Bio-Physical Chemistry
Dr. Pramit Chowdhury
Department of Chemistry
Indian Institute of Technology, Delhi

Lecture - 05
Tertiary Structure

Hello everybody. Let start today's class. So, today you know we have been looking at these different structural aspects right. We started from the primary structure, secondary structure then we looked at these helical angles access and all, we look at the different secondary structural elements right. Last time we were looking at something known as motifs.

Now, what we are doing what we are going to do today is you know rather focus in this class is kind of take the next leap, you know go to another structural level which is referred to as the tertiary structure.

So, what you will realize soon is as we go to the class is that once we have you know once we have the idea about these secondary structures then essentially it is the way that these secondary structures pack, you know thereby giving rise to the tertiary structure ok.

So, we will look at this tertiary structure in some details right, you will soon see. It actually gives an idea of how you know different secondary structural components are oriented with respect to each other in different proteins ok.

Hopefully we can realize that or be able to appreciate it a lot more after the class is over. So, when we talk about the protein tertiary structure then there are you know two things we are talking about one is the packing.

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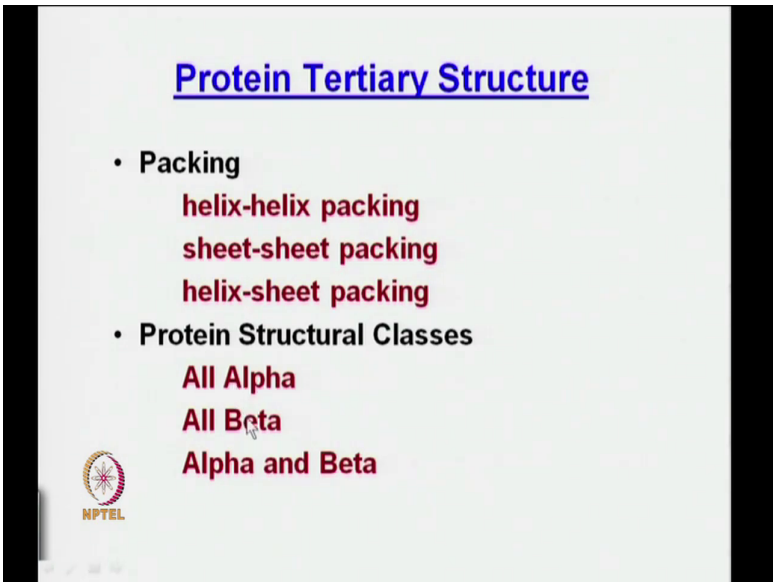

When you talk about the packing what are the different types of packings? Well, it is very simple. The two major secondary structural elements are helix and beta. So, obviously, one would be helix-helix packing; the other one would be beta sheet. So, sheet-sheet packing and the other one would be the third one is an obvious choice is a mixture of these right. It is a helix sheet packing.

Now, what are the different structural classes? So; obviously, if you are going to have a helix-helix packing then it is going to be all alpha. So, that is what you have as all alpha right. If you follow my arrow then if it is going to be sheet-sheet packing then it is going to be all beta and if it is going to be mixture then it is going to be alpha and beta.

So, in today's class what we are going to talk about primarily are the helix-helix packing giving to all alpha or giving rise to all alpha rather and the sheet-sheet packing giving rise to all

beta. Now, the alpha and beta I leave it up to you to read and as I was tell you later this alpha you know beta mixed is the most commonly structural element and I want you to read this yourself in details.

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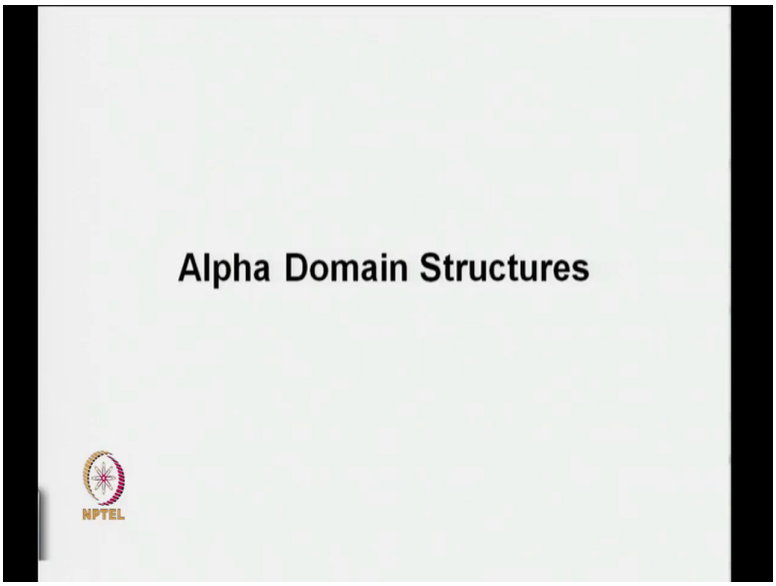

So, going forward let us look at you know the alpha domain structures. So, why do we want to look at the alpha domain structures?

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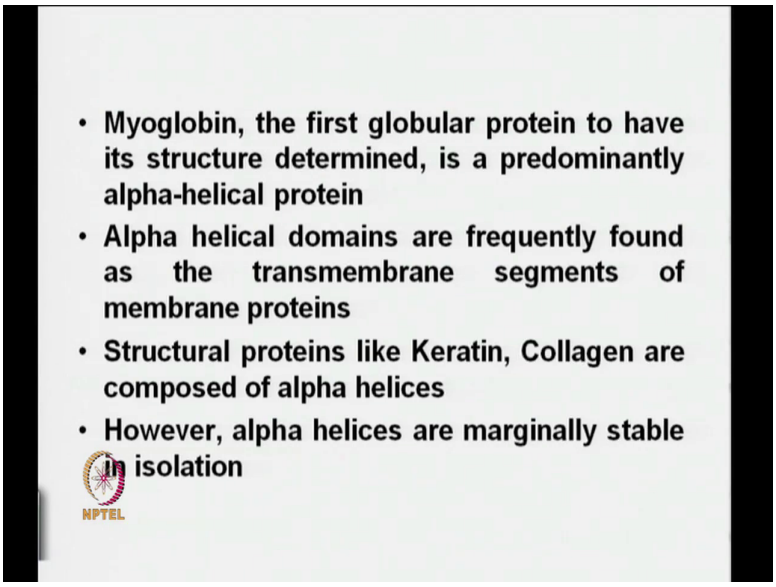

Well, all of you know myoglobin, this is the first globular protein to have it is structure determined. It is a predominantly alpha helical protein. Hence, the importance of you know alpha helical packing or alpha domain structures. So, these alpha domains are or alpha helical domains are frequently found as the trans membrane segments of membrane proteins. You know you must have seen this.

Then structural proteins like keratine, collagen are composed of alpha helices. However, alpha helices are only marginally stable in isolation. What I mans is that if you have say a protein having you know 8 or 10 alpha helices and if I take 1 alpha helix out of it, all these alpha helices together say 10 alpha helices together make a stable protein.

But, the moment you take 1 alpha helix out of that you know arrangement of that packing then that alpha helix in almost all cases in all probability would not be that stable which means

that alpha helices are marginally stable in isolation. So, hence you can understand that alpha helices have to be stabilized.

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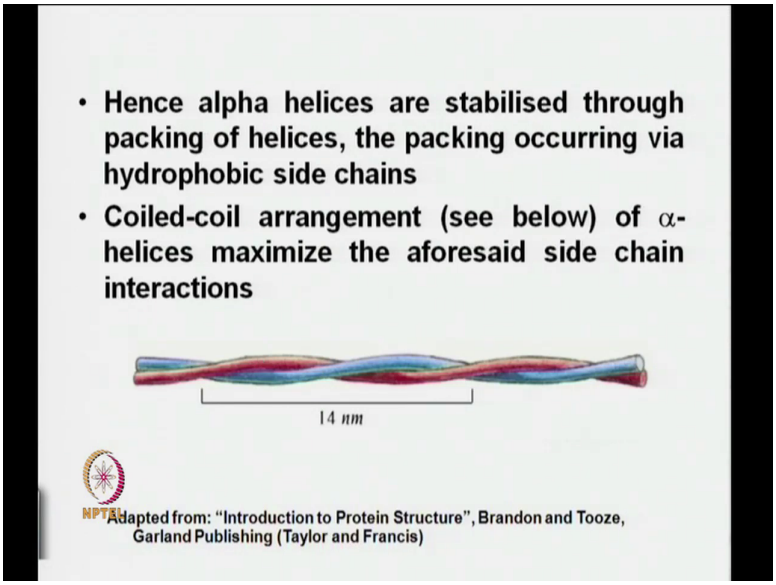

So, how they stabilized? If you read the first point, alpha hence alpha helices are stabilized through packing of helices, the packing occurring via hydrophobic side chains. Now; obviously, if you are going to pack you know if you are going to pack then what is going to happen is you will be having a certain surface of the protein which is going to be turned away from water which is your physiological you know solvent right, in your physiological environment water is in your physiological environment and then you would be having hydrophilic residues which are on the outside.

So, a coiled coil arrangement as you can see at the bottom the coiled coil arrangement of alpha helices maximize the aforesaid side chain interactions, right. So, this is an example of a typical

coiled coil and what you can see is if you know if this distance is about fourteen nanometer from one to the other what you can see is here you can see the way these two different alpha helices or kind of wrapping around each other, coiling around each other.

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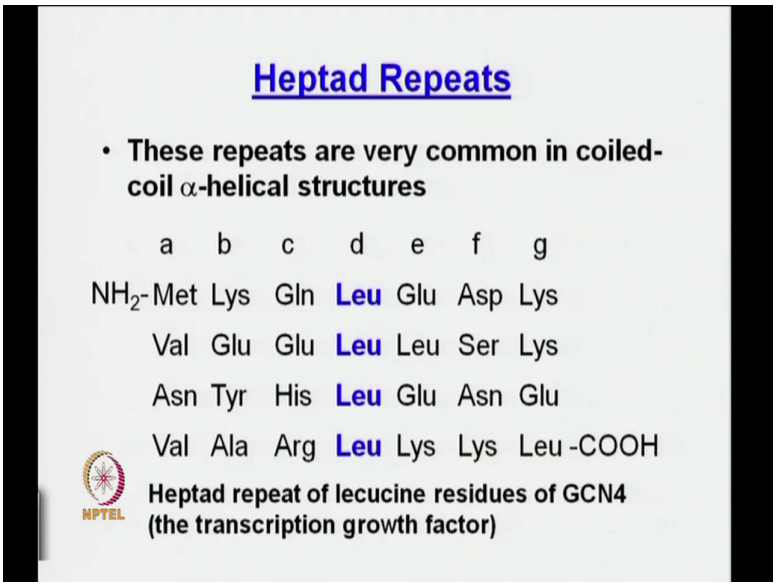

Now, going into this structural aspect in a little more detail of very common aspect of this coiled coil structure is a repeat or is you know sequence known as heptad repeats right. Now these repeats are very common in coiled coil alpha helical structures. And what I mean by heptad repeats I will soon tell you, but first let us look at these amino acids ok.

So, you see how many amino acids we have. We have a b c d e f g. So, you can understand; so; that means, a b c d e f g these are 7 amino acids; hence comprising of the heptad or this is what the heptad is made up of, specifically for the protein I am talking about.

Now, if you look at the different amino acids what you will soon see is if you go from a to g, well a, so, if this NH2 is if you follow my arrow this is N terminus and then COOH this is your C terminus. Now, this as I was telling you this is for a specific protein. Now, this heptad repeat is of lecucine residues sorry this c should not be there, it is a typo of this GCN4. It is a transcription growth factors, it is a coiled coil peptide helical coiled coil; it is a transcription growth factor.

Now, what you see is one of the most important things as is colored in blue, the d position the d position is occupied by leucine which is a hydrophobic amino acid ok. Now this has severe consequences and very interesting consequences that you will see very soon right. Also, if you go to you know if you look at the other amino acids like even e, you have your kind of share of charge or polar non polar residues too. But then what is the use of this heptad repeat? Let us look at that.

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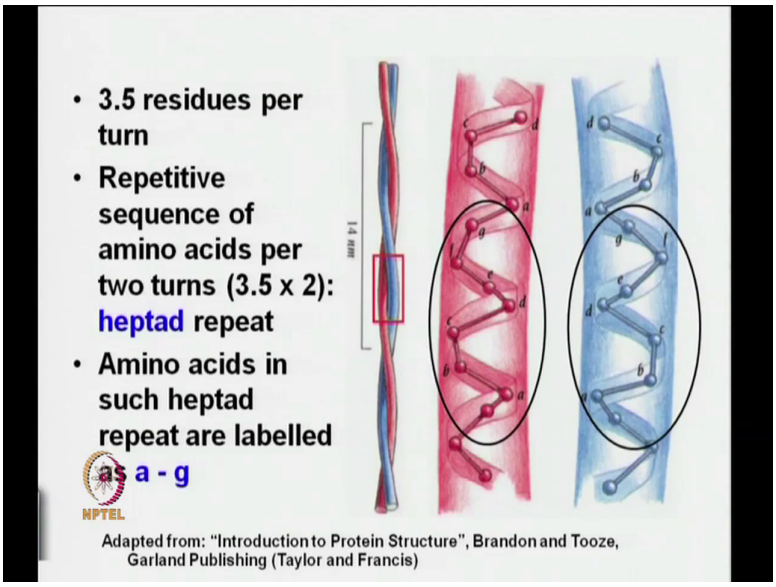

So, if you would remember this you know this coiled coil which we looked at couple of slides ago, now what you are doing is you are taking a cross section of it and then they use zooming into it.

So, once you zooming into it what you see is you know you remember in the last slide we talked about this heptad amino acid right a to g ok. So, this is what you see. This is the red one is one helix right as 1 helix out here the red helix and the blue one; obviously, this is the blue helix and you are looking at this portion right. So, this is how it looks like. It is kind of an; it is kind of a bent portion.

Now, moving forward, it says that there are 3.5 residues per turn. Now, if you talk about helix turn, if you remember our alpha helix there were like 3.6 residues per turn. However, here we have a little bit less. Now, the reason being if you know if you kind of go back to that alpha

helix discussion, the alpha helix was kind of a straight smooth cylinder right. There were no kinks; we never talked about those in 1 alpha helix.

But here you see the structure is not straight, both the alpha helices are to a little bit distorted and hence this brings about a decrease in the number of residues per turn. So, there are 3.5 residues per turn. So, you can understand. Then the repetitive sequence of amino acids per 2 turns 3.5 into 2, it is called the heptad repeat. So, 3.5 into 2 makes it is 7 amino acids the heptad and so, every 7 amino acids you have kind of a sequence which is repeated.

So, the amino acids in such heptad repeat I labeled as a to g as I was telling you and as I mentioned and also showed to you in the previous slide. In terms of the sequence this a to g amino acids constitute what is known as the heptad repeat and to kind of show it on these two helices you can see out here.

So, this is one this you know this enclosed portion, this black line we the portion this enclosing, it is showing you the heptad of the red helix coil and the other one you showing it for the blue one.

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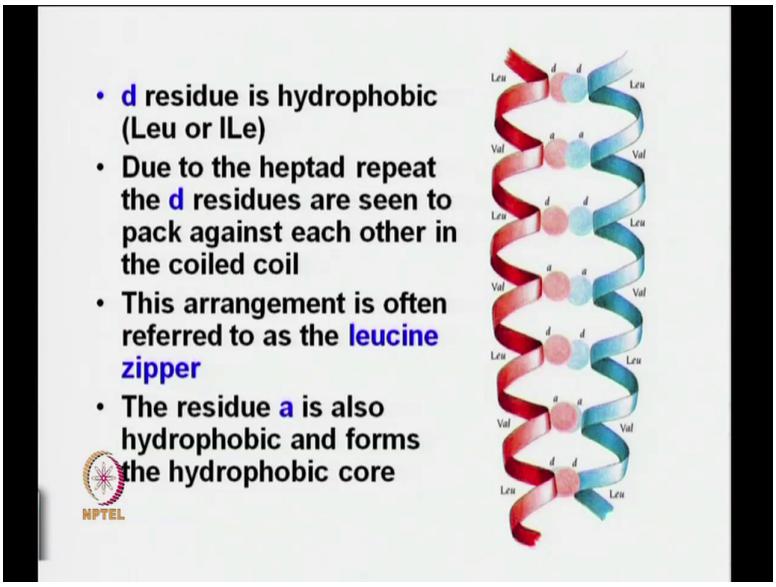

Now, how this packed? So, if you look at this now you are you know before you are just looking at in the in the previous slide you were just looking at you know the amino acids right, a b c; you are not looking at the actual interactions. But now if you if you think about in terms of the helix right, then what you see is as you go through as you go through; it does not matter which you go through you go from here to here or from here to here down.

You can see this d amino acids which I said was leucine or hydrophobic, a typically packed against each other; which means remember again a few slides ago we discussed that the helices cannot be stable and isolation. This means when they are stable they have to be packed and the packing has to stabilize this whole arrangement, right.

So, in this case you can see there is one component which is hydrophobic right. This is hydrophobic packing because a due amino acid was essentially a hydrophobic amino acid

which is leucine. So, the d residue as I said is hydrophobic it is either leucine or isoleucine, the one we are taking in this case is called leucine.

Due to the heptad repeat the d residues are seen to pack against each other in the coiled coil ok. Say this is because of the haptad repeat that repetitive sequence of 7 amino acids. Now this arrangement is often referred to as the leucine zipper. So, you know you know what you know zip is. You can zip it up like this like zipping change. So, you can see if I am going to zip down the helix coil what you are going to look at essentially are leucine residues.

And, hence it is referred to as the leucine zipper model or motif. Now, the residue a is also hydrophobic and forms the hydrophobic core. So, the residue a is also hydrophobic. Remember we talked about this residue a to g. The a typically also comes out to be hydrophobic.

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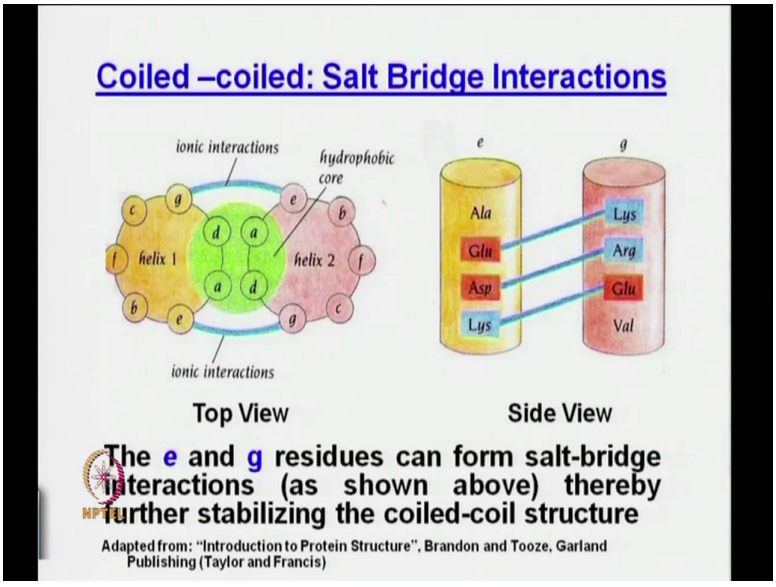

So, what are some other interactions that are present now? It cannot be only hydrophobic interactions; it must be something more than that because we have so, many other amino acids.

So, if you look at this what we had you know what we had bring now is if the helices we looked at what we are you are looking at from the top view; that means, these are the helices right. So, the so, if I go back, so, these are the helices. You looking at the helices from the top ok, you just looking at them from the top these are tops section.

So, because of the top section you can see how these are arranged. Now, you see this green portion these green portion you can see out here the green portion is made up of these a and d residues which are forming the hydrophobic core.

However, you have also other amino acid which are non-necessarily hydrophilic sorry hydrophobic, but hydrophilic. So, you then you will be having ionic interaction which essentially salt bridges between g and e; e and g of these two helices and that is what you also see in the next panel which is the side view.

So, this one is the top view and this one is you are looking at from the side. So, that is what it says the e and g residues can from the salt bridge interactions as has been shown above thereby further stabilizing the coiled coil structure.

So, again you know it pace to kind of look into this matter little bit more. And, what you have just realized is once we looked into this matter we see that how this isolated alpha helices which were actually not stabilized isolation are being stabilized in presence of other helices.

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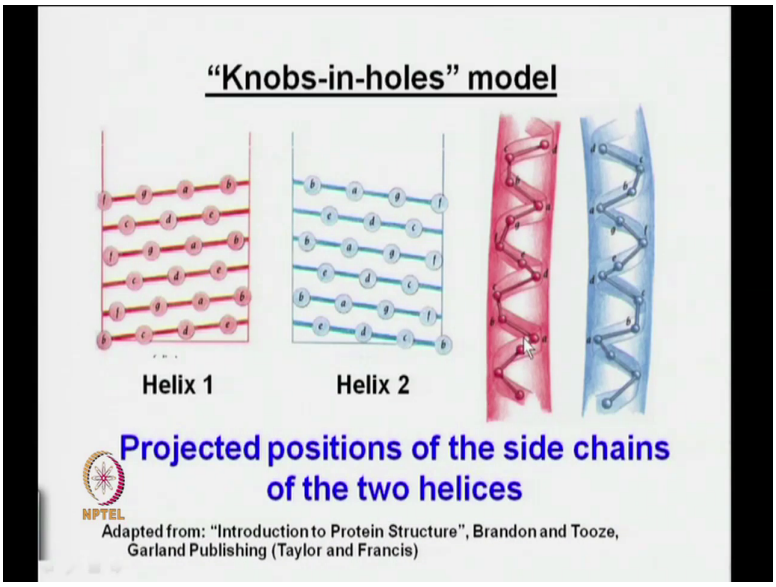

So, this is one type of packing we have. So, now, when we talk about this packing, this packing the leucine zipper packing I mean it is given to you know it is given a special name. It is called a knobs in holes model because helices have to pack and this is one type of packing. So, what I mean is so, this is one helix; helix 1 the red one; this is helix 2 the blue one. So, what you are seeing is as it says these are the projected positions of the side chains of the 2 helices.

So, you are taking this you are taking this the red one and the blue one in your taking this side chains and putting them along the helical axes. Now, what you see out here is please note. So, here the inclination and here the inclination is different.

So, you can understand if this helices have to pack against each other one of them will be have you know we have we will have to tilt or just move in an angular orientation one of this helices with respect to the other. This is exactly what happens.

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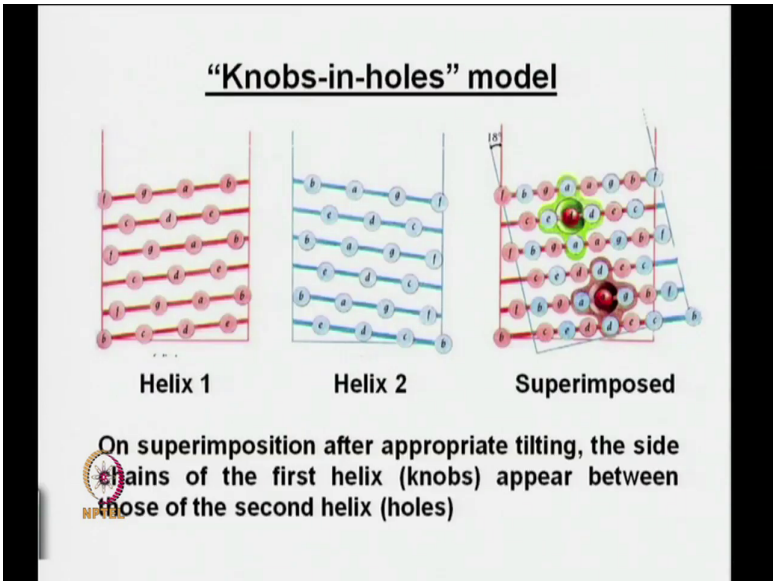

If we go to the next slide again you look at helix 1 you look at helix 2 and then you try to superimpose them. You can also superimpose them just like this in this form as it shown in helix 1 and helix 2; you will have to do certain transformation right. What do we see? So, this is what you see. We can see this blue one has been tilted a little bit with respect to the red one which is helix 1, the angle being 18 degrees. Not only that look at these look at this d out here if you follow my arrow this is d and this a right.

So, this d is surrounded by 4 other amino acids and this a is surrounded by 4 other amino acids ok. To explain it you know a little simply, what happen this on superposition after appropriate tilting; that means, now you are tilting it the side chains of the first helix.

So, these are the side chains of the first helix. You can see these are the side chains of the first helix. They are known as knobs right as written in brackets. They appear between those of the second helix. So, these are the side chains of the second helix.

So, what happens is the moment you tilt it, you can see this f g a b, the red ones which belong to the red helix they are now as knobs appearing in vacant spots or holes between this the in the ones in the blue; b a g and f ok. So, this is important now.

So, the way you have packed it, you just cannot pack it arbitrarily. You go to pack it so, that you have vacant spaces and then you maximize the interactions. This is the way the interactions are maximized and also you make use of the vacant spaces that is why it is call the knobs in holes model.

Holes means you have vacant spaces, knobs means these are the amino acids which were from one helix chain which can go inside the vacant spaces or holes of the adjacent or the other helix chain giving rise to this coiled coil structure.

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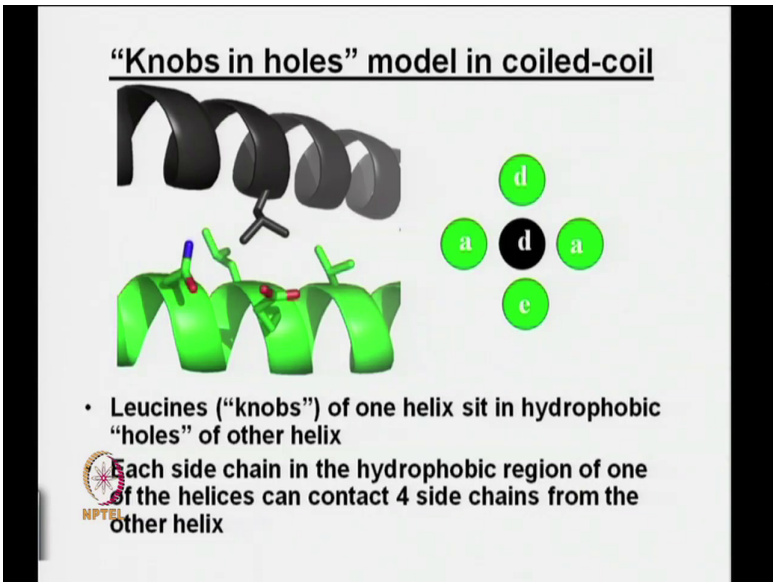

So, the knobs in holes model again. Now, if you try to zoom in to that you know remember we talked about this. So, if you go back we talked about this d and a right. So, if you now try to zoom into this and see then what we see is.

So, this is typical. So, this is your d right. So, this is a d which is a leucine and these are the respective amino acids from the other helix right that is why these two helices are so, colored differently colored. This is one helix this is these are the helix these two together forming the coil.

So, as it says leucines these are knobs of one helix sit in the hydrophobic holes of the other helix that is what you see. The d is leucine; d a a are primarily hydrophobic, but e is you know

hydrophilic. Now, each side chain in the hydrophobic region of one of the helices can contact 4 side chains from the other helix.

Now, this is exactly what you see out here. So, in a nutshell, this is the packing; this is the packing this is the knobs in holes model that goes on in a typical leucine zipper motif ok.

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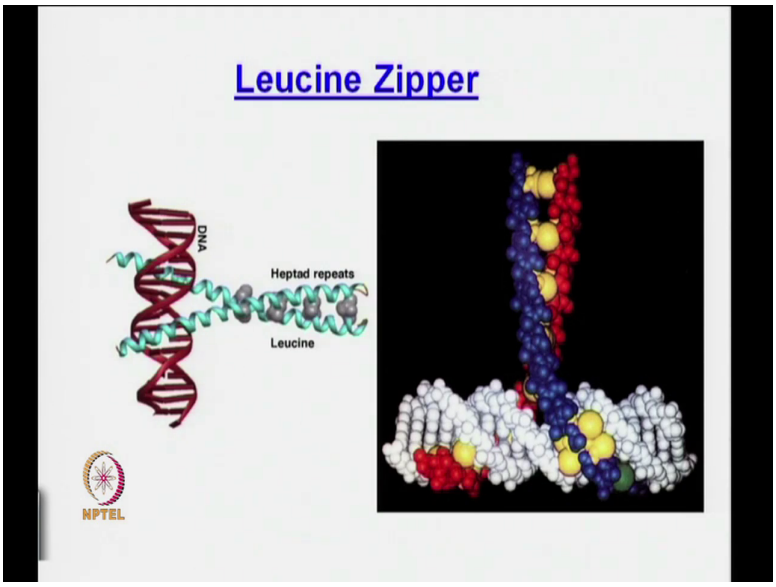

So, this is again we are looking at the leucine zipper. This leucine zipper binds. So, the leucine 4 coil it binds to DNA. So, this is the DNA you can see this heptad repeat and these are the leucines right. These grayish face of the leucines and there is one more way you can look at it. Again you can see this DNA out here and these are the two coiled coil helices with the leucines in between ok. So, this is typically what we have been looking at in the previous few slides.

But the question is this you know the only type of arrangement we have when it comes to helix packing. So, let us look at it. So, another very common helix packing is this 4 helix bundle.

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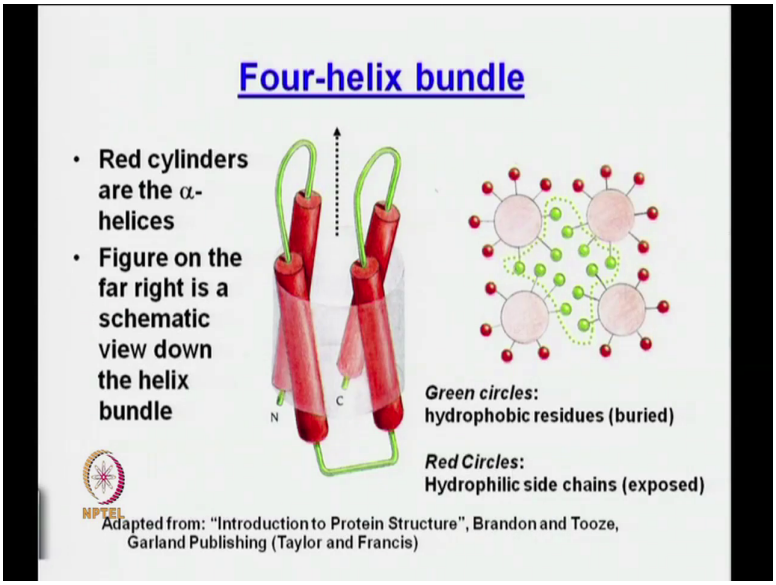

So, you can see this here there the red ones are your helices and what you have done is these are like the topology diagrams. You are representing helices as cylinders and you have 4 helices forming a bundle. So, the red cylinders are the alpha helices as I said.

Now the figure on the far right is a schematic view down the helix bundle. So, what do you mean is if you looking at the helix like this if you looking at the helix like this and this is the arrow we just saw then again you are looking at it is kind of a top view right. You are looking at or the bottom view which away you look at it.

So, what you see typically is see these green residues out here these green residues are packing in the core. Packing in the core means this is the core. This is the core. This is where the green residues are packing; that means, they are giving rise to something known as a hydrophobic core, but what about the other residues? These red residues.

These red residues are jetting out into the surrounding solvent interacting with the solvent surrounding solvent and hence this is a hydrophilic. This is the ones which are helping which are on the surface they helping to keep the total motif or the total 4 helix bundle protein solubilized in water.

So, it is says the green circles or the hydrophobic residues buried. So, this dotted line actually tells you the section of the hydrophobic residues and the red circles are the hydrophilic side chains which are predominantly exposed to this surrounding solvent.

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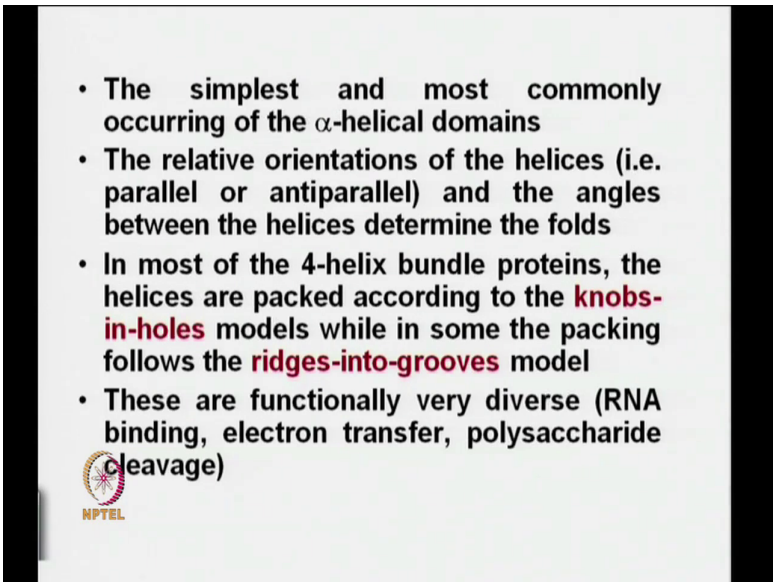

So, this is the simplest and the most commonly occurring of the alpha helical domains, the 4 helix bundle it is. The relative orientations of the helices and the angles between the helices determine the folds. Now, this is the important. How the helices will be oriented and in the what angle we will finally, determined what fold thus protein domain or this alpha helical protein domain takes.

In most of the 4 helix bundle proteins, the helices are packed either recalling to the knobs in holes models while in some the packing follows the ridges into grooves model. Now, the knobs in holes model I have looked at. Here we just discussed. By the ridges into grooves model what is that? That is what we are going to come into you know discuss about next.

Now, these are functionally very diverse starting from RNA binding, electron transfer say cytochrome base protein then polysaccharide cleavage and so, on.

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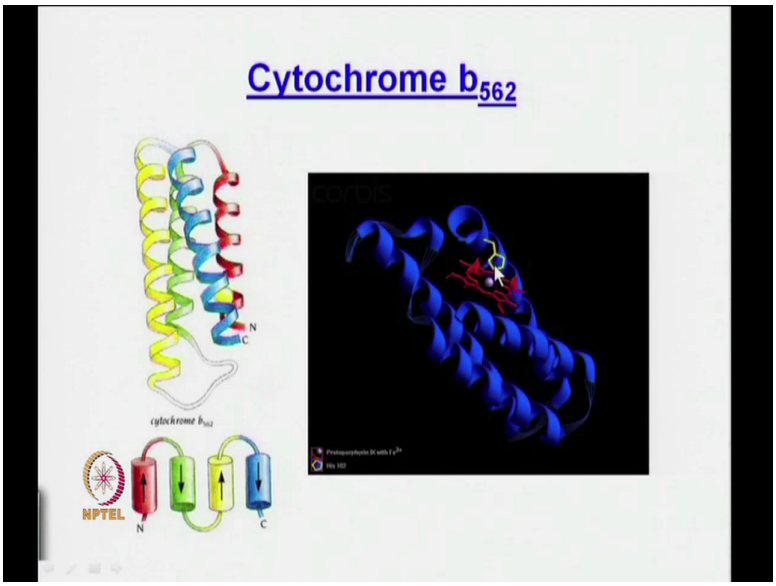

So, this is this cytochrome B562, it is an electron transfer protein as I was telling you. It is a typical 4 helix bundle ok. This is a you know a better picture of that it shows. So, not only that this cytochrome it is a heme protein; that means, it has this heme moiety, if you see this heme moiety and this is a histidine ring which actually is binding to the Fe iron out here.

So, anyway what it means is this 4 helix bundle of the cytochrome (Refer Time: 19:52) a heme moiety. Also what you see is if you look at this topological arrangement, so, this N goes like this from N it goes like this then it comes like this; that means, it is an antiparallel arrangement of your adjacent helices ok, so, antiparallel helical arrangement. So, some of the structural

features; remember we wanted to talk about the ridges into grooves model right because you have already seen what the knobs in holes model is.

So, but before going there let us look at some of the structural features so, that we can get a better feeling of this.

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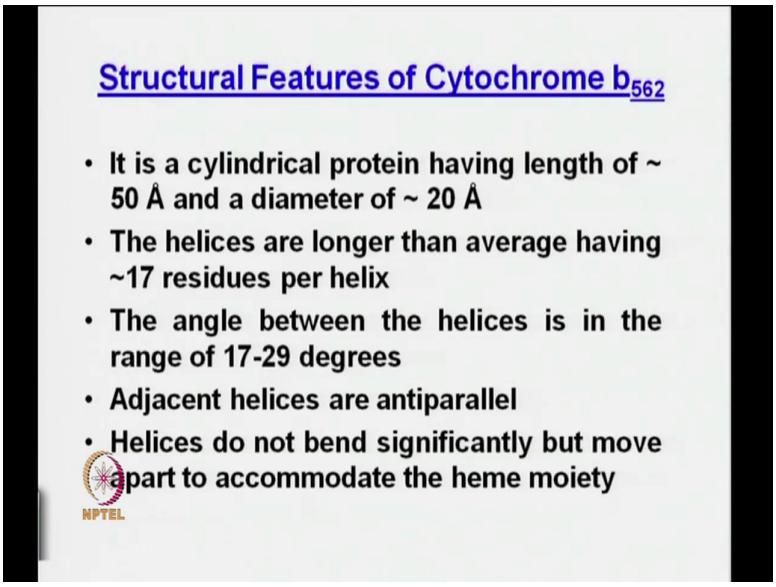

So, it is a cylindrical protein having length of about 50 angstroms and a diameter of 20 angstroms. So, this is the length and then you have the diameter. So, the helices are longer than the average ones they have about 17 residues per helix. The angle between the helices in the range of 17 to 29 degrees.

Now, this means, so, this angle is essentially again see later at what angle; that means, at what orientation are these two helices with respect to each other. The adjacent helices are

antiparallel, we saw it and the helices do not bend significantly, but move apart to accommodate the heme moiety. So, they are not bending. What essentially they doing is they just moving apart a little bit so, that the heme moiety can come in and be station out there.

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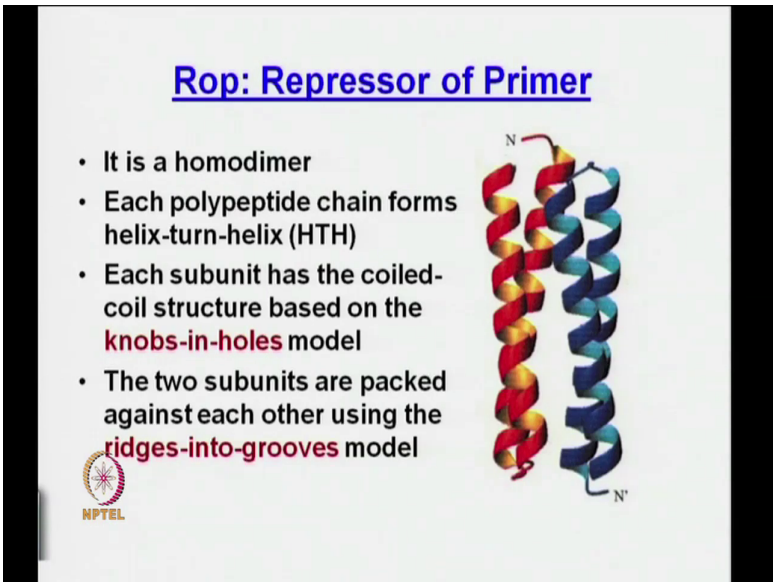

So, this is another protein, it is called the repressor of primer or the rop protein. Now it function is to bind to the RNA. So, it is a homodimer, it is a homodimer. So, homodimer means what you can see is see this see this red one and the blue one. So, these two, so, this the red one.

You can see there are two helices, one dimer, two helices another dimer and this is a homodimer; that means, the same dimmers. I mean the same sequence they just coming and packing against each other.

Each polypeptide chain forms the helix turn helix. So, helix turn helix is very simple if there is a helix then you have a turn and goes to the next helix. Now each subunit has the coiled coil structure based on the knobs in hole; holes model right that is how the helices are coiled remember that is how the helices are coiled. And the two sub units now are packed against each other using the ridges into grooves model.

So, what it means is if you just go back one point, so, these two helices or these two helices right, this is the two dimers, they then come together and form the homodimer. So, what it saying is that these two are packed against each other using the knobs into holes model. The other two are also packed against each other using the knobs in holes model.

So, the two red helices knobs in holes, the two blue helices knobs in holes among themselves. However, when these are; that means, when the red dimer and the blue dimer they are packed against each other what we get is the ridges into grooves model that is the model the use to vacuum.

You know that was with regards to this 4 helix bundle. Now, where does this ridges into grooves model really come into existence. It is becomes you know really evident right. So, before actually talking about the ridges into grooves model, let us look at this globin fold. This globin fold is very common fold, why? So, you talk about hemoglobin, myoglobin these are the ones these are the globins where this fold is typically found.

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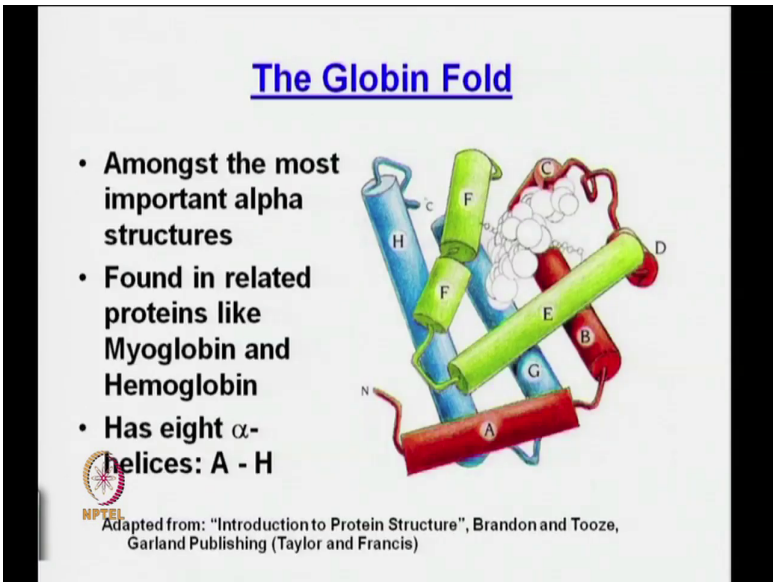

So, what you see out here is this is amongst the most important alpha structures good. Then these are found in related proteins like myoglobin, hemoglobin; I just said and there are 8 alpha helices A to H.

So, you can see A, B then if you follows C then it is D right then E, F right F sorry then G then H ok. So, this is F. So, this is F then you can have you have you see a G out here then you can see H out here. So, there are 8 alpha helices and what is this space filling diagram?

So, this is your heme moiety and it is connected it is connected using via a proximal histidine covalent proximal a proximal histidine to the iron atom to the F helix. Now, what is different here as compared to the others; that means, the ones you have just come across? You look at specially you know is first of all look at the orientations, you look at this helix P and look at

helix E. You see these are not just like this right. So, go back. So, these are not just like this. So, what the they are almost orthogonal to each other.

So, they are almost orthogonal to each other. If you can see they are almost orthogonal to each other. So, if this is B then E is coming like this ok. So, there are different arrangements. It is not regular coiled coil arrangement or the 4 helix bundle arrangement that you have just come across.

So, what are the features of the globin fold? So, this would a explain it a little more detail. The pairwise arrangement between alpha helices are different from those of the 4 helix bundles that is what I just said.

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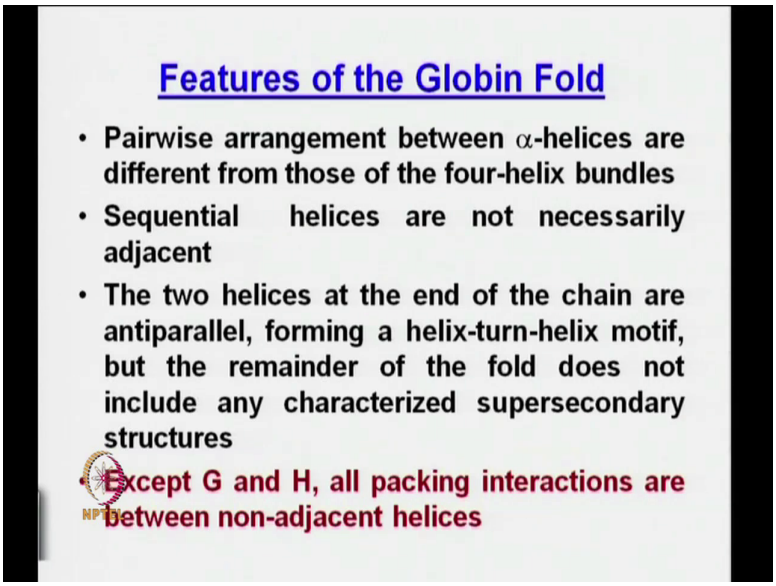

The sequential helices are not necessarily adjacent ok. So, if you go back what it means is you can see A, B then C then D, but E is more closed to B then C right. So, that means, this sequential helices are not necessarily adjacent or the way it looks is there H is very close to A rather than B being close to A.

So; that means, you will be having a sequence the amino acid sequence right you number it. This has 153 amino acids; myoglobin has 153 amino acids, but not necessary that one helix and the next adjacent helix adjacent in the amino sequence would be very closed to each other ok. Then the two helices at the end of the chain are antiparallel which is G and H forming a helix turn helix motif, but the remainder of the fold does not include any characterized or characteristics supersecondary structures. Except G and H all packing interactions are between non adjacent helices.

Now, this is really important. Just being you know adjacent in amino acid sequence does not guarantee that there would be adjacent in packing too. So, this is what how packing is different this is how packing is different from the sequential arrangement of the amino acids. It is not necessary that adjacent amino acids or adjacent helices have to be packed against each other; it is absolutely not necessary what we have just seen.

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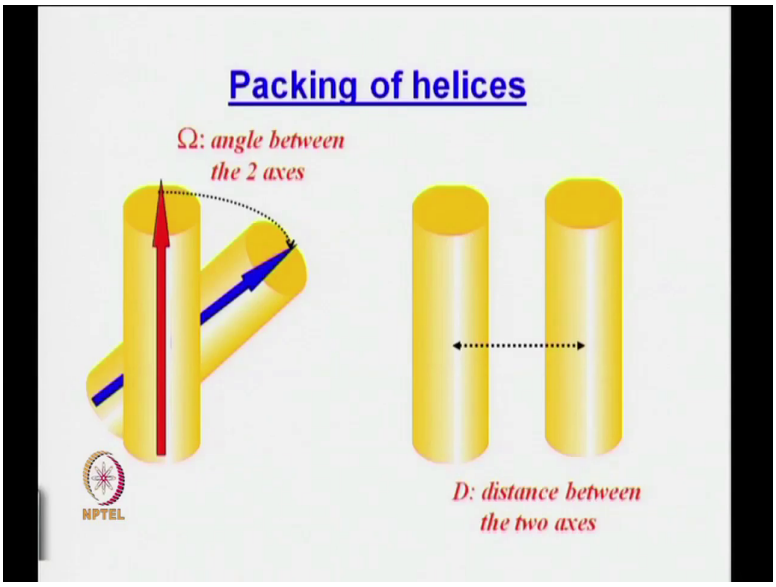

So, then as I was talking about the packing of helices, see in the packing of helices you will be having two things. One is this is one helix this is one helix this is the angle between the two helical axes and then the distance between the two helices.

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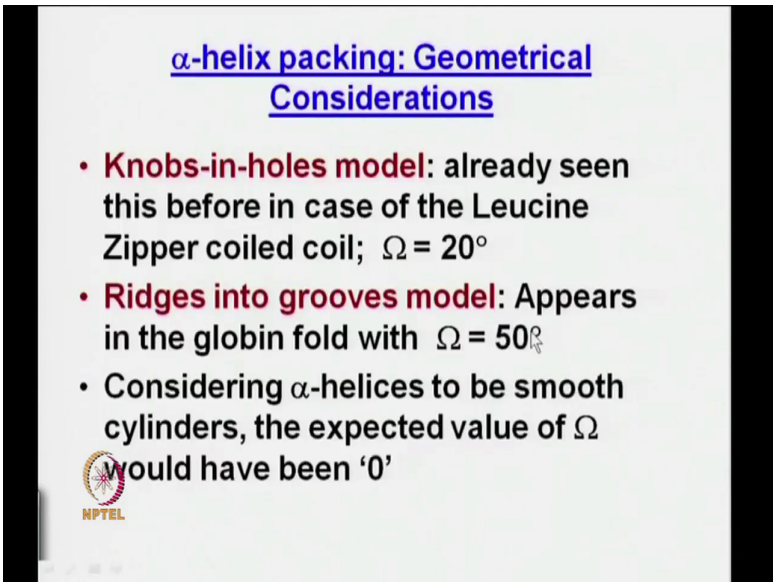

So, if you think about the different types of helical packing right then the one is the knobs in holes model. So, knobs in holes model, we have already seen this in case of the leucine zipper. Here this angle is 20 degrees. So, you can see out here. So, this is the angle between the two helices, it is about 20 degrees.

Now, for the ridges into grooves model which appears in the globin fold this angle goes to about 50 degrees. So, considering alpha helix to be smooth, now remember when we started talking about this that we never saw this when we are talking about a simple alpha helix right. Just the secondary structural element if you think about if you go back and think about the alpha helix we talked about we need not talk about these.

But what we just say these if you know based on the topology diagram alpha helices can be considered as smooth cylinders and hence we would expect that if you would be have an alpha

helix like this and another alpha helix like this side by side almost parallel they would be maximizing the interactions, but that; obviously, is not the case.

So, this is please be attention to this. This is where we start or try to see what this ridges into grooves model really means.

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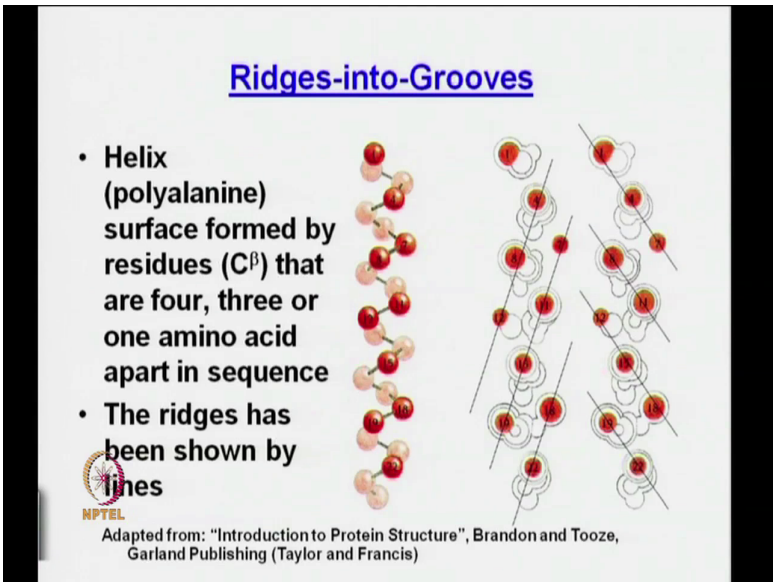

So, this is the helix, if you if you look at the first figure. So, this is the helix a polyalanine helix. The surface has the C beta residues that the carbon atom the beta position of the amino acids right. So, what you can see is this is the different amino acids. So, this first amino acid fourth amino acid and seventh eight eleventh 12 like that and the ridges has been shown by lines ok. So, please be attention.

So, for example, so, if these are the surfaces where I am looking at the C beta then look at this these lines correspond to ridges. So, these 4 8 12 are amino acids and they form one ridge. So, it is like if you have seen mountains ridges. So, it is like one is up, then you have a groove, then you have a ridge, then you have a groove. So, it is like ridge groove then ridge groove and like that.

So, that is what you seen. Ridge, then you form a groove ridge form a groove like that. So that means, here I have 4 8 12 these are the ridges. So, in between you will be having the grooves again you have 7 11 13 sorry 15 19 these are again ridge. In between you have a groove here. Again you have 18 22 right. These form again a ridge and between this line and this line you have a groove between this line and this line you have a groove.

So, the lines essentially your ridges and between the two ridges you will be having grooves right. It is like your valleys; same here, same here. If you look at the last figure you can see it is 1 4 7 8 11 12 15 18 and so on. Now, what is the difference though between these two? As I said if you look at these amino acid this 4 8 12, so, there are spaced by 4 amino acids like; that means, i i plus 4; same here 7 11 15; 7 plus 4 11; 11 plus 4 15; 15 plus 4 is 19.

The other hand the last one you can see these amino acid is which are on the ridge they are spaced out by 3 amino acids. Say for example, 1 4 7 spacing of 3; 8 11 again a spacing of 3 amino acids right.

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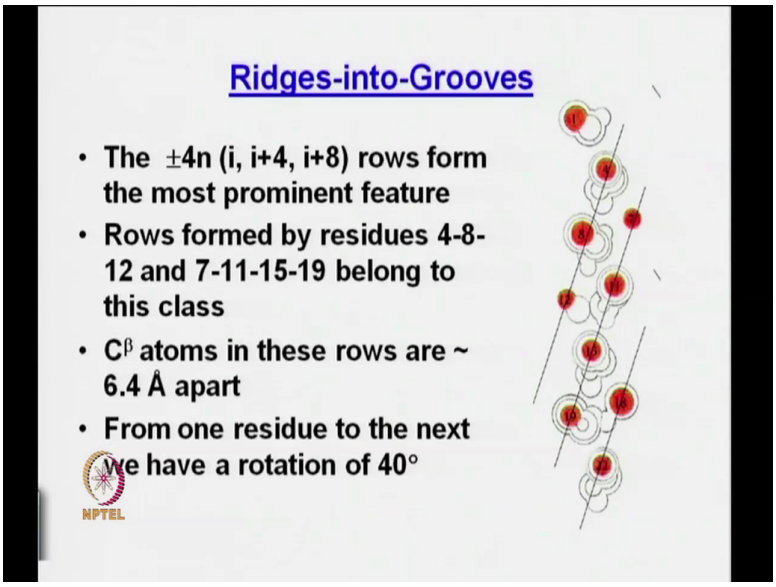

Now, this is important. What it tells you is this. If you take this you know the second figure where we have 4 amino acids; that means, you know there is a difference of 4 between the amino acids on the ridge. So, this is what we have. This is referred to as a plus minus 4n arrangement. So, plus minus means that if this is if we consider 8 then minus 4 would give me 4 plus 4 would give me 12.

So, this plus minus 4n arrangement, i i plus 4 i plus 8 i minus 4 rows form the most prominent feature; that means, this is the most important feature or the most common feature in the ridges into grooves model.

Then rows formed by residues 4 8 if you see 4 8 12 and 7 11 15 19 they belong to this class as i just said, so, 4 8 12 7 11 15 and 19. Then the C beta atoms in these rows are about 6.4

angstroms apart. So, these between these two there are the distance about 6.4 angstroms and from one residue to the next you go to have a rotation of about 40 degrees ok.

Now, please you know try to put this in the context of the rotation, we talked about for a screw access or the screw symmetry that a helix possesses. Now, if you got into ridges into grooves model now for the second case where now we have a spacing of 3 amino acids. So, this is what you get. This is plus minus 3n as it is i i plus 3 i plus 6.

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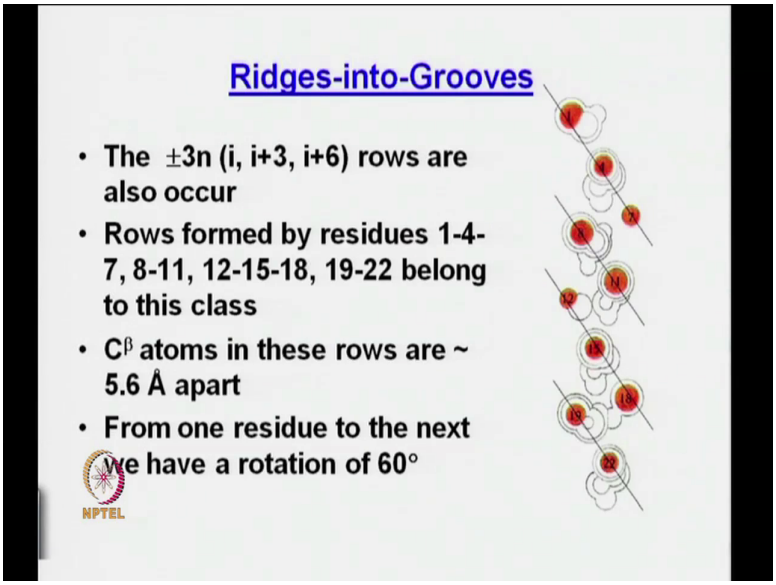

The rows formed by the residues, what is this; 1 4 7, then 8 11, 12 15 18, 19 22 they belong to this class. The C beta atoms in these rows are about 5.6 angstroms apart. So, you see they are closer than the previous one.

And from one residue to the next we have a rotation of 60 degrees; that means, you have to rotate a little bit more right. So, they are 3 amino acids they are they have to be rotate a little bit more. Now, so, the ridges formed a residues of one helix pack or intercalate into the groups of a second helix; obviously, right.

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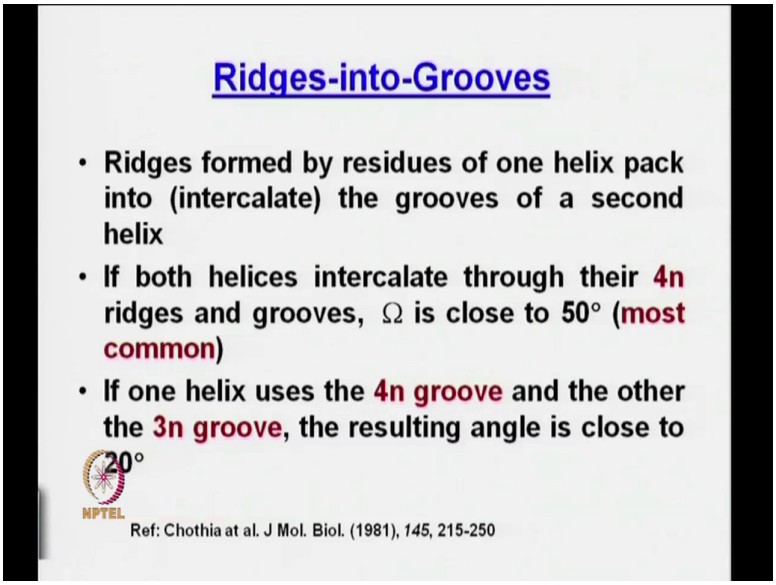

You will be having ridge of one. The ridge of another cannot fall on the ridge of another. So, it has to fall into the groove where you have the weakened space. If both helices intercalate through their 4n ridges and grooves then this angle is close to 50 degrees ok. Remember this angle we talked about this is the most common one. If one helix uses the 4n groove and the other helix this is the 3n groove then it is actually it should not be a groove, it should be a ridge.

There is a typo out here. This groove should be you know that is ridge, the resulting angle is close to 20 degrees ; that means, this is the groove and this is the ridge. So, you know you cannot have packing between two grooves; obviously. So, you can see the depending upon this 4n 4 n packing and 4n 3 n packing you give a rise to a different angles; one is 50 degrees the other is 20 degrees.

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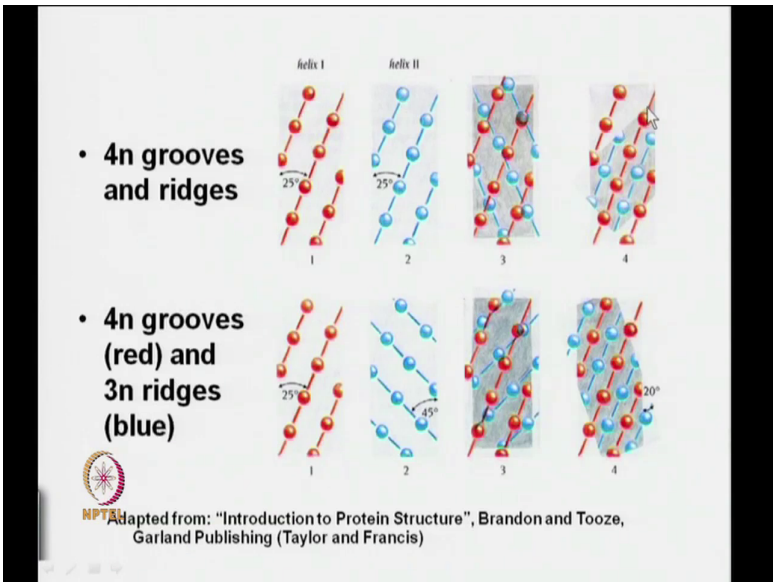

So, this is what I mean. So, this is the top row of figures. This is a 4n grooves and ridges. So, you can see. Here this angle is 25 here this angle is 25. Now if you go to the last one when we try to; you know actually what happens is if you try to take this and try to put this interface along with this helix then you have to take it out of the paper about 180 degrees which is what you do out here and then what you do is you flip it by an angle.

So, here, so, this angle out here is about you know this angle between these two this you know this shaded one and this red one is about 50 degrees ok, this angle is of 50 degrees. However, if you go to the other one which is a bottom one here is the 4n grooves are the red and the 3n ridges remember, I talked about the ridges. So, this is a groove and this is the ridges.

So, what we happen now is you can see the difference between this guy and this guy is these two helices were oriented in the same direction. But these two were oriented in the opposite direction not only that this is 25 and this angle is 45. So, you can see finally, when they are aligned the angle between them is 20 degrees is opposed to this angle being 50 degrees ok.

So, this means they are depending upon your 4n 4n or 4n 3n your orientation and hence the angle between these helices would be different ok. So, again coming back to the case of myoglobin, this is these are your cylinders this one is your heme this black one is your heme right this black line. You can see how this B and E are orient to with respect to each other.

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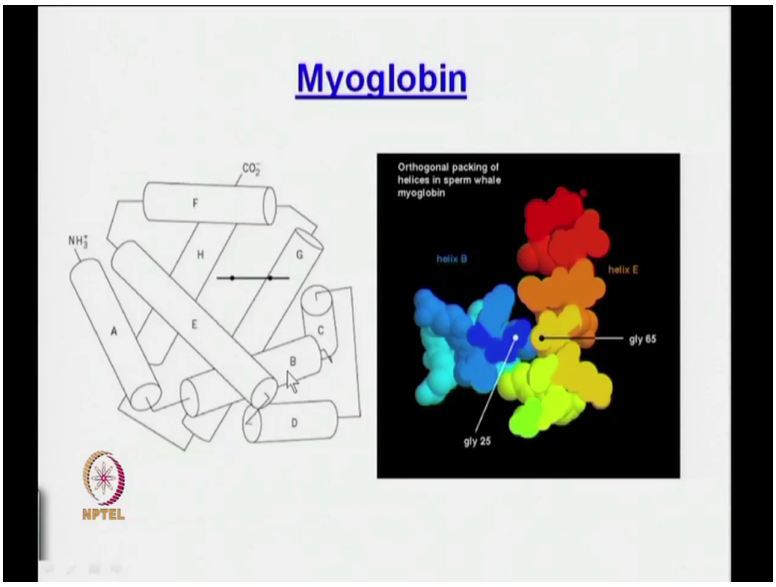

The same has been shown here ok. So, this is your helix B and this is your helix E and you can see almost having an orthogonal packing. This is in case of sperm whale myoglobin ok. So, this is what I wanted to tell you about alpha structures. Now, please keep in mind that there are other variations of this. There are other proteins which have you know many proteins which have different variations of these structures.

But, this is the principle that the packing is based on, obviously, one is your knobs in holes model right and the other one is ridges into grooves model. That means, one protein give as the grooves and the ridges of the; that means, the one helix as the grooves and the ridges of the other helix come and you know fall into that groove ok.

So, next talk would be beta structures. Here we have looked at all alpha helical structures. So, now, what about the beta structures? So, let us look at this. So, this is also very interesting.

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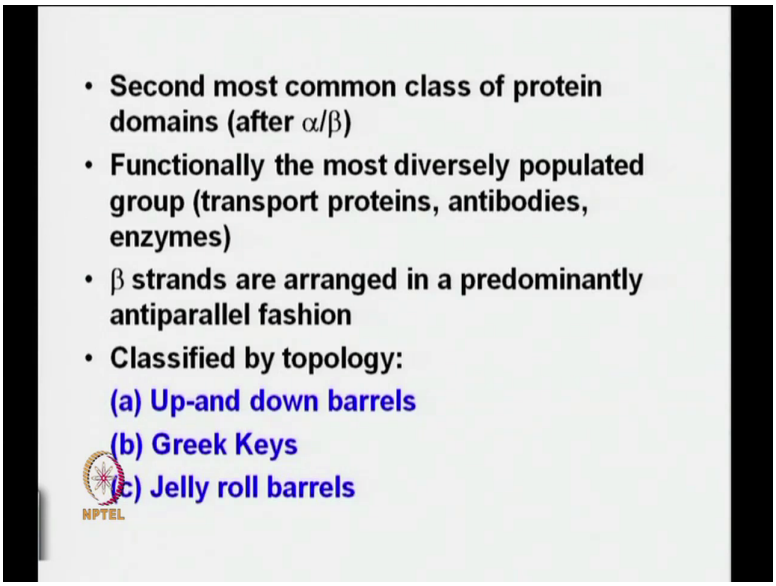

This is the second most common class of protein domains see the second most common class of protein domains. What was the first one? The alpha beta which I said you going to look up yourself, but I will be giving you questions though in the exam, please keep that in mind.

Now, this is the functionally the most diversely populated group. So, you see transport proteins antibodies enzymes and so, on. The beta strands are arranged in a predominantly antiparallel fashion.

So, whatever we discuss you will see it is an antiparallel arrangement. They are classified by the topology. So, what I mean by that? So, what are the different classifications? One is up

and down barrels. If you remember for a beta sheet, we were actually use using up and down arrows right. So, that is what I mean.

So, using those arrows we can see a what the topology is; what is the up and down barrels. The other one is your Greek keys and the other one jelly roll barrels ok. So, these are the 3 which we are going to look at in some details in the subsequent slides.

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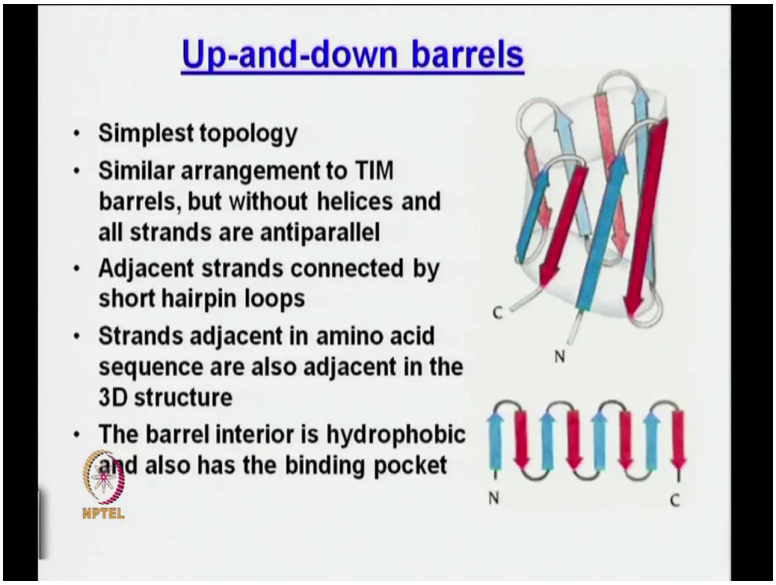

So, there we are concentrate on the up and down barrels. So, this is the one which is having the simplest topology.

Now, similar arrangement to TIM barrels, I have not discuss this, but without the helices and all strands are antiparallel. TIM barrel you can understand then. It says without the helices; that means, the TIM barrels would be a mixture of helices I mean alpha helix and beta.

Adjacent strands are connected by short hairpin loops. We will take this one by one. And then strands adjacent in amino acid sequence are also adjacent in the 3D structure.

The interior of the barrel is hydrophobic and also has the binding pocket. What is going to bind there we will come to it later. So, here what you see is ok, now let us let us talk about this. Adjacent strands connected by short hairpin loops right. So, you can look at this strands. So, suppose this is a N terminus right this is N terminus this is C terminus where it ending.

Now, you can look at these the beta strands, beta strands they are being monk by these arrows. So, for when you go here then you have this hairpin loop it comes and connects to the next strand. The next strand is connect to the next strand and so, on under you come to the C terminus; that means, these adjacent strands are connected by the short hairpin loops; almost these hairpin loops right. So, that is how the arrangement goes.

Now, you can see the strands adjacent in the amino acid sequence are also adjacent in the 3D structure. You can see what happens. So, these are adjacent in the amino acid sequence and these are also adjacent in the 3D structure. So, they are also the hairpin loops they are also adjacent close to each other in the 3D structure that is how they pack.

Now, because of this packing, now before I go there; if you take this and you will look at this. So, this is your topology diagram. So, the one you have at the bottom is a topology diagram. Here it does not actually show in the structure, it just show in the connectivity using the arrows.

So, this is what you see right. This N you start this is a adjacent one ok, if this hairpin loop then arrow is this direction then the arrow goes this direction and this is an antiparallel arrangement and each and every adjacent beta is strand is connected by this hairpin loop.

Now, the way this is arranged. What is what I will happen is if you look at this in the middle right; that means, if you look at in the middle you can see the weight is arranged, it is it is kind

of like this. So, that means, there is a pocket inside and hence this interior is quite hydrophobic.

So, this interior is quite hydrophobic and that is why it is called it is hydrophobic and also has the binding pocket. So, binding what? It must be bind to something right functionally important possibly. So, that is what you are going to look at in the next slide.

But before going to the next slide just understand up and down barrels. What it means is. So, you know see this is one is up this one is down this one is up this one is down this one is up this one is down and so on, essentially that is what it means; up and down up and down antiparallel arrangement

Adjacent strands also adjacent to each other in the structure as they are in the amino acid sequence. A typical example of this is this protein, the retinol binding protein RBP we call it ok.

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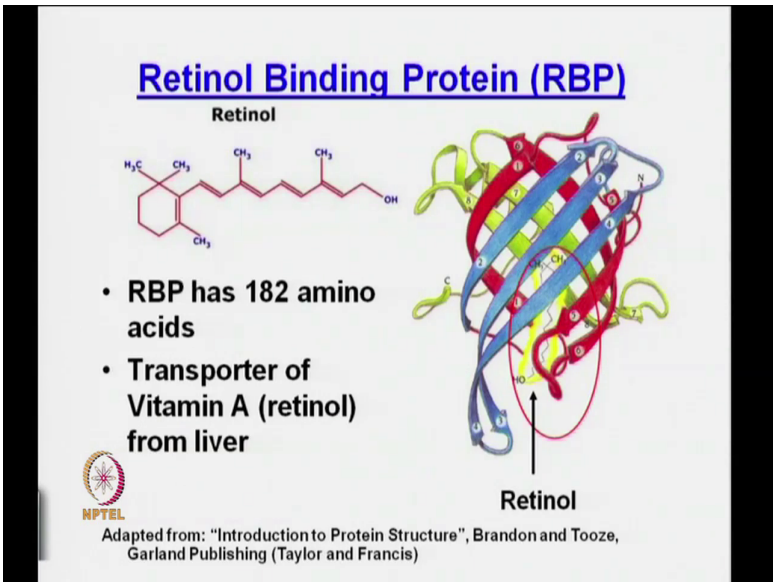

So, this is retinol ok. You see this protein you see this protein right the retinol binding protein. You can see this is 1 2 3 4 5 6 7 and 8 and then you will see this is the C terminus right. Now, what will happen is in the middle you see this yellowish color and you have seen this chemical structure this chemical structure is of that retinol. So, this is retinol.

So, this rest retinol is bound in the interior hydrophobic pocket of this up and down beta barrel that is why this you use this red you know circle portion right ok. So, the retinol what it does is I said that the interior is hydrophobic and it is the binding proteins the retinol goes and binds there and it is the arrow shows this is retinol.

Now, the protein has a 182 amino acids and it is a transporter of Vitamin A; obviously, because retinol Vitamin A which is bound which is getting bound to the hydrophobic pocket. It is being transported by this protein to tissues where it is needed from liver.

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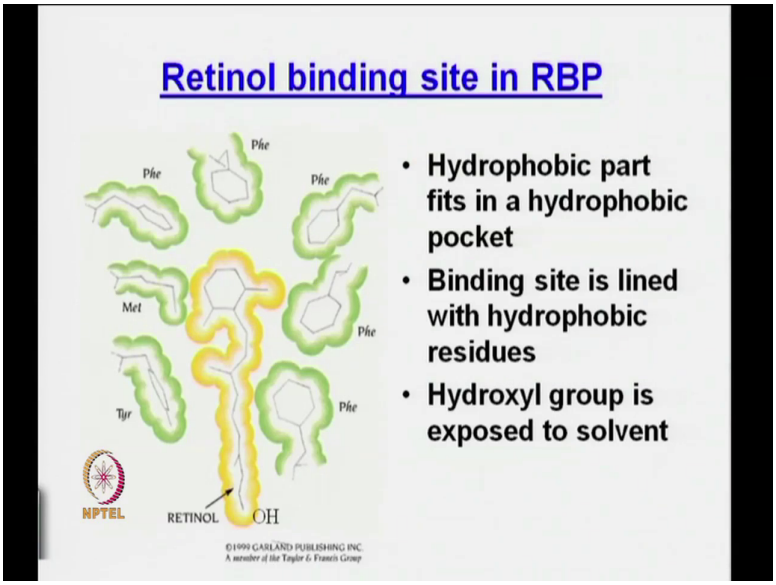

Now, looking a little bit like zooming into the zooming into the binding site a little bit more you can see this is the retinol right. See in the previous slide if you notice the retinol ends with an OH group. The other one is are primarily hydrophobic, the same thing happens here, you can see. So, these are the interior. So, you can these green ones are different amino acids which line the interior; hydrophobic core of your retinol binding protein.

So; obviously, this is hydrophobic. So, it would prefer hydrophobic what; hydrophobic groups right hydrophobic atoms out there. Hence the full almost the full electronic molecule is out

there inside the hydrophobic core except this OH which is polar and hence it is coming out getting exposed to a more polar environment.

So, this means that is a very good fit very good fit in terms of hydrophobicity in terms of this interactions between the hydrophobic retinol and the hydrophobic amino acids which aligning which aligning your core.

So, going forward the hydrophobic part fits in a hydrophobic pocket. Hydrophobic part means hydrophobic part of retinol. Now the binding site is lined with hydrophobic residues as we have seen see phenylalanine phenylalanine phenylalanine phenylalanine again phenylalanine methionine tyrosine; so, primarily hydrophobic primarily by phenylalanine residues.

Now, the hydroxyl group is exposed to a solvent as you can said, right. So, this; that means, that there is a very good arrangement or binding of this retinol inside the binding pocket stabilized by the surrounding hydrophobic amino acids or interactions with these hydrophobic amino acids

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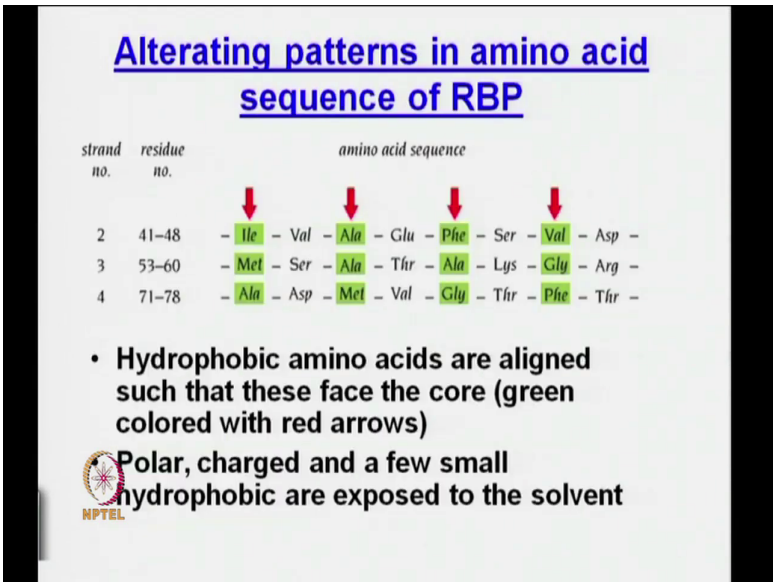

So, what I mean is then if you look at the alternating you know it should be alternating, alternating patterns in amino acid sequence of retinol binding protein. What you see is out here. So, this is a strand number this is a residue number then this is amino acid sequence; 2 3 4 this is strands 2 3 and 4.

You are talking about the amino acid 41 to 48, 53 to 60, 71 to 78. What you see is isoleucine alanine phenylalanine valine these are the hydrophobic groups. These are in between you have these hydrophilic (Refer Time: 43:08) glutamate aspartate and so on.

So, the hydrophobic amino acids are aligned such that these face the core, so; that means, they are facing the core, the green colored with the red arrows these are the hydrophobic amino

acids. The polar charged and a few small hydrophobic are the ones which are exposed to the solvent ok.

So, it is kind of alternate: one is hydrophobic goes inside the core the other one is staying outside, the other one goes inside the next one staying outside that is why it is called alternating sequence.

So, there was one about the retinol binding protein you know and one thing you would have also notice is that if you know when we are talking about this topology diagram, specially the one where we will just showing the connectivities it look a lot more simple, it look a lot more simple then the actual one where you had all those you know strands coming in and you are trying to look at the structure.

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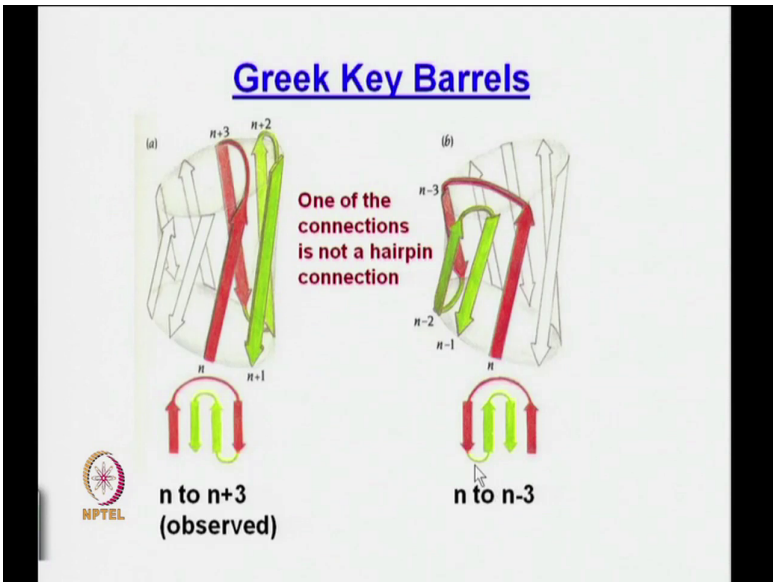

Then the next one is referred to as the Greek key barrel. Now, the Greek key motif is one motif which you know we have talked about before. Remember when we talking about the supersecondary structures and all, you know the beta alpha beta and all we are talked about the Greek key motif. Now, this is where you see it again.

So, it is essentially beta. Now, what you see out here is one of the connections is not a hairpin connection. Remember a hairpin connection is one where so, you take this and you take this the two adjacent strands are connected by hairpin loop. But, you can only get Greek key barrel where you do not have a such a hairpin connection right that is what it says one of the connection is not a hairpin your typical hairpin connection.

So, this what it means is if you look at this one if you look at this topology and if you look at these two strands this is n and this is n plus 3; that means, you have a linker. You have a linker between n and n plus 3 this is not a short hairpin loop. Why? Because, this is a not to adjacent strands; one is n and the other one is n plus 3 and if you look at the so, this is the one which is normally observed and if you look at the right one, so, this is n and n minus 3.

So, this is n and this is n minus 3 ok, but both of these tell you the same thing. That means, this is not a hairpin loop because it is not happening, it is not linking to adjacent strands. It is liking 2 strands which are 3 strands you know 2 strands which are 3. You know 3 strands apart like n n minus 3 or n n plus 3. But, in this case just to since we are already on this tell you and it says this is the one which is mostly observed right the n to n plus 3 is mostly observed the n to n minus 3 is almost not observed.

So, where is it found? It is found in a very important protein call gamma crystalline or crystallines as a matter of fact. Now, gamma crystalline or crystalline what it does is so, crystallines if you know if you have heard about it ,they are very important for eyes right. So, you know for retina for the transparency and everything for us to see properly.

Because what happens is you will see you must have heard or you must have seen your relatives or may be someone else that when people get aged they have problems with their eyes.

So, they undergo this operation known as cataract operation because; what happens is in that case so, they have a blurred vision they cannot see properly. You know why they cannot see properly? What happens is this crystallines they start aggregating, you know with age; something it is not a happen properly the body does not function you know to it is optimum.

So, the proteins start aggregating and the protein start aggregate, so, they become insoluble and then they start precipitating out there itself. Once it precipitate out there itself if it they are not getting removed by anything which they are not typically then because you are precipitating; that means, they no longer soluble they form an invisible screen for you or an opaque screen for you and hence your vision gets blurred. So, this crystalline has this Greek key motif as a part of it; that means it is made up of this Greek key motif.

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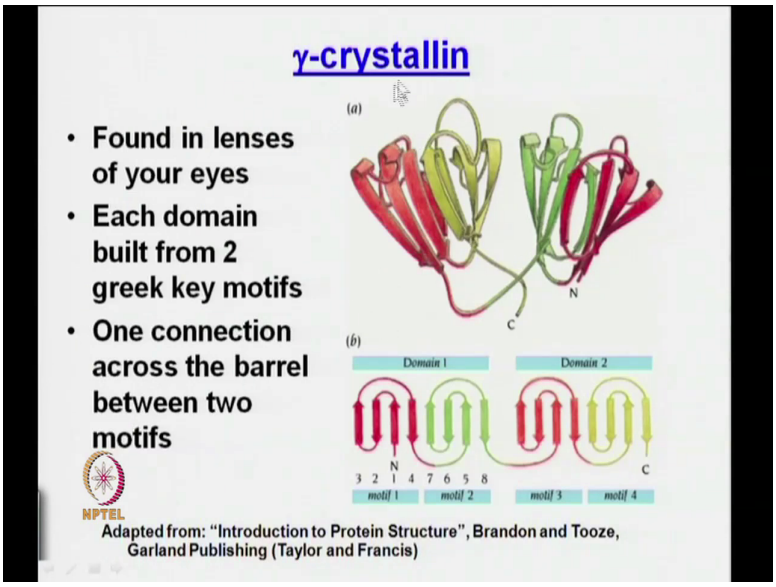

So, it says it is found in lenses of your eyes right, you can understand the importance of it all of us have this. Each domain is built up from 2 Greek key motifs ok. So, this is one Greek key motif, this is the other Greek key motif. One connection is across the barrel between the two motifs. So, there is one connection because you if you have this 2 motifs, you have the one connection which connects these two motifs. You can see this is a connection from here to this here.

So, this is one Greek key motif this is another Greek key motif and you can see right and you can see this is the connection. So, this is domain 1. So, this is domain 1, this is domain 2, the right hand side is domain 2, again this is a topology diagram.

So, what you can see in the topology diagram is let say what I have in the next slide. Well, what you can see in the topology diagrams I can I have showed that you is so, just look at this

here you can see this 8 beta strand. This 8 beta strand is linked to one strand in domain 2. So, this is the linker we are talking about.

So, again gamma crystalline is made up made up of two Greek key motifs. One is forming domain 1 and the other one is forming domain 2. What we will do in the next slide is we look at one of these domains in some detail at least the connectivity.

So, you can understand you can appreciate this topology, why this topology diagram is so, important, why people talk about this topology all the time in when you talk about protein structure or structural hierarchy and all.

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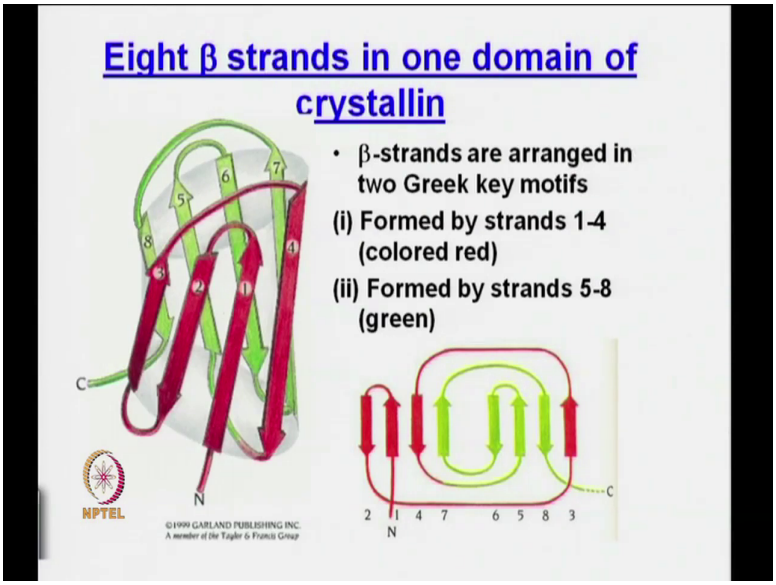

So, moving on let us look at this one domain this is one domain remember ok. So, I am just taking one domain, it does not matter which one you take whether this one or this one. I am

just taking one domain right. So, now, look at this. What I have is you can look at the strands. You have 1, strand 2, strand 3 then strand 4 then you have strand 5, 6, 7, 8; see how different this is from what, the up and down barrel. What do we see in the up and down barrel?

We saw in the up and down barrel was that the each strands or the strands which are adjacent in the amino acids sequence or also adjacent in the structure. Here it is not so, here it is 3 that is 4. Adjacent of 3 we have 8 ok; now 7 adjacent was 7; obviously, we have 6, but then 5, 6 7, 7 and 8 these are far apart. Are not they?

So, the beta you know strands are arranged in 2 Greek key motifs right 2 Greek key motifs these are 2 Greek key motifs, you can see. This is one Greek key motif and the green one is the other green key motif.

So, that is what it says. It is formed by strands 1 to 4 colored in red. So, remember we are looking at only one domain, 2 Greek key motifs, one domain; this is one domain. It is colored red; it is made up of strands 1 to 4. Then the other domain forms by the green ones strands 5 to 8.

Now, let us think about the topology right, how do we figure out the topology. Two ways, you have to figure out the topology. One is you are going to figure out you are going to figure out what are the adjacent strands. Once you have, so, that is what you focus on first. Figure out the adjacent strands start drawing the arrows. Once you have the adjacent strands then start putting in the connectivities. So, let see whether we can have it here.

So, suppose say I start with 8 ok. So, let us this is 8. If this is 8 which is a part of the green one then what is the one adjacent do 8? Obviously, this is 3, you can see out here sorry. Go forward. You can see this 3; hence 3 it is an adjacent to 8; got. Now; obviously, 8 is the last one. So, this is the C terminus one. Now this is 3. Now adjacent to 3 what do we have? We have to see this is where we have 2 right. So, this where we have 2 and this 3 is adjacent to 2.

So, this is the loop which is connecting 2 to 3. Now adjacent 2; obviously, is 1. So, this is 1, the 1 is N terminus. So, if you go from 1. So, this is 2, the 3 goes out there right then;

obviously, this is 4. So, if this is 1 adjacent this is 2. I have written 2 here; adjacent rates again 4, I have written 4 here right then 4. What do we have a adjacent of 4? We have a adjacent of 4 we have 7. So, see 4 is kind of in between 1 and 7 that is what we have 4 between 1 and 7 right.

Then if you have 7 then what we have is this is 7 then this is 6, right. So, this is 6, 6 and 5 you have this hairpin loop 6 and 5, you have this hairpin loop. Then look at this. 3 and 7, we have this connectivity. Remember we are talking about n and n plus 3. So, essentially this I am sorry. Let us go back on that sorry. So, this is 8, this is 7; you can see this is 7, this is 8 that is the connectivity you are looking at ok.

Then this is 5 this is 6 this is 5 this is 6 right. What else? Then we have 4 we have 3 that is we have already shown. So, here 4 you can see it is connected to 3 then this 3 is a internally connected to that is what we have. So, as I said if you start putting in the adjacent strands and start putting in the connectivities you can see how simple this thing becomes. You start at the N terminus with the 1 strand 1 and then we end at the C terminus with strand 8.

But, again adjacent strands are non-necessarily the ones where the amino acids are also adjacent in sequence. So, this is how a topology diagram kind of helps you to realize what the actual structure is without the connectivities or without the actual orientations. Again please keep in mind that here the arrangement is antiparallel in nature. The last one we are going to talk about in terms of this tertiary structure the jelly roll barrels.

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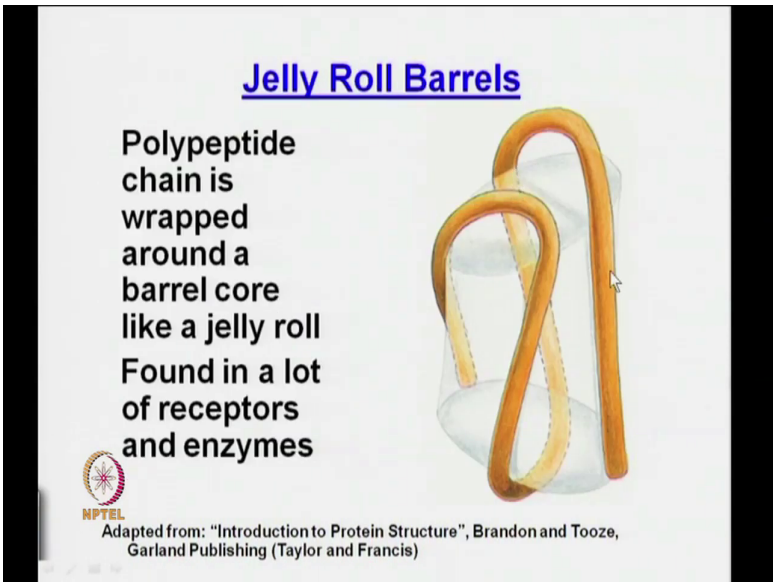

So, the jelly roll barrels, they are it is a polypeptide chain is wrapped around a barrel core like a jelly roll. So, you can see if this is you know this whitish one is a barrel and you can see it is kind of wrapping around like a jelly roll. It is found in a lot of receptors and enzymes.

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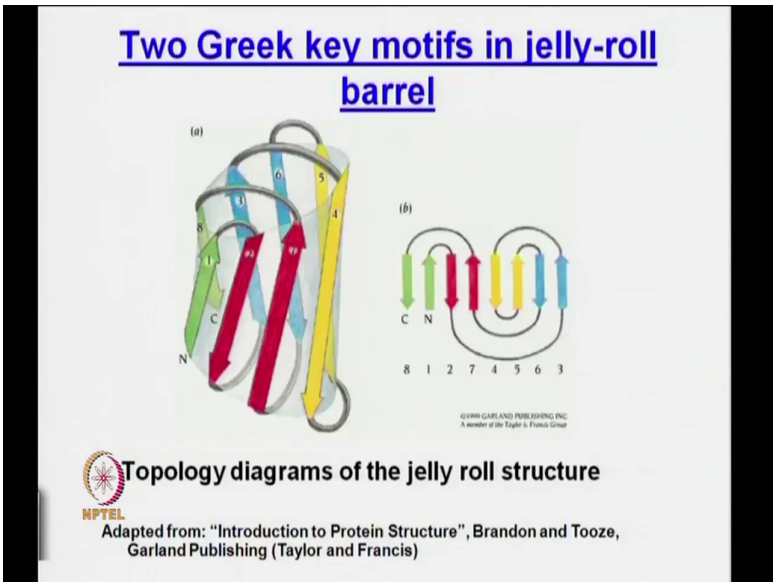

Now here also 2 Greek key motifs in the jelly roll barrel. So, you have 2 Greek key motifs. So, this is one motif. So, this is the other motif. So, this is the topology diagram of the jelly roll structure ok.

Now, I will come to this again, so, but just realize out here. What happens is it is kind of very similar to what we just did you know before that Greek key motif. You see 1, 2 and then you have 3 out here right then there is 4 5 6; there is 7 and there is 8 right. Again they are not adjacent; that means, the once which are adjacent in sequence are not necessarily adjacent to each other right.

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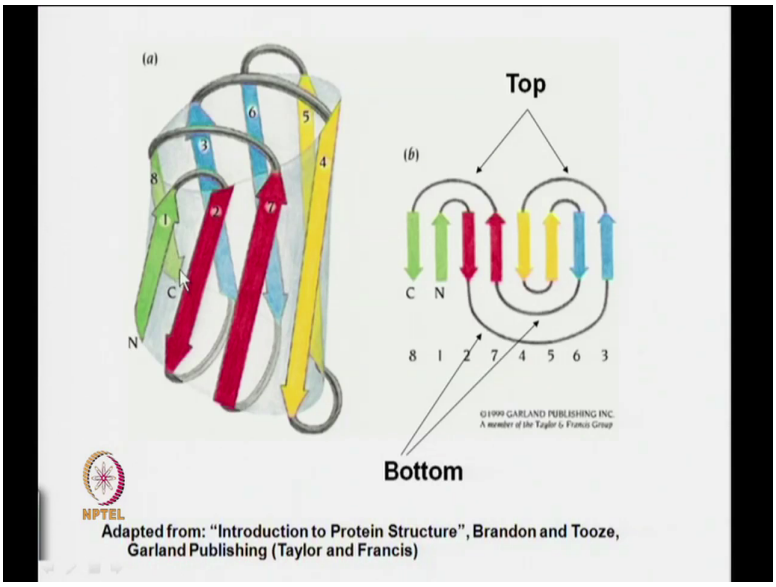

Now, there is a very interesting things out here. If you look at this one, if you look at this one and kind of zoom into it, spend little more time on it see what you have right ok. Remember if you look at this there are two wrappings.

So, this one wraps on top this one also wraps on top then this one also comes down; there is one the bottom two. Now look at this, do we have that in this topology diagram? So, you see 7 to 8 this runs across the barrel this is on top. Again 4 to 3; 3 to 4 this runs across the barrel this is on the top.

So, you can see this is what we have. This is 8, this is 7 this is running across the barrel just transverse across the barrel then the next one was 3. So, this is what we have 3 and this is 4.

You can see this is the top ones as I said this is the top ones which you running across the barrel. Then we also have 2 which are down.

So, these are the two ones which are looking at. So, this one if you follow my arrow it is this one and also this one. This one is between 2 and 3 and this one is between 7 and 6. So, look at 7 and 6. So, this is 7 and this is 6, you can see this bottom one and the next one is between 2 and 3.

So, it is very simple. So, you understand, you have just taken this. You can start from any you know any strand. You say you can start from 2, you can then after that you can put in this arrows up and down depending upon this antiparallel arrangement.

After you have done this antiparallel arrangement you just put in the connectivities right become so, simple, seemingly complicated structure. When you look at the topology diagram, it just looks a lot more simple without all the connectivities, without all the space filling models without you know the orientation the amino acids and everything.

So, as you see in the bottom we have 2 as we said top you have 2 and then we have these 2 these hairpins here and here ok. So, that is you know we have to I have you know told to you or talked to you about topology before, but not to this extent.

Here you can understand that in this beta you know sheet packing if this topology is really becoming handy, is not it; that means, using the topology you can you are being able to really simplifying the overall 3 dimensional structure.

Please try this yourself. I have shown this to you, but you know I guess what you should do is you should take this only and then try a figure out how I can connect these. So, if you compare of a you know compare all these beta barrels, it is B it should be beta B should be written as beta. So, you can see this up and down right.

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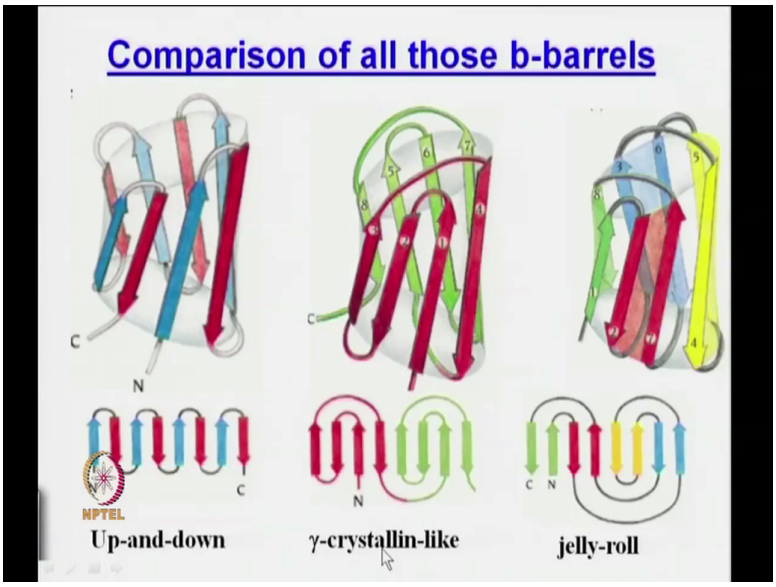

This is the gamma crystalline like and this is the jelly roll like. So, you can see in the you know in the in the gamma crystalline this is where you have these the top two. In the jelly roll you can see this is the top two and then you also have a bottom two. In this case you have one bottom also out here. So, this you know kind of brings us to the end of this class and to the end of the discussion of the tertiary structure.

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So, now what I needed to do is because I were always spend a lot of time on this is to look at these alpha beta mixed domains, look up this alpha beta mixed domains by yourself and you also look a coordinative structure.

So, coordinative structure is you know you guys are know this. I mean I do not have to go into that much details right, but I can just tell you that if you have different domains like different tertiary structures like you know considering hemoglobin, each hemoglobin is a tetramer.

So, each monomer is having its own tertiary structure and when these different monomers they combined they form this tetramer. This is a coordinative structure. So, essentially multi-domain proteins right where we have different domains and all these they are packed

giving rise to these coordinate structure ok. So, I guess that is what I will end. Please do look up these structures these are very fascinating.

You take up any you know look up this protein data bank; take up these beta structures or alpha structures of the mixed the one we have to look up. Try to see whether you can you relate you know their orientations specially for helix packing with what we discussed, the knobs into holes or the knobs in holes or the ridges into grooves. And, for beta sheet if you are looking at those structures then you think about the topology. That is why the topology makes it so simple that is why people are talking about the topology all the time ok.

So, we will meet next class.

Thanks.