Bio-Physical Chemistry
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Lecture - 04
Secondary Structure of Proteins
(Contd.)

Welcome to today's class. We will carry on with our discussion on Secondary Structure. So, last time, we were mainly talking about alpha helixes.

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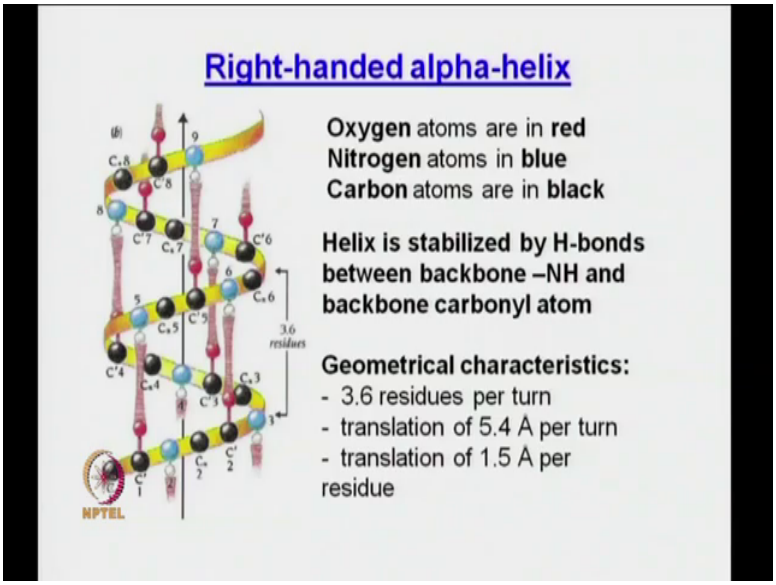

So, you know continuing with that just brief recap of what we looked at last class. So, this was you know the right handed alpha helix, we are talking about then we focus on hydrogen

bonds. Lot of symmetry operations for example, here the oxygen atoms are in red color nitrogen atoms in blue carbon atoms are in black.

And, then we have the helix which is stabilized by these intra helical hydrogen bonds, and we have this geometrical considerations and characteristics remember. We talked about this coordinates you know, we when derive these symmetry transformations ok.

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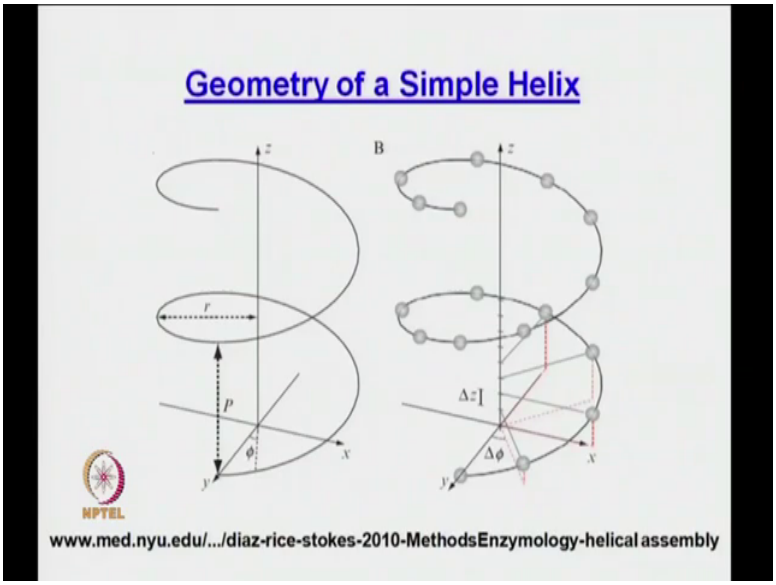

So, this was the geometry of simple helix that I was talking to I was referring to right now. Based on this, we look at those symmetry issues.

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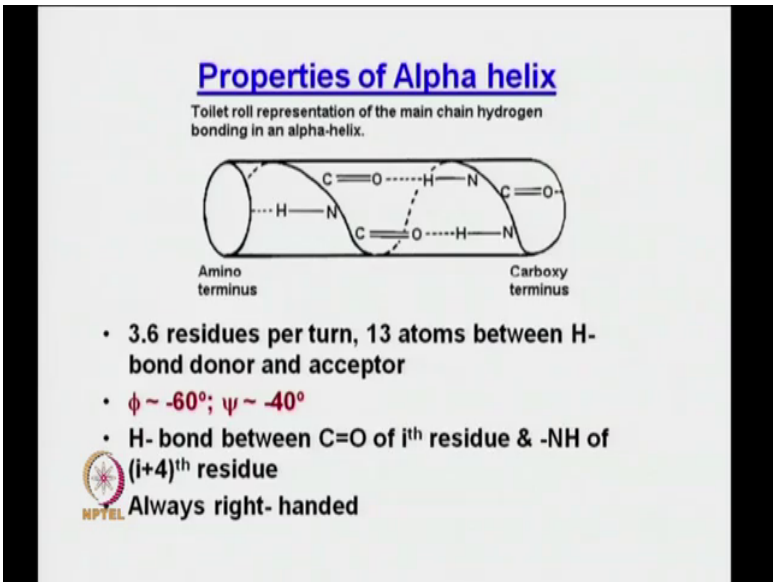

And you know some properties we looked at it always like a cylindrical appearance. And it has these properties like 3.6 residues per turn. This is the phi and psi values remember, these belong to the Ramachandran plot. Then you have hydrogen bond between the ith and i plus 4th residue and it is mostly always means mostly right hand.

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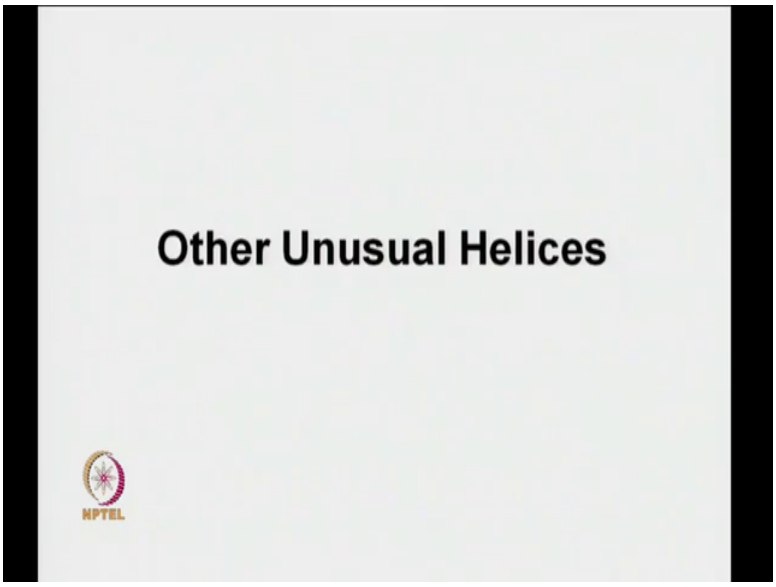

Now, the last you know slide where we stopped had this thing, you know this thing written out there which is the another other unusual helices. That means, we have talked about these right handed helix that is a most common one. What are the other helices possible?

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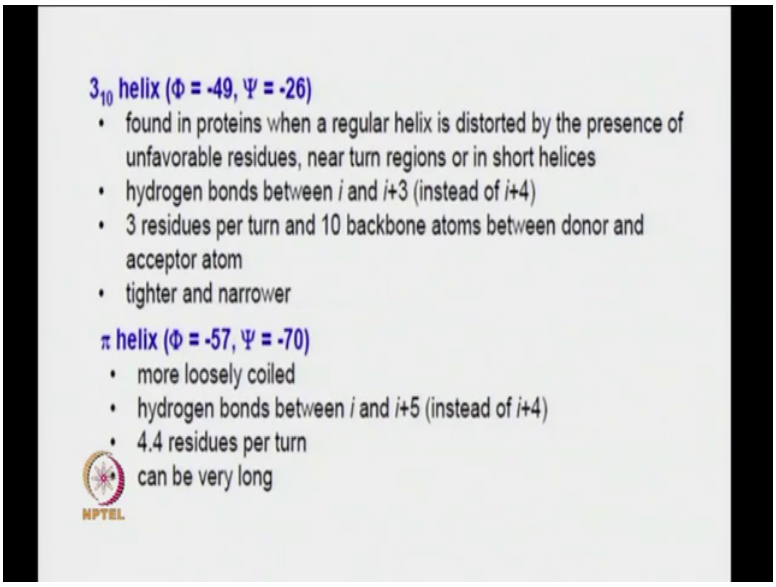

So, one is this is referred to as a 3 10th of helix it is you can see the corresponding phi and psi values. It is found in proteins when a regular helix is distorted by the presence of unfavorable residues, near turn regions or in short helices. The hydrogen bonds they are found between the i and i plus 3 residue instead of i plus 4 as in a regular alpha helix.

Now, the actual residues per turn and 10 backbone atoms between donor and acceptor atoms right. Try to refer to what we had in case of alpha helices, they were 3.6 residues per turn and then it is tighter and narrow than normal or regular alpha helix. The other one is referred to as a pi helix.

So, you can look at the phi and psi angles 1 phi is minus 57 psi is minus 70, helix more loosely coiled the hydrogen bonds occurred between i and i plus 5 at residue. So, i and i plus 5; it is

not i and i plus 4 neither i and i plus 3, which we just saw for the 3 10th of a helix ok. Then this is 4.4 residues per term and this can be very long.

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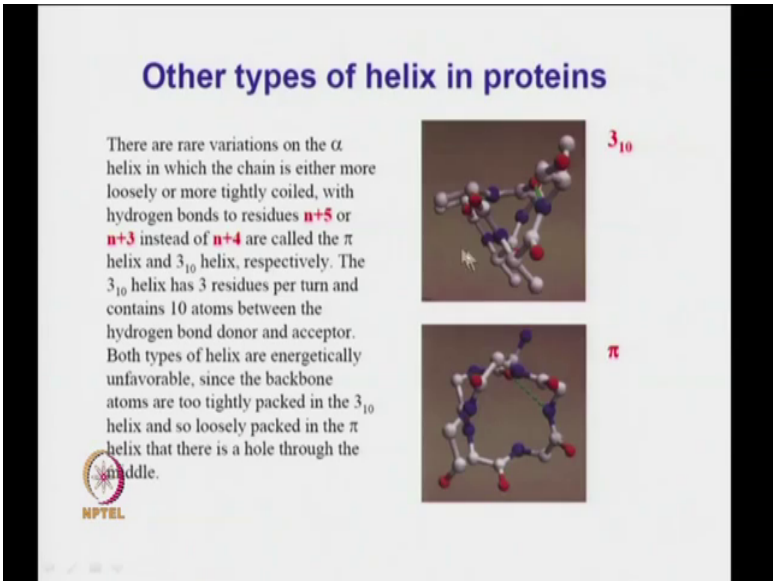

So, this is what I was talking about. So, if you look at it from the top a as you can see this is the rare variations. So, this panel you can look at my arrow this panel is 3 10th of helix this is the pith helix, you can see how lose if the structure looks at from the top right. And you compare it with the helical wheel, we had when we were looking at it just down the cylinder the down the helical cylinder from the top ok.

So, here as you can see with hydrogen bonds to residues n plus 5 or n plus 3; so, n plus 5 is for the pi n plus 3 for the 3 10th of a helix instead of the n plus 4 which is the regular alpha helix ok. So, as you said the 3 10th helix has 3 residues per turn that we just saw and that is what it means ok.

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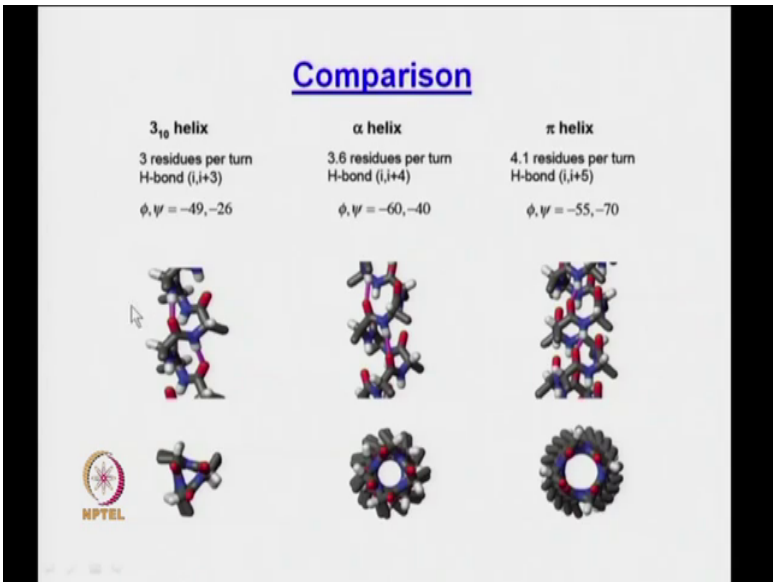

So, quick comparison; so, on the left here the 3 10th of a helix right phi and psi angles, then in a velve with the alpha helix you know remember. This is how what it look like when you looked at it from the top ok. So, you have the side chains with are protruding out and then this is a pi helix, you can see the pi helix is it is looking a lot loser ok and as a much looser coiled ok.

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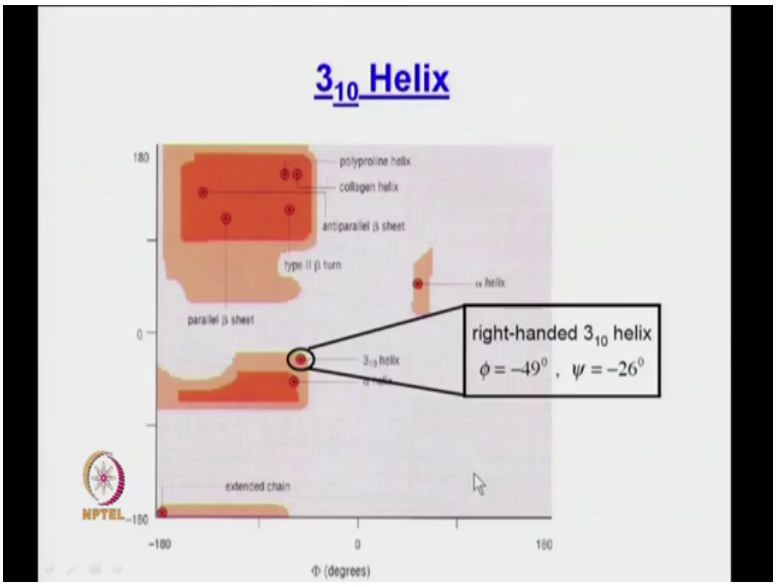

So, looking at the Ramachandran plot, you know this is where the 3 10th of helix comes. This is where the helix regular helix comes on average right.

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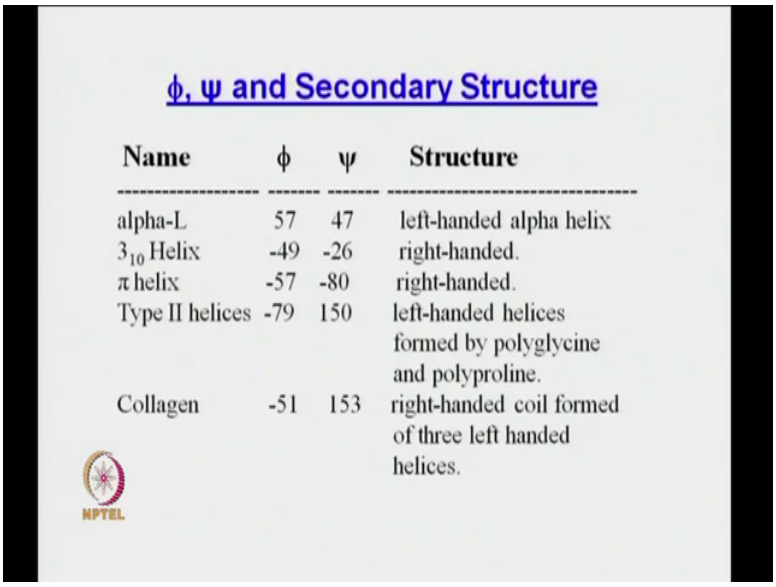

Now, a comparison of the phi and psi values then. So, this is the left handed alpha helix phi 57 psi 47 left handed it is where in the positive quadrant of your Ramachandran plot. Then these are the corresponding values of 3 10th of helix pi helix, then there are type two helices which are normal adapted by polyproline, polyglycine and then you have collagen.

So, collagen is something we will not talk about the phi and psi values in that details at least that is given out here. Collagen is something we will come to later ok when we talk about helix packing.

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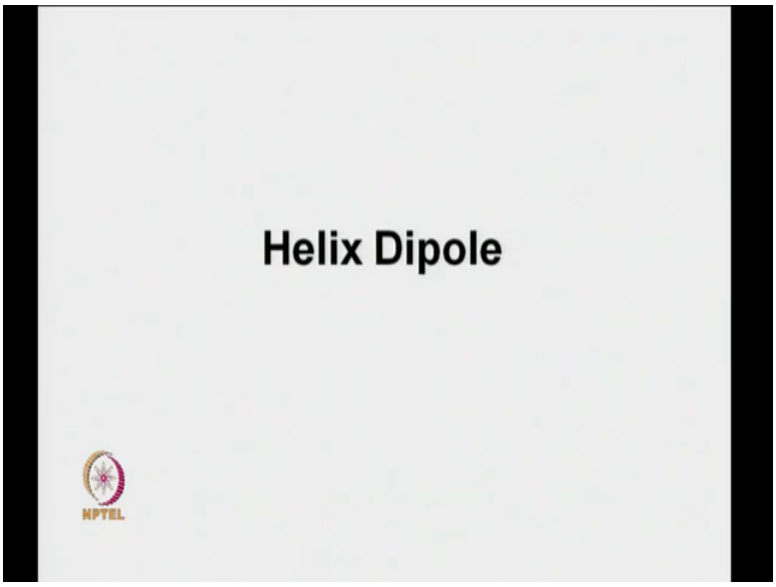

Now, an important feature of helical segments or helices is a dipole, commonly referred to as helix dipole or helical dipole or even helix macro dipole ok.

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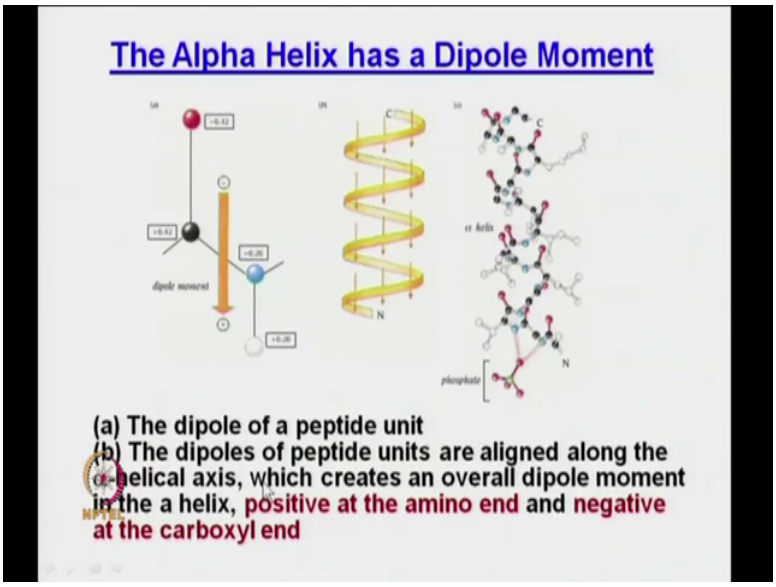

So, as the title of the slide says the alpha helix has a dipole moment. Now, if you look at the alpha helix, you see this is the peptide bond; you can look at the dipole moments out here. Then here this is the alpha helical you know secondary structure schematic this is a C terminus, this is N terminus. You can see all the dipoles you know pointing down towards N all right ok. And on the right side is the schematic of the helix with the phosphate out here attached to the N terminus.

Now, what is what happens is in this case the C terminus is negatively charged the N terminus is positively charged. So, the first one it is the dipole of a peptide unit; this is what you see, then the this is panel b of figure b.

So, the dipoles of peptide units are aligned along the alpha helical axis, which creates an overall dipole moment in the alpha helix this a refers to alpha actually. So, how does it do or

what is the charge distribution? So, the dipole moment is such that this positively charged at the amino end that this N terminus and negatively charged at the carboxyl end.

Now, keep in mind if you look at this figure. So, what we just say these this N terminus is positively charged, this C terminus is negatively charged right. Now, if you look at this alpha helix, you see there is a phosphate residue.

Here this is a phosphate group rather which is you know attached to the kind of interacting with the N terminus. Remember phosphate is negatively charged the N turn is positively charged. So, you have that several interaction out here so, then about the more about the helix macro dipole.

So, as it says we just discussed for this figure C negatively charged groups such as phosphate ions frequently bind to the amino ends of alpha helices. Now, the dipole moment of an alpha helix, as well as the possibility of hydrogen bonding to free energy group at the end of the helix favors such binding.

So, you can thing about this now. If you have any positively charged partners or negatively charged partners, which is supposed to bind to alpha helices to you know in the some sort of binding phenomena some site of some sort of signaling events.

Then based on the polarity; that means, based on the positively charged N terminus and the negative charged C terminus, they would know where to bind ok. And this is very important and that is why alpha helices really have been found to have a pretty well defined dipole, which is often referred to as the helical macro dipole ok.

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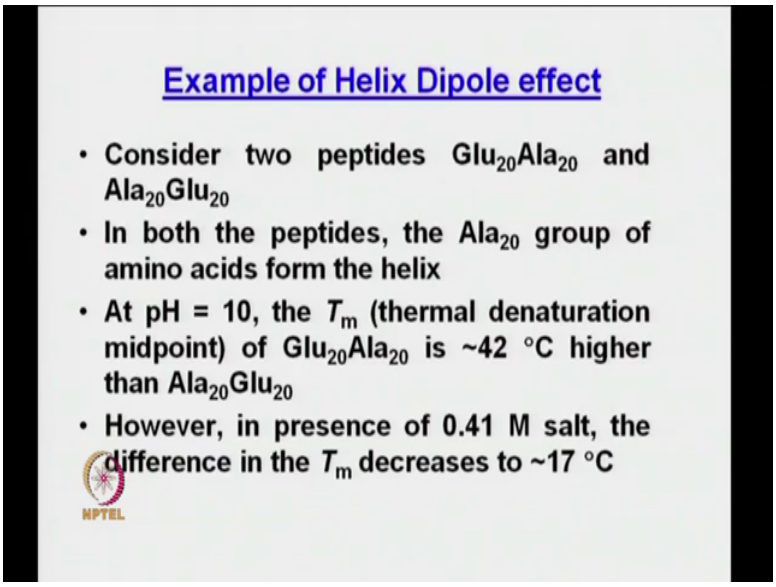

Now, let us you know taken an example of this dipole effect of the helix, how can we substantiate? So, one way so you know whether a many ways of doing it its one ways let us do comparison between two peptides. So, we have 2 peptides. So, the first one is glutamate 20 alanine 20 and the second one is alanine 20 glutamate 20.

So, one is so one is so, glutamate 20 is the N terminus, then you have the alanine 20 residues that is for 1 peptide, then for the second one you start with a Ala 20, there is a alanine 20 residues. This is starting from the N terminus and then at the C terminal end; that means, after you are done with a alanine residues, you have the glutamate residues.

So, that is the difference; that means, you have switched the positions of this glutamate 20 residues from the N terminus to the C terminus ok. Then both the peptides the Ala 20 group is

the one which is forming the helix; that means, this Ala 20 group of amino acids they are the ones which form the helix.

Now, at pH is equal to 10 there is an observation right, it is observed that at pH equal to 10. If you do a thermal denaturation; that means, you denaturing you are trying to denature this peptide by increasing temperature, these thermal denaturation everything, we will talk about in details; what a means we will also talk about in details.

Now, T m essentially mean is that when a denaturing the peptide, then there comes a certain temperature quality m which is called the melting temperature, that is what m stands for that means, you have half. So, here half there is if you know you have two states essentially; that means, half of the molecules are fallen half of the molecules are unfallen.

So, now what it says is at pH is equal to 10 the T m which is a signature of the stability, general signature of the stability of a peptide. For this the first peptide which is Glu 20 Ala 20 is about 42 degree Celsius higher than that of the other one right the glutamates on the other side.

So, what it means is; that means, the first peptides peptide at pH is equal to 10 is much more stable to an extend of about 40 degrees more stable, then the second peptide under these conditions. However, now if you are doing exactly the same experiment; that means, exactly the same thermal denaturation, but throwing in about 0.4 molar salt in the solution, you can see that the difference decreases to about 17 degree Celsius. Now, why this is so, do we have a reason for it?

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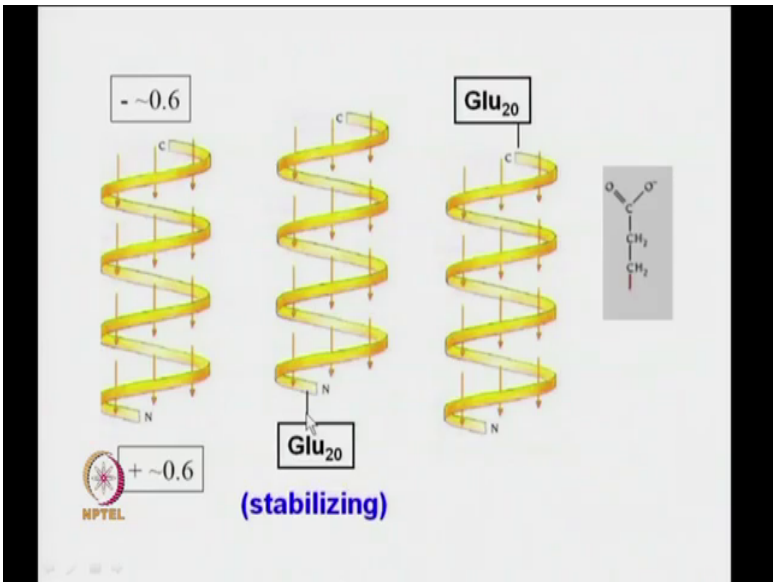

So, let us look at the reason. So, let us look at the peptide dipole moment again right; the helical dipole. So, again this is the helical dipole you can see here N terminus positively charged the C terminus negatively charged.

Now, then you have the helix, here the glutamate 20 out here; this is the first peptide and then so, the N terminus we have and then we have the this is the Ala 20 for you ok. Then you have the other peptide, where this helical part is the Ala 20 and you have the glutamate residues on the other side.

Now, see what you have done is see the here in this one in the first peptide; that means, in the glutamate 20 alanine 20, you see this Glu 20 is on the side where the helix is positively charged. On the other one it is on the side when the helix is negatively charged. Now, if you

try to remember what the side chain of glutamate is so, the side chain of glutamate is this so; that means, at pH is equal to 10 it is d protonate this is having a negative charge.

Now, for a moment think about this where would be having stabilizing interactions or why would interact favorably? Would it be interacting favorably in this one; that means a last peptide or would it be interacting favorably here? The answer is; obviously, here right.

Because here the glutamate residues or where at the N terminus which is positively charged. So, it can have several interactions; that means, this negatively charged carboxyl group can have favorable interaction with this, with this positively charged N terminus right. And that is why we say that this glutamate 20 outer is stabilizing.

So, now you understand why if you do not have any salt; that means, you would have taken both of these both this one and this one in pH 10, no salt remember no salt means not that high concentration of salt. I have not added extra salt except that coming from the buffer.

And then what you do is you do this T m and because, this is much more stable for the glutamate residues out here, electrostatically stabilizing your N terminus positive end of the dipole. Hence this is about 40 degrees more stable, then this one. That is 40 degree Celsius more stable than this one right.

Now, what happens in case of salt? So, now, you understand in case of the salt the difference decrease. Why does it decrease? Simple because, see when I am putting in salt you know the what the salt will do is salt will be having positive or negative charges. So, this salt will start screening this interaction screening this favorable interaction right.

Once you starts screen this favorable interaction, the stabilizing effect of that glutamate residues you know Glu 20 in this decreases, or we can also say like this. This glutamate out here was de stabilizing in the sense that it was interacting with a negatively charged dipole.

But, now once you start putting in salts what will happen is this unfavorable interaction would be screened to a huge extent. And hence the difference which was like 45 degree Celsius in the TMS comes down to about 17 degree Celsius. So, that is why now they are much more closer in terms of their stability. So, I hope you have understood this point this is an effect of your helix dipole coming into play.

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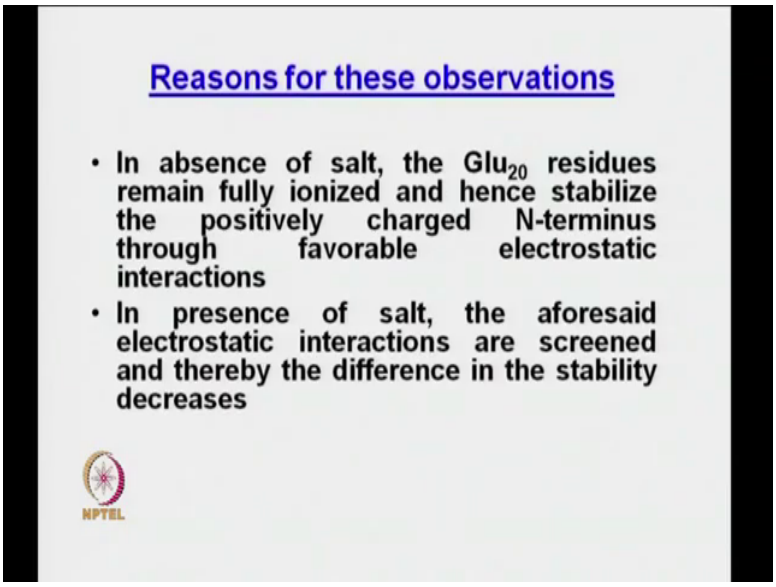

So, then the reasons for these observations in absence of salt the Glu 20 residues remain fully ionized. And hence stabilize the positively charged N terminus through favorable electrostatic interactions right.

In presence of the salt; however, what happen is the aforesaid electrostatic interactions are screened and thereby the difference in the stability decreases ok. So, again this is the influence

or the effect that your helical macro dipole can have on stability of a given system. In this case this glutamate alanine peptide ok.

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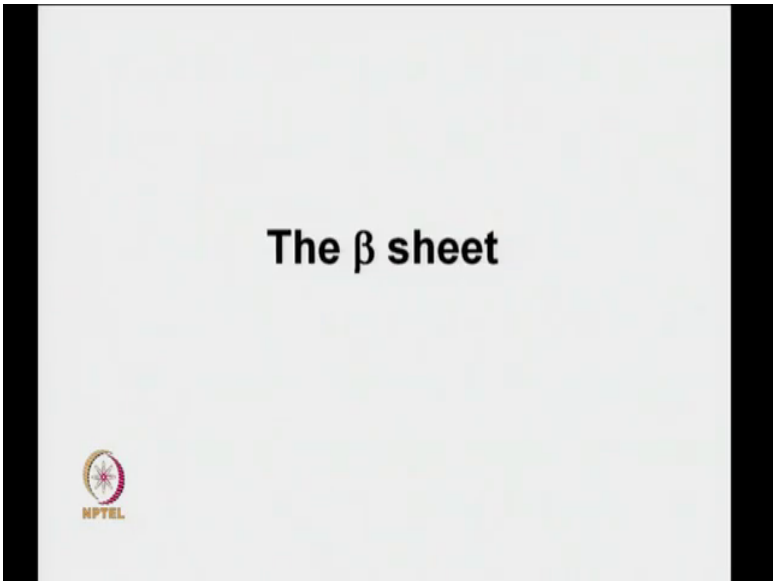

We have talked enough of about alpha helices; let us move on to the next most common secondary structural element, which is the beta sheet ok.

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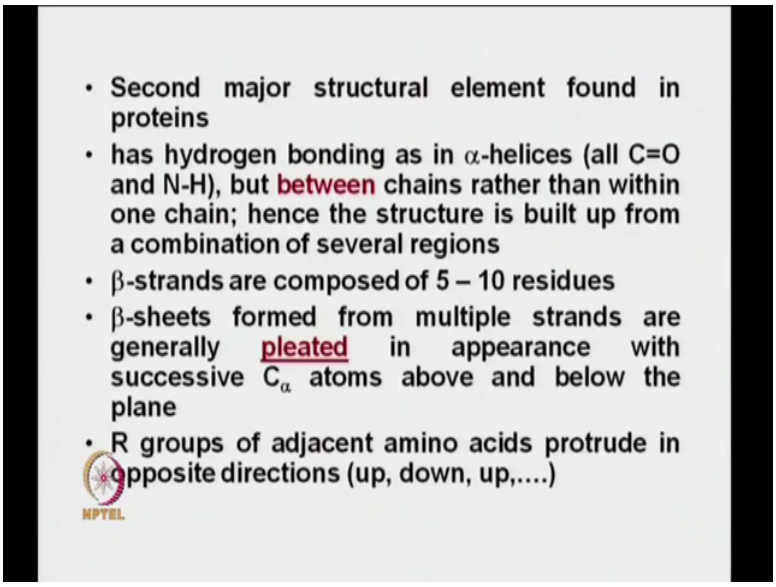

This is this is the second major structural element found in proteins. It is hydrogen bonding in has hydrogen bonding as in alpha helices; obviously, because alpha is also hydrogen bonding. But in this case the hydrogen bonding is between chains rather than within one chain remain alpha helix. We had everything in one chain it is like a cylinder, but not in beta sheet beta sheet is in between chains or in between something known as trance. Hence, the structure is built up from a combination of several regions ok.

So, the beta strands are generally composed of 5 to 10 residues, now you will see these specially the second point about these between chain the hydrogen bonds. Then beta sheets formed from multiple strands are generally pleaded in appearance with successive C alpha atoms remember this carbon alpha atoms.

Above and below the plane you will again see the structure very soon. And the R groups which are the amino side chains of the is a adjacent amino acids, in a beta sheet also protruding this opposite directions like the C alpha atoms up down like that ok.

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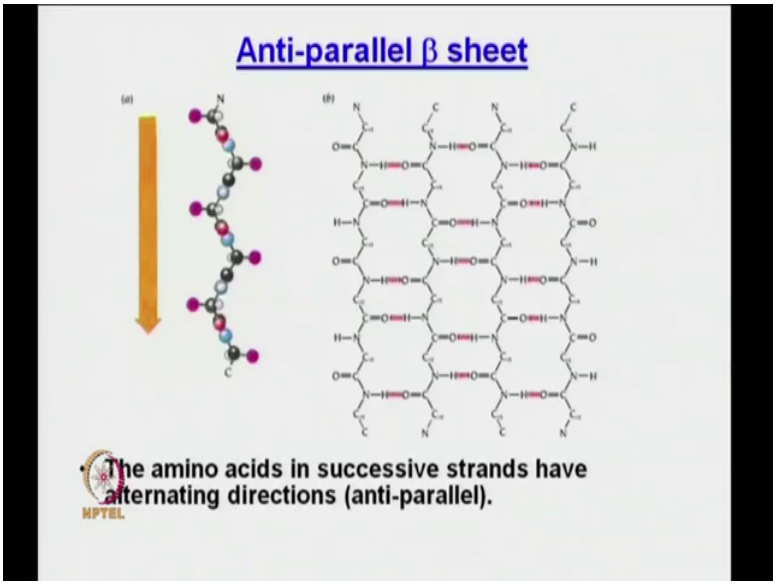

So, here this is the beta sheet now think about this a beta sheet is composed of minimum of two strands, and then you can go and adding strands. I will tell you what the beta strands are, but there are two ways of this getting oriented see. For example, if you look at this is a schematic of an anti-parallel beta sheet. So, what is happened is first of all.

Student: (Refer Time: 15:34).

What are we looking at? So, you look at this is from N this is C; that means, it is from the N terminus to this to the C terminus right this is one strand. Now, again here the second to the right there is another strand which is going from the N terminus to the C terminus.

Now, you can see this red ones which are the hydrogen bonds. Now, think about what we had talk in the previous slide, what we said in the second point was it has hydrogen bonds like alpha helices, but unlike the alpha helix where it is in one continuous chain.

Here you have the hydrogen bonds between two chains right and each of these chains individual like from N to C or N to C or N to C each of these chain individual is referred to as the strand. And the strands together form a sheet ok.

Now, that is one so; that means, we now know that you need at least two strands to form a beta sheet these strands have hydrogen bonds. As you can see the red ones, this is the hydrogen bonds, these are the hydrogen bonds right through out. Now, you also see one more thing see I can have an alternator arrangement too see the where the arrangement out here is it goes from N to C. Then here from here its starts N goes to C.

Then it goes again from N to C and it goes from N to C it is almost like see. If you follow my arrow you are coming like this, you are coming like this like this then you are making a U turn, you are going like this, then you are making another U turn you are coming like this. Then you are making another U turn you are going like this so; that means, in terms of the orientation the first strand is how is it orient from N to C the next strand is oriented from.

Student: (Refer Time: 17:14).

C to N is not it because here N; it is started from the N terminus was here. The second strand is ending at the C terminus; that means, each orientation is from N to C here the orientation is from N to C.

So, this means this is called anti parallel because, one is going like this. This is say this is N this is C the other one is going like this; that means, this is N this is C the adjacent strands. And, hence its anti-parallel that is why it is called anti parallel beta strand. Now, if you look at this schematic you can see how these things are moving up and down like this. So, this is what is referred to as a pleat you will see it again in the next slide. And this arrow is kind of the direction of this N to C; that means, this arrow is for the first strand say.

Now, if you go to the second strand this arrow would be reversed and hence (Refer Time: 17:57) anti parallel beta sheet. Now, not only that you look at this hydrogen bonds, you know this hydrogen bonds you can see they are formed.

They are pretty symmetric in appearance are not they are very symmetric in appearance, you can see here is the way the alignment is done is that H and O are very well aligned to form the hydrogen bond with minimum penalty; that means, with minimum confirmation strain.

Now, with respect to what I will just telling. But before that remember if you are having something as an anti-parallel beta sheet; that means, there is there must be something which is parallel beta sheet. We will look at it very soon.

Student: (Refer Time: 18:30).

But anyway just drive on the point the main difference between alpha helix and beta sheet, is the fact that alpha helix all the hydrogen atom the hydrogen bonds intra helical hydrogen bonds are in the same chain. But, in case of beta sheet it is actually in between two adjacent strands.

So, the amino acids in successive strands have alternating directions right. So, one is like this the other one is like this, then the next one is like this then the next one is like this. So, it is called anti parallel arrangement.

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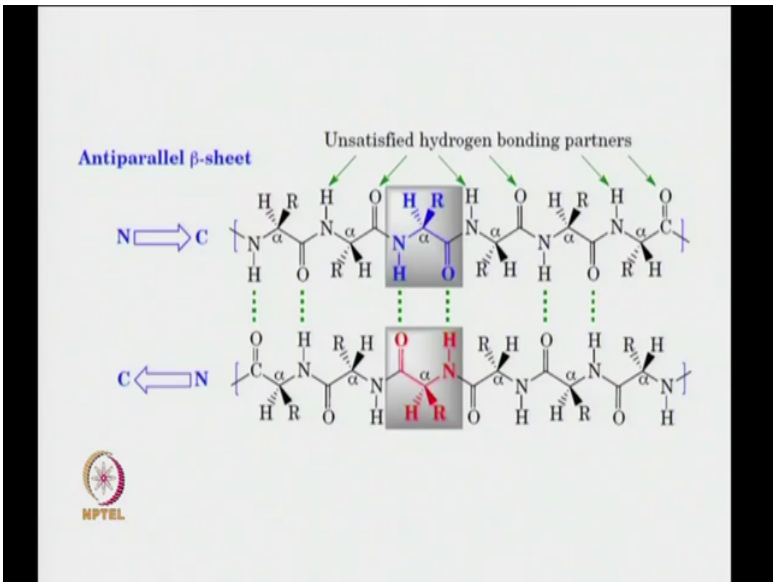

So, again looking at the anti parallel beta sheet. So, you can see this is the N to C this one is goes from N to C like this. This N to C in this direction the next strand is N to C in this direction. Here the green bonds with the hydrogen bonds again see they are so, well disposed to each other to give us the symmetric nature of the hydrogen bonds.

Now, there was one thing, but this one more important thing, you look at the flanking you look at this or you look at this see. See suppose I have not shown here suppose after this there is a third strand say, if there is a third strand. So, that but here there is no other strand. So, look at what is happened to this H this O this H this O look at all this green arrows. That it says these are unsatisfied hydrogen bonding partners ok.

Similarly, what happens on the other side, what will happen on the other side is in the other side we do not have anything, then again these would also be unsatisfied. So; that means, there

is at least say if we have 2 beta strands making 1 beta sheet; that means, there would be two sides which would be having this un satisfied hydrogen bonds right, like this you know there will be two faces.

Now, if we have three strands and in the middle strand is let us make out the previous slide. So, for example, if you look at these two strands these two middle strands they hydrogen bond to both right so; that means, they do not have any answer is for hydrogen bond donors and excerpters. However, the first and the last strand now they are not satisfied.

So, this is why if you would you know if you would go through papers, and if you look at characteristics of beta sheets or look at proteins which aggregate a lot because, protein aggregation is huge thing.

Then you will see that proteins which are having beta sheets or beta sheet corrected tend to aggregate a lot more because, see on this these faces; they have answer is for hydrogen bonds of bonding partners. So; that means, these at the first opportunity would like to interact with something. So, that these answer is bonds are getting satisfied ok.

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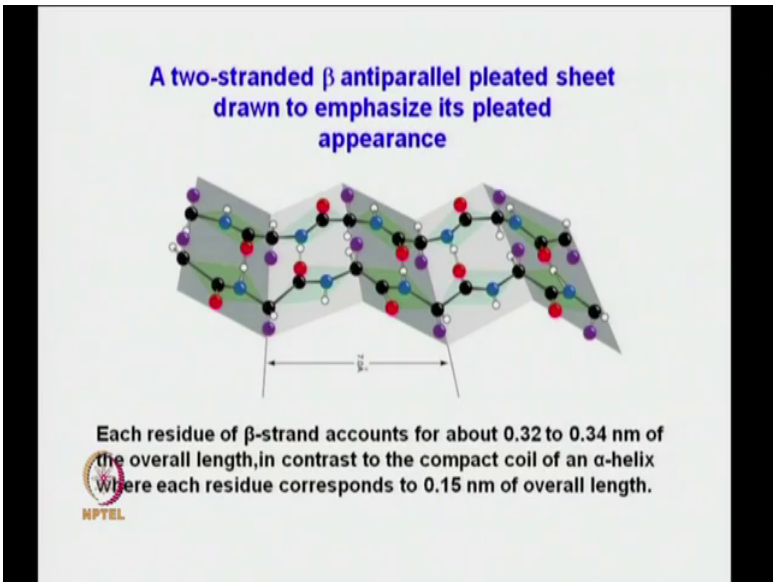

Now, let us look at a two stranded beta antiparallel pleated sheet to emphasize this pleated appearance ok. This is what I was talking about you can see, how it is coming from here it goes down it moves up it goes down it moves up. So that means, one is on top the other is on bottom. So, top of plane bottom of plane, top of plane bottom of this is what it gives rise to the pleated appearance.

Now, if you have looked at shirts and all you will sometime see the tailor puts a pleat in the shirt essentially that is what we are talking about. Now, also see between two residues between two residues your distance about 7 angstroms ok.

So, each residue of beta strand accounts for about 0.32 you know, 0.34 nanometer 3.4 angstroms or 3.2 angstroms of the overall length. In contrast to the compact coil of an alpha

helix were each residue corresponds to 0.15 nanometer overall length. So, think about this right.

In alpha helix is 1.5 angstrom, but in this case it is 3.2 or 3.4 angstrom 3.2 is essential, for a parallel beta helix 3.4 is for anti parallel beta sheet I mean ok.

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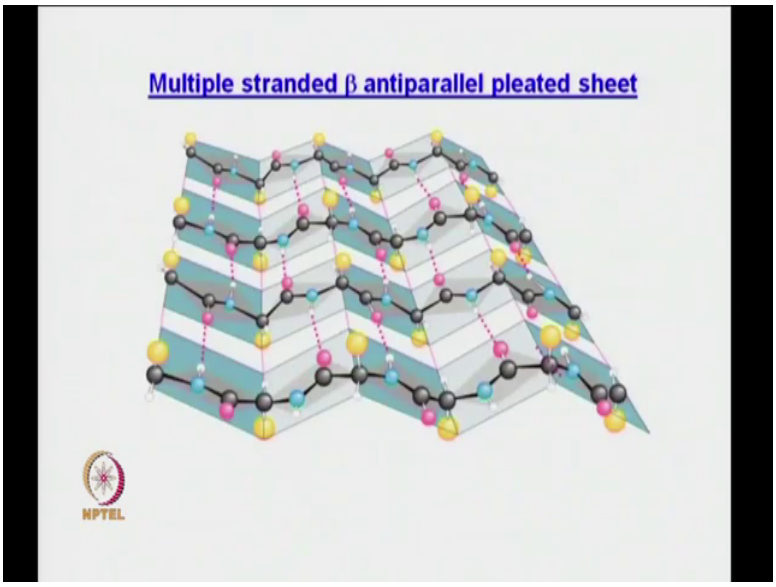

Multiple stranded beta antiparallel pleated sheet again. So, this is what again I was telling you now you have multiple stranded, before you have two strands. You can see how these strands are this one is coming down, this one is going up. Again you see the definitive pleated appearance and again these pink ones are your hydrogen bonds ok.

And here you can see the first strand out here or the first strand towards, you and the last strand which away from you these are again having hydrogen bonding partners acceptors and

donors both which are not satisfied ok. And as I was telling we have this anti parallel beta sheet arrangement, then whatever the parallel beta sheet what does it do for us.

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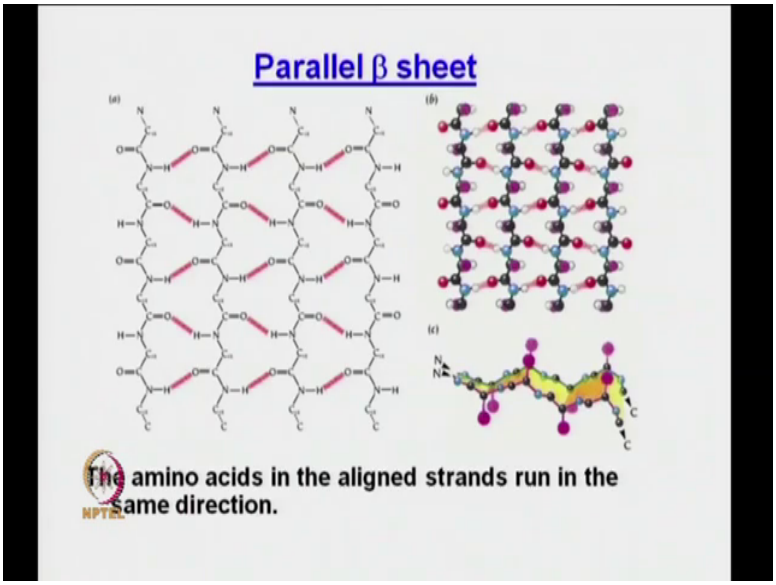

So, this is what the parallel beta sheet looks like just looks at the schematic. So, the parallel beta sheet right. Now, you can see first and most important thing you see this going from N to C in case of antiparallel beta sheet. The next one would go from N to C like this, but here you can see this goes from N to C; this also goes from N to C.

Again this one goes from N to C and this one goes from N to C; that means, all of them are pointing in the same direction; that means, parallel. Hence it is the parallel beta sheet. But then what is the result? As a result of this what you can see is that, you can see these hydrogen bonds hydrogen bonds out here. They are not they are at an angle now they are not exactly so,

the I mean the N H and the C O groups are not so, disposed that they can form these bonds in collinear geometry and almost collinear geometry.

I mean in this case they are at certain angles and hence you can understand that this set of arrangement, or this structure does have or does suffer from conformational stream. So, if you would ever think which one food, we found more in proteins then it would be the anti-parallel arrangement not the parallel arrangement ok. And this is the right side you have a schematic of how it looks like again this is the pleat coming from this parallel arrangement.

Now, you can ask this I have looked at anti parallel beta sheet, we looked at it just now we are on the slide where we are missing that parallel beta sheet. Now, can we have a mixture; that means, can we have the anti parallel beta sheet and the parallel beta sheet in the same protein as a matter of fact we do. So, here for the before I leave this slide for the parallel beta sheet the amino acids in the aligned strands run in the same direction ok.

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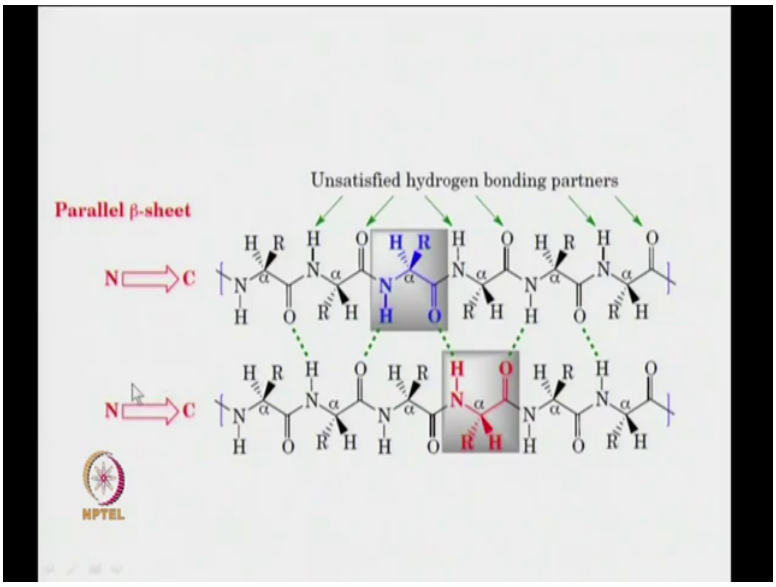

And again you see the parallel beta sheet you see this is N to C, this is also N to C and you have this un satisfied hydrogen bond partners ok.

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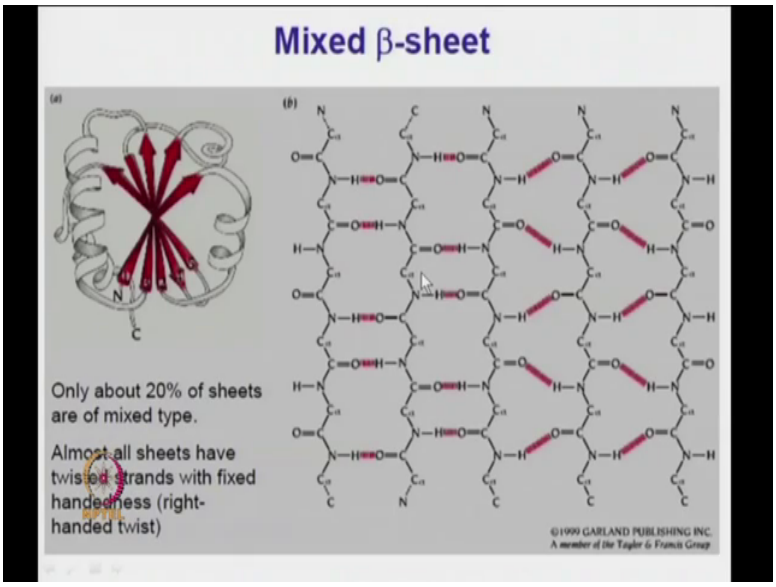

Now, this is the case of mixed beta sheet that I was talking about here you see 1 2 3. So, the first three of this antiparallel arrangement N C then N C again this N C, but then after that you have the parallel arrange. So, this is the case where you have a mixed beta sheet; that means, this one is antiparallel right. So, this one is antiparallel out here and this one is parallel ok.

But, you can see as your as you said on the left only about 20 percent of sheets are of mixed type. Because, you can understand that if you do not have any other stabilizing interaction it is very hard to have such a strain in the structure is not it. And this because you have this parallel beta sheet arrangement where the hydrogen bonds are at a strain and the anti-parallel beta sheet arrangement, where there were dispose.

So, this is combination the structure does suffer from significant amount of strain, which has to be stabilize by some other interactions. Only then can we have these things coexisting that is why it is only in 20 percent of the sheets it is found ok.

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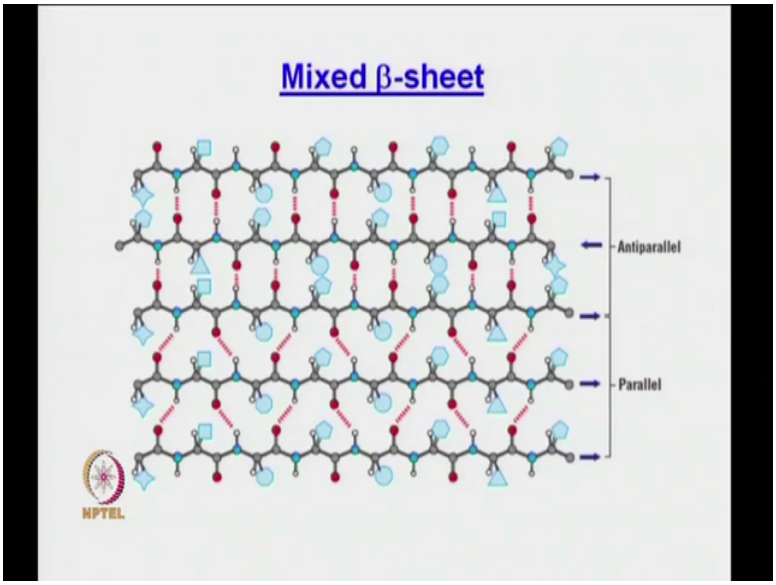

So, again this is an example of this mixed beta sheet. Just another prospective another way of looking at it here you have this anti parallel arrangement. As you can see you can look at the arrows these arrows go here, this arrow goes here this arrow goes here. And then they parallel these arrow goes in the same direction ok.

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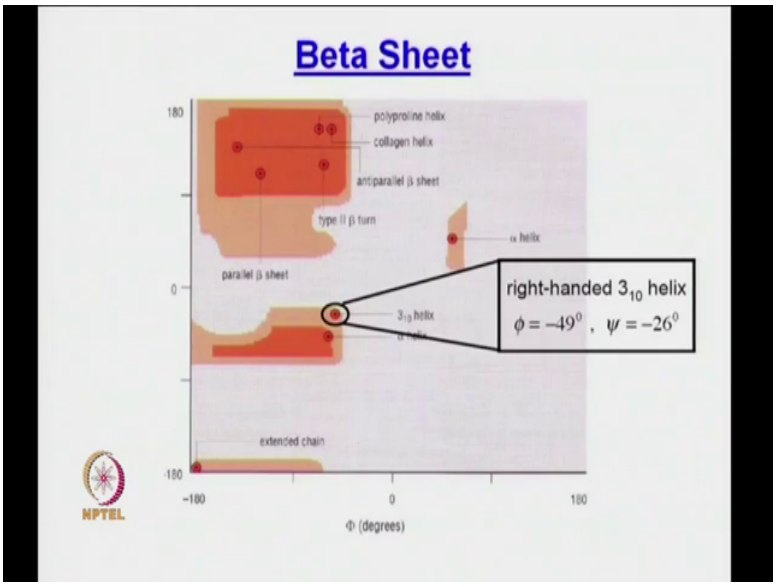

To look at the Ramachandran plot where does the beta sheet comes through. So, this is where the beta sheet comes through the anti-parallel and the parallel beta sheet right.

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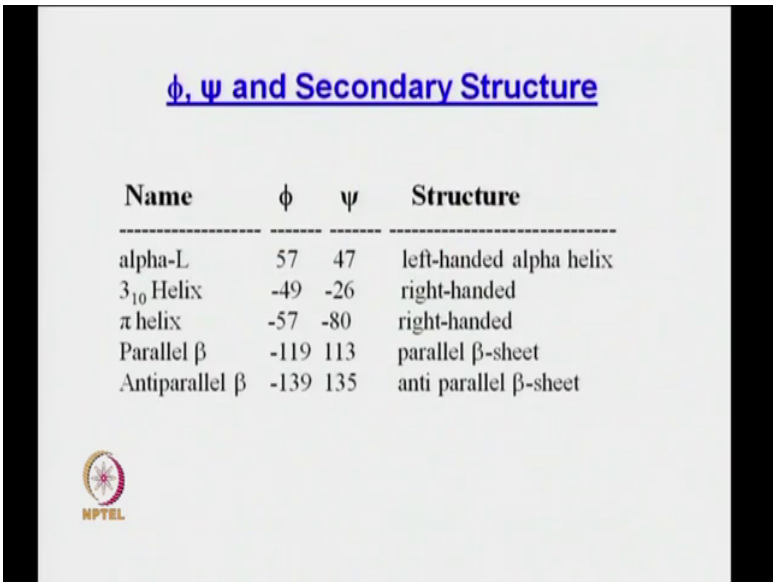

Now, we had looked at a table like this before let see incorporated the beta q. So, here if you compare with the alpha helices or 3 10th of the helix or even the pi helix the parallel beta has these phi and psi angles ok. If you can look if you look at this is the phi which is in the negative side where the size in positive now. So, phi minus 119 psi 113 for antiparallel beta it is minus 139 and psi is positive 135.

And as I was telling you again so, this is psi on the y axis, this is phi the x axis. So, you can see if it is in the region, then phi is negative for it because 0 is here for phi. However 0; it is above 0 for psi and psi is positive and that is what you see here ok. And that is what you see here psi being positive for both parallel antiparallel beta sheets. But phi is negative for both of this ok.

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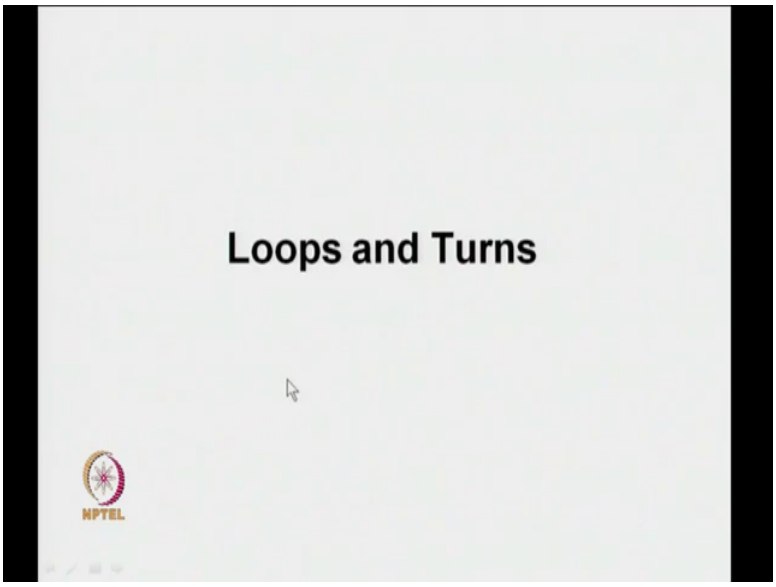

Now, these are all the two most important secondary structural elements right, they are almost present in each and I mean in almost all proteins or major (Refer Time: 27:18) proteins let me tell you ok. You pick a 1 protein in you know there is very good chance of, it having a either a alpha or a beta or a mix mixture of that ok. Then what do you mean by loops and turns; other some other secondary structural elements.

Now, think about this if you remember those anti parallel or parallel beta sheet arrangements say. Let us you know think about the anti-parallel arrangement. I want like this strand like this and I have the other strand going like this. Now, see you have to make a turn as there you see you have to make a U turn right. If you are going to make a U turn you better have something which connects these two.

That means, you are actually form a loop forming a loop you know hairpins, if you have seen they look like this. The this part where you were connecting both the arms is bend the same thing is going to happen here. So, that is where loops and terms coming; that means, the kind of acting as a bridge between your strands or a between your different secondary structural elements.

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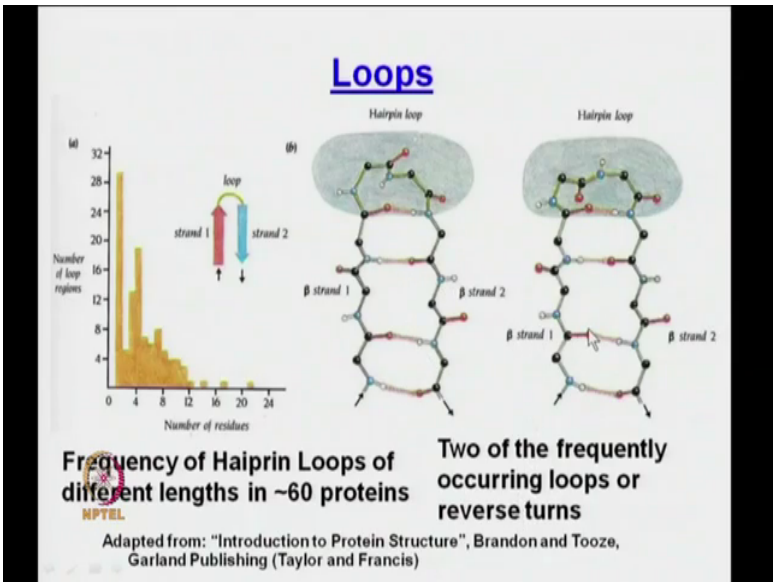

So, here loops we can see to your right always start from here. So, this is this arrow signifies one strand; this other signifies, another strand of beta say 2 beta strands. And you can see this one arrow is in this direction arrow is in this direction, this is an antiparallel beta sheet and you can see it is connected by a loop region.

So, the loop essentially connects these two strands. So, on the right side you have two examples of that two examples of hairpin loops. Because it looks like an hairpin you can see,

you follow this arrow it looks almost like a hairpin that is what it looks like a hair is not it looks like a it looks like hairpin. That is what it looks like ok?

So, you have beta strand 1 beta strand two here also beta strand 1 beta strand two these are two different ways of looking the hairpin loops I will come to this in the next few slides. But what is the major thing is if you look at this. What is this one show? This one shows the frequency of hairpin loops of different lengths in 60 proteins. Now, the question is going to ask is ok, I am having this strand and I am having this strand and I am going to connect it with the loop of amino acids.

Now, what how many amino acids? Can I have in a loop? Is there a statistical number? Is there a statistical representation to be know which one is most; that means, do we have 4 amino acids in the loop to be occurring the most do we have 2 do we have 3 so on.

So, that is what this histogram tells you can see this is the number of residues and this is a number of loop which is you can have, and you can see how it goes right. So, number of residues which is 2 it is almost the highest. And then I think it is a either 4 little have a 4, but anyway that is the significance of this figure. And then as I said these are two of the frequently occurring loops or reverse turns as we saw here.

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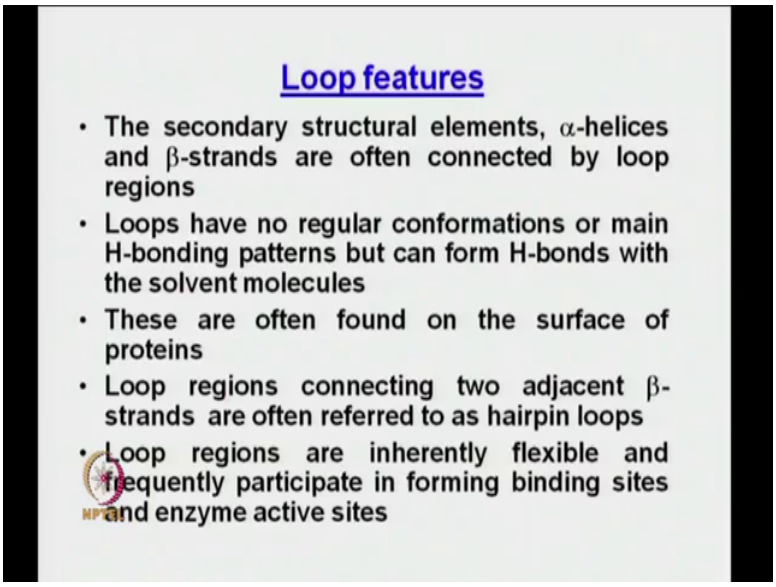

Now, going a little more into this loop thing. What are the features of loops? Some of the features now the secondary structural elements alpha helices and beta strands are often connected by loop regions, as I was just telling you or we just saw. The loops have no regular confirmations or main hydrogen bonding or main chain it should be, it should be main chain hydrogen boding patterns.

But can form hydrogen bonds with solvent molecules ok, these are often found on the surfaces of proteins right. And loop regions connecting 2 adjacent beta strands are often referred to as hairpin loops that is what we just talked about.

The loop regions are inherently flexible and frequently participate in forming binding sites and enzyme active sites. So, you can understand right you have it is like a hinge you have this you

have this right and it is forming a loop. So, this loop region is pretty flexible, you know has to be flexible and hence it is. So, frequently found in binding sites and enzyme active sites ok.

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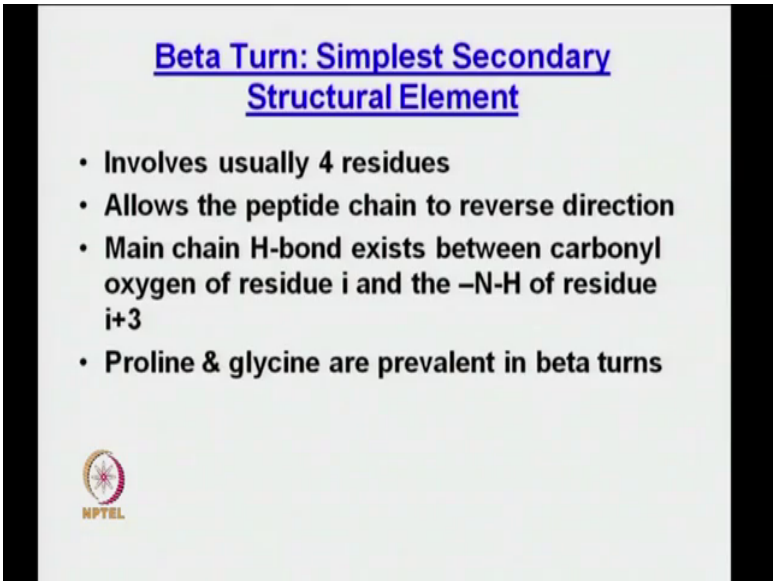

So, then related to the loop well it is the loop we give it a different name it is a beta turn it is essentially, the simplest secondary structural element it involves usually 4 residues, it allows the peptide chain to reverse direction as I said. If you are going in one way then you form a loop or form a turn and you come down the other way. Then you have the main chain hydrogen bond that exist between the ith oxygen residue and the i plus 3th hydrogen of the nth residue.

Proline and glycine are quiet prevalent in this beta turns ok. Remember glycine has only this H as a side chain hence it is very flexible proline; however, does not have that H and it is very

rigid. So, it actually ends up forming a bend or a kink and that is why this proline and glycine are so, favorable out there.

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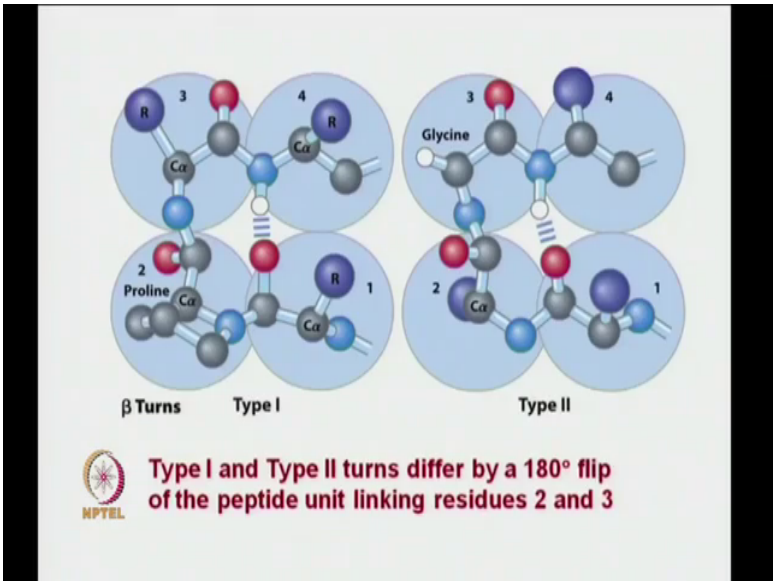

So, this is an you know an in depth look into these two turns. So, you can see this is a type one turn this is a type two turn both of these are beta turns. So, the type one turns and type two turns how do they differ they differ by a 180 degree flip between the peptide unit linking residues 2 and 3. So, this is residue 1 this is 2 this is 3, you look at this red, it is fair out here. And look at the type two what is happened is you can see this is again 1 2 3.

But here it was pointing down here it is just pointing up ok. Often in type I here the residues proline. As I was as I was telling you because, it gives rise to this kink. You know it is structure rigid and then you have the glycine out here ok. The glycine again is very flexible and it is oftently found in 3.

So, these turns are very important they are only present because, you go to have these you know two different secondary structural elements. If they go to be linked they have to link by loops right or turns ok.

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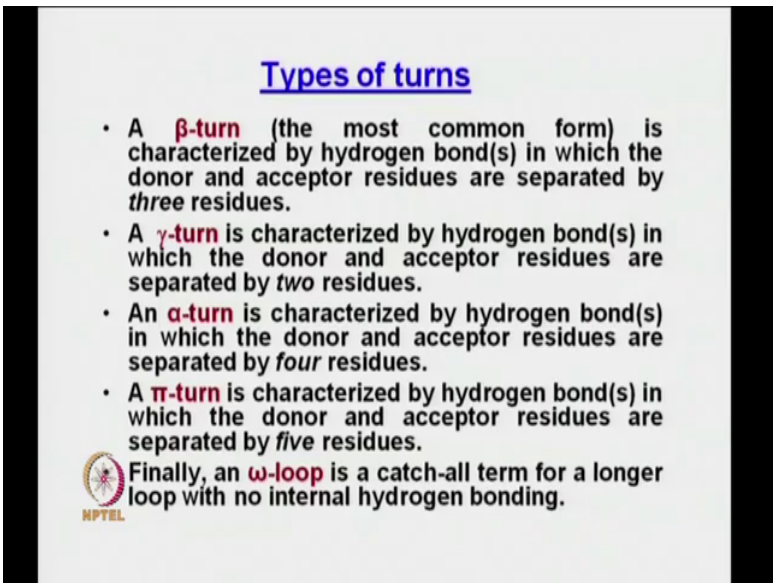

Then there are different types of turns I will just quickly go through it one is a beta turn, we just looked at, you know separated by our 3 residues; it is the gamma turn right. It is characterized by hydrogen bonds in which the donor and acceptor residues are separated by 2 residues. Then we have the alpha turn you already know it is by 4 residues like an alpha helix.

And there is a pi turn, it is characterized by hydrogen bonds, where it separated by 5 residues remember that pi helix it was i; i plus 5, it is almost something like that. And anything beyond that is covered by this terminological omega loop; it is a catch all term that is why it says. So, for omega loop I mean finally, an omega loop is a catch all term for a longer loop with no

internal hydrogen bonding ok. So, this is the loop in the real sense it has no internal hydrogen bonding no i plus 3 and so on ok.

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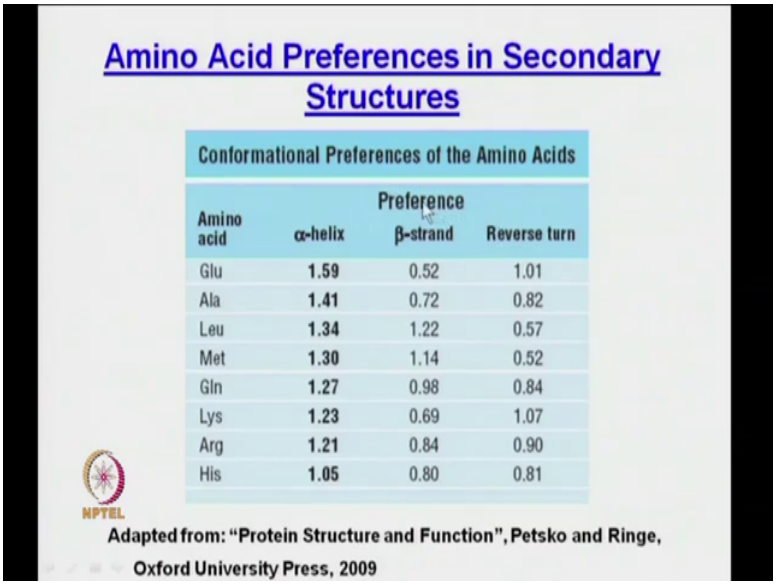

Now, this is the another thing you must have heard about ok. If I take a beta sheet if I take an a an alpha helix. And if I do a statistical interpretation; that means, I take many proteins where I (Refer Time: 33:49) alpha helices (Refer Time: 33:49) beta sheets, or mixture of these.

Would I find different amino acids occurring more frequently in the alpha helical region and, would I find different amino acids occurring more frequently in the beta region? That means, would this amino acids be having preferences to come either as a part of an alpha helical region, or as part of a beta sheet or as a matter of fact turns loops or turns too ok.

So, here this is some conformational preferences of amino acids. And you look at this is the amino acids on the first column, then you have the alpha helix second column the beta strand and the reverse turn. So, the preference is given like this. So, the glutamate you can see 1.59, it is the highest preference then alanine 1.41 and as you go down this one is.

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Decreasing and so, this black ones are the ones which are most favored for an alpha helix.

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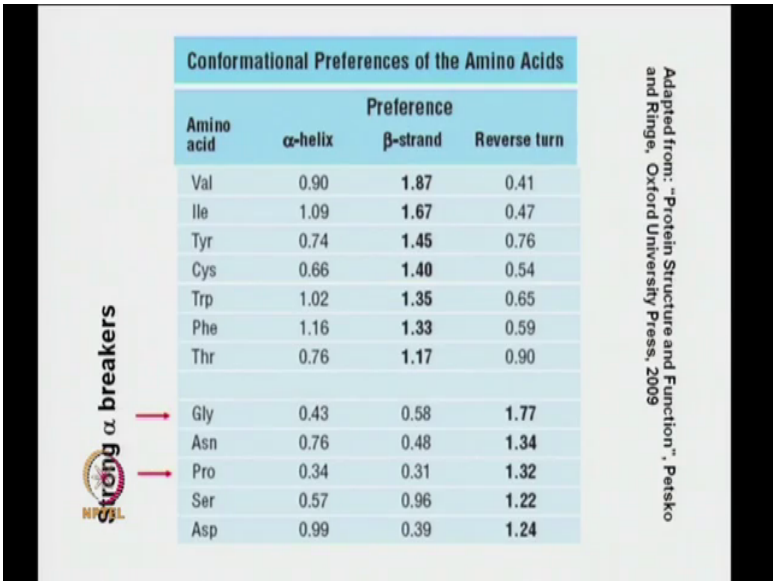

Now, whatever the other two so, if I go here I am talking about the beta strand now. You can see this valine, isoleucine, tyrosine, cysteine, tryptophan, phenylalanine, threonine these are more preferred in the beta strand with valine being the most preferred ok.

And then when I go to a turn this reverse turn; that means, where I am going to form this bend because, I have to do a reverse turn. Then you can see which is the ones more preferred glycine 1.77 it is very high, then your proline 1.32 asparagine it is kind of close and so on.

So, what we can see is these glycine and proline are often referred to as strong alpha helix breakers. So, now, you will have seen something; that means, if I go to the previous slide really quick here, you can see these amino acids they have strong preferences for alpha helices, but not that strong for beta strands snd also not that strong for reverse turn. Now, if I go for these amino acids these are these are more preferred for beta strands and if I go for these are more preferred for the reverse turn.

Now, you remember we talked about this right, a couple of slides ago there glycine and proline are better be found in this. In this turns whether type whether type I and type II and that is what exactly see glycine has the highest propensity. After that this asparagine proline which are very similar specially proline comes very often ok. So, there also means that these are alpha breakers its glycine and proline, then they better not be found in alpha helices ok. Otherwise they would randomize helix; that means, they would break the helix.

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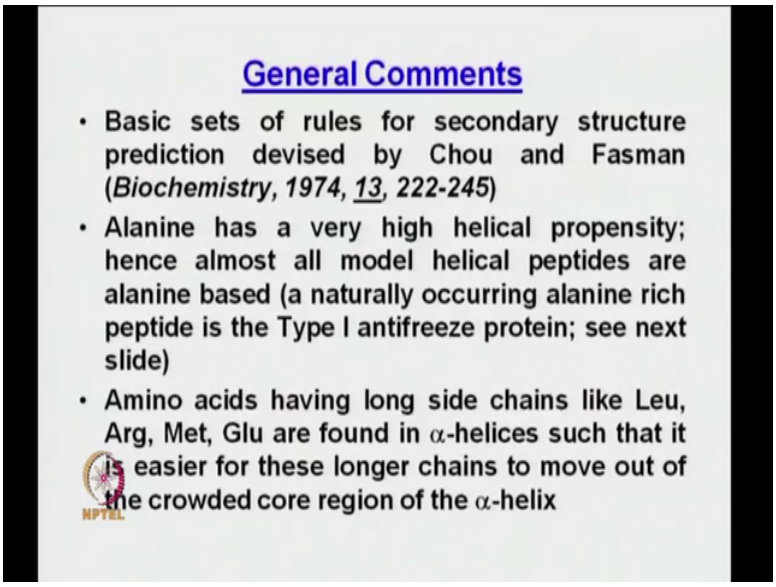

So, then some general comments, the basic sets of rules of for secondary structure prediction devised by Chou and Fasman; so, some I mean you should really look at this paper. So, what I mean is see once I know the preferences right; that means, what a secondary structure preference amino acids have.

Now, suppose I am given a sequence of amino acids and I must can I predict can I predict the structure based on the sequence of amino acids have; that means, would it be alpha helical, or would there be a stretch which is beta, or would there be alpha helix better together which would be loop and so on. Remember we have this preferences right.

So, then what we do is we say that alanine has a very high helical propensity, which just saw it was 1.41. And hence it is very hard to find helices in isolation; isolation, means you cannot

find 1 helix you can you cannot just take it out from protein and think that it will be stable as it was inside a protein.

So, that is why if you are going to use models for helices you better have helices which have a preponderance of alanine residues or predominant by alanine residues, that is why it says. Almost all model helical peptides are alanine base, because alanine has such a high helical propensity.

Now, naturally occurring though alanine rich peptide is the type 1 antifreeze protein, we will look at in the next slide. So, amino acids having long side chains like leucine, arginine, methionine, glutamte this is find in alpha helices y such that it is easier for these longer chains to move out of the crowded core region of the alpha helix.

So, remember the helix is the cylinder ok. So, I have to have these chains longer chains which can either trained point, they can easily just move out right they are free they just move out of the helix, core and the core remains essentially hydrophobic non perturbed ok.

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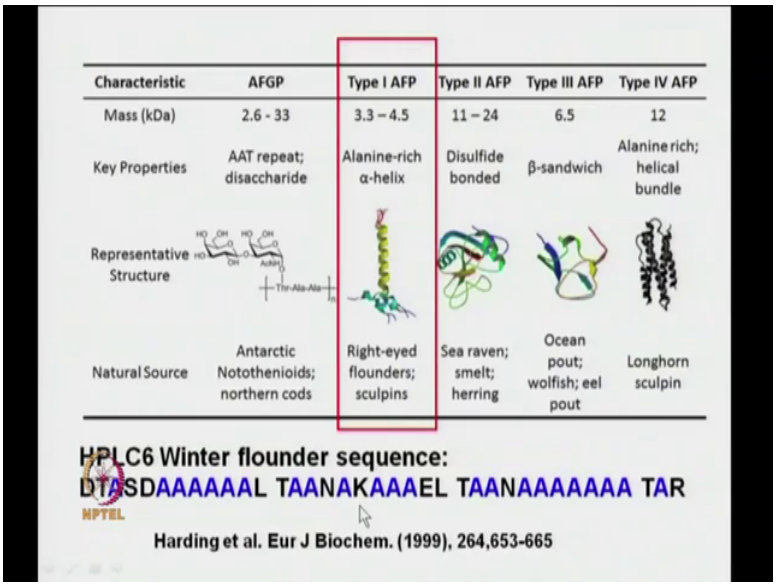

So, this is the antifreeze proteins. I am talking about and the one I am interested in this type 1 AEP AFP refers to antifreeze protein ok. This is alanine rich right and you look at the sequence now, this is a winter flounder sequence rights, fish you can look at the sequence you can see how many amino acid. It is had this is about a 36 residue protein; I can see all these blue ones has a alanine rich. So, it is a prepoline its a valine residues ok.

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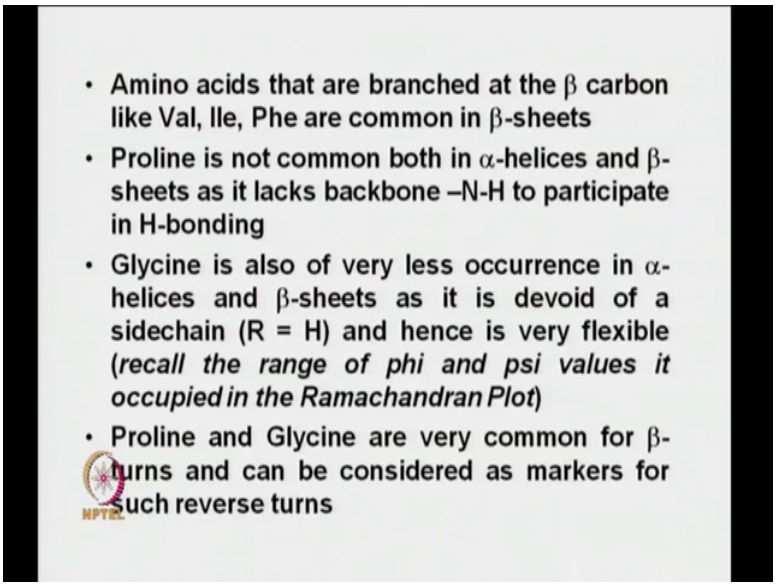

Now, amino acids that are branched at the beta carbon like valine, isoleucine are common in beta sheets. Now, proline is not common both in alpha helices and beta sheet as it lacks backbone N H to participate in hydrogen bonding remember, we told this before.

Glycine is also of very less occurrence in alpha helices and beta sheets as it is devoid of a sidechain. Here R essential is equal to H, and hence it is very flexible. So, you know if when thinking of flexibility try to recall the range of phi and psi values, this glycine had it almost had it you know it was coming which is almost every quadrant of your phi and psi plot, isn't it?

Now, proline and glycine are very common for beta turns and can be considered as markers for such reverse turns ok. So, now, think about this you are trying to predict right you are given a signals you are trying to predict. How do I predict? Now, what I do is I look for a

stretch of amino acids or I look for the amino acids say, I find a stretch where each and every amino acid has a very high propensity to form an alpha helix.

Then I know may be that one is an alpha helical stretch. Similarly I look for say like proline and glycine occurring, then if I look at a stretch where I see proline and glyicine occurring then maybe that is a loop. Then if I have this valine, isoleucine, phenylalanine occurring in adjacent residues or in adjacent strands then possibly I would know that there is a beta region ok.

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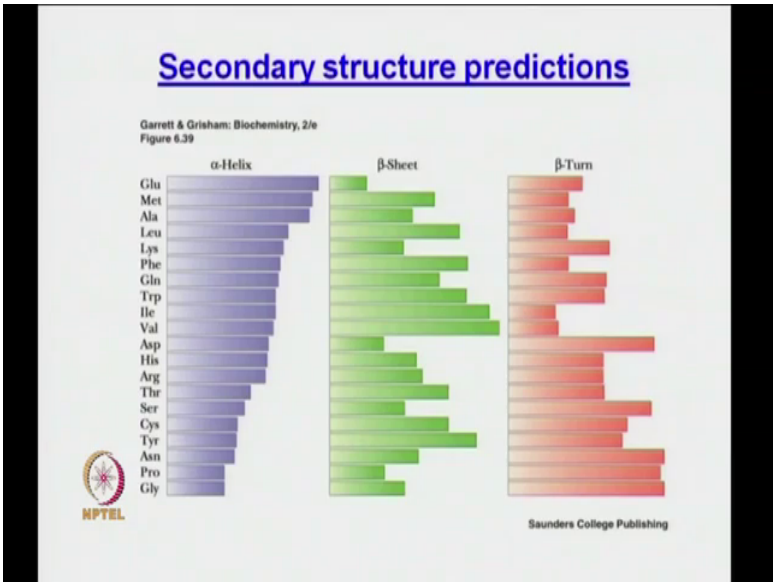

So, now with this in mind; that means, you are trying to predict what the secondary structure of a given sequence would be as I said secondary structure predictions I based on this. So, this is essentially histogram representation what we just saw a couple of slides before. You can see

alpha helix, glutamate is a maximum the alanine, then you have beta sheet valine is a maximum, then beta turn you can see here aspergene, proline, glycine these are very high.

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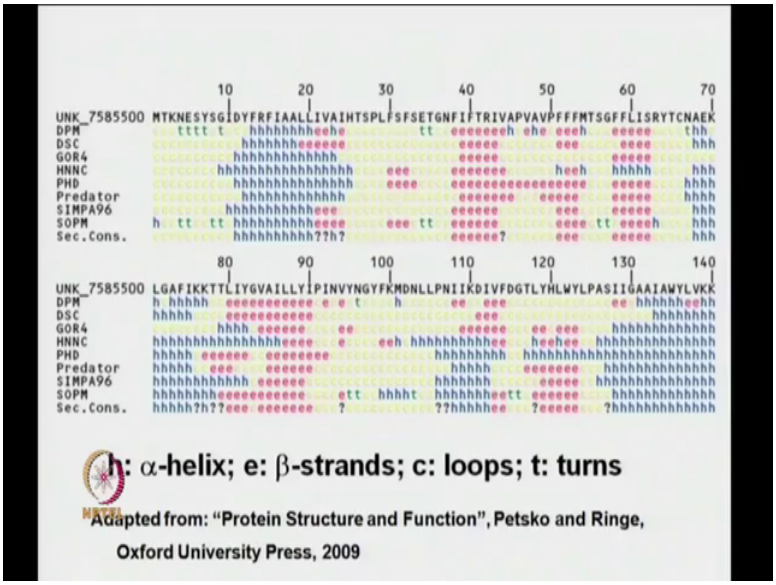

So, once you have this information or these sets of values, then you use this for secondary structure prediction. So, what we are looking at is you can see these 10 70 140. So, this is a 490 amino acid protein and out of that I am just showing you 140 amino acids. What I am trying to do is out of this 140 amino acids. I am tying to predict whether I would be having any helix or any turn or any beta something like that.

Now, what are these DPM DSC and all these thing these are different programs, which gives you secondary structure predictions ok. So, we will go into that much, but let see we can see all these h's, t's, c's, e's occurring. So, what does h stand for h stands for alpha helix, e stands for beta strands, c stands for loops and t for turns right.

So, we now know what loops are what turns are we have already understood this. So, then you can see here typically if you go through you can see this blue ones at this alpha these helices. Then this e's are the strands right again the c's are the loops the coils essentially or the loops and this t's are the turns.

Now, if you look across all the different programs of you know all the different you know the secondary structure prediction programs, you can see well they differ among themselves, but overall they do a decent job in terms of a comparison among these different programs ok. Now, as Chou and Fasman said, what do we do? So, what do we do is we look at P alpha. Something referred to as alpha helix potential. So, potential of amino alpha helix is referred to as P alpha good ok.

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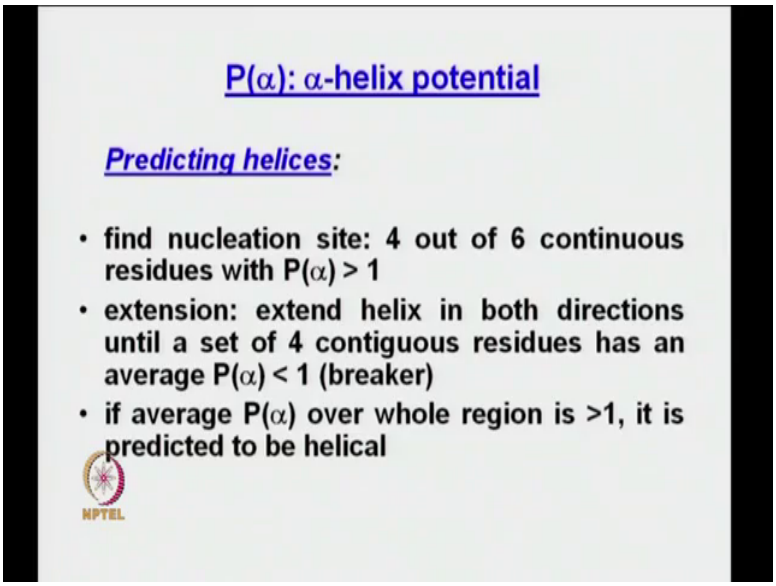

So; that means, we are predicting helices. Now what do we do is we find nucleation site. That is say as I way saying you look at a stretched amino acids say 4 out of 6 continuous residues adjacent residues like this, with P alpha greater than 1 ok. Good. So, P alpha means it is a potential of amino and alpha helix which is greater than 1.

Now we extend; that means, is it going to extend forever or what happens on the other sites. Now, extend helix in both directions until a set of 4 contiguous residues adjacent residues are found, which have an average of P alpha less than 1. If P alpha is less than 1, then it is definitely helix breaker. So that means, we know that there is no longer helix formation there right.

So, initially we had this helix formation, now we are looking for helix breaking by extension. If average P alpha over whole region is greater than 1, it is predicted to be helical ok.

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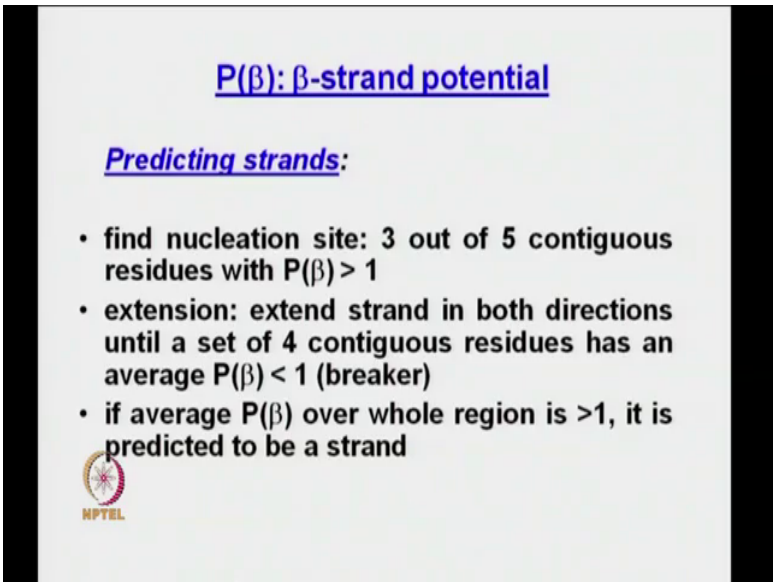

For beta strand again the same thing will happen, find nucleation site 3 out of 5 contiguous residues with P beta greater than 1, extension extend strand in both directions. Until a set of 4 contiguous residues of 10 with P beta less than 1, when I am talking about P beta less than 1 what I means, I am talking about beta breaking that means, it is not conducive for beta sheet formation. If average P beta over whole region greater than 1; it is predicted to be a strand.

So, essentially Chou and Fasman said that these are some guidelines based on which you can predict your secondary structure. See it is you know it is not so hard. Once you know, which are the alpha which are the amino acids which are prone to form alpha helices or we found in a alpha helix and beta sheet. Then you can go you know go about doing these necessary justifications or logical conclusions ok.

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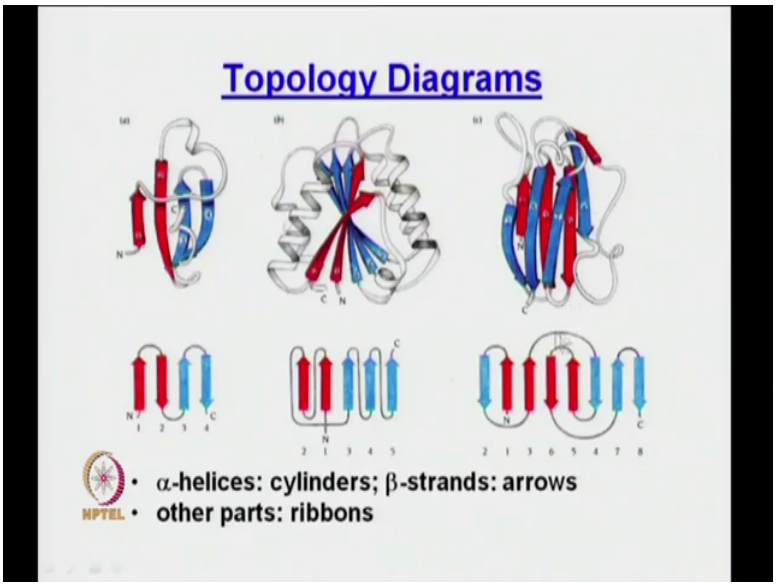

Now, that is what you know I wanted to talk about in terms of secondary structure. Remember secondary structure is very important alpha and beta, we have talked about then we have also looked about turns. Then we have also looked about some amino acid preferences and whether or how we can try to predict based on the given numbers or based on the preferences what sort of secondary structure sequence can take ok.

But you have realize one thing. It is not always easy. So, suppose you are trying to teach me and you are trying to tell me ok. So, this is how the protein looks like and if the protein has alpha helices and beta sheets and everything. And then if you are trying to tell me how a protein looks like, just by drawing with your hand pen or pencil, then you really have to draw the alpha helix or draw the beta sheet now that is not always easy.

So, doing of easier ways of trying to depict these things the schemes; so, this is what the topology diagram was look like you look at this. So, I am mainly looking at the beta. So, this is N so, these arrows. So, this is a beta you know this is a beta strands given beta sheets all these are beta strands.

Now, when I go for the topology see how it looks like. So, this one is looking at this was strand, this is just a loop I am showing again it is like this; like this. So; that means, I have simplified these very I simplified these very you know terrifying looking a structures to very simple structures. As alpha helices can be denoted as cylinders beta strands as arrows I just showed you, I am just showed alpha helices here I will show it to you later.

So, then this is what topology means topology essentially means nothing, it just tells you ok. I have this complicated protein structure if going to tell you how protein structure looks like in easier terms, I would rather go by the topology rather than the individual you know stereo chemistry and all that is what you lease in topology.

You do not know about the individual facets or the individual we know characteristics, but what you know is essentially the topology the overall scheme or the overall structure. And it becomes really handy if you are trying to explain something to somebody.

So, the other parts apart from alpha helix and beta strands, the other parts essentially ribbons that is what you see this ribbons and all out here ok.

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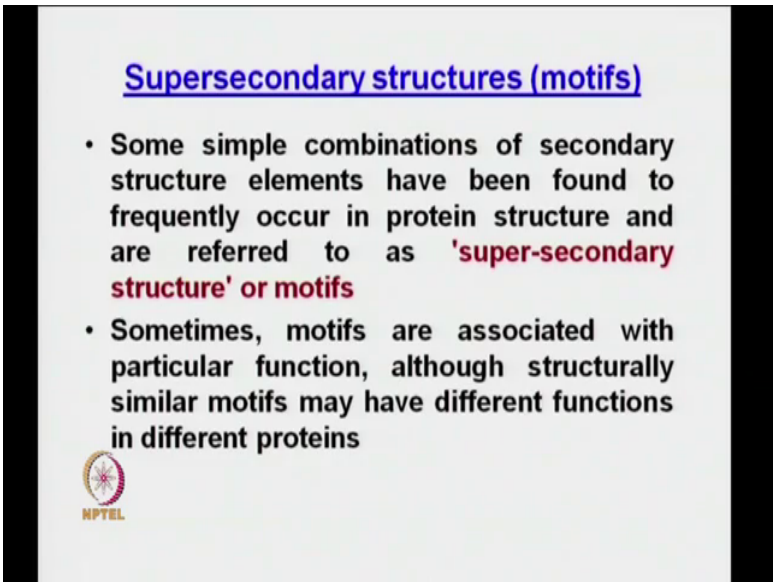

Now, what about super secondary structures? We have talked about secondary structures turns and all. So, what is super secondary structures? Remember when we talk about secondary structures secondary is the alpha helix or beta sheet ok. Those by individual; that means, after like one continues beta sheet it needs to have two strands, then you have the loop and all.

So, here when we are talking about is we are talking about a combination of secondary structure and you will see what I mean here. Some simple combinations of secondary structure elements; have been found to frequently occur in protein structure and are referred to as super secondary structures or motifs. You must have heard this what protein motif, if you have you know gone through you know paper related proteins and all. So, motif is a very commonly

used word and this is what it means simple combination of the secondary structure elements ok.

Now, sometimes motifs are associated with particular functions although structurally similar motifs may have different functions in different proteins ok. So, motifs again we often talk about motifs and this is what a motif means.

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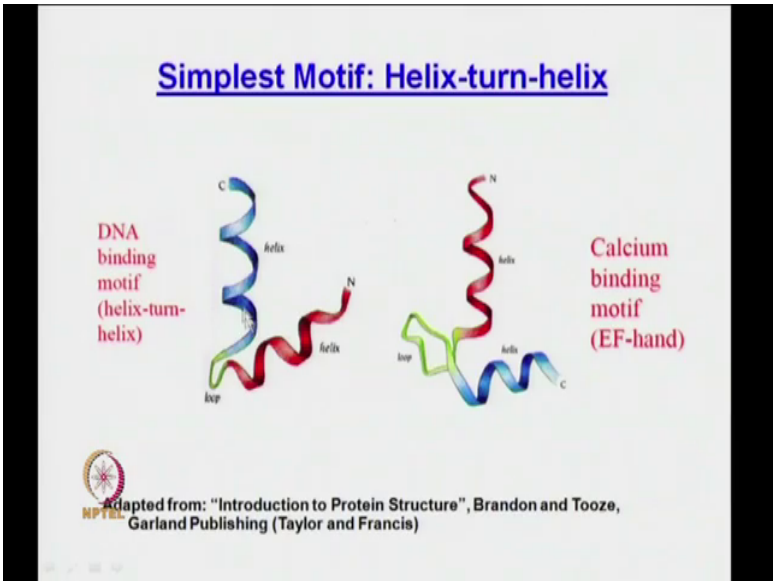

So, let us look at an example. So, here what I see out here is on the to your left, this is an helix, this is a loop, this is a helix, its helix, turn helix; I can say it is a DNA binding motif. So, this motif in DNA binding proteins you were often see this motif being present. Then this is the calcium binding again this is helix, this is helix this is the turn the green one again this is helix right; it is the calcium binding motif often known as the EF hand.

So, this is what the helix turn helix is; that means you have a helix. So, this helix write this helix red one then, this green which is turn and then the blue one which is the helix same here. This is the red one then the turn then the blue helix ok.

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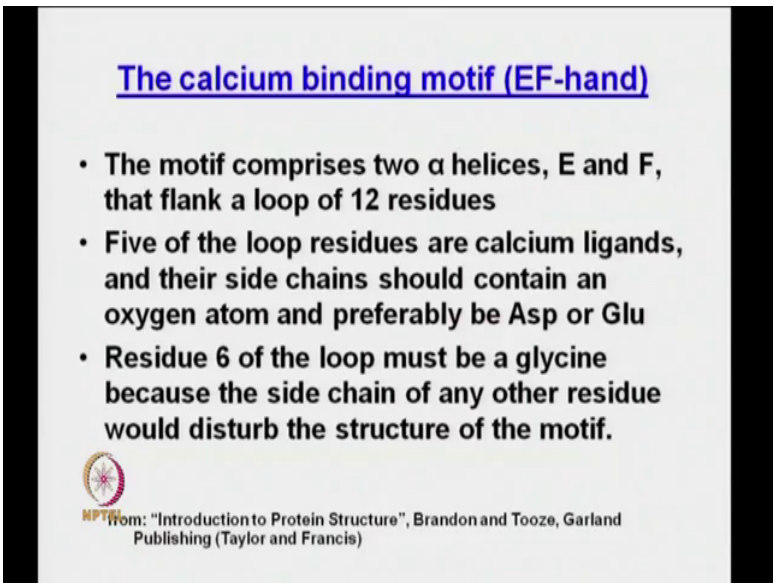

The let us concentrated on one the calcium binding motif that is the EF hand. So, this motif comprise of two helices, E and F that flank a loop of 12 residues. Now, five of the loop residues are calcium ligands and their side chains should contain an oxygen atom and preferably be either aspartate or glutamate.

Now, residues 6 of the loop must be a glycine because, the side chain of any other residue would disturb the structure of the motif. Now, remember glycine is very flexible. So, here glycine is needed.

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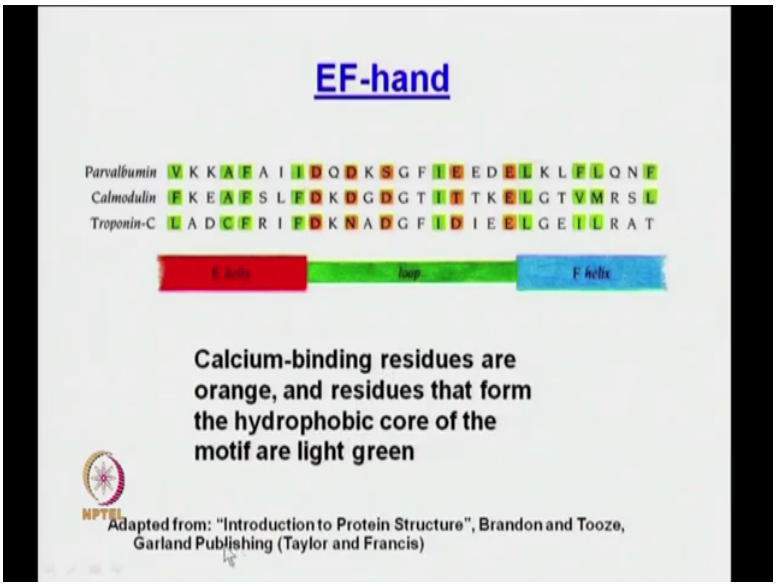

Now, the EF hand if you look at the sequences this parvalbumin, calmodulin and troponin C. So, these are calcium binding proteins, they are characterized by this presence of this EF hand motif ok. The EF hand I will show you what the EF hand means again.

So, here you can see this is E helix that is why it is E then loop, then the F helix ok. So, you know this motif is found by the E and the F helix helices with the loop in between. So, this loop these are the corresponding amino acids which have which correspond, you can see this under this stretch.

The amino acids of the loops we can see this is aspartate right aspartate out here that is what we needed, you also have a glycine which we need to have then we have this glutamate out here right, which was necessary this oxygen atoms ok.

So, the calcium binding residues are orange and the residues that form the hydrophobic core of the motif are light green. So, these proteins are calcium binding and the residues which is shown in orange other ones which actually bind a calcium or core into calcium how. Let us look at the next slide.

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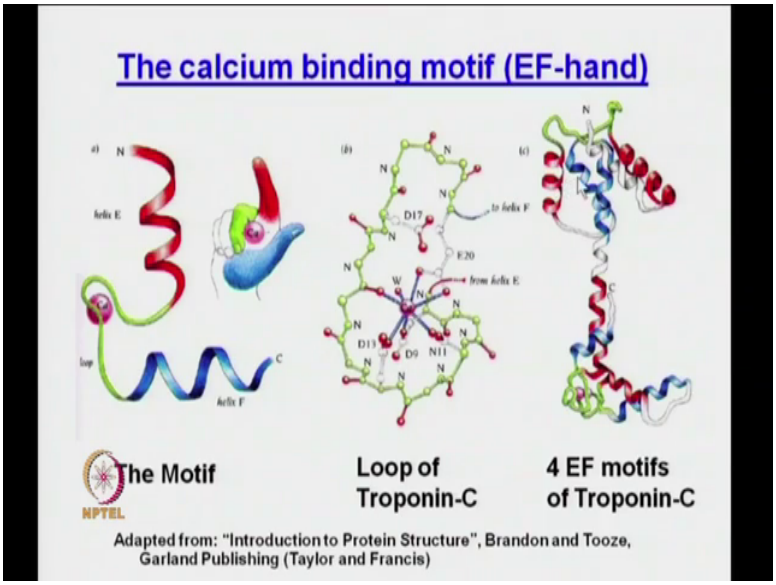

So, this is what I was talking about you can see this is helix E this is helix F, this is the calcium where it is bound this is the loop where calcium is bound ok. This is call the EF hand, you can see this is the calcium right, this is helix E red this is helix F blue and this is your loop region and the calcium is bound here.

Now, how is the calcium bound as I said this is the motif now this is the loop of troponin C. The calcium binding protein you can see this calcium here ok. There is a D 13 aspartate, there is a D 9 and aspartate, there is then and N 11 and asparagine then here water molecule W out

here. Then you can see this is E 20 glutamate which is also kind of here ok. So, this is helix E this one is helix E and this one is helix F.

So, this is how the calcium is coordinated too ok. So, the calcium is coordinated to all these different surrounding atoms, that is why the presence of those atoms was really necessary ok. And here you looking at troponin these are the four EF motifs you can look at the 4 EF motifs.

So, 1 2 3 4 green regions, these are the calcium atoms out here you can see this is 1 helix this is other helix. So, these two these so, this one this one or this one and this one for example, this one and this one they form the EF hand this one and this one they form the EF hand and so on.

So, there are two more on the other side ok. So, this is then an helix turn helix motif or EF hand which is responsible for calcium binding. The other one remember was responsible for DNA binding, that we looked at the first slide when we started talking about this motifs.

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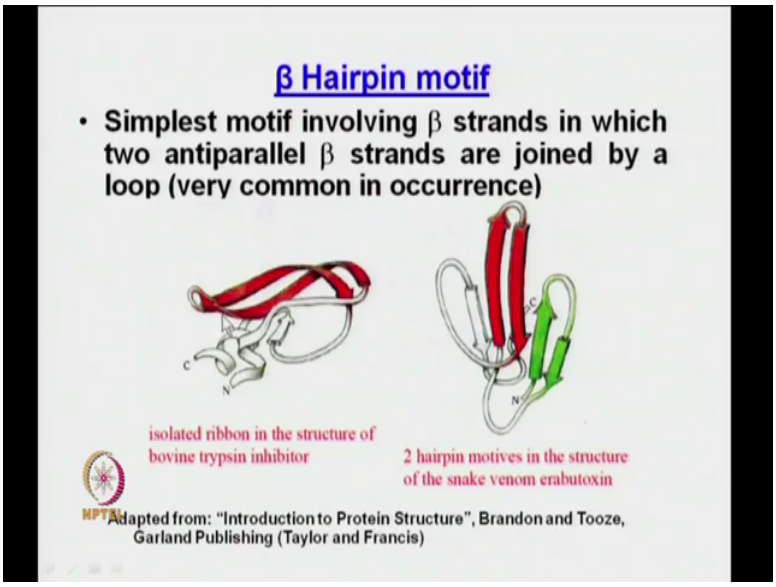

Then what is the other kind of motif? It is a beta hairpin motif remember the beta hairpin, we talked about its the simplest motif involving beta strands in which 2 antiparallel beta strands are joined by a loop. It is a very common in occurrence ok.

So, that is what you see. Here also you can see this is 1 beta strand, you can see the arrow out here this arrow pointing this direction for this one. It is pointing in this direction; that means, these are antiparallel here also this one is pointing in this direction this one is pointing this direction. This is antiparallel ok, and these you can see this hairpin loop this hairpin loop or this hairpin bend ok. This is from the bovine trypsin inhibitor and this is from the snake venom erabutoxin.

So, this is what I mean by a beta hairpin motif so nothing much you just need know. If you look at this you know this is what beta hairpin motif is nothing big, I have 2 antiparallel beta strands connected by a bend loop.

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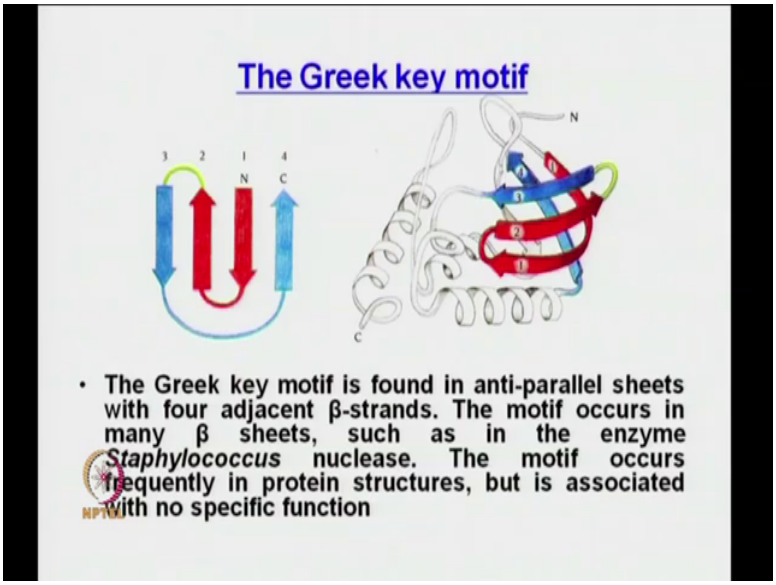

Then the Greek key motif: So, this is how Greek key motif looks like. So, the Greek key motif is found in anti-parallel sheets with four adjacent beta strands the motif occurs in many beta sheets. Such as in the enzyme staphylococcus nuclease, this motif occurs frequently in protein structures, but is associated with no specific function.

So, this is important it is know that every motif has to be associated with the function it is like the EF hand the calcium binding it was calcium binding, then the DNA binding ok.

Here this Greek motif it does not have a function, but you can see these are four adjacent beta strands that is what we are saying. So, all these are antiparallel and that is how we are having these loops coming through, remember these are also topology diagrams ok.

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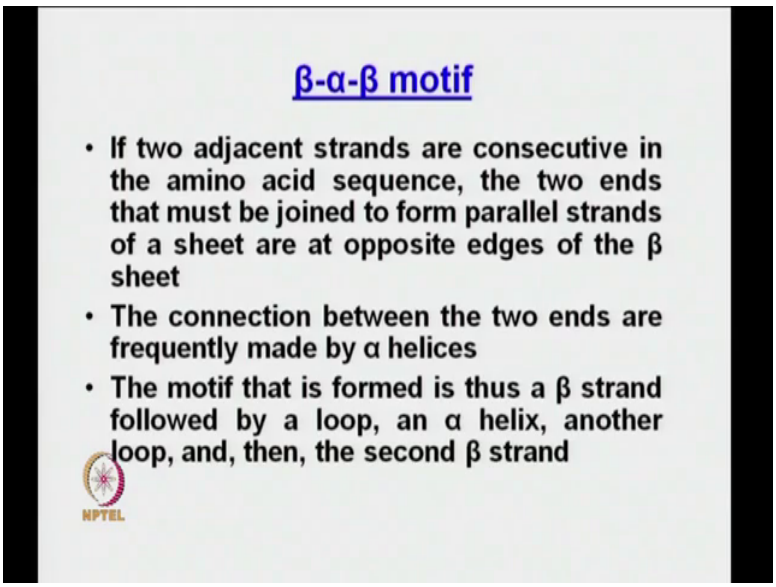

Then the beta alpha beta motif: So, if two adjacent strands are consecutive in the amino acid sequence the two ends that must be joined to form parallel strands of a sheet are at opposite edges of the beta sheet I will just tell you. So, what I means is if you have one thing like this and if one thing like this, they are going like this.

So, but they are not being connected here, what will happen is you have to come from here, you come all the way here from 1 edge to the other edge and then connect is not it. So, that is

why you need this alpha and that is why the beta alpha beta motif is so, important and you will see very soon.

The connection between two ends are frequently made by alpha helices and the motif that is formed is thus a beta strand followed by a loop an alpha helix another loop. And then the second beta strand, this is what I was talking about ok.

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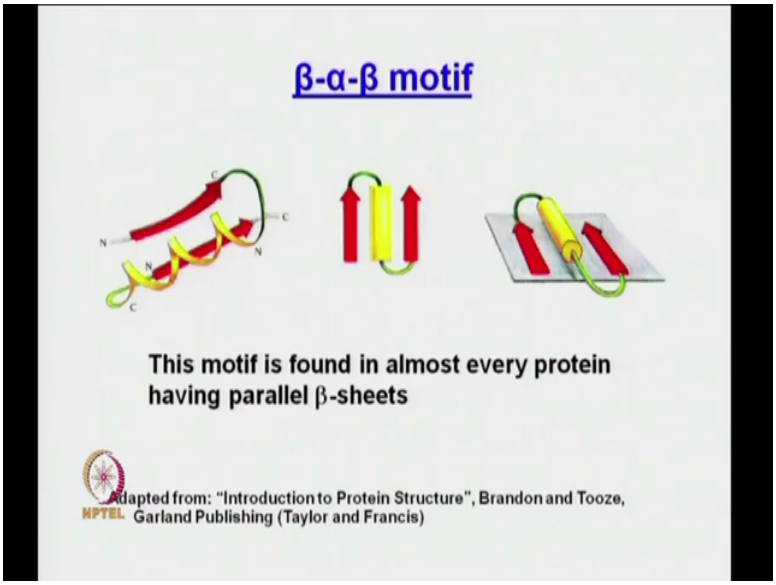

See this is NC going the same direction again this NC going the same direction, but for this one to be connected with this one I cannot disconnect here right that is not that is not the way the sequence goes like N to C, then again N to C.

So, what I do is I have to take this whole loop, I have to come here from one edge and I have come to the other edge and connect to the back which is here the N terminus. And how do we

connect what we do is we use a alpha helix that is most commonly used to connect between two parallel beta sheets right. And that is what I it is called a beta alpha beta motif.

Now, you can see the topology diagram this is the first time I am showing you how an alpha helix is depicted remember, alpha helix is depicted as cylinder that is what you see. That is not a big deal because alpha helix look like a cylindrical coil ok. So, this is 1 beta second beta two strands parallel and then they are connected by this alpha giving rise this beta alpha beta motif.

So, this motif is a found in almost every protein having parallel beta sheets ok. So, I guess we have talked enough about the secondary structure, super secondary structure I mean after that and then which are these motifs.

So, what I will do is in the next classes what I will do is I will talk little more about, you know other things like domains. And this helix packings difference sorts of packings how you know in the tertiary structure, how these helices or beta are they are packed in this each other. So, you will try to look at this in little more detail in the next class. And after that we start or you know or we go on with the journey. And look at the forces that are available in proteins right that would be very important.

So, after having looked at structures some structural aspects, after we have got you know some idea of what different structures are possibly, if you take a protein would be able to recognize ok.

This is the structural element we talked about or this is the sort of motif we had we talked about right. You would possibly you know it resemble something we had discussed in class. And then the next; obviously, big thing is what are the things what are the different forces, that hold these different secondary structural elements of the whole protein unit together ok.