Fundamentals of Protein Chemistry
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Module - 02
Protein Architecture
Lecture - 07
Secondary Structure

In the second lecture for module 2, we will be dealing with protein architecture, we will look at the secondary structure of proteins. We looked at the primary structure which was the sequence of the amino acids and from the sequence of the amino acids we found out that the connectivity, that is the peptide bond, is the covalent linkage that holds the sequence together and in addition we have the disulfide bonds.

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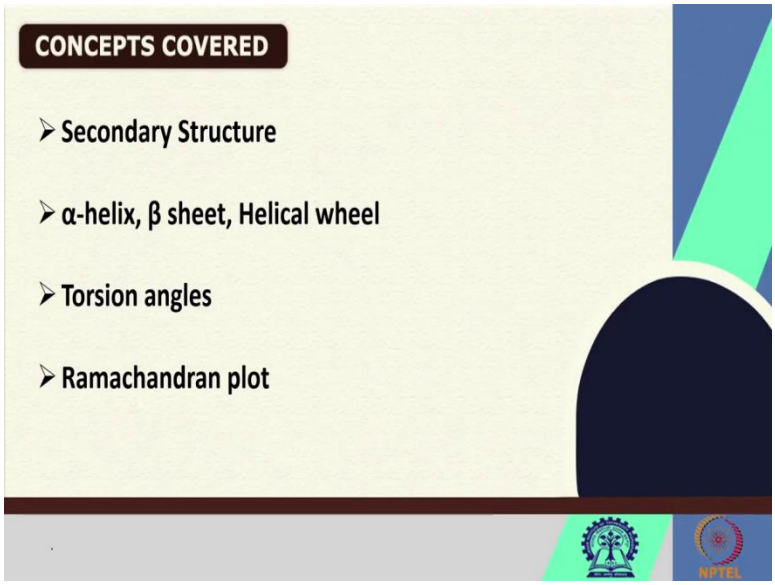

In the secondary structure component what we are going to understand is, we are going to study what we mean by an α-helix, a β-sheet and construct a helical wheel and see what this means. In addition the most important being the geometry of the backbone in the specific torsion angles, which we touched upon a bit in the last class when we also spoke about the rotamers for the amino acid residues, that is the rotation about the single bonds that are possible. And here we will discuss the important Ramachandran plot.

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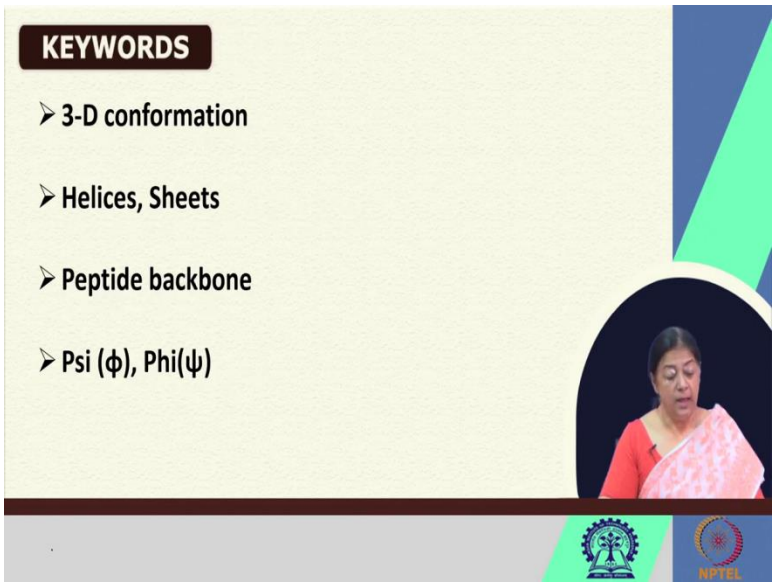

The 3-D conformation will come later, when we consider the three dimensional structure of proteins and how they are formed in protein folding. And we will look on helices sheets, the peptide backbone and the important torsion angles-the φ ψ angles.

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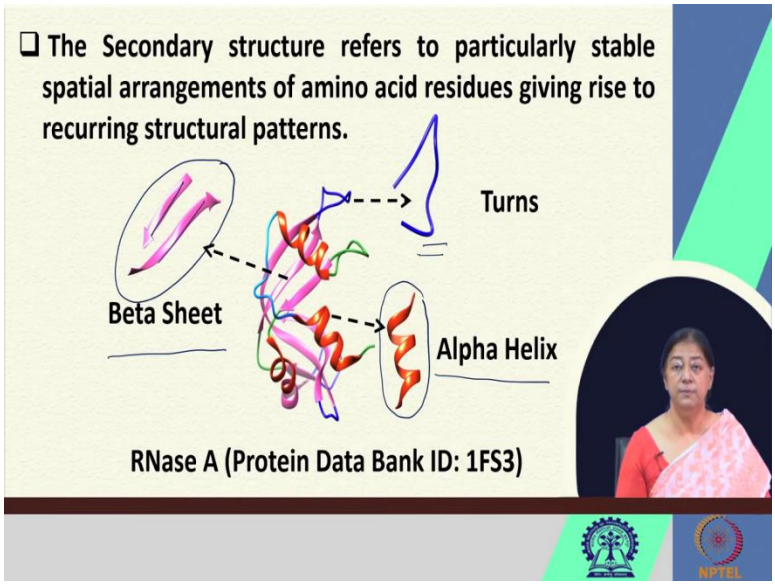

Now what do we mean by the secondary structure of proteins? We understood that the primary structure is the linking of the amino acid residues together through the peptide bonds and that this could form just a straight chain. But we know from our knowledge that it does not remain as a polypeptide chain in a straight form, it folds.

Now this folding can have specific patterns. One of these geometric patterns that is very unique is the formation of the secondary structures. This secondary structure can be what is known as the α-helix or the β-sheet. What we see in this particular protein [refer to slide] that is RNase A, this is a segment of the protein that forms a specific geometrical pattern. This is another specific form that is present in the protein known as the β-sheet, that is formed from separate strands and these give rise to recurring structural proteins or patterns that are observed in proteins. We will look at these in a bit more detail and another factor is the turns, that actually are linkers to these secondary structure elements as they are called.

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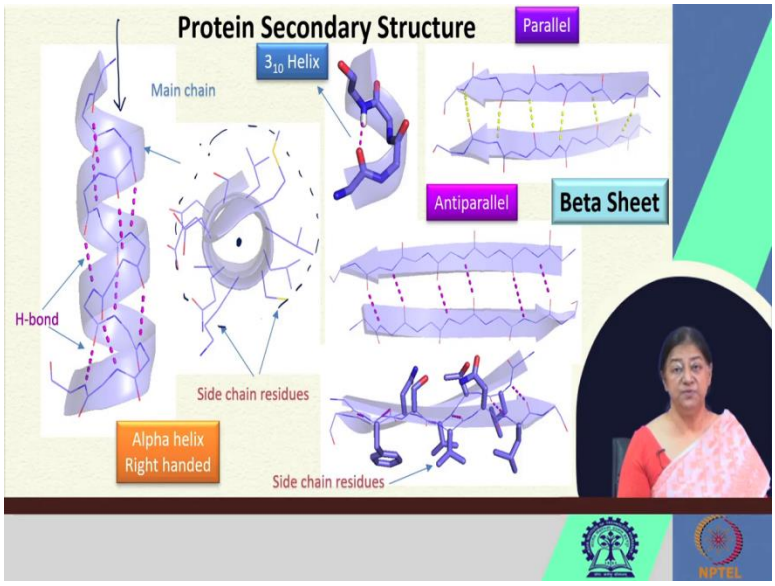

So when we look at protein secondary structure, we look at a helix, the α-helix. The most common α-helix present in proteins are known as the right handed helix and we know that it is a right handed helix by its direction of propagation. If we consider our right hand and we consider the direction of propagation, we know that this is a right handed helix.

Now, there are specific characteristics to these helices and there are reasons why they are held together in such a spiral fashion. This is because we have the main chain form this spiral form and the interaction is through specific hydrogen bonds. They are formed between specific amino acid residues which we will see in a moment.

When we look down the helix axis what do we see? We see surrounding the helix axis are all the side chains protruding out from the helix axis. We will see how this is important in several aspects.

The other secondary structure element is the β-sheet that is formed of strands. These strands can be parallel in fashion or they can also be anti parallel in fashion. And in addition to this when we look at the side chain residues, the connectivity in this case is also through hydrogen bonding interactions. This is what holds secondary structural elements together.

So the common feature of the α-helix and the β-sheet is that they have hydrogen bonding interactions that hold the helix the way it is or the strands, be it parallel or anti parallel. Another type of helix that also can be formed is the 3_{10} helix. We will look at these specific types of helices in a moment.

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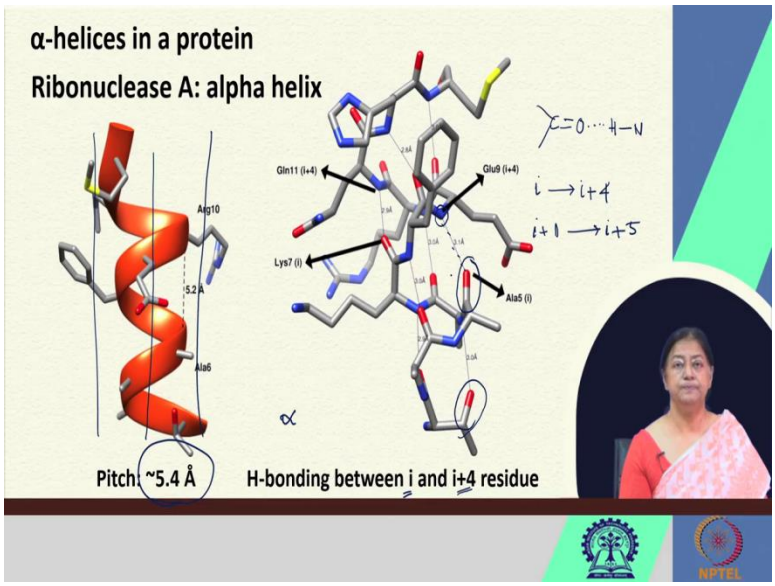

When we look at the hydrogen bonding patterns of these helices, the most common hydrogen bonding pattern is the one that we usually refer to as the α-helix. It is the hydrogen bonding between the i and the i + 4.

[Refer to slide] so we are looking at a hydrogen bonding between the peptide backbone residues. This is the C = O and this is the NH behind there and this is another C = O and NH.

So this bond is the hydrogen bond that holds the helix together, in this particular type of orientation. So, when we have this i to i + 4 bonding, then i + 1 will have a hydrogen bonding to i + 5 and so on and so forth, that will hold this in this particular fashion where we will see the side chains protruding out.

Now, this means that there is a particular turn of the helix and there are 3.6 residues per turn of the regular right handed α-helix that has the hydrogen bonding from i to i + 4. This means that there is a pitch to the helix which is 5.4 angstroms in length. The hydrogen bonding distances are usually around 3.4 angstroms.

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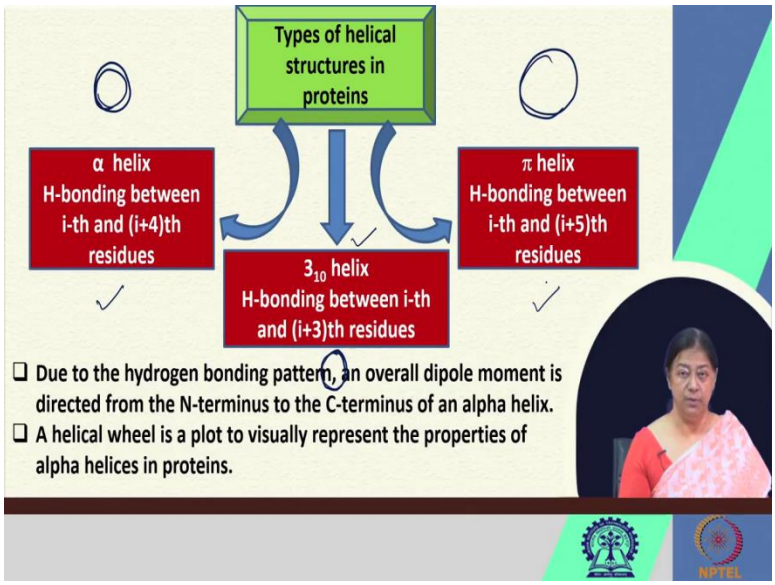

The types of helical structures that are present in proteins are the α-helix that is the common right handed α-helix, that has the hydrogen bonding between the i and the i + 4 residue, there is the 3_{10} helix that has the H bonding between the i and the (i + 3)rd residue and then there is also a helix called the π helix which has a hydrogen bonding from the i to (i + 5)th residue.

So it means that if we look down the axis of each of these helices we will see that the 3_{10} helix would be tighter because the rotation would be such that there would be a pitch that is smaller, the α-helix would have the i to i + 4 and the π helix would be a bit larger because we would have an i to i + 5.

Now due to the hydrogen bonding pattern, there is also an overall dipole moment because we know that there is a directionality to the hydrogen bond. The hydrogen bond as we learnt is formed between the C = O and the NH; the C = O of the ith residue to the NH of the (i + 4)th in the α-helix, i + 3 in the 3_{10} helix or i + 5 in the π helix and that is how we have the hydrogen bonding pattern in the specific helices.

Now what we can do is, we can plot what is called a helical wheel to visually represent the α-helix which we will see in a moment after our discussion on β-sheets.

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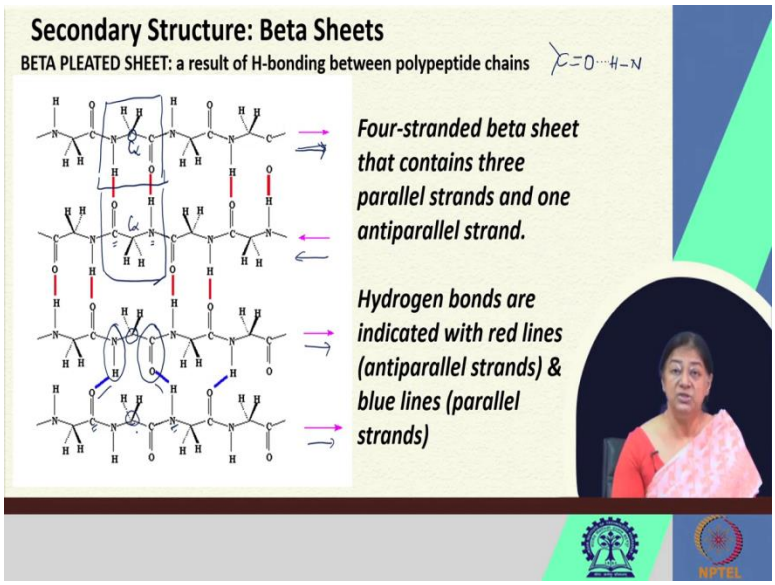

For the β-sheets what we observe is the hydrogen bonding pattern again between the polypeptide chains. Now what we observe in the hydrogen bonding pattern is a pattern that looks at a specific set where we have again the C = O hydrogen bonded to the NH.

Now when we look at the pattern that is shown on the left [refer to slide] it is a β pleated sheet that has specific strands to it. What do we see? We see 3 of these strands that are parallel in nature and one that is anti parallel, in the sense that it is going in the opposite direction. When we look at the hydrogen bonding pattern of these, we first try and observe the directionality of the backbone. We know that we have the direction of this particular polypeptide chain in this direction. Now when we have the Cα we try to recognize the Cα, saying that this is one particular amino acid. We know this because the Cα is connected to the NH and the C = O.

Similarly, if we look at the strand that is going in the opposite direction, this [refer to slide] is also one amino acid. This is the beginning of the amino acid, this is the Cα and this is the end of the amino acid because it is going in the opposite direction. Now, if we look at the hydrogen bonding pattern between these two specific strands, we will observe that in this amino acid the C = O is connected through hydrogen bond to the NH of this amino acid and the C = O of the same amino acid is linked through hydrogen bonding to the NH of that same amino acid on the other strand.

This is unlike what we observed for the parallel case, where we will see that again if we look at the Cα here and the Cα here what we observe is that the NH of this particular amino acid or the C = O of this particular amino acid, is hydrogen bonded again to the same type of chemical moiety, but to different amino acids. So this is the hydrogen bonding pattern difference that we observed for the parallel strands and for the anti parallel strands.

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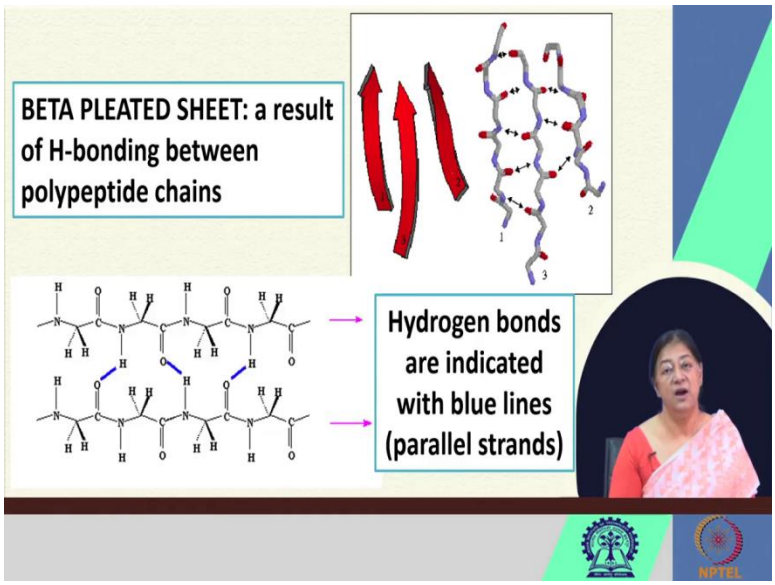

For the β pleated sheet where we saw the hydrogen bonding pattern we realize that this particular hydrogen bonding pattern is different for the parallel and the anti parallel sheets and while we look at the hydrogen bonding pattern, we can distinctly see what kind of strands are forming this particular β-sheet.

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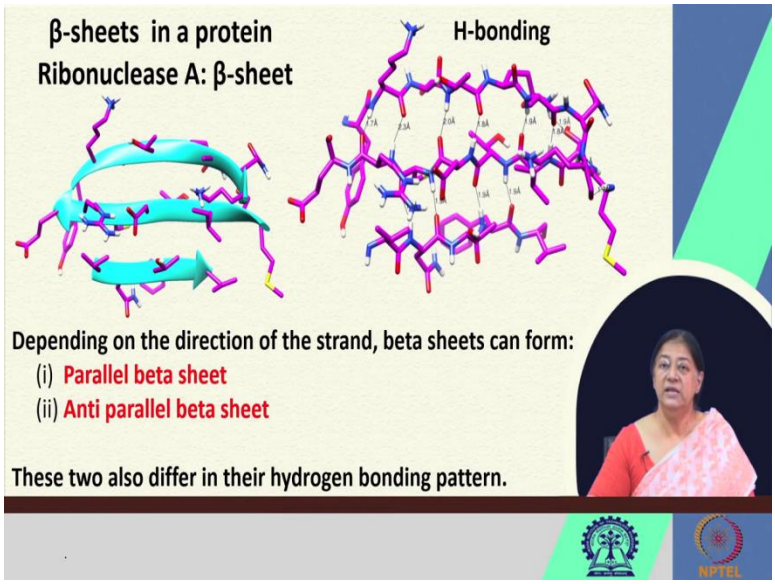

The direction and the hydrogen bonding pattern of the strands are important. Now how is this important? We realize that when we are looking at the two different types of secondary structures that is the α-helix and the β-sheet, the strands in the β-sheets, they are held together by hydrogen bonds.

This is what is common for the α-helix and the β-sheet, but what we realize in the β-sheet type of characteristic hydrogen bonding, the strands whether they are parallel or anti parallel will have

different patterns. Similarly, the hydrogen bonding nature for the α-helix would depend upon the type of helix that we have in terms of whether its going to be i + 4, i + 3 or i + 5.

Now, another major difference between the α-helix and the β-sheet is their specific locations in the polypeptide chain. While in the formation of the α-helix, we realize that we have to have a continuous chain, in the β-sheet case the strands can come from different parts of the protein and they may not be continuous in nature, in the sense that we could have even a helix between the strands of a β-sheet.

So, while we are looking at an α-helix, we know that it has to be continuous in the polypeptide chain, but the strands in the β-sheet can come from different regions of the polypeptide chain, bringing them together in specific patterns that are known as the secondary structure β-sheet.

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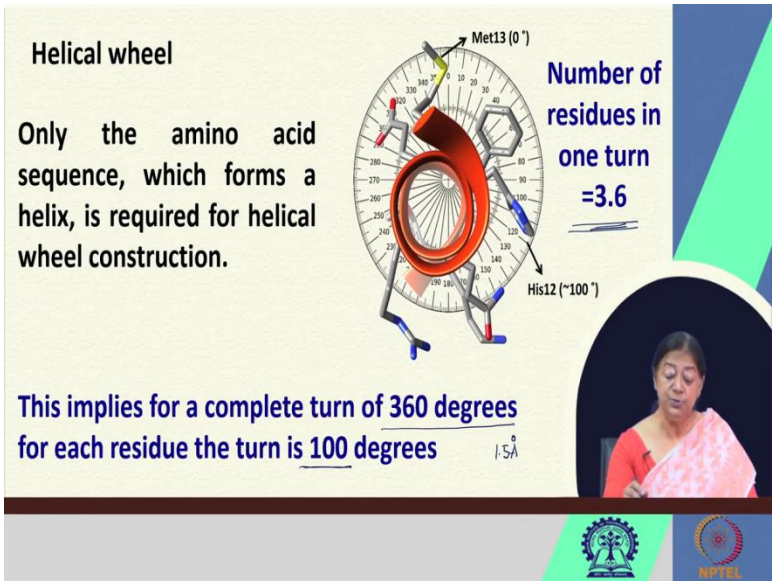

What we will now look at is what we mean by a helical wheel. Now, the construction of a helical wheel is very simple and it is very interesting as to the information that it can provide us with. What we need for this is, we need only the amino acid sequence. So, a knowledge of the amino acid sequence which is forming a helix is enough for a helical wheel construction.

Now what we know from looking down the axis of the helical wheel is that, the number of residues that we need for one complete turn for a regular α-helix is 3.6. So given that a complete circle is going to be 360 degrees, we know that when we go from one residue number 1 say to residue number 2, we are going through a rotation of one point or a 100 degrees and the rise is 1.5 angstroms. This implies that for a complete turn of 360 degrees; given that we need 3.6 residues for a complete turn, for each residue the turn is 100 degrees and the rise is 1.5 angstroms, that when we multiply by 3.6 gives us the pitch equal to 5.4 angstroms.

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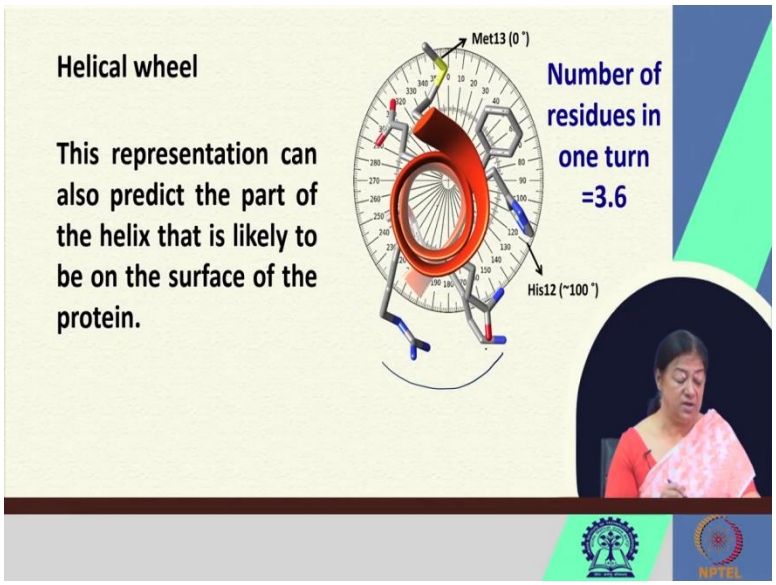

Now if we look at the disposition of the side chains, we see they are all away from the helical axis. What do we mean by this? If we have a specific residue and then we want to look at the location of the next residue in the helix, it is going to be a 100 degrees away. So, the representation can also actually predict which part of the helix is likely to be on the surface of the protein.

When we look into more of protein folding, we will realize that for regular globular proteins that have a solvent like water around it, what happens is the hydrophobic residues tend to be in the center of the protein; which means that the hydrophilic ones would be on the surface of the protein.

So if we look at a specific helical construction we will actually be able to predict in a manner which ones are likely to be at the surface or which face of the helix rather is near the surface.

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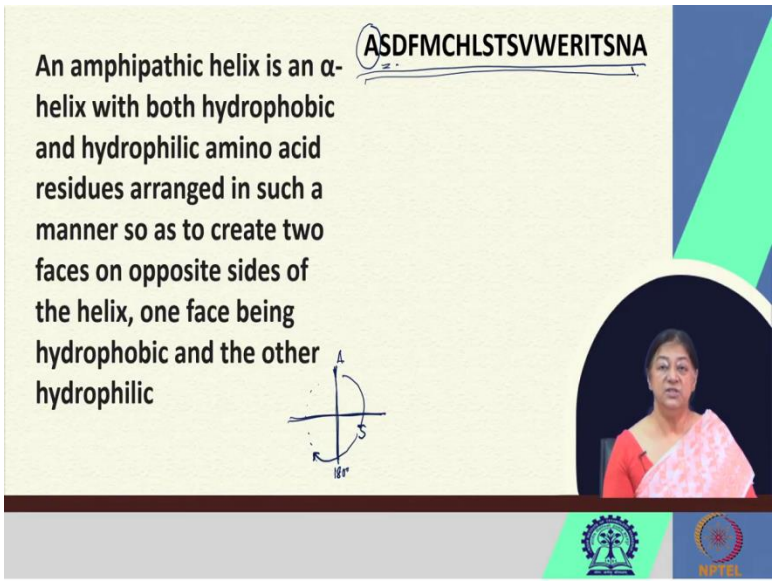

That means that we have an amphipathic helix in a sense that one part of it is going to be hydrophobic in nature and one part can be hydrophilic in nature, but then we can also ask the question whether this is always true. May not be so. We will answer this question as we go along.

An amino acid sequence is the all the information that we need to construct the helical wheel. What do we know? We know that residue number 1 A is going to be at the top say, the second residue serine is going to be a 100 degree. If we just draw an axis like this [refer to slide], we know that this is 90 degree. So we can say that this is around 100. If alanine is here it means serine is here. D, that is aspartic acid is going to be 200 degrees. We know that this is 180 degrees, so 200 is going to be somewhere around here. So the next amino acid will be here. Similarly we can go on doing this construction till we cover the whole sequence of amino acid residues that forms the helix.

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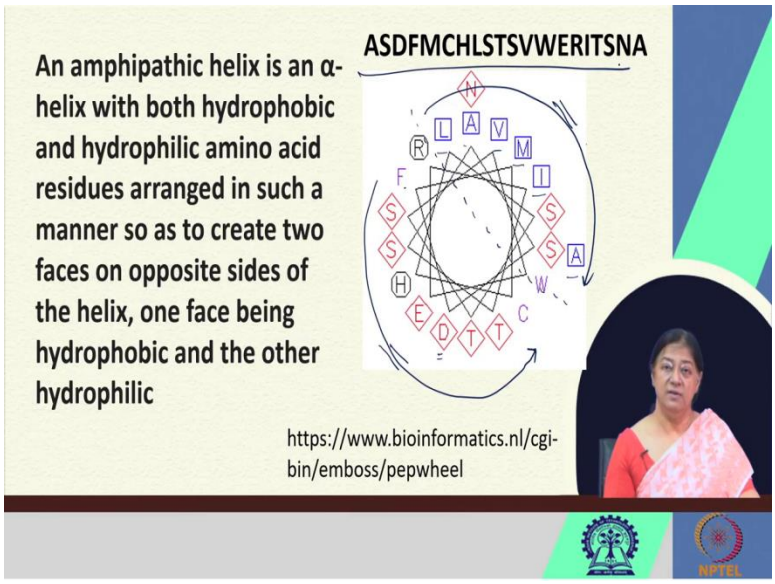

What do we see? [Refer to slide] this is what we see and this can be done very simply by using specific web or internet resources that are available to do this. Now let us look at the pattern. So this was the first amino acid the alanine, then the serine, then we can see the aspartic acid and so on and so forth. Now, if we look at the pattern, we see that the ones that are boxed are the ones that are hydrophobic in nature. We see some sort of a pattern in the sense that if we draw a dotted line along say somewhere around here, this part is hydrophobic in nature and this part is relatively hydrophilic in nature.

So what would we expect? If this helix were part of a protein, we would expect the hydrophobic region to be near the center or near the core of the protein and the hydrophilic region to be on the surface of the protein. So this is a very interesting way, just from the sequence, to identify which part of the helix is going to be present where in the protein.

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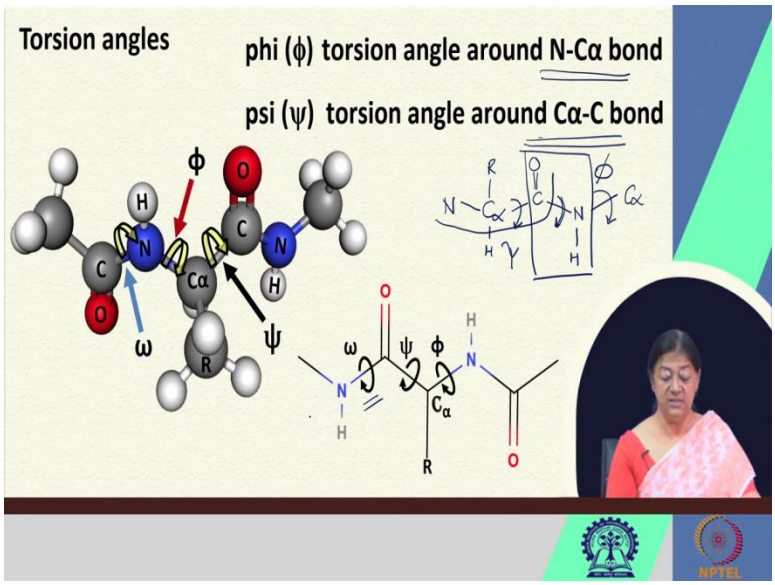

Our next important aspect of understanding the geometry of the backbone, which we touched upon in the previous class for the primary structures, is an understanding of the rotation about the single bonds. Because when we look at the rotation we are looking at a specific geometry and when we look at the specific geometry, we can try and correlate this with the pattern that is observed in the strands and in the α-helix. And this was done by Professor G.N Ramachandran which is now known as the Ramachandran plot, an extremely important plot in protein structure that decides upon some specific allowed regions and allowed angles for proteins. We will see what we mean by the allowed part.

So here is our ψ angle [refer to slide], it is the torsion angle around the Cα-C bond as you can see here, the φ angle is the torsion angle around the N-Cα bond. So, when we are look at the N-Cα-C=O we must remember that this is the backbone. We do not consider the H or the R for this right now, but this is how we would consider our C double bond. So this is the N-Cα-C=O and the next N comes right here. Now when we have the rotation about the Cα-C bond, this is the ψ angle. When we have the rotation about the N-Cα bond, this is the φ angle. What is this? This is our peptide bond. Our peptide bond rotation is known as the ω angle. So, we have the ω angle, the φ angle and the ψ angle.

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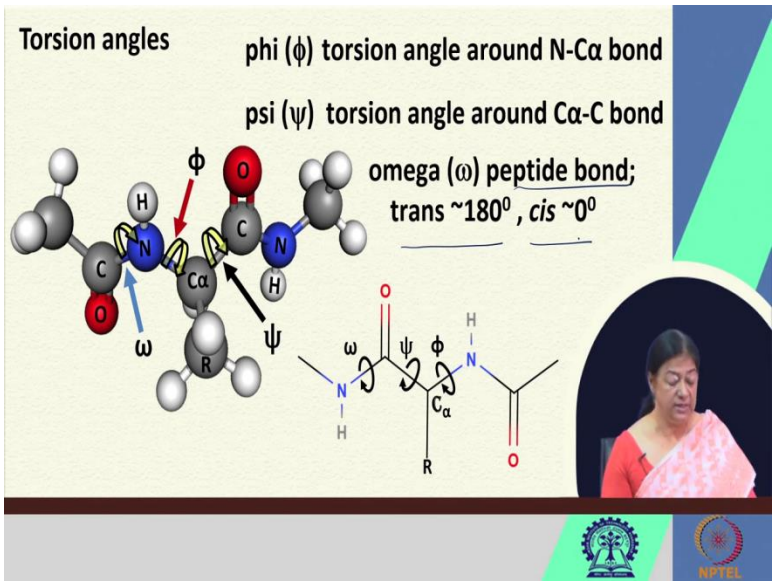

So the ω angle that we see is for the peptide bond. It is trans when it is 180. We know what the trans peptide bond means, we know what the cis peptide bond means; meaning an ω angle of 0, where the Cα is pointing in the same direction.

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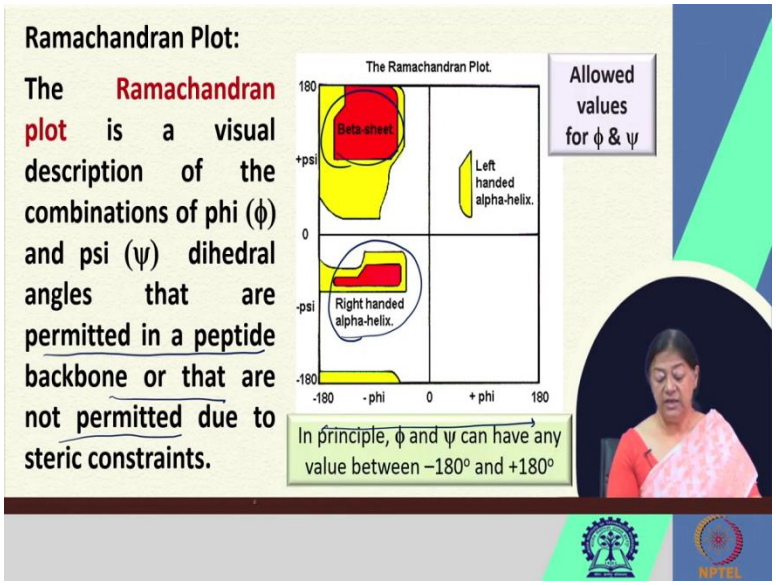

The Ramachandran plot is now a visual description of the combination of the φ and the ψ angles that are permitted in a peptide backbone, but are not permitted due to specific steric constraints. And interestingly the helices are [refer to slide] in this region, the β-sheets are in this region. Meaning that their φ ψ angles, because of the structural constraints, because of the specific geometrical patterns, occupy specific regions of the Ramachandran plot and some of the regions are not allowed.

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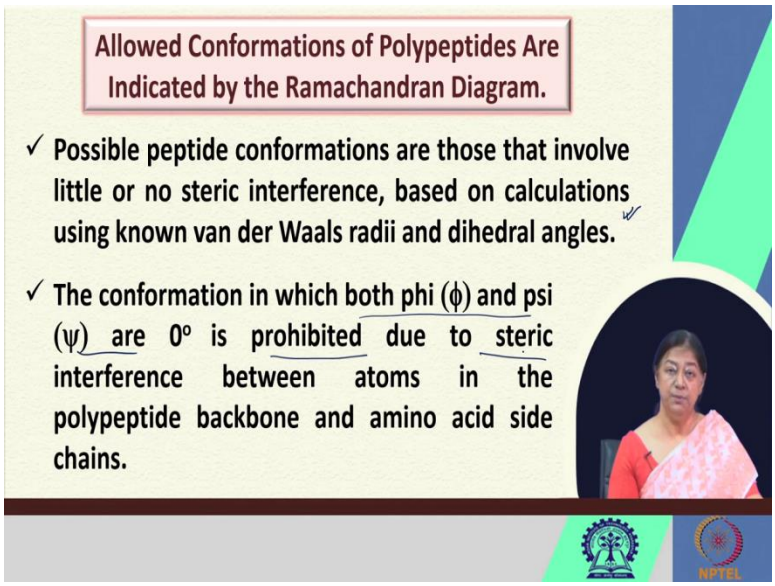

Why are they not allowed? [Refer to slide] these are the allowed values and when we look at the allowed conformations, there are possible peptide conformations that will not have steric interference. We do not want this rotation about the single bond to result in a clash and the conformation in which both φ and ψ are 0 is not possible, because of steric interference between the atoms in the polypeptide backbone.

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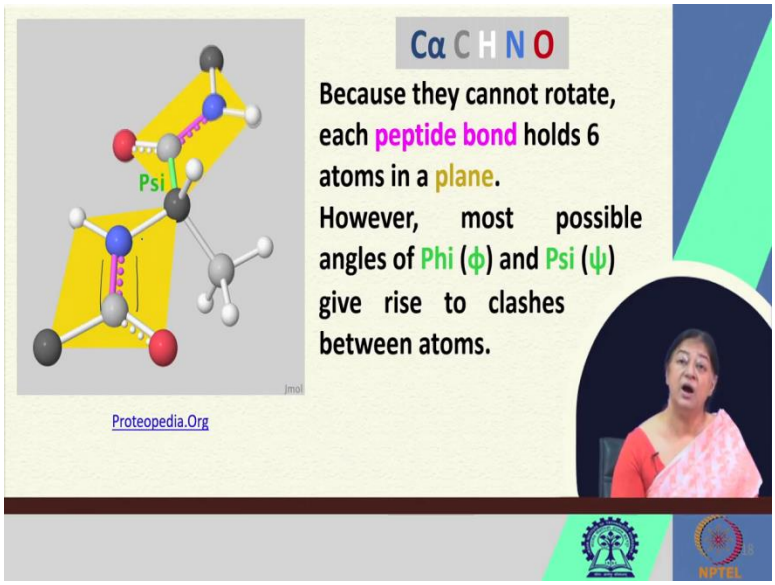

So, when we have the rotation they cannot rotate. We know that the peptide bond is planar because it has that partial double bond character, so it is not allowed to rotate by itself in the ω angle. However, there is a possibility of φ and ψ rotations.

But again when we look at the specific Cα attachments or rather the side chain attachments, we realize that when that bond is moving there is a possibility of steric clashes.

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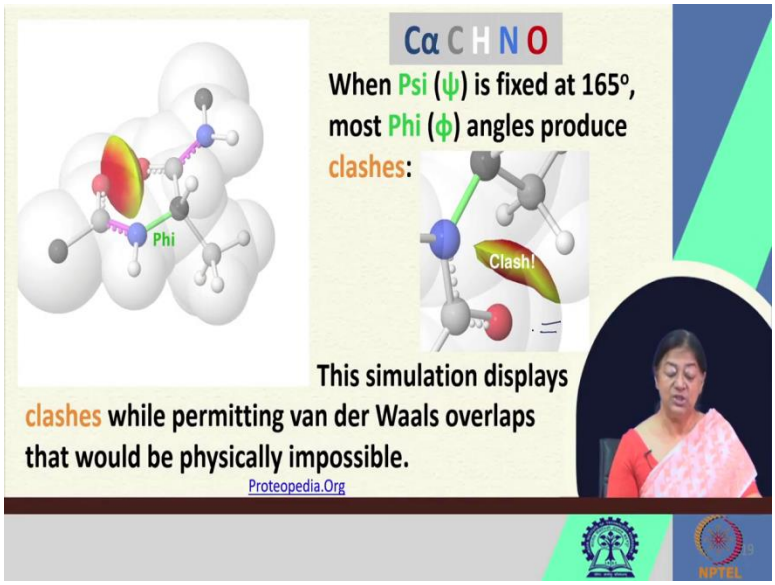

Now when we have the movement about the φ or the ψ angles we see the clash. With that clash comes φ angles, that cannot be allowed due to the steric interactions.

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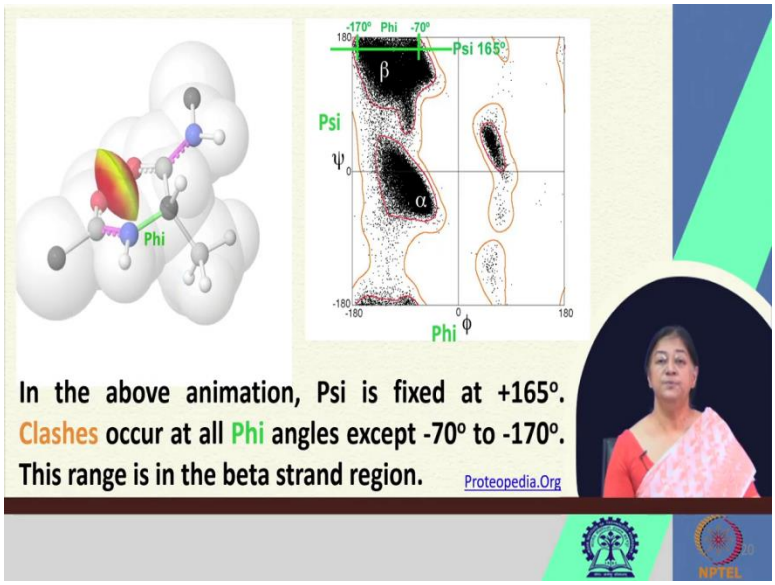

So the φ ψ interactions again give us specific regions of the Ramachandran plot, that can be occupied where clashes will occur for other possibilities. This is a very important feature of the backbone of the protein structures and this allows only specific orientations to be possible.

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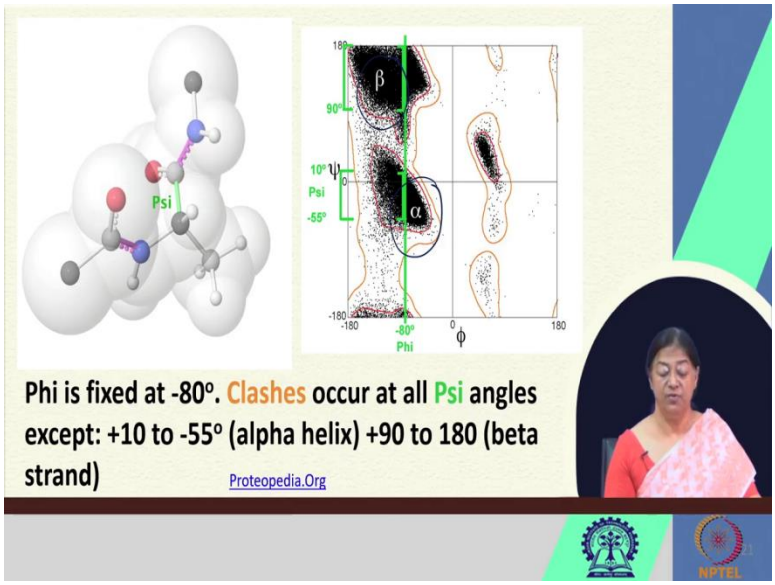

Again if we rotate about the ψ angle we see the same distinct possibility, where we see other regions that form clashes and some regions that are allowed. [Refer to slide] these regions will be allowed for the β and these regions will be allowed for the α-helix, the important secondary structural elements of proteins.

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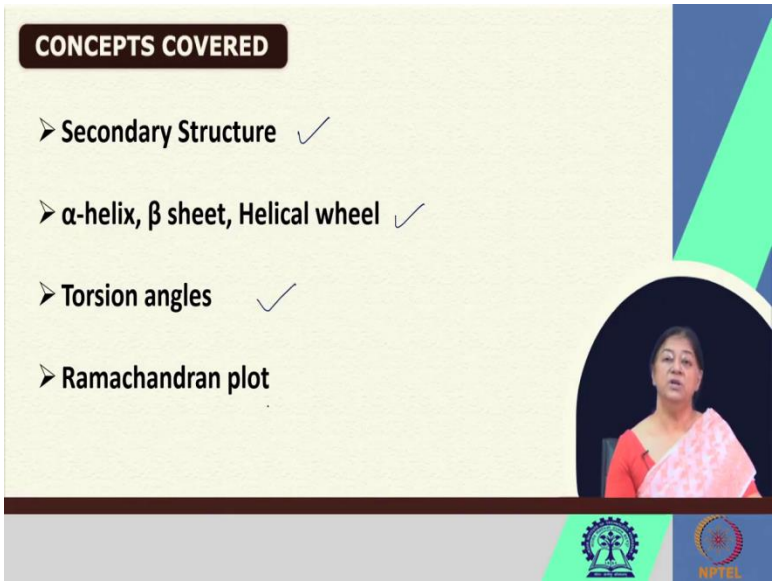

So the concepts covered are the secondary structure of protein, the α-helix the β-sheet and the helical wheel construction, specific torsion angles and most importantly the Ramachandran plot.

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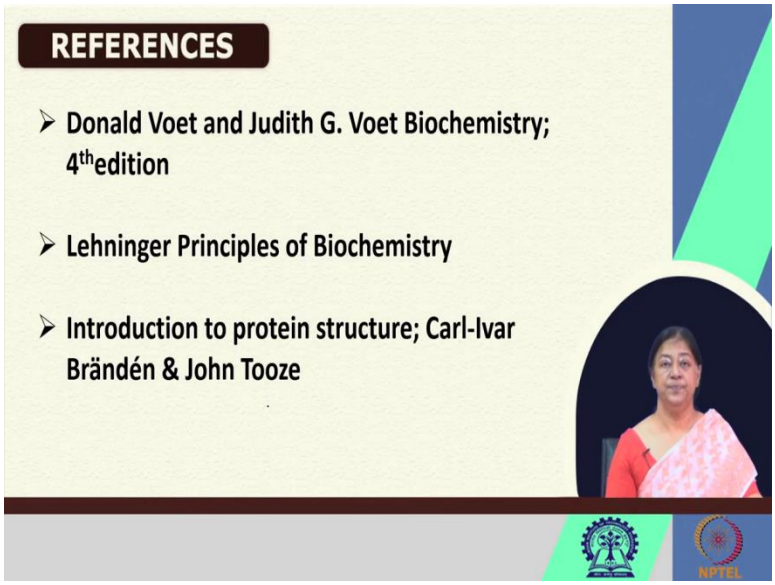

Thank you.