Structural Biology
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Lecture - 08
Protein: Dihedral Angles, Peptide Bond, and Ramachandran Plot

Again, welcome to the course of structural biology. We are continuing with the module on protein. Today in this class, we will talk more about the dihedral angle, peptide bonds, Ramachandran plot, which is critical in determining protein structure.

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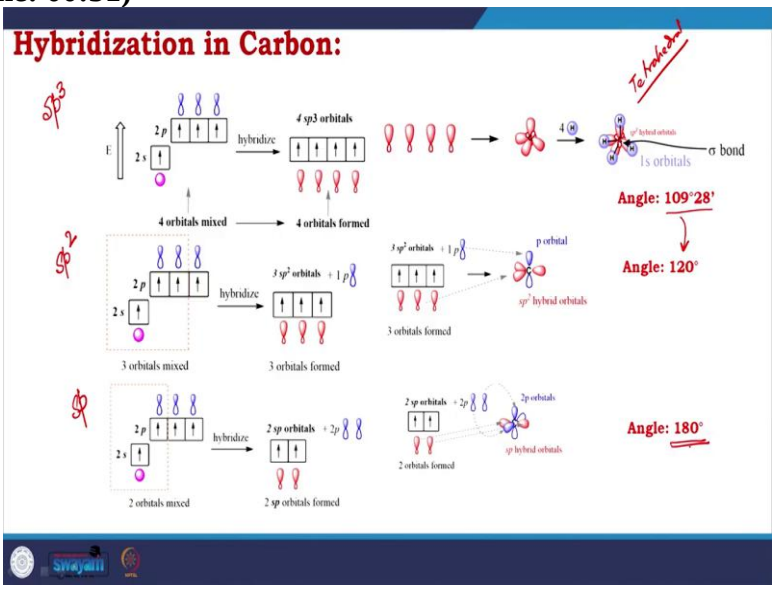

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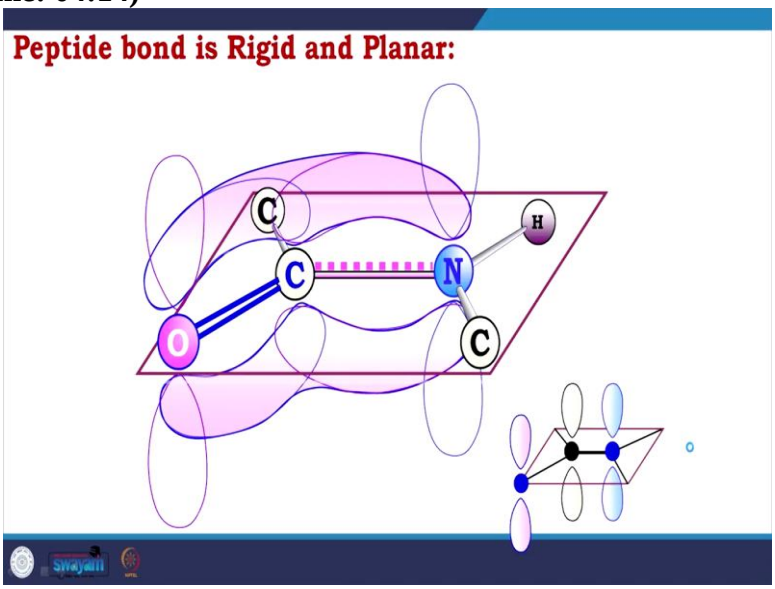

The same thing you will see here in the peptide bond involving carbon and nitrogen and oxygen could form sp2 hybridization and form the sigma and pi bond. The pi bond is

delocalized between oxygen and nitrogen. So, you get them into the same plane, and what we discussed earlier, you get the character being a partial double bond.

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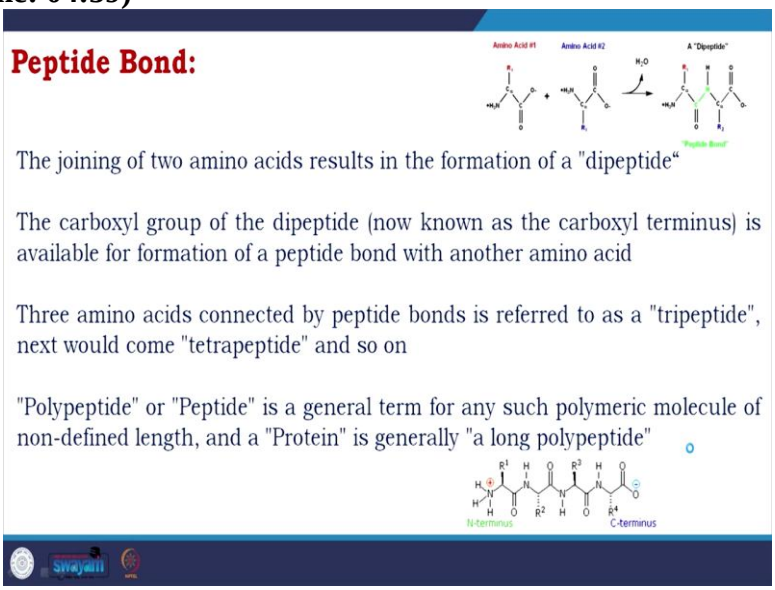

So, we will talk about the peptide bond, in which you see the amino acids 1 and 2 join and form the dipeptide. The joining of 2 amino acids results in the formation of a dipeptide. The carboxyl group of the dipeptide is now known as the carboxyl terminus. On the starting side, it is the end terminus and elongated. The new side is a C terminus. There is carbon available for forming a peptide bond with another amino acid. So, it continues like that. Three amino acids connected by peptide bonds are referred to as tripeptides. Next would come tetrapeptide and so on. Polypeptide or peptide is a general term for any such polymeric molecule of non-defined length, and a protein is generally a long polypeptide.

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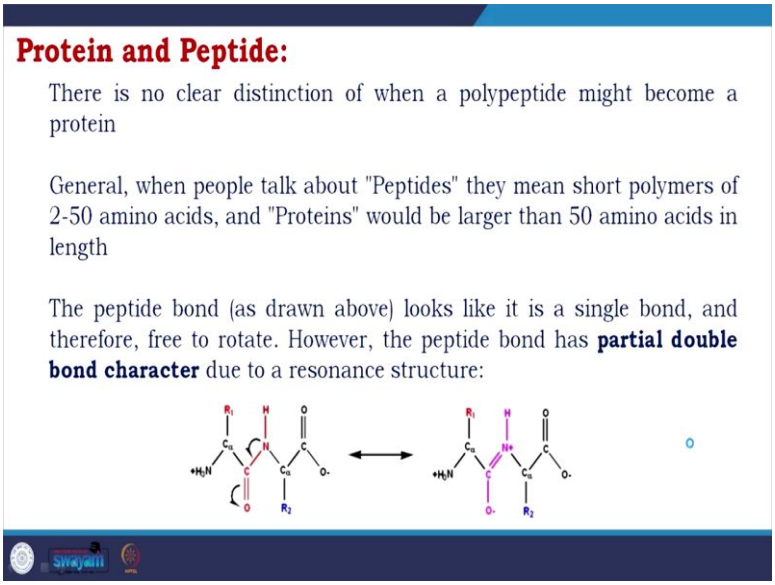

There is no clear distinction of whether a polypeptide might become a protein. But in general, when people talk about peptides, they mean short polymers of 2 to 50 amino acids, more than 50 amino acids we call protein. The peptide bonds look like a single bond and are free to rotate. However, as we talked about, the peptide bond has a partial double bond character due to the resonance structure.

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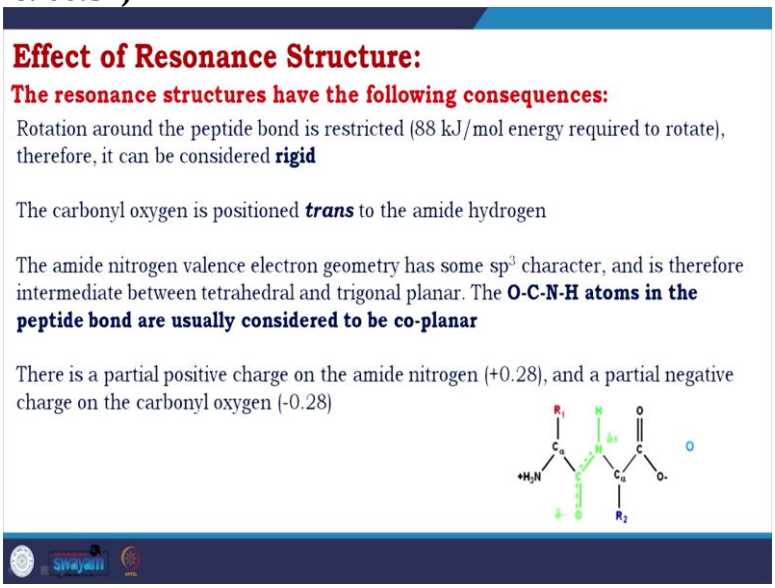

The resonance structure has the following consequences. Rotation around the peptide bond is restricted by 88 kJ/mol energy required to rotate. Therefore, it can be considered rigid. The carbonyl oxygen positioned trans to the amide hydrogen. The amide nitrogen valence electron geometry has some sp^3 character. Therefore, it is intermediate between the tetrahedral and trigonal planar the O-C-N-H atom in the peptide bond is usually considered co-planar. There is also a positive charge on the amide nitrogen (+0.28) and a partial negative charge on the carbonyl oxygen, which is -0.28.

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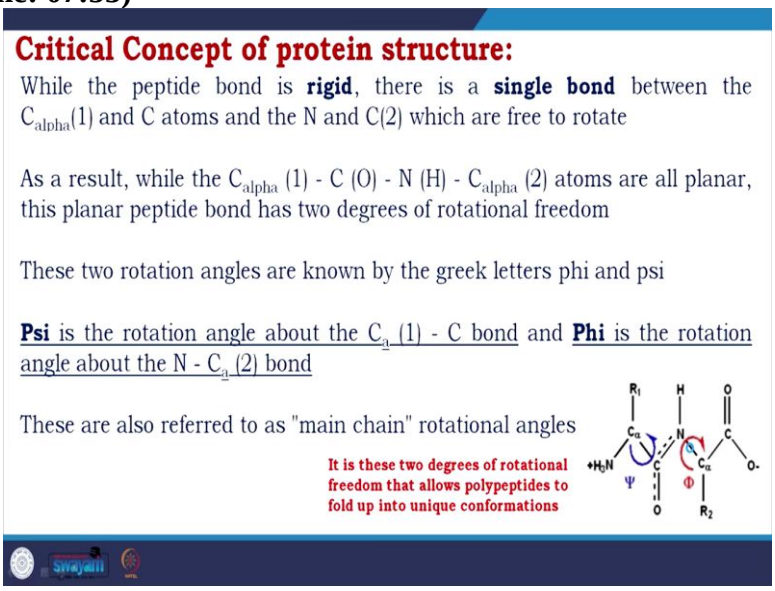

I repeatedly talk about our journey is from the sequence with the next-generation sequencing. We have got many sequences, and now from the sequence, we want to get the function of the intermediate structure. The concept we discuss here has a huge role in determining the protein structure. So, while the peptide bond is rigid, there is a single bond between the C alpha and C atom and N and the C 2 alpha, free to rotate.

So, one is C alpha C. So, when you say C alpha C is ψ(psi) bond and C alpha N is φ (phi) bond. As a result, while the C alpha C-O-N-H C alpha 2 atoms are all planner, this planner peptide bond has two degrees of rotational freedom. These two rotational angles are known by phi and psi, psi is the rotation angle about the C alpha C as I told when you say C alpha C uses C is psi. Phi is the rotation of N and C alpha second, the next one.

These are also referred to as main chain rotational angle. It is these 2 degrees of rotational freedom very important that allows the polypeptides to fold up into unique conformation I repeat, it is these 2 degrees of rotational freedom that allows polypeptides to fold up into unique confirmations. We talked about the difference of protein with other macromolecules. This is where they are differentiating. They have the junction connected partial double bond rigidity and because of the rotation, 2 degrees of freedom along with the phi and psi, they take unique conformation so each of the protein have their unique 3-dimensional structure.

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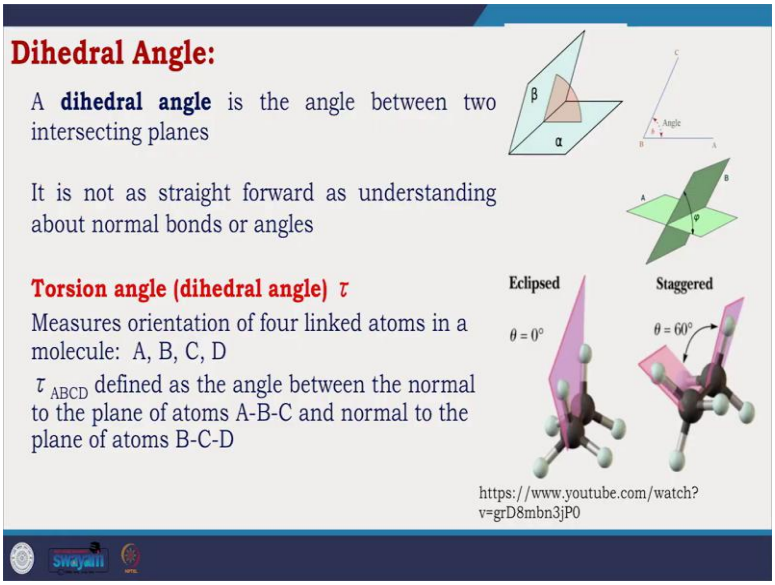

And that brings me to the concept of dihedral angle again a very very important concept a dihedral angle is the angle between 2 intersecting planes. As I say it is a angle between 2 intersecting planes, but I realized that it is not straightforward as understanding like normal bonds or angles. So, this is a dihedral angle, but when I say it is difficult to differentiate with that normal angle.

So, I try to put some more discussion if you look around you will see in the place you are sitting there are walls. So, if you look at the connecting wall, you see that there is a wall A and wall B connecting. Now, if you have one point here in this you have a line and in these you have a line the angle between them is the perpendicular coming from this line and perpendicular coming from that line, where they are intersecting making a angle that angle is called dihedral angle.

If you see here, you will get the idea a little bit more clear. But from here, I will seek to somewhere in the atomic description of dihedral angle which I hope will give you a much better idea it is also called torsion angle tau. So, what is torsion angle? It measures the orientation of 4 linked atoms in a molecule A, B, C, D if you see this molecule, the angle created by them is called the torsional angle or dihedral angle.

Tau A, B, C, D defined as the angle between the normal to the plane of the atom A-B-C and normal to the plane of the atom B-C-D which means if you see this now, you will understand in this it is called eclipsed or anti and syn and this is called gauche or staggered. So, when

you have these head-to-head the theta equal to 0 they are called eclipse or sim and when you make a 60 degree angle, it called staggered gauche.

Now, I have given a link here is a beautiful video which will help you understanding how a dihedral angle is measured you could see that for your reference, but I will do that in my way. So, if I put it like that and changing the angles, so this is anti where they are in opposite direction, but and this rotation is called the rotation of the torsion angle. If you remember, I talked about 3 secrets in the covalent bond, chirality conformation configuration.

This is called conformation where you get this structure and this structure without breaking any bond only by using the operation which is called rotation. Remember, just in the last slides when we were discussing about the peptide, we talked about phi and psi giving degrees of freedom which is rotational degrees of freedom because of this. Now, one thing you could have understood, here we have carbon, 3 hydrogens? So, if you put it like this or this, you do not get much difference in the energy. First of all, how these things what I am showing you would be projected in your paper?

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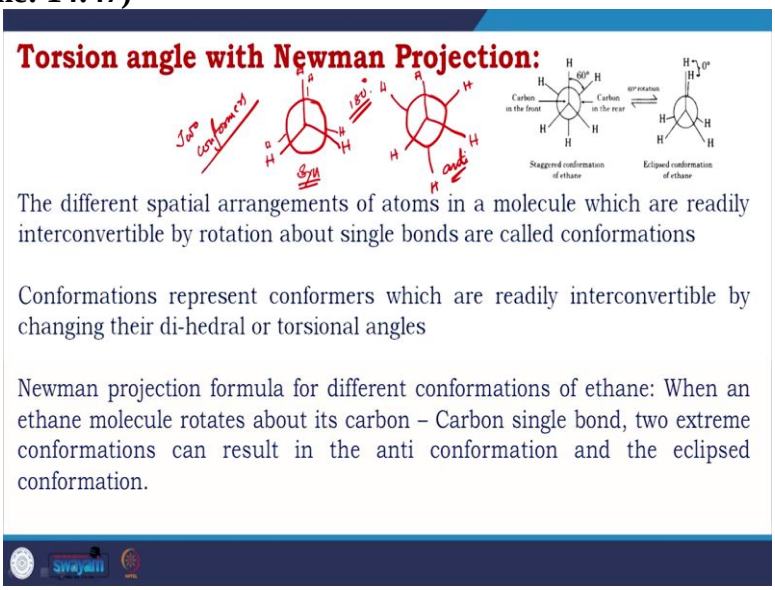

This is called the Newman projection and if you see these things could be drawn in such a way. This is the front carbon with hydrogens and this is the back carbon with the hydrogens, if this is called syn, the other one would call anti. So, these are 2 conformers. So, the difference spatial arrangement of atoms in a molecule, which are readily interconvertible the rotation about single bonds is called conformation. So, these and these they are readily convertible.

So, the different spatial arrangement of atoms in a molecule which are readily interconvertible by rotation about single bonds or about a single bond are called conformers, syn or anti are conformers. Conformations represent conformers which are readily interconvertible by changing their dihedral or torsion angles. This angle you see, here it is not touched. These angles are called torsion angles. Newman projection and it is also called Fisher Newman projection in formula for different conformations of ethane.

This is ethane, when an ethane molecule rotates about it carbon-carbon single bond 2 extreme confirmation can result in the staggered confirmation in the eclipsed confirmation that is I was talking about this is ethane, you move it you get a different confirmation syn and anti the staggered eclipses the 60 degree rotation here I make a 180 degree rotation. So, you get syn and anti you understand it.

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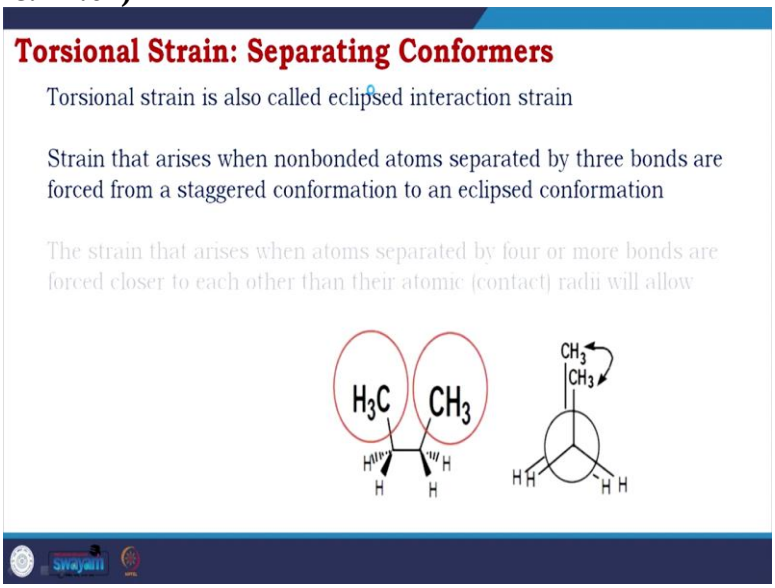

So, we are going to the next one, which is torsional strain, we understand the torsional angular dihedral angle. Now, we will go for the torsional strain separating the conformers. Torsional strain is also called a eclipsed interactions strain. Strain that arises when nonbond atoms separated by 3 bonds are forced from staggered conformation to an eclipsed conformation. So, what it is talking about here, you have note, a lot of differences because all are small, instead of this difference if I put a methyl group here.

So, there is a methyl group and here is another methyl group. Now, if you see, when they are sync, there is a steric class and that is why these conformers and this conformer are

energetically different, this conformer and these conformers are energetically very, very different.

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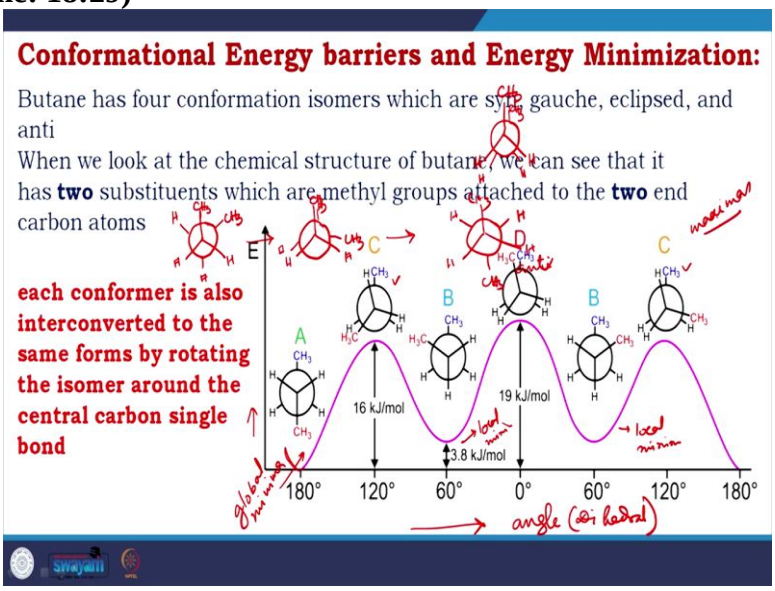

So, conformational energy barriers and energy minimization. So, I will not go for energy minimization, but I will introduce the concept of energy minimization here. Butane, this is butane now 1, 2, 3, 4 it is butane, how it could be presented, it could be presented in such a way he will be CH 3 H H. If this is syn; then this and with that here, here. So, this is the Newman projection, you have syn, gauche, eclipsed and anti 4 conformers. when we look at the chemical structure of butane.

We can see that it has 2, substituent which are methyl group attached to that to end carbon atoms. Each conformer is also inter converted to the same forms by rotating the isomer around the central carbon single bond. So, how the conformer would happen, these you have these parties. And now, it would come here. So, syn, gauche, eclipsed and anti. Interestingly, if you now put them the angles starting from 180 you rotate in this way.

So, get 120, 60, 0, 60 in that way you plot it with the angle which is dihedral or torsion angle with energy you get this at. So, this is anti. This is the minimum. So, this is called global minima, this is another minima. This is called local minima. This is also local minima and these are maximas. So, what do you learn from here that this is only butane containing 4 carbons?

But by changing the relative position, you could get them free from the steric classes and that make each of the conformer in different energy states, which give rise to maximas and minimas. So, if you have the molecule here, where there is possibility of maximum strain and you make it converted to this where minimum strain is there you are actually pushing it from highest energy to lowest energy.

The process by which are you develop a code by which a computer would do this process is called energy minimization because from a high energy conformer you convert to a low energy conformer. But, now, I want to tell you a few things like if you have replaced them with different molecules like this and this, so, now, these base carbons are having to heavy atom replacement like in 2 positions then it becomes even more complicated.

Also, instead of doing this if you do a separate thing like you have attach let us say oxygen molecule along with a nitrogen molecule. Now, they are heavy atoms still, but they might be happy in their syn conformation because there is a possibility of hydrogen bonds. I am not saying this is obvious, but it is also another complex possibility that they could love to form this hydrogen bond and want to stay in syn conformation because of this hydrogen bond, they get extra stabilization.

So, in summary, we have just talked about starting from a molecule where 2 carbon is there, change it by replacing another 2-methyl getting a normal butane develop a energy profile and replaced by other groups and see that in the limitation of all these 6 relatively heavy atoms like carbon and all. They are already development of complex situations there are already developments of local minima, global minima, local maxima, global maxima and all these things.

First of all, we understand the conformational stability, conformational energy barriers, the different conformers which we could achieve by rotation. But more importantly, we could also imagine how complex it would be for a protein where many of such carbons, nitrogen and oxygen are together and every one of them have their own rotational freedoms.

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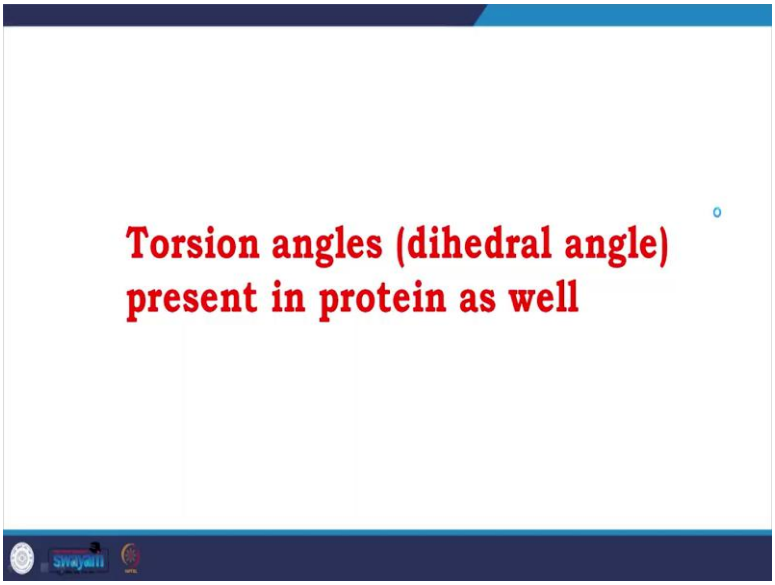

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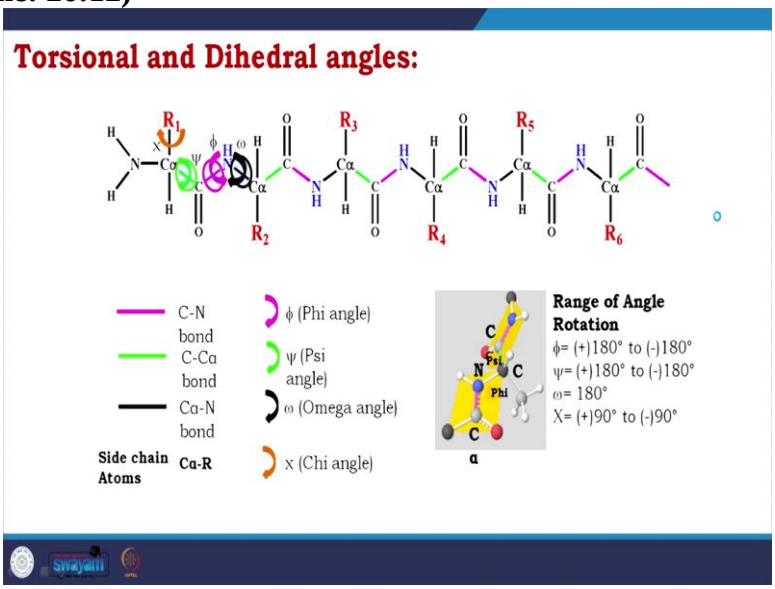

So, we will come to torsion angle which are present in protein. So, this is a peptide where we see that the C N bond which is phi angle, the C C alpha bond which is the psi angle the C alpha N bond which is the omega angle and the C alpha R which is the chi angle the range of angle rotation for phi is +180 to -180 psi is +180 to -180 omega is 0 or 180 and chi is +90 to -90, Who is chi? Chi is very important, as ~~we are talking about,~~ we are talking about 2 degrees of freedom.

We are talking considering the main chain of the peptide backbone, but chi's are angles which are associated with the side chain. For example, if you consider glycine there is only one hydrogen whereas if you consider alanine there is a CH 3 group, you consider a as part it CH 2 CO 2 H glutamate, CH 2 CH 2 CO 2 H. So, in that way with the difference in the

amino acids, there would be different distribution of chi angles, which would have their individual effects on the movement of the backbone.

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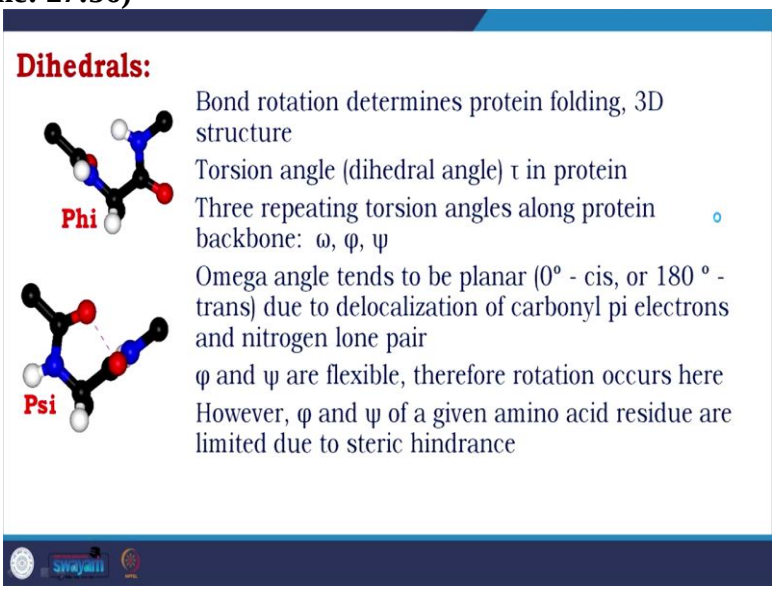

So, this is phi and psi which we are going to talk. So, this is the rotation of phi and psi. And when you look at them, you could easily see that there are restrictions when steric classes happen. So, bond rotation determines protein folding as I talked and the unique 3D structure of the protein, torsion angle tau in protein, 3 repeating torsion angles along protein backbone omega phi and psi omega angle tends to be planar 0 degree for cis or 180 degree for trans.

Generally, it is transit considered as 180 due to delocalization of carbonyl pi electrons and nitrogen lone pair. Phi and psi are flexible therefore rotation occurs here. However, phi and psi of a given amino acid residual are limited due to steric hindrance.

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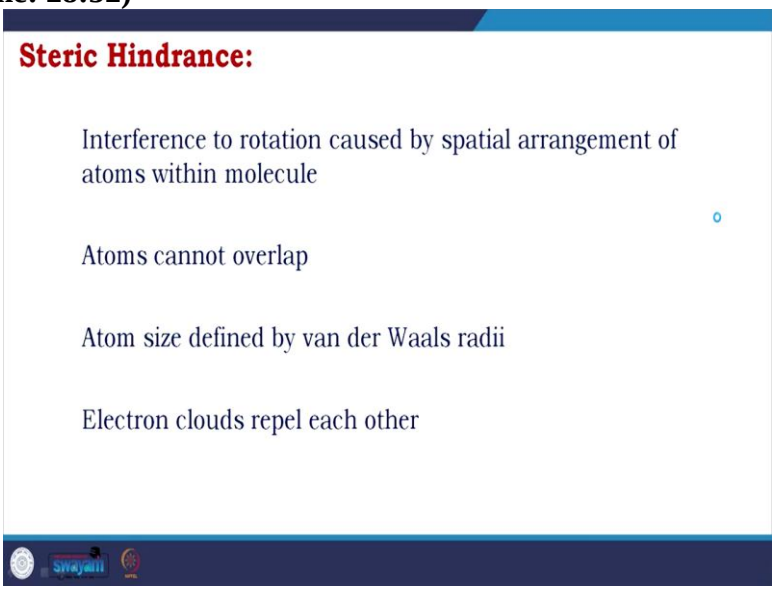

So; steric hindrance which takes a very critical role, interference to rotation cost by spatial arrangement of atoms within molecule when they are rotating if the common to the class that is steric class and that make the rotation disallowed. Atoms cannot overlap atom size, but how they will do that it depends on the Van der Waals radii. The electron clouds repel each other.

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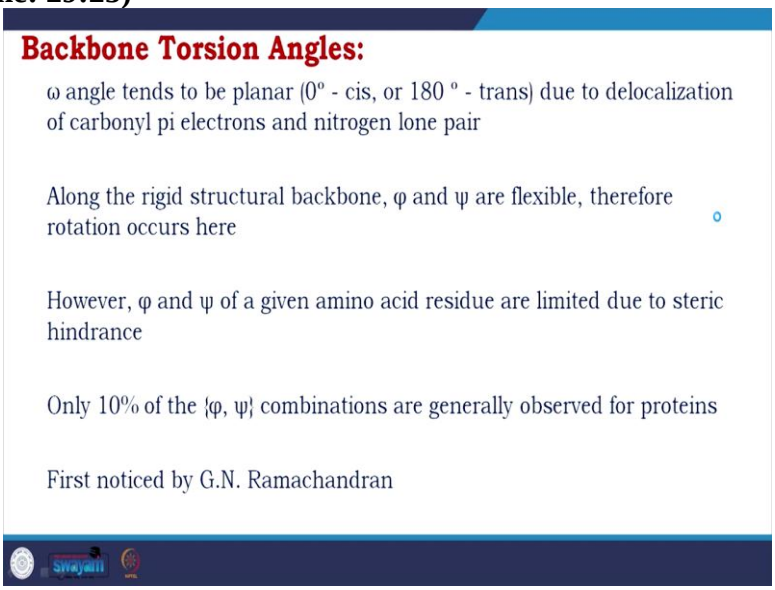

So, omega angles tend to be planar as I told 0 degree for cis or 180 degree for trans due to the delocalised carbonyl pi electron and nitrogen lone pair along the rigid structural backbones psi and phi are flexible they are for rotation occurs there. However, they are restricted because of the steric hindrance coming from the amino acids which means the rotation and the spatial position of the atomic residues which are in the side chain which are rotating with the chi angles.

Very important now, only 10% of the phi and psi combinations are generally observed for proteins, first noticed by G.N Ramachandran, we are very proud that this thing was observed first by G.N Ramachandran and group and they have worked on them and developed some principle which is called Ramachandran plot.

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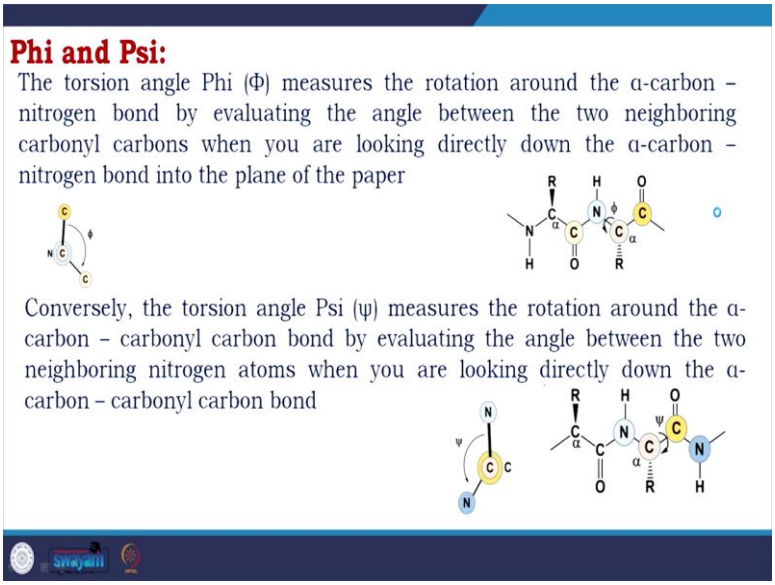

So, phi and psi the torsion angle phi means that the rotation around the alpha carbon nitrogen bond up by evaluating the angle between the 2 neighbouring carbonyl carbons when you are looking directly down the alpha carbon nitrogen bond into the plane of the paper. As I talked about, you see this you will understand better. Conversely the torsion angle psi measures the rotation around the alpha carbon-carbonyl carbon bond by evaluating the angle between the 2 neighbouring nitrogen atoms when you are looking directly down the alpha carbon carbonyl carbon bond like this.

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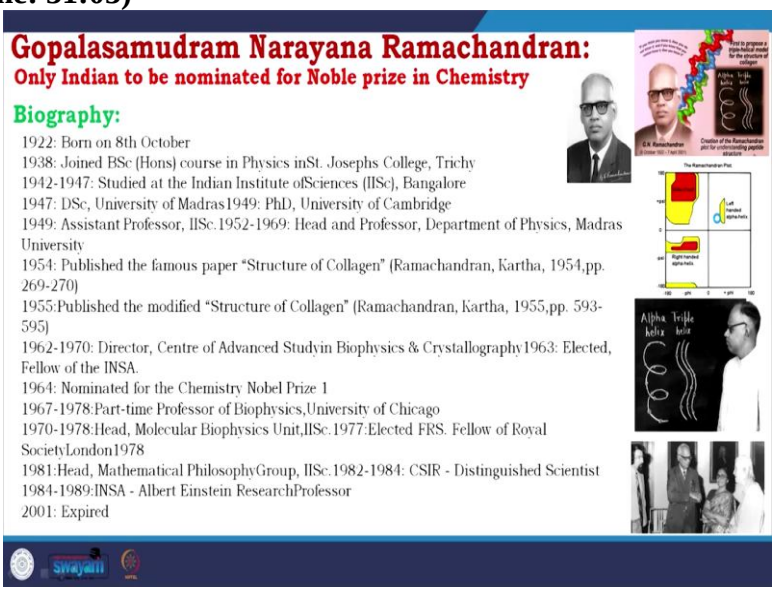

Before going to the details, this slide represents a small biography and highlighted walk-up Ramachandran you know you all have heroes in the film we have Shahrukh Khan Amitabh Bachchan in the in cricket we have Virat Kohli, Dhoni and all. So, this is my fan moment, this is my Amitabh Bachchan. So, I want the newcomer to know about him, this man was the only Indian to be nominated for a Nobel Prize in Chemistry had done immense impact not

only respect to Indian science, but in respect to International Science. I have put a little bit glimpses of his work here.

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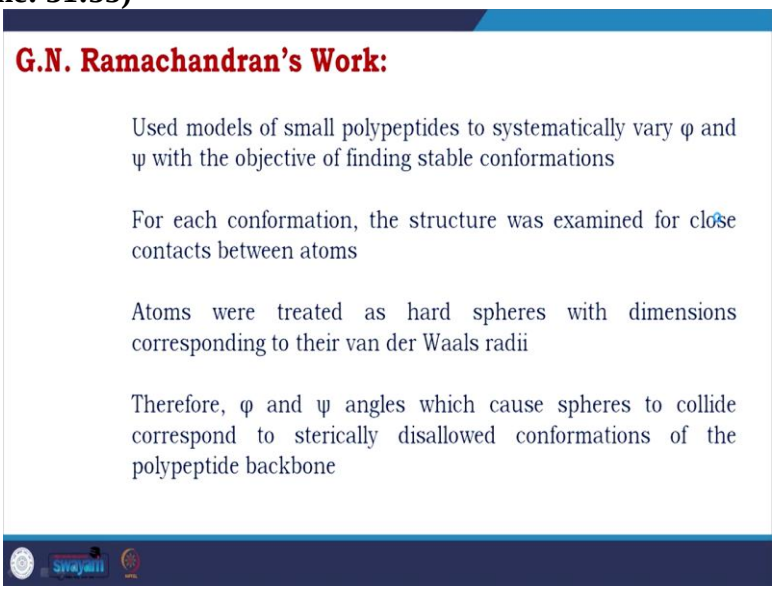

So, for these works, what is Ramachandran's model, they have developed models of small polypeptides to systematically vary phi and psi with the objective of finding stable conformations. See this line and you understand. In 1960s it was not possible for them to look at the rotation of back and tap protein. So, they have developed model of small polypeptides and these models were very crude they developed them by using initially using cardboards.

And in a proctor compass they measured and then they synthesize some of them they develop computer models. For each conformation the structure was examine for close contact between atoms to measure all the details that atoms treated as hard sphere with diamonds and corresponding to their Van der Waals radii. Therefore, phi and psi angles which cause spheres to collide correspond to sterically disallowed confirmation of the polypeptide backbone. So, what they did? They take them, they did the rotation and see where the steric classes are happening or not happening.

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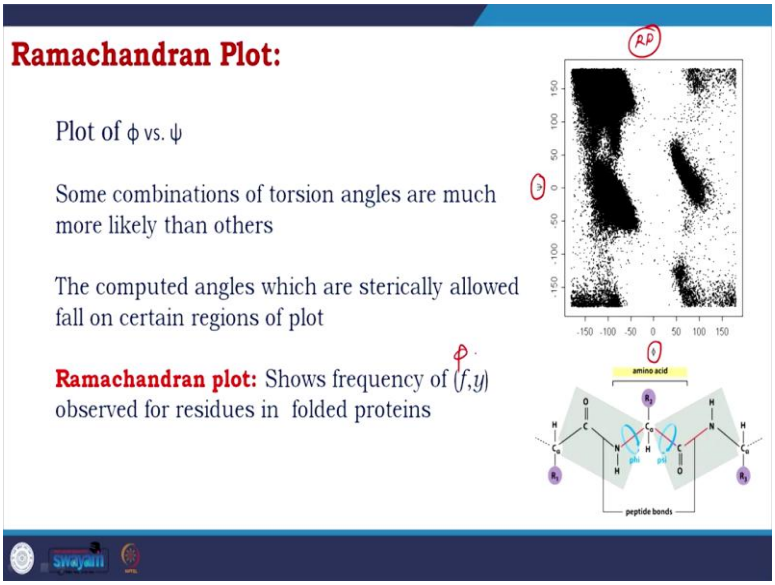

This is a Ramachandran plot what you see here. Ramachandran plot what they did, they make a plot of phi versus psi. So, phi from - 180 to + 180 in the x axis and psi in the y axis again – 180 to + 180 in the y axis. Let us say 2D plot. Some combination of torsion angles are much more likely than others they calculate them out the computed angles which is sterically allowed fall on certain regions of the plot, which shows frequency of phi and psi observed for residues in the folded protein if and why actually this is a like crypt representation of phi and psi.

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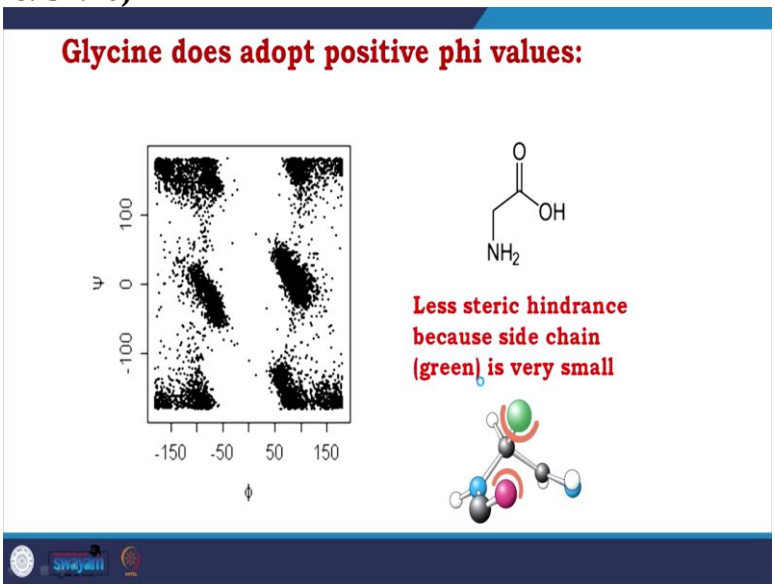

So, when you consider glycine you see because of the list steric hindrance of sidechain being only hydrogen, you get the plot mostly everywhere when you consider glycine.

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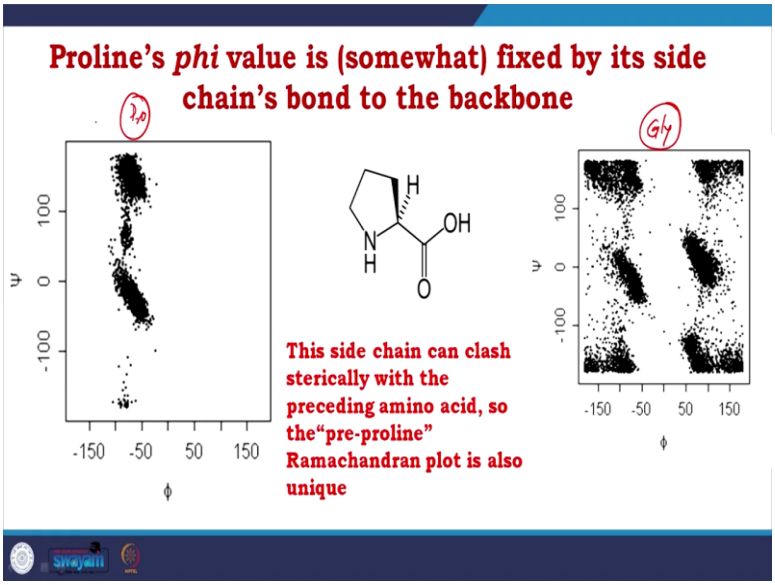

It would be very interesting when we put proline because, if glycine is the smallest proline is definitely not the bigger but because of its locked chain. It shows some restriction. So, proline phi value is somewhat fixed by its side chains bond to the backbone see it is fixed. So, it is only in one axis and other axis you do not see the change. So, this side chain can clash sterically the preceding amino acid. So, the pre -proline Ramachandran plot is also unique try to understand that.

Proline it locked. So, the residue before proline would also be interesting. But before that, I just want to show you this is glycine. This is the plot of Gly and this is the plot of pro and you get the clear difference.

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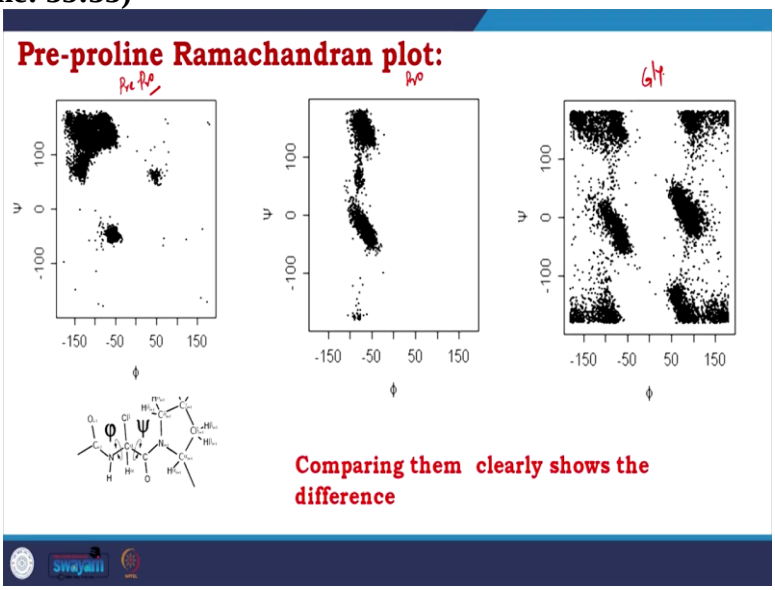

This is pre-proline Ramachandran plot, you see movements, but still you see the restrictions. This is so, this is pre pro and this is pro this is glycine and the difference is very clear.

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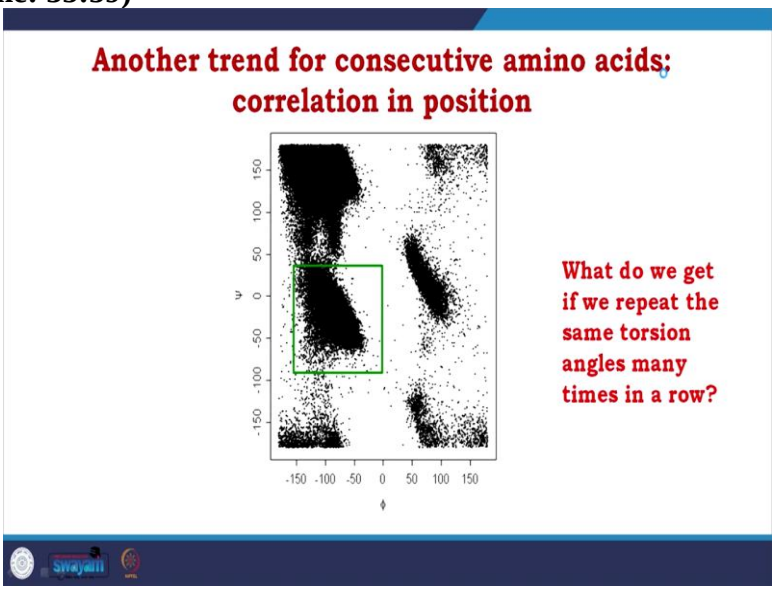

Another very interesting trend in the Ramachandran plot, if you see, this is the position of amino acid, let us say n the n + 1 amino acid would be very close to that. So, there is a correlation of their position. So, what do we get if we repeat the same torsion angle many times in a row? So, let us see that.

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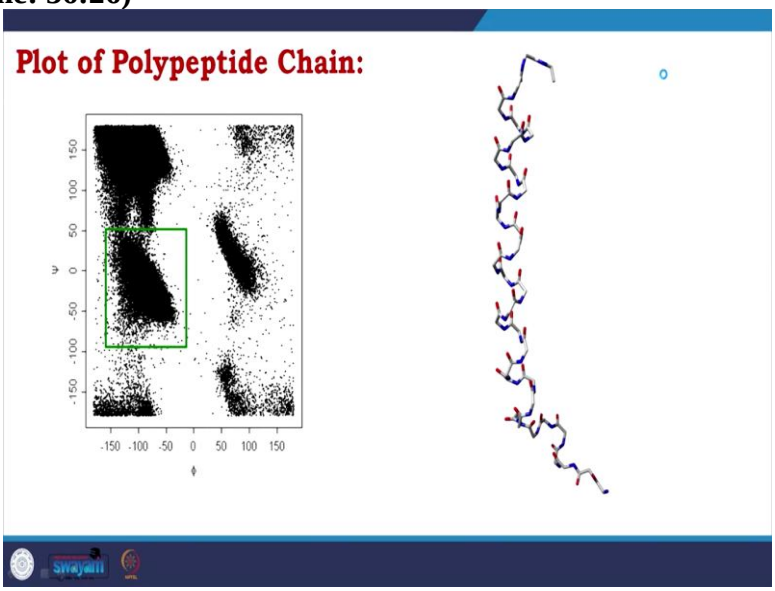

So, we put a polypeptide chain, which actually shows the trend to be restricted in the green highlighted region.

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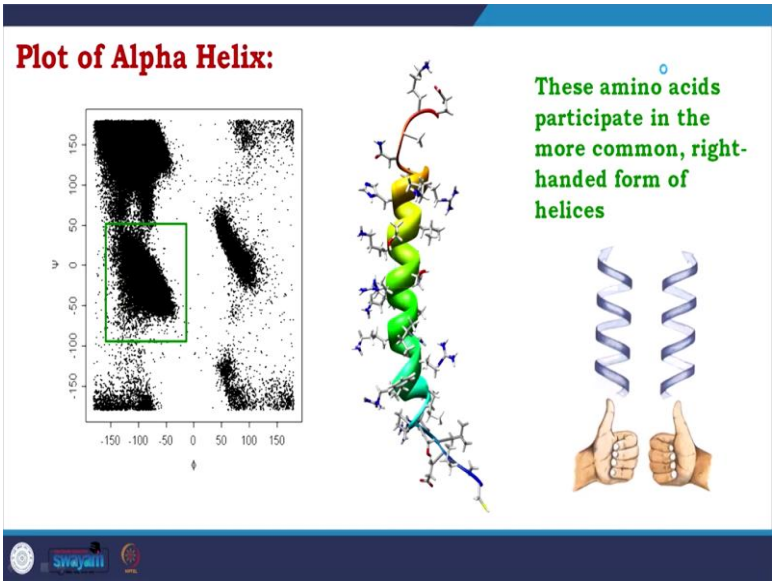

That is that alpha helix. So, these amino acid chain participate in the more common right-handed form of helices. So, if it is coming here in the phi psi plot Ramachandran plot, it is representing the secondary structure alpha helix. So, now, from amino acid we are shifting to the polypeptide we are understanding the correlation we are understanding the positions.

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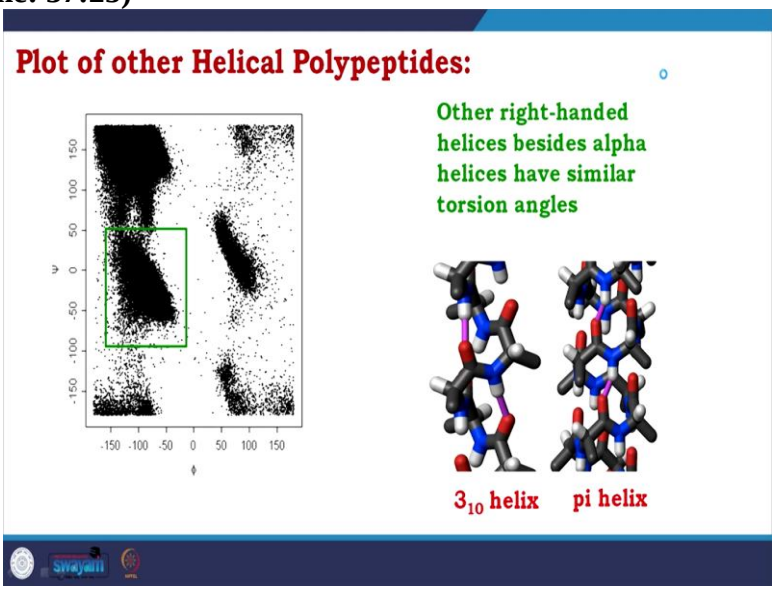

The other right-handed helices, which we talked about that 3-10 helices on phi helix, they also have similar torsion angles. So, you see that they are in the same place.

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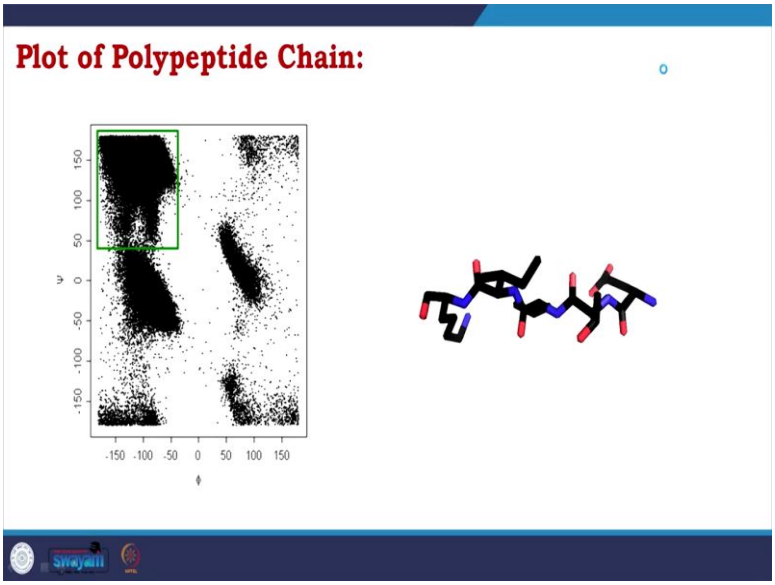

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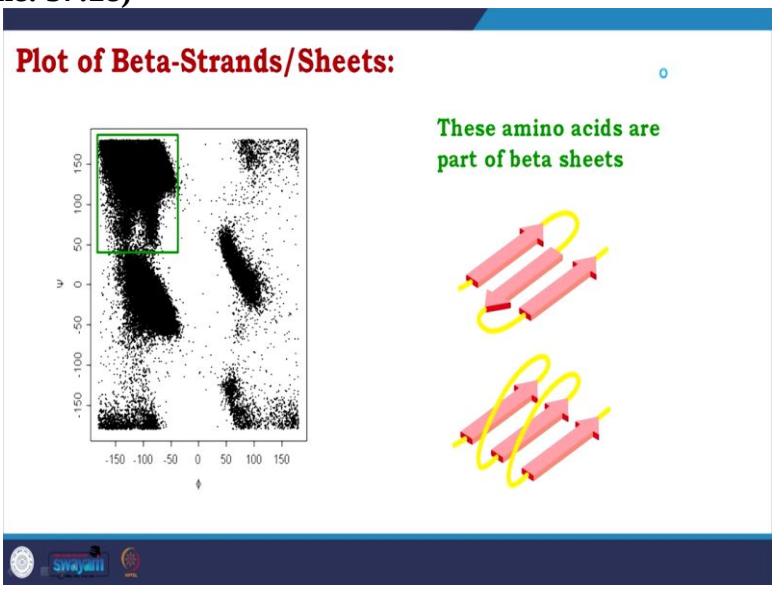

Then we put a different polypeptide chain and it shows that when it goes in the other region, it is representing amino acids, which are part of the beta sheets beat parallel or antiparallel.

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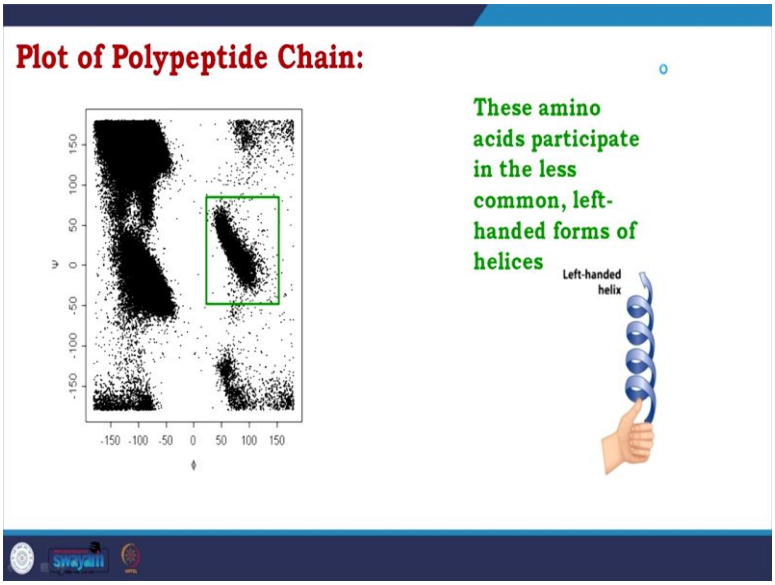

Now, another region where we see there are clustering of plots and these amino acids participate in the less common left-handed form of the helices.

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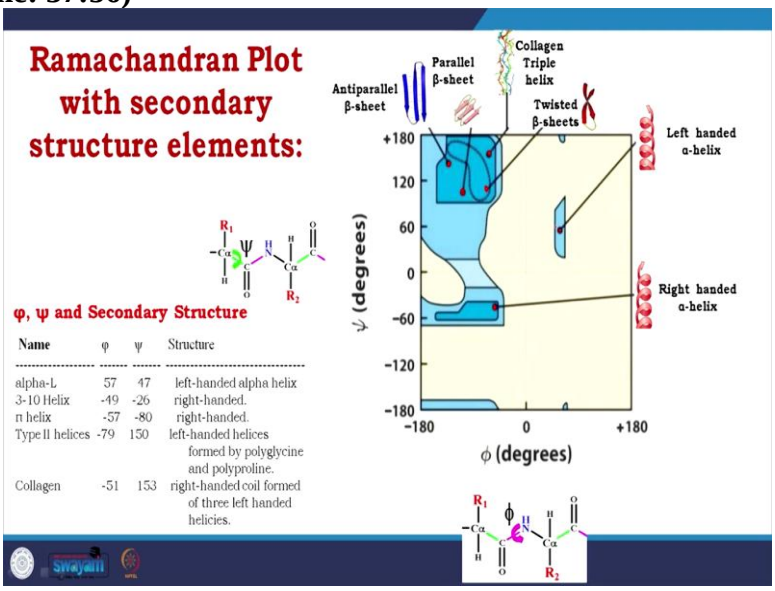

So, when we make a summary, we see that for alpha L comes in between phi 57, psi 47. The structure is represented left-handed alpha helix. For 3-10 helix says it is - 49 for phi and - 26 for psi, it is right-handed we know pi helix -57 for phi and -84 for psi for type 2 helices – 79 for phi and 150 for psi. These are left-handed helices formed by polyglycine and polyproline. Collagen – 51 for phi 153 for psi right-handed coil formed of 3 left-handed helices.

So, if you see here, this is the plot as I told the plot is made by putting the phi in the x axis psi if you see this is phi this is between this C and n and this is between C alpha and C. C alpha is psi which is put in the y axis and C n input on the x axis. Now, this portion which is here

highlighted this is the right-handed alpha helix. So, if the amino acids could be a part of the right-handed alpha helix, they mostly cluster in this region.

If they are part of the parallel beta sheets, they are part of this region. If they are part of antiparallel beta sheets, they are part of this region. If they are part of collagen, triple helix they will be part of this region. If they are part of twisted beta sheets, they will be part of this region. And if they are part of left-handed alpha helices, they would be part of these regions. So, we have started our journey from the understanding of the peptide bond the torsion angle, where the hybridization happened.

How this hybridization makes the things rigid and planner and this planner at the associated bonds which are phi and psi are rotating and how they are rotation are restricted by the influence of the individual amino acids, their side chain the side chains movement by different chi angles. And because of that, how glycine and proline we have seen very differently projected in the Ramachandran plot. And now, we see when they are forming alpha helices or beta sheets, how they are putting themselves.

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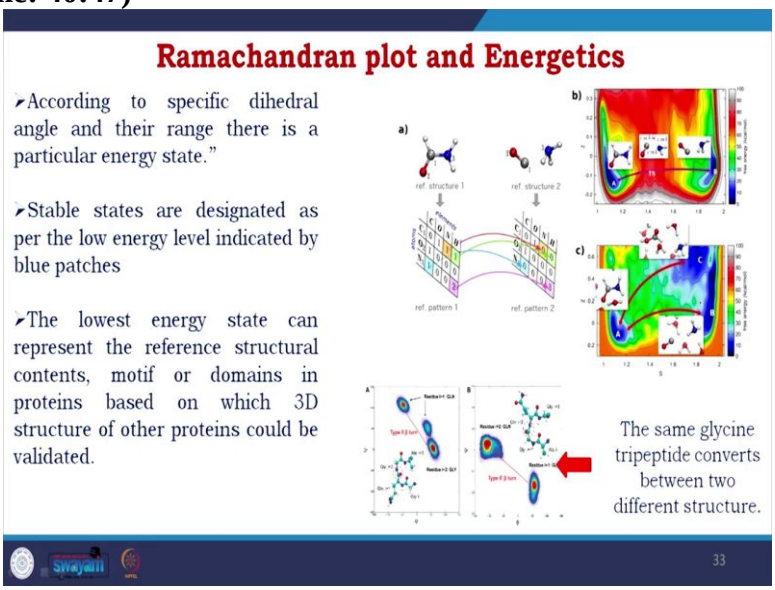

We could also understand Ramachandran plot and energetics. So, according to specific dihedral angle and their range, there is a particular energy state. So, because of the rotation, they would be in a sterically allowed position and that rotating state that state where they are the conformer state have its own particular energy state. Stable states are designated as part of the low energy level indicated by blue patches which you will see here.

The blue patches up showing the low energy state the lowest energy state can represent the reference structural contents, motifs or domains in protein based on which 3D structure of other proteins could be validated, very interesting point. So, the lowest energy state present the reference structural content, you already have a lot of already established structure in the protein databank. So, you get them you calculate their position in the Ramachandran plot by calculating their phi and psi plot them and calculate their energy.

This would be very interesting and very informative as a reference for validating a new structure. Also, if you see the story of the glycine here it is a same glycine tripeptide and they convert between 2 different forms. By rotation they convert into 2 different forms they are giving 2 conformers and hence they are position on the different part of the energy state in the Ramachandran plot.

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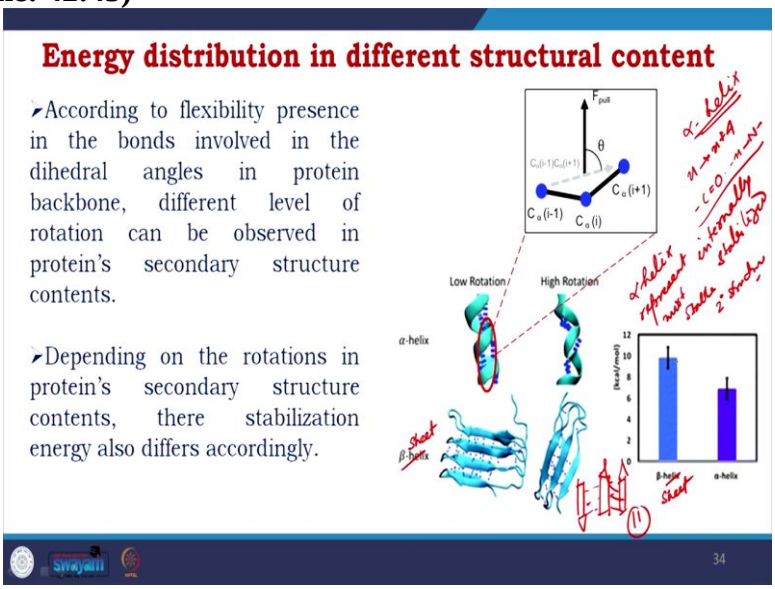

According to flexibility presence in the bonds involved in the dihedral angles in protein backbone, different level of rotation can be observed in protein's secondary structure contents. Depending on the rotations in protein's secondary structure contents, there stabilization energy also differs. So, when you see that you are considering those secondary structure alpha helix, I think beta sheets individually when you considering alpha helix what they have they are stabilized by n to x + 4 C double bond O N H bond.

So, though are internally stabilised. So, as an individual unit alpha helix represent most stable secondary structure. But now, you see the energy profile and you argue with me you told that alpha helix is the more stable but I see that this beta sheet is better you see that this because

this is beta sheet not the beta strand. Can you understand what I am saying? So, beta strand individually have no ability to stabilize themselves through hydrogen bonds.

So, they bring one beta strand, bring other beta strand be it the parallel or n-type parallel. So, they are together and together they are having more energy. Is it clear? So, individually one alpha helix is having better energy is most stabilized, but beta strand could not be stand together you cannot measure a single beta strand, So you have to measure the total beta sheets and as a sheet they are having more energy than alpha helix itself.

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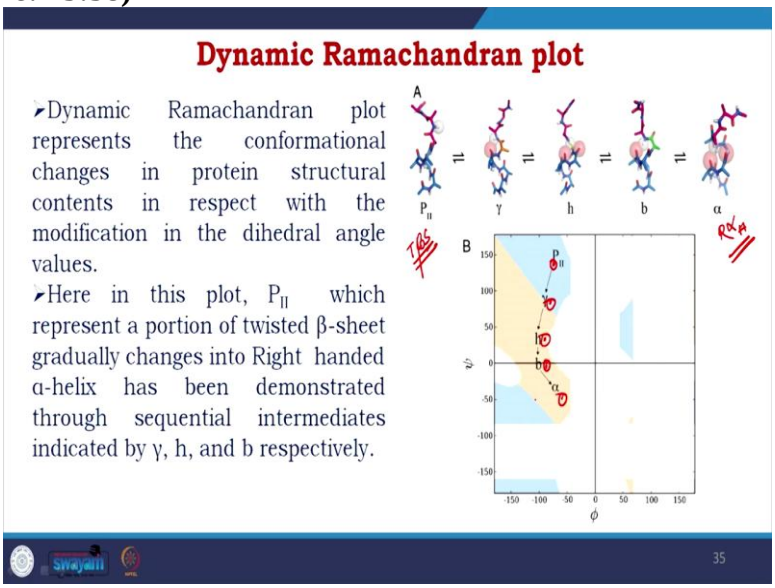

Coming to Dynamic Ramachandran plot which is a modification; modern modification. So, dynamic Ramachandran plot represent the conformational changes in protein structural content in respect with the modification in the dihedral angle values. Here if you see in this plot, this is also Ramachandran plot with the change here you see the changes in the peptide and you see P 2 which represent a portion of it.

Twisted beta sheet gradually changes into right-handed alpha helix has been demonstrated through sequential intermediate indicated by these 2 gamma 2 h 2 beta 2 alpha. So, when you are changing a structure, the same structure have changed it is state and changing from a twisted beta sheet. This is a twisted beta sheet to a right-handed alpha helix. So, this journey is reflected by having points.

Initially starting from this P 2 coming to this gamma state coming to this h state coming to this b state and ultimately coming to the right-handed alpha helix. So, when you go for

dynamicity, which we could see in this course, I have a small portion, I will talk about the dynamics. So, you have a structure, you have its force field and you have a equation for the force field which is influencing the bonds you put a force and gradually it would be changing its state.

And it would have a journey from the twisted beta sheet to the right-handed alpha helix and that journey could be reflected in a dynamic Ramachandran plot.

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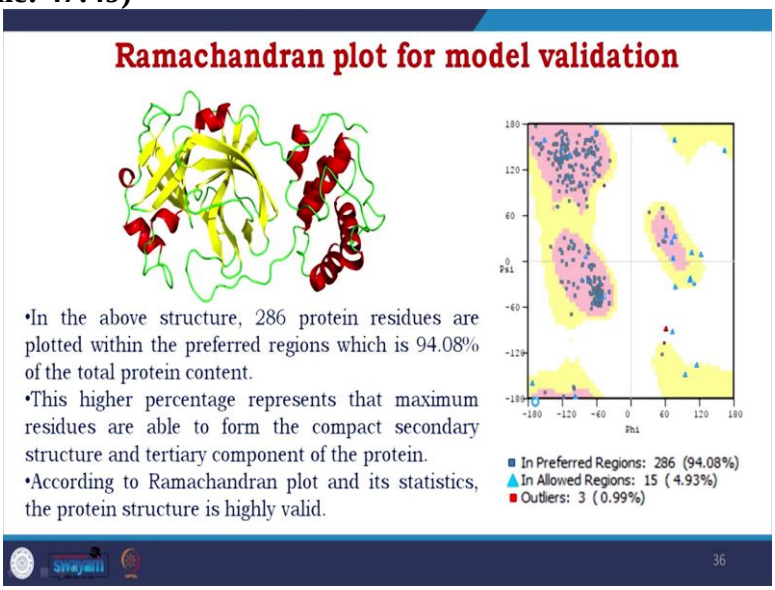

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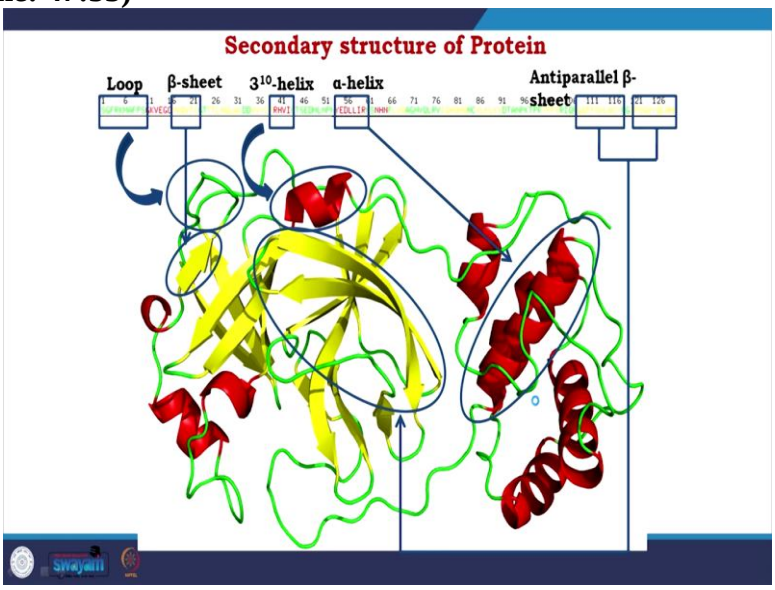

So, Ramachandran plot for model validation before I talk about, I want to show you this secondary structure of protein if you see that, you will see that alpha helix here, you see 3-10 helix there is a beta sheet here, you see antiparallel beta sheet here and loops here. So, this is

a protein which have many secondary structures and taking that we are going to validate it what is mean by validation?

Remember, I was talking about so, there are many experimentally solved structures by taking that experimentally solved structure putting them into Ramachandran plot. Now, we know that which 3D conformers what type of this torsional angle distribution are representing a valid structure. For example, if you see this structure, when we plot the torsional angles 286 amino acid residues among 304 residues are plotted within the preferred region, which is 94.08% total protein content, what is a preferred region.

So, as I told if you go to the protein databank, you will get the experimentally solved structure which are experimentally solved structures show they are validated structure that torsion angle distribution of amino acids are calculated they are clustered and from this cluster, we have developed a preferred zone which is favourite and then after that, it comes to the allowed region and then it comes to the out layer or disallowed region.

So, now we have this standard database and for a new structure, we are plotting its torsional angles and calculate the preferred region allowed region out layers. So, if you see in the above structure, I told among the 304 residues of the full length 286 amino acids are plotted within the preferred region, which is 94.08% of total protein allowed region that is 15 which is 4.93% in the out layer, there are 3 which represent less than 1% or 1%.

This higher percentage represent that maximum residues are able to form the compact secondary structure and tertiary component the protein according to Ramachandran plot and its statistics the protein structure is highly valid. So, up to now, I was talking about plotting this is what in wonderful application of Ramachandran plot, remember, we start this course, to putting the problem in front of us where we have to like next generation sequencing a lot of sequences.

So, DNA sequences, which would be easy to convert to protein sequence, now, you have to convert this protein sequence getting function out of that and which needs to understanding the structure. But, unfortunately, to the next part of the course, we will discuss, I have given you some glimpse in the last module, that how this structure solutions are extremely critical,

how they are expensive and time consuming. So, a gap is there and it is very difficult to bridge the gap.

But, one easy solution not an optimum solution, easy solution is to do predictions predictive models by using computational biology method, but when you are doing prediction you need a validation and here Ramachandran plot is working doing an amazing job so, that it gives us confidence the model that this model is validated.

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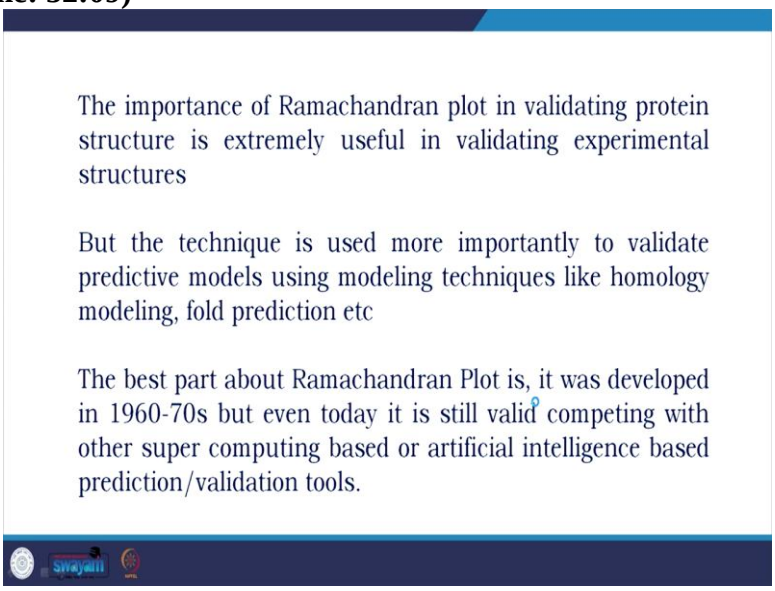

So, the importance of Ramachandran plot in validating protein structure is extremely useful in validating experimental structures that is one thing at the earlier stage. Now, that technique is used more importantly to validate predictive models using modelling techniques like homology modelling for predictions etc. I will finish this chapter today's class by saying that and I am very proud to say because he is my hero.

The best part about Ramachandran plot is it was developed in the 1960-70s. But even today, it is still valid competing with the other supercomputing best or artificial intelligence best prediction and validation tools till today, your model you need to validate you have to go for Ramachandran plot and do the validation based on them. So it is that important it is that critical.

And now you think about a innovation done in 1962 likes, it is actually 69, but the work done 60-70s which helped the entire world initially to solve the initial protein structures. But even now, we are taking help of this plot this technique towards the validation of predictive model.

Thank you very much. In the next class, we will continue this concept towards understanding the super secondary structures, which are also critical in development of structure or understanding function. Thank you.