Bio-Physical Chemistry
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Lecture - 02
Protein Structure

Hello everybody, welcome to the second class right. So, this class we are going to talk about Protein Structure. As you will see it will be an introduction to the protein structure without going into too much of details. But some of the some you know some details like amino acid structure and the outcomes of the amino acid structures or the peptide bond that is these are things we are going to look at in some detail right.

But before we go ahead with today's lecture, what we are going to do is, we are going to do a brief recap of what we did last class.

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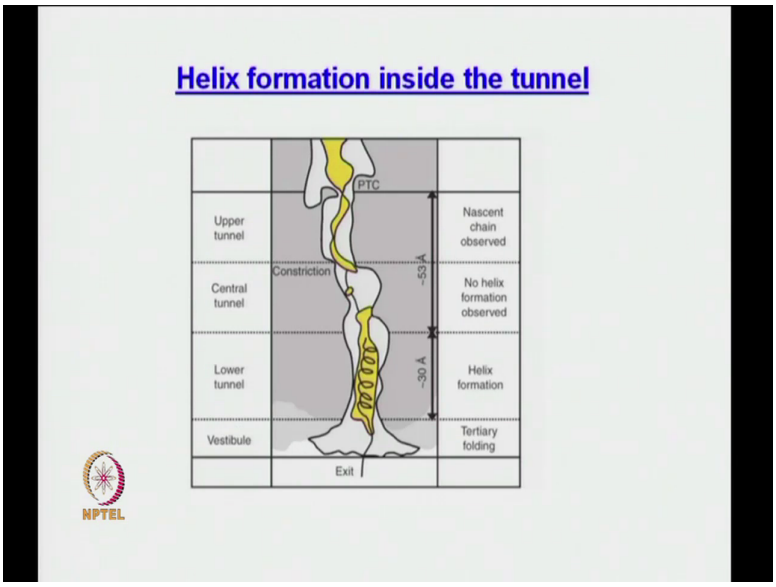

So, remember in last class we were talking about the protein synthesis on the ribosome and we were you know dealing with different aspects of the synthesis. So, we had this the peptide tunnel that is the figure or the diagram you are seeing is belonging to that ribosomal tunnel. This PTC phenomenon was a peptidyl transferase centre and you know this is the exit side, through which the elongating nascent chain there is a chain which is being formed in the ribosome is coming out.

Now, not only that apart from this peptide getting synthesized or you know the protein getting synthesized, when this protein or this elongated chain comes out through the tunnel that also many interactions it faces right or it experiences.

Then there is a constriction at some place in the tunnel right and we looked at why we have a constriction out there, because of you know to specific proteins being present there giving rise to the constriction.

And then after it comes out to the exit site which is you know a little bit wider, because that is where the tunnel ends. And, if you remember we discussed about the presence of the trigger factor which was a chaperone and the trigger factor made sure that the peptide that was coming out or the domain axis are coming out they are well protected from having bad interactions with the surrounding environment.

So, and we also I think talked about one aspect where we said that you know within this tunnel, a question can be asked is does a protein start having structure or start you know getting structure inside the tunnel itself yes evidence is there.

And as we were discussing in last class using fret people have shown that means this donor acceptor fluorophores people have shown that yeah, in there is development of some sort of secondary structure or even tertiary structure inside the ribosomal tunnel.

So, it is not only a place where the through which the you know the elongated, elongating chain comes out, is also placed where the protein starts you know gaining some shape which possibly helps it later on when it goes to the folded form.

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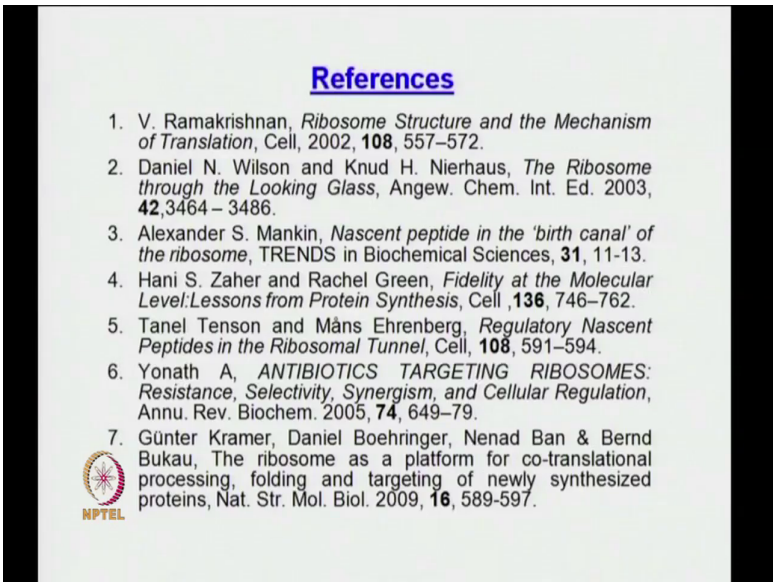

These are some references that I also put up in last class, but I want just want to you know go through these couple of slides really quick.

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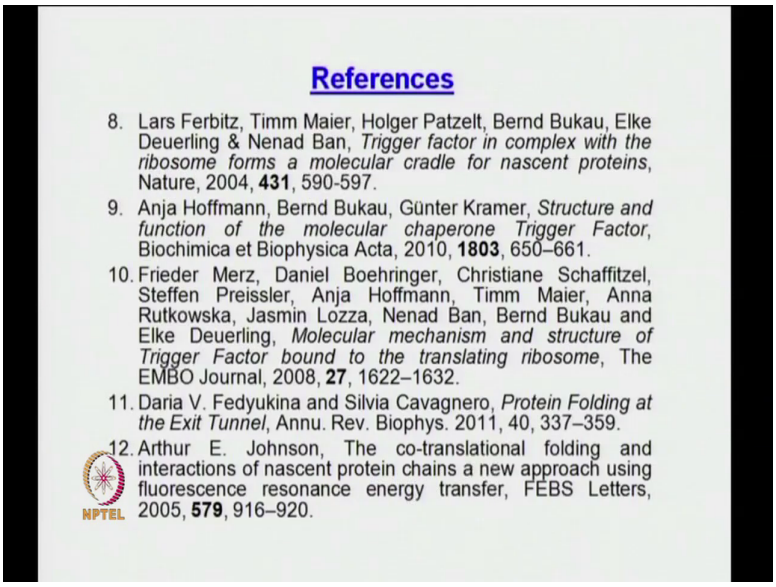

If you have an a hopefully you will be having time please do go through these references, these most of the materials we talked about have been taken from these references. And to have a much better idea it is really worthwhile to go to this go through this references each and every reference is excellent in it is own respect.

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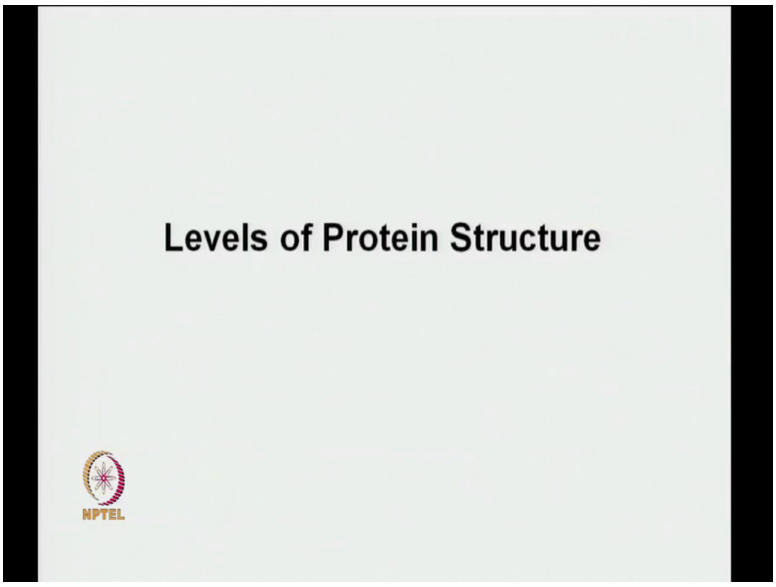

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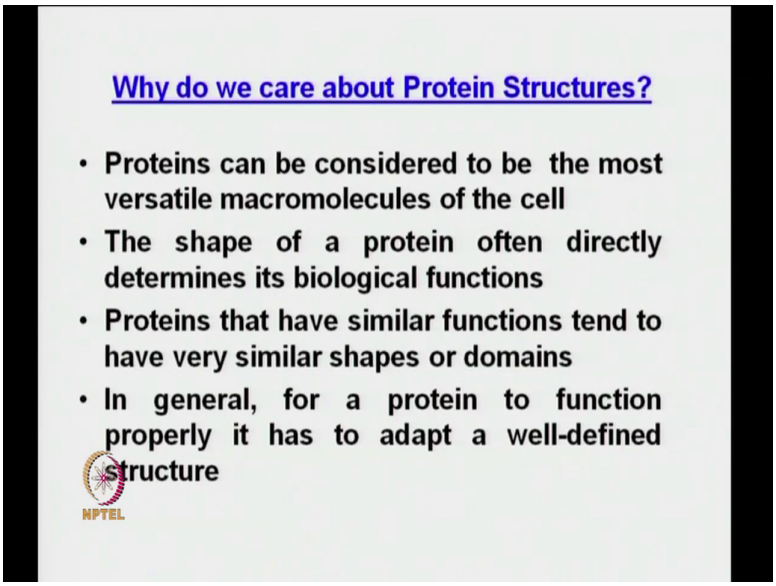

Now let us now move forward and talk about what we hope to discuss in today's class it is about the levels of protein structure right. Now the first question that comes to a mind is why do we really care about protein structures why should we care? Then this we all know it is just reputation of a well known fact that proteins can be considered to be the most versatile macromolecules of the cell.

That being the most versatile macromolecules of the cell you can understand that the proteins would be involved in various functions. Typically each and any function you can think of and you would see a protein component being there. So, hence protein is so important to us.

Then the shape of a protein often directly determines its biological functions right. What are the types of functions is going to follow or we going to perform? For example, each and every proteins is not going to be able to perform all types of functions. So, there has to be something

in the protein structure on the or in the protein shape itself, which makes it perform the function it is supposed to carry out right and hence the shape of a protein is so important.

And if you are going to talk about the shape of a protein, then the shape of a protein comes because of the structure it develops when it finally goes to the native state right. Then proteins this is also known fact that is there you know families are proteins, which have similar functions and they have been found to have similar shapes. So, again this is an important point with respect to the structure of the protein or why we should study protein structure.

And in general for a protein remember please focus on these two words in general. What I mean is, that in general for a protein to function properly it has to adopt a very well defined structure right.

That means, if it has to perform of function properly it better have a structure well defined shape. But this does not mean that each and every protein we have within ourselves has a structure, there are full proteins known as unfolded proteins which remain unfolded they do not have any structure.

But even in the unfolded form they are functionally active and this class of proteins are referred to as intrinsically unstructured proteins or intrinsically disordered proteins. Now, this is a class of proteins will not be talking about much, but these are also very important because of their flexibility you know because they are unstructured ok.

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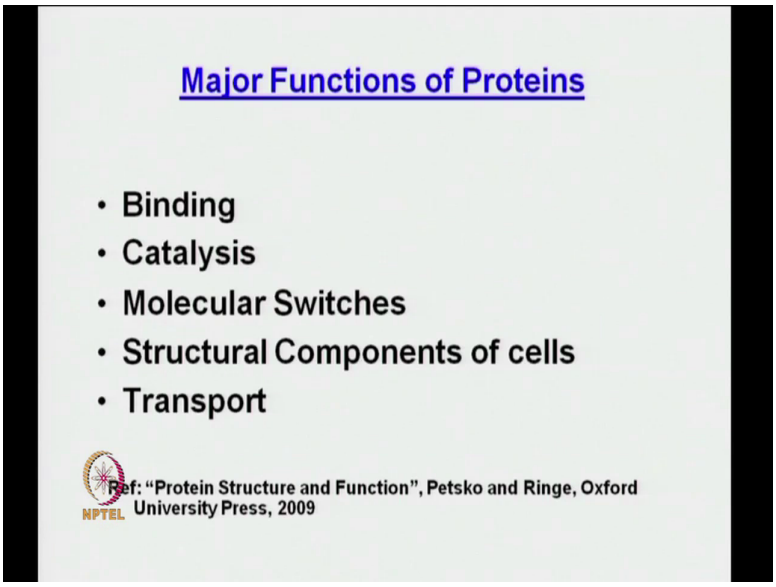

So, let us look quickly at some major functions of proteins. So, one is binding we will quickly look at a couple of examples of these, then it is catalysis that it will help in catalysing certain reactions.

As molecular switches often on switches maintaining structures of cells that means helping in the in maintaining the structure, that means a structural components of the cells then transport. And when we talk about transport you know the protein that first comes to your mind is haemoglobin right ok, it is a you know it is a former protein it is a tetramer.

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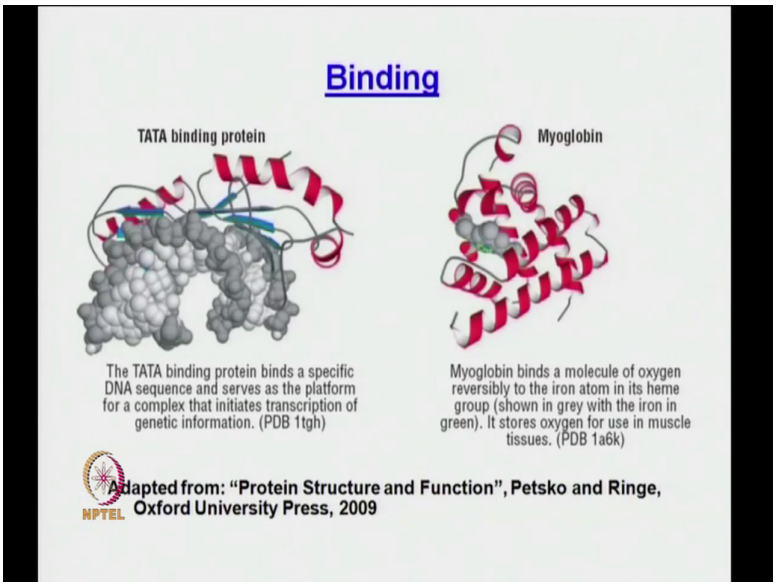

So, what about binding them well here two examples? The first one is a TATA binding protein. Now this protein you can see so this is the protein and this is a DNA sequence at the bottom part. So, if you follow the arrow this protein recognises a specific DNA sequence or binds to a specific DNA sequence and this serves as a platform for the initiation of transcription of genetic information you know transcription is so very important.

And hence this forms this binding this binding of the TATA of the you know the TATA this TATA binding protein. I mean that is a protein which is binding to this TATA box is the prelude to this transcription initiation.

Then we have the well known protein called Myoglobin. So myoglobin is known to bind to oxygen, it is found in muscle tissues right. And what it does is if you look at the centre this greyish space filling model this is a model of the heme cofactor.

This has an iron atom in the centre where the oxygen comes and binds right and myoglobin reversibly binds the oxygen. That means, it binds the oxygen it also releases it in times of need that is why it said is it stores oxygen for use in the muscle tissues right. That means, it keeps it there and when seen it let us the oxygen go that is why it is called reversible.

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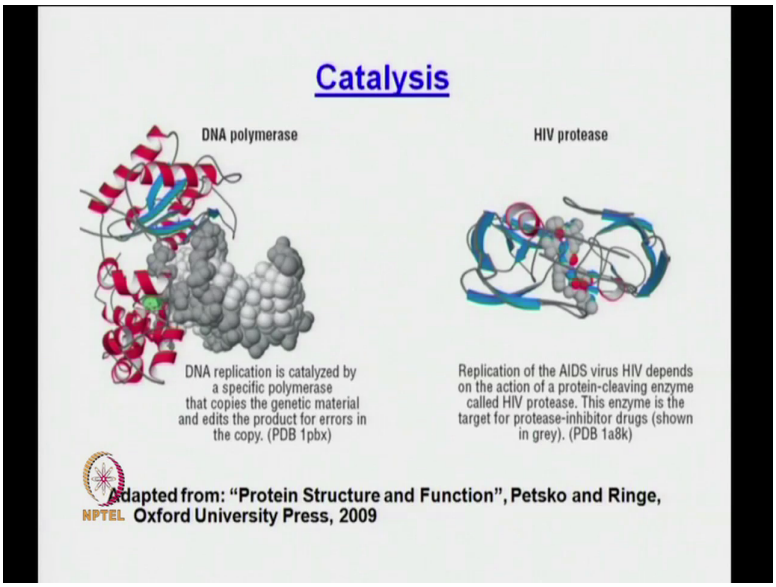

Then we have catalysis the first one is DNA polymerase. So, DNA replication we are talking about DNA replication in this is catalysed by a specific polymerase that is known as DNA polymerase right. This copies the genetic material that edits the product for errors in the copy.

So, essentially this one helps in proof reading of what you are typing out or what this complex is typing out.

Then we have HIV protease, the HIV protease this replication of the AIDS virus HIV depends on the action of this protein cleaving enzyme called HIV protease. So this enzyme therefore you can understand this enzyme has been the target for many protease inhibitor drugs, hence it is significance.

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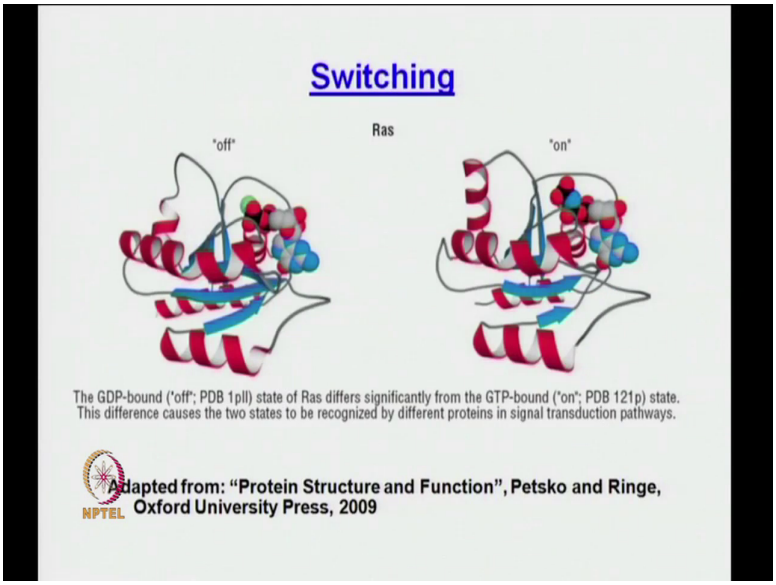

Then the switching on and off; so, the Ras group of proteins is are extremely important in signal transaction or signalling pathways. So, on the left it is called the it is referred to as the off you know state and the on the right is the on state. So, the left what we see is that it is a GDP bound state which is the off state and this conformation of the off state significantly different, differs from the on state where it now the Ras is GTP bound.

So, you can see if you are trying to figure out where the confirmation is difference. So, follow this arrow of mind you look at this portion and then you compare the portion out there you can see it is definitely drastically different. Then you compare these two strands out here and then you also compare this one out here again this is different.

So that means, when GDP and GTP are bound then the protein is respectively in it is on and off. Confirmations which accordingly is sensed and then transfer signal through the signalling pathway by combination with other proteins ok. That is why it says the difference causes the two states to be recognised by different proteins in signal transduction pathways.

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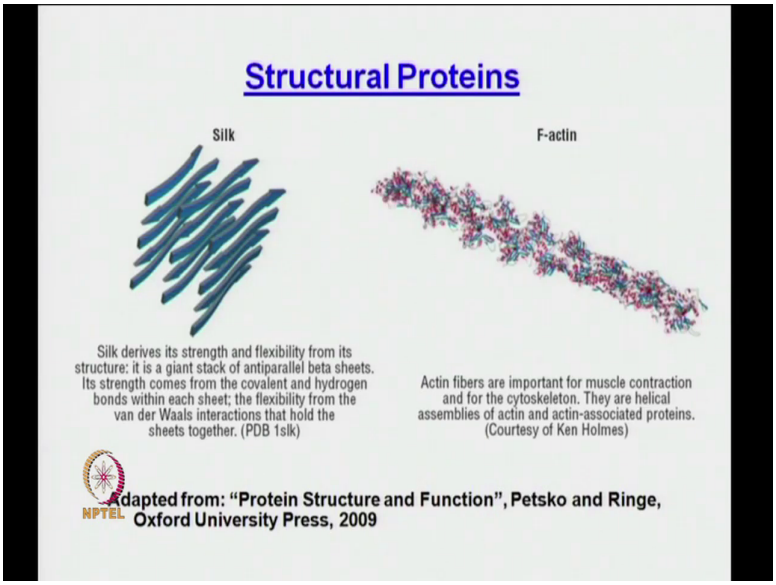

And last but not least we have the structural proteins one is the Silk you have heard about silk, silk derives it is strength and flexibility from it is structure it is a giant stack of anti parallel beta sheets it is written out here. It is strength comes from the covalent and hydrogen bonds

within each sheet, the flexibility comes from the van der Waals interactions. And then the we have the Actin fibres which are so important for muscle contraction and the cytoskeleton.

So, this is structural proteins they give structural balance or they held the cell in the structural aspect. Now, having looked at some different functions right, I mean I am not saying that these are all the functions that able to cover, but at least these come under very and four very broad classes. The other one I did not show was a transport and I said that haemoglobin does do that right we all know that.

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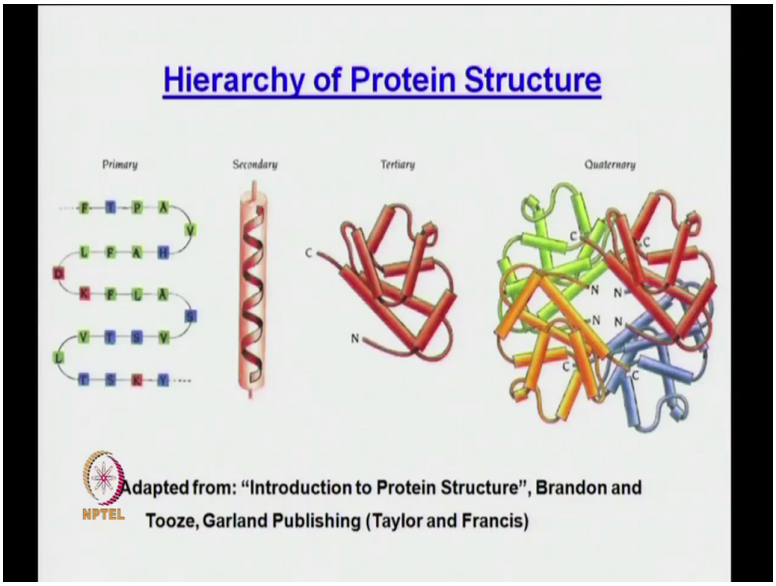

So, whatever the different levels or the hierarchy of protein structure; so, all of us know that there are 4 levels of protein structure, I am sure you have all of you have done some preliminary course in biochemistry. So, will not be going through this in very details, but you can see the first one which is the primary structure.

This is essentially your amino acid sequence right it is the sequence or the way in which the amino acids are placed in the peptide chain or way it is synthesized you know by the ribosome. Then you have the secondary structure there is mostly your alpha helix beta sheet or a combination of these.

Then you have the tertiary structure which what is tertiary structure it is essentially a packing of a different secondary structural elements and then you have quaternary structure. The quaternary structure itself is like a packing of a tertiary structural elements right. So, these are the 4 levels of protein structure and this is what is referred to as the hierarchy. So that means, you have the primary first then the secondary then the tertiary and then the quaternary.

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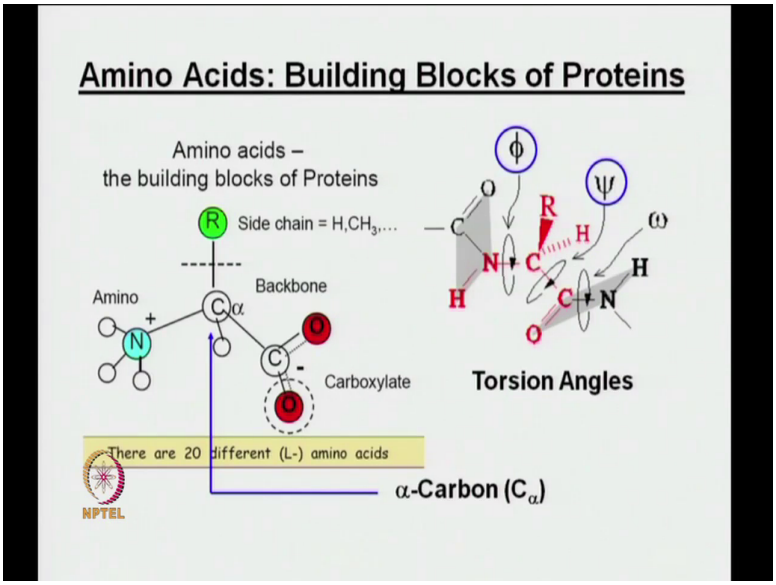

Let us start looking at the amino acids what is Amino Acids, the amino acids are so essential for us why because these are the Building Blocks of Proteins. So, let us look at the amino

acids structure. So, what we have is you know here we have it is an amino acid right. So, we have an amine group out here, so here it is protonated NH3 plus then it is an acid. So, we have a carboxylic acid out here it is deprotonated.

Now, we have a side chain which is R and here we have a carbon atom right ok. So, this carbon atom is very important it is called the alpha carbon atom and this alpha carbon we will see we will mention it quite often as we go on to today's class. Now this R is a side chain and depending upon the R; depending upon the R your nature of amino acid would vary.

That means, whenever you have a different R you have a different amino acid and that is how you have 20 different amino acids that will just depend upon the R right this for it stays in the bottom. There are 20 different amino acids and these are L amino acids these are the naturally occurring 20 different amino acids.

So, depending upon the nature of the R also the nature of the amino acid will change, nature means whether it is polar whether it is non polar whether it is charged neutral all will depend upon this. So, what you can understand is this is the basic structure of the amino acid. And, here what you have is each amino acid has this Amino group, the Carboxyl group and R group and which gives itself identity and then you have the C alpha in the middle, this is essentially your cradle centre ok.

Now, what do we have on the right. So, here this one what we are seeing here are the torsion angles. So, what do you mean by torsion angles is; if you look at this N C bond or the C C bond. So, this N this N bond this C is called the C alpha see the C alpha here, so this C alpha and this N right. So, this is referred to as the N C bond, then the C alpha then the C alpha and this C this is the C C bond.

So, if you have a rotation on the N C bond this rotation this torsion angle is referred to as phi and if you have a torsion angle if a torsion angle along the C C bond this is referred to as psi. And, then the C N the C N or this C N which and you see the you know it is kind of looking like a plane is shaded like a plane, this is your peptide bond. So, this one is a peptide bond we

will look at it in the next slide in a little more detail and any torsion around that ball is represent by the angle omega.

So, why we are looking at it right now well possibly it is the best place to look at because we have just started, you know looking at the amino acid structure. Because this leads to one of the very famous plots in biology or biochemistry is referred to as a Ramachandran plot and we will come to it later. So, what we will mainly be focusing on are these two angles the phi and the psi right ok.

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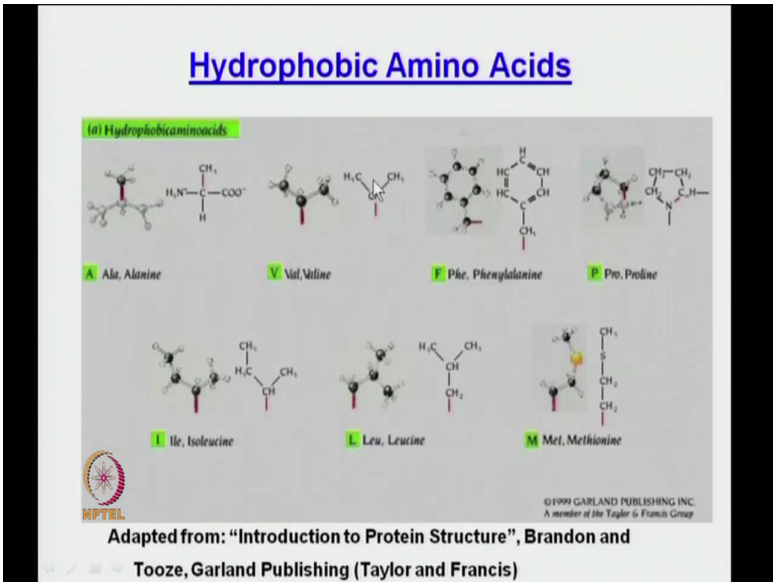

So, let us look at the different types of amino acids there is the natures and as I said how are these natures different this nature depends upon the type of all you have. So, if it is Hydrophobic amino acids then you can understand your R is essentially hydrophobic in nature.

So, let us look at the hydrophobic amino acids. So, you can see the first one is Alanine what is R CH 3 then your valine it becomes more hydrophobic. So, this is the group then you have phenylalanine, then proline then isoleucine, leucine and methionine right. So, this is your hydrophobic amino acids or the class or the you know the different amino acids coming under the class of hydrophobic amino acids.

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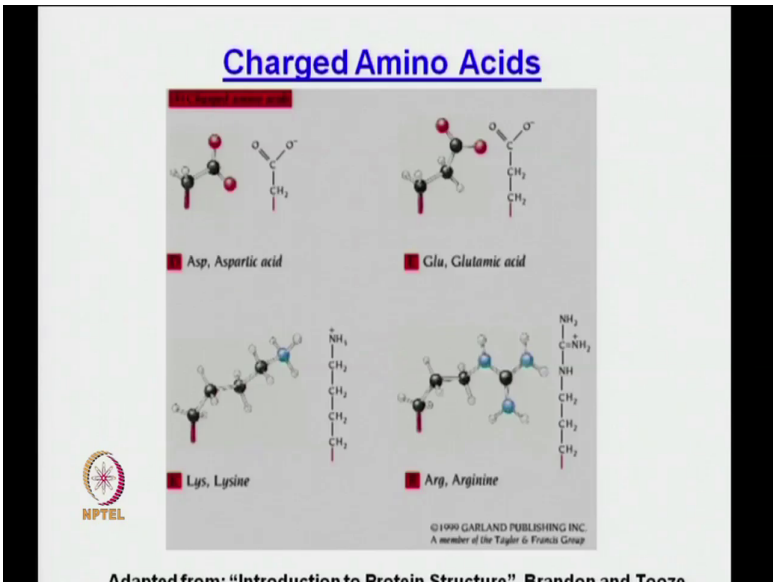

Then we have the charged amino acids; that means, the R group is charged out here. So, we have aspartic acid we have glutamic acid we have lysine we have arginine this charged acids.

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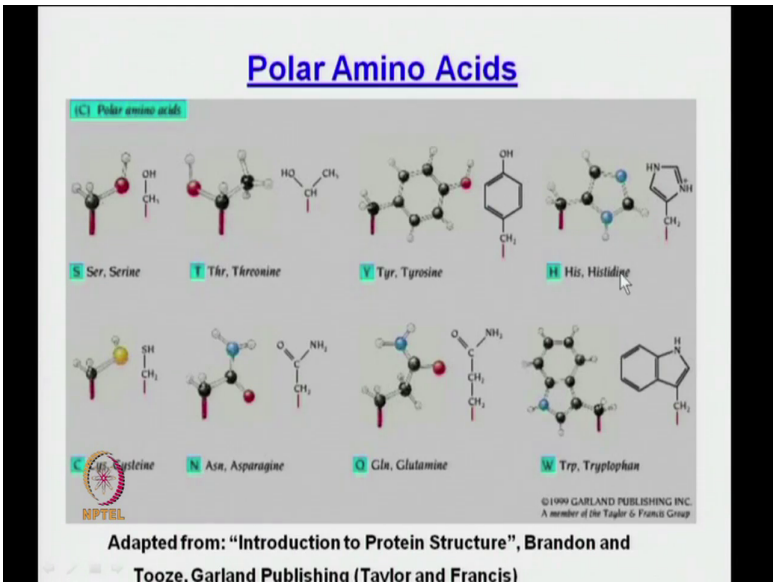

Again this charged amino acids mean that the respective side chains or the R groups they have charges. Then we have the polar amino acids. The polar amino acids, so I serine, so these are not charged but these are polar ok.

So neutral but they are polar then you have threonine then you have tyrosine, then you have histidine then a cysteine, asparagine, glutamine, tryptophan ok. So, then what we have is we have three different classes amino acids, one is hydrophobic, one is charged and then here the polar amino acids.

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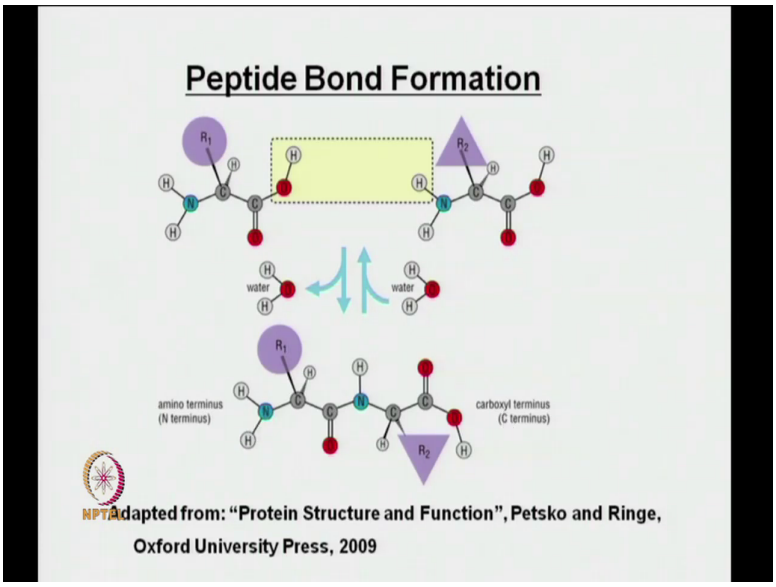

Let us look at the peptide bond formation. So, what do you have is how does the peptide bond form? Obviously, to have a peptide bond form we will need 2 amino acid units right. So, one amino acid a say one amino acid b or it may be the same amino acid a they come together in the form the peptide bond, so this is how it happens. So, you can see out here.

So, this is one it is coming like this on your left, this amino acid is approaching from your right here the N group then combines or reacts essentially with the C group out here the; C atom there is a loss of water molecule and then you form the peptide bond right. So, that is why it is a reversible arrow.

So, if you lose you found the peptide bond, then if you are trying to hydrolyze it will go the other way. But it does not mean that you if you put you know protein what it will really get hydrolyzed, it just tells you what goes on.

Then when you are forming this peptide bond, what do you get if you look at very careful is from the structure is on one side you have this amino group and the other side you have the carboxylic acid group right.

So, this amino acid group this amino group comes from the amino acid to your left and this carboxylic acid group comes from the amino acid to your right. So, the amino acid to your right as the chain as a side chain R 2 and the amino acid to the left has the side chain R 1. That means, if R 1 and R 2 are different, then obviously these are different amino acids.

So, then to a left you have the N terminus and then to your right you have the C terminus the carboxyl terminus. The C terminus because, it ends with the COH group the N terminus, because it starts with a NH2 group or the mean group.

So, that is why this is referred to as the N terminus and this is referred to as the carboxyl on the C terminus. Now, these are some terminologies you have to be familiar with throughout this course, because we will use a you know often ok.

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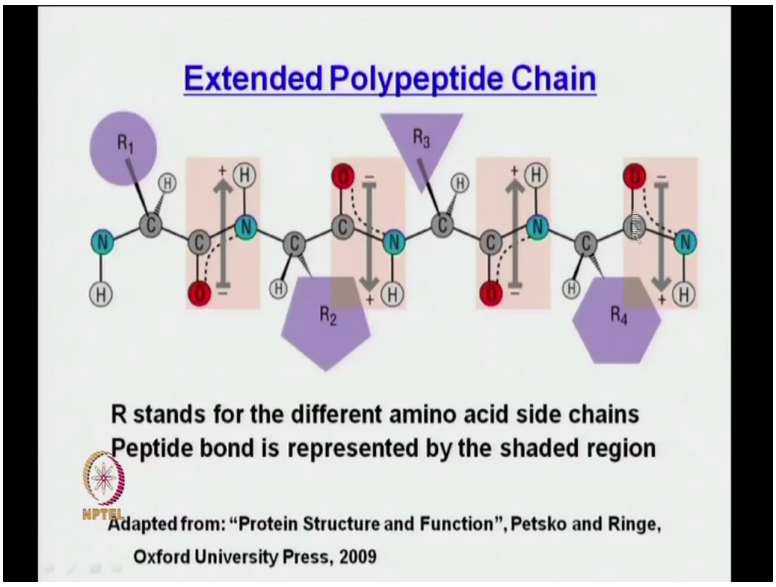

Now, what about an extended polypeptide chain. So, what you mean by an extended polypeptide chain here what we saw is we just had two amino acids coming together the main group of one reacting with the carboxyl centre of the other one, loss of water molecule we form peptide bond.

Now, when we get a protein right when we get a protein like myoglobin say has formed 53 amino acids, that means you can understand there are 153 amino acids which are forming which are coming together and being a part of the molecule forming the peptide bonds. So, this is typically how peptide bond looks like right, you can see you have 1, 2, 3, 4 different amino acid subunits are the R groups R 1, R 2, R 3, R 4 these again give specific identities to the proteins or to the amino acids we are combining.

So, here this dotted line if you look at this dotted line or before going to the dotted line if you look at this shaded regions right, as it says R stands for the different amino acid side chains then the peptide bond is represented by the shaded region.

So, this shaded region is which the shaded region is essentially a plane which contains the peptide bond you can see here also here and here. So, there are a 3 peptide bonds here and this the last amino acid is making another peptide bond.

Now, what you can also see is the C O and N, N is in blue O is in red through out what happens is here is the dotted line, what it means is, this C N has a certain double bond character. And because of this it is it you know it is a symbol of delocalization electrons are delocalized. So, if you remember there was this omega torsion angle around this C N bond and this omega is important because the C N being having partial double bond character, it is very hard to rotate around and this gives rise to many cis and trans configurations you will just see. And then what are these arrows these arrows essentially tell you your dipole moment vectors ok.

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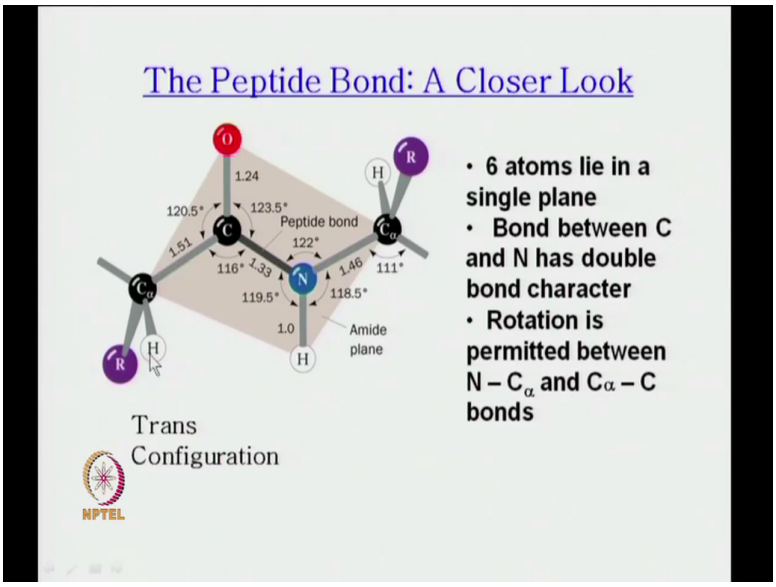

So, taking a closer look at the peptide bond let us look at it again. So, here we have the C alpha, I told you it is very important then the C this is N again the shaded region is your peptide bond or this is the plane which contains a peptide bond plane. Because the peptide bond essentially is planar as we just talked about it has double bond character.

So, some specifics there are 6 atoms lying in a single plane. So, you can understand C alpha C N C alpha these make 4 then H then O 6 atoms lying in the single plane. Now the bond between C and N has double bond character.

So, this one we are talking about C and N, this is the one which has double bond character right and this is a trans configuration. So, you can see this part and this part. So, if this is the one which is having the double bond character.

So, this is on one side of the double bond, this is on the other side of the double bond. So, this is a trans configuration. Now, whatever rotation being permitted so, rotation or the flexibility. So, rotation is permitted between the N C alpha and the C alpha C bond. So, what I mean with this is the N C alpha this is the N C alpha you can see this is a single bond rotation is permitted around it, then you also have the C alpha and C again. So, this is these are the different angles we are talking about, we will come to this come to that again and this C alpha C again this is a single bond rotation is possible around this bond ok.

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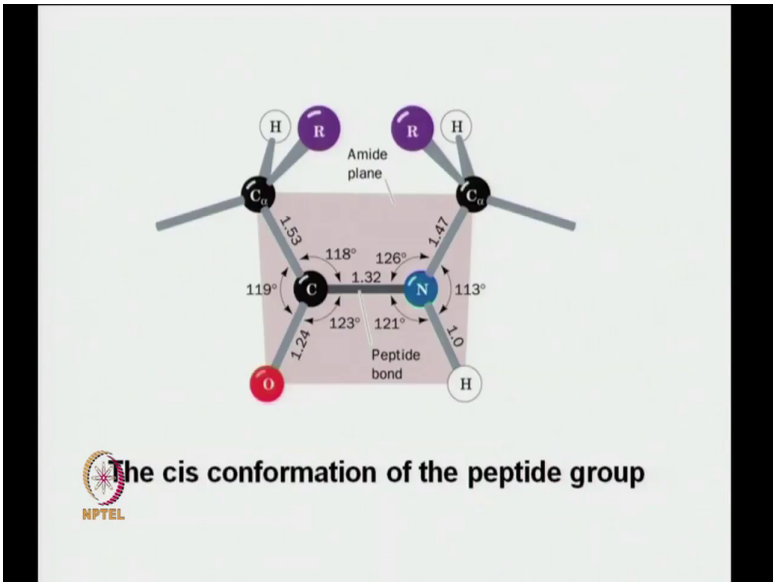

Now, once we are talked about the trans configuration which was in the previous slide, then the other configuration obviously is your cis configuration. Now you can see this again being the amide plane we have the same 6 carbon atoms on the plane. But now what you have done is you have taken the trans configuration and brought this.

So, you can see so if you go back you can see you these two were on different sides of the bond now you have taken this and brought it to the same side and that is what we have this is your cis conformation of the peptide group, ok.

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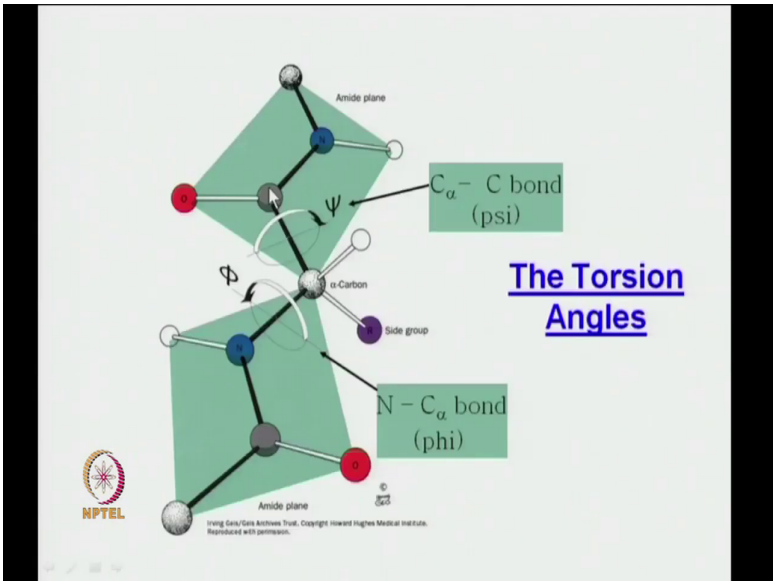

So, let us look at some details into the Torsion Angles. So, what do you mean by the torsion angles? So, this is what we mean. So, what we have out here is if you look at carefully is so this is the N this is the C this is one amide plane, again this is another amide plane.

Now, what we have is this is the alpha carbon, remember I will come back to this alpha carbon as I told you many many times. This alpha carbon it is very important because this is like a hinge this is like a pivot point along around which both these planes can rotate. So, you

understand so this one is restricted this guy is restricted too because this C N; this C N peptide bonds double bond characters cannot freely rotate.

However this plane and this plane they are hinged at one point which is a C alpha and along the C alpha they can freely rotate. How this C alpha and this guy this C; this C alpha and this atom which is N around these are single bonds right, so around these the planes can rotate ok.

So, then any rotation around the C alpha and N this bond there is a C alpha and N please follow my arrow, this is referred to as the phi angle or the phi torsion angle and any rotation on the C alpha and C this this one is referred to as the psi torsion angle and that is what this one says.

The N C alpha bond is your phi and the C alpha C bond is referred to as the psi torsion angles. And if you have I am I am sure already know these two in combination if you plot it gives rise to the Ramachandran plot and it actually tells you what are the possible or admissible psi and phi values that a protein or a polypeptide chain can take.

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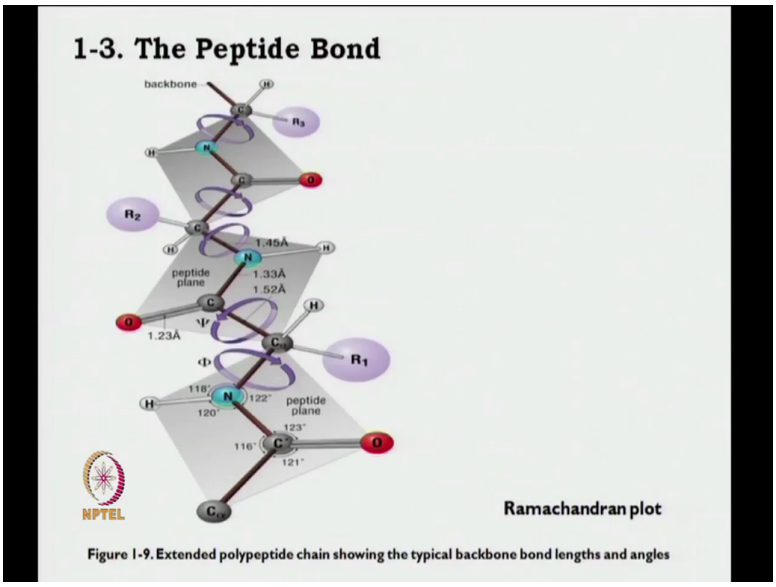

Figure 1-9. Extended polypeptide chain showing the typical backbone bond lengths and angles

So, again this is a peptide bond you are you are seeing the same thing only just from a different angle, you have this phi along N C alpha if this psi along C alpha C and it just this 1.45 angstrom this is tells you the different bond lengths involved.

So, here in the last so I mean in this slide you had 2 planes, now what you are seeing is you are having 3 planes. That means, 3 peptide bonds 3 planes having this peptide bonds and again you are looking at the torsion angles right.

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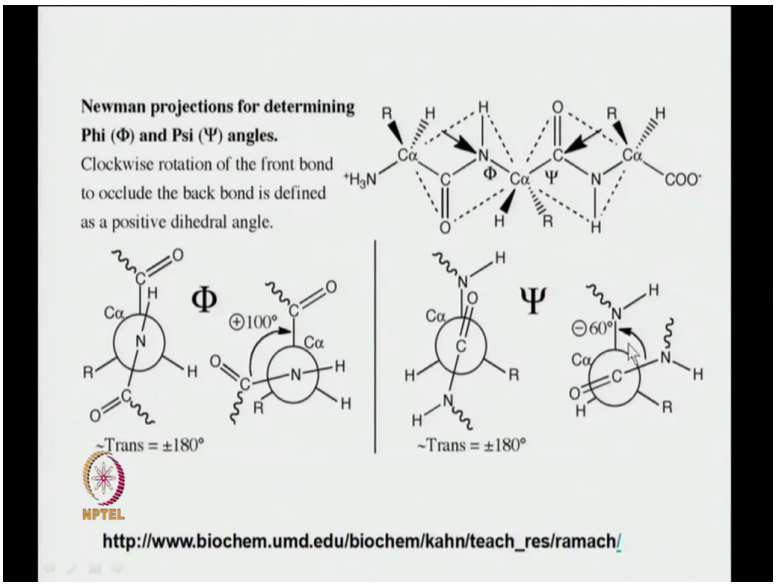

So, let us now go into little more details how do we visualise the torsion angles. So, let us try to look at it. So, what we have is here again we have the extended chain these dotted lines they constitute the planes, the planes are the those planes where you have the amide bonds housed or amide bonds located.

Now this is your phi this is your C alpha please again follow arrow or the pointer this is your C alpha, then this is the C and this is the N. So, any rotation around the C alpha N any rotation on the C alpha N is referred to as the phi, any rotation on the C alpha C will be referred to as the psi.

Now, how would you look at it? So, you have so what it says is if you are if you are having a chemistry background you will realise this word Newman projection you are trying to project it.

So, when you are trying to project it how do we look at this bonds around which you know the torsion or around which we are rotating? So, if I going to look at this phi angle, what we do is we look at the N C alpha right there is a C alpha stays at the back right and here we have the N out here.

Now, if so this is the arrow this is how we are looking at. If you are looking if you are looking at psi angle this is what we go. So, you look at this this is the arrow we look at it from this side we look at this carbonyl first this this carbonyl first and then we look at the C alpha at the back ok; so, the C alpha always at the back here.

Now, here what we see is so this is to the left of your screen we are looking at the phi angle you try to visualise it a little bit more. So, again go back to this arrow, this is what we are looking at we are looking along the N C alpha bond.

So, here this is the N this is the N this is the same N as this and then this is the C alpha this big circle. So, the N is N represent that point of N and the C alpha it is a big circle right, this is that is how a Newman projection is drawn.

So, you now looking along the line right, the once you are looking along the line then see what happens is if you look at this N C carbonyl and this carbonyl you can see there on opposite sides right ok. So, this one is pointing on the other on one side, this guy is pointing on the other side and this is referred to as the trans conformation. Remember the trans conformation where the C O was pointing on the two sides ok, so this is what you are looking at.

Now, what happens now do not worry of this positive sign right now. So, now we start turning, that means we start moving around this N C alpha bond ok. Now you can see we are

moving we are doing a torsion, that means you are moving. So, this is a plus 100 degrees do not worry about the plus, the plus I will come to the convention of what is plus what is minus.

So, here we can see when we have moved this, so this the angle between this guy and this guy is now 100 degrees. So, initially what I said the angle between this one and this one is 180, now the angle between this one and this one as this as this arrow is showing is 100 degrees. So, this is how you look at your phi right and these are the types of changes you are the are happening when you are moving around the C or N C alpha bond.

Now whatever the psi now again we do the same thing, we look at it along this bond right along the C C alpha bond. So, when we when we look at it in the C C alpha bond so this is what we have. So, this is the C again this is the trans, because here what you see this is the N H when we are saying trans here we are talking about the N H values.

Now if we go back to the phi when we are talking about the trans C, in this case the trans was about the CO right the C O is pointing in this direction the C O is pointing in this direction. But when we are talking about the psi, so there was about phi; but when we are talking about the psi now the trans is with respect to the N H bonds. So, this is N H is pointing in this direction this N H is pointing in this direction.

So, again this is a trans configuration you can see this is the for this is apart and then you do a minus 60 degree rotation ok. So, you are rotating like this you do a minus 60 degree rotation you can see like this and what you get is this one coming closer to this. So, this is how a rotation would be done or a torsion would be done, if you are doing if you are moving or if you are rotating along the C C alpha bond ok. So, this is one of the steps of trying to visualise it ok.

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Let us look at some better representations ok, let us go forward. Let us pick torsion angle phi, now how do we look at it. So, this is some conventions this angle is measured by looking along the bond with the nitrogen atom in front and the alpha carbon to the rear ok. Now, remember what does it say? It says that if you are looking at this bond if you are trying to see what this phi is; then you would rather look through the nitrogen right.

That means, through the N C bond with the nitrogen atom in front and the alpha carbon and back this is exactly what we saw here right N is in front C alpha it is back good. Now, what is generally measure it is varies from minus 80 to minus 180 to plus 180 right full rotation with the positive direction being when you turn the rear group clockwise. So, that the rear carbonyl bond is clockwise of the front carbonyl bond ok.

Again so this you see the range of measurements that can be done. Now or the range of angles it can take, now with the positive direction being defined as what when you turn when you turn the rear group clockwise, so that the rear carbonyl bond is clockwise of the front carbonyl bond.

Now, let us go back so this is what I said. So, the rear carbonyl bond so this is a rear bond. So, this is a rear carbonyl bond so that means this rear carbonyl bond is clockwise with respect to the front carbonyl bond, the front one is the bond which is here attached to N and the rear one is the rear one here attached to the C alpha ok. So, this so this C you know you know this C is your rear one which is C coming out from C alpha and this is directly attach to N. So, this is your front this is your rear.

So, what this one is saying is that you move it clockwise, when you move it clockwise that means when the rear carbonyl bond is always clockwise of the front carbonyl bond ok. So that means again so this is a rear carbonyl you can see it is clockwise in front of it is clockwise of the front carbonyl, here also the rear carbonyl it is clockwise of the front carbonyl ok.

So, that is why it is called plus 100 because that is the angle, but then this is how you know this is a convention of giving you the positive sign right. Or when you turn the front group counter clockwise or the rear carbonyl bond is always clockwise of the front ok.

So, these two mean the same right, either you start tweaking the rear carbonyl or we start tweaking the front carbonyl. Rear carbonyl you tweak in a clockwise fashion and to get the same effect with the front carbonyl you rotate it in the anti clockwise fashion or the counter clockwise fashion, so this is what I am saying. So, this is the rear carbonyl right or this is the rear carbonyl you can see this is the rear carbonyl.

Now, if I have to move it in this direction. So, I have to move in this direction this direction would be counter clockwise right, this direction would be clockwise. So, for the front carbonyl this direction is counter clockwise and that is what we have done here we have moved out here.

So, that is the convenience that is given to it ok. So, that is your positive direction, so positive in a nutshell for a phi torsion angle is where your rear carbonyl is always clockwise orient in a clockwise orientation of a front carbon right. Keep that in mind please.

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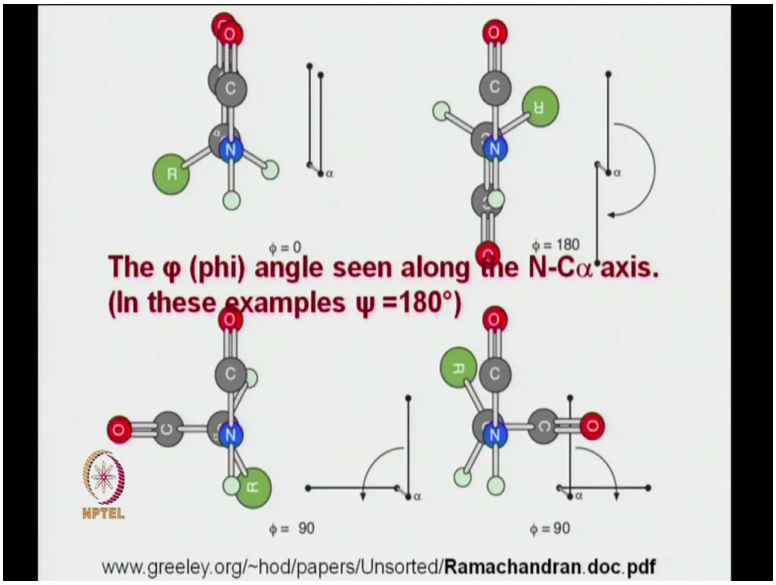

So, now let us look at some angle orientations. So, what we are doing is here this is so these are different representations phi is equal to 0, phi 180, then phi 90 phi 90. How do we look at it? So, here you can see this is N this is the C alpha right is exactly the same thing we are looking at right. This is where phi 0. So, why is phi 0? You can see why phi 0 because C O, C O they are top of each other same direction right and so you know this is what you are looking at.

Now, what happens is you start rotating, what do you do is, you can see what you have done is you have rotated you rotate one of these ok. So, this see this N C O this is out here, but this one you have rotated there the C C O you have rotated you can see.

So that means, this is the back there is a back N C O you are seeing out here. So, if you look at this these N C O and the C alpha C O these are given these are represented by two lines; the first line is N C O the backline is the C alpha C O.

Now, what you are doing is here doing a phi 180. So, when you are doing a phi 180 has come like this, so that means clockwise you have done it phi 180 will be clockwise like this and that is why it has come here. So, see it you have given it a clockwise flip right.

Then what can be the other one of looking at? So let us see out here. So, again this is the N C O right. So, this is N C O this is the C alpha C O now this is we are talking about 90 degrees the same thing happens here, this is N C O and this is C alpha C O.

So, again here you can see this we are going like this right, you can see the direction it is a positive y because this back carbon is always clockwise with respect your front carbonyl. Now look at this what is happened here is what has happened here is your back carbonyl; your back carbonyl is what? Your back carbonyl is behind your front carbonyl is not it by 90 degree.

So, this is a 90 degree in the other direction so essentially it is essentially negative right. So, this is a positive direction that is how it is rotated, but this one is rotated the other way like this. So, there is a negative direction.

So, what do you do is, this is a very good way of looking at it, you just keep in mind you just keep in mind the way this stick this stick or this line has been moved around ok. So, if it is moving around like this with a C coming like this it is phi is equal to 180. Again you move the stick in this direction. So, this is clockwise with respect to this right, it is again positive 90.

However, here we move this back stick anti clockwise that is a counter clockwise then it is negative, because positive by convention is what where the C alpha C O is clockwise to your N C O clear. Please go through it again you know think about it with a very cool head this is an excellent representation of the same. The phi angle this seen along the N C alpha axis that is what we are looking at and this we have kept psi as 180 degrees.

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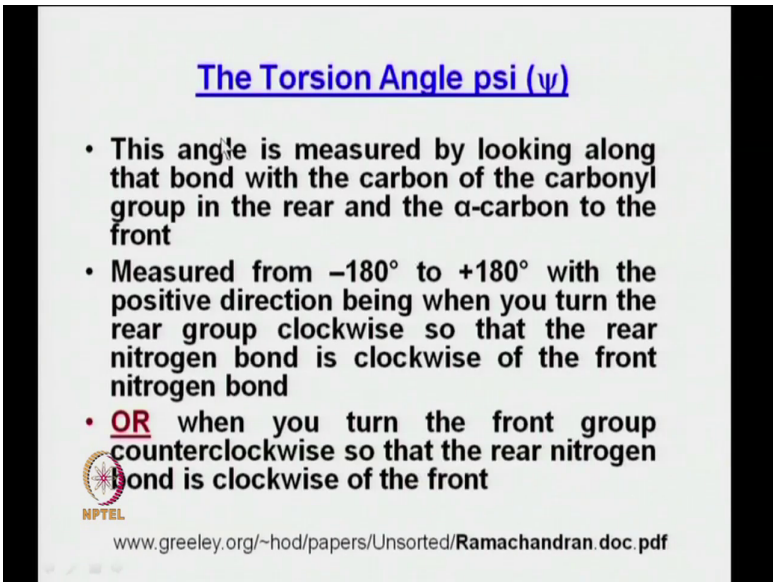

Now then once you have talked about phi, now let us talk about psi what are the torsion angle psi? So, this angle is measured by looking along that bond with the carbon of the carbonyl group in the rear and alpha carbon to the front. So that means, with the carbon of the carbonyl group in the rear it does not matter how you look at it there I think we had the other way around.

Now, measured from minus 180 to plus 180 with the positive direction being when you turn the rear group clockwise. So, the rear nitrogen bond is clockwise or the front nitrogen bond ok.

So, remember in that case we talked about the carbonyl bond or the carbonyl group. So, here what we are talking about. We are talking about the nitrogen N H. So, here it says what is the positive direction or what is your definition of positive?

So, when you turn the rear group clockwise, so that the rear nitrogen bond is clockwise of the front nitrogen bond right or when you turn the front group counter clockwise, so the rear nitrogen bond is clockwise of the front one. So, let us try to take a quick look at this, so here it says the positive direction being when you turn the rear group clockwise. So, that the rear nitrogen is clockwise of the front nitrogen bond.

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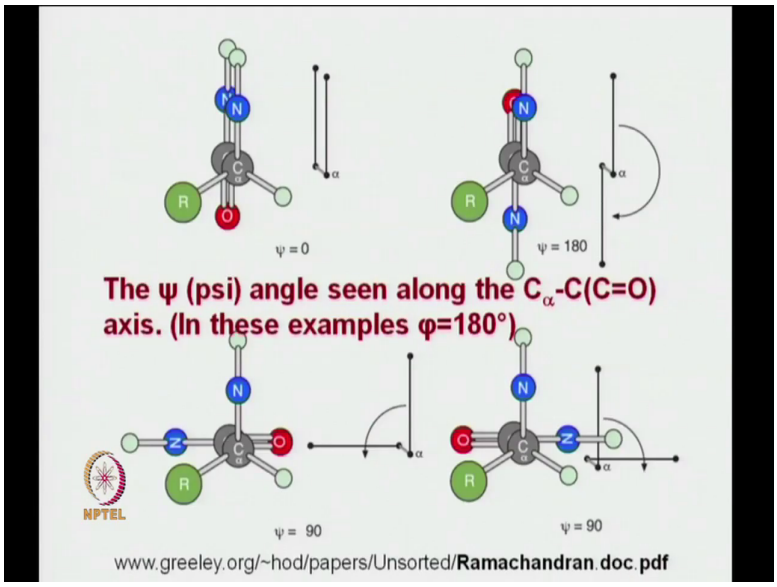

So, here let us look at this representation again. So, this is a C alpha right so this is a C alpha and this is the carbonyl that is what we said that is the convection we are going to follow ok. So now, what we see is here you can see what has happened is I have taken this now go back and talk about it, with the positive direction being when you turn the rear group clockwise. So, that the rear nitrogen bond is clockwise of the front nitrogen bond ok.

Let us see so here again you see these stick representations or line representations the first one again is this C alpha N and then the back one is this C N right. Now what you are doing is, you are doing a positive flip. If you are doing a positive flip, remember this N at the back N has to be clockwise with respect in a clockwise rotation respect to your front N this what is exactly I have done, you have rotated like this that psi is equal to 180 ok.

See it says with the positive direction in being when you turn the rear group clockwise. So, you are turning the rear group clockwise. So, the rear nitrogen bond is clockwise of the front nitrogen bond that is what you have. So, this is now in clockwise of the front nitrogen bond ok.

Now, how do we define 90 now? Again we do the same thing out there so this is how we are flipping. So, this rear group you see now comes here again it is clockwise in front. But what is a negative 90 right, that means doing a minus 90 then what happens is you are taking the rear group and just flipping in the other direction and because you flip it in the other direction then it is essentially going the other way right.

So, this is what we were trying to also see out here ok. This is what this minus 60 represented is not it? So, the minus 60 represents what? Remember this is your C alpha C so actually we have it the other way around the C should have been at the backside the C alpha should have come in front, that is why it is given a minus 60. Please try to you know go back try to take a look at it and realise why this is minus 60.

However I think we have had a you know decent idea of these torsion angles, how to take a look at these. So, here what we are doing is we have looked at the C alpha C bond and you have kept phi is equal to 180 in this case. Like in the last one we were looking at this phi we kept psi is equal to 180 right.

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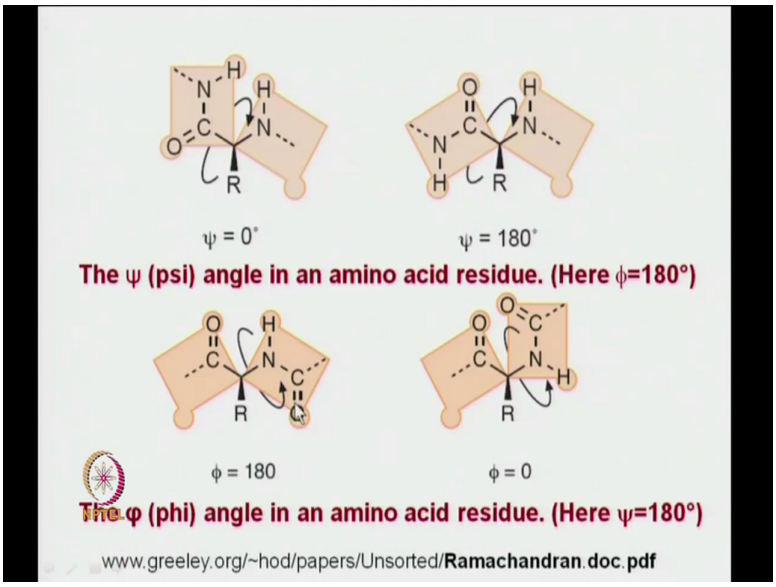

So, then a couple more examples we take an amino acid right, here phi is equal to 180 the psi we are looking at the psi angle. So, this is psi is equal to 0, you know when psi is equal to 0 remember psi is equals to 0. So, the N S N H is were on top of each other and when psi is equal to 180.

So, they are away from each other that is what you are seeing out here right, same thing out here now you looking at the phi this is phi is equal to 180. When phi is equal to 180 remember when phi equal to 180 the C is are pointing away from each other. And when phi is equal to 0 you can see the C 0 are almost pointing the same direction ok. Again here psi has been kept here 180.

So, this is when we you know when you think about the torsion angle this is what you should always think about, that you are rotating on one bond or you are rotating other bond you can

keep the other one fixed. And then what you do is you try to look at different orientations. That means, a certain value of psi or certain value of phi and that is how you get or give rise to the Ramachandran plot.

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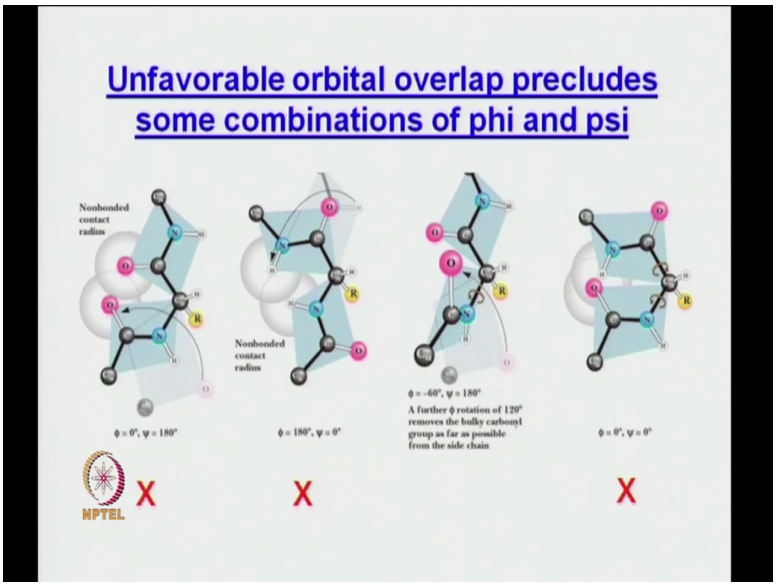

So, then we have looked at these aspects of what the phi and psi mean or how we can take a closer look at the at them. So, let us look at some unfavourable combinations. So, this is what we have we can see, what is this values? This is phi is equal to 0 psi is equal to 180 right and what has happened here? What is happened here is, you can see this C O and the C O they are very close. So, it is non bonded contact high energy it will be unfavourable, then so that is why it is unfavourable then you look at this again.

Now, phi is equal to 180 psi equal to 0 what are you having? See remember when we are talking about phi skipping the psi fixed when you are talking about the phi we are always

talking about the carbonyl groups looking at the orientation of the carbonyl groups. When you are talking about the psi keeping phi fixed you are talking about the orientation of the N H groups is not it?

Now, here also N H come very close to each other because psi is equal to 0, non bonded interaction revulsion high energy hence this is unfavourable right ok. Let us go to the last one here psi is equal to 0 phi is equal to 0 psi is equal to 0. So, what you have huge amounts of interactions out here and because of huge amount of interactions out again this is not favourable.

So, what you can see out in between there is a third one, so this is phi is equal to minus 60 psi is equal to 180. Where you see here the amount of non bonded interaction that is why you do not see these big space. You know these big space represent the non bonded interactions the repulsive interactions you are having m is you are having this steric congestion or steric repulsions.

But here you do not see those that means this is the one which is possibly one favourable conformation for a particular protein ok. Remember this depends upon the proteins right or the secondary structures these amino acids take ok.

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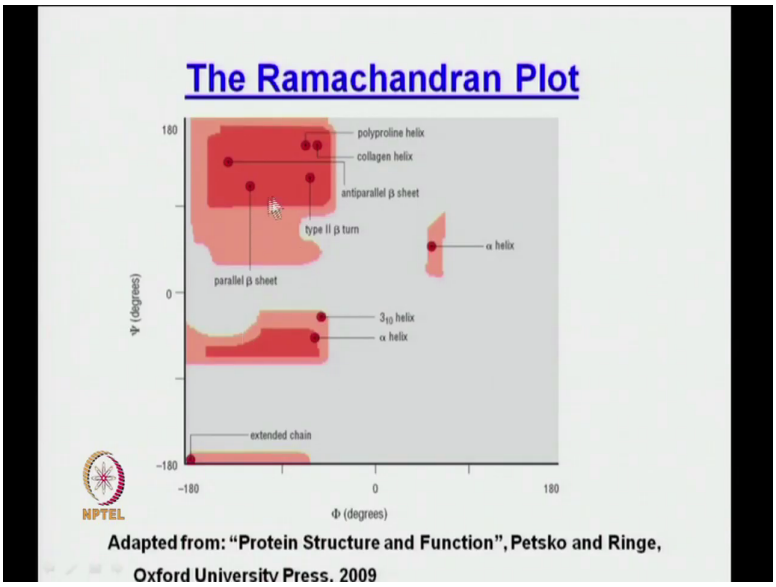

What did we do all this exercise for? We did this exercise because we want to look at the Ramachandran plot and this is what the Ramachandran plot looks like. So, you can see it is a plot of psi against phi both being in degrees right ok. So, this area is essentially dominated by a helix, this is your extended chain.

This area it is a big one it is dominated by your beta type of secondary structures; this was alpha helix and this is here another alpha helix. But can you tell me what? This one belongs to yeah you right, it is the left hand alpha helix or alpha helix are right handed that is the normal one we look into.

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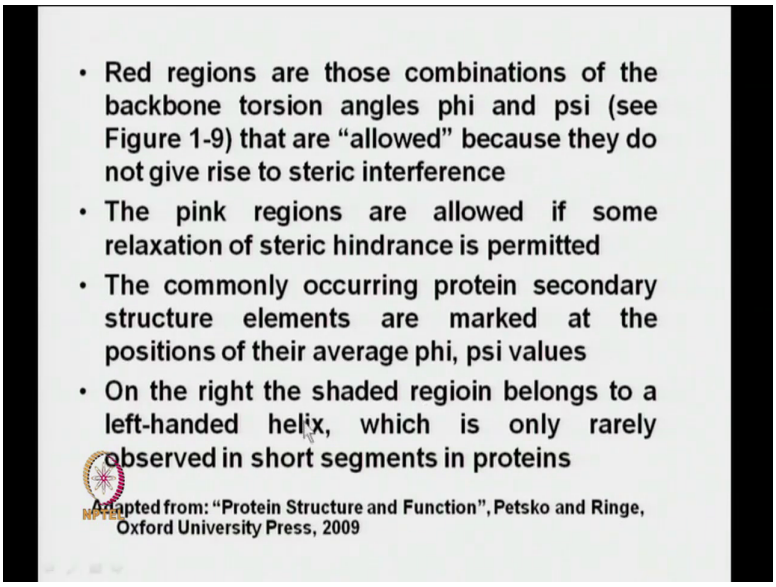

So, some quick but significant aspects of this. So, the red regions are those combinations of the backbone torsion angles phi and psi that are allowed, because they do not give rise to any steric hindrance.

So, this is the figure we are talking about right. And so this is the red the red ones are the ones which are really allowed favourable no steady clashes. The pink regions are allowed if some relaxation of steric hindrance is permitted, that means they are partially allowed.

Here you have to have you have to relax your steric chemists a little bit. The commonly occurring protein secondary structure elements are marked at the positions of positions of their average phi and psi values will go back go back to it again. And on the right the shaded region we have it belongs to a left handed helix which is only really observed in short segments

in proteins. There is only short segments in proteins to really observe it otherwise it is not that common.

So, then going back we take a look at it again you can see this is the beta this is the left hand alpha helix this is the right hand alpha helix this is extended chain. Now here itself you have different regions right, here you here you can see these different circles.

See each and every point each and every point on this corresponds to a definite psi and a definite phi. And we look at these values this this psi goes from one plus 180 to minus 180 this also goes from minus 180 to plus 180 right.

You can see for all these the only the only positive values of phi we have it is mainly for the left hand alpha helix, otherwise for all of these essentially phi is always in the negative right.

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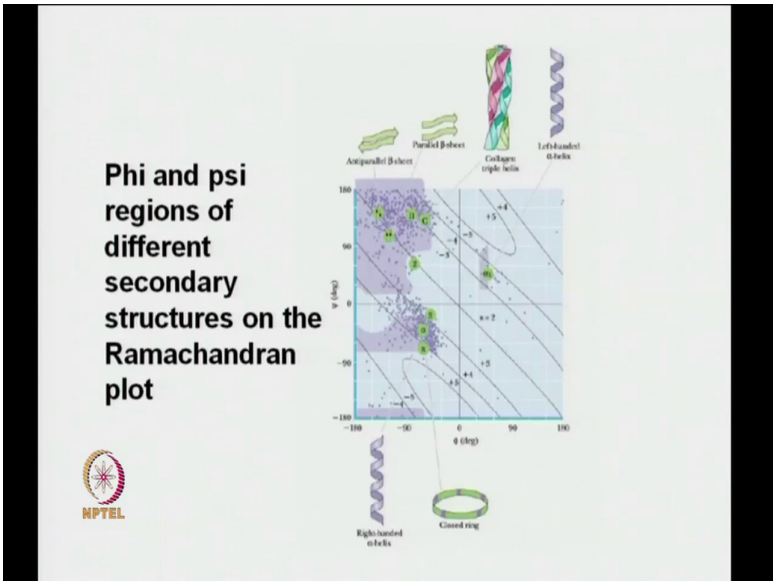

We go ahead and take a closer look, so these are the phi and psi regions of different secondary structures on the Ramachandran plot is an you know another figure of the same thing ok.

So, this kind of tells you a little more detail get everything written down there. So, this is the alpha l which is left hand alpha helix. Now this is your regular alpha helix which the right handed this is a pi helix you know it is the type of helix, this is like a threatened of a helix that is why it is called 3; this is your right hand alpha helix then this was the helical region right.

What do we have in the beta region? What we can see is you can see this one arrow pointing up and one arrow pointing down, these two should have had made it a little bigger. But if you kind of strain your eyes what you will see is these two are pointing in different directions right and hence this is an antiparallel beta sheet. So, this is the region with antiparallel beta sheet phi and psi angles come in.

So, this you can see now these two arrows are pointing the same direction. So, then this is what is referred to as a parallel beta sheet. So, this is the anti parallel these two arrows pointing different directions is parallel beta sheet both in the same direction, this is where they come in right.

Then what we have is, here the see this is a collagen triple helix, we will come to this arrangement of helix is later. So, these are in a triple helix this is at these are the psi average psi and phi angles that the triple then amino acids take in the triple collagen helix, on the collagen triple helix rather.

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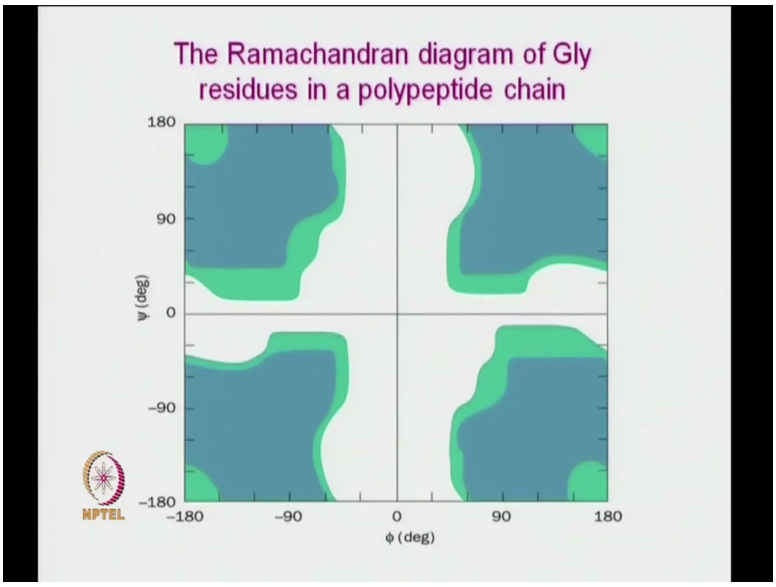

And to end this discussion about the structures of amino acids let us look at the Ramachandran plot of Glycine. Now, why is glycine simple to remember glycine the R; what is R? R was H 4 glycine was not it? So, think about the extent of freedom it will have. So, you can see throughout, the phi and the psi and phi plane or the psi and phi plot you have this glycine.

Whereas that means glycine can or has the ability to what spread around a range or cover a range of psi and phi values. Now that is why glycine is so unique because it is so flexible it just straight away comes from the flexibility.

So, then in a nutshell what is the Ramachandran plot? The Ramachandran plot in a nutshell. What it does is, it tells you this it tells you is it gives you a plot on the phi and psi angles right and it tells you ok.

I cannot have all the possible psi and phi angles why? Because as you saw in the previous slide, that means; a slide previous to this there would be combinations where the steric conjunction would be so high. The non bonded interactions would be so high that the structure would be very unstable and next would be very high energy and would sell them be populated remember talk. Remember I mean if you think about the Boltzmann distribution.

And hence those regions would not be occupied those regions in the phi psi angle or the psi phi plot would not be occupied. Instead we only have to concentrate on these regions on these shaded regions, because these are the ones which are covered by the different secondary structural elements in the different proteins whether it is beta alpha or mixtures of those right.

I think this was what I needed to discuss with you about the Ramachandran plot, see we do not think too much of the Ramachandran plot. But if you kind of you know tried to think about it or if you kind of try to dwell on it for some time, you know if you have the psi and phi angles you essentially have the conformation of the protein and that is exactly what G N Ramachandran did ok.

Now, let us take a first step towards protein structure, initially I mean we have looked at the building blocks we have looked at the Ramachandran plot, we looked at the hierarchy of structure. But this is an unique this is the unique thing that we are going to look at right now.

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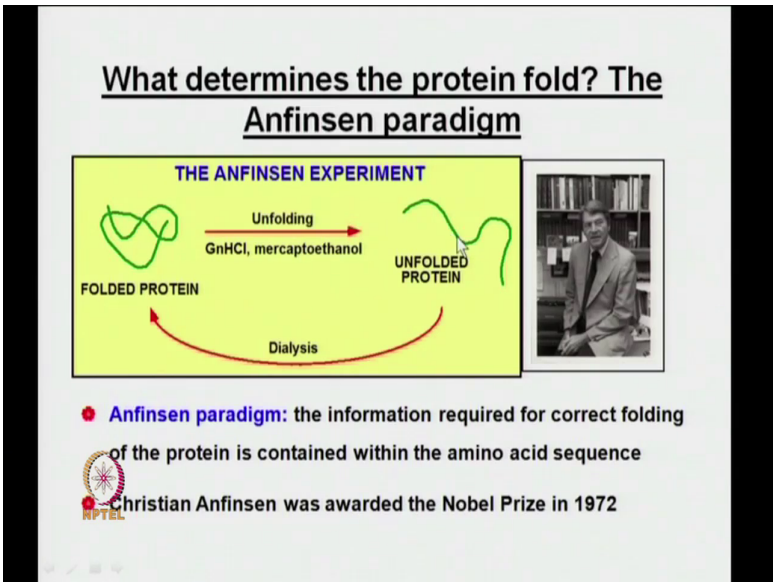

The first question is and I am sure which has come to your mind is what determines the protein fold? That means, whether the final protein is like myoglobin which is you know fully alpha helical or like lysozyme which has mixed alpha and beta or you know some beta sheet protein.

How does it know? How does the protein know what structure what native structure it has to take? So, Christian Anfinsen you know try to and his co-workers and some other groups try to address this problem.

So, he did you know a very simple experiment, what he said is he took a folded protein he unfolded it went to the unfolded you know it got you got the unfolded form of the protein

right. And in a dialysis and then what he saw is that he got the folded protein back with almost the same activity.

Now, what activity I am talking about I will tell you within a very short time just hold on. This is Christian Anfinsen he was awarded the Nobel prize for his pioneering work in 1972 and what he said is this is his this his parroting. It says that the information required for correct folding of the protein is contained within the amino acid sequence. What is mean is, it is a primary sequence.

See it is a primary sequence which is the most basic that is why it is called primary sequence, it is a primary sequence where that information is encoded that what type of structure the protein should be going for once it is folded with all it is tertiary or quaternary contacts?

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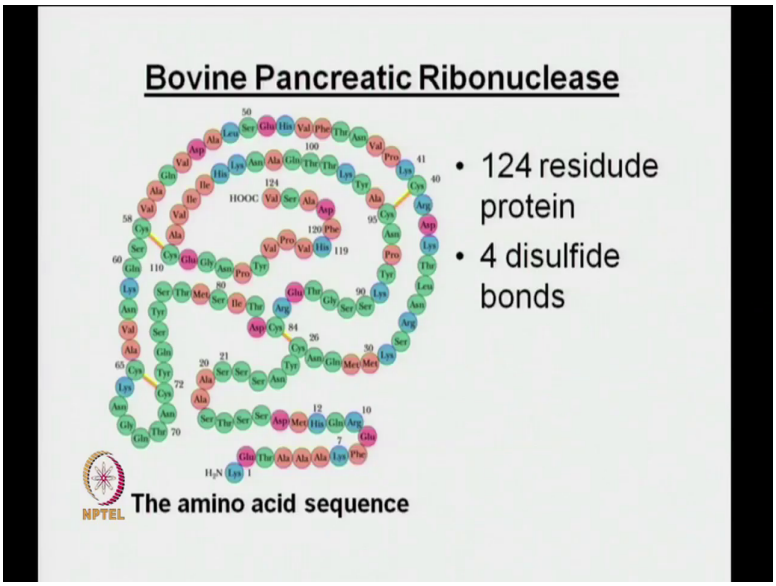

So, how do he do it? So, what he did was he took this 124 residue protein bovine pancreatic Ribonuclease right also called RNA's. So, this one has 4 disulfide bonds that means 8, 16 groups you can see, the different groups are out here.

So for example, if we start from say 1 out here this is so here is one group here is one here is one and here is one. So, there are about you know like this 4 disulfide bonds. Now, what do you do with this and why this disulfide one so special right.

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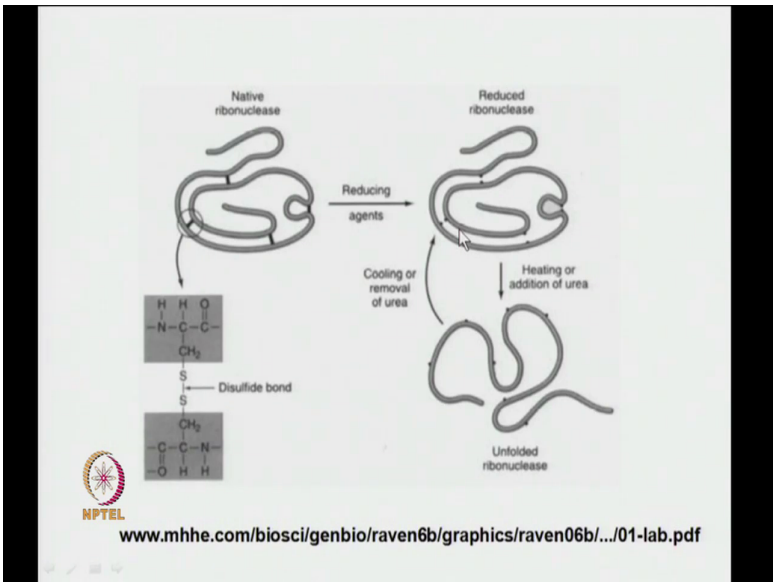

So, this is what he did. So, what he did was he took this native ribonuclease remember these lines are essentially a 4 you can see these 4 lines these are your 4 disulfide bonds and he added reducing agent. So, once he added reducing agent, what happened was the reducing agent

reduces disulfide bonds and converted the S S bonds to S H S H; that means the disulfide bonds do not exist.

So, the protein is little more free because, you can see these contracts no longer exist. And the principal has no he denature the protein. That means, he added either heated or he added urea. So, he added urea in this case and then he unfolded the protein.

That means, this disulfide bond you can see out here this was reduced with the reducing agent. This was reduced with the reducing agent and once it reach this reduced ribonuclease he unfolded the ribonuclease with the urea. And then when he removed the denaturant he saw that it went back to the reduced ribonuclease.

Then what did he do? But this was a scheme, the scheme was essentially that he started from the native which is having all the 4 disulfide bonds; he reduces the disulfide bonds; he adds a denaturant urea he unfolds the protein, then he takes the urea or he does something and he sees what happens. Let us look at his two different experiments.

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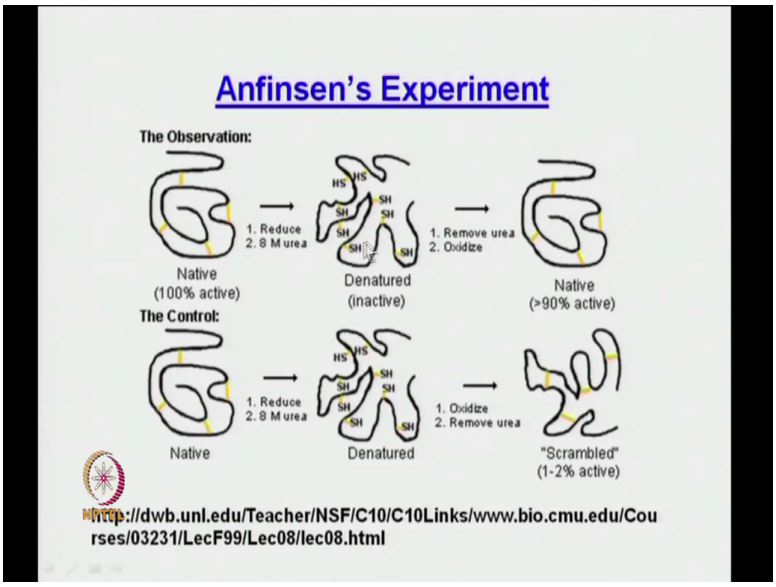

The first observation so this is the native form, the native form means the one which is found which is 100 percent active right. See it is ribonuclease that means, it is an enzyme, so it will be having enzymatic activity. So, it is good because then you can whether you know it has attained it is native structure or not, how would you know? You would know by it is activity right.

So, this guy is 100 percent active then you look at this arrow. So, this is first experiment he reduces it, he reduces it by a reducing agent by beta mercaptoethanol then he adds 8 molar urea chemical in nature. So, you can see all these are you have these 1 2 3 4 5 6 7 8 right this 8 SH groups.

Now, what he does is he removes the urea ok, see it says denatured inactive. That means, now the protein is denatured it does not have the active side intact and as it cannot act as a as an

enzyme. So it is fully inactive, now what he does is he removes urea. So, once he removes urea then he oxidizes, once he oxidizes now what happens is then he gets back this 90 percent active state.

So, what it tells you is that, he removes the area he oxidizes and the protein he waste for the protein automatically goes back to his 90 percent active state, almost 90 percent I mean almost 90 percent greater than 90 percent.

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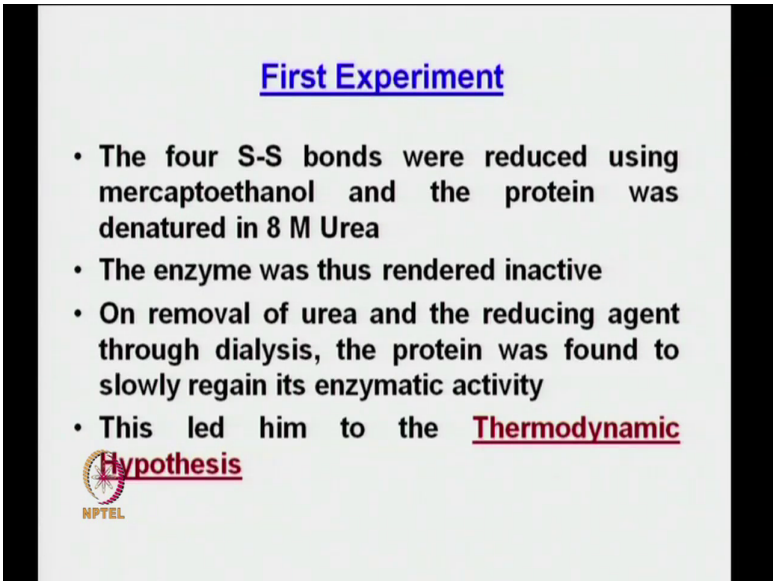

So, the first one is this there are four SS bonds reduced using mercaptoethanol and the protein was denatured in 8 molar urea, the enzyme was just rendered inactive on removal of urea and the reducing agent through dialysis.

The protein was found to slowly regain it is enzymatic activity, this led him to the thermodynamic hypothesis I will come to it later; what I mean by the thermodynamic hypothesis. So, this is a couple more slides before I end.

Now what is the what was the other experiments? Look at the other experiment he takes the native form. Again the first step is essentially the same right he reduces it then he denatures it, so it is the denatured. Now what he does is he look at this step he removes here then oxidizes, but here he does different thing he first oxidizes and then he removes.

Now because now he oxidises before removing urea; the protein is in a denatured state. So now it is actually a double bond it is scramble it cannot form all these native bonds all the time, maybe a small percentage would form. But mostly it would be scrambled, that means you would be having different cross links. That means, say for example, this s h my cross link with this is this SH this is assume icon this is SH and so on.

Now, what he found was after oxidizing when he removed urea, that means after he has fall these form the scrambled disulfide bonds when he is removed urea it is not get activity, it is only 1 to 2 percent active. That means, maybe only 1 or 2 percent of this molecules actually gained the native structure, where it had the correct disulfide bonds right that is why the activity is so low.

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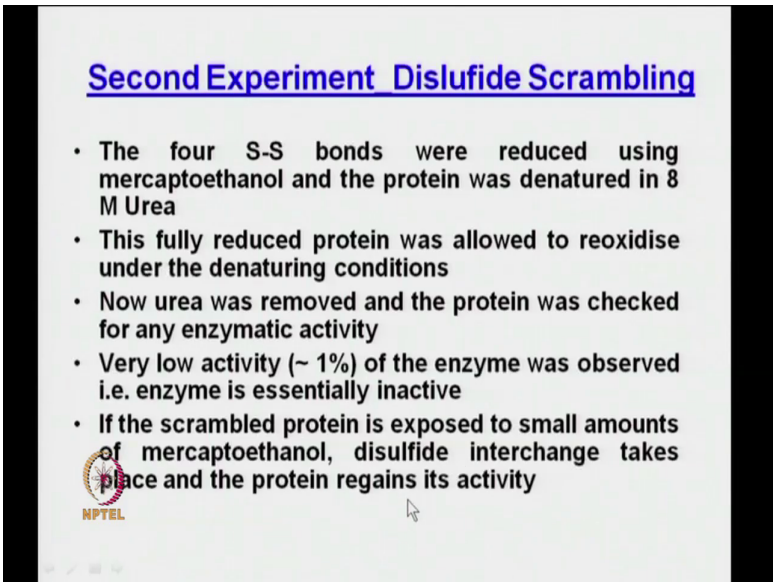

So, then the steps of his second experiment it is called the Disulfide Scrambling. The four SS bonds were reduced this was the first step and the protein was and the protein was denatured. The fully reduced protein was allowed to reoxidise under the denaturing conditions ok.

So, initially we removed urea and then we allowed it to oxidize, but now we are allowed to oxidize and then we removed urea. So now urea was removed and the protein was checked for any enzymatic activity, very low activity of the enzyme was observed that is enzyme is essentially inactive.

However, if the scrambled protein is now exposed to small amounts of mercaptoethonol; so, this is a very neat experiment but a brilliant idea. So, what he does is he has a scramble protein right. Now is the protein is rearranged remember the protein can be rearranged right, that

means you have to break the disulfide bonds. So, what he now does is he just expose it to small amounts of disulfide bonds.

And you know small amount reducing in this beta mercaptoethanol and the disulfide which is not takes place and protein regains it is activity. Now, this is really nice it is really important, what message does it deliver.

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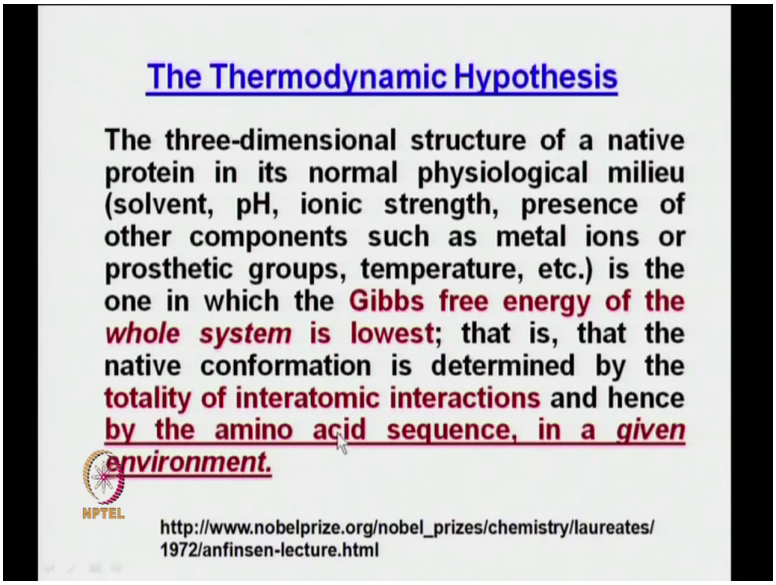

This is what the Thermodynamic Hypothesis says. So, this is essentially the last slide. The three dimensional structure of a native protein in it is normal physiological milieu that solvent, pH, ionic strength, presence of other components such as metal ions or prosthetic groups, temperature etcetera.

It is normal physiological environment is the one in which the Gibbs free energy of the whole system is the lowest right; there is most stable. That is that the native conformation is determined by the totality of inter atomic interactions and hence by the amino acid sequence in a given environment ok, sorry, So hence by the amino acid sequence in a given environment.

So, in a nutshell what is what is telling is that ok. Going back to a second experiment, when I oxidize this before removing the urea I found the scrambled disulfide bonds. But the moment after I remove after I remove the urea it was essentially inactive, but only 1 to 2 percent activity.

But the moment I allowed it to rearrange; that means, I added the small amount of reducing agent for it to rearrange and the protein immediately rearranged. The immediately I am not talking about the kinetics here. So, do not quote me on that, that means the protein rearrange over the function of time until it gained almost that 90 percent to 100 percent activity. What does it mean?

That means, the protein actually went to a thermodynamically more stable state when it was allowed to, this is where it says by the totality of inter atomic interactions and hence, by the amino acid sequence. So, this is amino acid sequence which defines the totality of the inter atomic interactions and which finally determines what structure at the end the protein is going to have ok.

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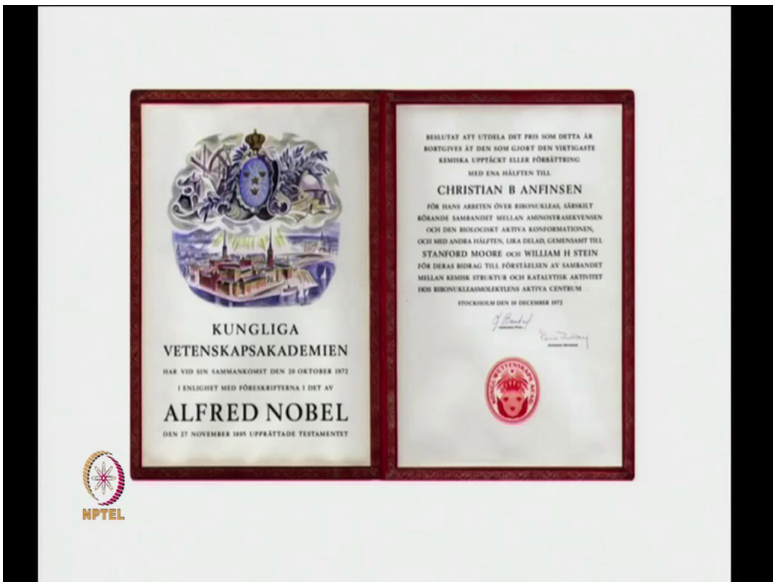

So, this is the noble pluck that was given to Christian B Anfinsen in 1972 for his pioneering work.

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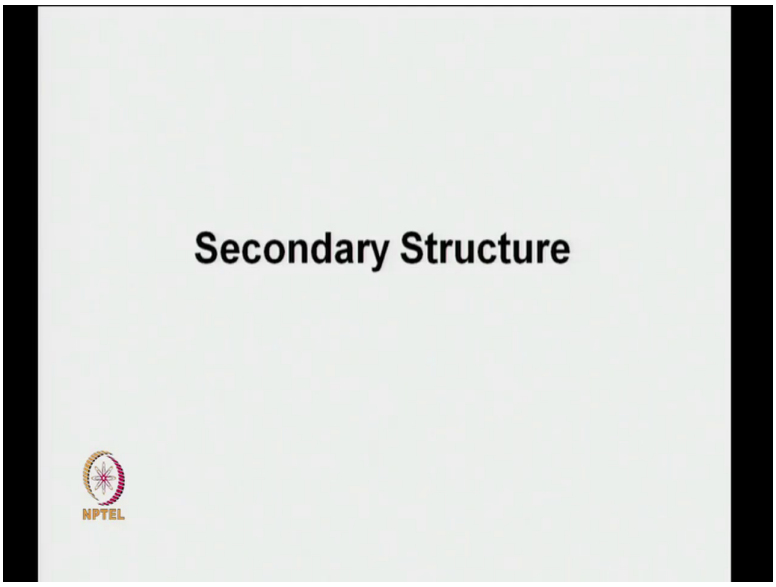

We will stop here, what we will do is, next class we will start talking about the secondary structure. And, I just want to end this class with the requests that you guys take please take a close look at this Ramachandran plot, look at the phi and psi angles. And try to understand the importance; try to understand the different non bonded interactions you guys you know that take place. And why we have that only those small regions in the Ramachandran plot, which the proteins in different confirmations actually cover.

Thank you.