Introduction to Cell Biology
Professor Girish Ratnaparkhi
Professor Nagaraj Balasubramanian
Department of Biology
Indian Institute of Science Education and Research, Pune
Secondary structure of Proteins: Ramachandran Plot - Part 2

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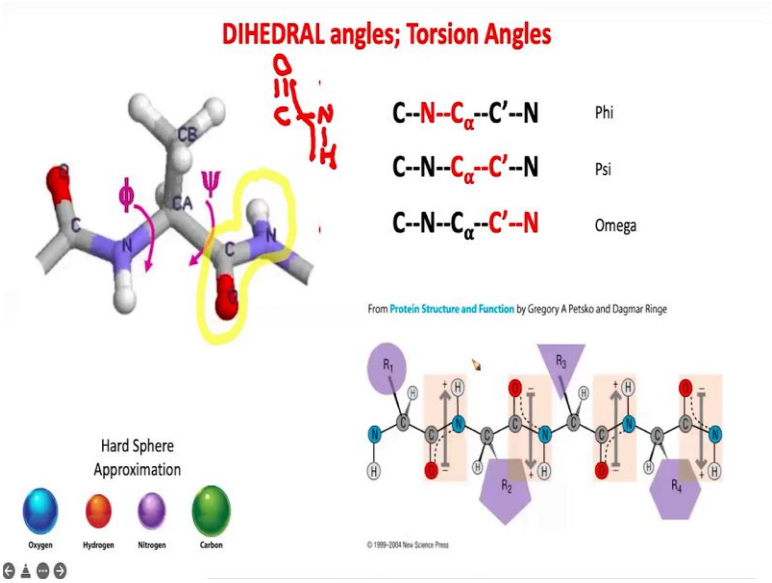

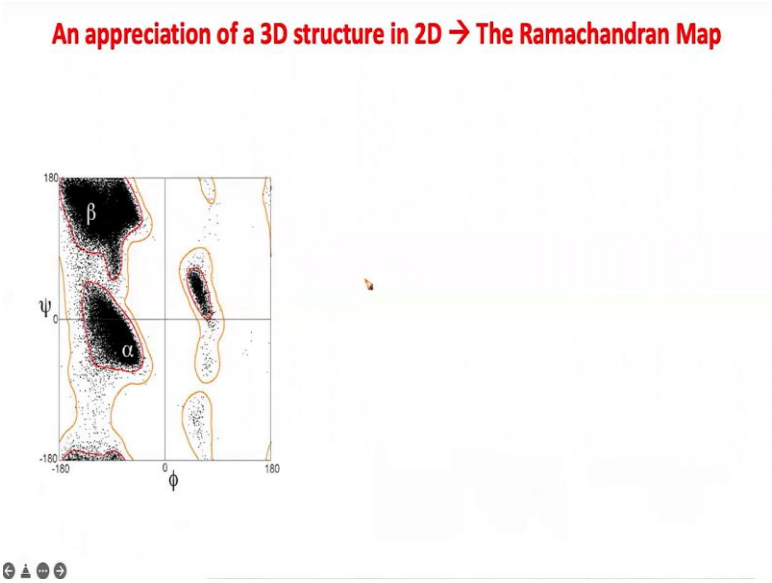

Student: Sir, could you just go with dihedral angles again, because you said that already you have four different, different carbon and oxygen atoms and you talk about their positions being

arranged in a certain way that is how you describe, whether this angle is going to be phi, whether this angle is going to be psi or whether this angle is going to be omega.

Professor: So, there are definitions of these angles which you need to understand four atoms in space. A triangle is, sorry, an angle is usually three atoms in space. So, you have to remember a dihedral or a torsion angle needs four atoms in space. And you can take coordinates of any structure and you can calculate phi and psi for them.

Student: So, sir like these four angles could be, these four atoms could we any random four atoms like in a continuous sequence in the polypeptide.

Professor: So, every each dot over here, each dot you see over here, for example, represents one peptide. So, basically, it is one unit one phi, one psi right next to each other. So, the next phi-psi will be plotted separately, next phi-psi would be plotted separately. So, each dot over here represents one amino acid is the way to look at it.

Student: And could we say that all those, all these 2D maps are basic of all those 3D structures we saw before like in the previous slides we saw the different 3D structures, could we say like that?

Professor: So, there are over 10,000 structures, let us say, in the protein databank. I do not have the correct number. There are probably many more. And this represents, this is a modern picture. It represents, God knows, 100,000, 200,000 atoms. There are lots of dots over here. So they represent all the structures solved till date in terms of their phi and psi. And it tells you that phi and psi cluster only around three major regions, the alpha region, the beta region, and this is the left handed helix region.

And the reason it turns out, you can model this in today's day and age we model it using energy calculations. But in the 1960s when GNR did it, he used plastic models and he used a hard sphere approximation. He gave a certain radius to each atom, which is shown over here, and he built physical models and then he turned these models around the torsion angles, because phi and psi were the only two things which could be rotated. Omega could not be changed, it was at 180 degree. And of course the side chains depending on how big or small they were, were also

important. But it turned out for secondary structure. The phi and psi were the main critical parameters, the rotation of the phi and psi.

Student: Sir, why omega was fixed at 180 degree?

Professor: Because the, this particular combination of atoms over here, here, I am going to show you is basically this group. This particular variant is basically partial double bond in character. And because of this partial double bond, the electrons freely roam between these four atoms, which makes it very rigid. And this partial double bond does not allow rotation around the C-N bond over here.

Student: Sir, what was the reason for the restrictions in that plot piece?

Professor: So, are you asking me why is there a lot of space over here?

Student: Yes, sir.

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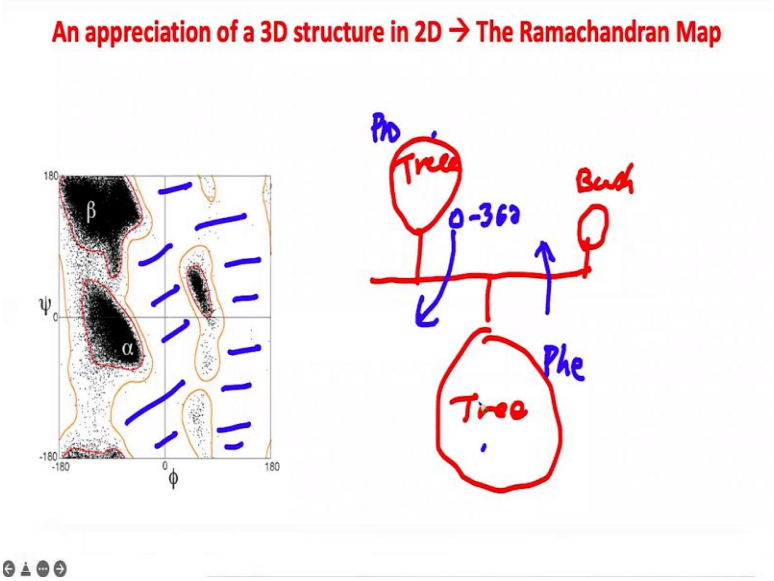

Professor: I will try and give you an idea or here. Let us just take, this as an analogy. So, let us say that this is a tree and this is another large tree. And let us put this as a bush, a small bush. Now, if you rotate this particular, consider this is the main chain of an amino acid, and if you rotate things around this main chain, this rotation and this rotation, at some point, this tree and this tree are going to come close to each other and they are going to hit each other.

Whenever they come to close each other, come close to each other, energy-wise they will repel each other. And in terms of physical space that they occupy, they will hit each other if you rotate this blue around from 0 to 360 degrees. Now, it turns out when Ramachandran modeled polypeptide structure using simple idea of large atoms, so remember if this is a phenylalanine residue, it is very large, and if you rotate it, and if this is let us say, a proline, this is going to come and hit the side chain. And whenever there is physical proximity or physical contact, this is energetically very unfavorable. So, all these regions over here are regions where the phi-psi angles are not possible because of clashes between the side chain.

Student: Excuse me, sir. In this map only does each dot represents a molecule with a certain psi value and a certain phi value?

Professor: No, each dot represents. So, for 100, let me try and explain this to you. It is good you are asking questions. I realize it takes time to get this concept.

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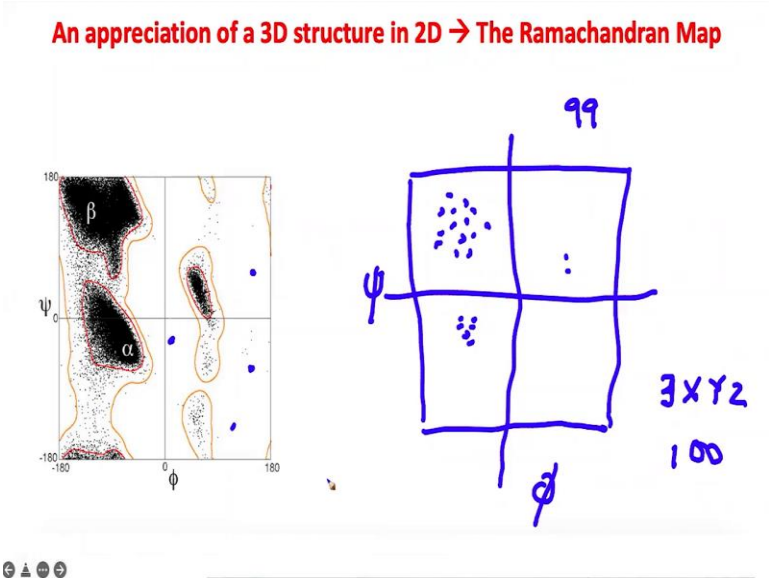

If this is a Ramachandran map, which you and I are drawing, let us say for one protein, which we take from the PDB, let us say it has a PDB ID of 3XYZ, we know that this protein has 100 amino acids in a linear sequence and it is folded into a state. The folded state has been captured by x-ray crystallography. And we have a file with coordinates in space, because it will have 99 unit, it will have 99 pairs of phi and psi. Is that clear enough?

Student: Yes, sir.

Student: Sir, even if we do have a 2D map of the proteins, we still need more information like what the nuclear, what the amino acid is and what the order it is in? So, what is the big deal of this Ramachandran map?

Professor: So, the big deal, so when initially it came out, it was a nice representation of proteins. So, it was nice, people commented on it. It was not a nature paper. It was published in journal of molecular biology. Then as we started getting more and more structures in the 70s, 80s, there came a time when every day there were about 10 to 20 structures deposited in the protein databank and this ramped up dramatically in the 90s and in 2000.

Now, the people who are managing the protein databank, they were overwhelmed with this large number of structures, and they realized that many of these structures were wrong, either the solution was wrong or there was something, somebody was basically not, some of these structures were falsified. That also happened in a small fraction of cases.

Now, it turned out if you take these, took these coordinates which were submitted by, let us say, a group in Harvard and you drew a Ramachandran plot and you got dots over here, you knew that the protein structure was wrong. It was as simple as that. So, it became very popular in the 80s and the 90s as a quick way of clearly defining whether the protein structure which was solved was correct or not. So, it became a quality control check, a phi-psi map. And that is one of the reasons it became extremely popular at the end of the last century. Does that answer your question?

Student: Yes, sir, it does. Makes a lot of sense.

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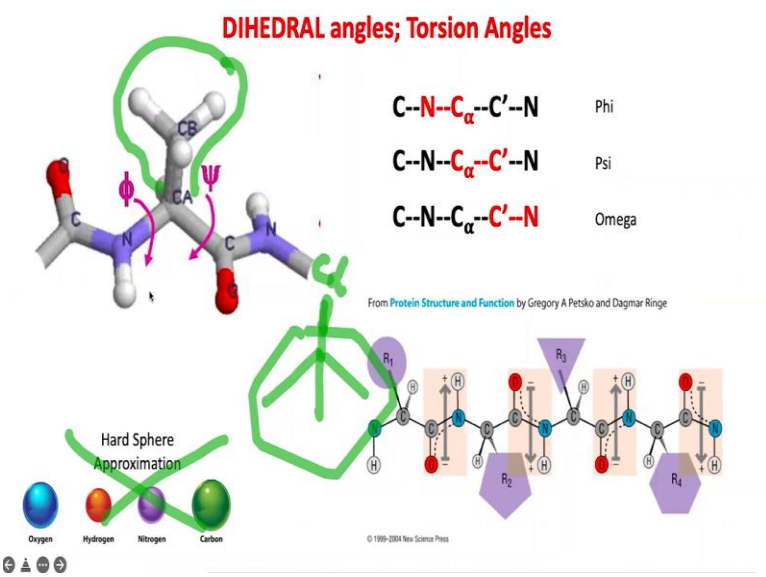

Student: Sir, can you show the hard sphere approximation slide.

Professor: So, the best way to appreciate the hard sphere approximation is to in fact look at this picture.

Student: Sir, I had a doubt. Sir, why is the size of oxygen greater than that of nitrogen?

Professor: I just copied this from somewhere. These are not the correct sizes. So, maybe I should very specifically say that these are not the correct sizes. This is just to give you an idea. Look at this. This is a CH3 group. Now, there will be a C alpha over here and let us just imagine a CH3 group over here also. So, when you rotate around CN, at some point this CH3 group and the CH3 group on top will come close to each other and this will not be allowed.

So, when you take the correct hard sphere approximations, physically these two side chains will knock into each other. And since this is, disallowed in space this will represent the blank regions on the phi-psi space.

Student: I have a doubt that is am I right in saying that the GN Ramachandran plot of different rather proteins will be different.

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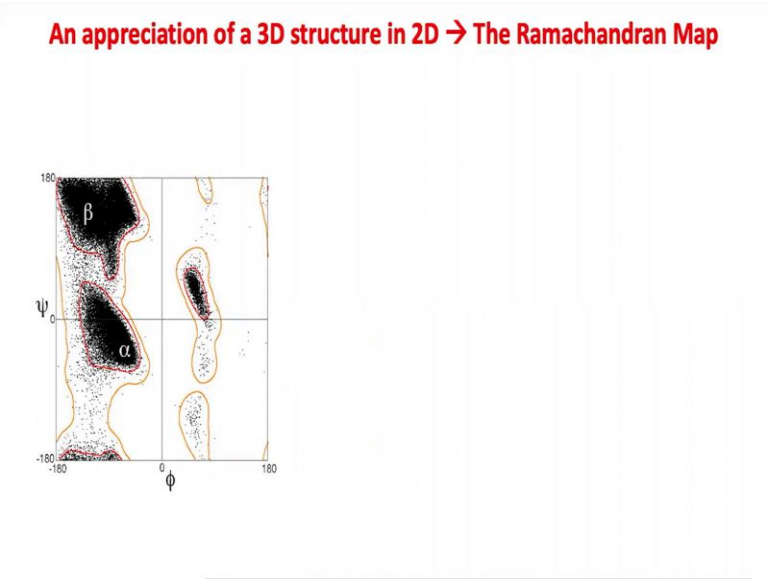

Professor: It will be different in the sense that it will have different dots. But what we have learnt from the…

Student: The distribution of the dots will be different.

Professor: Distribution of the dots will be different, but all of these structures will have alpha helices and beta sheets. So, they will all be crowding this area of space.

Student: For different proteins the graph will be same or different?

Professor: Well, the dots will be slightly different, but they will all crowd over here. It is like VT Station over here. So, you cannot look at a pattern of dots and say, oh, this must be this protein. That is not possible. But if you have a coordinates of atoms in space and you build a picture using ribbons or any of the different techniques you will be able to identify the protein based on the sequence and on the structure.

Student: Sir, if different amino acids land in beta region and alpha region how do we finally determine that which structure the protein is?

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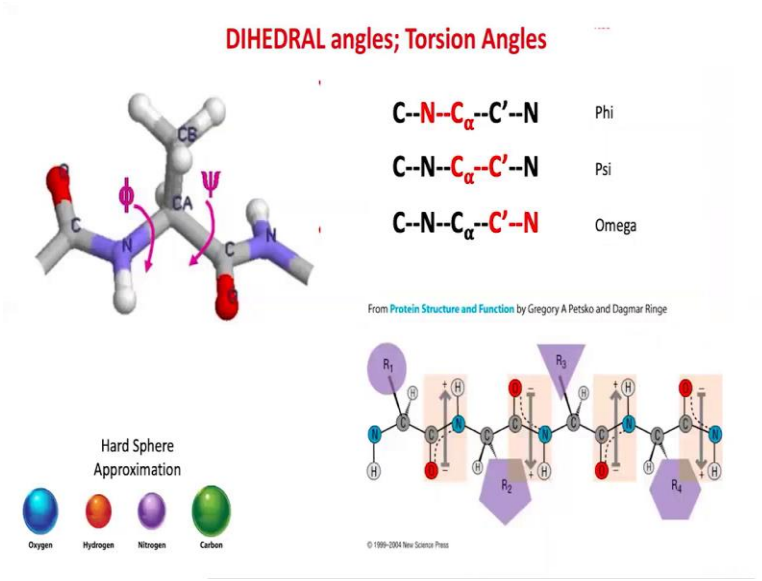

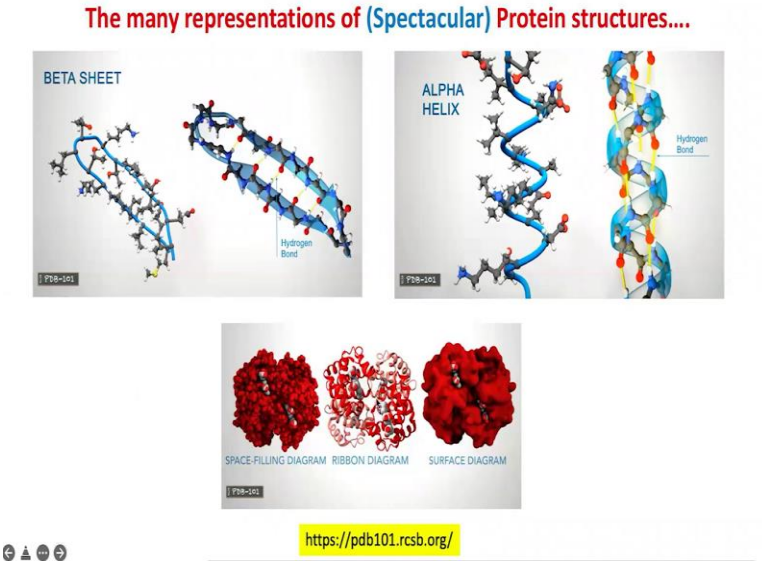

Professor: We just plot that. So, the solution of the structure comes from x-ray crystallography. And when we plot it, you will get structures like this. So, Ramachandran map comes downstream of a structure as of today. When Ramachandran first started making the map, he was building models. But what we are seeing are not models. They are actual atomic level protein structures.

Student: Sir, how do we measure psi and phi angles?

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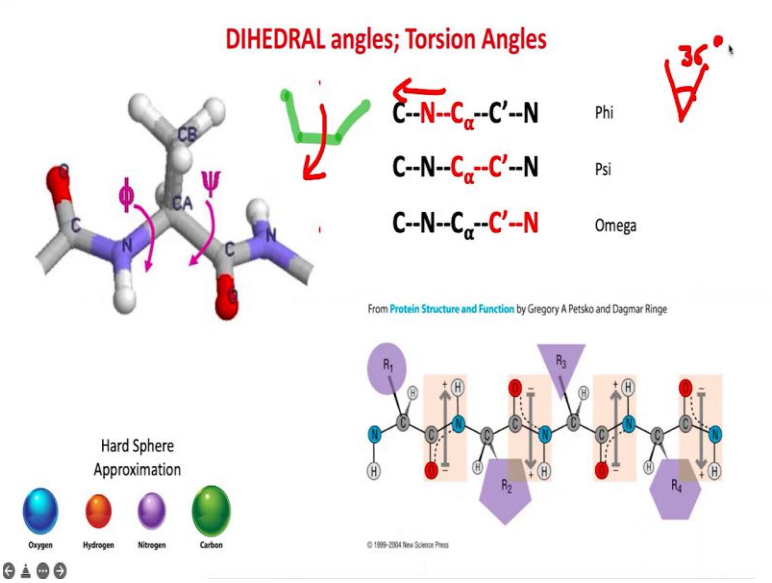

Professor: We measure psi and phi angles by looking at the coordinates of these four main chain atoms in space. It will give us four dots in space dot one, dot two, dot three, dot four. And then using these four dots, we will basically measure this as the phi angle or the psi angle. So, if I look at it from this direction, it will be something like this with the bond hidden over here and if this is 36 degrees that is the angle phi.