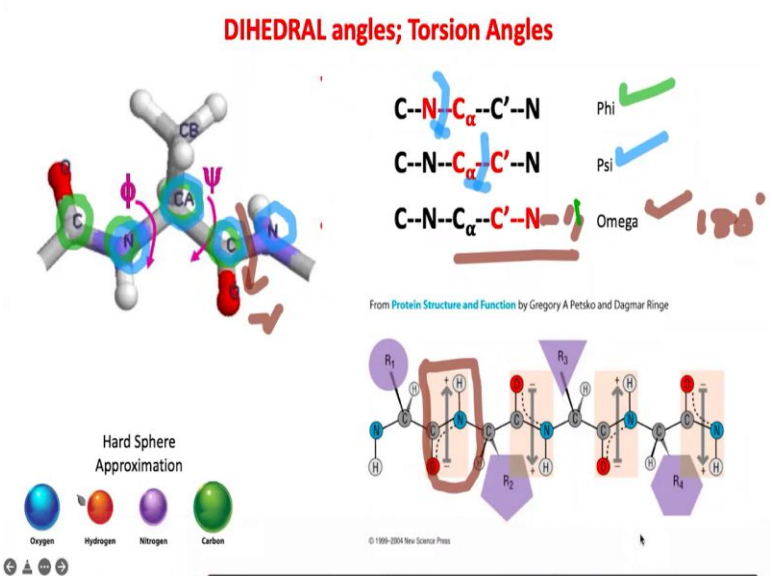
Introduction to Cell Biology
Professor Girish Ratnaparkhi
Professor Nagaraj Balasubramanian
Department of Biology
Indian Institute of Science Education and Research, Pune
Secondary structure of Proteins: Ramachandran Plot - Part 1

(Refer Slide Time: 00:15)



So, now, let us go on to the concept of what is the dihedral angle. Now, here you see a small stretch of a ball and stick representation. Now, this is another representation of a small stretch of a polypeptide. And again the main backbone is always the same C prime, N, C alpha, C prime, N. So, the backbone is always like this.

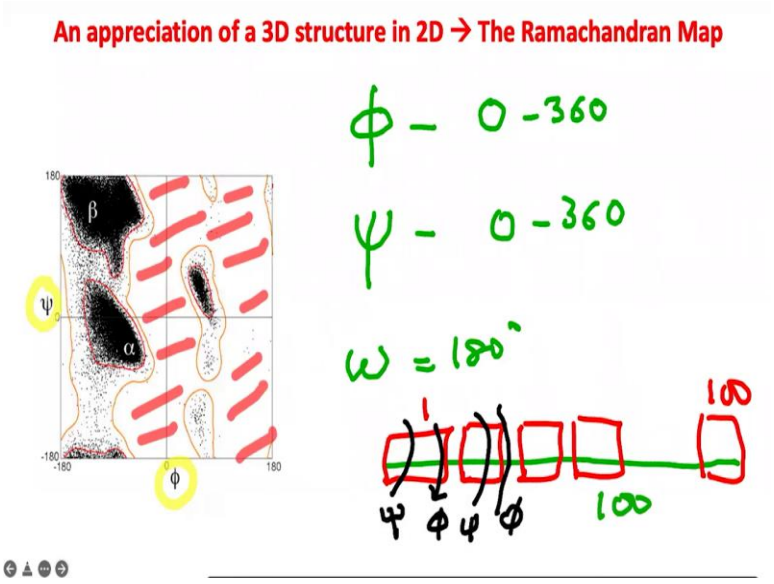
Now, over here these four atoms, now I am going to circle them, I want use green as a color, this atom, this atom, this atom, and this atom, these four atoms define the phi angle, which is C, N, C alpha, C prime. The psi angle, now I am going to change color and try sky blue, N, C alpha, C prime, N define the psi angle. So, we are saying four atoms are defining a dihedral angle or it is also called as a torsion angle with the angle phi between these two atoms, angle psi between these two atoms and the third angle is the omega angle and I will change color here again this is omega and omega is basically this angle.

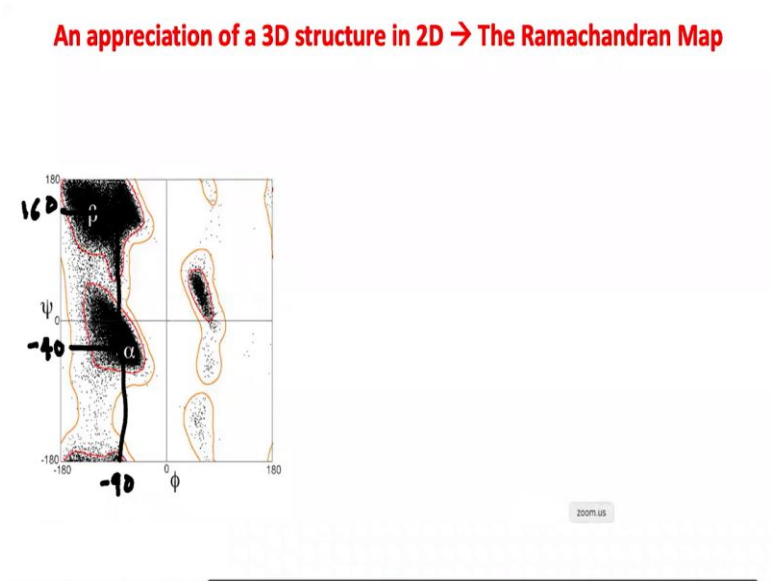
And again N, C alpha, C prime, N, C alpha, so these four atoms, this does not look like C alpha sorry, somehow this pen is not working very well, these four atoms define the omega angle. Now, the CO-NH group which is shown over here, which is repeated again and again across the

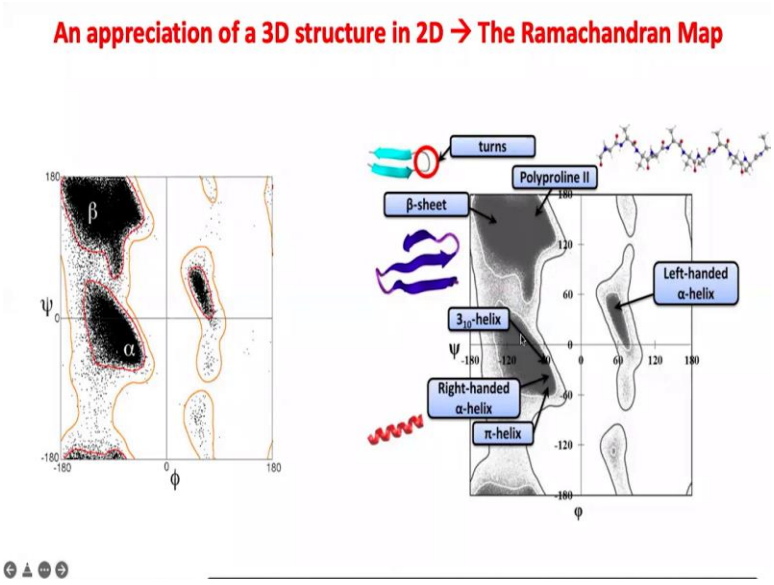
main chain, and this is the group shown over here has a very special geometry. It is a very flat plane. And that is because the CO-NH bond and I will talk about this, I will repeat this in the future, is a partial double bond, not allowing this particular angle to move.

So, this angle pretty much always is at 180 degrees. Whereas, the phi angle and the psi angle, these are both torsion angles, have the ability to rotate across the central bond by about 360 degrees. Now, remember the concept of phi, psi, omega and their definitions and remember that one can approximate each of these atoms which we are talking about carbon, nitrogen, hydrogen, oxygen, as hard spheres. And the sphere basically dictates the electron cloud around these atoms. This is called as a hard sphere approximation and I will come to why this is very important.

(Refer Slide Time: 03:21)







So, let us go to the next slide and see why this is important. Now, many years ago, Indian scientists called GN Ramachandran tried to build models of proteins, because this was in the 1960s and 70s after DNA structure was solved, modeling of structures was very popular and crystallography for proteins had actually taken off much before the crystallography for DNA. So there were a few structures which were solved out there. I think myoglobin was the first structure.

And, but the question was that was just one protein. There were hundreds and thousands of proteins which had been purified. Could we predict the structure of these proteins based on the linear sequence of the amino acids and what GNR along with a few of his students did, is that he

said, let us start making models of proteins. He was particularly interested in the protein collagen, which he was working with. But generically, he and many others were looking for the basic rules by which you could predict the folded state of a protein from just the linear sequence of amino acids.

So, what Ramachandran did is he took, sorry about that, he basically took, made models and he took, in these models, he kept on changing. Remember, these were models of the kind you saw Watson and Crick make. These were large scale models which basically with each atom being as large as, let us say, the palm of your hand, and you could put them together knowing the length of the bonds, because Pauling had already defined the alpha helix and also the length of the bonds and you could try and build structures in space.

And the phi and psi angles are very well defined. And as I have already told you, omega always is pretty much 180 degrees. Omega does not really change in most of the structures. So, if you varied phi and psi through, from a range of let us say 0 to 360 degrees, you could basically visualize these models like LEGO-models in three dimensional space.

And what he basically found is that if you now take this three dimensional representation, ignore omega which as I said is always at 180, and just keep on varying phi and psi, you can convert a three dimensional information of a folded state of a protein into two dimensional information, where you plot in the x, y axis, phi shown over here, sorry, phi shown over here, and psi shown over here with phi on the x-axis, psi on the y-axis and the range being from minus 180 degree to plus 180 degree, which is basically a 360 degree rotation along the torsion angle.

And this is not Ramachandran's data, this is the data which is basically about two to three years old. What you see over here is when we take all the hundreds and thousands of structures, protein structures in the protein databank and we, let us say there is a structure of an amino acid, which is let us say 100 amino acids long, and let us say, for this 100 amino acids, long structure, remember, there is a peptide unit along, which is a repeating unit along the structure, it goes from 1 all the way to 100. And for each peptide unit, there is a phi angle, sorry, psi angle, and there is a phi angle, there is a psi angle, there is a phi angle.

And if you plot for each unit, phi versus psi torsion angle, you get these dots in a two dimensional representation. And what Ramachandran basically found that these dots did not

occupy all the space available, all the three dimensional space available. Remember, this is a two dimensional representation of a three dimensional object. And he found that many, many places, and I am going to use red as a color, all these spaces were not occupied at all.

So, phi and psi could never in a combination be any of these angles at all. Most of the phi-psi plots, the angles, were in two major areas, which is let us call this, let us I am being a little weird over here, but let us call this North America and let us call this South America. So, a large number of dots were in North America, and a large number of dots were in South America, there was a little bit in Antarctica, and let us say a little bit in Asia, but the rest of the plot was completely empty.

And when he looked closely at these dots, he realized that all alpha helices had torsion angles roughly over here. Let us try and see what this torsion angle is. So, for example, the torsion angle over here is, let us say, minus 90, and over here it is minus 40. So, a phi of minus 90 and a psi of minus 90, if you had a torsion angle like this, you were pretty much in alpha helix. So, the location of that particular peptide, main chain peptide was in a alpha helix.

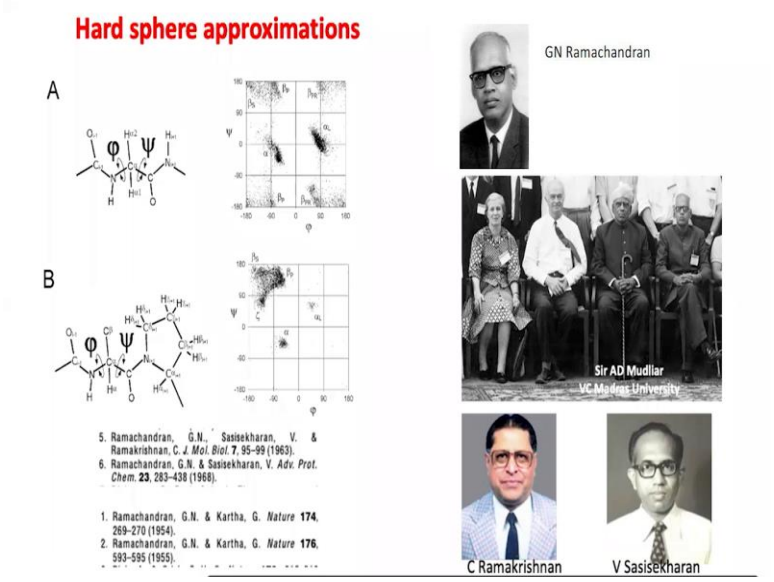
If you had, let us say, a psi of 160 and a phi of minus 90, it was pretty much in a secondary structure which was a beta sheet. And there were very few dots on the right hand side of the grid. And this basically turned out to be a right handed alpha helix. So, it is a alpha helix which is not the same handedness as this alpha helix. So, GN Ramachandran basically found out a way to do the following.

He used a hard sphere approximation, he built models, he found a simple way to represent in two dimensions, three dimensional models, he used the phi-psi angles to plot and he found to his surprise that these phi-psi angles were restricted in space, they could not occupy any region, they could occupy only specific regions. And when you extrapolate back to three dimensions, you realize that this meant that when you turn torsion angles around each other, there were many places where they would not, which would not be allowed. And why were they not allowed?

They were not allowed because while turning these torsion angles the R groups of the side chains were coming too close to each other. And if they came and knocked against each other, those particular angles were not allowed and that is why a huge amount of this tortion space is completely empty.

So, this is the representation we have now. We know that the left handed helix basically comes over here, beta sheets have phi-psi angles over here, right handed alpha helix is pretty much over here, so are pi helices, 3 10 helices, these are all different kinds of helices.

(Refer Slide Time: 10:28)



So, we now have a map as defined by GN Ramachandran, Sasisekharan and Ramakrishnan these are the three scientists who worked in Indian Institute of Science for this definition. And this became a very famous plot using very simple models and hard sphere approximations and just turning in models torsion angles around each other and realizing that the amount of rotational space available in a three dimensional model was restricted and these restrictions were specific torsion angles of phi and psi and they then could be related to secondary structure elements in the protein structure.

Shown here is a very famous picture. This was the visit of Sir Linus Pauling shown over here and Dorothy Hodgkin she is from the United Kingdom and you will hear a lot about her. She was also a crystallographer and here is GNR. This is a crystallography meeting held in Chennai, Madras, God knows, maybe 60 years ago.

And over here are the famous papers in bottom which are the 5 and 6 for example are the two papers which defined rotation around phi-psi space and the development of a simple way which is the Ramachandran map and one and two are also very famous papers by GN Ramachandran, where he basically solved a structure of collagen, modeled the structure of collagen would be a

better idea, because collagen like DNA is basically a fiber and they use fiber diffraction to solve these structures.

These are very exciting times for Indian biology because Alex Rich who you have heard of as part of the RNA club and also somebody who was a very famous crystallographer was competing with GN Ramachandran for the correct structure of collagen and some of the, who was right arguments are still going on in this century about, who really got the correct structure for collagen, was it Alex Rich or was it GN Ramachandran.