Introduction to Cell Biology
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Protein Structure, Folding and Function - Part 5

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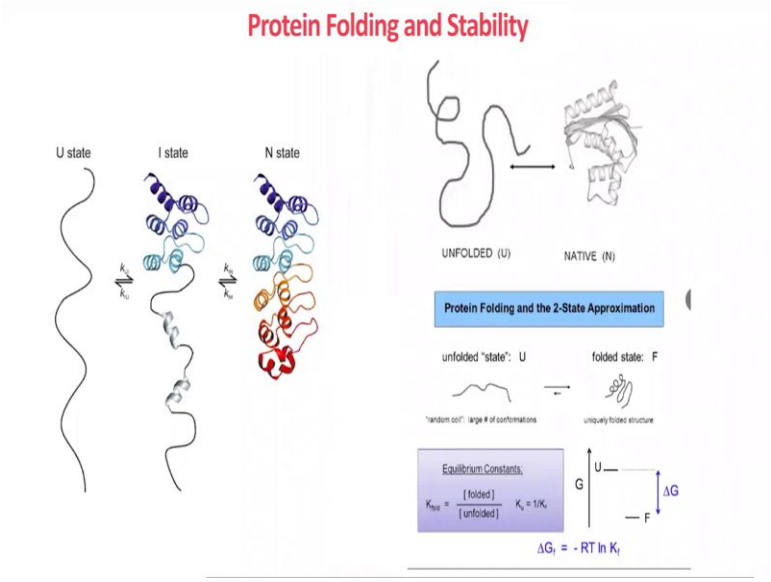

Professor: Is there a question?

Student: I would like to ask something. Could you go back a few slides to where you had led through the bond lengths?

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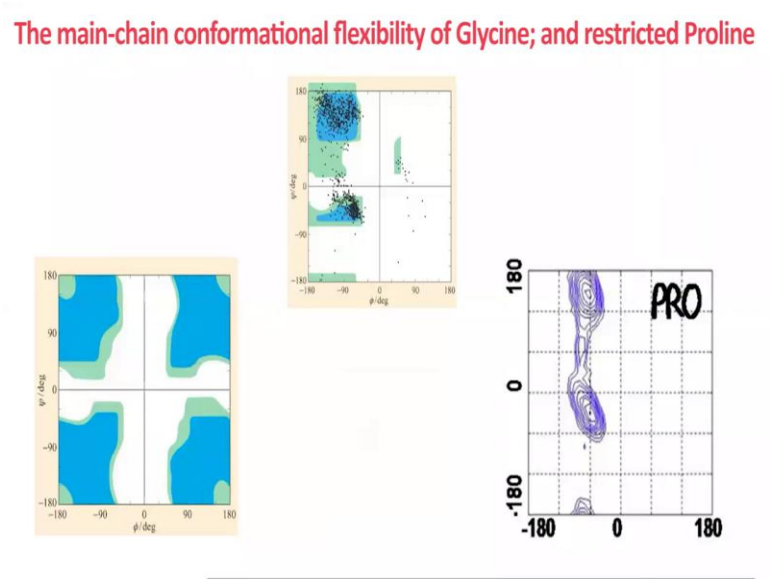

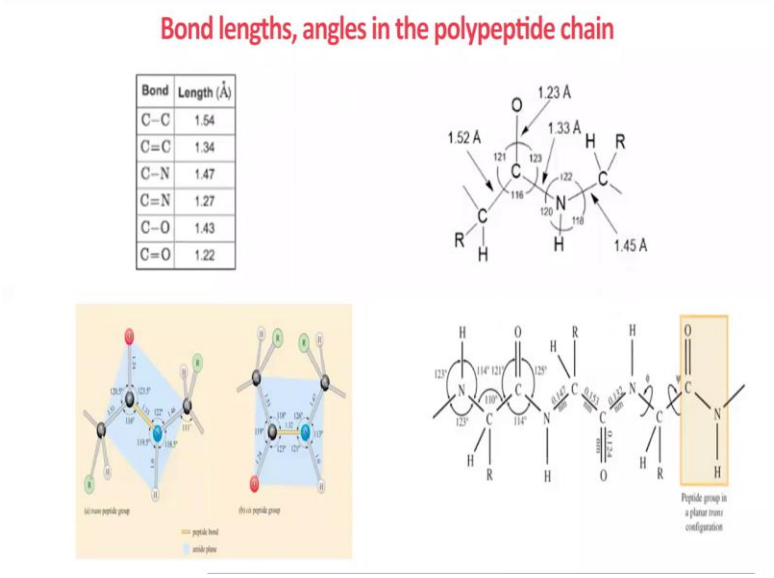

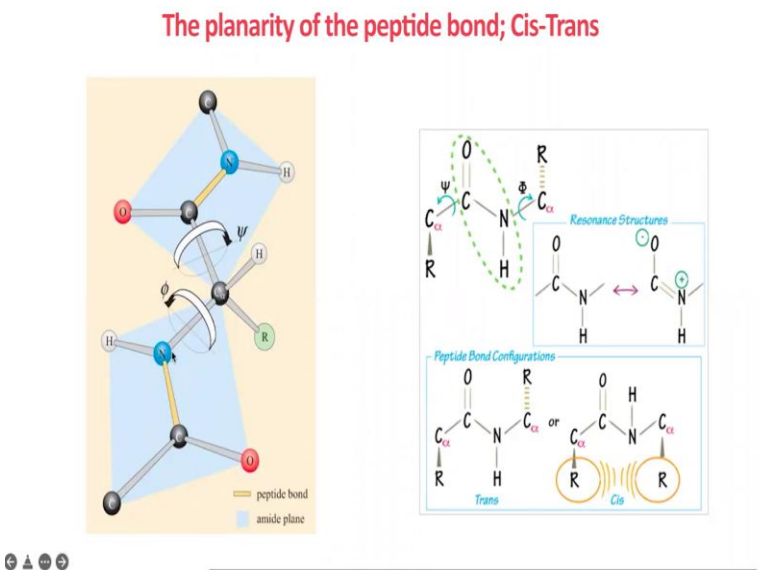

Professor: So, can you, I do not really pay attention to the chat window. Can you just go ahead and ask.

Student: Sir, what I was asking was these are canonical bond lengths for specific bonds such as a carbon oxygen double bond or the carbon-nitrogen bond. But you said earlier that there are, there is a bit of residence, there is a bit of delocalization in the amino acids. So, would not these be more indicative than accurate?

Professor: No, so all the lengths will be closed for covalent bonds. The lengths will be very close to the canonical. There is no reason for them to behave differently. The chemistry of the polypeptide is just chemistry. There is nothing particularly special about that. The specialty is coming from the order of atoms which are covalently connected to them.

Student: Sir, what is the difference between these angles and the dihedral angles?

Professor: So, the dihedral angle, as I have told you, is basically an angle defined by four atoms. These angles are defined by three atoms. So, there is a difference between a dihedral angle defined with four atoms and a normal angle which is defined by three atoms. So, the angles over here are angles defined by three atoms. The phi-psi is important for proteins, because by defining these phi-psi angles, we can literally assuming that the omega is at 180 degrees, which it always is more or less with very few exceptions.

We can define the position of any atom in space by just knowing phi and psi. So that is why these phi-psi angles are so important. And by definition, phi-psi angles are defined by four atoms. And these four atoms have to be main chain atoms. We do not use side chain atoms for defining phi and psi.

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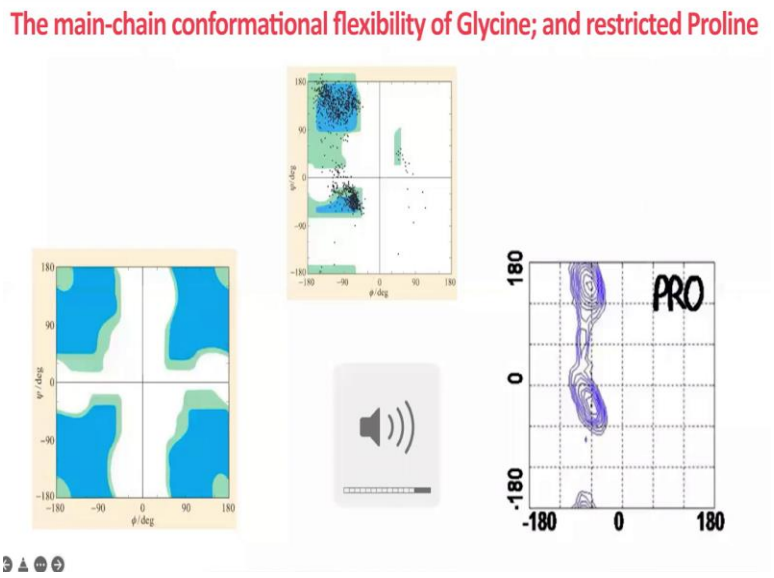

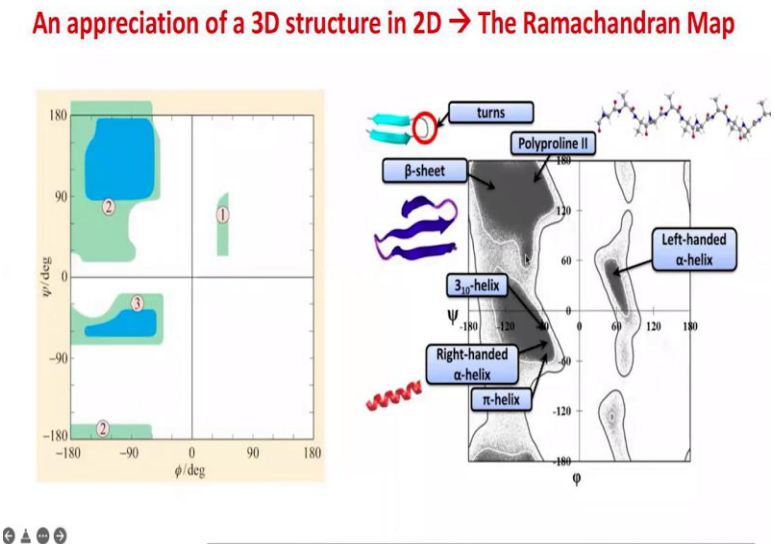

Student: Sir, if the Ramachandran map for glycine was different from what we had already seen, then when scientists discovered this Ramachandran map, how do they know that it is just an exception or not a wrong assumption?

Professor: No, so you have to understand that Ramachandran map initially was a way to plot a three dimensional structure in two dimensions. And first plots came from models. Then the first crystal structures were solved. And let us say you had 10 crystal structures. First 10 crystal structures, let us say, you plot on a Ramachandran map. You get about a few thousand dots on the Ramachandran map and you realize that they are all clustered together. They are not evenly distributed all across conformational space.

And you also realize through Ramchandra's work, that when you build a model of, let us say, of a peptide with 10 amino acids, and you take this physical structural model, and you realize that omega is fixed, so the CO-NH bond is a flat plane and it cannot really vary. So, the only way you can rotate the main chain is phi and psi. And then you make a model and you hold it in your hand and you start rotating it and you realize that if the side chain is anything bigger than a proton and as you turn it 360 degrees there are angles, phi-psi angles in which the side chains come too close. They either clash or even if they are coming close, they are energetically unfavorable, because they repel each other.

Whereas for polyglycine stretch, since there is no, there is not a bulky amino acids, just a small proton, the degree of flexibility is very high. You can move, you can change the torsion angles, phi and psi, into many, many different combinations in phi-psi space, which relates to actual turning of these torsional angles in your models. So, glycine is the only amino acid where if you have four glycine, peptide, you can move the phi-psi quite a lot around 360 degrees, which is shown by the map on the left. But the moment you put even a single CH3 group over there which is an alanine, the amount of rotation you can do is heavily restricted. And it is even more heavily restricted the bulkier the side chain. And proline, which I am showing on the right hand side, is even more restricted than any other amino acid. Is that clear?

Student: Yes, sir.

Student: Sir, when you talk about proline being even more restricted than any other, is that the reason why instead of having dots for your 2D Ramachandran map it has spirals?

Professor: No. So, I am sorry. Getting the right picture is not very easy. There are multiple representations of Ramachandran map. So, for example, if you look over here, you will see that the dots genuinely represent a pair of phis and psi which is basically one amino acid in a

polypeptide chain in a structure which has always been, already been solved. You will notice that even though this part and this part is completely black, there are dots in these gray areas.

That is because modern ideas relate not to two physical atoms banging against each other, but about the energy, but it is relate to, relates to the fact that if you bring two atoms, for example, two CH3s too close to each other, since they do not bond to each other, they repel each other. And because of this repulsion, these light gray areas over here and the light gray areas over here can also have, these are also occupied in phi-psi space, but they are statistically unfavorable.

Much of the favorable spaces are the blue spaces over here, and the black spaces over here. But the other spaces are possible, but statistically unfavorable. So, for example, if you look at a dot over here that is in all probability a glycine. If you look at a dot over here, that is in all probability a glycine. So, glycines can come in the so called unfavorable zones. But tryptophan cannot. It is too bulkier residue. And proline cannot be on the right hand side of the Ramachandran plot at all, because it is restricted due to a covalent bond between the side chain and the main chain. Is that clear enough?

So, I have been showing you dot plots. I have been showing you classical representations of Ramachandran plot. And I have been showing you energy diagrams. This is a energy diagram over here. But they all conceptually are the same thing.

Student: Where the contours are close together, can we say that the capability of protein being, the amino acid being there is more?

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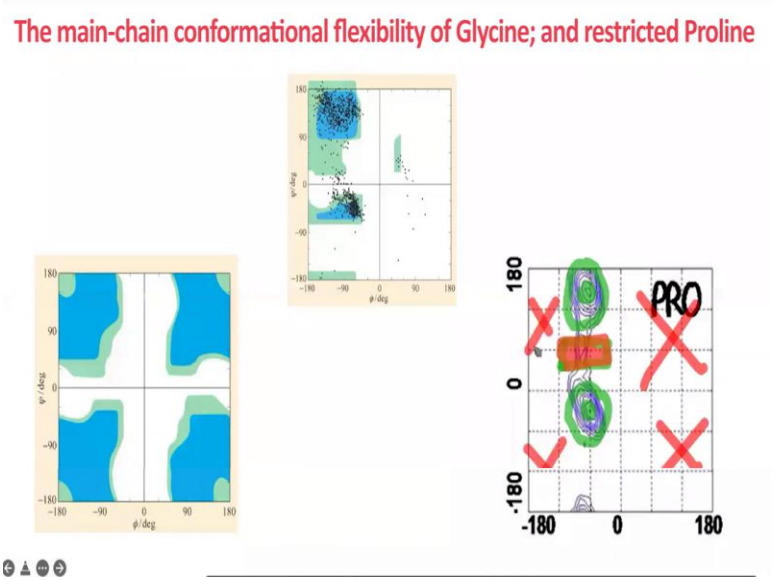

Professor: Yes. So, for example, highest possibility of the amino acid in structures when you plot them are, is here. These are the two spots where the densest dots will be there if you plot. The ones outside are less favorable means less chance. They are there. And this part is very rare. Very few proteins will have, will occupy a phi-psi space in this area. And of course, nothing here, nothing here, nothing here and nothing here. Is that clear?

Student: Yes, sir.

Student: Sir, how do we define positive and negative phi and psi, like which atoms do we define?

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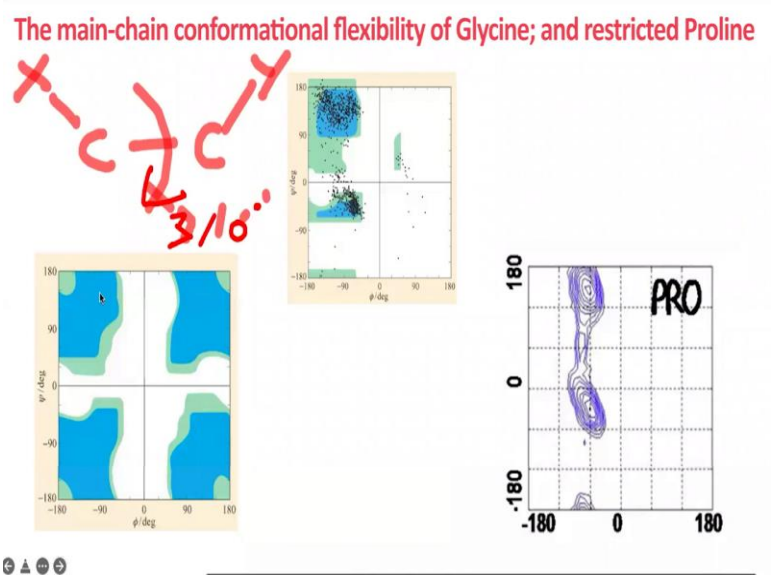

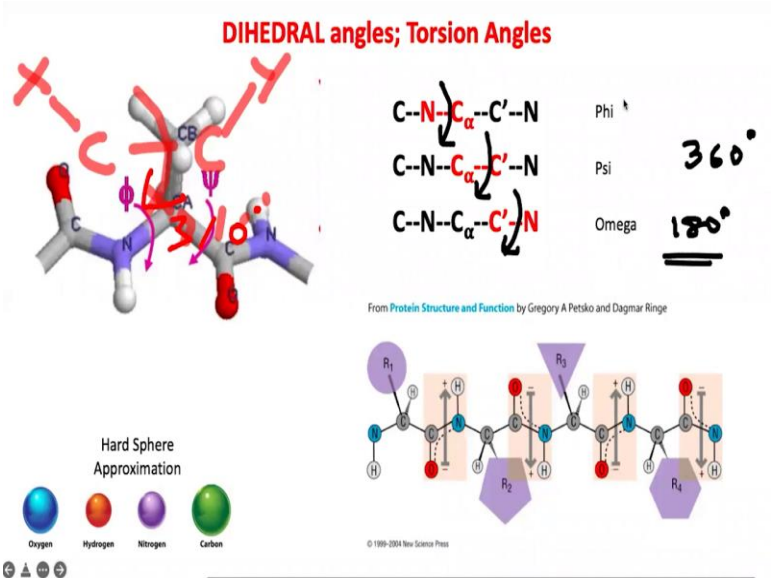

Professor: It is a 360 degree rotation. So, if you have, let us just take, this is not the best representation, take a carbon-carbon, I am calling this x, I am calling this y. So, this carbon-carbon can rotate 360 degrees, but 360 degree, sorry, it does not look like 360 degrees. Let me try again 360. So, this rotation can be 360 degrees, and all phi and psi representation is, representing is a 360 degree rotation around the bond, which as defined over here. Let me go back.

So, here are the phi bond. So, this is the phi rotation, this is the psi rotation, this is the omega rotation. All of them can rotate 360 degrees. Omegas most of the time, almost always stays at

180 degrees, whereas a phi and psi in theory can rotate from 0 to 360 degrees, which is pretty much what the Ramchandran map is showing.

However, even though it can rotate, it turns out, it cannot actually take certain positions. And the reason it cannot take certain positions is the R group of amino acid one clashes with the R group of amino acid two and because of this even though theoretically the bond can rotate 360 degrees practically in protein structures, there are limited phi-psi locations where you find the phi-psi for each residue. And because of this limited restriction in terms of where phi-psi's can be, proteins tend to fold in certain structured manner, alpha helices, beta sheets and so on and so forth.

Student: Sir, can we say that in the graph, the scattered dots are probably of glycines?

Professor: The scatter plots are what?

Student: The scattered dots which are in the open space are of glycine.

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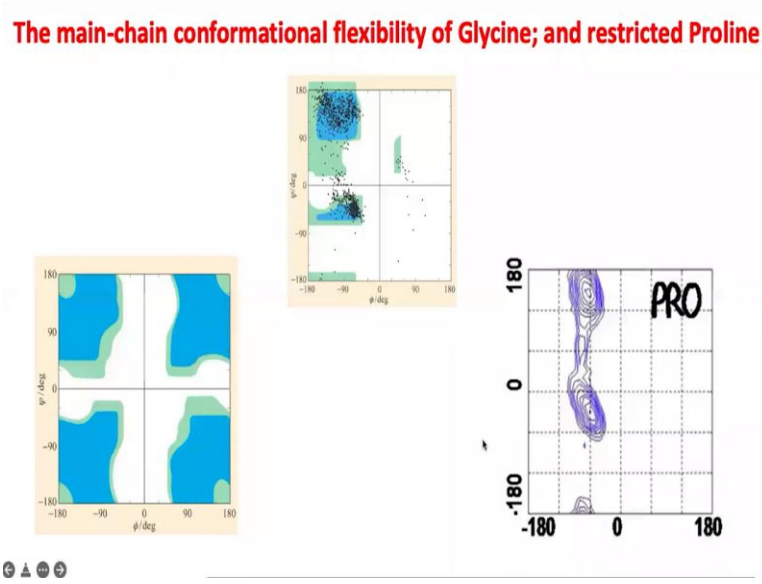

Professor: Yes, so these should be glycines, either there should be glycines or there is something very strange happening in that local area. So, for example, if you have an amino acid in active site of hemoglobin, there is a possibility. I am not saying it happens. There is a possibility of because it is interacting, that main chain is interacting with iron atom that there may be a little bit of torsion or twist over there which causes it to take what are defined as unfavorable confirmations. So, there can be special cases, but 99 percent this is glycine.

Student: Sir, when we say that we are plotting a phi-psi graph of protein, we mean to say that every, that four we are in the protein chain, we are taking every possible four combination of that atoms and we are plotting the phi and psi for, like if I get phi and psi 0, 0, I am plotting that and then I go to the next four chain, and then I am plotting again a phi and psi. So, are we plotting that like for the entire protein or.

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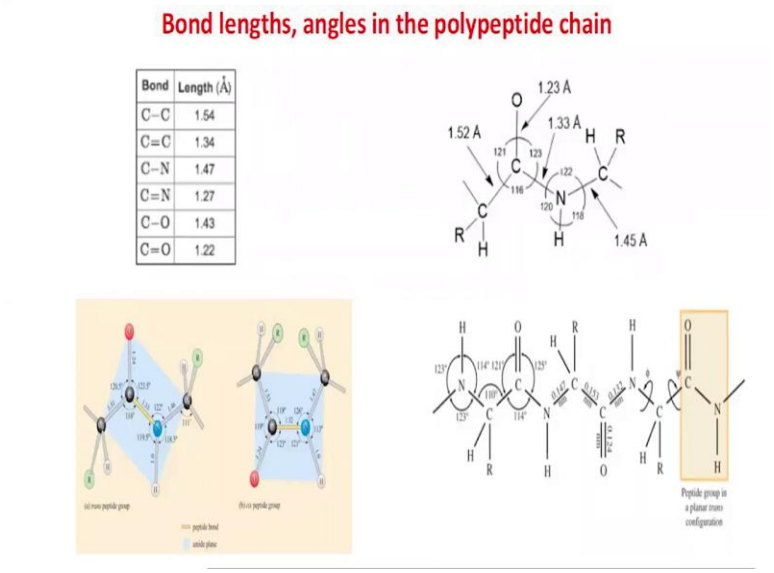

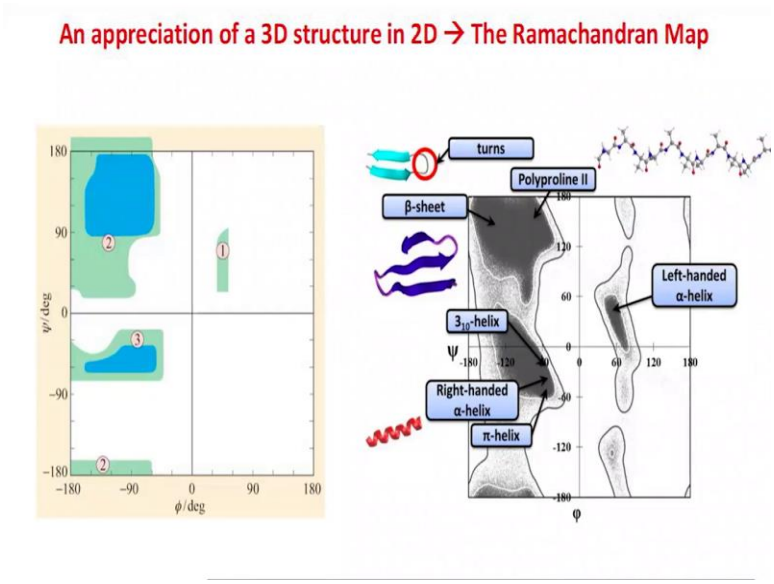

Professor: Exactly, so, not only are we plotting it for an entire routine, we are also plotting it for all the 1 lakh proteins which are there in the protein databank. We can and which is what this is

showing you. If you look at this very densely crowded picture, there are thousands and thousands of proteins over here. So, what you are saying is…

Student: All the proteins are over here.

Student: Sir, proteins or amino acids, like all the amino acids of your triple helix.

Professor: All the amino acids in a protein are being plotted over here. So, is everybody facing problems in understanding this concept?

Student: Yes, sir. Yes, sir. Yes, sir.

Professor: So, I do not know why this is complicated. Let us try this again.

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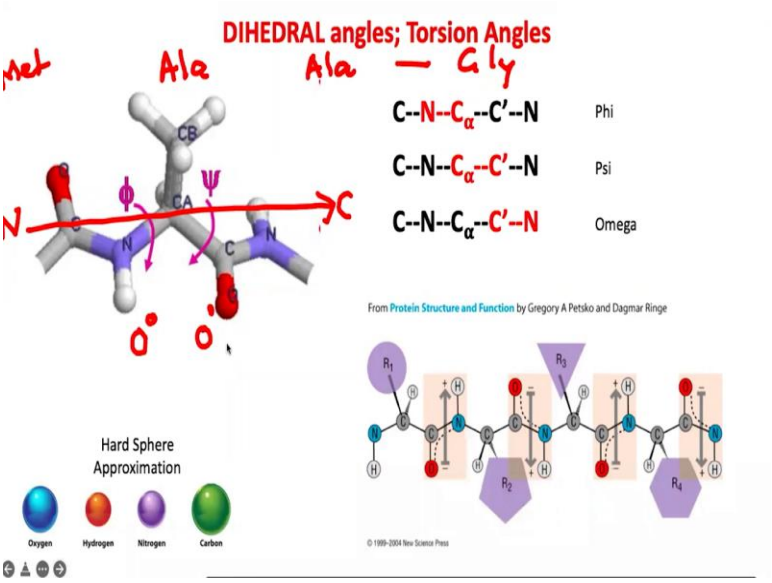

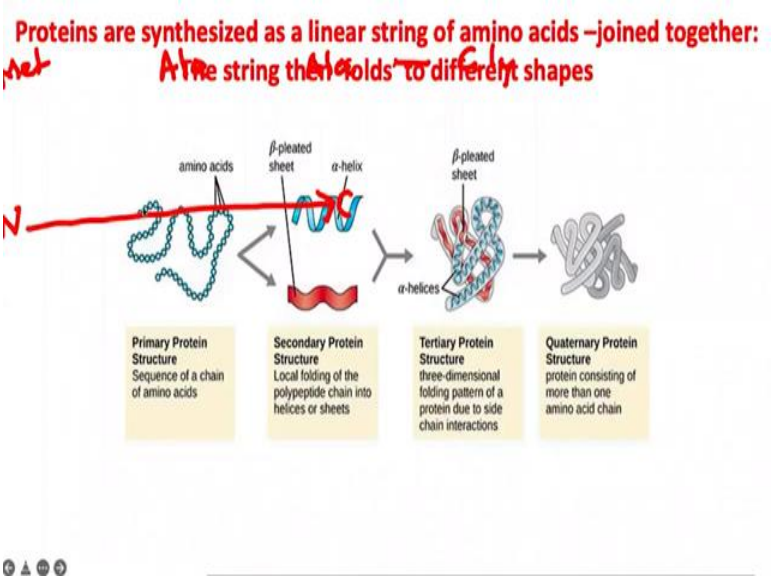

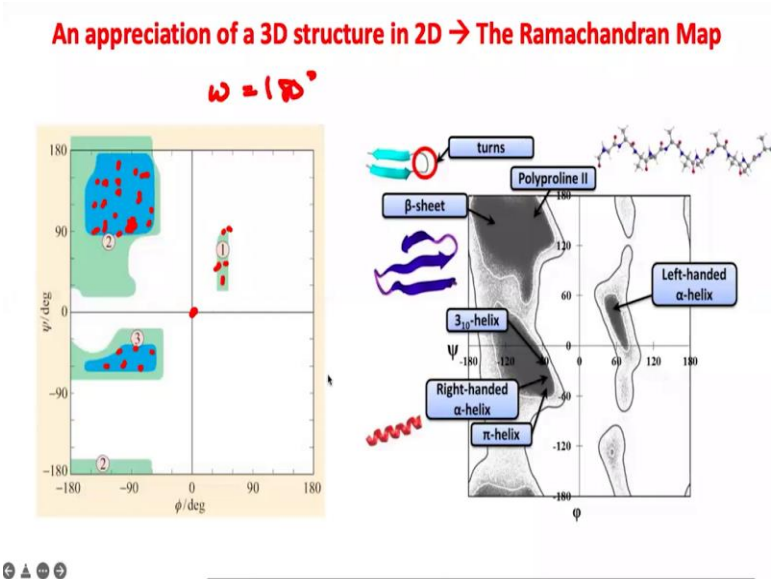

Let us say that this is a polypeptide chain going from N terminal to C terminal. This is alanine over here, which is the, let us say, there is a methionine before this. There is a methionine, there is alanine, let us say, there is another alanine and let us say there is a glycine, so it is just a sequence of amino acids, as shown over here, just a sequence of amino acids, like this 1, 2, 3, 4, 5, 6.

Now, all we are doing is for every amino acid pair, so for a single amino acid, ala, you take phi, you measure phi from the atomic structure, which you have solved. And just like one of the students said, let us assume that the angle you get is 0, then you measure phi. And again, let us

say, you get the angle, the dihedral angle which you measure is 0. All you do is you go to the Ramchandran map, which is over here. And you say 0, 0. So, you basically plot a dot over here.

Then you go to the next amino acid, let us say, the next amino acid is another alanine, but it is phi is minus 90 and psi is plus 90. So, you plot a dot over here and the next amino acid. So you keep on doing that. So, for any protein, when you keep on plotting the dots, as you grow from amino acid to amino acid using the structural model, you find that all these dots almost always are in the regions defined by the Ramachandran map. It is a restricted region and it does not go beyond these regions. And remember omega is always going to be 180 degrees. Is that clear?

Student: Yes, sir.

Professor: Think about this.

Student: Could you please go to the page where you showed the glycine Ramachandran map?

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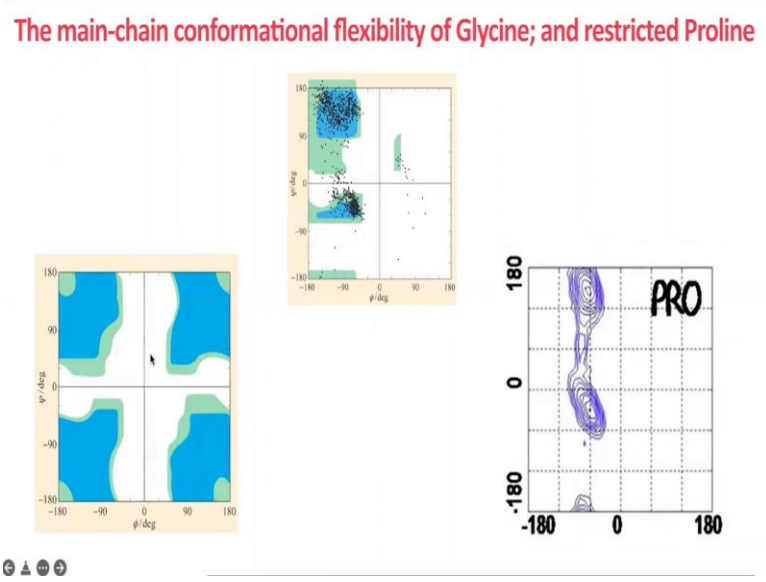

Professor: Yeah.

Student: Is there any significance of the symmetry in that about the, like y is equal to minus x line, there is a symmetry in this second and fourth quadrant.

Professor: So, remember, you are just looking at a two dimensional representation of what is basically a rotation around a bond. And the rotation around the bond is basically 360 degrees. So,

it is telling you that the white regions are not going to be energetically favorable. So, you are not going to have even a glycine at 0 phi and 0 psi.

Student: I got that part, but unlike all other graphs this is symmetric.

Professor: Because it is freely, it can freely rotate without any problems in phi-psi space, because the proton has a side chain does not restrict anything.

Student: Then why does it still have some limitations?

Professor: So, remember, look at the dark blue as more favorable, light green as possible, but unfavorable and the white as probably not.

Student: So, 0, 0 is like a fully eclipsed composition type.

Professor: Yes, the chances of getting 0, 0 in a protein structure is 0 effectively.

Student: Okay. Understood sir.