Bio-Physical Chemistry
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Lecture - 14
Hydrogen bonding

Well start with the topic of Hydrogen Bonding which we just touched yesterday. One of the very important forces stabilizing a protein in its native state right because secondary structures like alpha helices, beta sheets they all have hydrogen bonds like intra helical in case of alpha helix or intra strand in case of a beta sheets.

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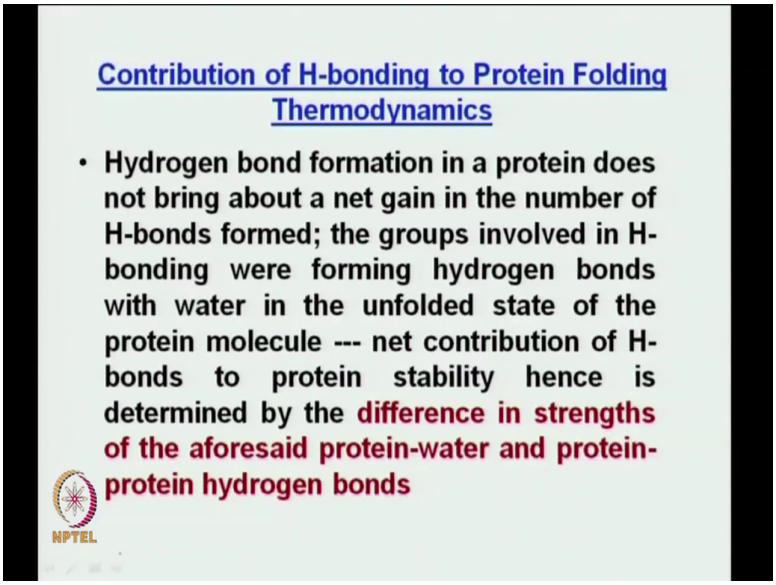

But before we talk about hydrogen bonds though there are two things we have to keep in mind. For example, you know hydrogen bond formation in a protein does not bring about a net gain in the number of hydrogen bonds formed. Essentially because when you had the

protein in the unfolded state, the groups that were making hydrogen that are making hydrogen bonds in the stable state of the protein on the native state of the protein were also making hydrogen bonds with the surrounding water molecules in the unfolded state.

So, essentially there was still making hydrogen bonds. It is not that you are introducing a new system or a new condition where they start to make hydrogen bonds so; they are already making hydrogen bonds. So, the final stability is actually a balance or it is determined by the difference as its said of here in strengths of the aforesaid protein water and the protein-protein hydrogen bonds.

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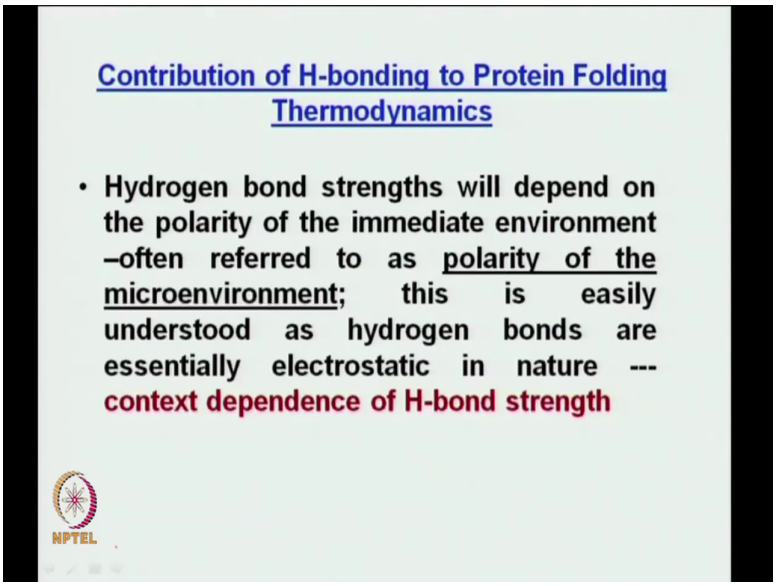

Now, that is one. Now, the next is depending upon the protein in question or any protein as such your hydrogen bonds can be at different places. For example, you can have a hydrogen bond which is the surface of the protein; you can have a hydrogen bond which is in the

interior that is a core of the protein. If it is on the surface then, you can imagine it to be more exposed to the surrounding water molecules hence; it will be interaction with water.

If it is in the core then, the obvious effect is that the dielectric constant in the core of the protein would be small right because it is essentially a non-polar core. And it might be that the hydrogen bonds, the strength of the hydrogen bonds or even the length as a matter of fact would be depending upon where these hydrogen bonds are actually formed inside a protein.

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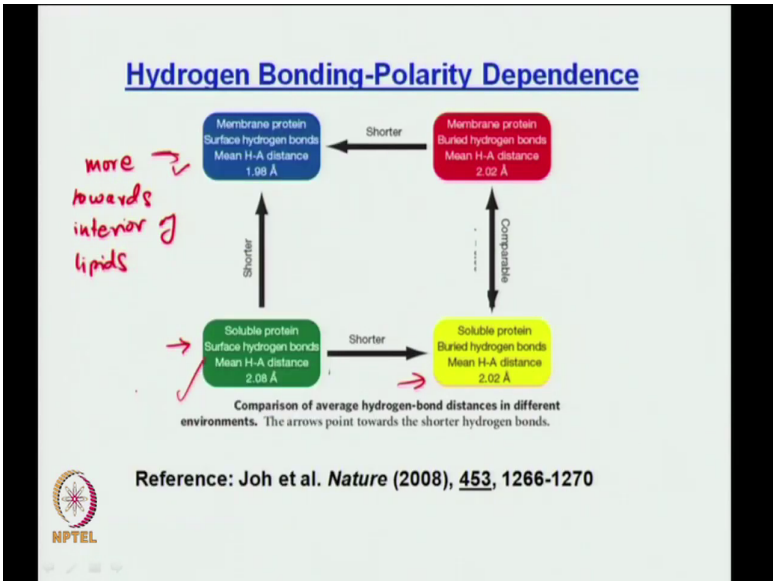

So, there was a very nice paper in Nature which talked about this effect and what they said was which we saw yesterday, that if you start from you know this very bottom you can see that out here, you have the soluble protein surface hydrogen bonds. Now, these hydrogen bonds have an average distance of about you know 2.1 angstrom 2.02 angstroms.

Now, when you go to the same soluble protein, but now you have you have hydrogen bonds which are buried, so I am talking about this now, you have buried hydrogen bonds. If you have buried hydrogen bonds; that means, there the dielectric constant is low and immediately what you see is the mean hydrogen bond distance has decreased to 2.02 angstroms.

Now, hydrogen bonds can still essentially be considered to be electrostatic type bond. Hence, if the dielectric constant is low; that means, the screening between these two charges or electrostatic interaction would be lower and thus, the hydrogen bond strength would be more. Thus, the distance of the hydrogen bond is lower.

Now similarly, if you go to a membrane protein so, the bottom one is constant is we are talking about a soluble protein if we go to membrane protein now, you know membrane proteins typically would be in hydrophobic environments. For a membrane protein, if you talk about the surface hydrogen bonds I cannot show you now, but the way it goes is the surface hydrogen bonds are oriented in such a way that they point towards the interior of lipid molecules ok. So, this so, these ones are more; so, these ones; so, these ones are more towards interior of lipids.

Lipids are the ones which actually constitute membranes. So, lipids you know are highly nonpolar and if you compare this to a protein core, in protein core you can still have some polar side chains or some polar amino acids. But in case of a lipid, its hydrocarbon tail right, fatty acids hydrocarbon tail and that is why the dielectric constant in this case is the lowest among all the four and you can see accordingly, the hydrogen bond length is 1.98 angstrom it is the shortest.

And the last one is membrane protein buried hydrogen bonds, see whether you are talking about a membrane protein buried hydrogen bond or a membrane protein or a soluble protein buried hydrogen bond, the hydrogen bond is buried; that means, it is in the core of the protein and the core of the protein both the cases essentially the same.

So, there is not much of a difference. That is why, this buried hydrogen bonds and this buried hydrogen bonds in both membrane soluble proteins typically have the same mean hydrogen bond length. So, this is from I have taken this picture from following reference given below ok.

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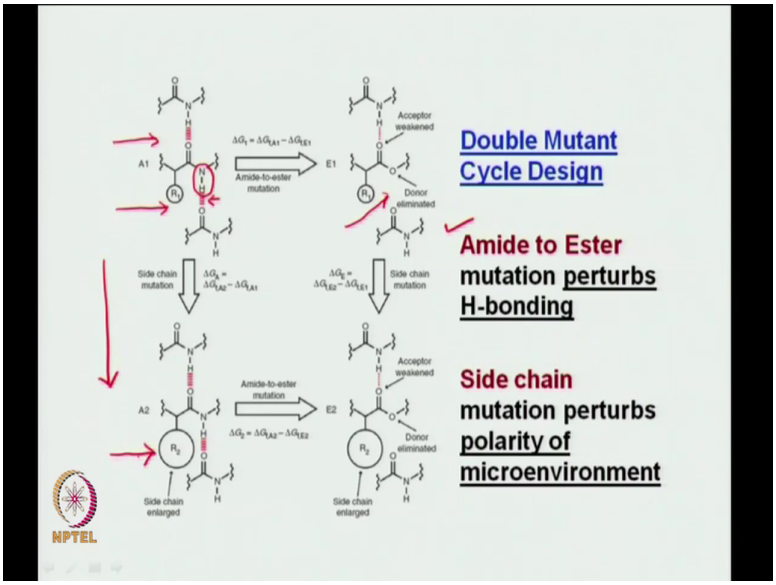

Now, what is the next discussion to be on? So, we have just seen the context dependence of hydrogen bond structure, but how can we prove it? To understand it, you know it is easy to understand, but how easy it is; how easy it is to prove or can we actually prove it.

So, group of researchers essentially from the group of Jeff Kelly is its Scripps Research Institute what they did was, they did a double mutant cycle design right and we will discuss

this at some length. So, he did two things, one was why is it called a double mutant and if you look at this, I will it will be more clear in the next slide.

There are two aspects one is there was he did an amide to ester mutation. Now, if you look at; if you look at this portion so, if you look at this portion right and say if you look at this portion. So, I am talking about the hydrogen bond here ok, I am talking of the hydrogen bond this one. So, this is all amide; that means, you have NH, CO and all those things.

Now, what they did was, here see this NH, this particular NH was replaced by an ester linkage O and the moment you do that, you lose H right because you do not have H now and hence, you can see you have disrupted the hydrogen bonding here, there is no hydrogen bonding here right so that means, here as it says donor is eliminated you now have no hydrogen bond. So, this is you have done, you have disrupted the hydrogen bonding by doing an amide to ester mutation.

Now, that was you have distorted the hydrogen bonding right. The other thing is, if you come down; if we come down you can see this R 1 is being replaced by another bigger side chain R 2. If it is a bigger side chain, a more hydrophobic side chain what does it mean? That means, R 2 gives rise to a more non polar environment than R 1 see here we are coming to a context dependence term and also on the same one R 2 you do the same amide to ester mutation and then, you look at the thermo dynamics.

But what it means is, why is it called a double mutant? Because there are 2 mutants. What are the mutants? One is the amide to ester mutation the other one is R 1 going to.

Student: R 2.

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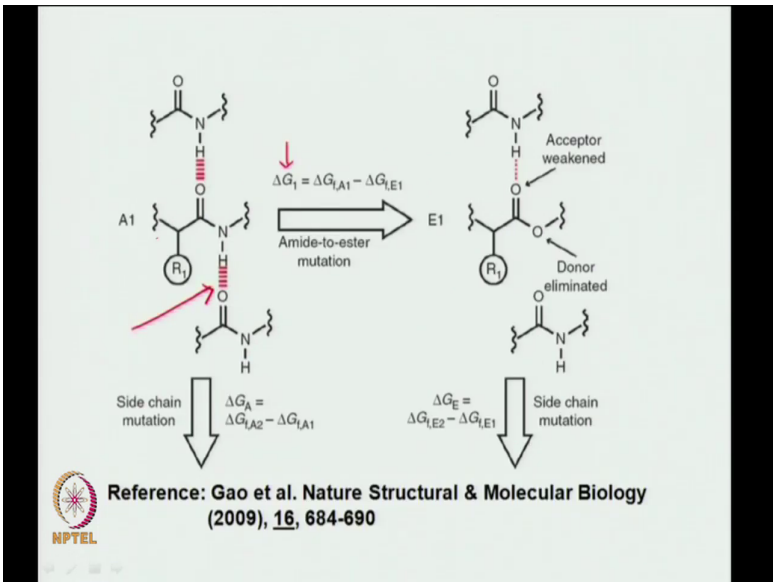

R 2. That is why it is called a double mutant ok. So, it will be a little more clear in the next slide, we will zoom into it now. So, here so, again look at this, this is the one we are talking about the hydrogen bond perturbation right. So, we are perturbing the hydrogen bond by forming the ester, that is why it says donor eliminated on this side right and it says the acceptor is weakened, but you know let us not worry about the specifics, but look what they doing here so, this is important this delta G. What is this delta G?

So, this delta G 1 is equal to delta G f, A1 minus delta G f, E1. Now, f stands for folding free energy, we are not unfolding the protein what are we are doing is, we are essentially looking at the difference instabilities of the two proteins in their folded states but in one case, you have the amide in the other case the amide is replaced by the ester that is what you are looking at. So, that is one right.

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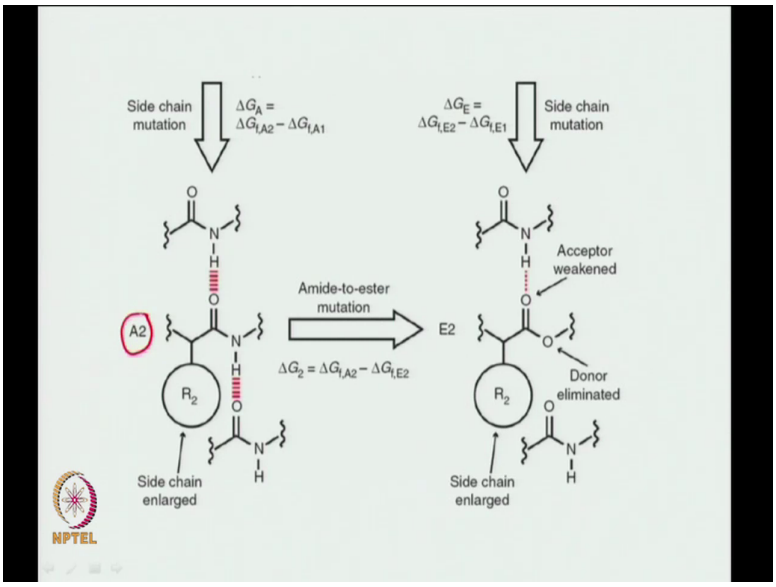

Now you can understand so, that is what; that is what delta G 1 is. If I go to the next slide, I do the same thing, but now in case of the amide, what I have done is, I have replaced R 1 by R 2 and then, on that amide, what I do is, I replace this hydrogen bond again by an ester. So, I have R 1 going to amide to ester, R 2 going from amide to ester again two mutants.

In this case, what is the delta G 2? I can see the delta G 2 is essentially the difference in free energy between A2 and E2, E stands for the ester mutation and this stability essentially comes from what? Mainly the hydrogen bond disruption and if you look at this, if you go from when you are going from R 1 which is A1 to A2; that means, when we are going from A1 to A2, what are you disrupting? You are not disrupting in hydrogen bond, essentially what you are tweaking with or playing with is the.

Student: Surrounding environment.

Surrounding environment, the remember the micro-environment we were talking about. So, that is why that is called delta G A; A stands for amide and we are only sticking to amide same amide, but we are replacing R 1 by R 2 and similarly, you can understand you will be having this delta G E on this side right delta G E; E stands for the ester mutant and also you are doing the same thing you are replacing R 1 by R 2 ok. So that means, you are go to amide to ester right and also you go for amide to amide R 1 replaced by R 2 also in the ester to ester R 1 is replaced by R 2. So, essentially you have this parallel stuff coming out.

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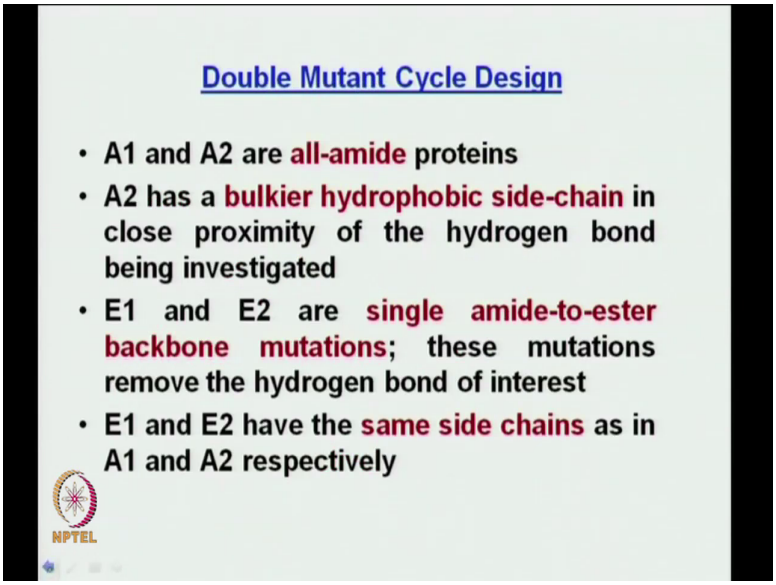

So, just to make it a little more clear what does this say? So, this slide says that A1 and A2 are all amide proteins understandable. A1 is the one we started with where we at R 1, A2 is the one we had replaced R 1 by R 2, but A stands for amide we have not done ester mutation.

Now obviously, A2 has a bulkier hydrophobic side chain. So, whenever we talk about A2 under whatever context, A2 always resembles that R 2 which is bulkier than R 1. E1 and E2 as E stands for ester, these are amide to ester mutants right.

So, these mutations essentially, we have removed the hydrogen bond of interest ok, whichever hydrogen bond we are trying to probe here and again like A1 and E2, E1 and A2 will have the same side chain meaning. So, E1 corresponds to R 1 the less hydrophobic or the less bulky one E2 corresponds to R 2 having the?

Student: More bulky.

Less bulky, I am sorry.

Student: More bulky more bulky.

More bulky R 2 clear the terminology ok.

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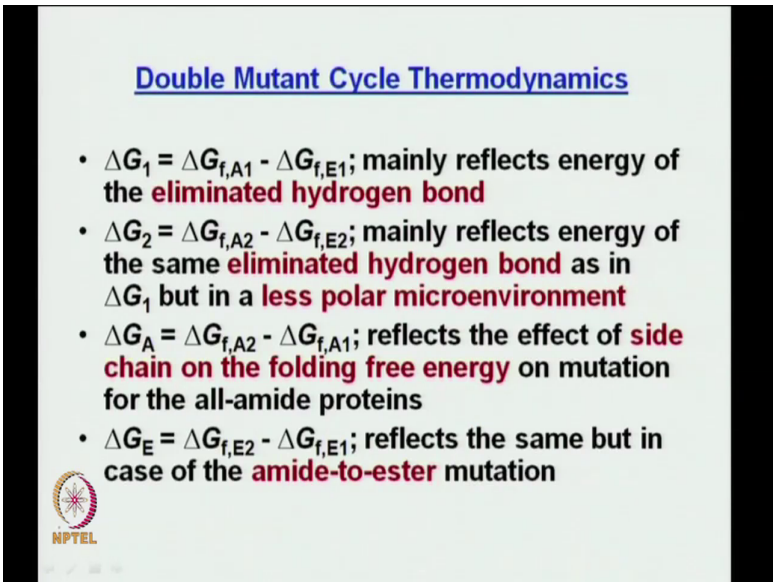

Now, let us look at thermodynamics. So, if you are talking about delta G 1 as we briefly did in the other slide. So, delta G 1 is the folding free energy difference between your amide 1 and your ester 1 where 1 stands for R 1. What have we done is, we have disrupted the hydrogen bond. So, essentially it is this difference in free energy will come mainly from disruption of hydrogen bond.

Now, if you have disrupt the hydrogen bond, we also start disturbing the structure. So, there are many other aspects that come into like solvation all those things, but let us not worry about those you know let us make keep it very simple this paper has talked about those aspects and said that you know those things really do not matter.

Now, if you go to delta G 2; delta G 2 also it is the same thing exactly like delta G 1. Only in this case, R 1 is replaced by R 2 right. Now, what is delta G A? Delta G A reflects the effect

of side chain on the folding free energy. So, delta G A essentially is the delta G f, A2 minus delta G f, A1 you have not replaced any.

Student: Hydrogen.

Hydrogen bond, instead what you have done is you have replaced R 1 by R 2. I am repeating this so that, you can make the connection always. So, this essentially is just because you are looking at a change in free energy where you have replaced a less hydrophobic, a less bulky side chain by a more bulky side chain and the same thing is happening for delta G E ok.

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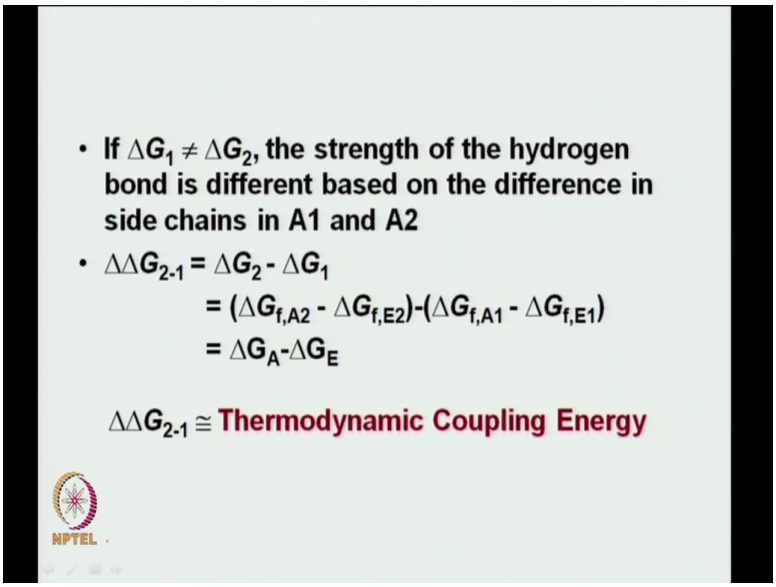

Now, look at the beauty of this approach. If delta G 1 is not equal to delta G 2, then the strength of the hydrogen bond is different based on the difference in side chains A1 and A2

and why do we say that? You look at delta delta G 2-1 what is delta? You know delta G is difference between two and delta delta G is the difference in.

Student: Delta G.

Delta G's right. So, this delta delta G 2-1 is essentially the difference in delta G's of delta G 2 minus delta G 1. What is delta G 2? We know this from before, it is essentially delta G A2 minus delta G E2 and what is delta G 1? It is delta G A1 minus delta G.

Student: E 1.

E 1 this we have defined before and if you do the math, you can see I can write this one as delta G A2 minus delta G A1 right, I can also write this one as minus delta G E2 minus delta G E1 so, that essentially comes to what delta G A and delta G E is this cleared right. If you remember the previous, see this is this is what delta G A was delta G A2 minus delta G A1 and delta G E was delta G E2 minus delta G E1 right and that is what we have used here.

So, the difference in delta G's; that means, the delta delta G's essentially boils down to what? Delta G A minus delta G E. This double differential essentially this delta delta G 2-1 is referred to as the thermo dynamic coupling energy.

That means, if you see a difference; that means, if delta G 1 is not equal to delta G 2 you know does not matter what the sign is of the value you are going to get. It means that because of the change in a side chain in R 1 R 2; R 2 R 1 you have brought about a change in the strength of the hydrogen bond essentially, that is what you are trying to prove by this thermodynamic cycle.

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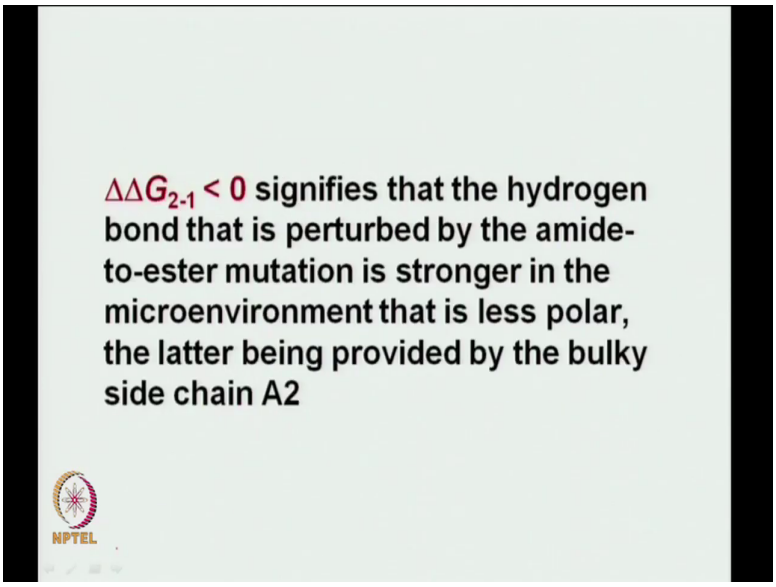

Now, what experiment did they follow? So, this is the experiment they did. So, as I was saying, this delta delta G 2-1 if it is less than 0 it signifies that the hydrogen bond that is perturbed by the amide to ester mutation is stronger in the microenvironment that is less polar, the latter being provided by the bulky side chain A2. Essentially, that is what is the outcome of your double mutant thermodynamic cycle; that is what they are trying to prove.

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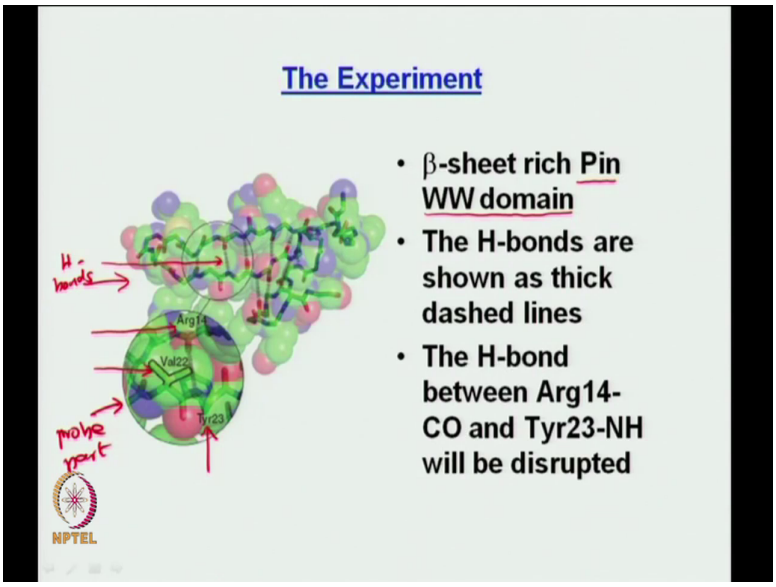

Let us come to the actual experiment, what they did was, you know they did with a series of proteins, but let us you know stick with one, take one as an example. So, this is a protein it is called a Pin WW domain you can see out here, it is a Pin WW domain and this is the one I am talking about.

So, this is the protein it is essentially beta sheet right not alpha helical its essentially beta sheet and this is visible from this figure right, is not it? You can see the beta strands, you can see this is one beta strand, this is one beta strand and these two are intra.

Student: Bonded.

Bonded through hydrogen bonds right and these are the corresponding you can see these are the corresponding hydrogen bonds right. Now, which hydrogen bond are we going to

perturbed like the amide to ester so, what they did was so, that is what this is the zoom into portion, this is our probe part of the molecule of the protein.

So, what they did was you can see this is arginine 14 so, this is arginine 14, this is tyrosine 23. The arginine CO is hydrogen bonded to tyrosine NH. So, what they did was, they replaced tyrosine by the corresponding ester. So, you have done the amide to ester mutation.

Now, you look at this presence of valine out here; so this is a valine. So, valine has this isopropyl side chain and this valine is very close to the hydrogen bond now that is why they pick this hydrogen bond. I so, you know if you are going to look at this contextual dependence, you need to have some amino acid or some amino acid as a side chain which is kind of bulky provides a hydrophobic environment and that is why they focused on this hydrogen bond. So, valine has this isopropyl group which kind of provides a non polar environment to the hydrogen bond we were talking.

Now, what they did was, they replaced valine; they replaced valine by the amino acid alanine so that means, valine is now bulky, which is your R 2 and when they replace it by alanine it becomes.

Student: R 1.

R 1 ok. So, as it says, the hydrogen bonds are shown as thick dash lines. So, they just showed and the hydrogen bond would be arginine 14 CO and tyrosine 23-NH you know will be disrupted.

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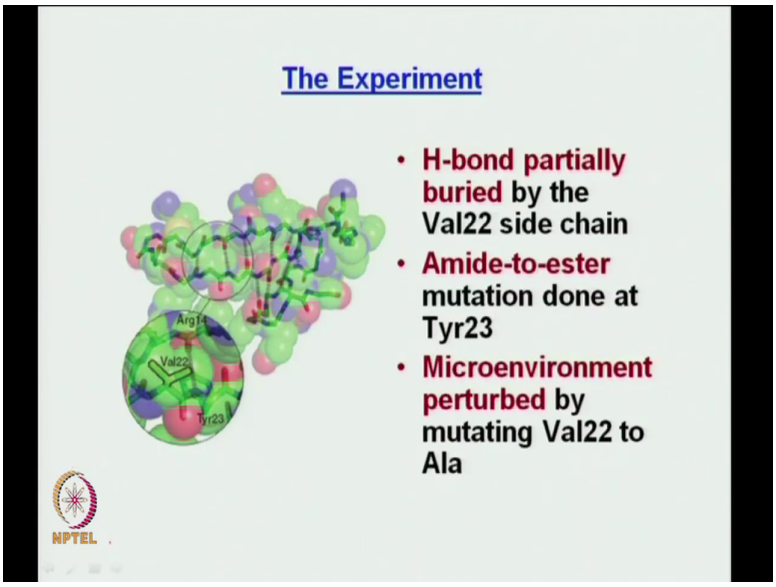

Again as we just discussed, the hydrogen bond is partially buried by the valine 22 side chain because valine 22 essentially its hydrophobic. Amide to ester mutation done at tyrosine 23, the microenvironment now, all the hydrogen bond is perturbed by mutating valine to alanine, now that is the mutation you are doing.

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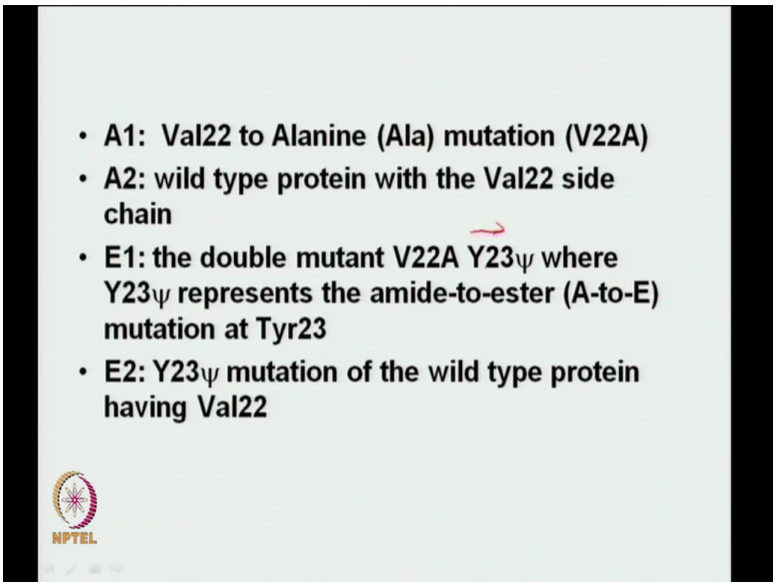

So, based on the terminology we had before A1 should be the one where I have done the mutation why because A1 was R 1 and R 1 was the smaller one which is alanine right. So, that is what it says A1 is valine 22 alanine mutation, the mutation is refer to V22A; that means, you have replaced valine at the 22nd position where is the 22nd amino acid by alanine.

Then A2 is essentially your wild type protein. Wild type protein means the protein which is found in nature just like that, but this is the one which has valine so, this is R 2 valine has a biggest side chain.

Then when I do E1, E 1 should correspond to A1; that means, I am doing the corresponding amide to ester mutation on what not the wild type, but the V22A protein is not it? Because that was my R 1 so, when I do the amide to ester mutation that becomes my E1 and when I do

the E1, you can see this E1 is this is what I am talking about. So, this is the one I am talking about this E1 is called Y22 33 psi where the tyrosine at 23rd position is replaced by the corresponding astro analog, that astro analog is represented by psi that is the mutant.

And then, Y the E2 is Y23 psi mutation of the wild type valine 22 ok. So, essentially you can see you are going like this V22A, then V22A amide to ester, then you have V22, then V22 amide to ester.

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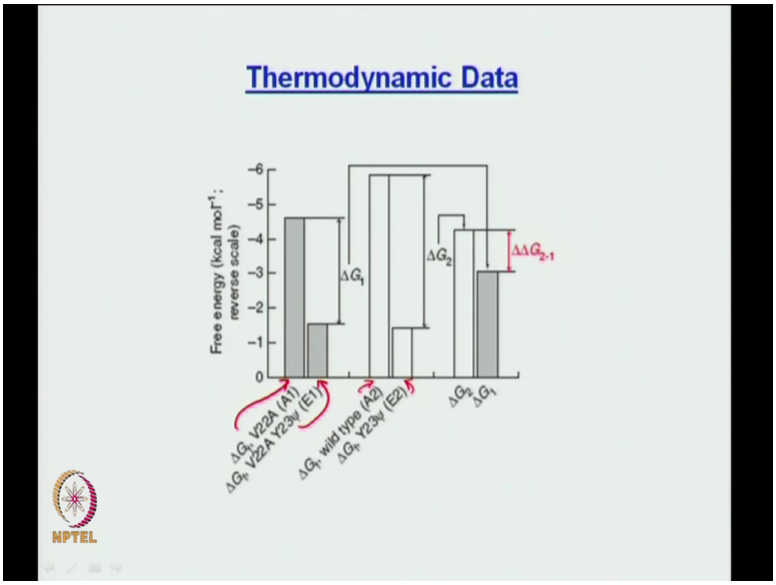

So, this is the thermodynamic data I will give you the values later. So, look this is delta G 1 right so, this is delta delta G 1 is this guy right for A1; for A1 and this is delta G 1 for E1. Now you remember what delta G 1 delta G 2 where right; do you remember delta G 1 delta G 2 where? So, go back so, this needs a little bit of tweaking.

So, delta G 1 was delta G A1 minus delta G E1 right and delta G 2 is delta G A2 minus delta G E2 ok. So, what now they are doing is, this is the corresponding delta G for A1 this one so, this is the corresponding delta G for A1 and this one is the corresponding delta G for E1 and if you take the difference between delta G A1 and delta G A1 what you get you get delta G

Student: 1.

1. Similarly, on this side you can see, you have the delta G f remember everything is a folded this is delta G f for A2 and this is delta G f for E2 and the difference between these would give you what delta G.

Student: Delta G 2.

2 and obviously, now since you have got delta G 1 and delta G 2 finally, that difference will give you what? Delta delta G 2-1 you know that is the one you are going for, to see whether the hydrogen bond has any contextual dependence ok.

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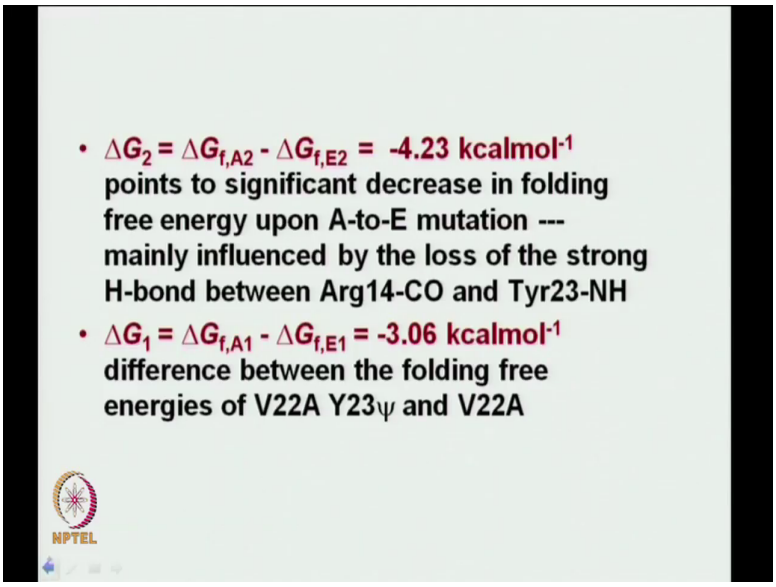

Let us look at the values now. So, delta G 2 you know they have done these experiments delta G 2 is minus 4.23 kilocalories per mole right. Now, this points to a significant decrease in folding free energy upon A to E mutation now that is understandable. Why is it understandable; why is it understandable?

Because you have disrupted the hydrogen bond. Hydrogen bond is necessary for the stability of the protein. When you disrupt the hydrogen bond, even if even if you are disrupting one hydrogen bond possibly it is a major hydrogen bond, major in terms of the structural preservation and hence, you have disrupted it and that is why you can see the delta G gives rise to so much of a difference now this is a huge difference take it from me.

Delta G 1 also the same thing, but its minus 3.06. Now, see in both the cases you have disrupted hydrogen bonds isn't it delta G what is delta G 2? It is essentially delta G A2 minus

delta G E2. What is delta G 1? It is essentially delta G A1 minus E1. What you are looking at is, you are not looking at between A2 and A1 what you are looking at is between A2, E2, A1, E1.

If in both the cases the hydrogen bonds strength had been the same; that means, the extra extent of disruption would have been the same then, delta G 1 would have been equal to delta G 2, is not it because you are disrupting a hydrogen bond, you are doing nothing else now do not; now do not worry about the side chains, what essentially you are doing is just disrupting hydrogen bond you do not have this thing in mind that hydrogen bond might be contextual, might be having a contextual dependence; that means, a dependence on the surrounding environment. If you do not have that in mind, what you would think is if I am going to disrupt a hydrogen bond, I would expect delta G 1 to be equal to delta G 2 as simple as that clear.

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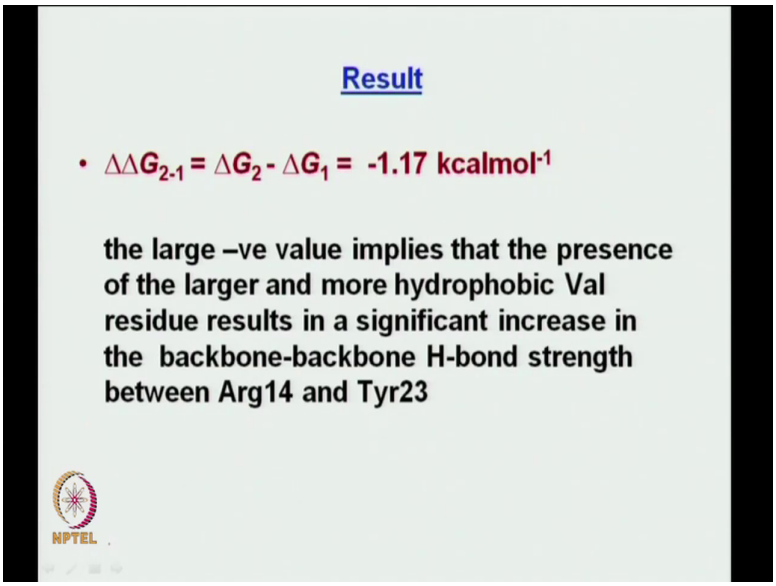

But obviously, this is not the case, you can see there is a distinct difference and why does the difference come in? Because you have replaced valine by alanine or the other way around. So, what this is saying is delta G 2 minus delta G 1 is minus 1.17 kilocalories per mole. Now, what was there 2? A2 was essentially the valine one right the wild type.

This is the one which at the more hydrophobic side chain.So, it immediately tells you, if the difference in free energy is negative; that means, that mutant in that mutant or not that mutant in that protein let us talk the hydrogen bond is actually more stable than the other one and why is it more stable?

Student: Hydrophobic.

The only reason it is more stable because you have a hydrophobic your microenvironment is more hydrophobic for the valine one as compared to the one where your mutated valine to.

Student: Alanine.

The alanine ok. So, this is; this is a direct evidence; a direct evidence of your hydrogen bond being dependent upon its microenvironment.

Now remember, when I talk about its microenvironment, I am not talking about the whole environment I am talking about micro means very small that means, very close to where the hydrogen bond is. Because if we are doing some perturbation which is far apart from the hydrogen bond, it might not feel its effect that much.

Hence, you have to be very close this is why you call that is why it is called the microenvironment ok. So, again the driving point of this is yes hydrogen bonds are necessary because you have seen if you disrupt hydrogen bonds, you have this change in free energy right. So, that is what we saw last slide.

See if you disrupt the hydrogen bonds where delta G A2 minus delta G E2, then delta G E2 is less stable right which means hydrogen bond are important. Not only that, you will also have to consider what are the nearby side chains the side chains which are proximal to the hydrogen bond. Because that will also finally, determine what the strength of your hydrogen bond is right.

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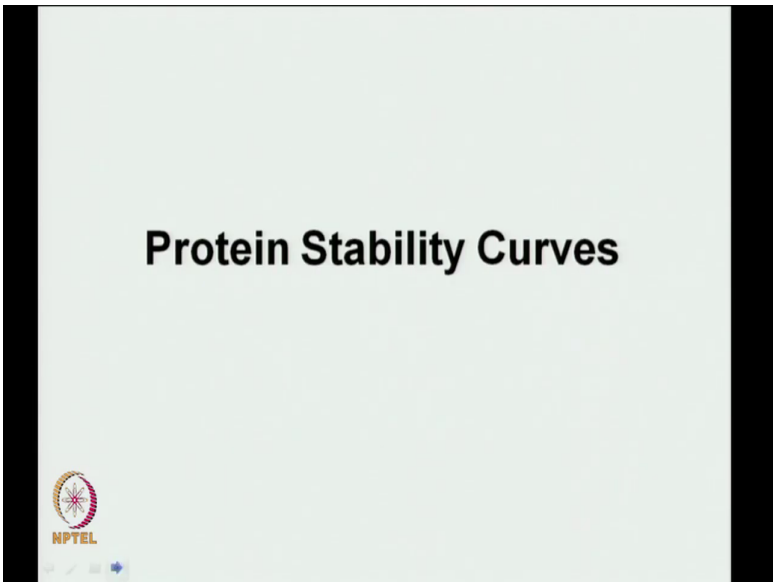

So, you know having said this, this brings us to the end of our discussion on the forces involved in protein folding. Now, whenever I would take any specific case, I would actually maybe come back to this or give you some more examples relate to this, but right now we have already spent a lot of time on forces in protein folding so, let us move on now.

What do these forces in protein folding finally, give you? This forces in protein folding finally, make sure that when you move from a folded state from an unfolded state to folded

state, that the protein remains folded right. When if you are talking about this difference in free energy between the folded form of the protein and the unfolded form of the protein, what are you talking about is the protein stability because the protein can only be stable, if there is a decrease in free energy when you go from the unfolded to the folded form. So, that is the next topic of a discussion which is protein stability curves.

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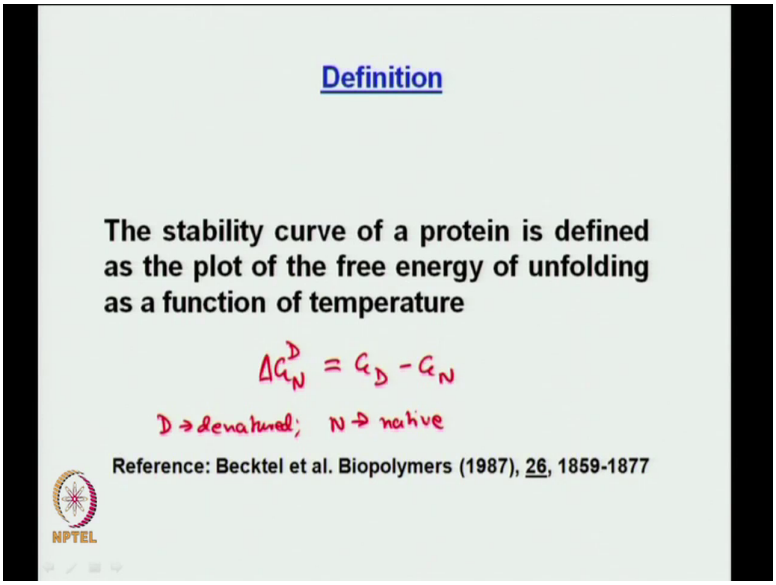

Now, what do you mean by a protein stability curve? What I mean is this; the stability curve of a protein is defined as the plot of the free energy of unfolding as the function of temperature. Again, it is the plot of the free energy of unfolding as the function of temperature; that means, what am I looking at? I am looking at the free energy of unfolding which is given by delta G I can write this delta G as well just keep it like this N to D I will tell you what N and D are is equal to G of D minus G of N. What is D?

Student: Denatured.

D stands for denatured and N.

Student: Native.

Stands for native and that is why it is called the unfolding free energy, right. I am not looking at the reverse, I am trying to unfold the protein and that is the cost the free energy I am looking at and if you plot the free energy as the function of temperature that is your stability curve.

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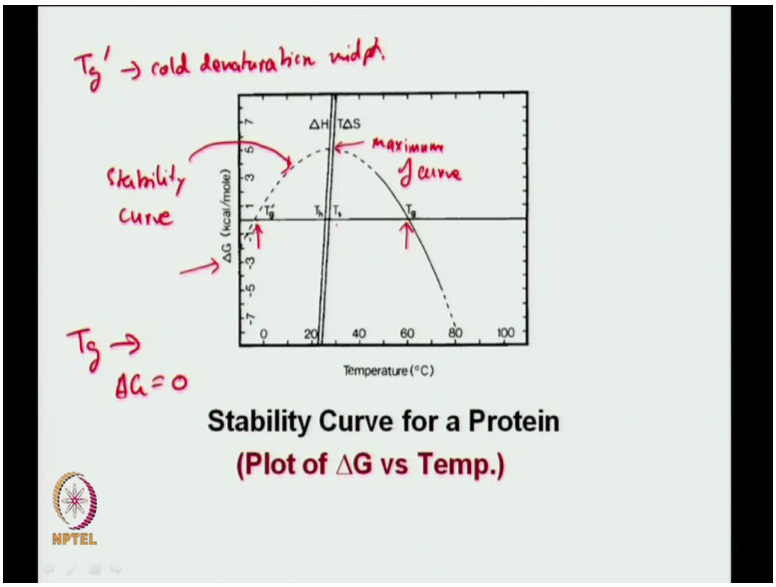

Now, this is something you have seen before, but will now will kind of look at it in a little more detail. So, look at this delta G. This is actually your molar Gibbs free energy you can understand right it is kilo calories per mole. So, you know whatever I am we are going to talk right now is essentially molar Gibbs free energy, I am just not going to write any in a symbol or try to specify anything else by any symbolism that is molar Gibbs free energy or molar enthalpy or molar entropy everything is essentially in molar you are going to talk about.

Now, see this line; this line is your stability curve. What you are looking at again? You are looking at delta G unfolding right; that means, G D minus G of N. You look at the temperature; you look at the temperature this going from 0 to 100 and look at the how the curve goes, how does the curve go? You see the curve goes from here, it maximizes so, this is the maximum of the curve somewhere here maximum of your stability curve and then, it again comes down. There is one more important point, point is you look at these two temperatures, these 2 temperatures are those points where delta G is what?

Student: (Refer time: 30:38).

Equal to 0 right. If your delta G is equal to 0, see if your delta G is equal to 0 what do you mean?

Student: Equilibrium.

Equilibrium, in that sense, if a delta G is equal to 0 you do not have any preference for any of these right, your delta G is 0 there is no free energy difference between G U and.

Student: G N.

G N; that means, either one should be present and that to present in equal bias you have no bias right that means, in equal concentration and if they are present in equal concentration;

that means, k is equal to 1 and hence, delta G is equal to 0 for that because delta G is equal to or delta G naught is equal to minus R Tln k equilibrium.

So, when you have an equal amount of folded and unfolded molecules, that is native or denatured protein molecules, those points are referred to as your melting temperatures. So, melting temperatures are those points where you have equal concentration of unfolded and equal concentration of folded protein molecules right. But that is not the only thing.

When you think about see this is this plot is of Gibbs free energy as a function of temperature is not it. So, when you think about trying to disrupt a protein structure and going to the unfolded state or the denatured state from the native state what you essentially doing?

You are putting in thermal energy because increasing temperature and you are destroying and that is understandable and that is why, you can see at the higher temperature you have a Tg right, there is a Tg at the high temperature this corresponds to somewhere close to 60; that means, you have a melting point for this protein the melting point or the Tg, g is for transition if you remember Tg refers to that point where delta G is equal to.

Student: 0.

0 ok. So, let me write that, Tg refers to that point where delta G is equal to 0. This is very similar to two other temperatures do you remember what two other temperatures we discussed before Th and Ts. So, T h was defined as the bond where.

Student: (Refer time: 32:47).

Delta G 0,Ts was delta is 0 and obviously, then Tg it means delta G is equal to 0 and the moment you take delta G equal to 0; that means, you are talking about the midpoint thermal melting midpoint. So obviously, this the higher end Tg is your high temperature; that means, you have increased the temperature, you have disrupted the protein and you have started melting it.

But you look at the shape of the curve, the curve is crossing this 0 line at two points, one is Tg and the other one is Tg prime. So, this Tg prime; this Tg prime right here, this Tg prime is referred to as the cold denaturation midpoint. It is little hard to think right, we have always we always think of proteins being disrupted when we increase the thermal energy, but we never think of the reverse; that means, we are going to when we are going to decrease the temperature, you are also going to unfold the protein that is why this Tg prime is referred to as the cold denaturation midpoint so, proteins will have the tendency to undergo cold denaturation.

In other words, if you would decrease the temperature a lot, the protein would undergo cold denaturation. It is just so evident from this stability curve because it just crosses the delta G is equal 0 at two points ok. One more thing, you look at this Tg prime what do you think is approximate temperature it is coming here? It is coming at less than 0, right.

Student: (Refer time: 34:28).

Right less than 0 see normally, it is in degrees Celsius right. So, normally when you do your protein unfolding temperatures, you say essentially look at it say from 5 to 90, 95 whatever you cannot go to 100 and above because water will start boiling anyway. But what it says is, that if you have to look at the protein which is getting denatured in the cold form; that means, at a low temperature for this protein; this protein I have to go to a temperature which is below 0, if you are not going to go below 0, you are not going to you are not going to absorb what you are not going to observe that Tg prime ok.

Well, there are other ramifications of this, there are the other meanings of this depending upon the protein this Tg prime can be below 0, can be above 0 and all these things, but at least the take home point of this is the stability curve gives you two points of denaturation. One is a heat induce denaturation and the other one is a cold denaturation. So, Tg is heat induce denaturation, Tg prime is?

Student: Cold.

Your cold denaturation please keep that in mind and this is so, evident from the stability curve ok.

One more thing, you look at this delta H and T delta S right, this T H and T S were seen before, but look at the slopes, the slopes are really steep isn't it, the slopes of delta H and T delta S this is really steep why is it so? Because we are talking about a protein and the change in what? Heat capacity delta C p is really high and that is why the slopes are.

Student: (Refer time: 36:01).

So, steep ok.

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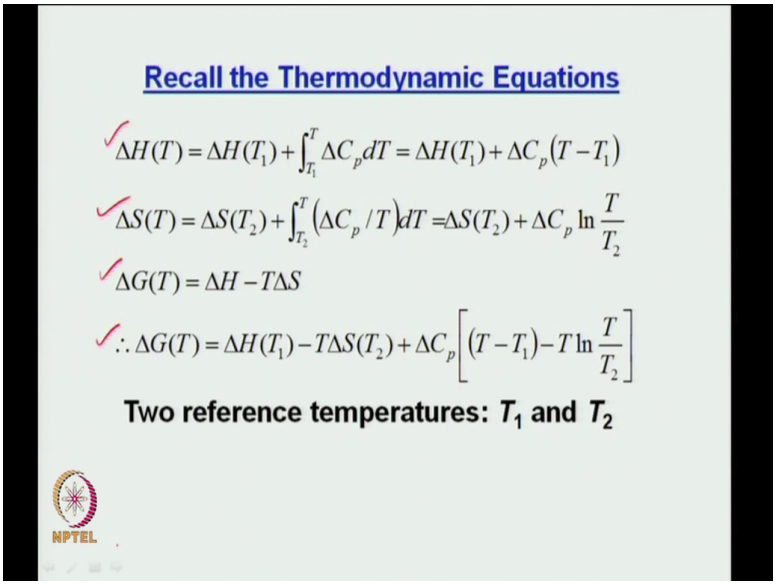

Let us move on. So, let us recall this thermo dynamic equation, these thermo dynamic equations we you know derived before when we were talking about the hydrophobicity right, the hydrophobic effect and we will come back to these again.

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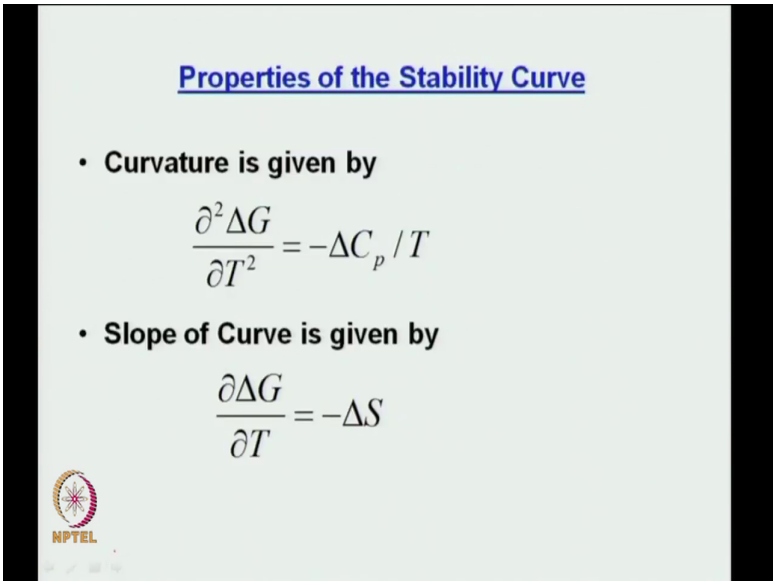

The curvature; this is also what you have looked at before. The curvature which is del 2 delta G over del T squared is equal to minus delta C p over T. So, the curvature is defined by your change in heat capacity and the slope of the curve is given by the change in interval, that is del of delta G over del T is equal to minus delta S now this we know.

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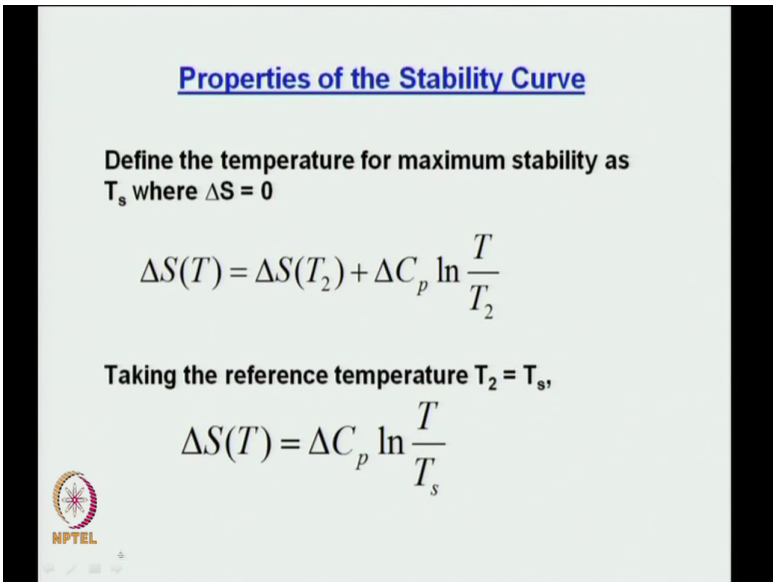

Now, where do we go from here so, where do we go from here? Let us actually, now go to pen and paper and start deriving some basic thermodynamic equations ok.

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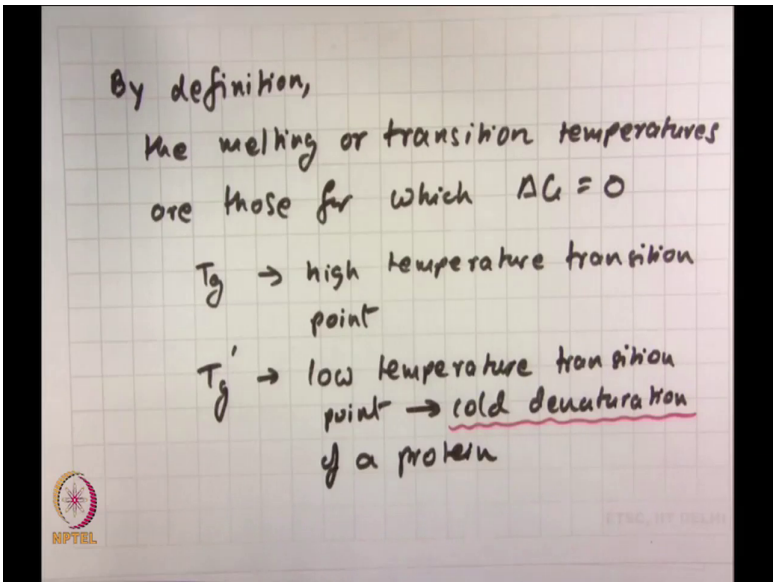

So, by definition; by definition the melting or transition temperatures are those for which delta G is equal to 0, right because when the transition is occurring, that is where you are at Tg and Tg prime, delta G is equal to 0 that is what it means.

So, Tg is the high temperature transition point and Tg prime is the low temperature transition point and this refers to cold denaturation of a protein ok, this refers to cold denaturation of a protein, now this is important.

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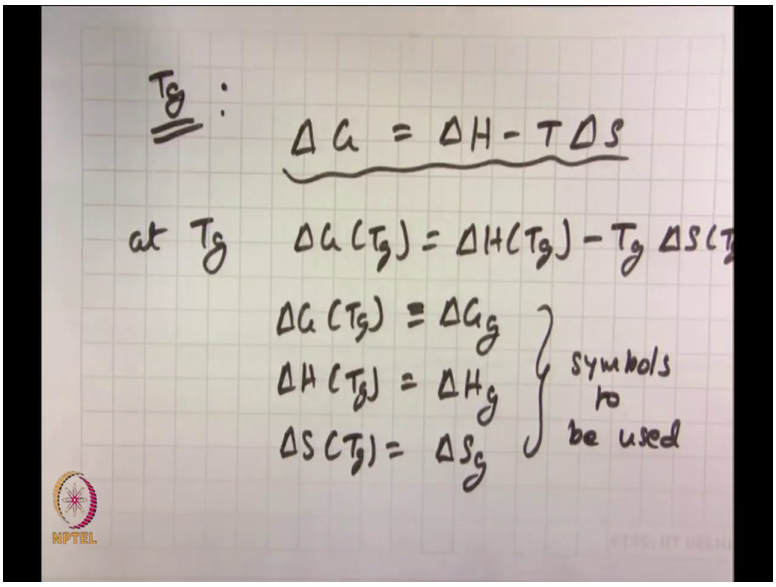

Now, let us see what we know based on this midpoint. So, let us look at focus on Tg; let us focus on Tg. At Tg, well I know from my Gibbs and duhem equation, delta G is equal to delta H minus T delta S right this is known to us.

Now, when we are considering Tg; that means, at Tg I can write delta G T of g is equal to delta H T of g minus Tg delta S T of g. Now, what I will do is, I will just make a small change in symbolism I will say delta T of g is actually delta Gg, delta H T of g is delta Hg, delta S T of g is actually delta Sg. So, these are the symbols I will be using hence forth.

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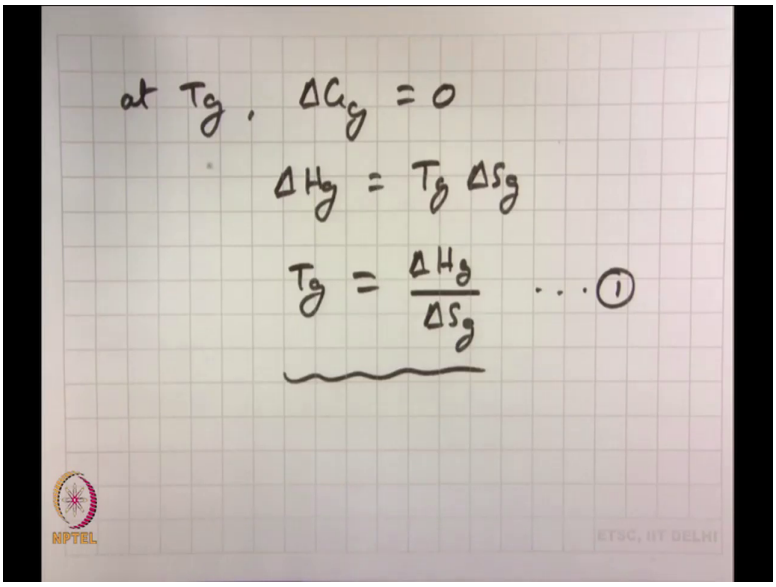

Now, this we know it is not a big thing, but along with this what we also know is; what we also know is at T of g delta G of g is equal to what?

Student: 0.

0 excellent. So, what we have is, then what we have we have delta Hg is equal to Tg delta Sg right or we have a relation for Tg in terms of delta Hg over delta Sg this is equation 1; that means, your transmission temperature where delta G is 0 is essentially equal to the change in enthalpy over the change in entropy good.

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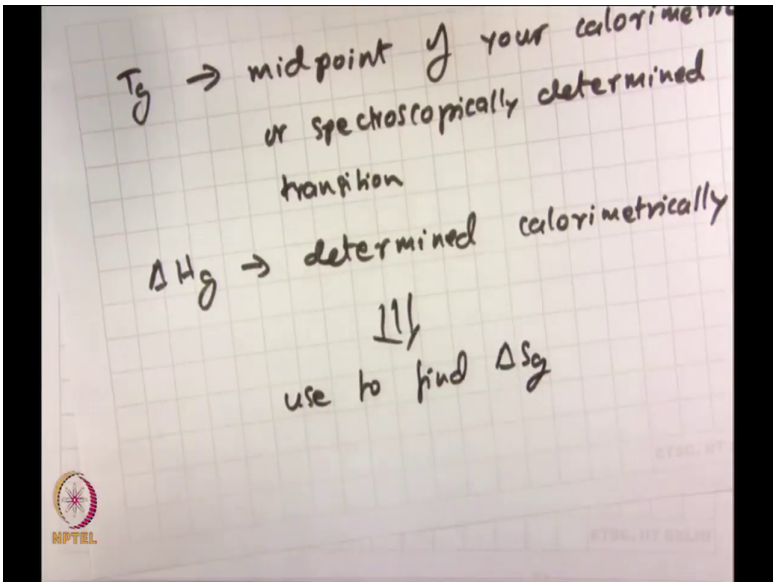

Now, Tg how is it determined it is essentially the midpoint; it is essentially the midpoint of your calorimetric or spectroscopically determined transition. Now, what I mean by this is not immediately clear to you, but just keep this in mind, we will come back to this very soon.

See if you ever look at a protein, a protein depending upon what type of protein it is, it will always exhibit a transition from the folded to the unfolded state. You have the you have heard of the word cooperative right either cooperative transition or non-cooperative transition essentially that is what it talks about the nature of the transition and we take the midpoint of that.

Now, delta Hg can be determined calorimetrically. So, what we have done is based on equation 1 you can see, based on equation 1 I have I already can determine Tg, I can

determine what delta Hg so then, what can I find? Using these I can find delta s right so, then use to find delta Sg straight forward.

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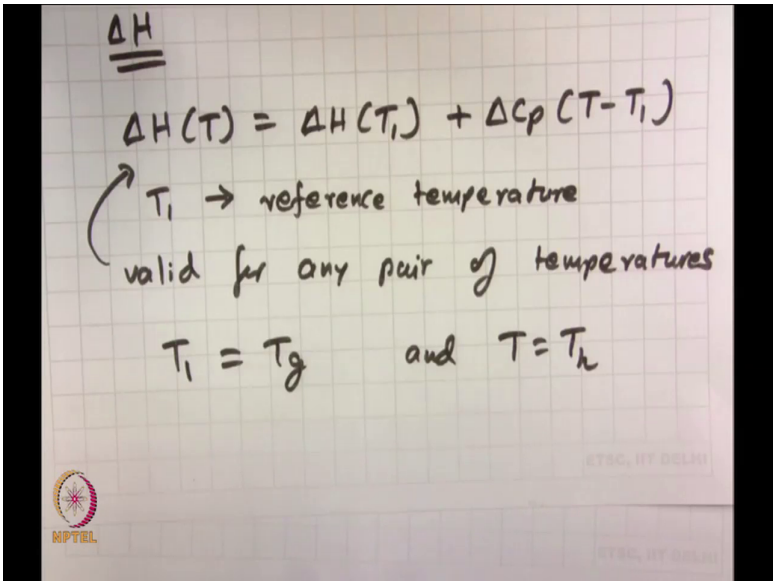

Now, let us look at this again. So, remember now we are focusing on delta H you are focusing on delta H. Now, remember this delta H at a certain temperature T was given as delta H T 1 plus delta C p T minus T 1 where I said T 1 is a reference temperature isn't it? When I say t 1 is a reference temperature.

Now, let me do this, this equation; this equation is valid for any pair of temperatures. So, this equation is valid for any pair of temperatures so it does not matter what T and T 1 is right. T 1 can be any reference; T can be any temperature that I am doing that I am interested in.

So, what I will do is in this case? What I will do is I will say that T 1 is equal to Tg; T 1 is equal to Tg and the temperature I am interested in T is equal to T of h. See Tg is what? Tg is the transition temperature. Now, why I am taking Tg to be the reference one is very simple because if I look a protein if I look at a spectroscopic transition of a protein or any calorimetric experiment, I can easily figure out where my what my Tg is because it is what it is the midpoint.

See if I have a transition something like this, I can you know if I have a transition I will show this to you later I can easily kind of look at where the midpoint of the transition is somehow. And then hence, I know Tg easily that is why Tg is always the easiest reference temperature right and then I say ok, I also know what T h is the T h has a significance of what the delta H at that point is.

Student: 0.

0 see I am trying to get some relations.

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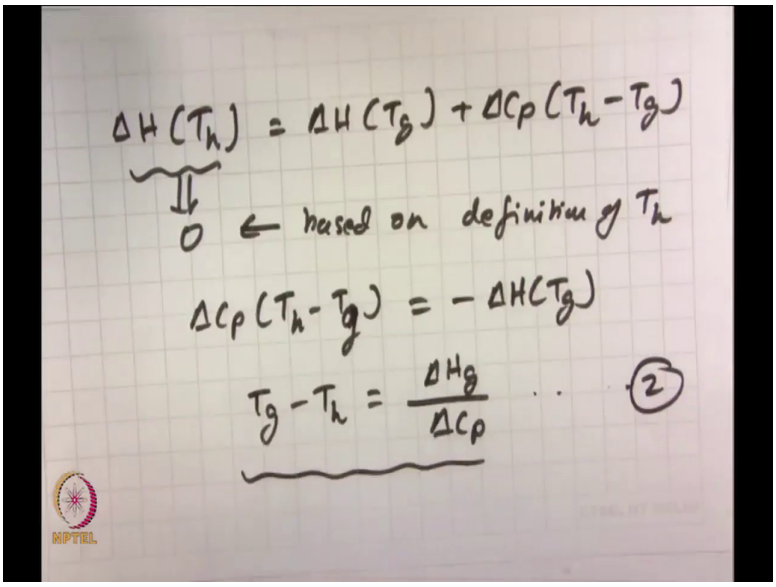

So, then based on this, I can rewrite as delta H T of h is equal to delta H T of g plus delta C p, now this T is T h minus T of g clear. So, for me T 1 was Tg and for me again T is T h.

But, based on the definition of T h, what do I know this is equal to what 0 based on definition of T h. Hence, what do I have immediately? I have that delta C p T h minus Tg it is Tg is equal to minus delta H T of g or I can write T of g minus T of h is equal to see I have reversed the order, I have taken care of the sign is equal to delta H I write delta H g over delta C p say so, this would be what equation number 2, I guess.

So, what are we done now? What you have done is, you know what the sequence of T h is, you know what the sequence of Tg is and you have managed to relate this Tg and T h by what

through delta H and delta C p both of which can be measure, both of which can be experimentally found good.

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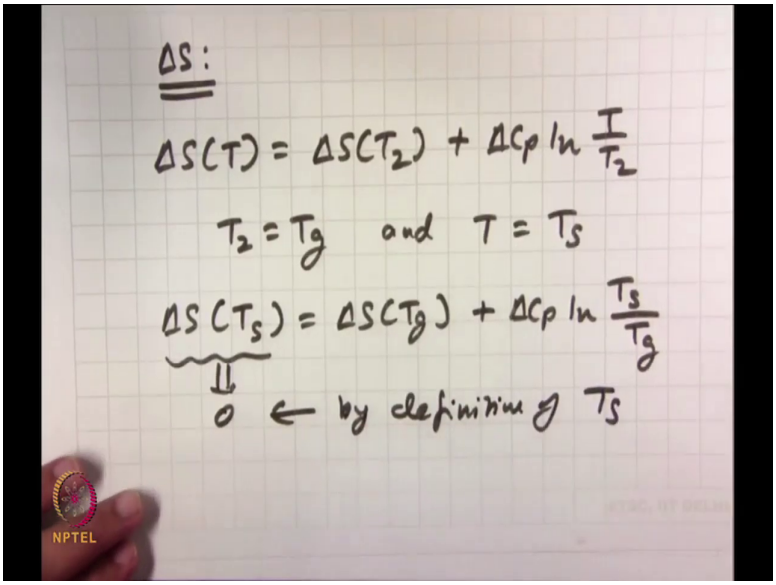

Now, let us do the same thing with entropy. So, let us look at delta S. So, what was delta S for me? Delta S at a certain temperature T was equal to what? Delta S I said T 2 right plus delta C p natural log T over T 2 wasn't it? That is what we had before ok. Now, I say T 2 again is equal to Tg because that is my reference temperature and T is equal to what?

Student: T s.

T s ok. So, therefore, I have delta S I can write T s is equal to delta S Tg plus delta C p natural log and look at this, this T is T s by Tg. Again by definition, what happens to this guy? This is 0 by definition of T of s clear.

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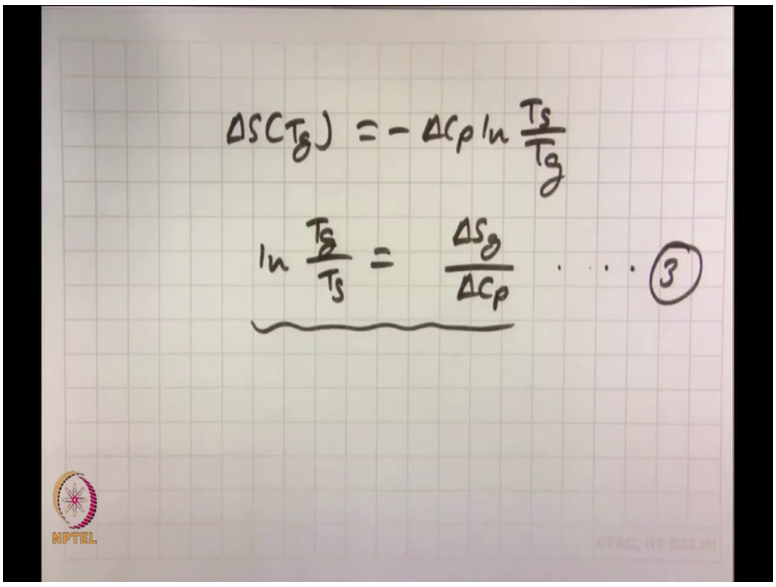

So, based on this, I mean just go one more step and say that delta S T of g is equal to minus delta C p natural log T of s over T of g or I can take care of the negative sign and write natural log T of G over T of s is equal to delta Sg over delta C p this should be equation 3 clear.

So, now, what you have done, one you have to relation between T h and Tg. What does this equation give you? This equation gives you a relation between T s and Tg now. So, you see you have 3 major temperatures one is Tg, the other one also is Tg prime well, but let us talk about Tg, you have Tg where g is equal to del G 0, if T h where delta H is equal to 0, if T s were delta S is equal to 0 and we have managed to relate those two pair wise through these two equations good. Now, this is one set of equations. Now, let us do one more thing.

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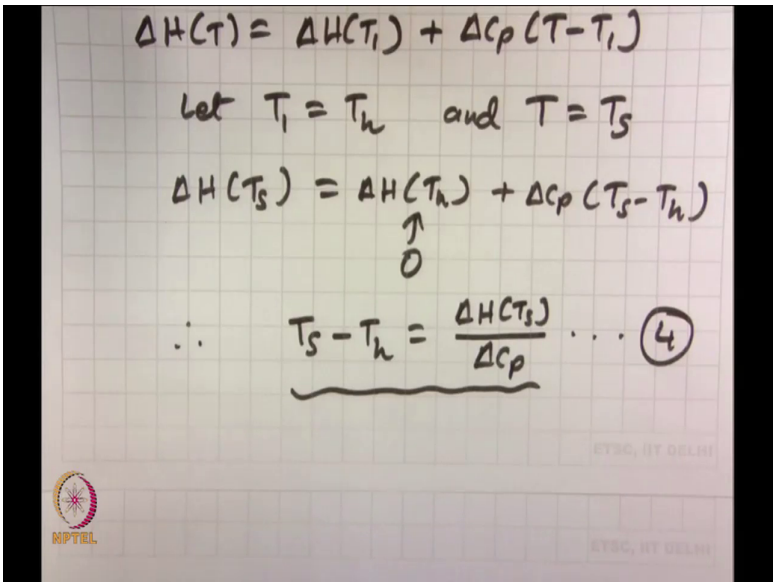

Let us go back to delta H again. So, for example, delta H I know T, I will always start with this is delta H T 1 plus delta C p; plus delta C p t minus T 1. Now what I do is, I say let T 1; let T 1 be T of h; let T 1 be T of h and T equal to can we say what would be here?

Student: T s

T s yes T s if I have that, then I can write delta H T s is equal to this delta H T of h would be what?

Student: 0.

Let me write it again T of h this is 0 plus delta C p, then T s minus T of h or I can write T of s minus T of h is equal to delta H T of s by delta C p say this is a question 4 ok. Now, can someone tell me the importance of this one, this equation? See the other two, we had related what? T s to Tg.

Student: (Refer time: 51:36).

T s to Tg, in this case what we have related? We have related.

Student: T h to T s.

T h to T s why? Why do you need it?

Student: Tg can be calculated; Tg can be calculated.

Tg can be calculated ok, there is one more thing Tg can also be calculate from the other ones, but there is also one more thing what was that?

Student: Without bringing T s (Refer time: 51:59).

Well not exactly, you need to think about this what was the main difference between your you know these delta H, T delta S curves for a hydrophobic molecule say neo pentane as compared to one where you have of protein what was the main difference?

Student: Mainly 2 difference T s minus T h.

T s minus T h right and we said that in case of protein that T s minus T h was very close I mean they are very close to each other, but it was far apart for a hydrophobic molecule. See if we can somehow get a relation between T s and T h in terms of the thermodynamic

parameters, it will be easier for us to reason out why? Now this is what will lead us to that. You can see T s minus T h is equal to delta H T s by delta C p.

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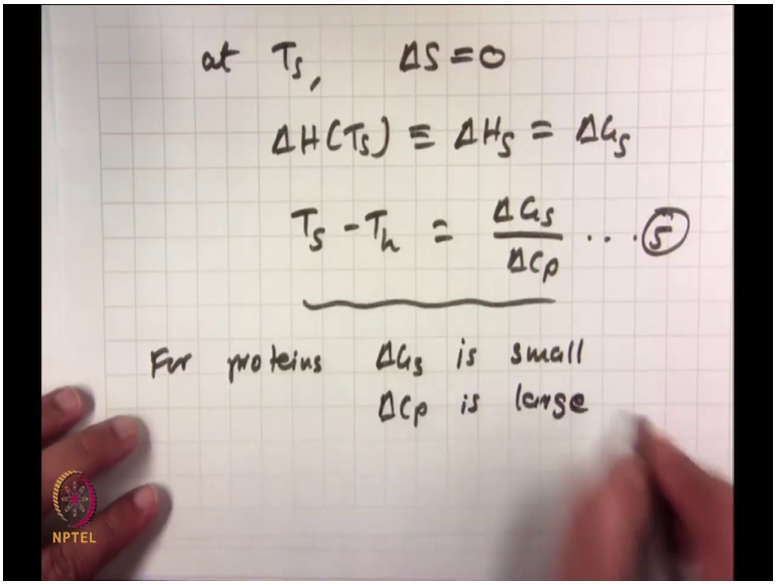

Now, what do I mean by delta H T s; what do I mean by delta H T s? At T s what happens to delta S?

Student: (Refer time: 53:01).

0 therefore, I can write delta H T of s; s I can say its delta H s right this is equal to what?

Student: Delta G s.

Delta G s is not it? Because delta G is equal to delta H minus T delta S, delta S is 0 for you at T of s. So, delta G is directly equal to delta H s. So, then what will happen is T s minus T of h is equal to delta G of s by delta C p this is number this is what number 5 I guess ok.

So, here the magnitude of the difference between T s and T h is directly correlated with the ratio of the change in free energy over the change in heat capacity. Now, let us try to see why this T h and T s are so close to each other for a protein.

Student: (Refer time: 54:03) C p is (Refer time: 54:04).

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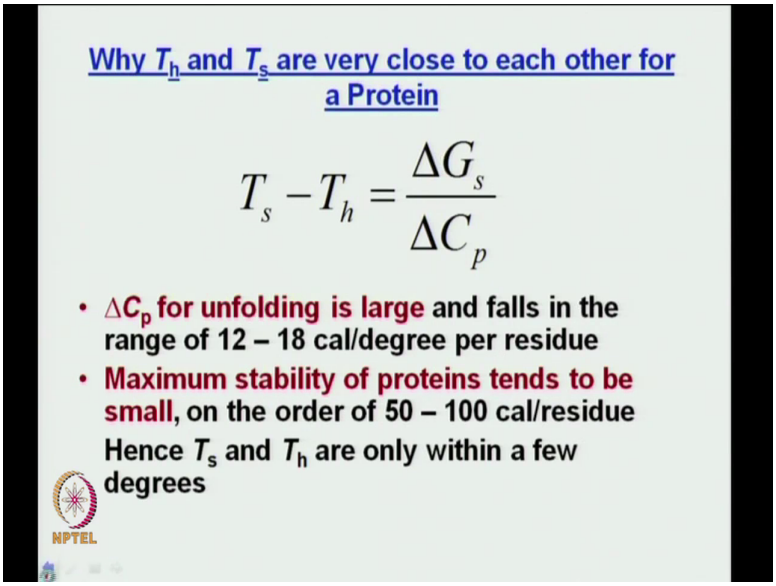

Yeah look at this the delta C p for unfolding so, this is what we derived just now. Now, the delta C p for unfolding is large and falls in the range of 12 to 18 calories per degree per residue this delta C p. The maximum stability of proteins how about not that high, it is only of

the order of 50 to 100 calories for residue, it is not high at all. Typically, for proteins the stability range is from say 5 to 10 to 20 kilocalories for mole which is not high by any standards.

So that means, if your delta G is not high, but if your delta C p is very large what happens to the difference between T s and T h?

Student: It becomes small.

It becomes small.

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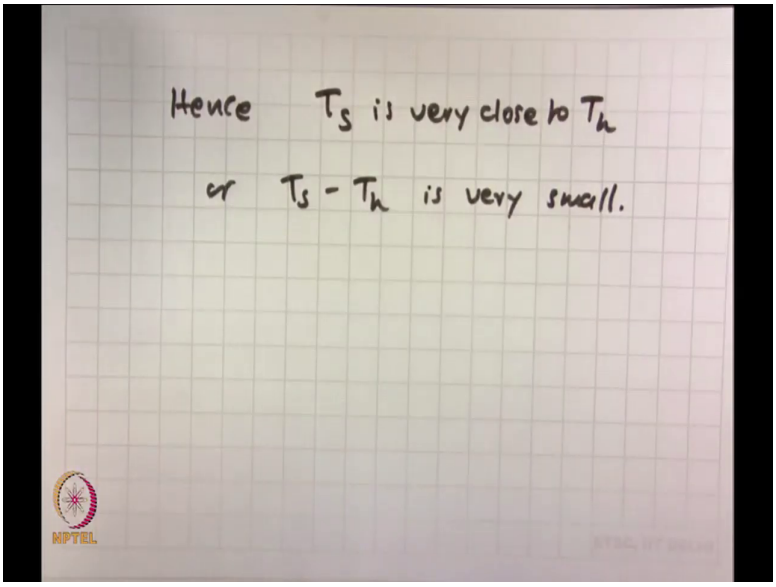

And essentially, that is why so, for proteins delta G s is small delta the C p is large. Hence, T s is very close to T h or T s minus T h is very small. This is why, derivation of this relation was so important.

Second, based on this, can you rationalize the huge difference in T h and T s for a hydrophobic molecule like neo pentane?

Student: Delta C p is very smaller than.

Delta C p is very small. See for a hydrophobic molecule if you remember, if you remember the comparison when we were taking neo pentane in water, the delta C p was like 363 or 368 or something like that, but neo pentane; in neo pentane like gas phase gas neo pentane in liquid neo pentane that delta C p was what?

Student: 38.

38, 40 exactly. So, huge difference from that make sure of the fact that T h and T s for a hydrophobic molecule are far apart from each other. So, that is you know that is where; that is where a protein is so different from a normal hydrophobic molecule ok. So, this is about you know T s and T h.

Let us look at some I do not know how much time we have for today oh already we are out of time, but I think will stop here, but before stopping I just forgot tell you one thing let me mention that stress that point.

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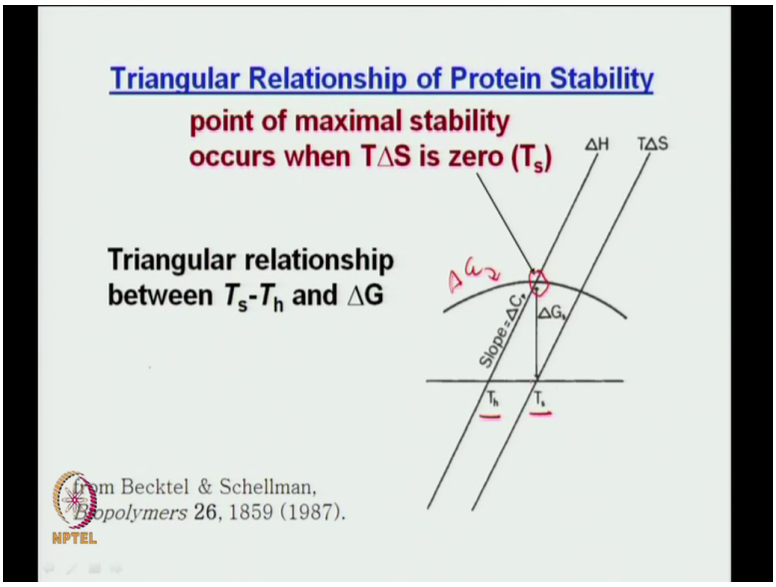

See we talked about this right, we talked about this ok. We talked about this T s minus T h right. So, we talked about this T s minus T h being equal to delta G s over delta C p you know that is what we talked about.

Now, what we can do is, this gives rise to something known as our triangular relationship; this gives rise to something known as a triangular relationship and if you would look at it; if you look at it is not coming there I guess so, what the triangular relationship will look like is I can draw this or it is there.

We can draw it the way we can draw is this, you can see this is T h here you have T h right and you have T s and this is your delta G it almost looks like a triangle and this is the triangular relationship we are talking about and this triangular relationship has been maintained obviously, for proteins and that is what the relation we have derived. So, this also

you can see this we have described this one was; this one was the point where you have the maximum stability and where do you have the maximum stability? We have the maximum stability where it corresponds to.

Student: T s.

T s and what time is the T s? Your enthalpy sorry your entropies since 0. So, essentially maximum stability arises mainly from the difference in what? Enthalpies between the folded and the unfolded states or denatured and native states right. Again, please keep this in mind it is called a triangular relationship between T s minus T h and delta G and this serve to be a very important way of trying to rationalize why in case of proteins T h and T s are so close to each other ok.

So, we will stop here and from next class, we will start with your some more thermo dynamics with relation to protein stability.