Circular Dichroism and Mossbauer Spectroscopy for Chemists

Prof. Arnab Dutta
Department of Chemistry
Indian Institute of Technology – Bombay

Lecture – 27
Examples of Circular Dichroism - III

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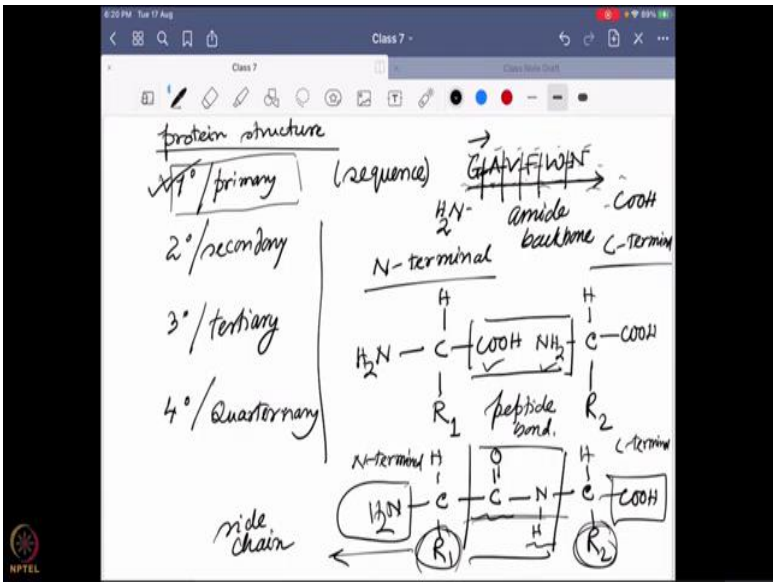

We are going to do the following, we are going to look into the difference of protein structure and how CD spectra can give me the idea of a protein structure? So, before I go into the details of the protein structure, let us have an idea what are the different variations or different levels of protein structure I can have? So, protein structure can have four different layers.

And they are written as 1 degree, 2 degree, 3 degree, 4 degree or also known as primary, secondary, tertiary and at the end quaternary structure. So, these are the different structures present. So, what do I mean by 1 degree of primary? 1 degree or primary structure says what is the sequence? We have already gone through there is a 20 or 20 odd natural occurring amino acid, we have gone through there three letter word and one letter word description.

And over there what are those amino acids? So, for an example I am saying I have a sequence of G A V F W N so these are the different amino acids present. So, what is G? G for glycine, A for alanine, V for valine, F for phenylalanine, W for tryptophan, N for asparagine so these are the different amino acids are present. So, what is the sequence? That is going to give you the primary structure. And over there what is that primary structure basically means?

Basically, means that you have one amino acid and generally when we write that sequence, we write it such a way that this is going to have the free amine group at the end and this is going to be the free carboxylic acid group at the end. So, this is known as N-terminal and this is known as the C-terminal. Anything you are drawing automatically from left to right it is the N-terminal to C-terminal that is how do we draw.

So, what do I mean by free amine group, free carboxylic acid group or N and C-terminal respectively? So, if I want to draw an amino acid how do I generally draw? Particularly like this I am not drawing it in the original four coordination just drawing everything in this line structure. So, this is how one amino acid looks like. Now, one amino acid if it reacts with another one, so say it is R_1 react with another amino acid, what is going to happen?

This carboxylic and amine group is going to react, acid group and basic group they actually react and what they do? They form this amide bonds, the rest of the things remains the same. Now, over here you can see that one amino acid uses carboxylic acid group, one amino acid uses amine group. So that means you are going to have one free amine and one free carboxylic acid group.

So, this is going to be the N-terminal and this is going to be the C-terminal and this is known as the amide bond or the peptide bond. And when I draw such sequence of G A V W N, whatever so in between each of them you have an amide bond. each of them you have an amide bond So, over there is sequence of 1, 2, 3, 4, 5, 6 amino acid you are going to have five different amino acid, six different amino acid connected through five different amide bonds.

And this R_1, R_2 the side chains will be hanging and this particular amide will create the backbone of this chain and that is known as the amide backbone and this hanging R_1, R_2 are known as the side chain. So, in a primary or 1° sequence we actually just look into the actual nature of the amino acids which are actually forming that one. We are not looking how the overall structure looks like? First, we look into the sequence so that is the primary structure.

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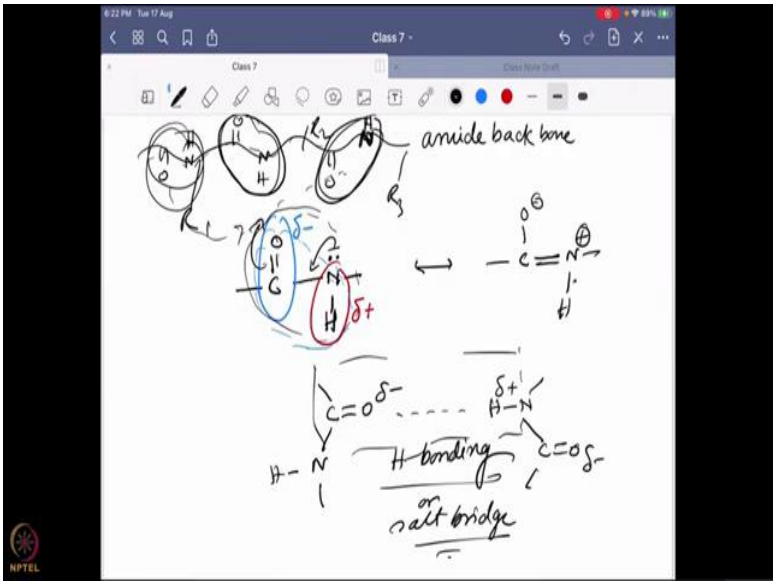

Now, once we have the primary structure then we look how the overall structure of the molecule looks like? And over here the molecule can have some secondary interaction coming in. So, what can happen? You have a sequence of different amino acids so this is the amide backbone I am drawing as a cartoon. So, this is there is a -CO-NH- bond over there then there is another -CO-NH- bond over there, there is another CONH bond over here so on and so forth.

So, you have different amide bonds present and that is forming the amide backbone and the other groups R_1, R_2, R_3 are hanging around. And now, these amide backbones are actually polar because you have a C double bond ONH group(-HN-C=O) and over there this NH bond you can imagine that it should have a delta positive charge whereas this carbonyl group will have a delta negative charge.

Because they these are all going to interact among them and form a planar structure so the different uh change in the electronic distribution. So, this lone pair of this nitrogen can come over there and create another form of the molecule so these are possible. So, all together you are going to have a planar structure around the amide bond and not only that this carbon will have a negative charge, a hydrogen should have the a little bit of positive charge.

And once you have this negative and positive charge next to each other there is a possibility that a carbonyl group coming from one particular end can have some interaction and in each part of another molecule and this will create a very nice hydrogen bonding or ionic interaction network. So, this kind of network can be possible and now, in the three dimensional how these interactions are happening?

Is it happening to two different ends of the amide points or the same amide bond? Is actually moving out a little bit and creating an interaction with this; amide backbone of the same chain or different chain.

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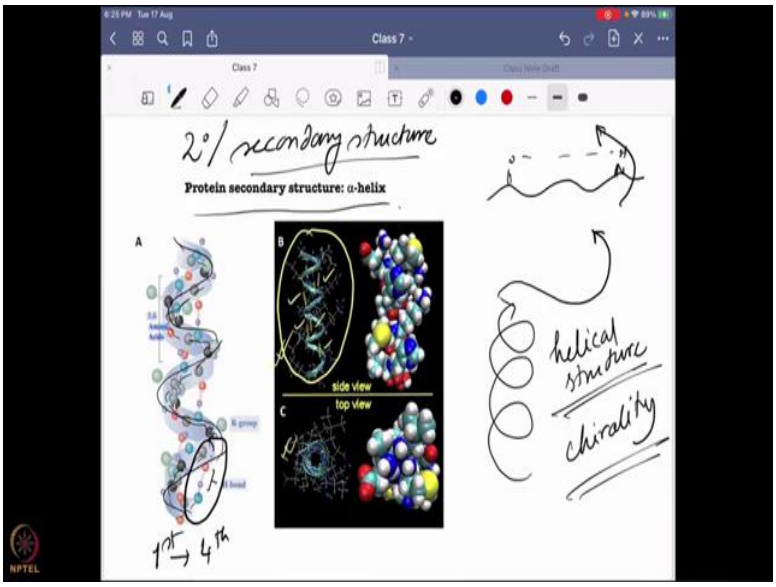

Depending on that we can have two primary orientation of interaction which are known as the 2° or secondary structure. So, what are those? So, let me find out what are those? So, the first one we can have is known as the alpha helix. So over here the alpha helix you can see this is the amino acid backbone over here. And over here this is there you can see a CONH interaction is happening.

And it happens such a way that the first amide bond interacts with the fourth one and by that it creates a turn. Because if you have a linear change; you cannot have this bond to have an interaction with that that is too far. So, it has to bend a little bit and for bending when it is doing it is creating a turn. So, this is the turn it is happening over there. And by that it actually ends up creating this kind of helical structure which can be seen very interestingly over here. which can see very interestingly over here

So, this yellow dotted lines over here these are the hydrogen bonding first to fourth then these two size is the first to fourth, these two this first to fourth that means if you take one as a number 1, the fourth one from that is going to create a hydrogen bonding. okay So, by that you can count the numbers and you can see it actually creates this nice helical interaction that is how it looks from the side view and that is how it can look from the top?

So, you can see and by that you can imagine it is actually creating a helical structure. And once you create a helical structure what does it mean that it means it can have a

chirality. So this chirality will be created by the helical structure in the secondary one. Not only because all the amino acid except Lysine are chiral. But because of the overall secondary structure has a directionality a helicity present over there so that is what is coming for alpha helix.

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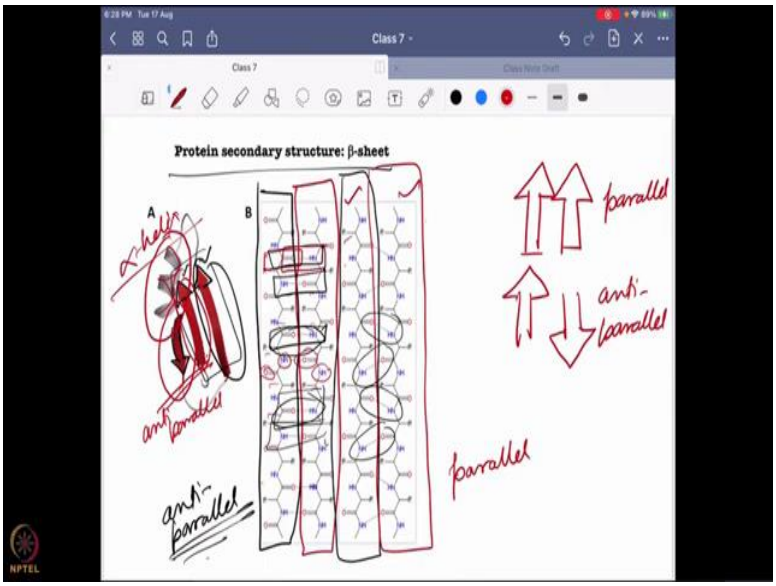

Now, the second one is possible is known as beta sheet. So, how is beta sheet looks like? So this is known as the beta sheet and that can have two different orientations. So over in alpha helix you can see that the same chain is interacting with on it is own, first to fourth. But in beta sheet two different sides of the chain interacts and it can interact you can see this is one particular chain and this is another particular chain.

And they are interacting in such a way that the amide bond of one and the NH bond of the one is interacting and they form these bonds in this particular way nicely. However over here you can see the orientation of this two chains are exactly opposite to each other, over here you can see that NH is in the middle, O is outside and over here you can see O is inside, NH is outside so they are actually oppositely oriented.

So that is why this is known as an anti-parallel chain. If some of you having issue to look into that look into the next one this is also two different chains. One is over here another is over here but over here you see the both the chains look exactly same they are similarly oriented this is known as parallel chain. But what is the problem with parallel chain? In the anti-parallel you can see the NH and carbonyl are actually next to each other.

And that is why they can form a much better hydrogen bonding network whereas in parallel chain they are a little bit skewed because if you want to put parallel the NH and CO from the next two chains cannot be in the same direction. So that is why you have to create a skewed bond. Whereas if you put it anti-parallel the NH and CO are perfectly positioned for hydrogen bonding network and that is what is actually happening.

And in a structure how it is actually shown over here? You can see there are these are shown as lines over here and you can see there is an arrow shown over here. So, this arrow is shown from there it is showing that which is the direction of the chain. Over here you can see those two arrows are in the same direction that means these two are going to create a parallel chain same direction.

Whereas this one and this one are opposite in direction that means these two are going to create an anti-parallel chain. So, just look into the actual structure of the protein and look into their directionality same direction parallel if it is opposite direction anti-parallel. And this one over here you can see this helical thing this is the alpha helix. So, these are the two primary sections beta sheets parallel, anti-parallel and alpha helix.

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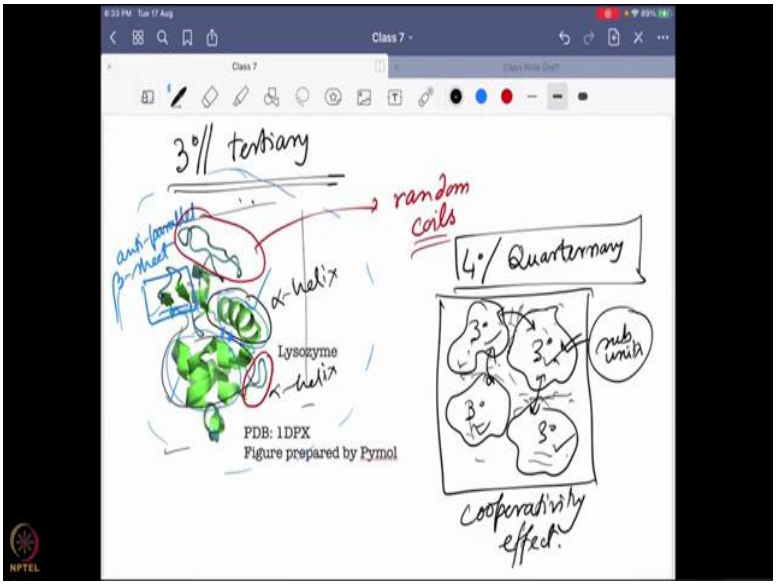

And now if I look into the actual protein structure it comes to a 3° structure or tertiary structure. Because a protein is not only having alpha helix and beta sheet but it can be a combination of a lot of things. So, if I give you an example of that so this is a protein name as Lysozyme. So over here in this protein you can see there is alpha helix present there is alpha helix present and there is also beta sheets present over here you can see over here and they are anti-parallel.

So, during the formation of the overall protein structure this alpha helix the beta sheets they actually interact among them through this side chains. okay So alpha helix, beta chain you can imagine they are the backbone amide interaction. But you also have the side chains the R_1, R_2 hanging around they also start having some interactions. And with respect to that you have an optimized orientation of the protein with respect to alpha helix and beta sheets and this full system is known as the tertiary structure.

So primary structure is only the sequence, secondary structure is only from the backbone you are getting alpha helix or beta sheet and if alpha helix and beta sheets are oriented in a particular conformation that is known as the 3° or tertiary. And over here very interestingly you can see there are some particular portions which are actually floating around without particular alpha helix and beta sheets those are known as random coils.

That means they are actually having some interaction among their backbone but they are not really well defined as alpha helix or beta sheets and that are also there. And they are also very important because they actually create the turns and connect the alpha helix and beta sheet. Because if everything is alpha helix and beta sheet; you are going to create a polymer without much of flexibility.

But as you know protein are much more flexible that means you need some portion which does not have very robust structure. Where the structure can be malleable into different forms and that is why this random call structures are also very critical. So that is the tertiary structure is known as. Now, the 4° or quaternary structure, what is that? So generally, 3° structure is actually going to give you one particular part of the protein which is very much stable on it is own.

But in some proteins very importantly not all proteins but in some proteins you can find there are different section of such globules of 3° structure. These are all 3° of tertiary structure and not only that each of this 3° structure have some interaction and between them. There are some interaction between this tertiary structure, tertiary structure on the interfaces.

And all together one part of the 3° structure flexibility or regulatory function affects all the others. So they have a closed connection through bonding with one 3° structure to the other 3° structure. If you find such structures we say it is a 4° or quaternary structure. And we call each of them sub units so there are different subunits onto each and each of them

actually have some effect on each other, some cooperativity effect you can say.

And once you have the cooperativity effect term comes in I know what comes to your mind when you talk about protein and it is true it is the haemoglobin.

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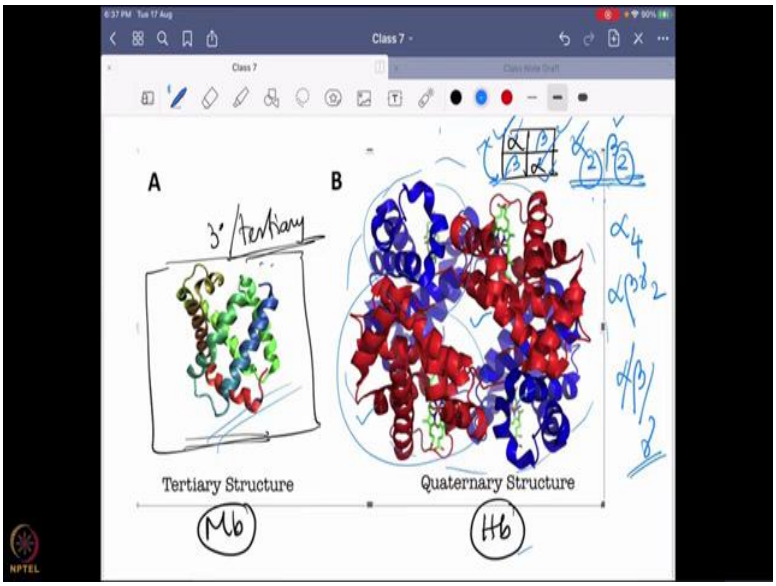

So over here I am going to show you the structure of haemoglobin and myoglobin which you already have seen. But now, today I am going to show you with respect to the tertiary and quaternary structure. So over here on the left hand side this tertiary structure what I wrote in as, it is the structure of the myoglobin and this is the structure of haemoglobin. Now, in myoglobin there is only one particular unit this is the 3° of tertiary unit.

Nothing else other than that and one myoglobin unit is not affecting other so, it is this functional units on it is own it does not require anything, no cooperativity effect. However, in haemoglobin as you learned earlier there are four different units and they can be defined in the following way. There are four different units 2α (2 alpha), 2β (2 beta) so it is an α_2, β_2 which is known as hetero-tetramer.

Because not all the 3° structure is same there are 2α, 2β and they all come together. So, these two red one is are same and two blue ones are same. They come together to create the overall structure of the haemoglobin and that is why it is known as a quaternary structure. Because this red one or the blue one on it is own already have a 3° structure. But it expands it is overall structure complexity little bit more and creates a subunit, sub unit interaction.

So that one sub units change structural change affects the other. So, they are not independent. Now they have a

cooperativity effect and that is why they known as the quaternary structure. okay So that is how it actually comes into the picture, any questions up to this point? Primary, secondary, tertiary, quaternary structure of proteins and what is that effect? "Professor - student conversation starts" Sir, what is the α_2, β_2 here? Ah α_2, β_2.

So, this is actually each of the sub units actually named. So, alpha is kind of like from the mathematical point of view if you say like the first term it is alpha then the next time beta, next term gamma, next term delta. So, similarly when people actually try to find out the structure of haemoglobin they found there are two different units. Which actually creates this overall four unit structure, two units are same and the other two units are different.

So, to name them you can write it as like A_2, B_2 similarly, you can write it as α_2, β_2. So α_2, β_2 is how it is historically named so that is why you are still following it α_2, β_2. okay So, two of them are alpha sub unit, two of them are beta sub units their sequence their overall numbers of alpha helix and beta sheet their orientation their overall 3° interactions are different for each of the alpha with respect to a beta.

The alphas are same the betas are same but in between alpha and beta they are not the same. So, when we say α_2, β_2 which should read in the following way there are four units, two plus two, four. And two of them are alpha, two of them are beta. okay And now say if I want to say it is an α_4, I would say it is a homo-tetrameric like that means there are four units affecting each other but all four are same.

You can have an α, β, γ_2 but that means there are four units, two of them are alpha and beta and two of them are gamma. And when I say alpha, beta, gamma, delta all these things what I mean to say? Is their primary structure, secondary structure the 3° structure are different, okay if all the things are same only then I say they are having the same overall tertiary structure.

Does it answer your question? Yes sir. Okay okay Any more question? So, if not. "Professor - student conversation Ends".
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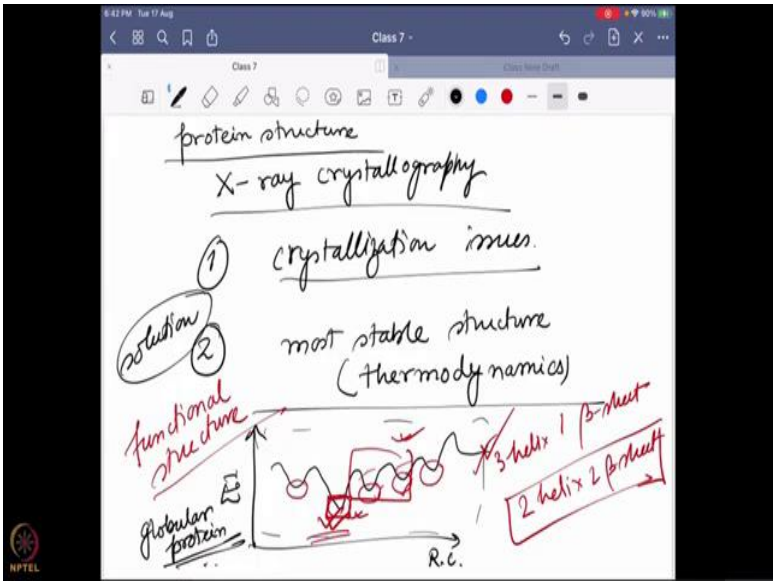

Now, we moved into how we should look into the protein structure? How many different ways I can find out what is the structure of the protein? So, whom we should call? "Professor - student conversation starts" So Raj deep, you are having a good connection today so far? um Sir, I am currently not in my home and I am in a cafe right now sir. yeah okay okay So, can you please tell me what are the different ways you can find out the structure of a protein any spectroscopic experiment?

I think x-ray crystallography is one of the main things okay and for others um I think CD can be a way but I do not I am not sure. okay Thank you. "Professor - student conversation Ends". So, x-ray crystallography is totally correct is the structure and it is one of the ultimate ah spectroscopy that we actually use to find out what is the overall structure? What is the overall orientation of a protein in 3D? But there are two problems.

First problem not all proteins can be crystallized very easily. So, if it is that easy we should not be doing also different many experiments because crystallization of a protein are very critical because not all proteins are found in a way that it can be crystallized very easily. Because some of them are actually water soluble and water soluble proteins are very tricky to crystalize because they absorb a lot of things.

Some of them are fat soluble they are relatively easy but most of our proteins which are actually we want to know more they are transmembrane. That means some of them are hydrophobic some of the part and some of the part is hydrophilic. So, they are very much challenging to crystallize it so that is one of the point, we cannot crystallize every one of them.

And second and one more fundamental thing is the following that when we crystallize a protein what we are actually seeing? We are seeing the most stable structure of the protein. That means I am staying with respect to the thermodynamics, thermodynamically the most stable structure I am seeing. And as we know if I draw the energy diagram of a protein with respect to reaction coordinate, reaction coordinates means if I am changing the structure a little bit.

Protein structures are actually having energy diagrams something like this and when we crystallize, so that means this are actually the troughs where we actually stabilize the protein. When you crystallize a protein we are going to get the most stable structure. However, activity wise that may not be the most active structure of the protein itself, it might be doing it is activity in this particular scale and we want the structure over here.

But we cannot get it because whenever I am trying to get crystallized it automatically goes to the most stable structure. Is thermodynamically driven I cannot do that I cannot make it always stable in a thermodynamically a little bit higher energy state where it is probably doing it is reactivity. So, for example, over here it probably have 3 helix and 1 beta sheet however, the most stable structure probably have 2 helix, 2 beta sheet.

So, if I get this particular structure I may not have the idea what is the actual structure? Who is the more functional one? So that is why the functional structure might be different which is also known in more colloquial term, the solution structure. In the solution the protein structure may have different orientations and we might not get the real picture if I only looking into the x-ray crystallography.

And this particular phenomenon where protein can be present in different orientations which are separated by a very limited amount of energy is known as the Globular protein structure. And that is one of the unique things about protein because that is very flexible. So that means it can do multiple functions with the same protein structure. So, you do not have to do the evolution multiple times, you have one protein which can perform three different functions.

However, in the thermodynamically probably one particular structure is probably more stable. So, you can get the structure but you might be missing very unique points how it actually behaves?