**Biochemistry - I**

**Prof. S. Dasgupta**
**Department of Chemistry**
**Indian Institute of Technology, Kharagpur**

**Lecture # 06**
**Protein Structure IV**

Welcome, today we continue our discussion on Protein Structures and just to recap on what we done so far.

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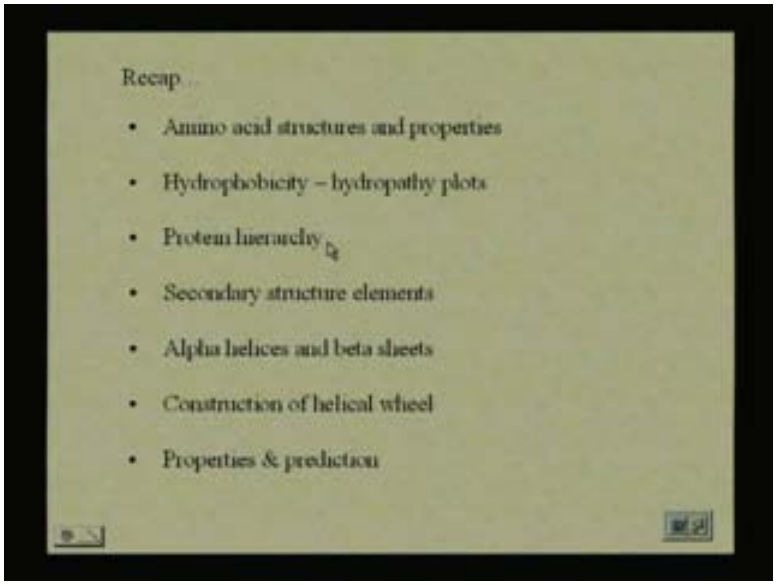


Initially we studied about the different Amino Acid structures and their properties. We considered the Hydrophobicity as the most important property of the amino acid and constructed hydropathy plots to determine which regions of the Protein amino acid sequence are actually hydrophobic in nature, which would occupy the core of the Protein.

Then we considered where not to the Protein hierarchy meaning the different structural properties of proteins from the amino acid sequence to the final quaternary structure which some proteins adopt. Then we considered the Secondary structure elements which may be the Alpha helices and the beta sheets and their different properties as to how they interact and what are their specific features be at the backbone or the hydrogen bonding properties. And then we constructed a helical wheel and to figure which regions of the α-helix would be facing inside the surface of the protein. Finally we went to the certain properties of the proteins and predictions of secondary structures(Refer Slide Time 01:54min) .

Now we know something about Proteins. We have to consider the different classes of proteins that are formed based on their structure and solubility. Initially we have gone though some of these, for example if we look at Mioglobin where it is a Globular protein so it is more or less shape like a Globule which is the case with the most proteins.

Now we understand that we will have hydrophilic residues on the surface and we will have hydrophobic residues within the core which tend to remain away from the solvent as far as possible.

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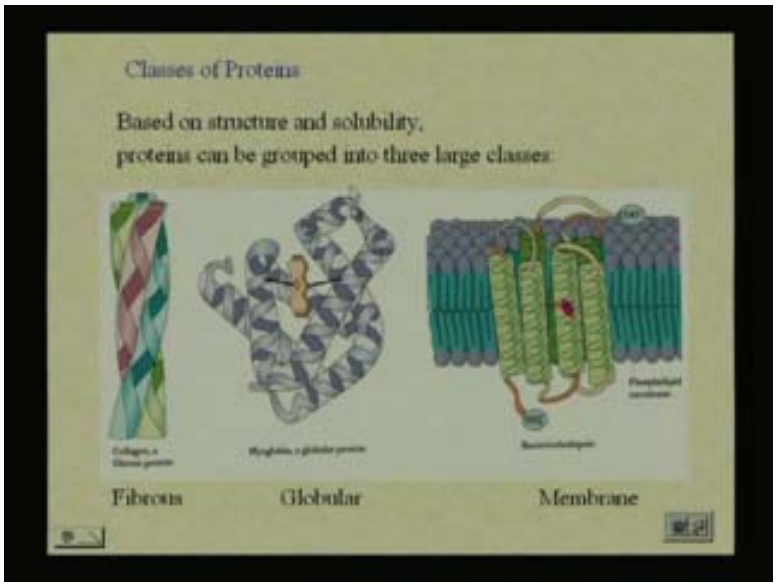


Here you can see the structure of the Heme. Here We have Membrane Proteins on the right hand side, as we studied earlier the Membrane Proteins are embedded in phospholipid bilayer that forms the cell membrane. And we have studied about the Transmembrane helices and we also did hydropathy plots to figure out how we can actually determine which of these helices are, where they are going to spam the membrane.

We also learned that it is likely to have hydrophobic amino acid residues on the surface of these helices so that they can interact with the hydrophobic tales of these lipids (Refer Slide Time 3:21 min).

There is another class of proteins that are Fibrous proteins. From the name itself you can figure out what thus construction of it will be, they are more or less fibers. And they are adopted to give strength to the structure of the protein. So what we have in fibrous proteins is as you can see here you can have a single α-helix or you can have a coil of two helices or you could have a triple helix (Refer Slide Time 3:51 min).

So here the interactions would result in a fiber. These long fibers or large sheets that are actually formed our parts of structures that required mechanical strength. α-Keratin and

Collagen are two such examples of fibrous proteins. For example you can find them in hair or in muscles because of their strength or because of their structure. And because of they are fibrous in nature they form as long parallel structure along a single axis so they give this longitudinal structure and they give a mechanically strong nature to this.

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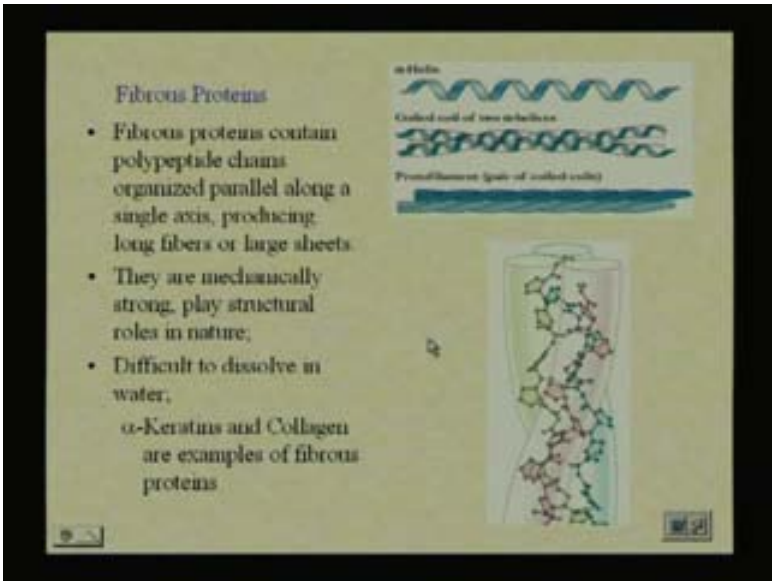


For example α-keratins, they are found in hair and their sequence consists of long alpha helical rod segments. β-keratins which we can see here are formed as β- sheets, they are found in silk which is also a fiber. But over here if you look carefully, you can see Alanine and Glycine repeat sequences as they are called.

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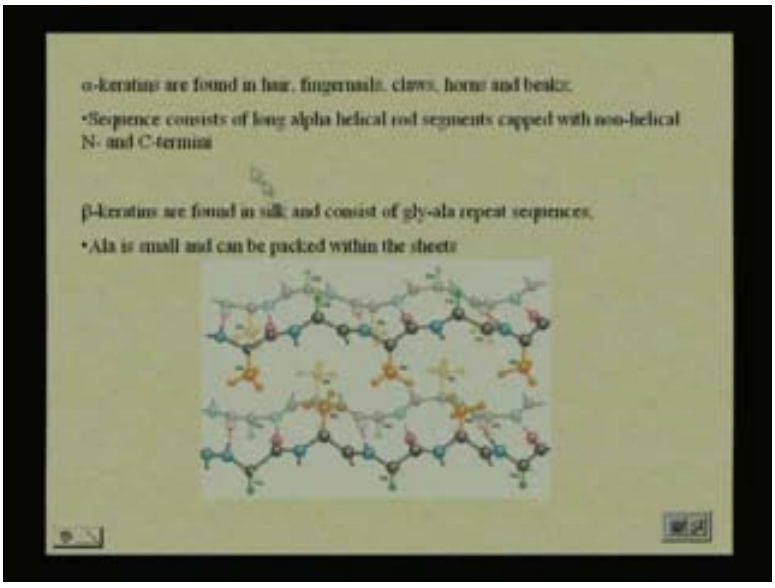

So you can have alternating Glycine and Alanine. The reason being is that they are small in size and they can be accommodated in the β-sheet structure of β-keratins.

So the α-keratins are the ones that forms the alpha helices, β-keratins are the ones that forms these beta sheets that consists of a Gly Ala repeat sequence. And in the α-keratins that are found in hair we will see longitudinal parallel alpha helices that give mechanical strength to the fibers that are found (Refer Slide Time 04:41 min).

Now considering the Globular Proteins, usually they are classified according to the type in the arrangement of the Secondary structure. So what you find is you will probably see anti parallel α-helix proteins that consist of only alpha helices. You might find β-sheet proteins, you might find α + β or α/β proteins. There are different types of nomenclatures depending on how the protein actually looks.

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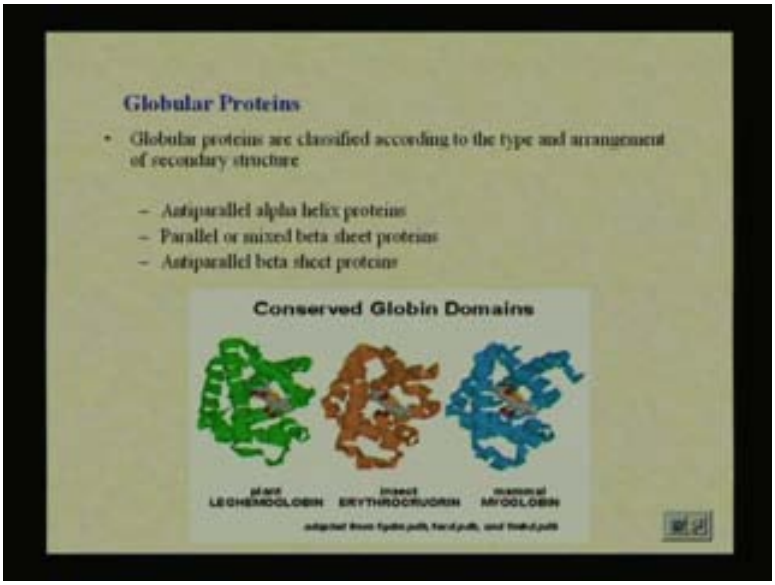


So in this case you would have a largely alpha helical protein. For example when you look at a Globular Protein you are looking at essentially the globular structure of the protein. Now if you look at this case these are all globins, they are all globulin proteins. This one is from plant, the middle one is from the insect and the last one is from a mammal (Refer Slide Time 6:45 min).

What do you notice here? You notice that each of them have very similar structure. The reason being is they are going to perform the same function. So whether it is from a plant or an insect or a mammal these globins perform similar functions because they have similar structures.

now today what we mean to study is what keeps the structure together because now we know that the only types of covalent bonds that we have in proteins or the linkages

between the amino acids that give the peptide bonds and the disulfide linkages that are covalently linked together which can bring different parts of the chain together (Refer Slide Time 7:38 min).

Now we also know the alpha helices and the beta sheets that also bring parts of the structure together and they are connected by the hydrogen bonds but not by covalent bonds.

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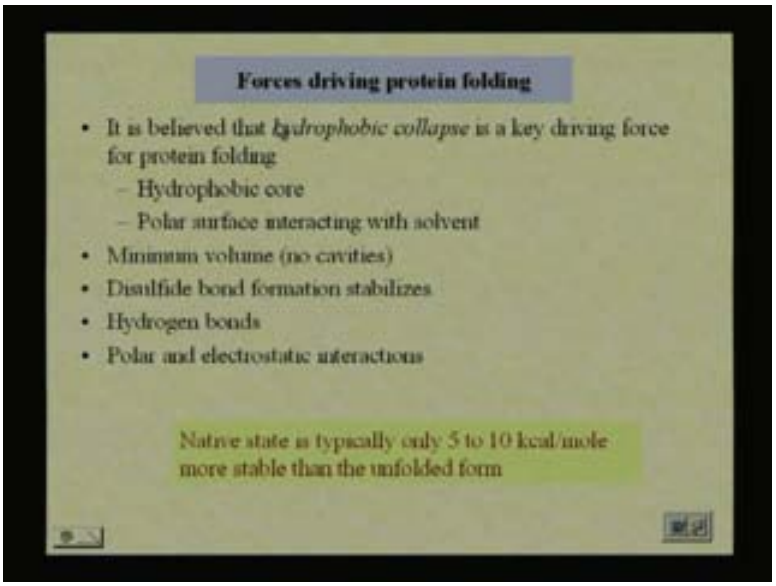


So now we need to know what the forces are going to drive protein folding. Because now we do not have polypeptide chain anymore we have only a folded protein structure. And what we need to know is how this protein folded structure is found.

Here there are certain terminologies that we have. In which one is called *hydrophobic collapse*. This means you know the core of the protein is hydrophobic in nature so it may so happen that initially when the polypeptide chain is formed then there is a hydrophobic collapse which means there is a hydrophobic core formed first and then the rest of the protein chain will gradually forms alpha helices or beta sheets or interconnectors such as are loops or turns to bring about the structure (Refer Slide Time 8:44 min).

So initially you will have a collapse, the polar surface is going to interact with the solvent. And we will have gradual formation of the alpha helices. There may be another case where the alpha helices are formed first. So first they would be local interaction followed by gradual collapsing of the overall structure.

Here what you are looking is for Minimum volume where you do not have very large cavities because that would open up the protein. The Disulfide bond formation will stabilize the protein because it will bring different parts of the polypeptide chain close to one another. Then you have Hydrogen bonds which will bring different part of the

protein structure together. Again we have polar electro static interactions. Then we will consider how actually form these ionic interactions or other polar type hydrogen bonding between amino acid residues. (Refer Slide Time 9:46 min).Now what we have is an unfolded form that was the polypeptide chain and we have a native folded form of the polypeptide chain.

If we look at the hydrogen bond see how it stabilizes the interactions. For example in alpha helices and beta sheets you recognize that here we do have a lot of hydrogen bonds and what happens is the accumulation of these hydrogen bonds renders a large amount of stability to the protein. Because you have not just one hydrogen bond you have a series of hydrogen bonds both in alpha helices as well as in beta sheets. And also you can recognize that in anti parallel case the hydrogen bonding is between similar amino acid residues.

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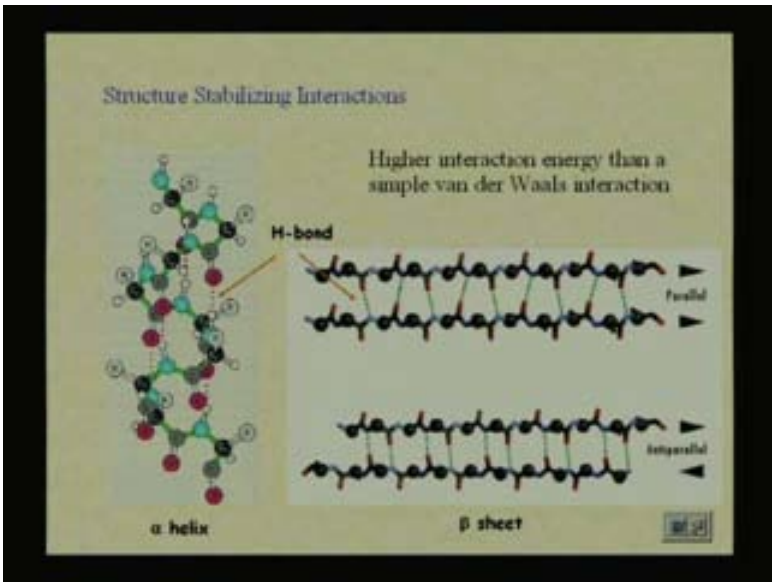


And what happens in the parallel case is they are between different amino acid residues. So what are the structure stabilizing interactions? The Covalent Interactions but now we also know that we have these Disulfide bonds which are actually formed from the link of two Cysteine amino acid residues in forming a cysteine bond where we have this SS linkage. These can be very far apart in the structure and bring the polypeptide chain close to one another.

So we would have a structure stabilizing interactions in the form of covalent bonds which are in the form of disulfide bonds of Cysteine residues. (Refer Slide Time 11:42 m min). The other cases are The Van der Waals forces that are weak electrical interactions, they are transient in nature. We have hydrophobic interactions where they are clustering of non polar groups together like we form hydrophobic core of the protein. Then we have Hydrogen bonding. What do have in that case? We have a donor atom that has hydrogen attached to it and we have an acceptor atom. We have hydrogen atom that is sort of

partially shared because of the acceptor atom and the donor atom having partially δ- charges and the hydrogen being δ+ charges.

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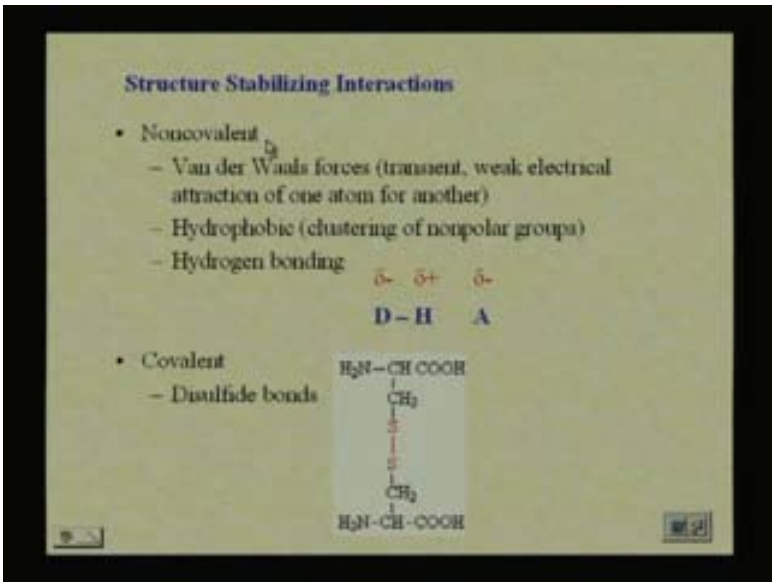


So now what are all these interactions? All these interactions are non covalent in nature, there is no covalent linkage. But what happens is when you add up all these you will have a substantial amount of energetic favorability for the folded conformation.

Now what do we have here is the hydrophobic collapse or a hydrophobic effect. When we have the non polar solute then there is no possibility of hydrogen bonding because we do not have any polar group or any group that can act as a donor. We should have a donor and an acceptor. But in the case of a polar solute we see that there is a possibility of hydrogen bonding (Refer Slide Time 13:23 min).

So what would be expected on the surface of the protein is all these polar residues that can interact with the solvent with preferentially be on the surface of the protein. However the non polar solutes that have no such interaction with the solvent around it would tend to be in the central part of the protein or rather in the hydrophobic core of the protein.

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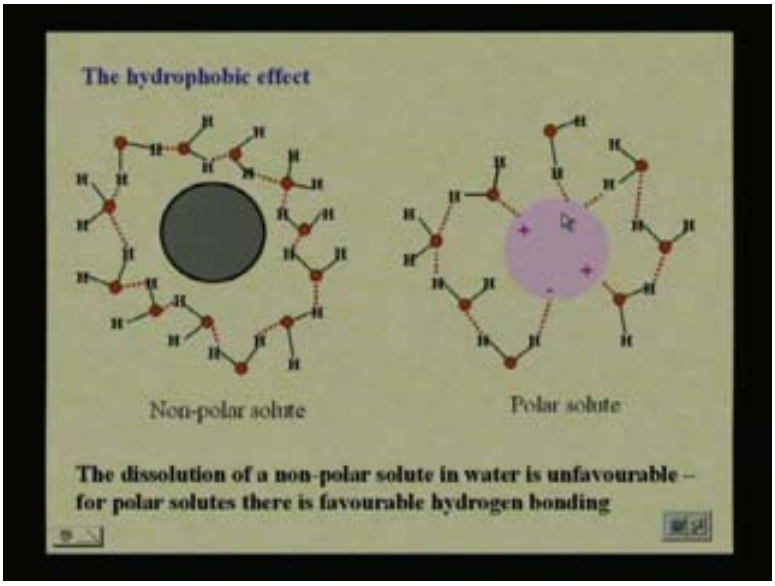


This is an example of a Structure Stabilizing Interactions. What do we have here? We have an ion pair. An ion pair would link a Lysine and an Aspartic acid together. It is not always possible for all the Lysines or all the Aspartic acids or all the Basic and Acetic charged amino acid residues to be on the surface.

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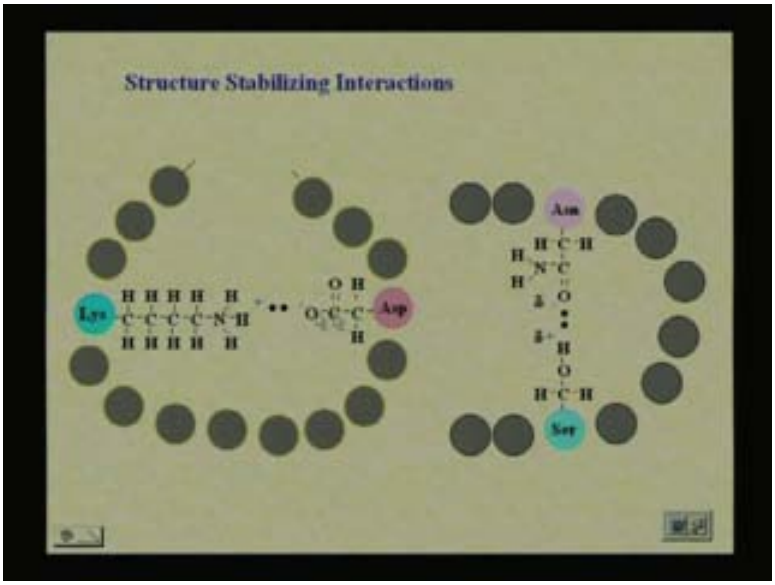


The reason being is that it follows a large hydrophobic region so that would tend to be in the core. Then the Covalent bond is not going to break. But the Lysine is there so what it is going to have is an ionic interaction or an ion pair formation with a charge of an opposite kind in the protein chain itself. So that will effect the folding because the Lysine

is going to be looking for an Acetic amino acid residue closed to it so that it can form this ion pair. So that is going to change the folding in a sense, it is going to effect the folding. For say you had all of these hydrophobic in nature then the nature of the folding would definitely have been slightly different than what you see here (Refer Slide Time 15:11 min).

What do we observe here is a Lysine which is a basic amino acid residue have an ionic interaction and ion pair formation with an Aspartic acid residue. This is a case where you can have hydrogen bonding. Here we do not have an ion pair formation but we have a donor that is Serine and we have an acceptor that has the carbonyl oxygen of the amide group of the Asparagine.

So this would be the formation of a hydrogen bond, this would be the formation of an ion pair. Both of these are extremely important in forming a final protein folded structure (Refer Slide Time 16:03 min). Now, we are considering the energy terms. We lets just look at what energy terms we can have. If we are looking at energy then we can have the total energy can be covalent or we can also have a non covalent component to this.

Now under the covalent ones we have normal bond formation that is going to contribute so we have bonds. What else we have? There is a contribution from angles and there is a contribution from torsional angles. So we have energy contributions from these three components under the covalent set because these are formed from direct linkages (Refer Slide Time 17:23 min). Under the non covalent ones we have listed a hydrogen bond, we listed an ionic interaction and we have listed Van der Waals interaction.

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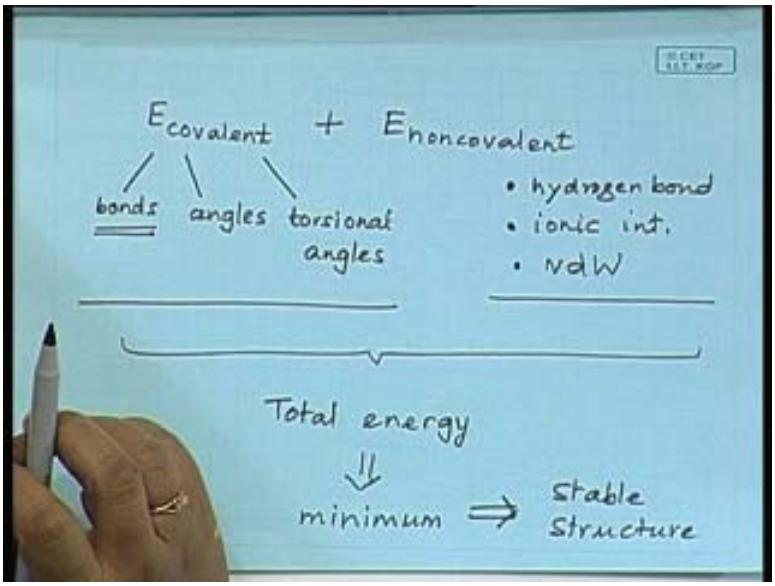


So these are the three that we could have in case of a non covalent formation and these are three that we are going to have in case of covalent bond formation. And summing these together we are going to get the total energy.

Now what we do we want this total energy for the protein to be? We want this to be a minimum. So we have a stable structure. So we have to get a minimum of total energy which is going to result in a stable structure and this structure is a final native folded form of the protein.

Now what are these in terms of energetics? If we look at the bonds, how do you represent bonds normally for a compound? You represent them as springs. So in case of bonds we represent these as springs and they follow Hooke's law. The Hooke's law is when you have two masses connected by a spring they are going to give some energy. So that is this energy.

What is this energy due to? It is due to stretching energy, this follows Hooke's law. Here r is the variation that you will get because the bond is not going to stick to one specific distance. It acts like a spring because it is going to form certain interactions. So it has to acts like a spring where it is going to have some favorable interaction which will try to adopt or it will have some unfavorable interaction which is trying to get away from. So what do we have here is we have covalent in which we have a bond and this bond is going to give us an energetic contribution to the total energy in the form of Hooke's law. We have a Bending for the angles that is going to contribute to the energy also. These are called energy terms that is added to the total energy that the protein is going to have (Refer Slide Time 20:27 min).

Now you understand that this is going to get extremely complicated if you want to do a computation because the number of atoms is huge. But nevertheless these are the components that found the total energy. Then we also have the Torsional energy. You need to know what the contributions are. You need to know that the covalent terms are come from the Stretching, the Bending and the Torsional. Then we need to know about how the non covalent terms are going to come. In these we will have an ionic interaction, we are going to have a Van der Waals interaction and we are going to have hydrogen bonding (Refer Slide Time 21:13 min).

So for Van der Waals interaction all of you know about Leonard Jones potential curve where we have an attraction due to the negative part of this. This is called as a six twelve potential where we have a contribution for attraction and a contribution for repulsion because you know that these atoms have repulsive force acting on them when they get too close to each other. Then we have the Coulomb interaction which is due to the ionic interaction between the two ion pair formers.

Finally we have the hydrogen bonding. So this is another feature where we have the hydrogen bonding and again we have ten twelve potential in the case (Refer Slide Time 22:13 min).

Here we are about the total energy that a polypeptide would have based on its energy terms? Now what are these energy terms? We have energy terms contributions from the covalent component and contributions from a non covalent component.

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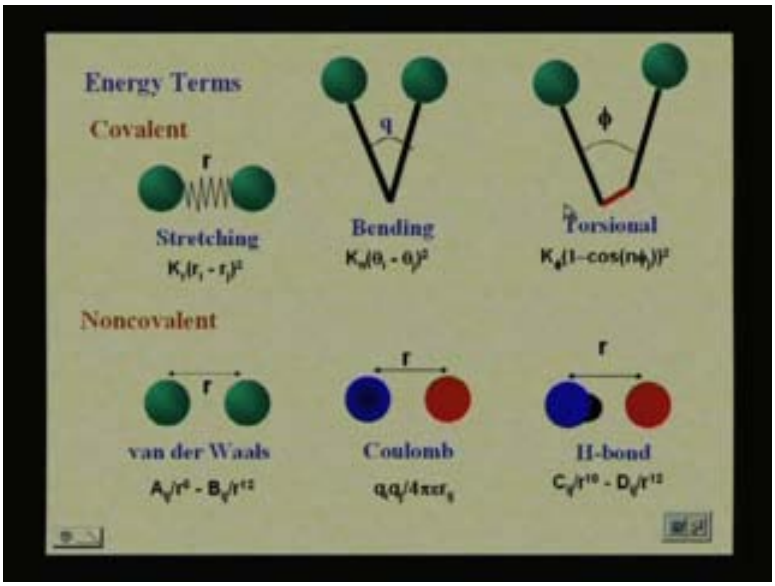


The covalent component is comprised of three parts and these three parts you have to remember from the name itself. These are covalent because they are directly linked. You have to remember that if we are talking about a spring means the two atoms are directly linked to one another, if we are talking about an angle bending energy these are linked to one another, if we are talking about a torsional energy the atoms are linked to one another. But none of them are linked in a non covalent formation. I do not have any bonds linking the two atoms that have been drawn in the non covalent set (Refer Slide Time 23:26 min).

So when we are talking about a covalent contribution we are talking about energy contribution from the parts of the protein structure that are linked to one another in which one is the bond, second is the angle and the third is the torsional part. We have the Van der Waals interaction, the coulomb interactions and the hydrogen bonding for the non covalent set.

If we look at the interactions and their approximate bond strength in kJ/mole you will see that Covalent bonds are extremely strong, Ionic bonds are 20 to 40 kJ/mole, Hydrogen bonds are approximately 5 to 10 kJ/mole, hydrophobic interactions are 8 kJ/mole and Van der Waals are approximately 4 kJ/mole. But when we consider the combination of the large number of hydrogen bonds, Van der waals, hydrophobic interactions that are possible they are add up together making it a strong folded structure because of the multitude of interactions that are possible (Refer Slide Time 24:41 min).

Now what I have listed here is an energetic contribution. Suppose you have to do a calculation to figure out what the total energy of a protein was then you would use a force field. Since we consider the different energetic terms, I just want to emphasize here that

when you are calculating the total energy you have to find the summation of all the possible variations from equilibrium bond length for bonds.

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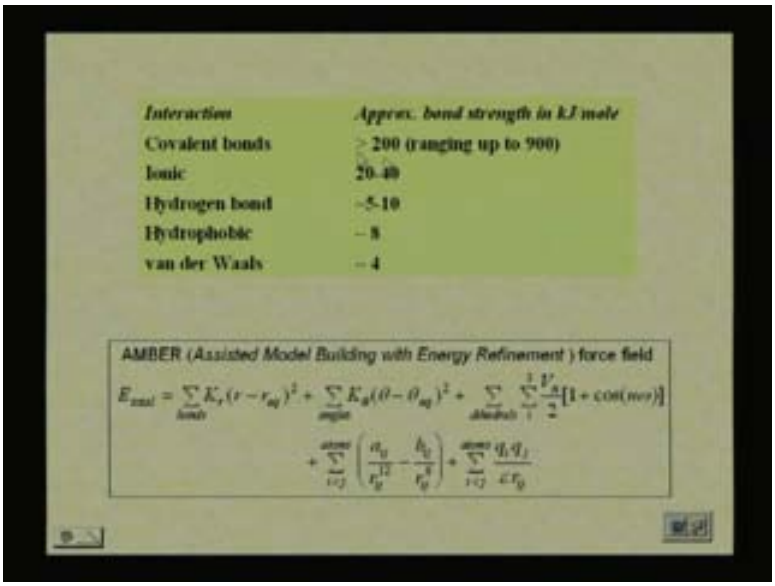


So now we are looking at all the possible covalent interactions the bonds, the angles, the dihedrals. In this is case this is a very crude one because here I have not considered hydrogen bonds. What do I have here is this is for Van der Waals and this is for Coulombic interactions. but here I am missing out hydrogen bonds because you understand that when you are doing a computational calculation it is going to be extremely difficult to try and calculate all the bonds because as soon as you form a dipeptide you can recognize how it is going to change or increase the number of bonds, increase the number of angles and increase the number of dihedrals. (Refer Slide Time 26:12 min)

So we have an extremely large number as we go from a single amino acid to a dipeptide to a tripeptide. If you consider even the smallest protein that would have approximately thirty amino acid residues would be extremely difficult to compute. Because you can understand the multitude of interactions that are going to form based on the number of atoms that the protein is going to have.

So we have a final native structure. What is this native structure? We have a well defined 3D structure, it has a specific function and we remembered we spoke about the specific *Isoelectric point* due to the type and number of amino acid residues it has (Refer Slide Time 27:05 min).

So what are we going from? We are going from a relatively unfolded structure to a folded structure. So basically I spoke earlier about levinthals paradox where they were a multitude of open conformations that was possible for the polypeptide chain but it forms the single native structure. So what is happening now is when it forms the native structure

we have all these interactions that are taking place which are hydrogen bonds in the alpha helices.

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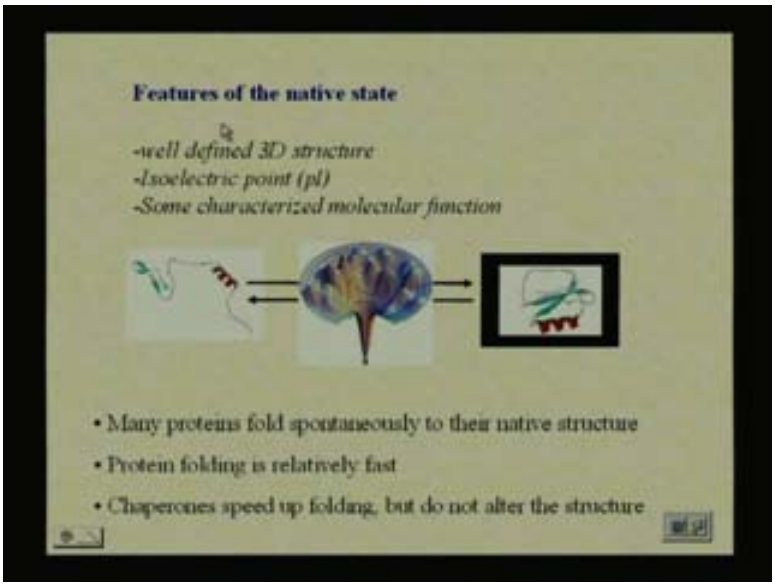


Hydrogen bonds between the strands of the beta sheets. Then we can have the other interactions that are going to form the covalent interactions are disulfide interactions, hydrophobic interactions, Van der Waals interactions and other Electro static and Coulombic hydrogen bonds and all others between the different amino acids in the protein (Refer Slide Time 28:10 min)

Now we will consider the Thermodynamics of protein folding. If we consider an initial and final state, what do we have is a $\Delta G$. What is this $\Delta G$? We want to find out the free energy change due to folding of the protein. So initially we had an unfolded state that is G initial. Then we have a folded state so that is G final. So how we find the $\Delta G_{folding}$? We know that the G final which is $G_{folded}$ minus G initial which is $G_{unfolded}$. We all know that $\Delta G$ is $\Delta H - T\Delta S$ (Refer Slide Time 28:55 min).

So I have a $\Delta H_{folding} - T\Delta S_{folding}$. So the $\Delta H_{folding}$ is again going to be the H final minus H initial so it is $H_{folding} - H_{unfolding}$. Now I am doing this at a particular temperature so the temperature here is constant.The $\Delta S$ are $S_{folding} - S_{unfolding}$ because the unfolded is again my initial state and the final is folded state (Refer Slide Time 29:29 min).

Now we are going to look at the specific terms of these. What happens to the $\Delta S$ which is $S_{folding} - S_{unfolding}$ when I consider the entropy. We know the entropy is the disorder of the system. If I have an unfolded protein and now I am having the protein fold I am bringing ordered into the system. Now if I will bring order into the system then will be $\Delta S$ is negative it is not positive any more because I have ordered the disordered polypeptide chain into an ordered folded native structure. So the $\Delta S_{folding}$ is negative. So what happens

to TΔS? Therefore -TΔS is a positive quantity because my $\Delta S_{folding}$ is negative. So -TΔS is a positive quantity which is I have drawn here.

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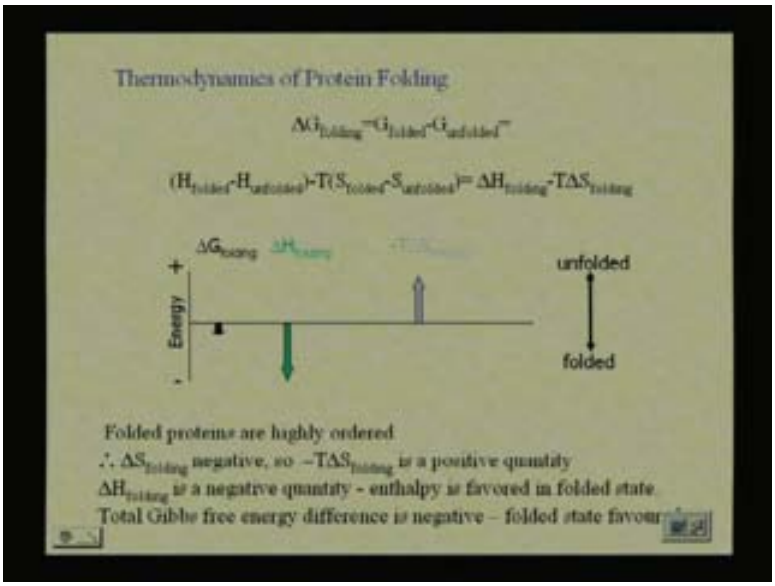


So if we look the energy on the Y-axis and just this axis is the energy axis here. We have an unfolded form here and a folded form here. Now obviously the entropy of an unfolded form is going to be much more than entropy of a folded form. So the overall -TΔS is going to be positive but the enthalpy or the folding interactions that are found are all favorable. Because the hydrophobic interaction is going to be favorable, a hydrogen bond is going to be favorable and ionic interaction is going to be favorable.

But what is not going to be favorable is if I have a specific hydrophobic interaction trying to make with the solvent. That is going to give an unfavorable situation. But for most of the cases in the folded protein I am going to have a large number of interactions contribute to the enthalpy and this multitude of interactions which makes this a high negative number (Refer Slide Time 31:44 min).

So what happens is these more or less compensate one another and I eventually have a favorable ΔG of folding. We have a ΔG of folding and ΔG of folding is going from an unfolded state to a folded state. So I have to consider in the thermodynamic quantities $\Delta H_{folding}$ - $T\Delta S_{folding.}$

I know that the entropy of a polypeptide chain in the open form will be much more than when it will be in its closed folded form. So the $S_{folding}$ is negative which makes my - $T\Delta S_{folding}$ is a positive quantity. Then I have $\Delta H_{folding}$ which compensates for this positive ordering of the system because of the large number of favorable interactions that are formed on protein folding. And we have a $\Delta G_{folding}$ that is a small negative quantity which means that this is a favorable spontaneous process. So I am going to get from the unfolded form to the folded form in a spontaneous process because the ΔS is not going to

make it spontaneous it is the enthalpy that is going to make it spontaneous (Refer Slide Time 33:12 min).

Now we move on to the Thermodynamics of the Protein Folding. If we have considered the $\Delta G_{folding}$ we have the initial part which is an unfolded part and the final part which is the folded part.

So when we are considering the $\Delta G$ we go from an initial unfolded form to a final folded native structure. So the $\Delta G$ is G final that is $G_{folded}$ minus G initial that is $G_{unfolded.}$ If we consider the form of $\Delta H_{folding}$ - $T\Delta S_{folding}$ we recognize that we can also open up these into the initial and final components. So the $\Delta H_{folding}$ is going to be H final that is $H_{folded}$ minus H initial that is $H_{unfolded}$. Similarly for $\Delta S$ we can have $S_{folded}$ - $S_{unfolded}$ (Refer Slide Time 34:17 min).

Now we considering the entropical considerations if we go from an unfolded form which is disordered to a folded native conformation so the entropy contribution $\Delta S$ will be a negative quantity because we are ordering the system. A positive quantity is when we have more disorder. A sciatic situation would give you favorable entropy. But we are bringing order into the system because we are folding the polypeptide chain. So our $\Delta S$ is negative so $-T\Delta S$ is a positive quantity which is what we have here it is positive in its energy contribution.

But the $\Delta H_{folding}$ is a negative quantity due to the different interactions that give favorable energy to the folded conformation. We have this positive energy which is due to the entropical considerations compensative by the enthalpy due to the large number of interactions possible (Refer Slide Time 35:30 min).

So the over all $\Delta G_{folding}$ is very small but negative quantity meaning that you have favorable spontaneous folding from the unfolded to the folded native protein. Now we will consider the native state and the denatured state, this is in an equilibrium situation. The beginning of the proteins begins at N and it ends at C so here we have a disordered orientation which is also called as an unfolded state or a denatured state and we have a native state in this case.

So if we have a $\Delta G$ associated with it then we are going to have an equilibrium associated with it. So we have a K that is -RTln[K], the K being the equilibrium between the denatured state and the native state. So the stability of the native state defined as difference in free energy between the native and the denatured states.

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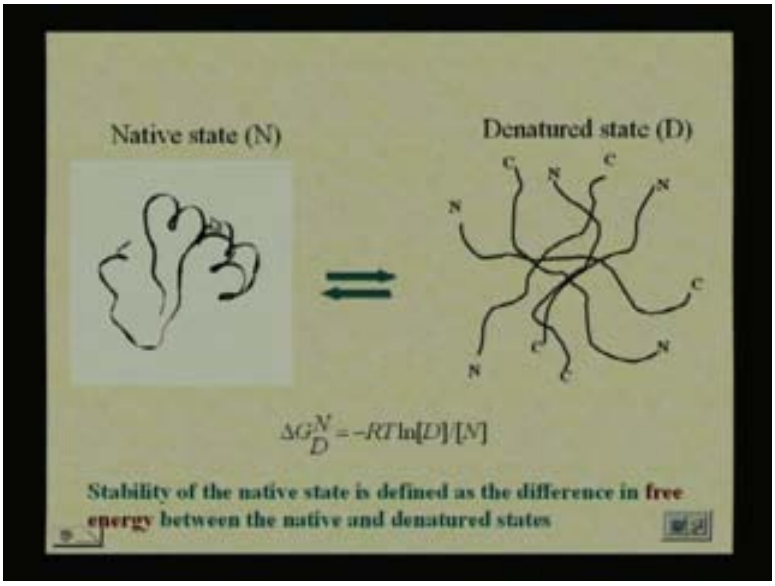


So what do we have in the native state? We have a compact structure, intra molecular non bonded interactions. And the entropy significantly decreased because of the well ordered conformation. What the features do we have for the denatured states? We have a non compact structure, we have inter molecular non bonded interactions and we have a large $\Delta S$ due to the large number of different conformations.

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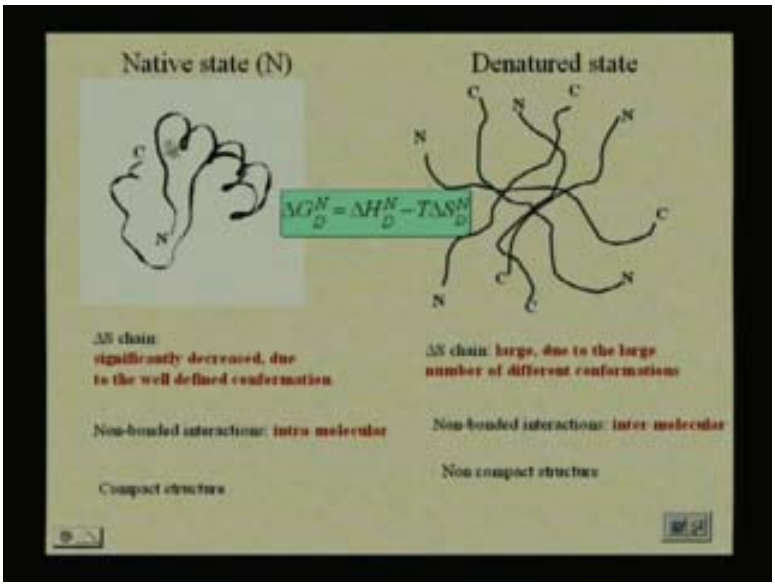


All of these contribute to the $\Delta G$ of the system, the $\Delta G_{folding}$ which is going to be a small but negative quantity because we are going to have spontaneous folding from denatured state to the native state.

Now we will consider the different interactions that we had. What were the different interactions that we had? We had Hydrogen bonding, we had Electro static interactions, we had Van der Waals interactions and we had Hydrophobic type interactions.

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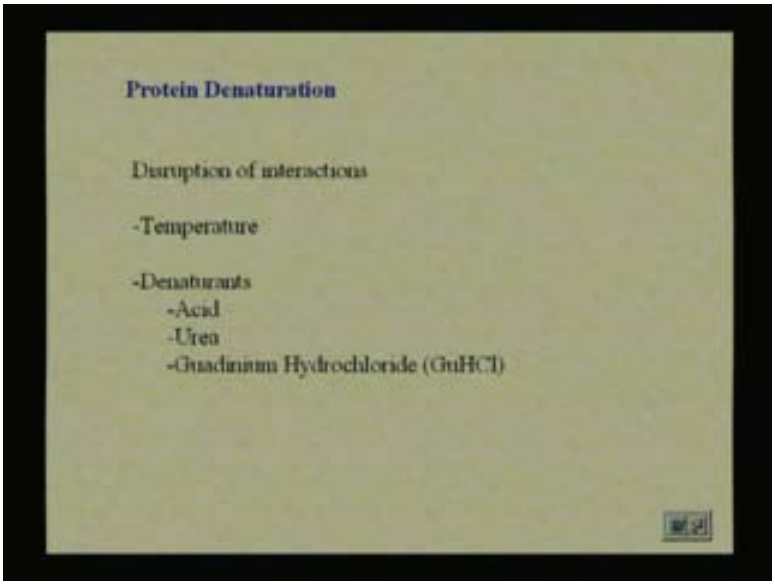


I can denature the protein if I add certain denaturants to it or increasing the temperature. We have to consider that the disrupting the actual interactions that were holding the protein folded together in the process of denaturing.

So if I look at all the different conformations that I had so if I had a protein helix structure and I had some sheets then in this folded structure as I add a denaturant say I add Urea where Urea disrupts the hydrogen bonding. Now if I disrupt the hydrogen bonding of an α-helix what is the eventually going to do is it is going to open up the helix because the hydrogen bonds that were present here would preferably found with urea. So urea sort of gets into the protein structure disrupts the interactions that were holding the folded or the protein polypeptide chain together (Refer Slide Time 38:44 min).

Now what happens if I heat it? Again if I heat this, for example you know that if you heat albumin like egg what happens you form a solid mass. Basically what you are doing is you are denaturing the protein. So if you have a solid globular albumin structure when you heat it up so you are decreasing the solubility or aggregating it because you are opening up the polypeptide chain. You will have this mass form by opening up the polypeptide chain. And so each of these structures that are supposes to look identical are opening up into a denatured form forming a solid mass of aggregate. And you have now a denatured protein. This is a denaturation due to the temperature. So here essentially what we are doing in protein denaturation is we are disrupting all the interactions that were possible. And these interactions were Hydrogen bonding, Van der Waals interactions and Ionic interactions.

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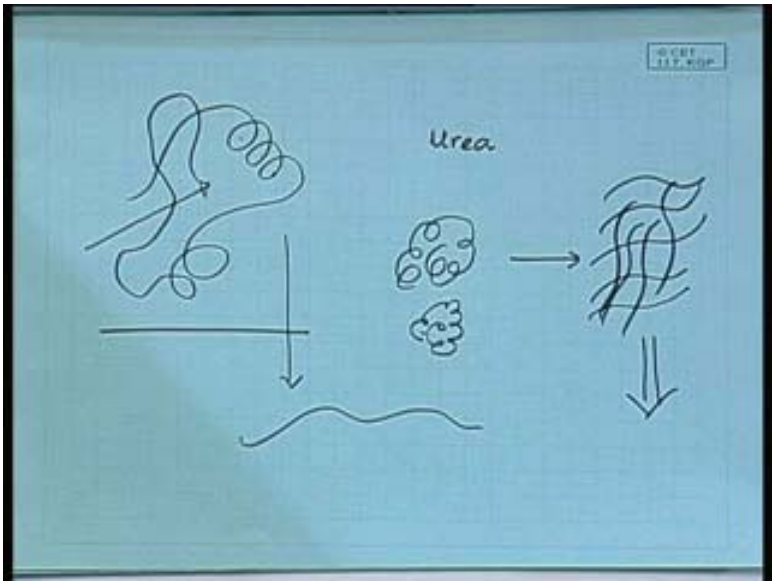


We can be denatured the Proteins by increasing the temperature. We can also add different denaturants for example we could add Acid or we could add urea or we could add Guadinium Hydrochloride.

What is acid going to do? How is acid going to change the components? How is going to disrupt the interactions? (Refer Slide Time 40:27 min). So if I add Acid means I am adding $H^+$. What happens when I add H plus? For example all the groups are acidic groups which comprise for example Aspartic acid or Glutamic acid so what is going to happen to them is they will get protonated. So if they get protonated on addition of acid then we will get this (Refer Slide Time 41:13 min).

Now if this Aspartic acid or Glutamic acid happens to be involved in ionic interaction with say Lysine then it would disrupt the ionic interaction. So now we are adding acid to our protein. When we add acid to a protein any acidic group that had the free carboxylic acid group in its ionized form would get protonated. And what happens is if this Aspartic acid or Glutamic acid that could form an ionic interaction with basic residues they would no longer form. Once I get into this form it is not possible for the ionic interactions to form (Refer Slide Time 42:13 min).

So any folded structure that would have this ionic interaction with an Asp and say Lys what would happen is this would no longer formed so that would open the chain. So eventually the idea here is that when you have a denatured structured you are disrupting the energetics. Usually denaturation does not mean that you are disrupting the covalent bond formation.

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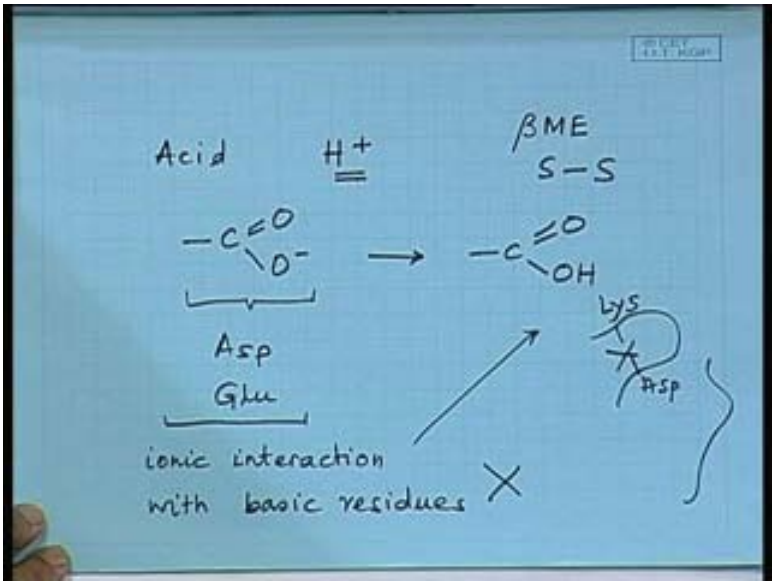


So if you had a disulfide linkage you would not actually disrupting unless you would put in same β-Mercaptoethanol or you would have some other reagents that would break the SS linkages because that you are remembered as a covalent linkages.

Also you are not breaking the peptide bond. The polypeptide chain is intact so what we can do is we can go from a folded form to an unfolded form by denaturing the protein by adding Urea. You can also renature the protein in some cases. So, what you would have to do is you would have to remove the Urea if you want the get back here and in some cases you can reform the Folded Protein.

So you are going from the native folded structure to an unfolded structure and you reform this back. So this would be +urea and this is –urea. Here again you could form folded structure back (Refer Slide Time 44:07 min). So what we learned today was we learnt about the Protein folding, The Energetics of Protein folding where we have Covalent and Noncovalent contributions where the Covalent contributions comes from bonds anything that is connected that are bonds, angles, dihedrals and Noncovalent contributions come from Ionic, Van der Waals, Hydrogen bonds. These all are going to contribute to the Energetic of Protein folding. (Refer Slide Time 44:57 min) Then when we have considered the Thermodynamics of Protein folding in which we have a $\Delta G$ associated to the folding, we have a $\Delta H$ associated to it and we have a $\Delta S$ associated to it.

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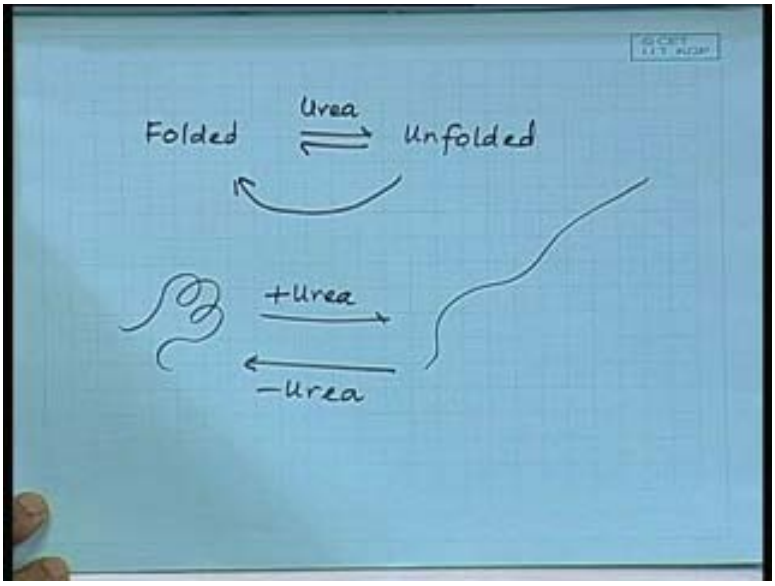


So all of these fall under the per view of protein folding where we are considering the Thermodynamics the ΔG, the ΔH and the ΔS and we have an equilibrium consideration here as well.

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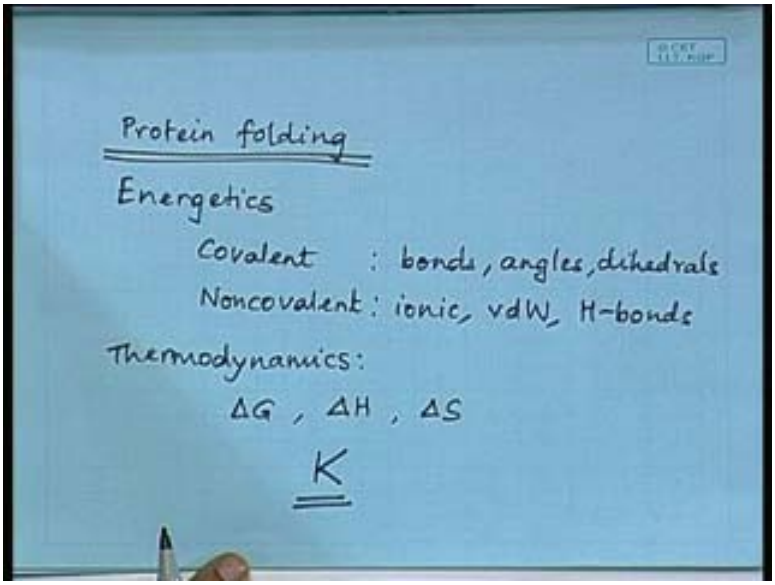


We will stop here. Thank you.