Experimental Biochemistry
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Lecture - 22
Protein Folding and Denaturation Summary

Welcome to the end of week 4. So, in this week we learned about Protein Folding and Denaturation.

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In this week we learned about the basics of protein structure, we learned about different the primary secondary and tertiary structures of protein. We learned how these different forms fold together to give a folded tertiary structure of a protein which is basically a folded shape of a protein. And, we also learned that this particular shape is very important for it is function.

And finally, we talked about the experimental technique where we denatured a protein and that gave us a lot of information about how proteins fold.

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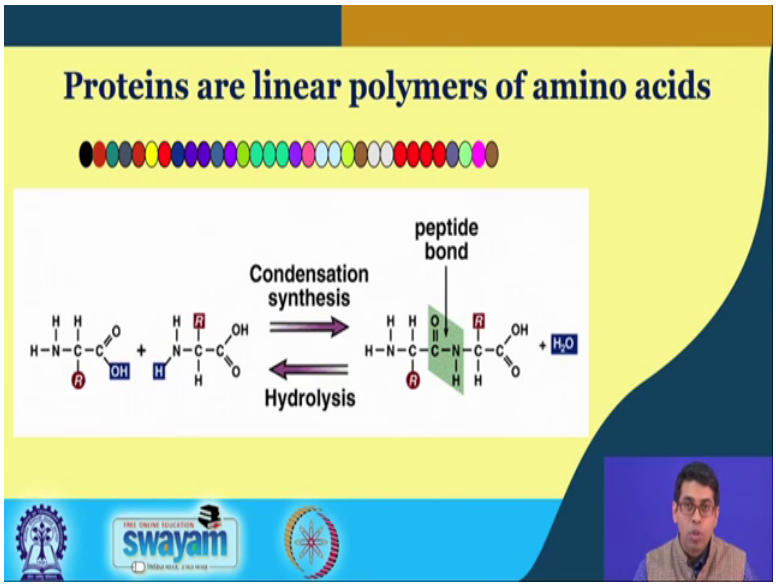

So, proteins are linear polymers made of amino acids. And, this is the basic structure of an amino acid where this unit has 2 functional groups; one is the amino group and the other one is the acidic the carboxylic group, it has also hydrogen atom and this r is the side chain. So, there are 20 amino acids in a natural protein.

And, these R group is the one that is different between these 20 amino acids. The linear polymer of amino acids is formed by a condensation reaction, where the carboxylic group of one ameno amino acid reacts with the immune group amino group of another amino acid, resulting in the formation of a peptide bond. So, these CO, NH group this is the peptide bond.

Now, this is a dipeptide this free carboxylic group is free to react with the NH 2 group of another amino acid and that will result in the formation of a tripeptide. So, that ways these peptide can keep on increasing in length resulting in a long linear protein, but that is just the chemical structure of the protein ok. This protein this linear molecule spontaneously falls into a tertiary structure, resulting in a particular shape of that protein.

And, this particular shape of the protein is very important for it is function. For example, enzymes have a particular shape so; enzymes are natural catalysts which can speed up reactions to a very high degree. And, the capability of catalyzing reactions depends on the arrangement of the functional groups in it is active side. And, this active side is maintained in a particular shape by the tertiary structure of that enzyme.

So, the tertiary structure of protein is very important.

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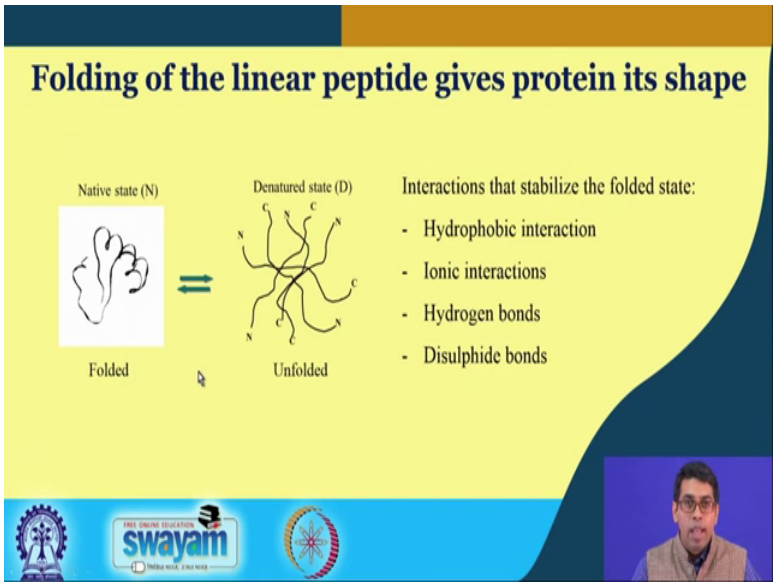

We also learned about certain terms that are very useful in studying how proteins fold. So, we learned about the native state or the folded state of a protein. These 2 terms are used interchangeably and they mean essentially the same thing which is the final folded structure of a protein. This is the form in which the protein is functional. The other state of a protein is the denatured state or the unfolded state. So, in this state, the protein exists as a linear or some random structure which is very different from this folded structure.

So, that is why you see that several different chains have been drawn here just to show that many different forms are possible in the unfolded or the denatured state. So, this is like up unraveled ball of wool. So, here the ball of wool is tightly shaped and here it is unraveled. So, that you can have any structure which is not very compact not very tight and also this one is non-functional.

So, we also learned that there are several interactions with stabilize the native or the folded state of a protein, the first one is hydrophobic interaction. So, this interaction happens between the hydrophobic or the non-polar groups, for example, methyl groups or the aromatic groups in the citation of aromatic amino acids. So, they want they interact with each other these are very bulky hydrophobic groups, and they do not like to interact with the polar solvent that is water. So, they can interact with each other and this

interaction these groups are found at the core of the protein which is away from the polar solvent.

The second type of interaction is ionic interaction there that is interaction between positive and negative charged groups. For example, the side chains of aspartic or glutamic acids are negatively charged, and they can interact with the side chains of arginine and lysine which are positively charged. So, you can have ionic interactions between these side chains. And, these interactions are found on the surface of the protein, because they do not have any problem to interact with the polar solvent.

The third type of interaction which is also very important is the formation of hydrogen bonds. So, this is a dipole, dipole interaction a very good example is the hydrogen bond formation between NH groups and the co groups. So, co is the carbonell group and NH is the amide group. So, they can form hydrogen bonds and stabilize the structure of a protein.

These 3 interactions are all non-covalent interaction; one covenant interaction that is also seen frequently in proteins is the formation of disulphide bonds, but this present only when they are 16 amino acids which have the site acid in the side chain and they can form a disulphide linkage. If, they are close enough to form a disulphide bond and that is a covalent bond and the formation of a disulphide bond can tremendously increase the stability of a protein.

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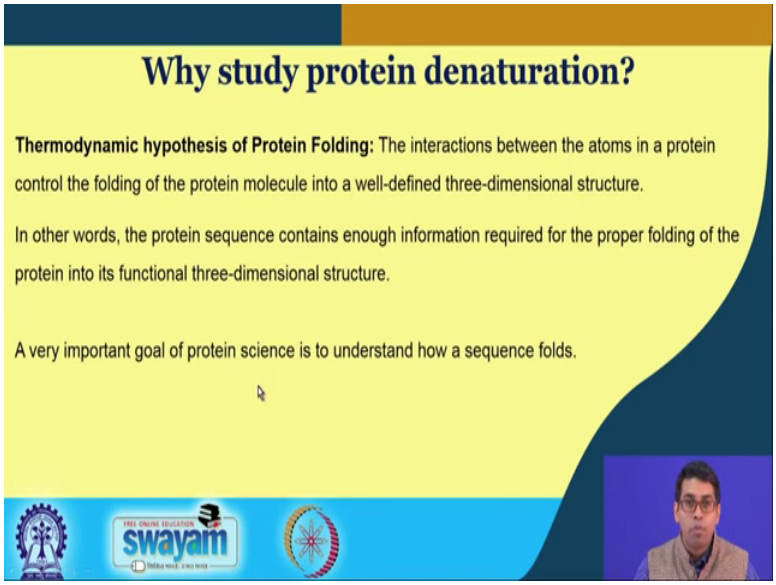

So, before we go into the study of protein by denaturation that we saw in the lab class last lab component of this week also, let me just reintroduce this idea of thermodynamics hypothesis of protein folding. Because, it is important to for us to understand why should we study protein denaturation? Because, we know that the native or the folded protein is the one that is functionally important, then why at all should we care about unfolding a protein or denaturing a protein.

So, what is the thermodynamic hypothesis of protein folding? It states that the interactions between the atoms in a protein control the folding of the protein molecule into a well-defined 3 dimensional structure, what does that mean. So, it means that the protein sequence, the primary sequence of amino acids contents enough information that is required to fold the protein into a 3 dimensional structure or shape.

So, the final structure or shape of the protein is determined by the primary sequence of that protein. This is a very powerful and very important statement, because what it means is that if I have the sequence of protein, and if I understand protein folding properly, I should be able to predict it is final folded form.

So, if I can predict it is final folded form I should also be able to predict it is function. So, if you discover a new protein and if you determine it is primary sequence, if you understand protein folding, you can immediately tell what will be, it is final folded form, what will be it is structure and what will be it is function? The converse also becomes true that if I want to design a protein with a particular function, I can come up with the structure that will be necessary to get that function.

And, if I can get that structure, I can determine the primary sequence of a protein that will give that structure. So, if I need a particular function I can design a protein that will have that function. Now, all of this can be done if we understand protein folding.

So, that is why understanding protein folding is a very important goal of protein science.

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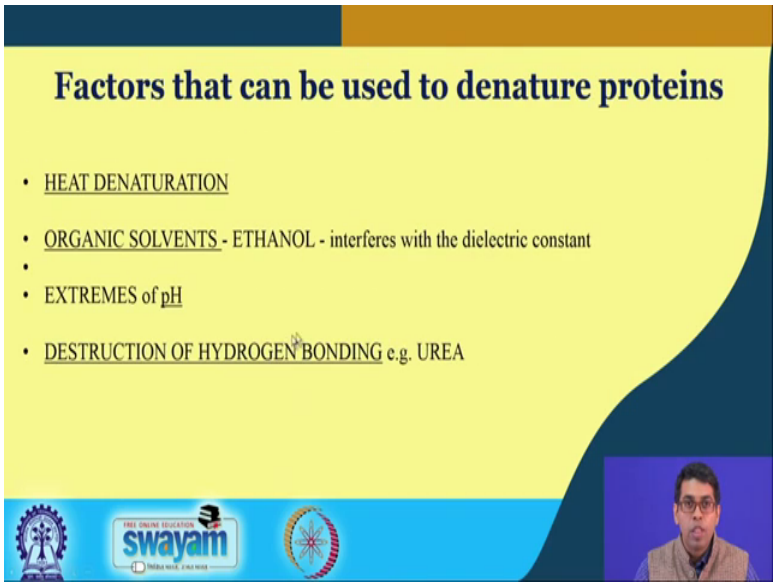

So, how do we study the denature? Denaturation of protein there are several ways. We can denature a protein by the application of heat energy. So, that is thermal denaturation, you can increase the temperature of your protein solve protein solution and you can follow how it is denaturing. You can add various organic solvents that will disrupt the dielectric constant that will disrupt the hydrophobic interactions.

And, it will denature the protein. We can also change the pH we can go at extreme pH to denature a protein for example, if you drop the pH of our protein solution to less than 2, then we know from our previous lectures that the electrostatic interactions will be hampered, because aspartic acid glutamic acid their side chains have PK have more than 3.

So, if I drop the pH to less than 2 then all these amino acid side chains will become neutral instead of negatively charged. So, the electrostatic interactions will be lost. And, if enough electrostatic interactions are lost then a protein can de denature or unfold. And finally, there are several chemical reagents which can be used to the nature protein a very good example is UREA. UREA is something that interferes with the hydrogen bonding pattern of a protein it also interacts with interferes with how the solvent the water molecules are arranged in around the protein. So, all these disruptions also lead to the unfolding of a protein molecule.

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In the lab component of this week we looked at denaturation of the human serum albumin. So, it is a protein and we just followed this one particular protein and we looked at it is denaturation by several methods. So, we used urea and guanidinium chloride these are the 2 chemical denatiuration denaturants and we also used heat to the denature this protein.

And so, that is the reagent that was used to unfold the protein and how did we observe. So, we used 2 different techniques; one was UV visible spectra and the other one was the tryptophan fluorescence. So, I will not go through all of these examples, I will just go through one and I will try to explain how once you have done the experiment, how you will look at the data and get some meaningful information out of this experiment.

So, I will talk about the deheat denaturation of the human serum albumin and it is observation by UV visible spectroscopy.

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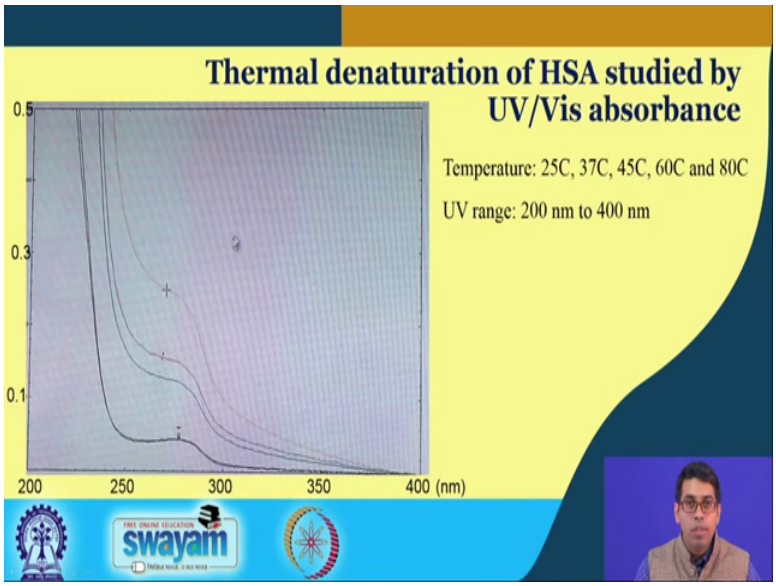

So, thermal denaturation of HSA studied by the UV visible absorbance. So, we saw pritam discussed this in details in the lab and what he did was he used 5 different temperatures 25 degree centigrade, 37 degree centigrade, 45, 60 and 80. So, he heated these protein sample at these 5 different temperatures and he collected the UV visible spectra in the range of 200 to 400 nanometer. This particular range was selected because beyond 400 nanometer there was no not much signal it was mostly noise. So, he selected this range which was good enough for this particular experiment.

So, what we saw? So, the spectra for these under these 5 different conditions are plotted here. And, for our further analysis what we will do is, we will only look at the absorbance at a 280, because at a 280 we sort of got the maxima for this particular curve. So, at 25 and 37 there was not much difference you can see that these are 2 different lines, if you follow it up here and you can see there are 2 lines ok.

So, there was not much change between 25 and 37 and then it changed a lot at 45 then there was again some change at 60 and finally, there was a lot of change at 80. Since, this was just a demonstration we followed the denaturation of this protein by only at only 5 different points.

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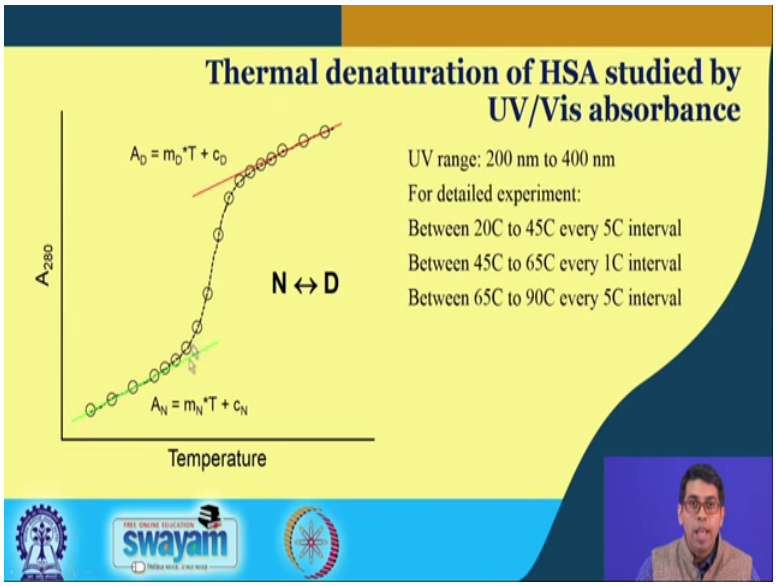

But, if you are doing a very meticulous experiment you will have to collect much more data points.

So, for a detailed experiment you will have to collect data at 5 degree intervals between 20 to 45 degree centigrade, between 45 to 65 you have to collect spectrum at every 1 degree interval and again between 65 to 90, you can collect spectrum at every 5 degree interval. The reason that we collect spectrum at every 1 degree interval is that for most proteins the thermal the denaturation happens between in this range. So, we want as much data point as possible in this most interesting region.

So, this is a typical curve that you will see if we perform an experiment like this. So, what we have done is, you will collect data you will collect spectrum for each temperature between 200 to 400 nanometer, and you will get a get spectra like this, but you will follow the absorbance only at one particular point in this case it is at 280 nanometers.

So, what I have done is, I have taken only the absorbance at 280 nanometers and I have plotted this. So, if I do an experiment like this and if I look at only the absorbance at 280 nanometers and if I plotted versus the temperature, it would look something like this now it is very important to note. So, we can draw curve through that. Now, it is very important to note that the absorbance at each point is contributed by these 2 different states of the protein.

So, the protein exists in 2 states either it is in the native state or it is in the denatured state. We do not know how much of it is contributed by the native state and how much of it is contributed by the denatured state? That is something that we need to figure out from this curve.

So, before we do that, what you will also observe is that initially there is increase in the absorbance with temperature, then suddenly there is a jump, and then there is again sort of a linear increase in the absorbance. So, linear means it is increasing in a straight line. So, it is increasing in a straight line here and it is increasing in a straight line here.

So, this is just the dependence of the absorbance a 280 on temperature. So, it turns out that even though the protein is folded the absorbance can change with temperature. And, again similarly even the protein is unfolded the absorbance will depend on the temperature. And, it can if it increases linearly we can actually fit these few data points and these few data points to 2 straight lines. So, that is the first thing that we need to do.

So, we can fit these few data points to a straight line and we will get a straight line equation for the dependence of the absorbance of the native state on temperature. So, these are straighten equation, where this a n is the absorbance of the native state this is the slope of the line and this is the y intercept of the line and this T is the temperature of each of these points.

So, once we determine m and c we know how the absorbance of the native state depends on temperature. And, we can calculate the absorbance of the native state at any temperature. Similarly, if we fit these few points to a straight line we will get the dependence of the absorbance of the denatured state on temperature. So, here again we have 2 other 2 constants this is the slope and this is the y intercept and we have to determine these 2 constants. And, then we will know the dependence of the absorbance of the denatured state on the temperature.

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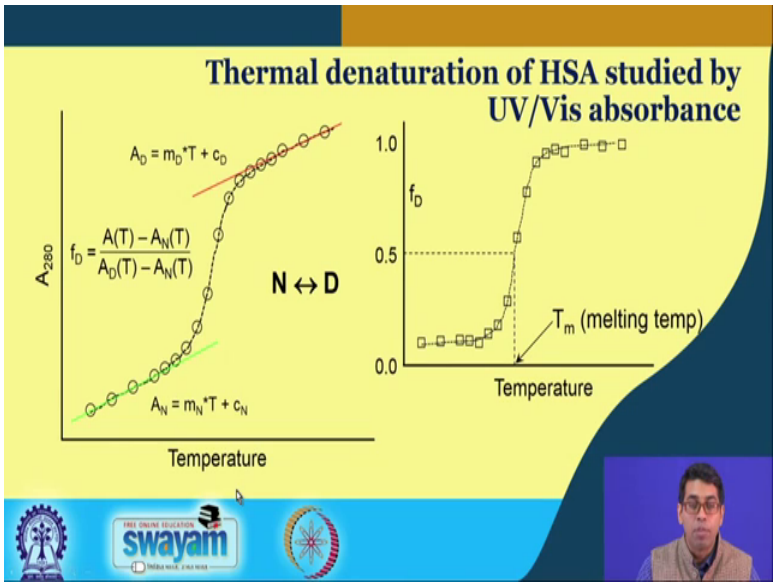

Once, we have these 2 equations we solve these 2 equations, we can determine the fraction of the denatured state present at any of these points. So, how do we do that? We use this particular equation. This A is absorbance at any temperature suppose we are looking at the absorbance at this temperature.

So, at this temperature we know the actual absorbance value, we will subtract this absorbance value from the absorbance of the native state. So, how will we get the absorbance of the native state we have this straight line equation. So, this straight line we will have an absorbance somewhere here. So, we will subtract this absorbance value from this absorbance value.

And, then in the denominator we subtract the same absorbance of the native state from the absorbance of the denatured state. So, what is the absorbance of the denatured state that will be somewhere here? So, the absorbance of the denatured state will be somewhere here. So, we subtract the absorbance of the native state from the absorbance of the denatured state and that goes in that denominator.

So, what we will get is a ratio and this will be something less than 1. And, that ratio gives us what fraction of these total molecules is present in the denatured state.

So, for each of these points that we have collected data we can calculate this f D and we can plot that versus the temperature and if we do that it will look something like this. So,

f D is plotted in the y axis and that gives us the fraction of the denatured state present. So, in the initial stages where the temperature is low the protein is mostly folded or it is in the native state.

So, you will see that this fraction f D will be almost close to 0 ok. As the temperature increases it slowly keeps on increasing, then suddenly there is a jump and then at high temperatures almost all of the protein is unfolded, because now the fraction denatured is almost one. So, this is a typical curve that you will see.

And, one of the most important parameters that we determined from curve like this is the temperature at which f D is 0.5. So, it means that at this temperature 50 percent of the protein is in native state and 50 percent of the protein is in the denatured state and this particular temperature is called the melting temperature and it is represented by T m.

So, melting temperature is the temperature at which a particular protein exists in 50 percent native and 50 percent denatured state. So, this T m is a very important parameter, that is used to characterized a protein, because if the melting temperature of a protein is high then that protein is a very stable protein. And, if the melting temperature of a protein is low then that protein is a unstable protein. So, melting temperature of a protein gives us a very good idea about the stability of a particular protein.

So, what we have done so, far we took the raw data we followed the absorbance at a particular value and plotted it as a function of temperature. Then, we fit the 2 extremes to 2 straight lines to determine the dependence of the absorbance for the native state and the denatured state on temperature.

And, then we calculated this fraction of denatured state present at each temperature and plotted that and from that, we determine the melting temperature. So, the same principle can be used for any denaturation studies. For example, if we are using urea instead of temperature here it will be urea. So, you will follow the absorbance of your protein under varying concentration of urea.

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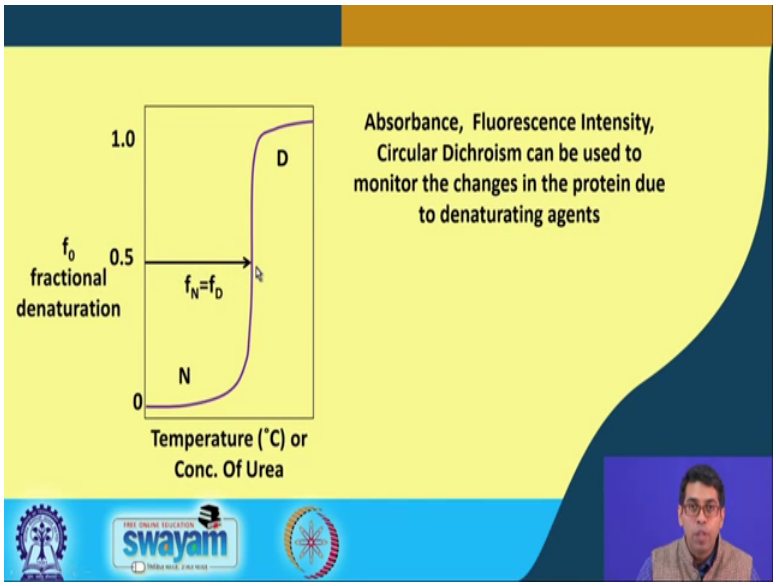

So, this will be a very general plot of all the experiments that were shown in the lab. So, the ultimate goal is to determine this fractional denaturation, and you plot that versus temperature, or the concentration of urea, or the concentration of guanidine hydrochloride. This fractional fractional denaturation is calculated from the observle observable parameter that we use.

So, it can be absorbance, it can be fluorescence, it can be circular dichroism, it can be anything. So, we will use that and from that we will calculate the fractional denaturation and then when you plot this at 0.5 is the, you get the value where the so, if it is temporary will get the melting temperature. So, once we have that from that we can go on to calculate the free energy of the protein, we can calculate the enthalpy and entropy of the folded state of the protein so on and so forth. So, that is all for this week.

Thank you.