Fundamentals of Protein Chemistry
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Module - 03
Protein Structure and Stability
Lecture - 14
Protein Denaturation

In our module on protein structure and stability, we will talk about protein denaturation today. What do we mean by protein denaturation? What are the factors that could cause a disruption in the protein folding? So far we have learnt about the specific forces that are involved in protein folding. And now we will see how we can disrupt those specific forces.

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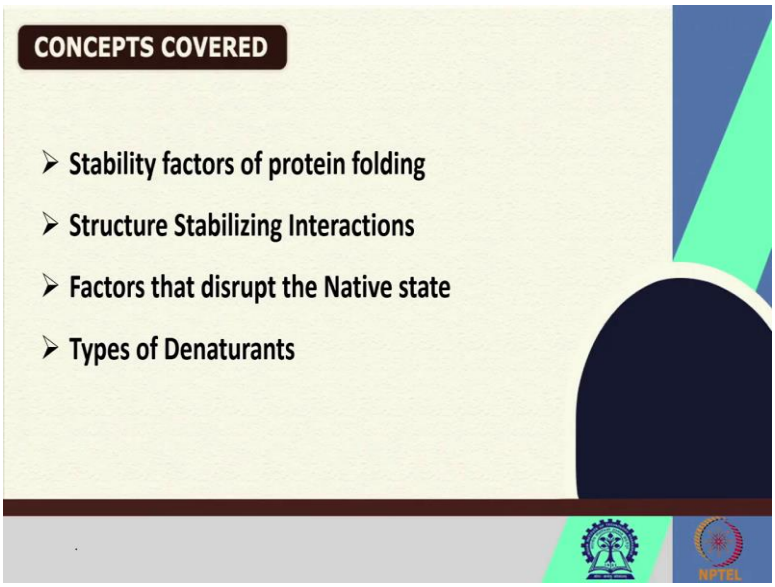

Initially we looked at the stability factors of protein folding, the structure stabilizing interactions, the specific enthalpic and entropic contributions to the overall folding of the protein, and also the thermodynamics of protein folding in one of the lectures. And now we are going to look at the factors that are disrupting this native state and the types of denaturants that are possible.

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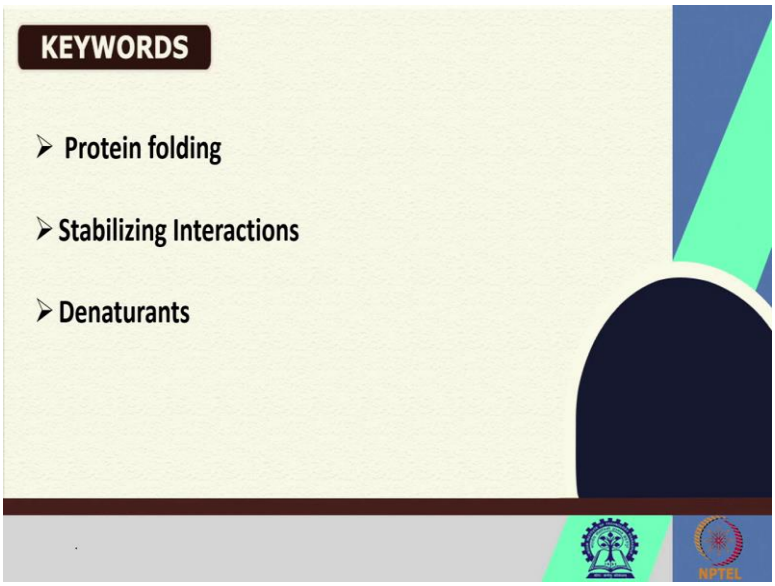

So these would be the keywords protein folding, stabilizing interactions and the denaturants.

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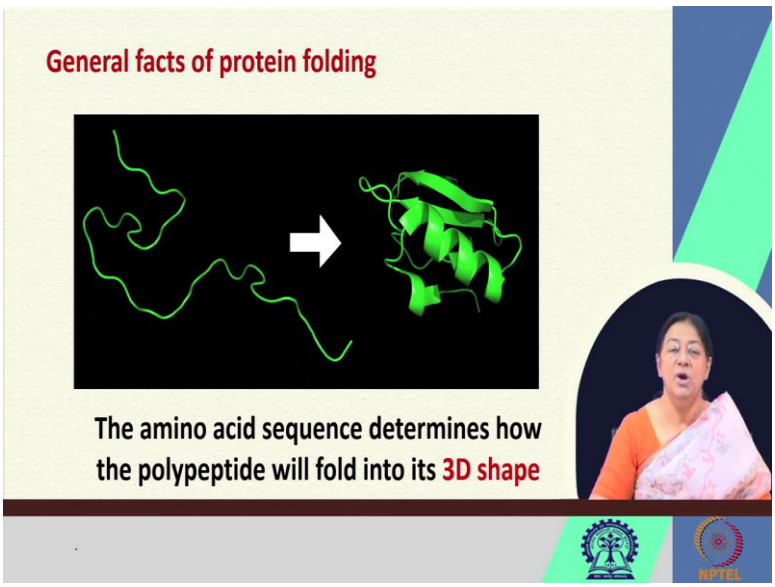

If we look about the general factors of protein folding, the idea is that we have this unfolded structure that finally falls into a native 3 dimensional form.

Now given that this occurs under specific conditions, in the sense that there has to be the right condition for the protein to fold to form its 3 dimensional structure and the factors that are involved in these are the amino acid sequence, which actually determines how

the polypeptide will fold into its 3 dimensional shape; which we learnt that the sequence actually contains all the information from Anfinsen's experiment that we studied that was done with ribonuclease A.

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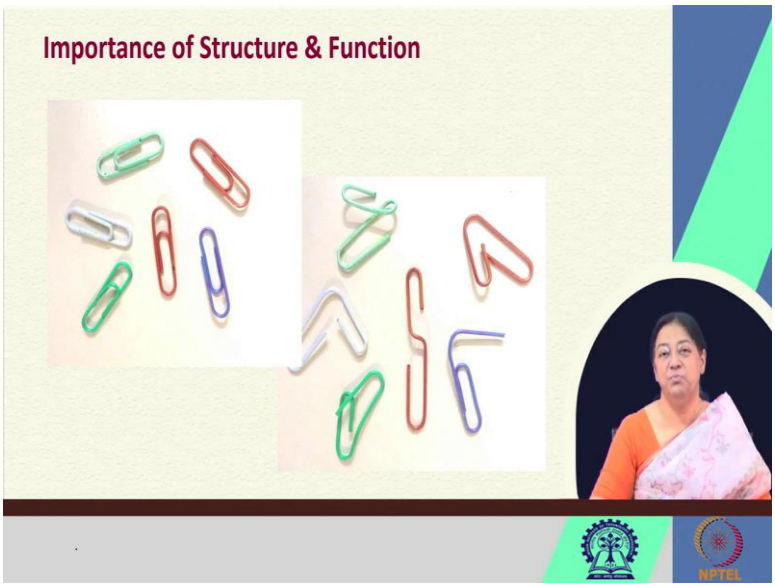

Now, when we look at the importance of protein structure and function, there is a beautiful way to understand it in terms of the general idea. For example, if we look at a structure of a paper clip, this was shown by Professor Ada Yonath who was a Nobel prize winner for protein Chemistry.

When we look at a paper clip, we know that this has to be folded perfectly in this particular fashion for it to perform its function. Its function being to hold pages together. Now if we have the unfolded forms of these specific paper clips, we know that it cannot perform its function. This is just a very simplistic idea of looking at how the structure and the function are important and this holds true for proteins as well.

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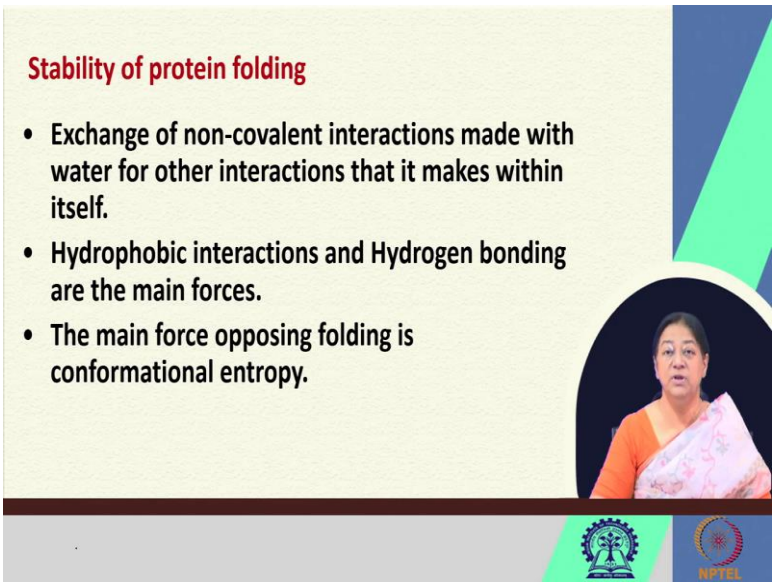

Now, when we look at the stability of protein folding and we try to understand what components are bringing about this folding, this is what is important.

We have non-covalent interactions that hold the protein together and initially when we have the unfolded form, it gradually folds into the specific ways in which we saw a hydrophobic collapse. A specific type of model where we may have the secondary structural elements formed first and a folding funnel where we finally get from the many unfolded structures possible, to the native structure of the protein.

This exchange of non-covalent factors or non-covalent interactions that are made with water, are made within themselves and these hydrophobic interactions, in addition to the hydrogen bonds, are the main forces that are going to result in this protein folding. In addition we found that the main force opposing this is the conformational entropy, where we would not want to bring about order in the protein. But again we found out that the destructuring of the water molecules is also very important in the protein folding scenario.

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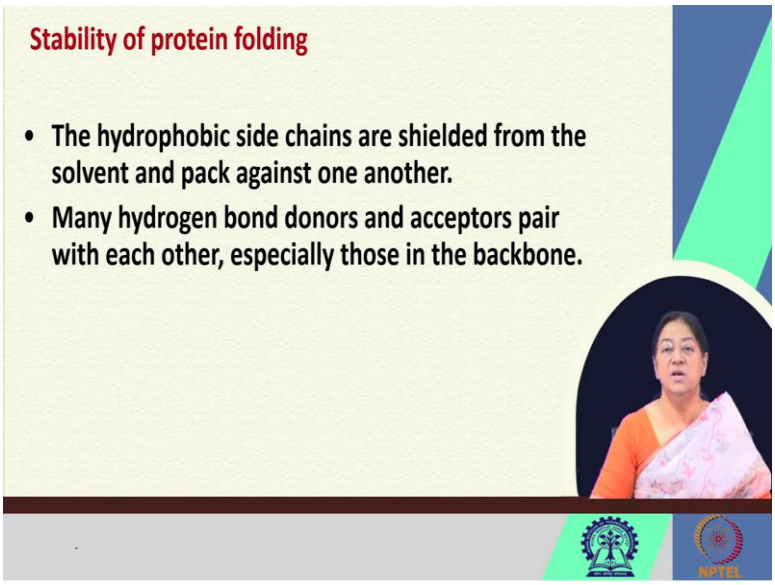

If we look at the hydrophobic side chains that are present, we know that these are the ones that have only the carbon and the hydrogen present in their side chain. They have to be shielded from the solvent in the polar solvent. When we talk about proteins in general we talk about regular globular proteins in polar solvents. So we want these hydrophobic side chains to be shielded from the solvent and what they do is they favorably pack against one another.

As mentioned, we have hydrogen bond donors and acceptors that pair with each other particularly in the backbone, where we see the C = O with the NH, which we have seen in the secondary structural elements as well as in the backbone; in addition to other hydrogen bonding possibilities or iron pair interactions that may be possible because of the basic and the acidic amino acid side chains.

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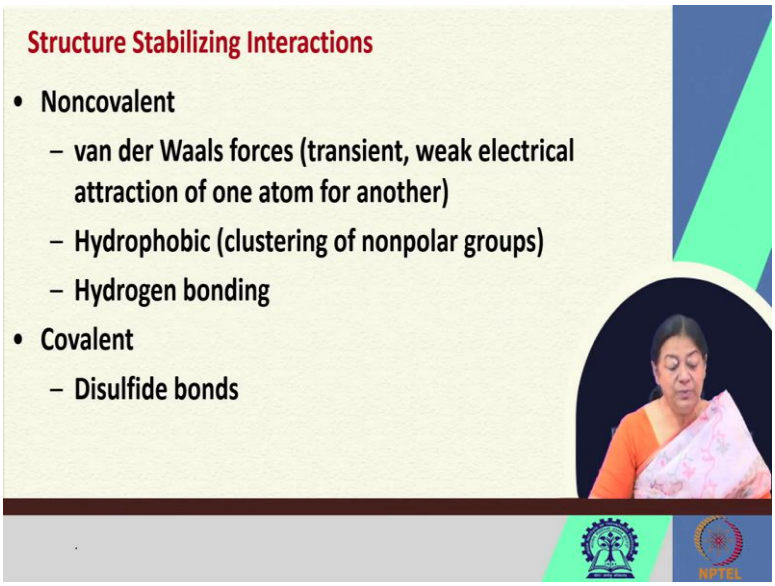

So when we look at the non-covalent types of interactions we have the van der Waals forces that are transient, weak electrical attraction of one atom to another because of the instantaneous dipole that arises in every atom; the hydrophobic interactions that bring about a clustering of the nonpolar groups that is going to account for a favorable interaction between hydrophobic amino acids; the hydrogen bonding and of course, the electrostatic interactions as well. In addition to the covalent disulfide bonds that are present, bringing different parts of the protein molecule to the polypeptide chain together.

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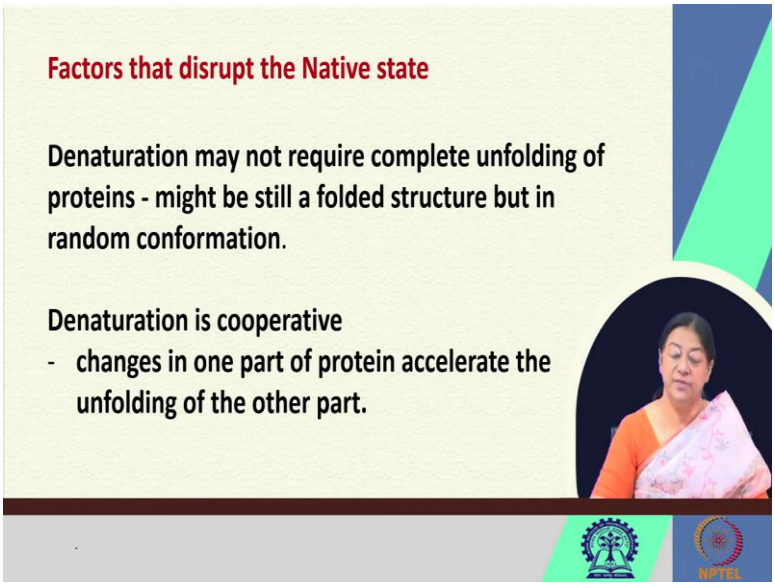

Now, that we know we have the formation of this native state, the folded form of the protein, we are interested to see what factors are capable of disrupting these forces. So, we would have to either disrupt the hydrogen bonding interactions or the hydrophobic interactions or the electrostatic interactions or the disulfide interaction. If we are able to disrupt these interactions we will result in an unfolded protein.

So the denaturation may not require complete unfolding of the proteins, but it may be in a slightly folded structure; in some random conformation that will not be able to perform its function, so that would also result in what we called a denaturation.

This is the cooperative effect. What do we mean by cooperative effect? It means that if there is a change in one part of the protein this percolates to the other parts of the protein, in a sense it accelerates the unfolding of the other part as well.

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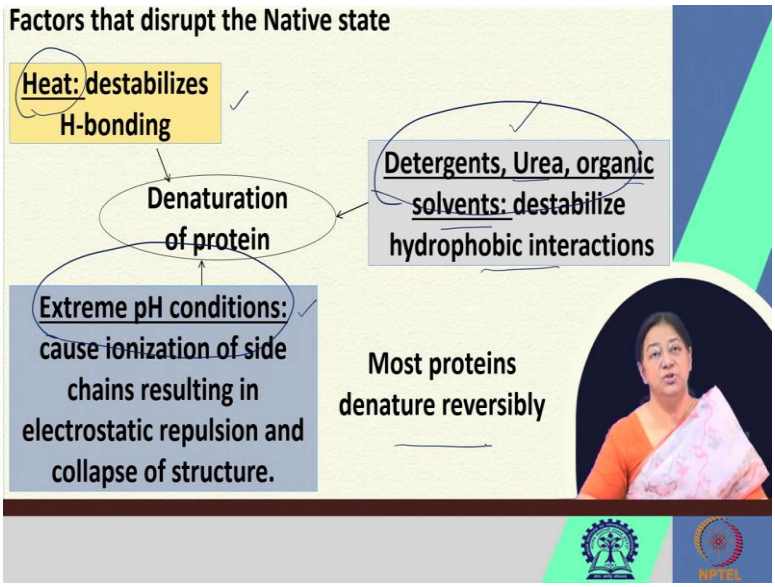

The factors that actually disrupt the native state of the protein are, the heat that destabilizes the hydrogen bonding; specific detergents, urea, organic solvents because what they can do is they can destabilize the hydrophobic interactions; and extreme pH conditions that can not only cause the ionization of the specific side chains that have electrostatic interactions, but can result in electrostatic repulsion and a disruption of the structure.

So, we are looking at a denaturation of a protein. What do we mean by the denaturation of protein? We mean the disruption of its 3 dimensional structure and this can be brought about specifically by heat. So we have to look at the specific factors that cause protein folding; hydrogen bonding, hydrophobic interactions, electrostatic interactions and disulfide linkage formation.

We have to break these in terms, so we can use heat, we can use detergents, urea or organic solvents or extreme pH conditions to exploit the specific types of interactions to bring about a denatured protein. And most proteins are known to denature reversibly, meaning that if we remove the denaturation causing agent, it may go back to its original form. But this is not always true.

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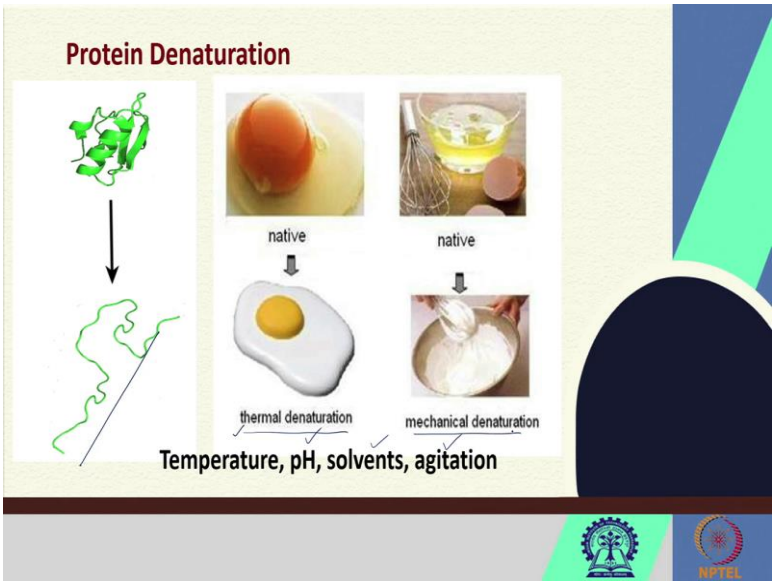

If we look at protein denaturation saying that we have the folded structure and now we are unfolding it into its primary sequence here [refer to slide], into its polypeptide chain. This can be brought about by temperature, by pH, by solvents and even agitation and we have examples where we can have the native form we know thermal denaturation that is very common in an egg or we can even have mechanical denaturation that will bring about a disruption in the protein structure which we are trying to aim at.

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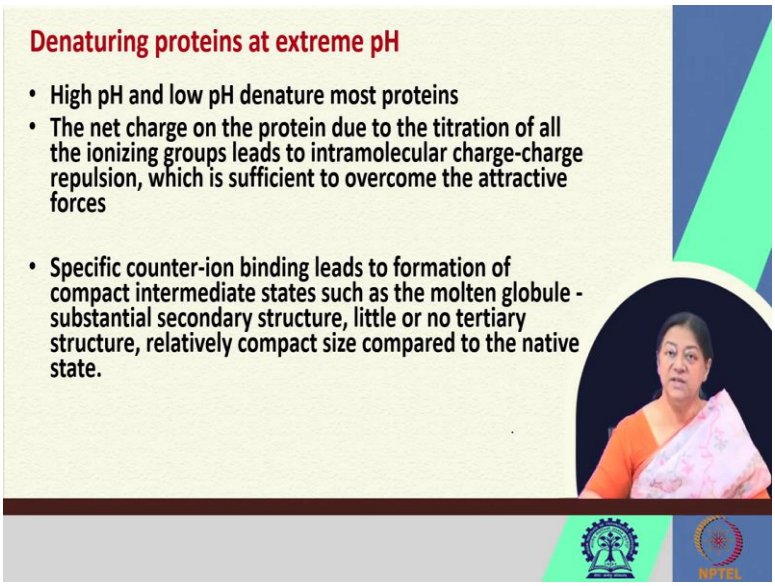

When we are looking at denaturation of proteins at extreme pH, we are looking at high pH and low pH that are going to denature most proteins. Why does it do this? Because the net charge on the protein, due to the titration of the ionizing groups (we learnt about the titration at different pH values), what happens is the charge to charge repulsion occurs. Because if we go to extremes of pH, we either have its high protonation or we have complete deprotonation, which is sufficient to overcome the attractive forces that are holding the protein together.

Then we also have specific counter iron binding that leads to the formation of the compact intermediate states for example, like the molten globule. And what happens in this case where we have a secondary structure element, we have less tertiary structure and a relatively compact size compared to the native state that readjusts for the specific components of interactions.

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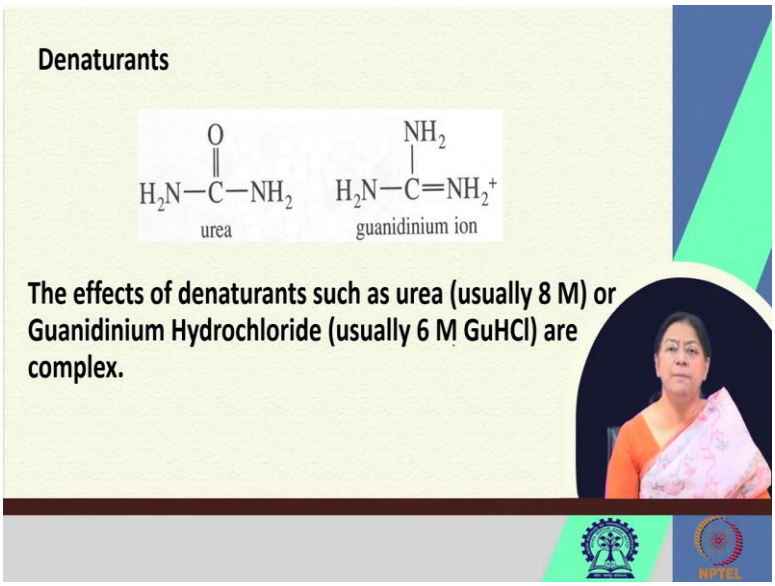

The denaturants that are commonly used are urea and guanidinium hydrochloride, where we have a Cl^- associated with the NH_2^+.

Now, the question is what do these specific denaturants do in order to bring about the denaturation? The effects of denaturation such as urea are actually complex, but nevertheless we know that they are disrupting a specific type of interaction in the protein.

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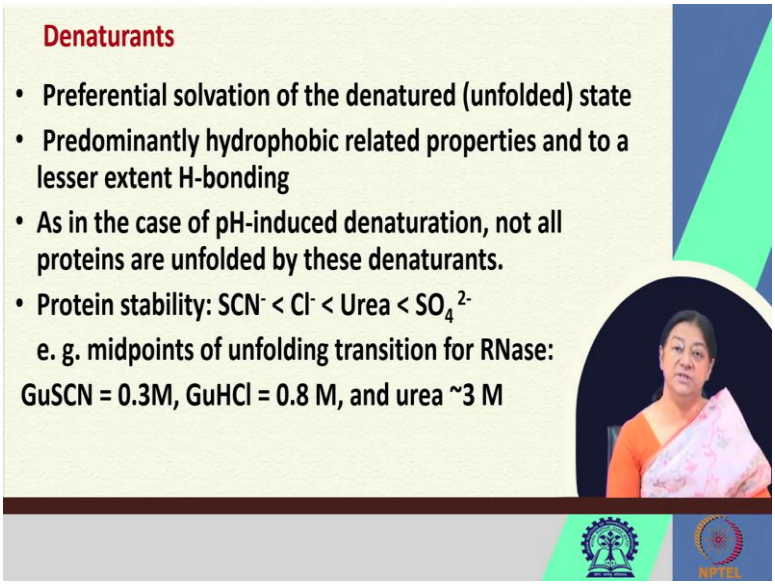

And we have a preferential solvation of the denatured, of the unfolded state. Predominantly hydrophobic related properties and to a lesser extent hydrogen bonding.

And as in the case of pH induced denaturation, not all proteins are unfolded by these denaturants. What happens is there is a protein stability; a protein stability related to the specific ions that have been mentioned here [refer to slide].

Now, we are looking at titration curves or we are looking at unfolding transitions in terms of addition of the specific denaturants.

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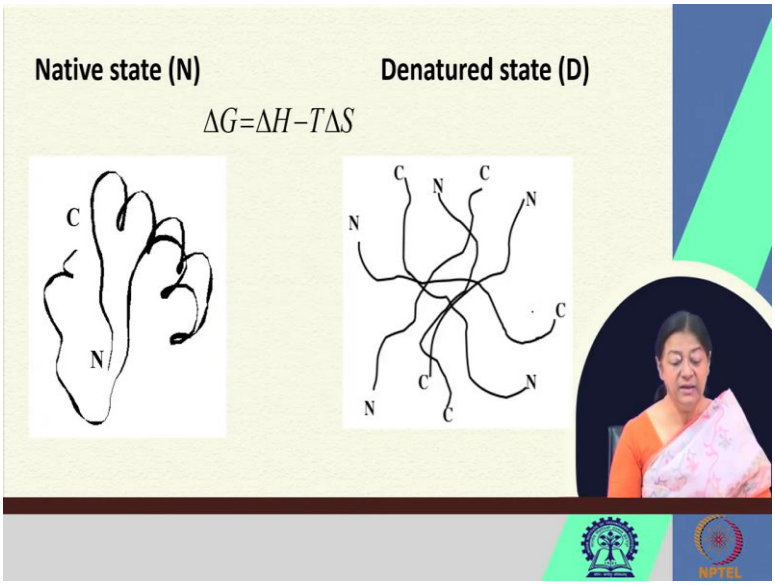

What do we mean by that? It means that we have a native state and we result in a denaturant state, because of specific denaturants that have been added here. And this is obviously going to have an effect on the overall ΔG. And what is this ΔG? If we consider this to be a specific equilibrium condition, then we are going to have our expression, $\Delta G = -RT\ln K$. We will have a look at what this means in a moment.

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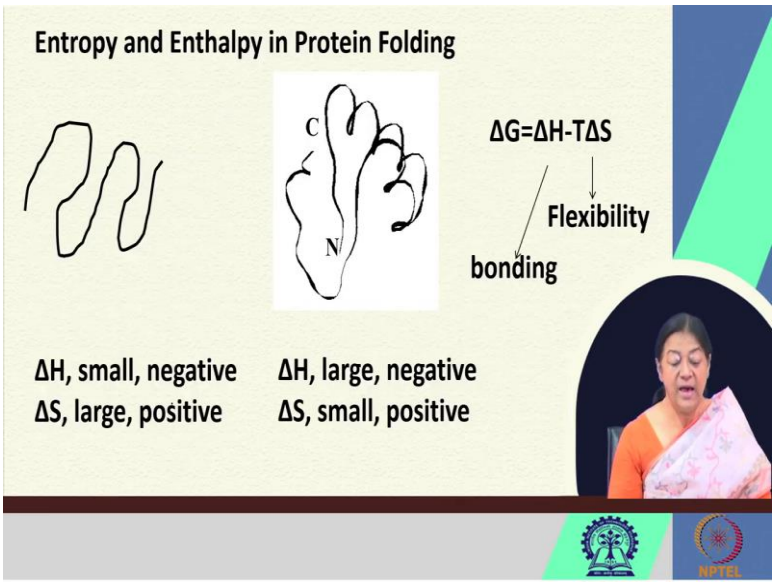

So, we have the entropy and enthalpy of protein folding that has been discussed earlier, where we look at the specific enthalpic contributions due to the different types of interactions that can occur for the ΔH and the $T\Delta S$. The entropic contributions that come from the specific parts of the protein coming together, to form the native structure from the unfolded form.

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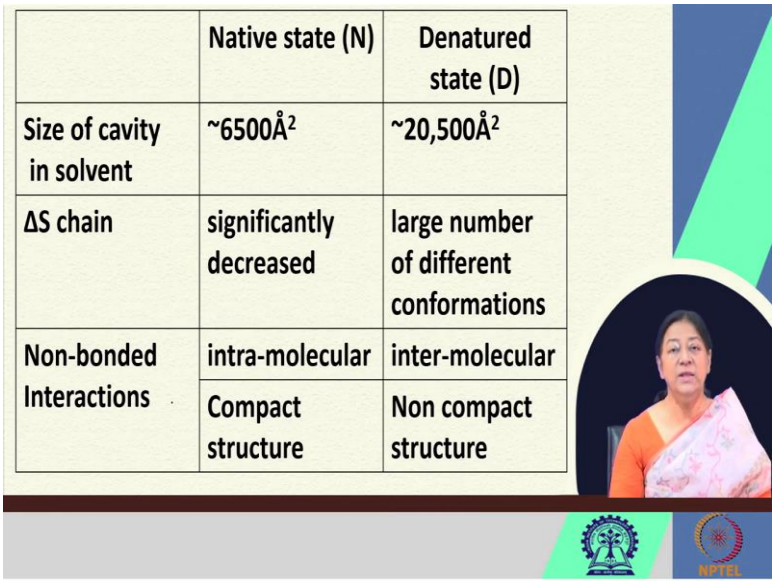

If we look at the native state and the denatured state and we do a rough comparison of the size of the cavity of the solvent present, in the native state the structure being more

compact will have a size of the cavity for the solvent much less than that for the denatured state.

And when we look at the entropic conditions or the entropic contributions we know that the denatured state is capable of having a large number of different conformations whereas, this for the native state is significantly reduced.

We can have non-bonded interactions, that for the native state are within the molecule, intra-molecular. However, in the denatured state when we have the hydrophobic parts of different protein polypeptide chains, they may interact with each other in inter-molecular combinations, giving to different types of structures.

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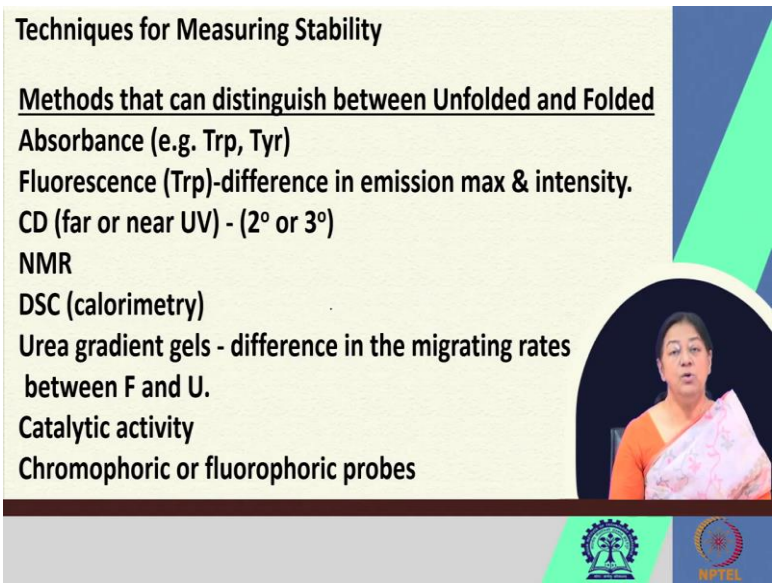

To measure the stability of the protein there are several factors, we will be considering some biophysical methods in a later lecture. But we know about the absorbance that we have studied, there is fluorescence emission, there is circular dichroism, NMR, calorimetry, urea gradient gels between the folded and the unfolded form and an understanding of the catalytical activity, that is going to tell us whether our protein is in its active form or not.

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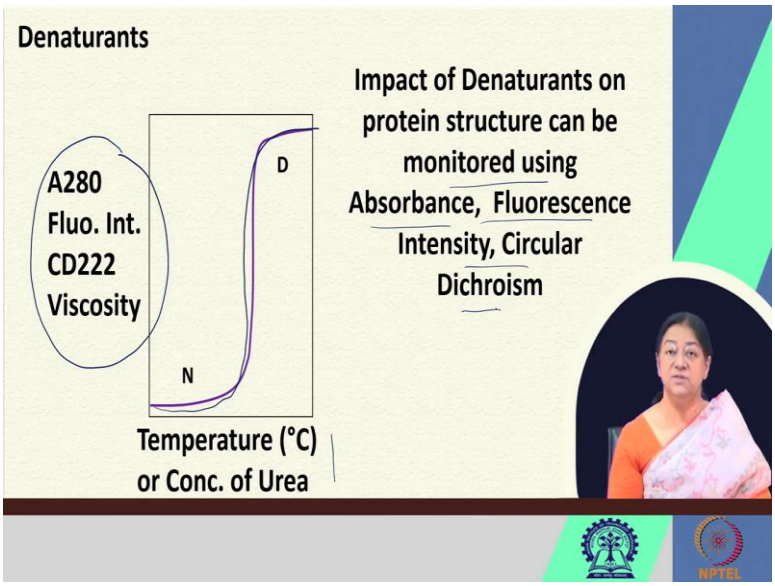

These [refer to slide] are the different monitoring effects that we can use to study the denaturation. Essentially what we have is, we have some signal that is going to tell us whether the protein is folded or denatured and along the x axis here we have the impact of a denaturant. In this case we can have temperature or we can have an increased concentration of urea.

And what we observe is, we observe a curve that has an effect like this, the sigmoidal effect, telling us that this is a cooperative effect that we would have the native form. When one part starts to get denatured, as mentioned before, it accelerates the denaturation of the rest of the polypeptide chain and we have this specific type of curve that can be monitored using absorbance, fluorescence intensity or circular dichroism or any such monitoring factor. Say the enzyme activity that is going to tell us that whether the protein is in its folded form or in its unfolded form.

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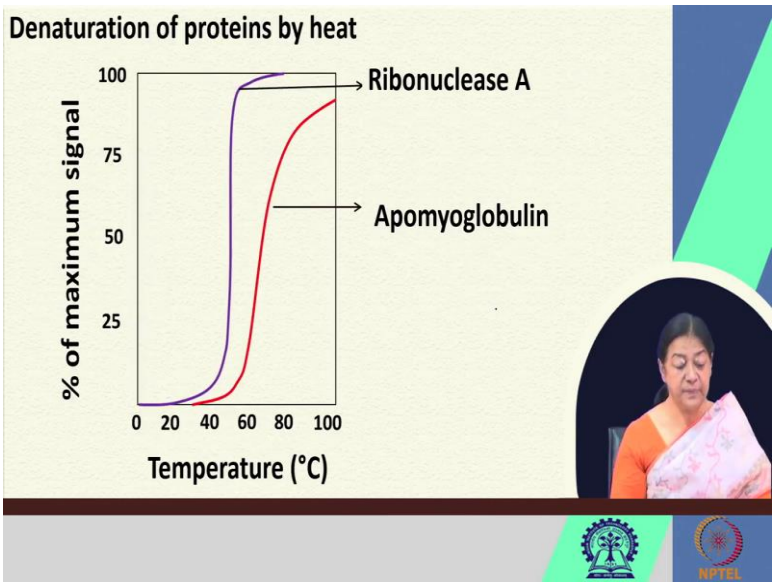

For example, [refer to slide] this is the percentage of the maximum signal and we have increased the temperature along this axis. There are two different proteins here. What we have is the 50% folded points. So this would be if we are looking at a 100% signal and say that the 0 corresponds to its completely folded form and the 100% says that it is entirely in its unfolded form.

Now we are looking at a feature that is going to give us what is called the melting temperature of this particular protein. And we realize from this curve right away, that apomyoglobin has a higher tm than ribonuclease A, rendering it more stable. So just by looking at the specific signal that we are monitoring, versus the denaturating agent, we can determine the stability of a protein which is what we are interested in.

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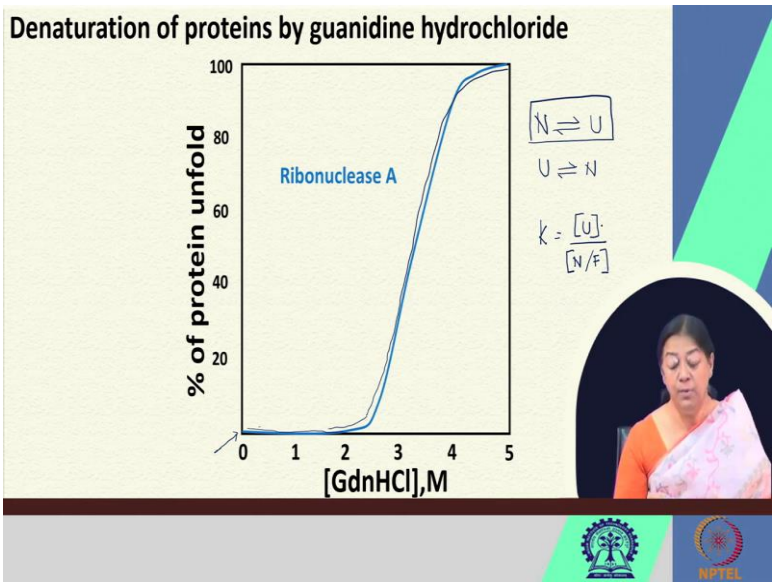

In this case we look at the denaturation of ribonuclease A by guanidine hydrochloride. Now what we have here is we have the process that has been monitored in terms of the percent of the protein that is unfolded.

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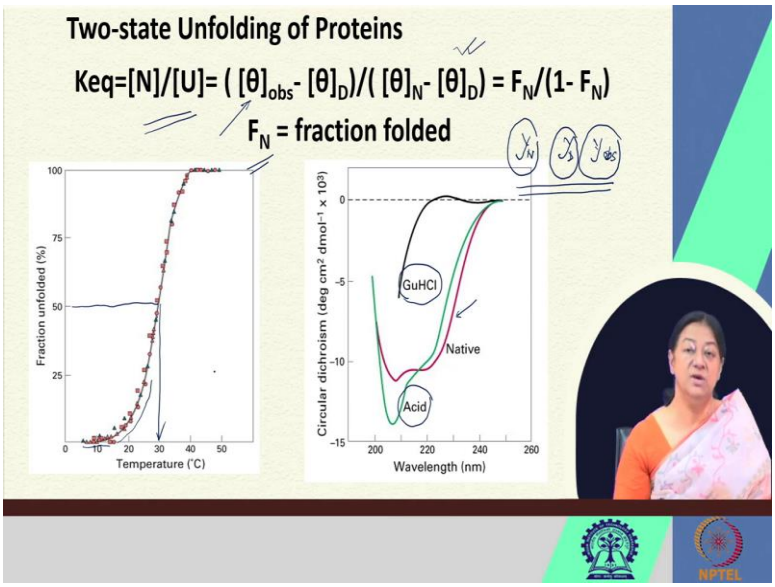

If we go back and try and understand what is the specific feature that we are looking at, we are trying to monitor the level or the extent of unfolding of the protein.

We observe that here we have 0 M concentration, meaning that it is in its native form entirely, we see that there is a flat form to the curve because our monitoring signal tells

us that there is no change in the protein unfolding characteristics. Then as we increase the concentration of the denaturating agent, in this case GdnHCl, what we have is, we have a curve that goes up like this and then is saturated saying that at this point we have the completely unfolded form.

So in this case we are monitoring the native that goes to the unfolded form. We can monitor the unfolded form forming the native form. Depending upon the K equilibrium for this particular graph that we have here [refer to slide], we are looking at the native form going into the unfolded form. And the K that we considered here the equilibrium constant, is going to be the ratio of our unfolded form to the ratio of, as it is called, the native form or sometimes also referred to as the folded form.

Now we have a monitoring signal that is going to tell us whether the protein is in its folded form or in its unfolded form. So, if we look at a situation like this where we are looking at the fraction folded, that is the fraction that is in its native form, we are looking at a two state unfolding of proteins.

Now when we are considering an unfolding of proteins, it means that we have the native state and we have the unfolded state. In this case we are looking at a native, divided by an unfolded form. The specific terminology that is seen here, corresponds to an observation; an observation that tells us about our monitoring technique.

For example if we call this signal y; we have a signal for the native form, we have a signal for the completely denatured form and we have an intermediate signal that we call y_{obs}, for every measurement that we take for the different say concentrations of the denaturant that we are adding or say for different temperatures that the experiment is being conducted in.

If we have these specific points that are mentioned here, we are looking at a temperature. These are calculations based on looking at a fraction of unfolded protein, that tells us again that, when we are at low temperature we have a major part of the protein that is folded. There is a gradual unfolding that occurs majorly after say 25 and definitely after 30°C. At this point we are talking about a 50% unfolded form of the protein that occurs at this specific temperature.

Now these specific points that we see here [refer to slide], are measurements that have been taken to give us an idea. When we look at the signal whichever way we monitor this, say by absorbance or by circular dichroism or even fractional activity; whichever

way we are looking at this, it is the same characteristic observation that we are going to get. We are going to get a signal for the native form, we are going to get a signal for the denatured form and we are going to get signals for the intermediate ranges of the unfolding of the protein.

In this case the circular dichroism has been shown here, with the specific wavelength. For now, since we have not looked at circular dichroism spectra so far, we understand that the red one that has been drawn here is the native form and we realize that with the addition of acid we do not have the native form, with the addition of GuHCl we do not have the native form which is shown in red.

There is a disruption of the structure and this can be conducted by different additions of acid, different additions of the GuHCl, that would result in a specific calculation that can be plotted to give us what the tm value is.

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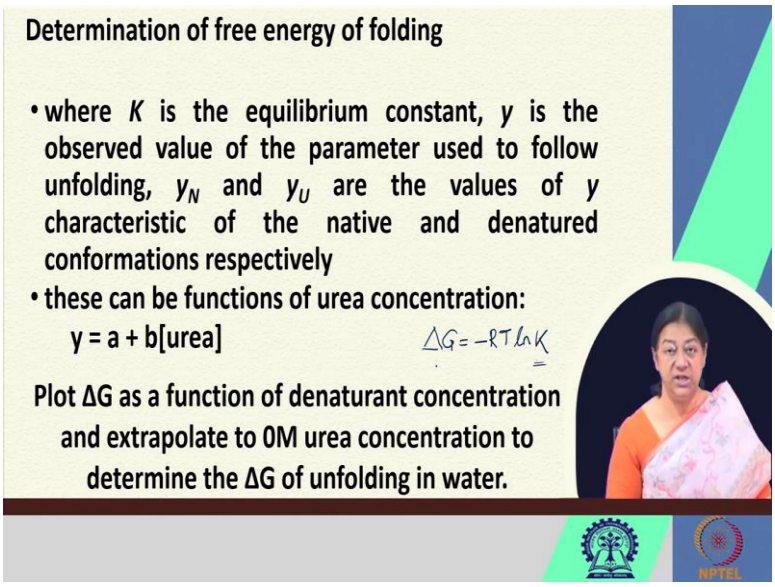

If we want to find the free energy of folding, what are the steps that we have to take? The first thing that we have to do is, we have to find out K, the equilibrium constant, we have y as the observed value of the parameter that is used to follow the unfolding, we have y_N and y_U, that are the extremes in the signal. One is characteristic of the native form and the other is the denatured form and these can be functions of the urea concentration.

So, our denaturant concentration is going to give us a measure of different values of y that can be monitored by spectroscopy. When we are looking at these, we look at a y that is our signal, sum intercept and we look at the concentration of urea.

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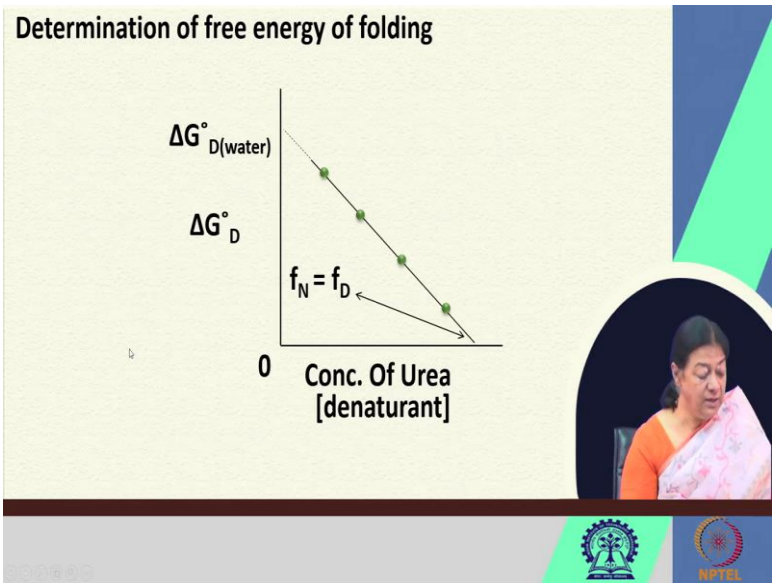

Now when we have the K value, we know that we can calculate the ΔG. How do we calculate the ΔG? We know from our thermodynamics that the ΔG = - RTlnK. So now, that we have a value of the K, we can find out what the ΔG is. We then plot ΔG as a function of the denaturant concentration, extrapolate to 0 M urea, where the protein is completely folded and determine the ΔG.

If we want to determine the free energy of folding, we calculate the ΔG for the different concentrations of urea and then we can find out the value of the folded form.

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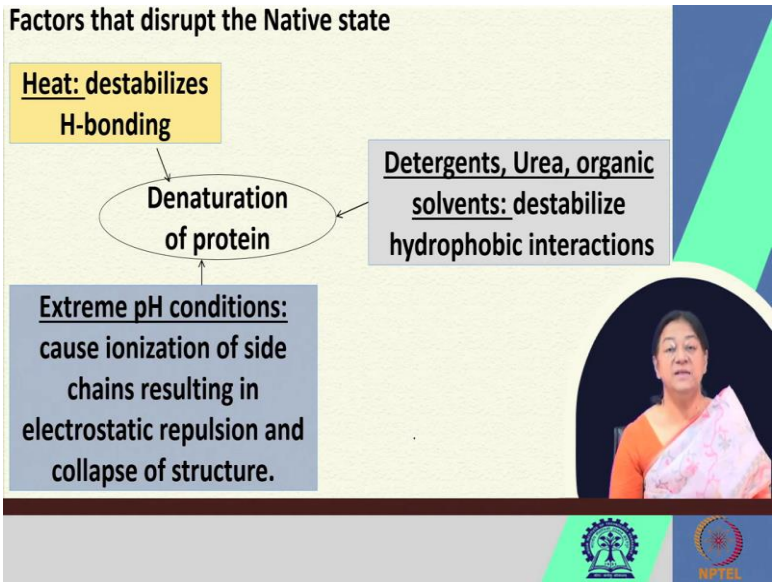

The factors that we found out that disrupt the native state are heat, specific detergents, urea, organic solvents and so on and so forth. And extreme pH conditions that result in a disruption of the overall structure of the protein, any of these agents will be able to disrupt the structure of the protein and we found out how we have a specific cooperative effect.

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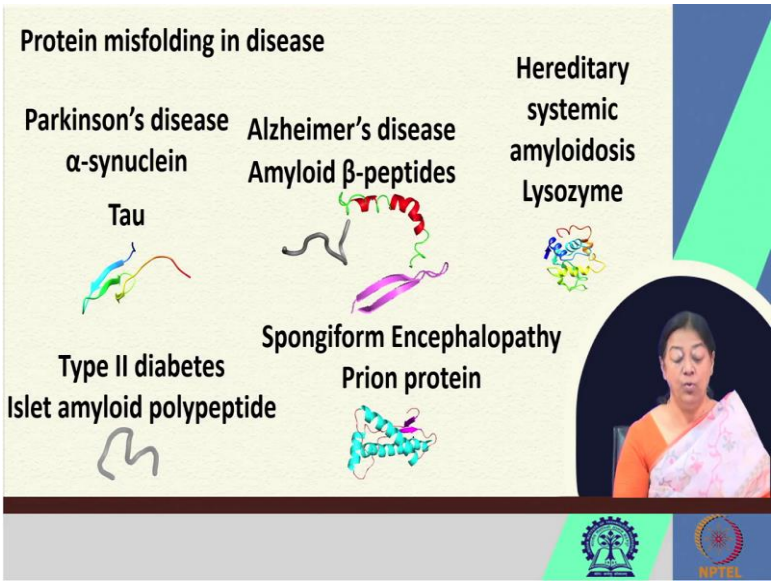

If we look at protein misfolding in association with disease, there are several diseases that occur as a result of misfolded proteins, where we do not have a proper folding occurring in the protein.

For example: Alzheimer's disease, amyloidosis in lysozyme, Parkinson's disease where we have a specific type of interaction that results in. For example, in Alzheimer's and plaque formation that has depositions in the brain and also type 2 diabetes and the prion protein misfolding that results in disease.

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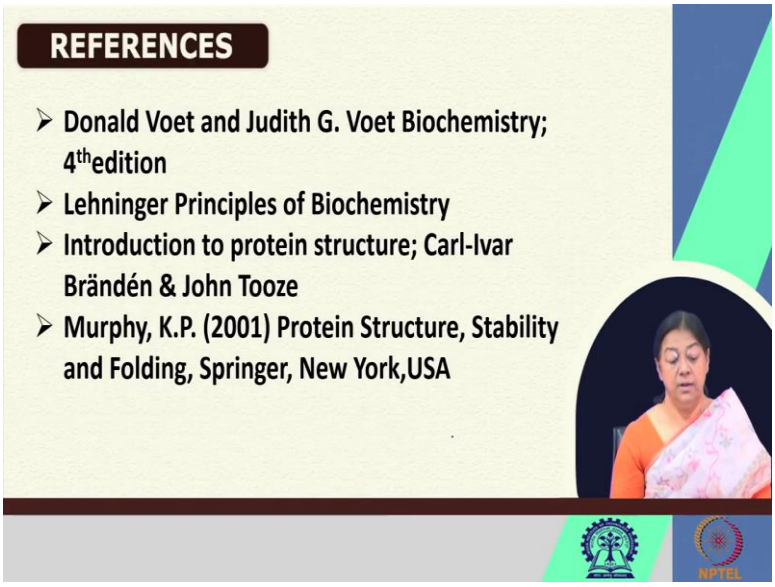

[Refer to slide] these are the references.

Thank you.