeptide chains, that would come together to form the protein in its quaternary structure. In a trimeric protein we would have 3 such monomeric units or subunits come together and in the tetrameric we would have 4.

Now, these polypeptide chains that form the quaternary structure may be of the same type. Say for a dimer we could have a homodimer, where we would have 2 units that are identical or we could have a heterodimer where we would have 2 dissimilar units come together.

What do we mean by a homodynamic protein? It means that the two monomeric units that come together are identical. In a heterodimeric unit the 2 monomeric units that come together are different.

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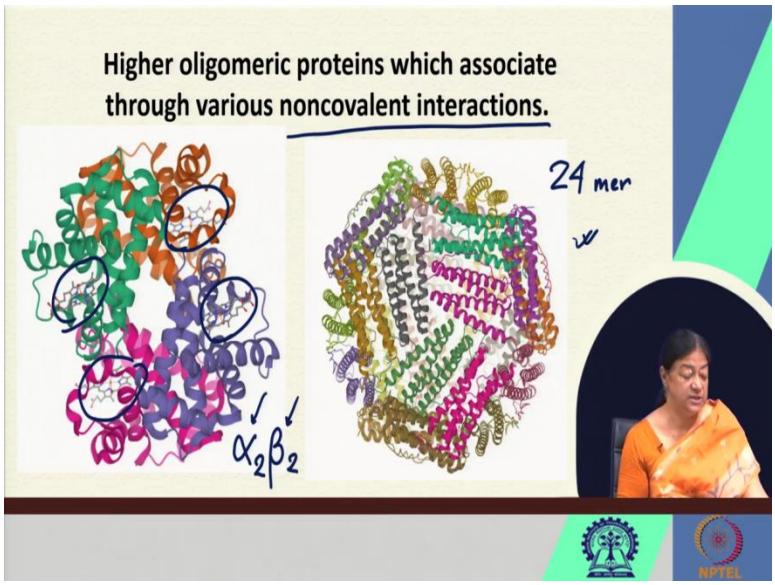

When we look at higher oligomeric proteins, which associate through various non-covalent interactions which are extremely important, this is the structure [refer to slide]. On the left is the structure of hemoglobin. Hemoglobin is a tetrameric protein. It has 4 subunits, 4 monomeric units, 2 of them are alpha and 2 of them are beta chains.

So, it is an $\alpha_2\beta_2$ tetrameric protein. If we look at the case of myoglobin we see that the monomeric chain holds the heme unit. In hemoglobin we have 4 such heme units which you can identify from the 4 subunits that are present and this also binds oxygen. But there are 4 monomeric units together; 2 of the alpha type 2 of the beta type and hemoglobin as we all know is an extremely important protein in our body.

Similarly the one on the right here [refer to slide] is ferritin. Ferritin is another very important protein that has the job of transferring iron, it is a 24 mer. So, there are 24 subunits in this beautiful structure that forms ferritin and there is a reason why the structure looks like this. There is a reason why the tetrameric units of hemoglobin are positioned in such a manner. So that is what the beauty of protein architecture actually is.

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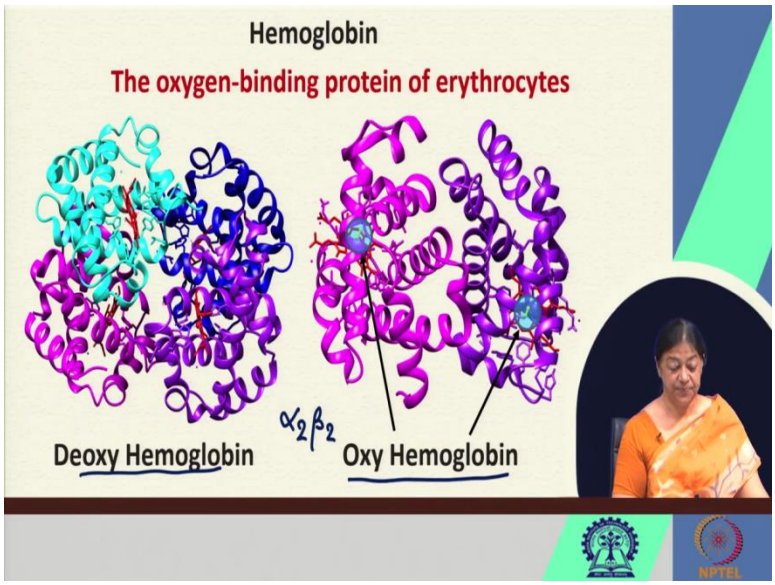

Now, when we look at the oxygen binding protein of erythrocytes that is hemoglobin, we realize the importance which we will study later in a course when we do more about protein ligand binding. We have oxy hemoglobin, we have deoxy hemoglobin and the structures though having the same $\alpha_2\beta_2$ units, they loosen up or they breathe as it is called in a beautiful manner.

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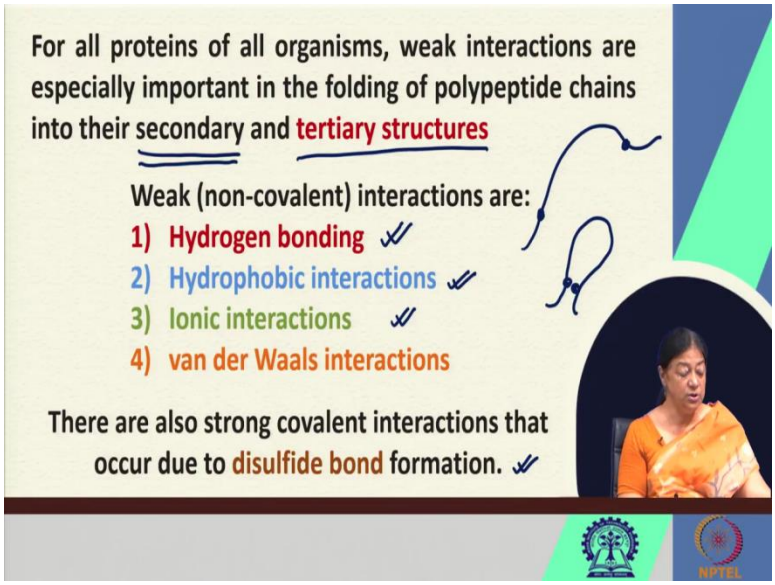

So for all proteins of all organisms we have weak interactions that are specially important in the folding of the polypeptide chains into the secondary and tertiary structures. Now, what we did learn is, we learn that when we are looking at the secondary structure of proteins hydrogen bonding is the important interaction that holds the helix together in its particular type of interaction, that holds the strands of the beta sheet together.

Now what is important? As we saw in the case of fibrous proteins in the tertiary structure, we have a lot of hydrophobic interactions. We have a lot of small amino acid residues that are present and hydrophobic interactions are predominant. Hydrophobic residues are also predominant because they are insoluble. The solubility matters in the specific class. We will also look at membrane proteins and see how they interact with their environment.

In the case of ionic interactions these are the ones that we would observe with the specific types of amino acids that are capable of electrostatic interactions, acidic and basic amino acids. And the van der Waals interactions are mostly seen through hydrophobic amino acid residues. The other linkage that brings different parts of the protein chain together or close to each other is the disulfide bond.

The disulfide bond as we learnt is forming the SS-bond. So, if we have a long chain of our polypeptide which is linked together as we know by peptide units and we have one cysteine group here [refer to slide] and another cysteine group here, forming a disulfide linkage. We straight away see that it is possible to form a part folded structure in three dimensional space because what is going to happen is these two residues are going to come together.

So the strong covalent interactions that occur due to the disulfide bond formation also bring different parts of the polypeptide chain close to one another. The important part is learning of how these interactions help in the tertiary structure.

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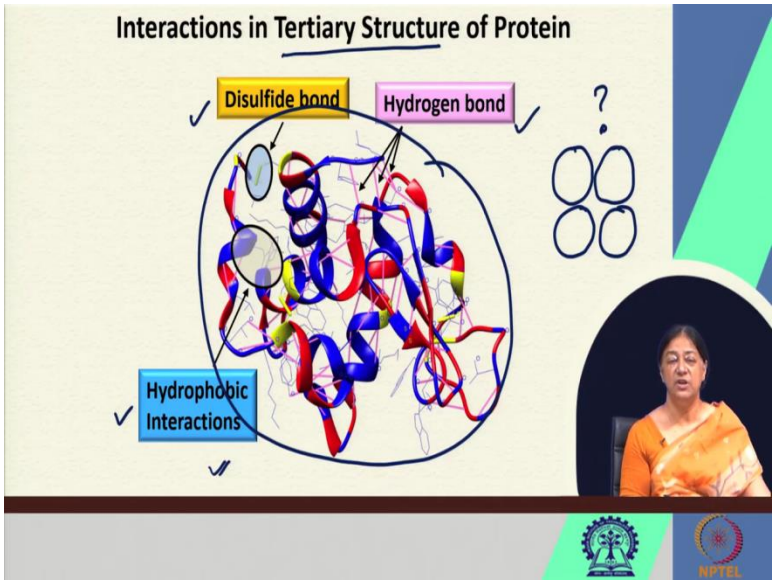

When we look at a tertiary structure we have the formation of the disulfide bond, we have the formation of the hydrogen bond. Not only between the alpha helix, within the alpha helix or within the strands of a beta sheet, but also inter strand or between the helix and the strand and so on and so forth and these are mostly through side chains.

So, which are the side chains that can be part or be involved in hydrogen bonding? Which are the side chains that we would mostly see for hydrophobic interactions? For a globular protein where would we expect these hydrophobic interactions? These are the questions that we are going to answer.

If we look [refer to slide] at this monomeric unit and say that these are the interactions that hold the polypeptide chain together or form the folded structure, then are these the same interactions that are going to hold the monomeric units together? If we do have a tetrameric unit where we have 4 subunits come together, are these the same interactions that are going to hold the tetrameric unit together?

So, these are the questions we will be answering when we look at protein folding in more detail, the interactions in more detail and how the protein comes together in its native three dimensional structure.

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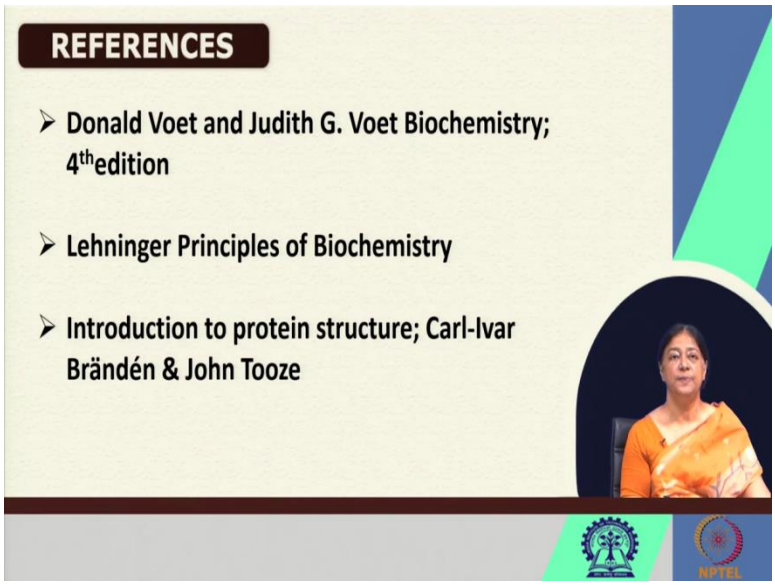

Thank you.