Bio-Physical Chemistry
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Lecture - 03
Secondary Structure of Proteins

Hello again everybody. Welcome to the class. So, we had started a discussion on the Secondary Structure of Proteins right. So, you know as usual we will just do a quick recap.

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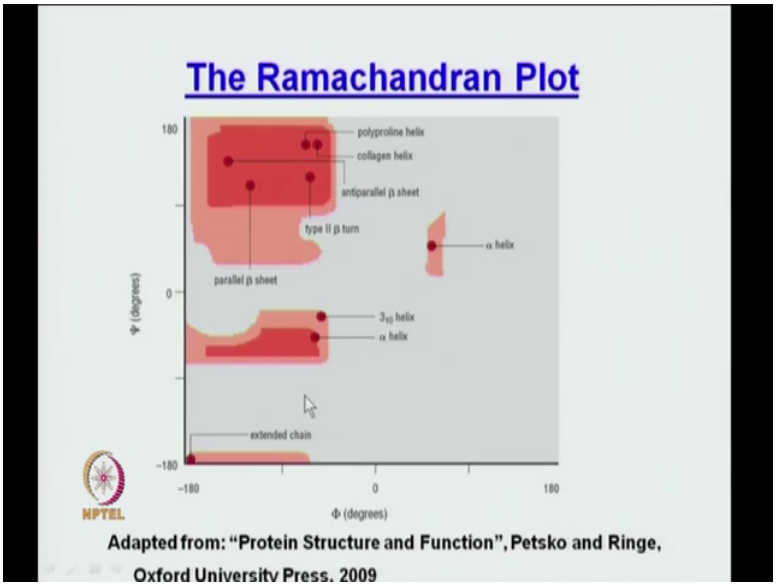

So, last time, we had talked in some detail about the Ramachandran Plot. I know you we talked about the torsion angles, we saw what the angles were, what they represented and you know how you could visualise the angles, what the different values of the angles meant, then we were looking through the Newman projections.

Then, we you know went ahead and looked at all also looked at those things in some details right. And so, the Ramachandran plot essentially what it tells you? It tells you the allowed psi and phi values. So, this is your psi in degrees and this is your phi in degrees and so, then you plot the value for a particular structure, you have a psi value and a phi value for the different amino acids right.

And so, what you can see here is this say if you look at this, this is essentially the helical region. So, and if you look at this area on the top this, is if you follow my arrow, this is your beta right to the beta sheet mostly right. Then, to the right you have the alpha helix, but this is a left handed alpha helix; generally, all the helices, most of the helices are right handed and this is extended chain.

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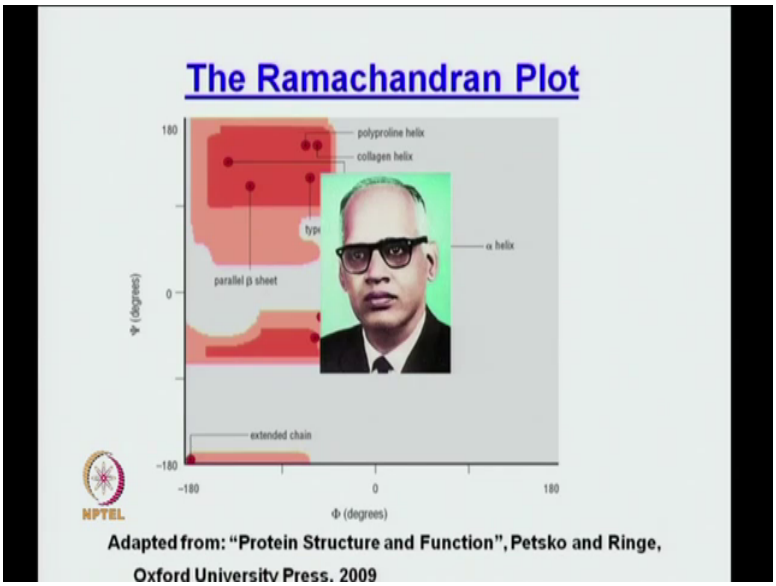

And then, we went forward and what we saw was well before going forward, so this is G. N. Ramachandran, who actually got this plot or derived this plot.

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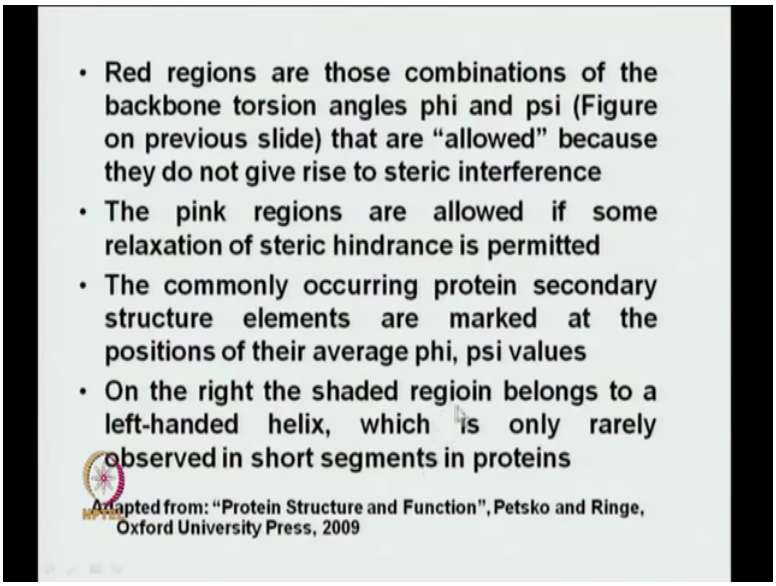

And what he said was then that the red regions are those combinations of phi and psi which are sterically allowed right; allowed is the key word. Then, if you look at the pink regions, the pink regions are the lighter regions which are surrounding the red region.

So, those means at there is some relaxation or sterical steric hindrance and then, we looked at the commonly occurring protein secondary structural elements as we were seeing. We will just go back to that slide again and on the right, so this is a small mistake out is. So, this you should read as region.

So, on the right shaded region, we have the left handed helix which is you know not so common that is why its really observed. So, again if you go back, as I was saying, one more; this was the red region, this is the red region for the beta and all beta ensemble, this is the red region for the alpha ensemble or the helix helical ensemble and then, this is these are the pinkish regions which are you know some relaxation is permitted ok, that is what we have said.

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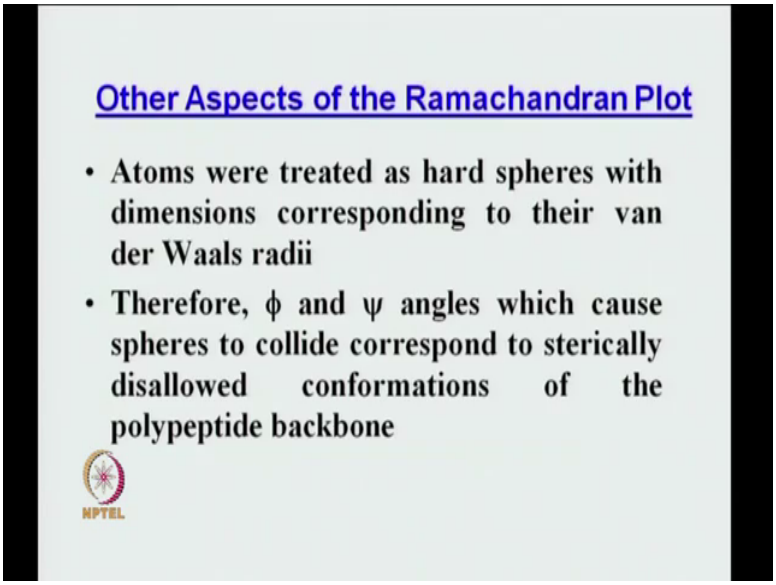

And then, there are some other aspects of the Ramachandran Plot; for example, the atoms when Ramachandran did that, he treated the atoms as hard spheres ok. So, it is essentially, these you know these are not soft potential; these are hard potential that is what we call it.

So that means, you cannot squeeze this spheres; these just collide, move apart that is where the steric thing is coming from. Based on that the psi and phi angles which cause spheres to

collide correspond to sterically disallowed conformations of the polypeptide backbone and hence, you get those shaded regions of the plots which or where the psi and phi values are allowed ok.

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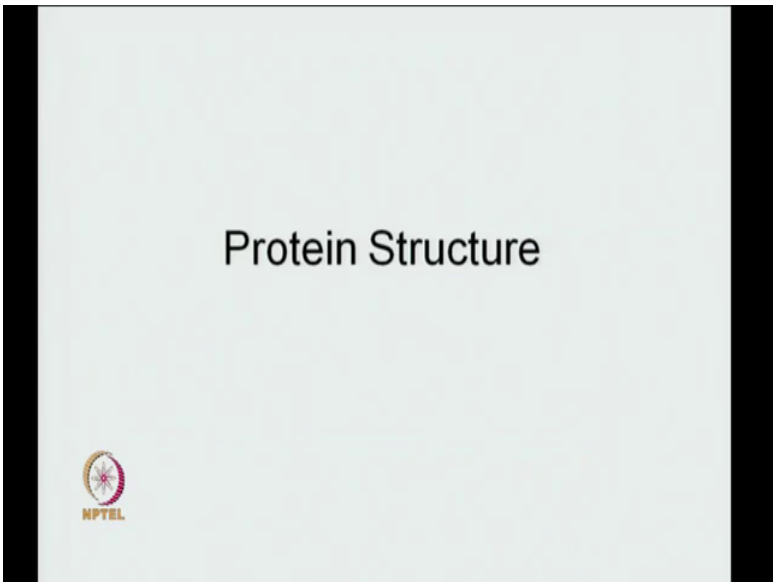

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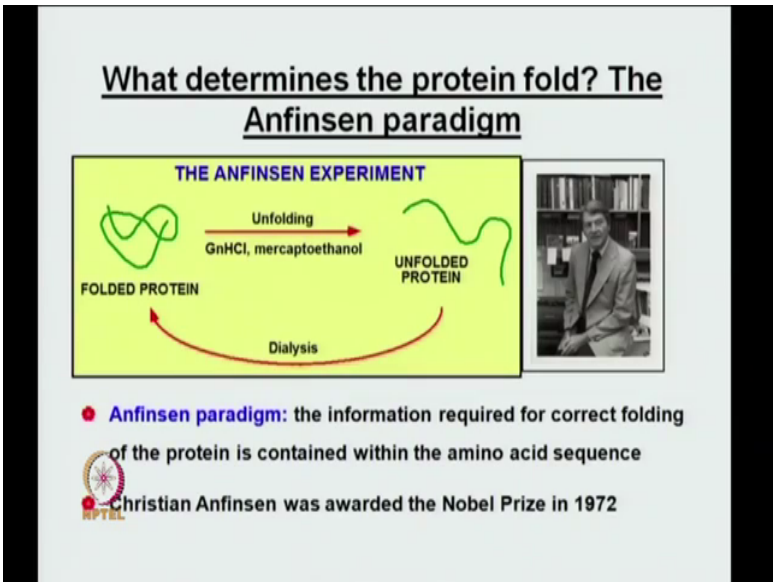

Now, let us talk about protein structure. So, this also we looked at Anfinsen, he won the Nobel Prize in 1972 and what he said was that the final structure, where protein is going to get is encoded in the primary sequence which is the way the amino acids are arranged right, that the sequence of amino acids.

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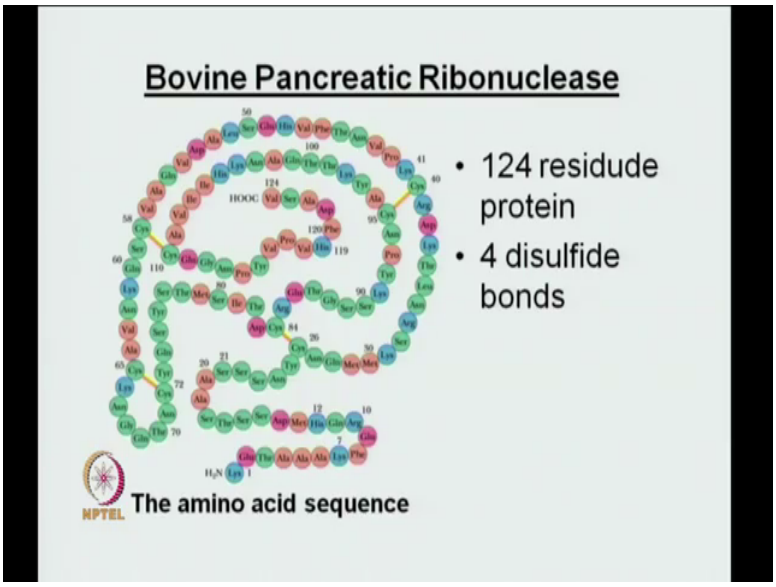

So, what you know the protein he took, this was the major protein took. It was bovine pancreatic ribonuclease.

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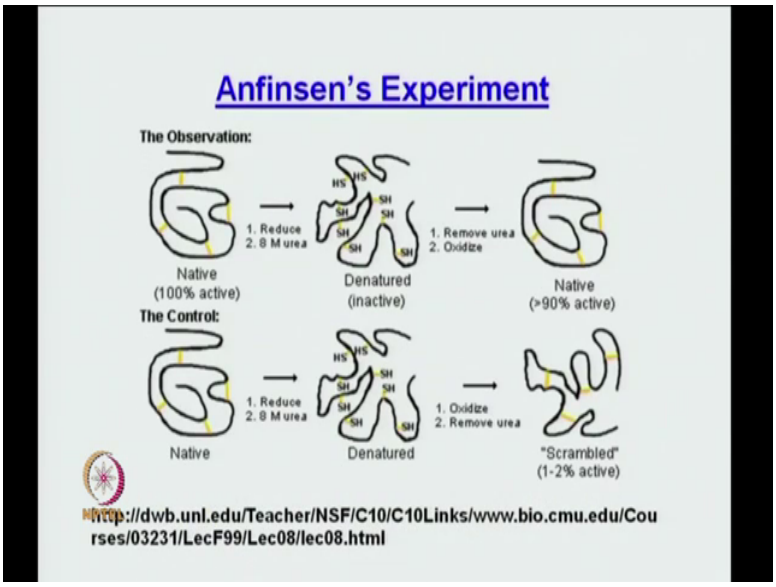

And then, we talked about this two experiments right. So, in one case, there are four disulphide linkages, it reduces with mercaptoethanol; then, denatures with 8 molar urea. Then, he removes the urea, oxidises it and he gets back the native like activity.

However, what he tried to do was he scrambled it in the next experiment; that means, he reduced. The first step remained the same alright, this step remains the same as the previous one and here, now before removing urea denatured oxidise them.

Now because it was a denatured protein, so what happen was not all the sulphide acids (Refer Time: 04:45) were in the proper orientations right. Obviously, they were not because it is a denatured protein, you have 8 molar urea in it. So, what he managed to do by oxide? He managed to scramble disulphide bonds.

So, at many different disulphide bond bonds formed and as a result, what happened was when he removed the urea now after oxidation, what he found was this scrambled one was only 1 to 2 percent active. That means, the protein had not achieved his native state and only because only 1 to 2 percent of the protein molecules actually had the correct conformation which could carried out carry out the enzymatic activity.

But then, a clever thing that they did was that on this they add a little bit of reducing agent. So, the reducing agent what it you know allows you to do is, it reduces this disulphide bond. So, goes to SH and then, now it can scramble.

Scramble in the sense now, it can exchange right and once the exchange happens, what he saw was after giving it some certain amount of time, then the native activity native like activity slowly comes back, back, back. It almost reaches you know the activity in its native state.

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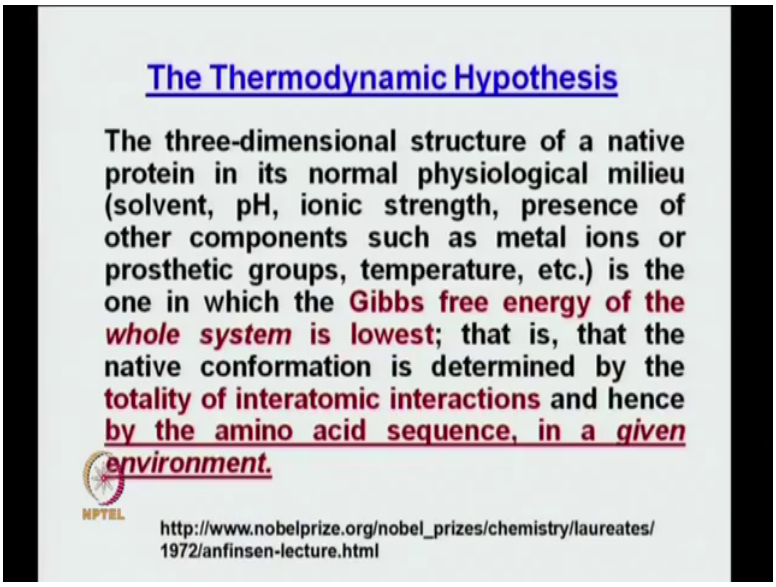

So, this led him to propose the hypothesis which he said is the three-dimensional structure of a native protein in its normal physiological milieu is the one in which the Gibbs free energy of the whole system is the lowest; that is, that the native conformation is determined by the totality of inter atomic interactions and hence by the amino acid sequence.

So, what would define that inter atomic interactions? Those would be the amino acid sequence because that is where the interactions are coming from in a given environment right. So, this is the so called Thermodynamic Hypothesis as proposed by Anfinsen and his co-workers right.

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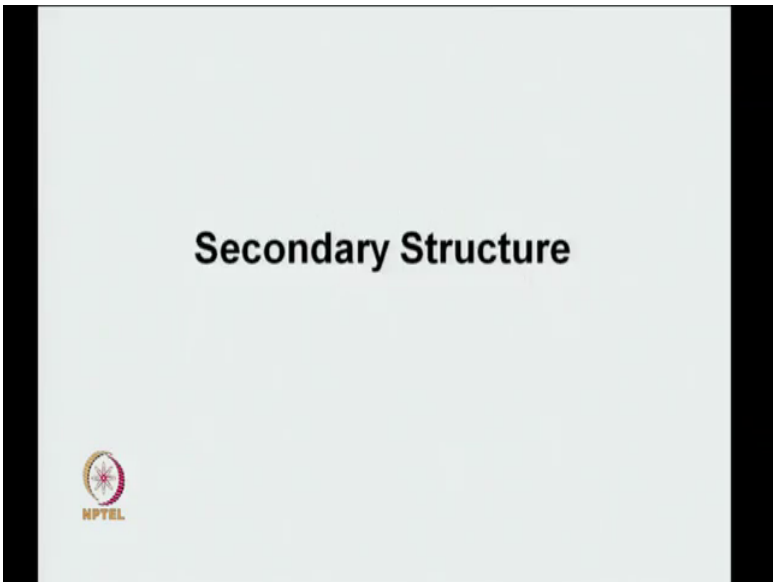

So, this is until you know what we had covered in last class. So, let us move on. Now, let us actually go deeper into the secondary structure; start looking at different components of the secondary structure. So, first question. You know remember, when we started talking about proteins, the first question we asked is or was why do we need proteins? Here, we ask the same question.

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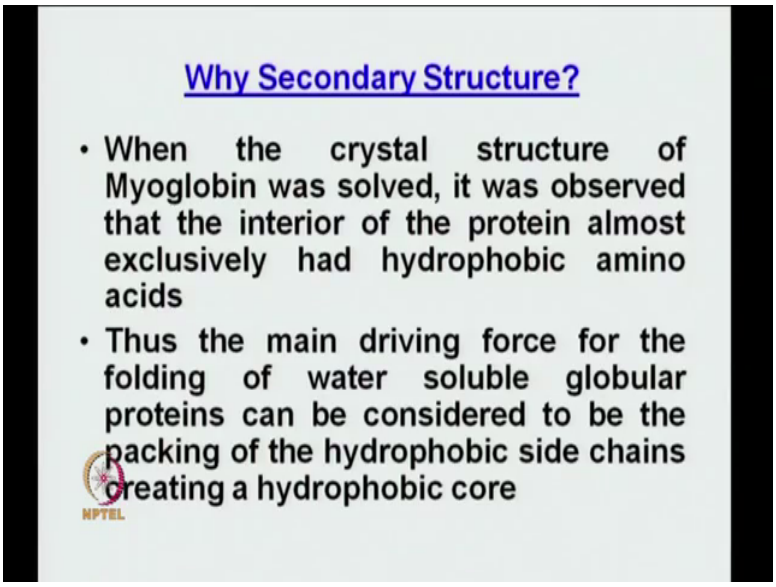

Why we need a secondary structure or why should we care about a secondary structure? So, let us look at it. See Myoglobin is a protein of which or is that protein whose crystal structure was solved the first by Kendrew ok.

So, what they saw was or what Kendrew and co-workers saw was when the crystal structure was solved of Myoglobin; that means, it was observed that the interior of the protein almost exclusively had hydrophobic amino acids ok. Now, what does it mean then? See remember, if you go back to the previous class, where we are talking about the different amino acids.

You have you know polar, non-polar, amino acid like hydrophobic amino acid side chains essentially right and so, what they saw was that all this, I mean the interior of the core after it

has folded right because its doing a good crystal structure or the folded protein; so, the interior mostly has hydrophobic amino acids.

So, what then what conclusion they reached was that is the main driving force for the folding of water soluble globular proteins can be considered to be the packing of the hydrophobic side chains creating a hydrophobic core. Now, this hydrophobic effect or hydrophobic core, you would look at in more details a few classes ahead.

But let us you know just take it very simply and see and you know think about it what has happened is that if you have taken Myoglobin soluble, you put it in water right. Now, what will happen is the hydrophobic amino acid side chains, they would not like water.

So, they would try to move away from water and once they move away from water, what they would do is they would try to go inside the interior of the protein being surrounded by the other polar amino acids right or charged amino acids. So, that is exactly what has happened. That means, the core of the protein is essentially hydrophobic in nature ok. So, what is the consequence?

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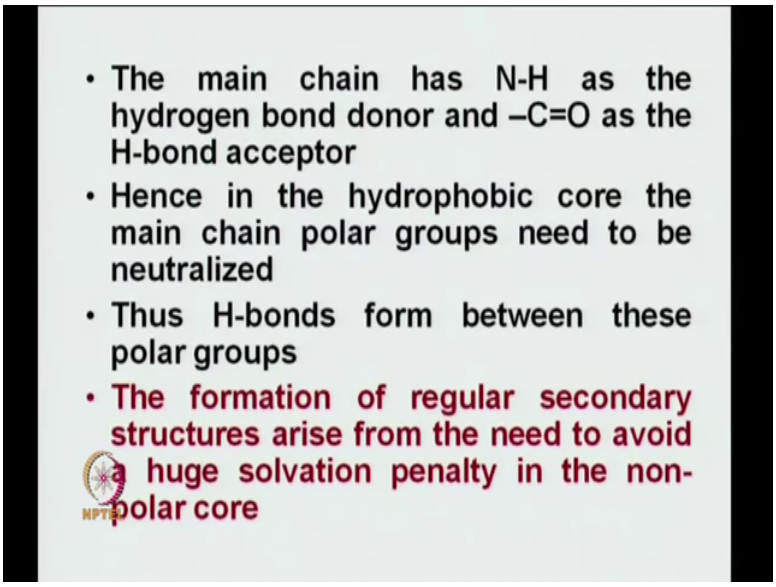

Now, if you think about the peptide backbone right, it still has the NH and the C-O groups. So, that is what it says the main chain has NH as the hydrogen bond acceptor and C-O as the sorry, hydrogen bond donor and C-O as a hydrogen bond acceptor. So, they are still there.

You remember, your core is hydrophobic right; but then, the core has to be made of amino acids right. It is only the side chains that make them hydrophobic, but you still have this NH and C-O groups. So, hence, in the hydrophobic core, the main chain polar groups need to be neutralised. What do I mean by the main chain is it is essentially the peptide backbone. So, you have this NH 2 group and the C-OH group.

So, then N, if you remember the way the peptide bond was being formed and makes a nucleophilic attack on the C-O, there is a loss of water. So, one C-O stays and there is one NH out here. So, this NH and C-O, this is from the peptide backbone, it still remains right.

But when you form the hydrophobic core, you cannot get rid of this these are polar. So that means, these polar groups are still there are in the hydrophobic core and these polar groups need to be neutralised. So, this hygiene form bonds formed between these polar groups right, simple.

So, let me see if the chart has to be neutralized or if that you know polar character has to be initialized, you better form a bond. So, the bond they formed is the hydrogen bond and this is very important the formation of regular secondary structure arises from the need to avoid a huge solvation penalty in the nonpolar core ok. Think about the statement again. It is a very important and a very significant statement. This is why we have secondary structures arising from regular hydrogen bonding patterns.

What is the secondary structure due to? The formation of regular secondary structures arise from the need to avoid a huge solvation penalty in the nonpolar core or the hydrophobic core. Because in the hydrophobic core you would rather not have these NH and C-O groups dangling around right.

Their polarity or their charges have to be on even the partial charges, those have to be neutralised and that is how the hydrogen bonds are found and that is why the hydrogen bonds are found and that is why you get secondary structures ok. So, this is the important secondary structures right. I mean this, we should really keep in mind.

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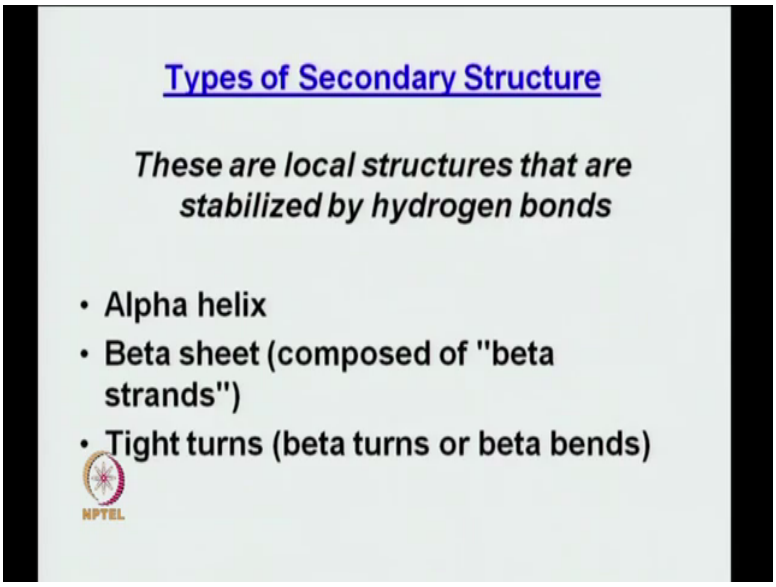

So, what are the different source of secondary structures ok; then, the types of secondary structure? So obviously, this is the local structures that are stabilised by hydrogen bonds. These are local because remember the first level is the primary, then you form the secondary, then the tertiary, then the co-ordinary. So, obviously, these are local structures means hydrogen bonds are forming locally that means, between adjacent or nearby residues; not exactly adjacent, but nearby residues ok.

The first one possible the most common one the Alpha helix; then, you have the Beta sheet which is composed of beta strands; then, you have Turns right beta turns or beta bends. We will take this individually and what you will see today in classes, we will mostly focus on the first one which is the alpha helix.

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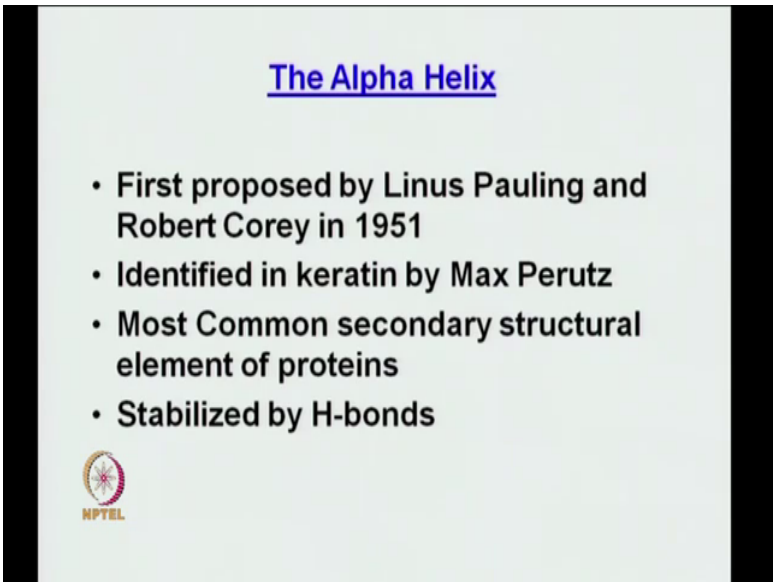

So, let us go on to it. So, this is the alpha helix, we are talking about. This was first proposed by Linus Pauling and Robert Corey in 1951. Now, it is it was identified in keratin by Max Perutz. It is the most common secondary structural element of proteins that is what I was just saying stabilized by hydrogen bonds. We will soon look at the hydrogen bond pattern, this is a famous pattern.

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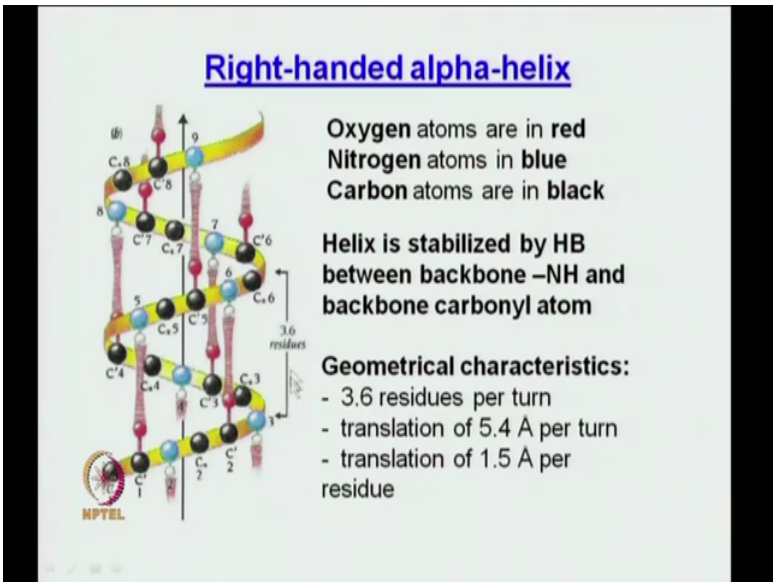

Then, we go and look at the Right-handed alpha-helix ok. So, this is what typically alpha-helix looks like right. This is your alpha helical you know arrangement ok. What do we see? The oxygen atoms, they are in red. Then, the nitrogen atoms, they are in blue.

Then, the carbon atoms, they are in black and if you look at this white ones, the white small space, these are the hydrogen atoms. Let us go with you know these go without same right. So, if you look at these things, these are essentially the hydrogen bonds that are taking place between the I and the i plus 4th residue right. Some other characteristics of the alpha helix, I am just giving a schematic of the characteristics.

We will come back because we will this is what we are going to discuss on or discuss spend time on in this class. So, the helix is stabilised by hydrogen bonds, HB stands for hydrogen bonds between backbone NH and backbone carbonyl atom.

So, you can see this is carbon right, this is the oxygen attached to it and then, you have this hydrogen bond which is accepting the hydrogen bond from where? This is the H and this is the corresponding N and you can see what the residues this is 9 and this is 5 right. So that you remember I and i plus 4, so this is 5 plus 4 is 9 and that is essentially what we are looking at ok.

Now, these some geometrical characteristics; so, alpha helixes have 3.6 residues per turn. That means, you complete 1 turn, you go from here to here; so, this is 1 turn from here to here, as it says. It has a 3.6 residues, you complete a full turn like this and you get 3.6 residues. It is the translation of 5.4 angstroms per turn; that means, it is a this is essentially a pitch; do not worry, I will come back to it again. This is the pitch which is 5.4 angstroms and hence, if in 1 pitch of 5.4 angstroms, you are having 3.6 amino acids per pitch or per turn.

Then obviously that means, for each residue in the 3.6 residues per residue, I have a movement of 1.5 angstrom right. That means, a translation of 1.5 angstrom per residue. So that means, we have 3.6 residues and I go from the first residue to the second residue, I have a translation of 1.5 angstrom; the second to the third 1.5 and so on ok. So, this in a nutshell is your alpha helix and also, if you look at it you know, if you look at it like this it just looks like a cylinder. Is not it?

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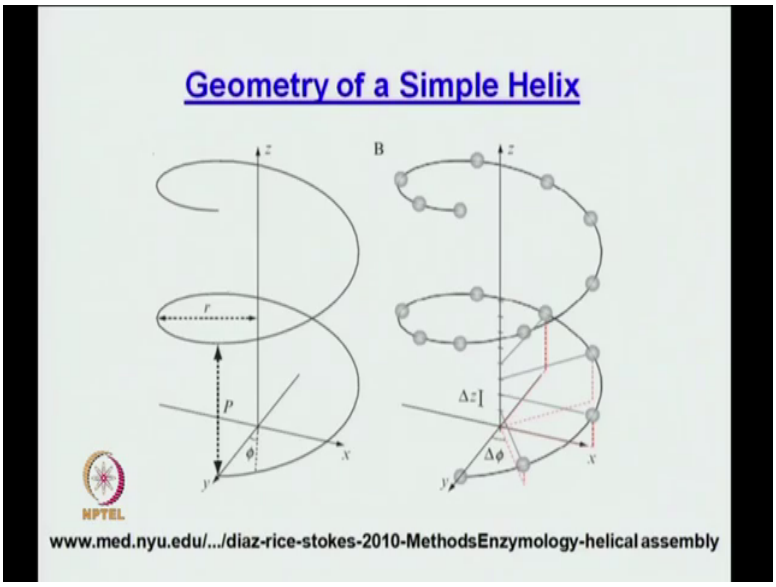

So, let us talk about the geometry. Now, this is where it becomes really interesting. So, on the left, you can see a continuous helix; that means, it is just in continuum, there is no break. On the right, what you see is I have put you know some space or some big dots or big circles.

Now, these big circles can be considered as amino acids. So, essentially now the helix is not continuous. Why what I mean by not continuous is that means, from here to here you have nothing in between; is not it? So, that mean this say this is the first residue, this is a second residue, this is a third residue, the 4th residue and so on; is not it?

Now, let us look at this. I hope you are with this right; no questions right ok. So, just to you know make the point again, this is a continuous helix means that wherever I move on this you know line, I have; something. However, if you look at the right one ok, if you look at this

right one, it is labelled as B. I have put in these circles that means, from this circle to this circle in between it, I do not have anything; its essentially discontinuous helix right.

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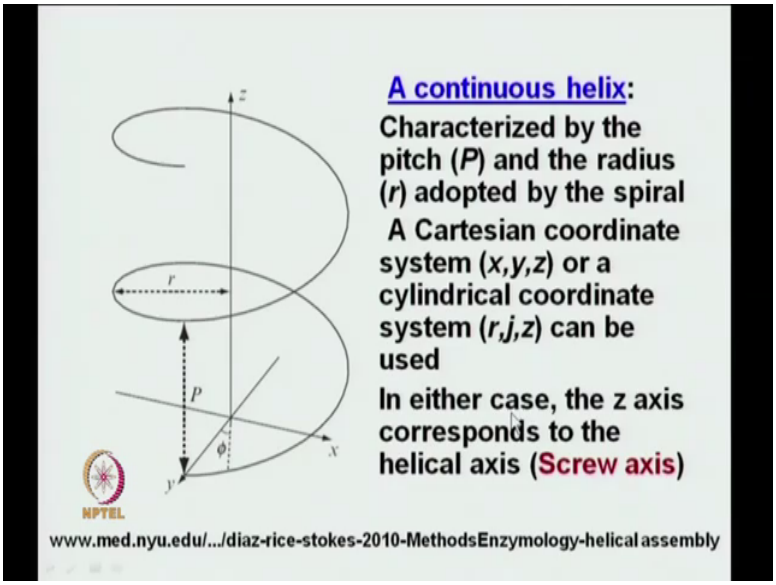

So, the characteristics of a continuous helix, let us look at it. It is characterised by the pitch P and the radius r adopted by the spiral. Because the helical spiral right, it is this is how it is going on and its going around the z axis ok.

So, this is the z axis. So, like if I take a pen, you know if I take a pen like this; if I take a pen like this, if this is my z axis, then the helix actually is moving around the z axis and if you can see; if you can see from your side, it will be different if I look at from my side. See when I move from this side, this to me is front if you look at this finger; but to you, its back right. Then, I go like this, I go like this, I go like this; then, it becomes in front and I come back here, this is my 1 turn ok.

So, then this is what P represents. This P essentially is the pitch, it represents 1 complete turn. So, you can see, I started from here right, I met 1 turn and I came back to the same position; but it I have translated by this amount of P which is the pitch ok. So, how we can describe the geometry of the helix right; so, we will talk about this in more detail. So, do not worry; I do not think you are getting lost, even if you are getting lost, we will find you. So, do not worry about that.

So, we can describe it with the help of a Cartesian coordinate like you know normal x, y, z system or we can use a cylindrical coordinate you know r, j, z. I will tell you what these are; you know these coordinates are, this r, j, z.

And in either case, in either case whether it whether it x, y, z that means, the Cartesian or the cylindrical, this one in between that is in the middle is essentially your z axis or the helical axis. It is also referred to as please keep in mind, the Screw axis. So, whatever we do, I have the axis that around which the helix is developing ok.

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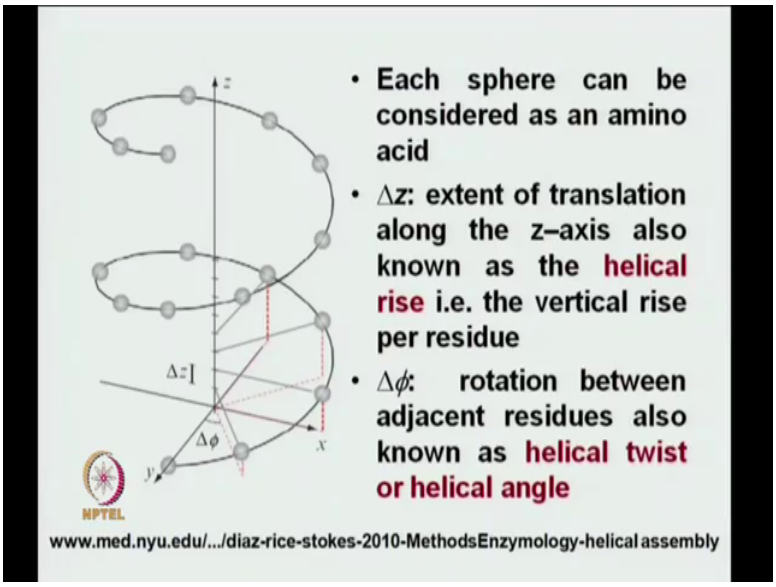

Now, let us look at this. Each sphere as I was saying, so you know this is the second figure, each sphere can be considered as an amino acid. Its good. Now, what is delta z? Delta z is the extent of translation along the z-axis also known as the helical rise. There is a vertical rise for residue. Now, look at it very carefully.

So, suppose, I am moving from say suppose, I am moving from this amino acid to this residue acid ok. You follow my arrow from this amino acid to this amino acid. Now, what I have done is see I am not on the same plane that means, I am moving; but I am also translating, I am also moving up, like it is not moving like this, it is actually moving like this right.

So, moving like this and hence, what do you see is if this is what the value around the z axis is, then for this one it has moved by this amount ok. So, it has moved from here to here after going to the next residue.

That means, as I move from here to here, I have translated in the vertical direction; that means, along z by I unit of delta z. So, that is what this delta z or delta z is referring to. That means, the it is the amount of vertical translation, I am having per residue movement. I move from one residue to the next residue delta z is the amount of transition, I moved in the z direction and then, what is delta phi?

Now, you know keep this in mind, I am moving like this. So, not only I am moving up, I am also rotating right. That is also moving a angular, in an angular fashion. So, then it is what is delta phi. So, you look at this.

So, to get you know delta phi, essentially what you are doing is you look at this, first amino acid and you look at this one, what do you do is. So, this is the x-y plane right and this is z axis ok, say the first residue is essential on your plane right and the next residue has actually moved up like this.

So, how to get the angle between this? You draw projection from this residue on to the x-y plane. So, that is exactly what I have done. What I have done is that remember from when I went from here to here, I have already moved by delta z ok. So, this is this by from here to here, I have moved that is my delta z right from here to here. So, it means I have already moved up. So, now I cannot place it in the plane, but what I can do is I can do a projection and I just. So, this is my projection, you see this red dotted line.

Now, this is x-y plane right. This is y, this is x, this is x-y plane and what I do is now I connect the to the origin and this angle; that means, the angle which this projection of the second residue mix with the first residue is referred to is your delta phi. So, this much rotation you have done on moving from one residue to the next one.

So, there are here there are two very important components. What are the two important components? One is delta z that means, the vertical translation and the other one is delta phi, there is a rotation as I move from one residue to the other.

So, in a helical movement, what am I doing? I am doing two things, simple. I am rotating and also translating. That means, if I ever have to bring about a coordinate system or try to you know get the geometry, I need essentially two operators; one is a rotational operator that will cover my rotation and the other one is a translational operator that will cover my translation right along the z axis.

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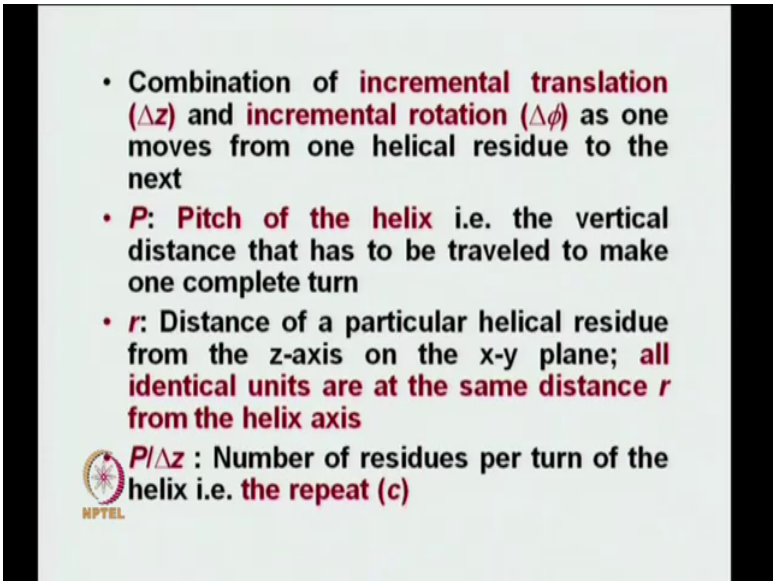

Then, there are some key points. Combination of as I just discussed, combination of incremental translation and incremental rotation as one moves from one helical residue to the next; so, this is what happens.

So, for to get a you know helical symmetry, I need a combination of incremental translation which is delta z along the vertical axis and incremental rotation is delta phi as one moves from one helical residue to the next ok. What is P? P is the pitch of the helix; there is a vertical distance that has to be travelled to make one complete turn. Remember, I was telling I you have this, then move from here to here, you make this one complete turn and you come back here.

Then, what is r? It is a distance of a particular helical residue from the z axis on the x-y plane right. All identical units are at the same distance r from the helix axis. I will just show you, I will go back and show you what I mean by this and what is P by delta z then. Remember delta z is the vertical rise per residue. What is P? P is the total rise I have gone through during one complete turn.

If I need to know the number of residues, which are there in making one complete P, then what do I need to do? I just need to do P by delta z. So that means, the number of residues per turn of the helix is given by C which is equal to P by delta z ok. So, this is what I meant, when I was talking about let us see. So, this is what I this was what I meant when I was talking about r and P.

So, you look at this; this is P. You are making from here, you are going here is one complete turn, now the odd thing. So, this r is you look at this one, does not matter what which one you are looking at. You can see this is my z axis right and suppose, this is the residue this residue.

So, you can consider this x-y plane. So, this sections of the x-y plane like this. So, you are going like this. So, this is the distance, this residue has from the z axis that means, is this is the x-y plane, the z axis is the residue, z axis is the residue somewhere here like this is the z axis

right, x y plane the z axis is the residue somewhere here; then, from here to here, the distance is essentially your r ok.

Now, what we will do is we will just make a small change of guess, I will be using I will be writing something down. So, that it is better for us to understand. So, you know what do I need. So, then what I can say is let us talk about cylindrical coordinates ok.

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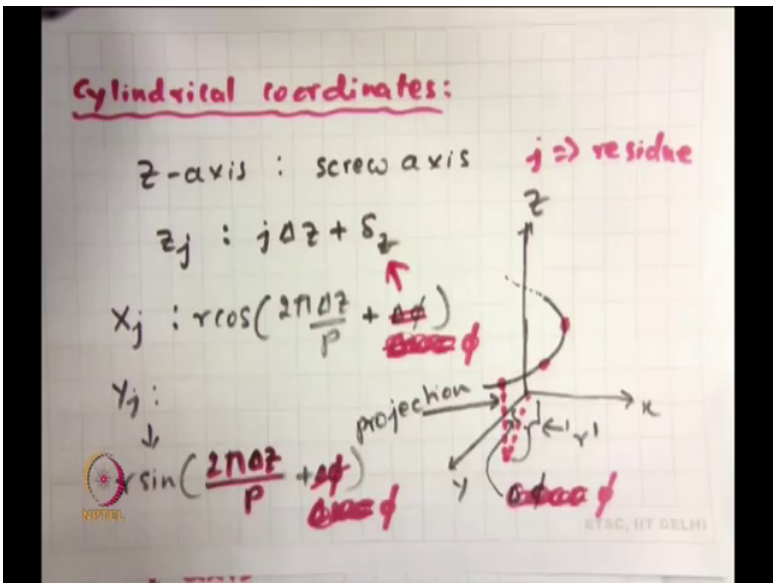

So, let us talk about cylindrical coordinates. Now, in cylindrical coordinates, what we remember, what we had is that the z axis was essentially my helical axis or the screw axis. Is not it? Now, you have so many residues ok. So, what can I do? So, what I can do is say suppose, I have you know many residues in helix right; then, what I can write is say suppose, the z coordinate right, the z of the jth residue. So, how many residues? Say I have j, j plus 1, j plus 2, j plus 3 and so on.

So, what I can have is this. This is what I can write. I can write is j times delta z plus delta z ok. So, what do I mean by this? What I mean by this is this. So, suppose I have something like this. So, this is my you know this is my z axis right and you know this is my x, this is my y ok. This is a rough schematic. So, this is how I am moving. So, I am moving is I am moving like this, this is my helix.

So, now what I can do is I can you know put my amino acids in one amino acid is here, one amino acid is here, one amino acid is here. Now, this is a very rough schematic. So, please keep that in mind ok. Now, what can I do? See suppose, this is my first amino acid, remember we talked about r. Let me show you show that to you. So, this if I draw a line on the x-y plane, remember this x, this is x-y plane right, I drop a line; now then, what I do is I connect this ok.

Now, once I connect this, what do I get this distance this distance. So, you see this is the projection of this amino acid right. So, if this is the projection, if this is the projection, see if this is the projection, then of this amino acid, then this distance is r as discussed in the previous slide and you talk about this angle, this angle ok; so, this angle is essentially your delta phi ok.

So that means, you know whatever it is as I moving, as I moving from one residue to the next ok, this is the type of angle I am moving through ok. You know this delta phi is essentially the angle it is forming with that axis ok.

Now, what else can I put in? What I can put in is this is my z z of j that is the z coordinate, but let me tell you what j is. j is essentially you know it is the jth backbone residue ok. So that means, I have say j minus 1, j minus 2 j minus 3 and so on right.

So, when I move from one amino acid to the other, I move by delta z, delta z; if I move by two amino acid, I move by two delta z; if I move by j minus is moved by j delta z and what is delta z? The delta z is very simple. So, if you look at this one, if you look at this one right, now this one what has happened is this first amino acid.

See this first amino acid is not starting from the x-y plane, see it is at a distance from the x-y plane right. So that means, the z it all it already has some z value and that are you know some distance some vertical distance as a z. So, that is what I have represented as delta z ok.

So, this is what the delta z is. You know, see from here to here, this is what your delta z is referring to ok. Now, you know the way, I look at it or the way we can formulate is once we have the z coordinate, we can also go with the x coordinate. So, the Xj, how can I write it?

Now this is where the cylindrical coordinates are coming in. So, I can write this is r cosine 2 pi delta z over p right plus delta phi and then, say I move to Y now. How can I write Y? So, I will write Y a little bit down here. So, Y, I can write r sin 2 pi then, delta z by p plus so this is delta phi ok. I am just running out of ink; but I will just use this. So, this is I can I think you can recognise, this is delta right and this is delta, this is phi, this is 2 pi and this is p ok.

So that means, what I have done is I have been able to have a coordinate system, where z is given by this. Remember j is the residue amino acid residue ok, then I have x of j and then, I have y of j right. What do we do next? What do we do next is, you just look at a simple feature of this. Now, what is the simple feature? The simple feature is see for example, if I do this, now keep this in mind ok.

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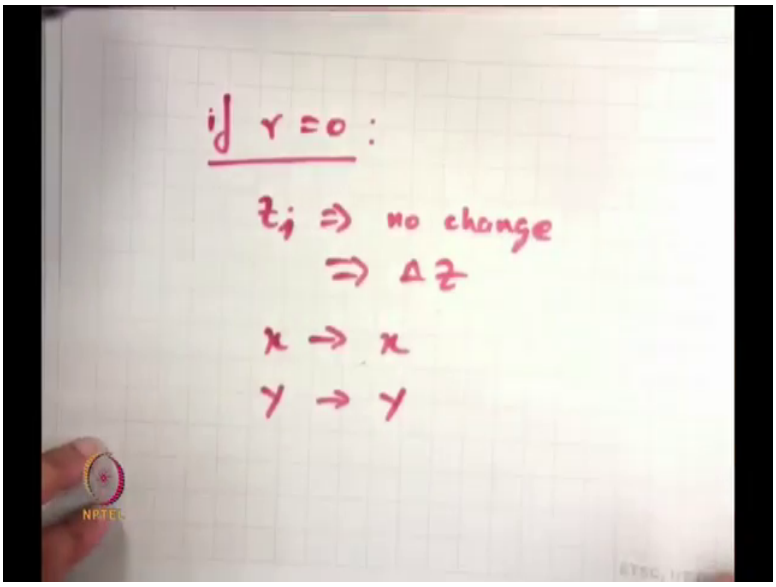

If I do this, what will happen? What will happen, if my r is 0; what will happen if my r is 0? If my r is 0, now look at this ok; z is z that is fine, z remains the same, no change ok. So, what I do is then because there is no change, what essentially happens it is not no change, then what it will happen is it will go as delta z right say I am moving one residue by one, then x transforms as x, y transforms as y. Why is so?

Because you look at this x and y, x has r times something, y is also r times something. Now, you are saying that r is 0. So, if r is 0, essentially what is happening? If r is 0, there is no distance of the residue; that means, the r is 0 that means, this quantity is 0. So that means, there is no distance from here to here. So, essentially are being 0, this it those that amino acid that residue is falling on the z axis.

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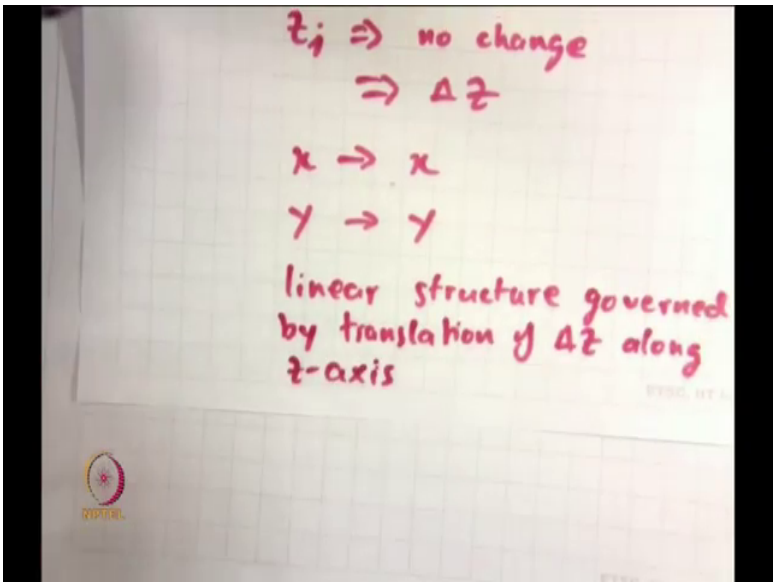

So, if it is falling on the z axis, what do you get? So, essentially what you get is you get a linear structure right governed by translation of delta z per residue along the z axis ok. So, this is what I have. Now, this is in cylindrical coordinates right ok. I hope you understand the significance of this.

So, z vertical, then we have the x-y plane, we have these to define. Now, what we will do is we will take a look at the Cartesian coordinate system. What can we get from there? So, when we will talk about a Cartesian coordinate system, now this is what we do right. We talk about a Cartesian coordinate system.

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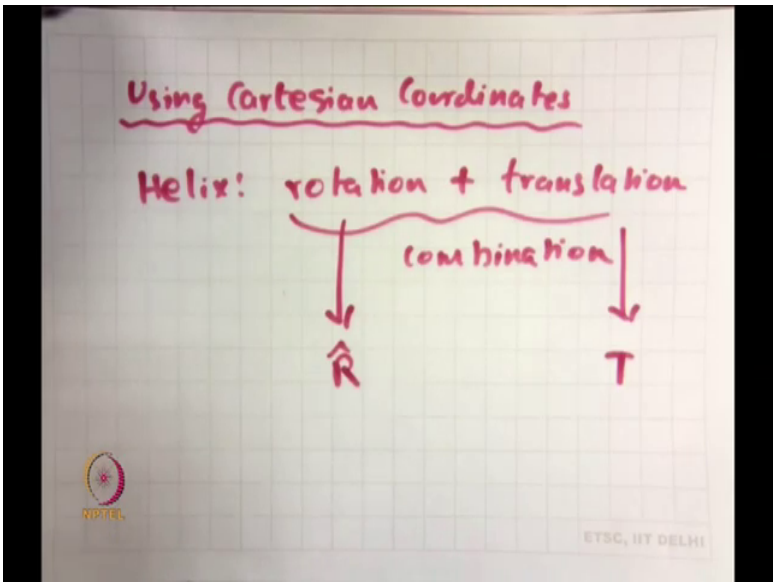

Then, in the Cartesian coordinate, so we can write using I am trying to use Cartesian coordinate right. If I am trying to use Cartesian coordinates, what we know is we know that in helix, what do we have? We have rotation plus translation. Is not it? Now, once we have that rotation and translation, this is a combination. So, you helix is what essentially a combination right that is why I have written the plus sign.

Now, if I am having the combination, what am I going to do? So, if I am having the combination, then what you would understand is this I would be having a rotation operator and here, I would be having my translation. So, if that is true; that means, I need a combination of two operators, R and T.

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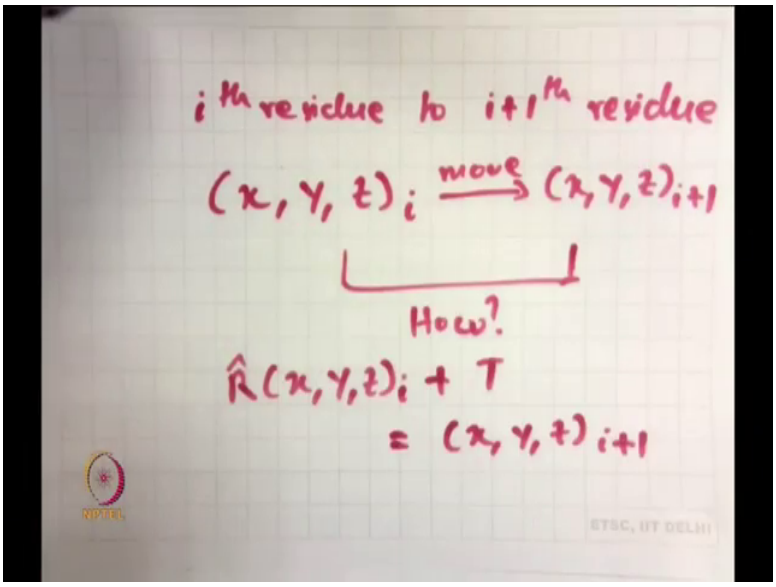

So, if that is true, what can I have now is then, if I have to move say I have to move from ith residue or jth residue to i plus 1th residue ok. This is what I have to do. Now, if I have to do that, what I can how then that means, what I am looking at is see my ith residue initially has these coordinates i.

Now, I move to what? I move to x, y, z i plus 1; x, y, z i plus 1. The question is how? Well, you know the answer. It is the combination of rotation and translation; is not it? So, what I can write is; so, what I can write is I can have my rotation operator rotation x, y, z; then, i plus I have my translation right. I am translating along the vertical axis.

Now, this is going to give me x, y, z coordinates of i plus 1. So, see what I am trying to do is if I know the coordinate of one; that means, if I know the coordinate of any atom in helix and I know that it is sitting in helical symmetry, its part of the helix.

Then, as I move from here to here that means, I move from i to i plus 1th residue, there is a next residue; can I get the coordinates and like this, can I get the coordinates of the whole helical system right, whatever amino acids are being a part of the helix ok? So, then if that is what it takes, what we have to do?

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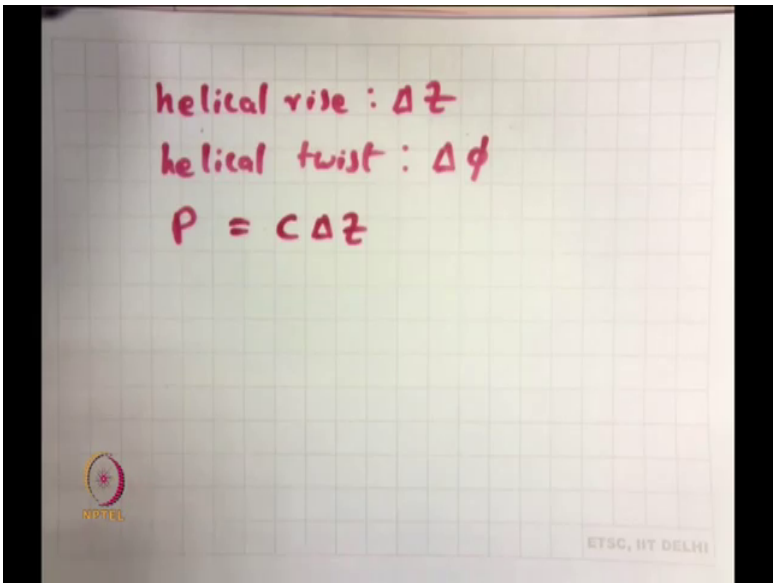

Then, what we can do is we define something we had, we know from before, we define the helical rise you know as delta z that is what we are defined ok. We have defined the helical twist. We have defined the helical twist as what? We had defined the helical twist as very

simple delta phi. So, once we have defined the helical twist as delta phi, you know we have this right not a problem; then, we also know some other things. What do we know?

What we know is that you pitch, a pitch that means, the total translation in one repeat is equal to or one turn is equal to the number of repeats which is c times delta z right. Now, what do you do is if I start looking at the coordinate system, what can I do? Remember, I have to do a rotation and I have to do a translation. So, I will just go to the next page because I do not have much space here. Then, what I can write is I can write a matrix representation. So, this is what I can do.

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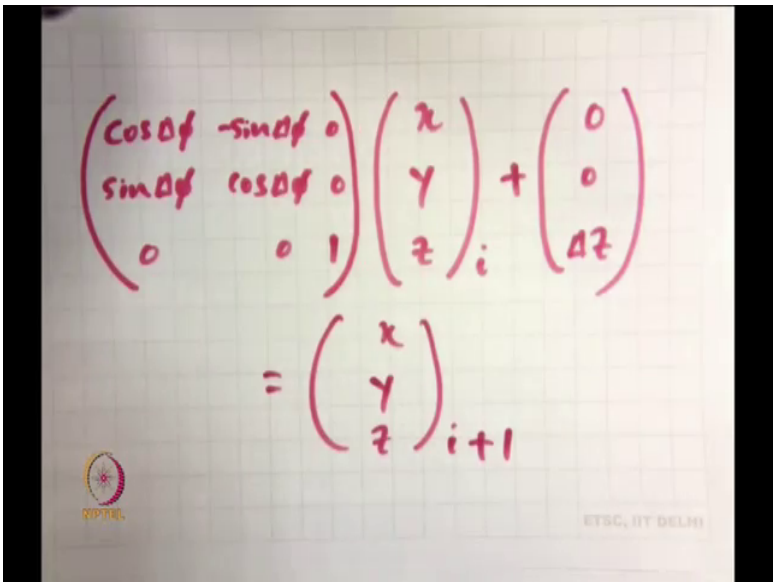

So, I can write cosine del phi ok. So, then I can write minus sin del phi, then 0, then sin del phi cosine del phi 0, then I have 0 0 1. Why? Because this is a rotation along the z axis in the x-y

plane. This I multiply on the coordinates of the ith residue. Now, once I have done that, remember what delta phi is?

Delta phi essentially is the angle between the two helical residue that is what that is how much I am moving by. So, if delta phi is you know if delta phi is the angle between the two residues, then I have just done a rotation by linear translation. So, to do a translation, what I will need is I will just move along the z.

So, this is what I will do. I will do a 0, a 0 because I am not moving, I am not you know translating along the x y plane, but moving along the z axis and hence, I am moving by delta xi and if I do this, if I do this, then I get my coordinates of i plus 1 ok. So, I hope you follow what we have done. So, we start from the very first thing. So, you know see this is what we did ok.

So, this is what we did. Just be careful about one thing though that you know this angle, now this angle is essentially the angle it is forming. So, when I talk about delta phi, when I am talking about delta phi, I essentially what I mean is you know this delta phi is actually the angle between the two residues.

That means, you move from one residue to the other residue, this what you get ok. Now, the z coordinate j the number of residues is equal to look at or that means, the number of residues are going to move from here.

So, if I move from here to here 1 that means, j is equal to 1, this plus delta z. Then, I have X j, this r cosine of 2 pi delta z by P plus delta phi. So, here this when you know when you talk about this delta phi, just keep this in mind that you know this what do I mean by this delta phi? This delta phi here actually has a different meaning.

So, this delta phi is suppose this is already what I have here. Now, this delta phi is essentially what you are seeing is here ok. So, this is you know this is what I am looking at this angle. So, I would be you know let me change it, let me change one thing. So, what I will do is instead of

doing delta phi, what I can write is delta phi I should maybe I can write. Well, I can keep it as delta phi i ok, delta let me just change it.

So, let me do this delta omega; let me do that. So, this is actually your delta omega ok, let me change that. So, what is. So, this one also could be then referred to as delta omega ok. What I mean is because there is no amino acid here, please do not get confused by this. It is just the angular orientation from with respect to the y axis.

So, but the delta phi that I am actually talking about is the angle, if you remember what we had seen in the previous one, it is the angle essentially what you are its very simple. It is the angle essentially what you are plotting is between this guy and this guy; so that means, here you have a projection out here, here is the projection out here. This is the amount of angle you have moved and that is what delta phi is ok.

So, this delta omega, I can just represent as phi because I was looking at phi and this is also phi and I can take this as phi. So, what I am saying is that this angle with this amino acid projection is making with y is phi and then, when I am moving to the next one amino acid, I am moving by delta phi. So, I had this phi and then, I am moving by delta phi. So, that is what this is representing. I hope you are with this.

So, what you do is you just replace this delta phi by phi or delta omega, does not matter and here also, you represent you replace this delta omega or this delta phi by phi or delta omega. Whatever it is just one angle. It tells you that this is a amino acid, this is at a certain distance from the y axis.

Now, so, this is the projection, I mean now from here I move to the other amino acid that movement is delta phi; but the amino acid makes a certain angle phi or delta omega with the corresponding y axis ok.

So, now when we talked about you know remember we talked about this matrix representation; that means, we have a set of coordinates x, y, z for any residue in the helical axis or in the you know amino acid residue in the helix, I tried to look for the coordinates for

the adjacent residue. This is what I do right. I do a rotation and I do a translation. Now, once I do that, I have some also other things in mind. What are the other things, I have in mind ok. So, these are some of the other things, I have in mind.

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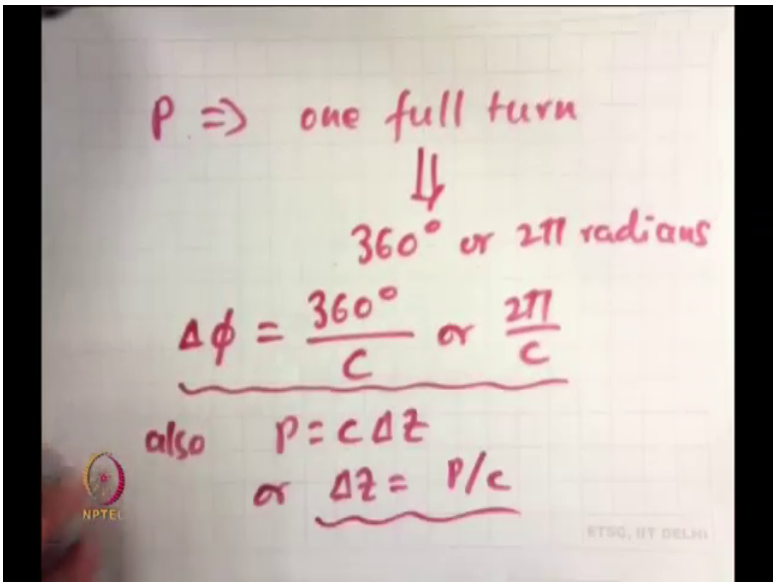

So, the other things I have in mind is that for one P represents one full turn. Now, this one full turn corresponds to a 360 degree rotation or a rotation by 2 pi radians; is not it? So that means, remember the number of repeats or so the number of residues was c. So, what I can do is I can write this delta phi is equal to the full rotation is 360 degrees for one turn over c that is my expression for delta phi or 2 pi over c ok, that is one expression. Also, I know p is equal to c times delta z or delta z can be written as p by c.

So, then what we can do now is I have this expression, I have this expression, I also have this expression; is not it? So, I can take these two expressions and then, I go back to this matrix in

place of delta phi, I put in this 360 degree by c or 2 pi by c and in case of delta z, I put in P by c provided these are known to me; good. So, that was the next step. Now, let us come to a normal helix or a regular you know normal helix means that regular alpha helix that is what I am talking about.

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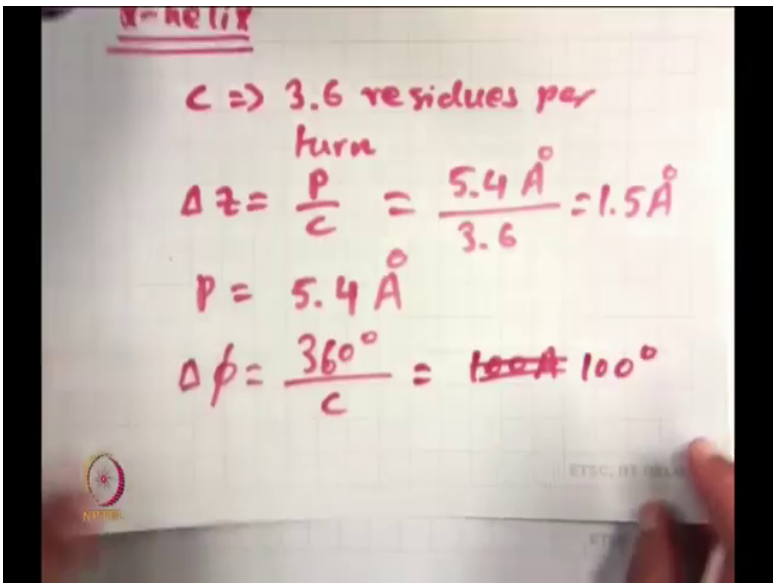

Now, the regular alpha helix we know; so, this is a regular alpha helix. What do you have in regular alpha helix? We have which is you know c 3.6 residues per turn. So, we know one thing; we know delta z. Delta z is equal to P by c right. Now, the pitch for a helix is, can anyone say? Alright. It is 5.4 angstrom. This is a total pitch. So that means, the delta z is equal to 5.4 angstroms over 3.6 which is essentially 1.5 angstrom. Is not it?.

So that means, what it says is that vertical distance I move, when I go from one residue to the other, the vertical distance whatever you know I move when I go from one residue to the

other is referred to as your you know its helical rise is 1.5 angstroms in a normal alpha helix. That is what P is ok. I pretty much have everything now you see.

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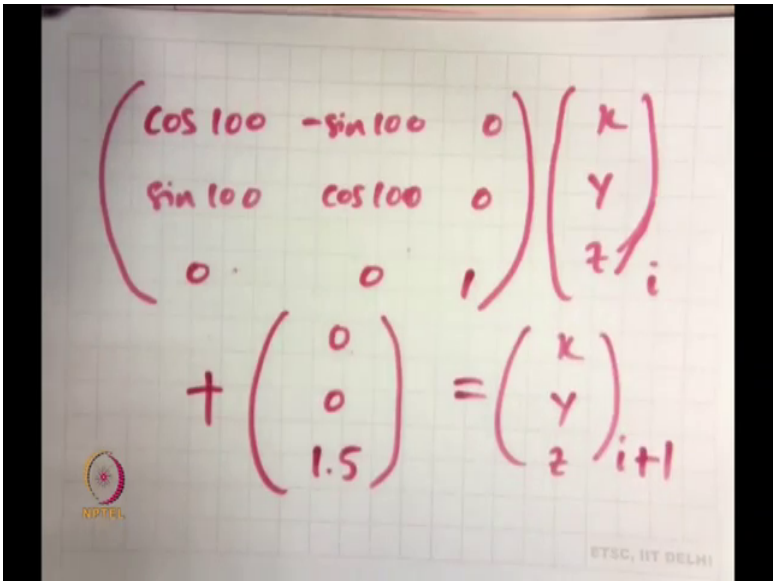

Now, what I can do is let me write the matrix again. Based on this, if I write the matrix, what will I have? I know what my delta phi is right ok. Do not talk about delta phi here. So, did I? So, now then delta phi, if you remember it was 360 degrees by this is 6 by c. So, essentially c is 3.6, then I get 100 right. Sorry, what I am write this is 100 degrees. If I am writing 100 degrees so that means, now I have everything.

So, what I do is I go back and I say this is cosine 100 minus sin 100 and 0 and sin 100 cosine 100 and this is 0, 0 0 1. Do it on x, y, z of the sorry of the ith residue ok. Once I did of the ith residue plus I have the translation. Now, what is my translation? Now, it is 0 0, then delta z

and what is delta z for me? Delta z is 1.5 angstroms. So, once I have that; is not it? So, this is equal to my x, y, z i plus 1 ok.

So, this is really significant. Why is this so significant? Because you see what you have done; what you have done is I am taking this, I have one residue, I know, one and new one residue, then if I have to go to the next residue, how do I know the coordinates?

I know the coordinates of one, now I do a transformation; that means, a rotation matrix followed by translation; I know the residue of the coordinates of the next one ok. In this way, you can generate the whole coordinate system of all the amino acids which are in that helical segment ok. So, you know a few more pointers on this.

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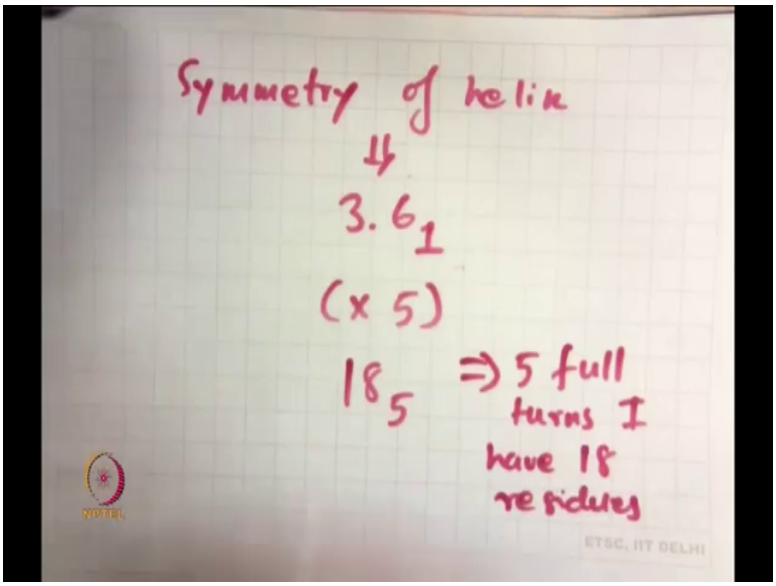

If you would sometimes see the symmetry of helix is often given as 3.6 1 so; that means, I made one turn and I moved by 3.6 residues, but you know integers are much better deal with. So, what I do is I take this and I multiply by 5. So, what do you get? I get 18 5. So, what does this mean? It means that if in 5 full turns, I have 18 amino acids or 18 residues.

I hopefully you will remember this exercise and just before you know completing this exercise, I just want to you know take you back to that you know confusing thing which you still find might find confusing is you know this one, which I was trying to tell you.

So, again this guy you do not it is essentially you know delta phi; it is phi or delta omega whatever it is. So, let us consider as phi, then when I move from here to the next amino acid here, that is my delta phi ok. So, that is why instead of delta phi you should be writing phi here ok. So, please keep it as phi. I might even remove delta omega, but well let me do that ok. So, let me actually get rid of this delta omega, let me get rid of this delta omega too. Just keep it phi, this also delta omega, this is just phi ok.

So, that was about you know the symmetry operations that we needed to talk about; but let us come back and look at the slides. So, in the slides, remember where we were? We were at this. See this is the phi I was talking about, I missed that out because I thought that you know that is the mistake I did.

So, this is phi right and then, if I am going to move from here to here that would my del phi. So, that is what I am doing. So, essentially this is how I am moving. So, anyway it does not matter; that means, we moving from this residue to this residue now it is del phi ok.

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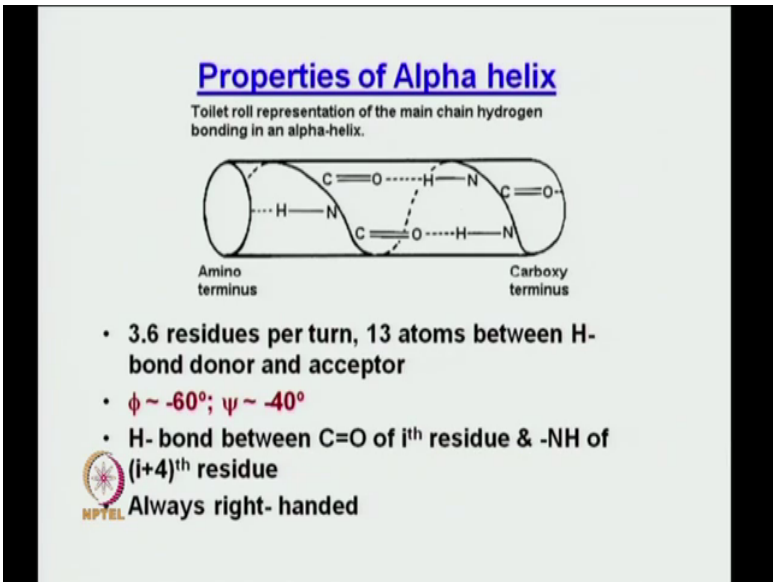

So, moving forward, then we can look at the properties now. So, this is remember the helix is looking you know it looks as a cylinder, there is (Refer Time: 50:39) in the I and i plus 4th residues ok, between the C-O and NH. There are 3.6 residues per turn, the 13 atoms between the H hydrogen bond donor and acceptor. So, this is the hydrogen bond donor and this is the hydrogen bond acceptor, the C-O.

What are the Ramachandran angles? The phi about you know you know this is some average values; phi is about minus 60, psi is about minus 40. The hydrogen bond you know the helical hydrogen bond, it happens between the C-O of the ith residue and the NH of the i plus 4th residue and it is essentially or most of the (Refer Time: 51:15) are always right handed ok.

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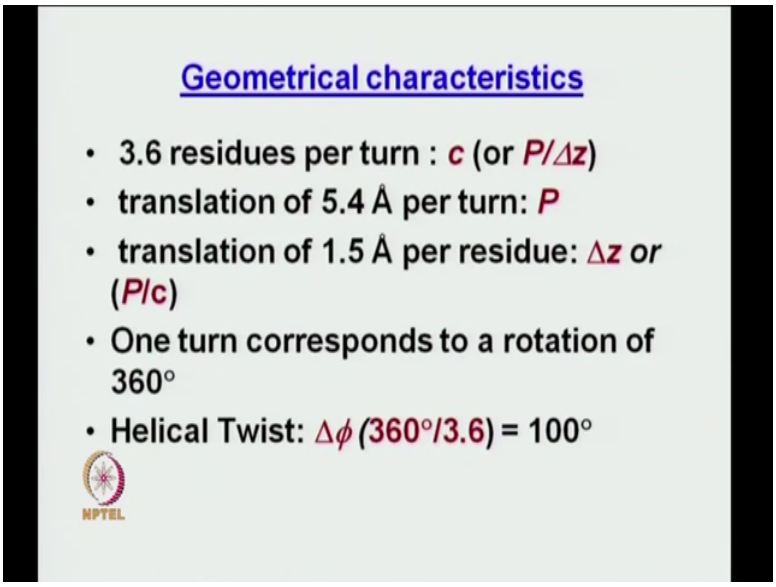

The geometrical characteristics, we have just seen the 3.6 residues per turn right, first point. The next point is the translation of our 5.4 angstroms per turn which is the pitch the translation of 1.5 angstroms per residue which is the delta 0, P by c. One turn corresponds rotation of 360 degrees right; the full turn. Then, the helical twist is essentially delta phi, this is given by 100 degrees; 360 degree over 3.6 ok.

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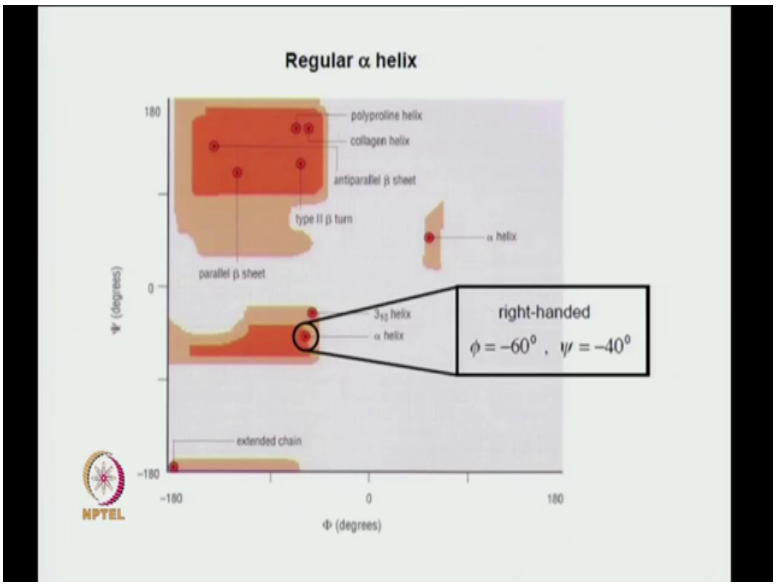

So, this is a regular alpha helix again I can, if you remember the Ramachandran plot, we have seen before this is where the regular alpha helix comes ok. Having minus phi of minus 60 psi of minus 40. So, you know this is phi just in the negative of phi and this is psi. So, this is 0 and you have the negative of psi ok.

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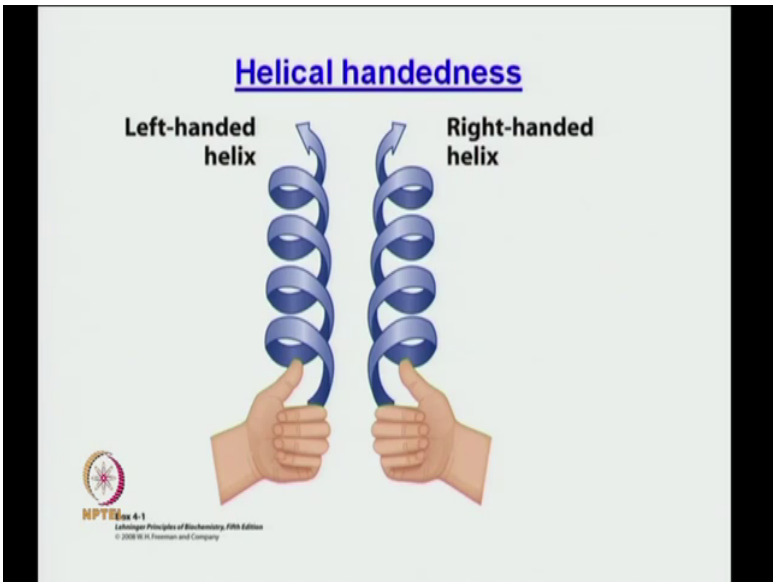

Helical handedness, I can have a right-handed and I can I have a left-handed. So, if you remember, so if you know this one is your left-handed alpha helix and mostly, what we have is you can just look at how the left-handed and the right-handed differs. So, this is how you know right-handed and then you have the left-handed. So, this is what we mostly encounter very rarely we have a left-handed combinations helix.

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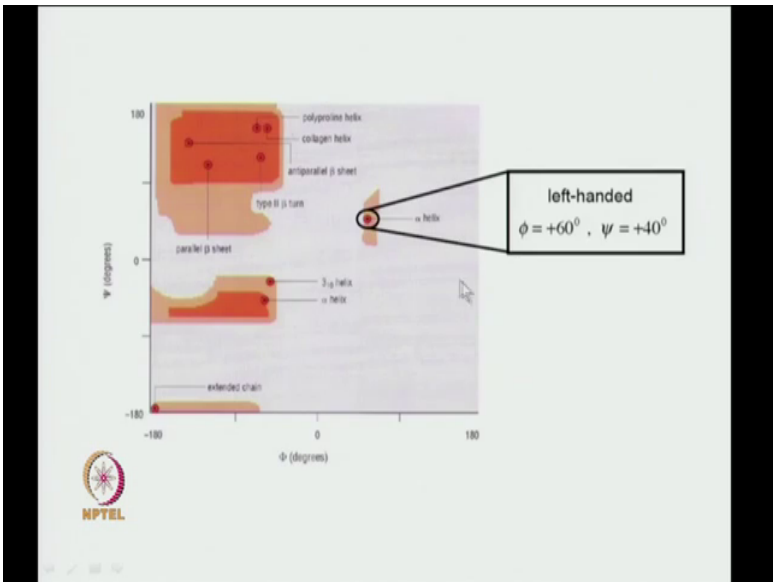

This is about the left-handed helixes both. So, both phi and psi angles are in the positive domain, you can see phi is plus 60, psi is plus 40.

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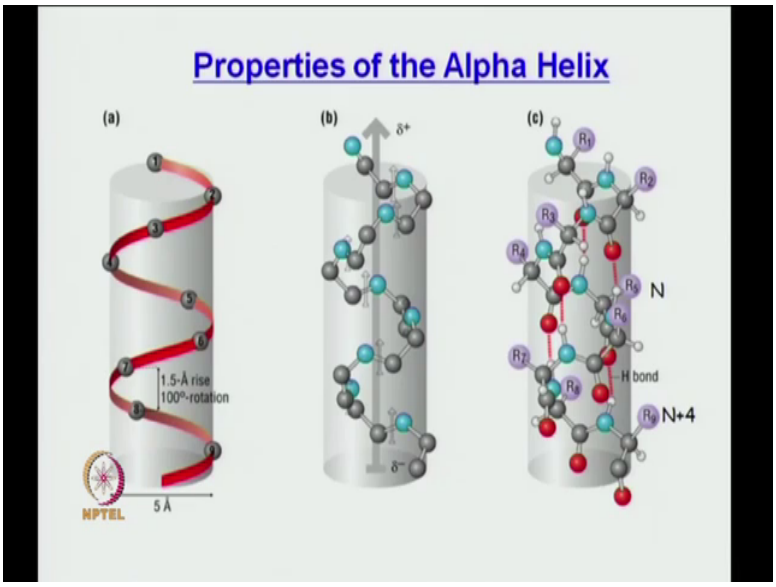

Now, some more properties of the alpha helix; so, you look at this. So, this is I just go you know through this one by one. So, this is some schematics and you can see the alpha carbon atoms. Here, I can see these small arrows ok. These are the helical dipoles right; the small dipoles of the individual peptide bonds and this thick arrow is a helical dipole and then, here is the third one, you almost see everything ok.

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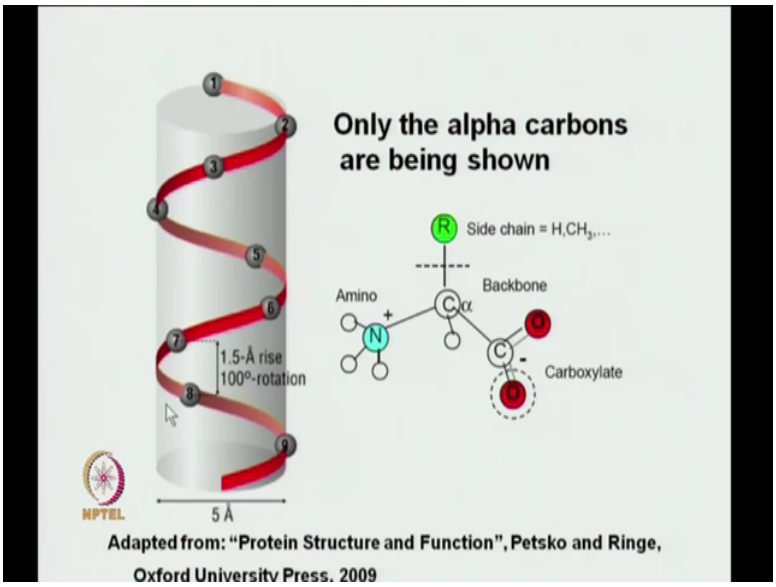

So, let us look at what I am trying to say the first one. So, in the first one, what we have is we have only the carbon atoms, the alpha carbons; I mean c alpha. If you remember, c alpha; this was what c alpha was. So, I am showing only the alpha carbons; is not it? Now and also keep in mind 5, 9, difference of 4 residues. 1, 5 again difference of 4 residues remember and hydrogen bonds would be between i and i plus 4th residue ok.

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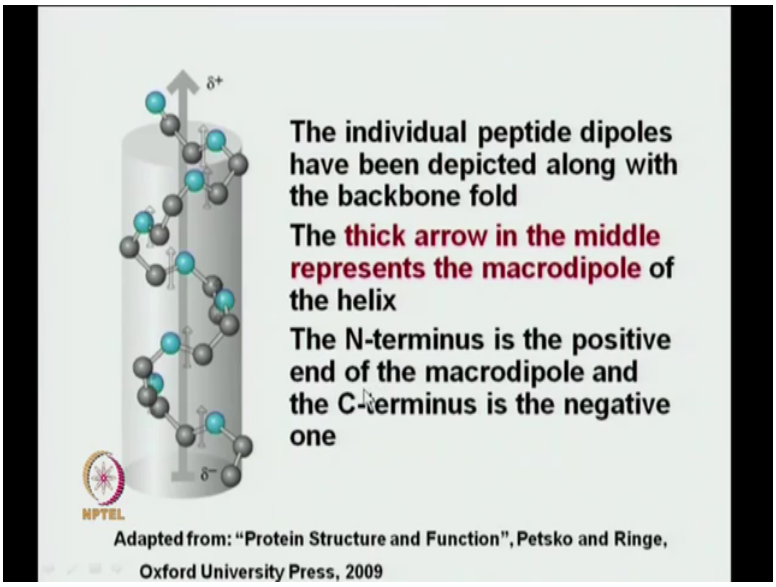

Now, if I look at the second one. So, here this is a small dipoles of each and individual peptide bond and then, all these since they are in the same direction, they sum up together to give this collective dipole. So, this dipole; that means, a helix has a micro dipole. So, the individual peptide dipoles have been depicted along the backbone fold as you can see.

So, these are the individual helical dipoles right. This is the tick arrow which is the arrow in the middle, this thick arrow, this thick arrow difference the macro dipole of the helix which is summation of all this small individual dipoles. As a result, what happens is see your this is the N - terminus and this is the C - terminus ok. So, the N - terminus is the positive end and the C - terminus gives you the negative end of the dipole right ok.

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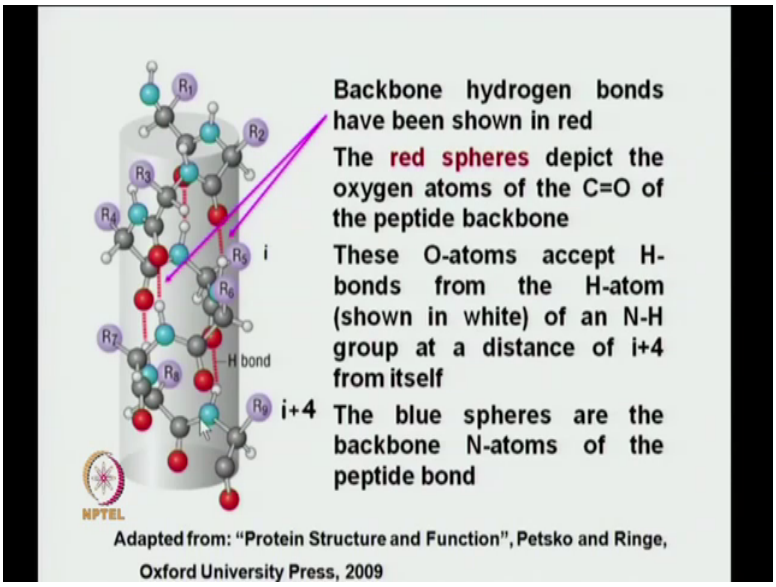

The third one, remember where we are seeing almost everything. So, these are the backbone hydrogen bonds which have been shown in red. Then, the red spheres so that means, these are the red spheres, these are the red spheres, these depict what the oxygen atoms of the C-O of the peptide backbone or there is a main chain. Then, the oxygen atoms, what do they do?

These oxygen atoms they accept the hydrogen bond from this H attached to the N of the H you know the H in the NH group at i and i plus 4. So, if you look at this R 5, say this is i, then you see R 9, this H is hydrogen bonded to the C-O of this R 5, the residue having the side chain R 5 say. These i and i plus 4, 5 plus 4 is 9. The blue spheres are essentially the backbone nitrogen atoms of the peptide bond ok.

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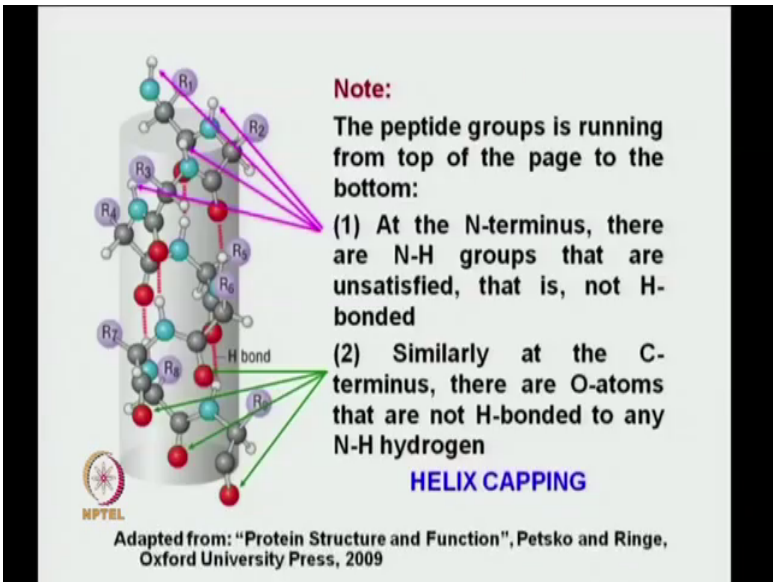

So, what do we note here special mention, the peptide group its actually group should be group is running from the top of the page; that means. So, this is R1, R2, R3, R4 so on; that means, is starting from the top and it is going towards the bottom.

Now, what you get is at the N – terminus, this is important, there are energy groups that are unsatisfied that is not hydrogen bond remember all NH and C-O groups are not supposed to be hydrogen bonded. You can see here, look at this 1 2 3 4; see these are without any hydrogen bonds to a C-O group because that is where helix is standard, there are no other things out there.

Similarly, you know you know a similar thing would be happened to the C - terminus. So, you can see there are also oxygen atoms like 1 2 3 4 which are free, which are not hydrogen

bonded now. This gives rise to something; it is an important concept in a protein structure, it is helix capping.

That means, you have some residues either at the N - terminus and on the C - terminus and which I am both I mean which help in neutralising or forming hydrogen bonds with these unsatisfied donors or acceptors.

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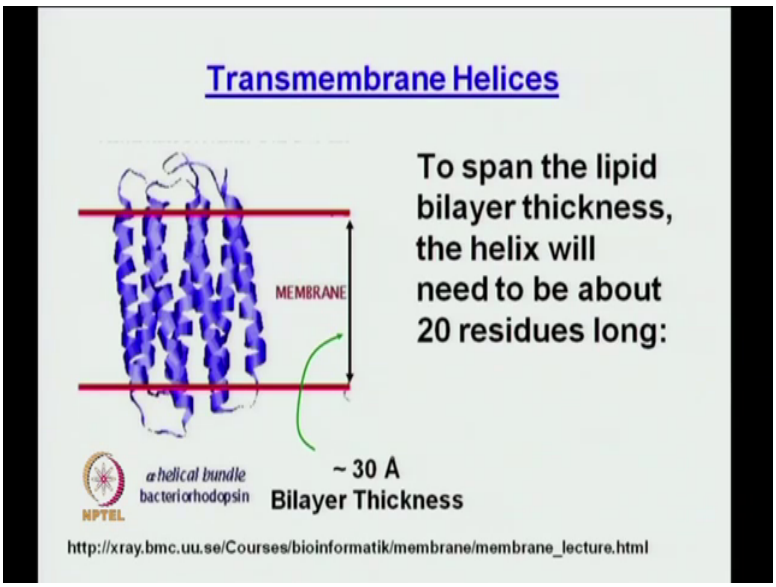

We talked about Transmembrane Helices because if you look at membrane proteins, there are essentially the segments panning across the across the memory is essentially transmembrane, that is what we call transmembrane.

So, this is what we talking about. So, this is the membrane ok. This is so this whole thing is a membrane from here to here right and you can see this helices encompassing the membrane,

this is called the transmembrane. This of the protein bacteria or hodopsin ok; thickness of the membrane is about you know 30 angstroms that is what we see out here. So, Bilayer Thickness.

Now, to span the lipid bilayer thickness, the helix will need to be about 20 residues long; is not it? Because it is about 30 angstroms. So, why does it need to have 20 residues long? Because remember, I move from one residue to the other, it is 1.5 angstroms. So, this is what I have 20 times 1.5 which is 30 angstroms ok.

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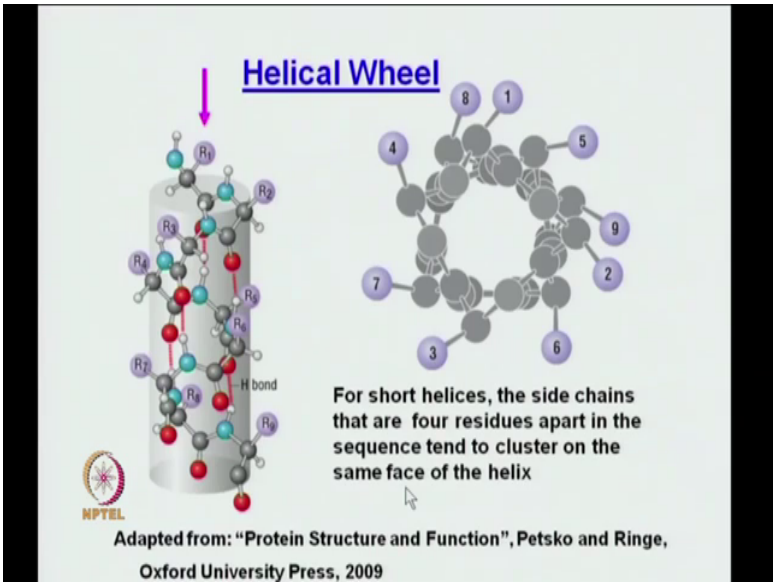

Now, what do you mean the helical wheel? So, look at this if I look at you know this helix from the top; that means, this what I am viewing it? I a m viewing it from the top right, I am not looking from the side. So, going back; so, this is you looking from and this is how it looks right.

So, you see R 1, this is 1; then, 5 9. So, you see this R 1, R 5 and R 9; these are almost on the same side. Are not they? And hence, here also they are occurring on the same side ok. Then, you talk about 4, 7; you can see 4, 7, they are occurring on the other side. So, this is helical wheel. So that means, you are looking like this through the helix from the top and this is how the wheel looks like.

So, for short helices the side chain, there are 4 residue is a part, in the sequence tend to cluster on the same face of the helix. That means 1, 5, 9. So, they are clustered in the same face. Then, 2, 6; then you can see 4, 7 right. You know they are kind of clustering on the same face or when I can say instead of saying 4, 7 maybe 4, 8 is better; i plus 4 remember, its 3.6 residues per turn right.

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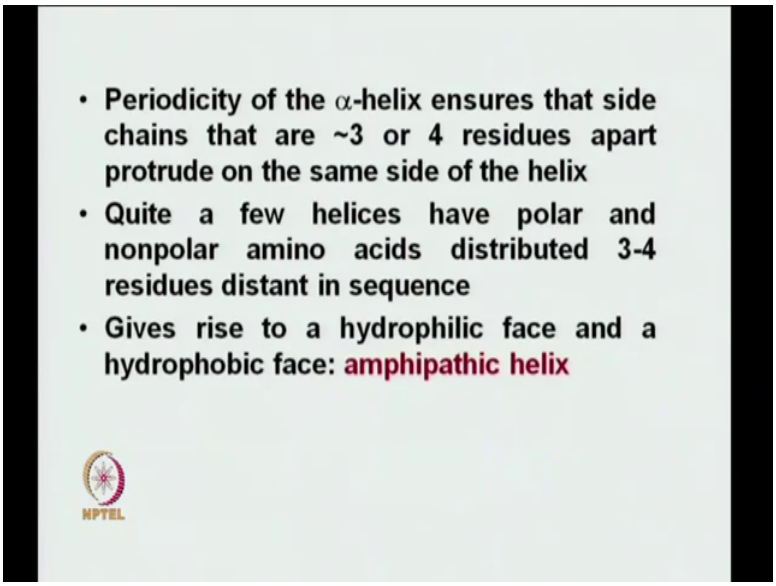

So, the periodicity of the alpha helix ensures that side chains that are about 3 or 4 residues apart protrude on the same side of the alpha helix right, that is what happened right 4, 7; 4, 8. Quite a few helices have polar and nonpolar amino acids is distributed about 3 to 4 residues distant in the sequence.

So, this gives rise to hydrophilic face and a you know hydrophilic face and this should be a ok. So, this should be hydrophobic, let me change that ok. It is a hydrophobic face that is what it should be. So, it is a gives rise to hydrophilic face and hydrophobic face, it is called amphipathic because its amphi pi; that means, it has both hydrophilic and hydrophobic faces. Now, such an arrangement can stabilise packing of helices and very well-known.

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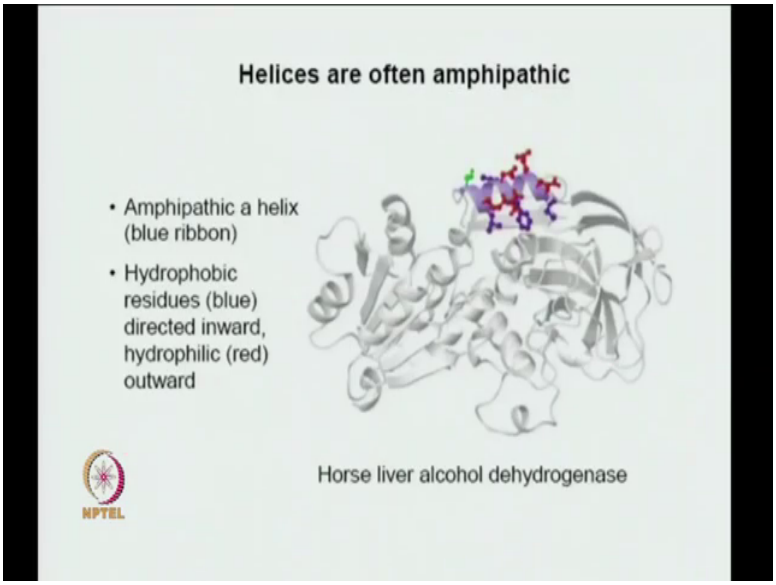

Now, this is an example of an amphipathic helix. So, this is your Horse liver alcohol dehydrogenase and you look at this helix. So, is an amphipathic helix and how do you know?

So, the hydrophobic residues are these blue ones; these blue ones are the hydrophobic residues and these residues are pointing inside.

That means, towards the protein and these the red ones, you can see these red ones, these are pointing out. So that means, towards the solvent. So, that is how they are. So, there is a blue ones hydrophobic, on the same side pointing inside; the red ones hydrophilic on the same side, they are pointing outside ok.

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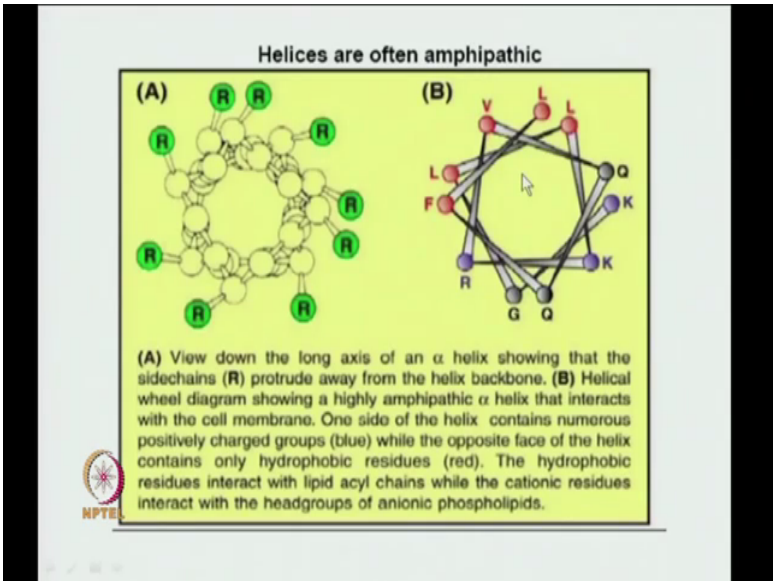

Again, an example of an antibiotic amphipathic helix; so, this is how the helix again is going to look like, if I look at it straight through. So, this is as I said a is view down the long axis of an alpha helix showing the side chain. So, all these R's of the side chains. Remember we had labelled them as R1, R2, R3, R4 like that.

So, these R's protrude away from the helix backbone. So, this is important. So, the way the helix arranged in itself is at the C-O in the NH groups, those main chains, they point inside where the all side chains; they are actually pointing outside ok, they are pointing outside.

Now, this is your helical wheel. So, the helical wheel diagram, it shows a highly amphipathic alpha helix. Remember we discussed amphipathic. It would be having both hydrophilic and hydrophobic faces. So, what would happen is on one side, you can see instead of one side of the helix, it contains numerous positively charged groups which are the blue ones, which are these ones.

So, R arginine Q, you should know what Q is, K lysine right, then you have G out here which is glycerine is not particularly charged. So, this face is essentially again a Q here. So, this face is essentially hydrophilic face. You go to the other side, you have F, L. What is F, L? An allen right, L leucine, V valine, leucine leucine; this is the hydrophobic face.

So, you have two faces amphipathic; this is hydrophobic, this is hydrophilic ok.

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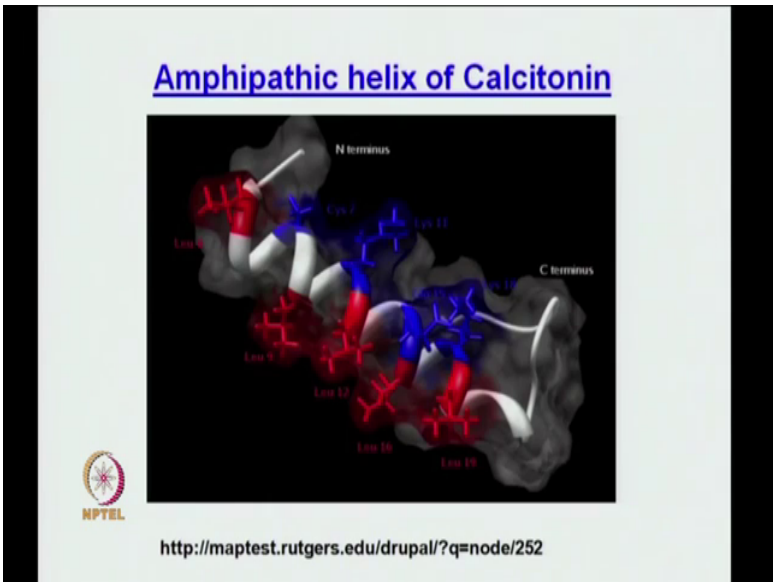

This is another example of peptide Calcitonin. This is an hormone, you can see out here again here the red ones, it is a different difference in colour. The red ones you will see leucine, leucine, leucine, leucine, leucine; these are all the hydrophobic amphipathic right; hydrophobic face the cysteine lysine right. What is G L U? You are right. This glutamate lysine, this is amphi, I am sorry hydrophilic right. So that means, hydrophobic face, hydrophilic face.

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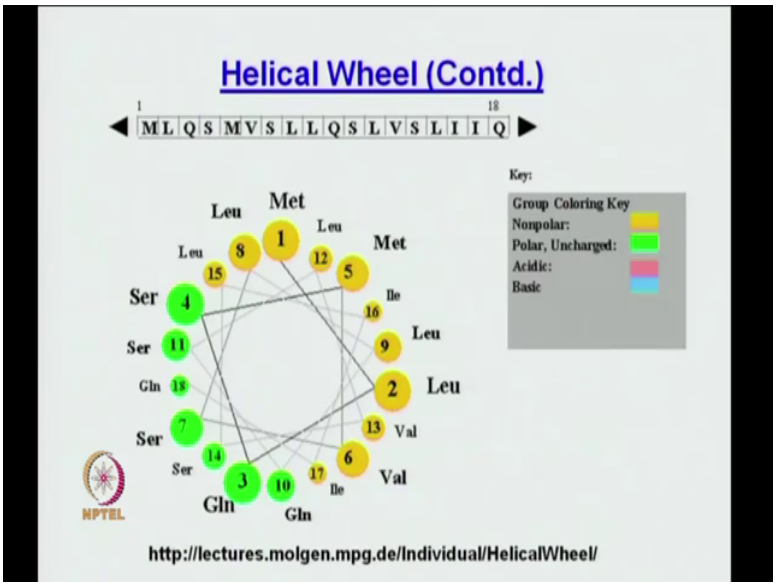

If you cannot do the helical wheel, again you look at this one. So, there is a group colouring. If you look at this helical sequence, what do you see out here is the nonpolars are represented by yellow; the polar uncharged represented by green; the acidic you know by this pink; the basic by this light blue.

So, you can see what has happened is on this side leucine, leucine, leucine, isoleucine, leucine, leucine, valine, valine; all these on one side and all the polar or uncharged all these like glutamine, serene, serene; all these on the other side. That means, this is the hydrophilic face again, this is the hydrophobic face.

So, this is you know huge consequences and hopefully, we can go through some of those in the next class. But this is what a helical wheel is supposed to look like and what the

importance of it is that you can look at through down and look at the different ways, then the all groups are packed.

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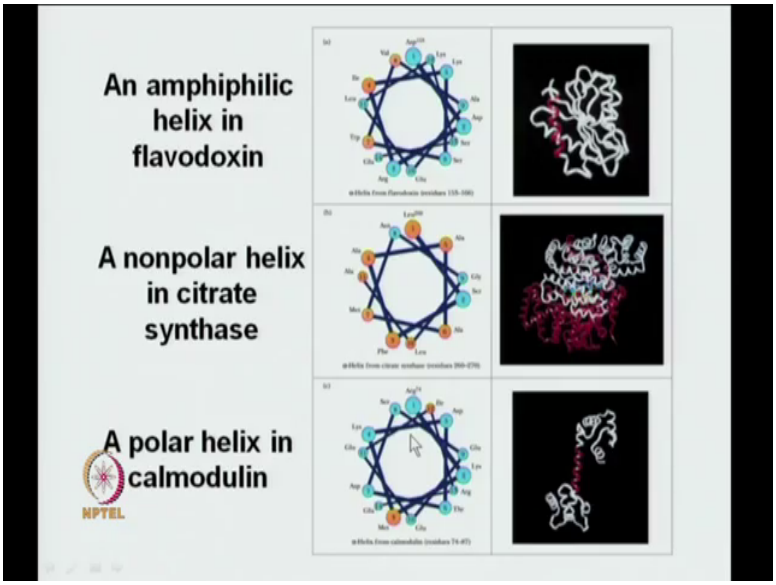

So, again this is another example. So, the first one it is an amphiphilic helix, you can see amphiphilic means. Well, you can have these these are isoleucine trip valine, these are its essentially amphipathic; that means, these are the hydrophobic, this is the hydrophilic. Then this is the nonpolar.

It is a nonpolar, why? You can see wherever you are looking at these, these yellow ones nonpolar is essentially non polar and this is a polar, polar because you have a predominance of these blue ones which are the polar residues.

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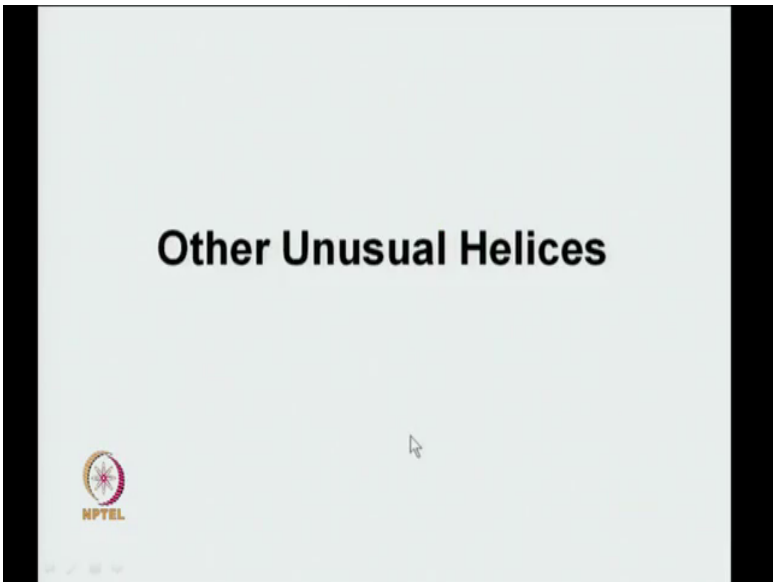

There are other unusual helices, what we will do is these other unusual helices; we will talk about in the next class. Then, we will look at some more aspects of these helices and once, we are done with this unusual helices, but just you know give me a couple of minutes, I will just well I will just keep it for the next class I guess.

So, once we are done with this, then we will look at some helix packing; then we will look at the second most important one, structure secondary one is a beta sheet and we will go ahead and look at several combinations of those ok.