Bio-Physical Chemistry
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Lecture – 28
Protein Folding: Mechanisms and Kinetics (Contd.)

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Protein Folding:
Mechanisms and Kinetics

So, last class we were discussing about this Protein Folding Mechanism and Kinetics. Mechanisms we had discussed briefly before, and right now we are going to discuss at length the kinetics part and that is essentially what we were going through in the previous class.

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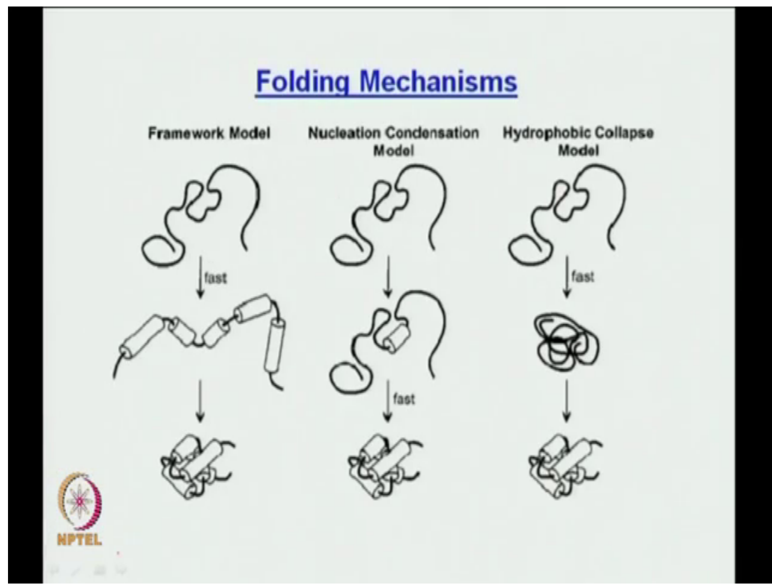

So, what we started with was I mean three different folding mechanisms one is the framework model. So, this is the framework model right this is the framework model. So, you can see you start from an unfolded protein right, then you have this micro domains. So, this is a micro domain.

So, this micro domains are essentially relatively well formed like helices and then they packed together to give you the native state right. So, then this one is an intermediate ok. So, the middle one is possibly an intermediate for you right.

Now, when you go for the nucleation condensation well before going to the nucleation condensation go to the extreme right which is the hydrophobic collapse model now. In the

hydrophobic collapse you see initially you have a very fast a collapse, where you have the clustering of the hydrophobic residues right.

So, if you consider this to be an intermediate I, and then what will happen is everything else starts to form and here we said once you have the hydrophobic collapse occurring it is the tertiary interactions which define your formation of the secondary structural element ok. So, that was for the hydrophobic collapse. So, this also goes to an intermediate right.

Now, what about the nucleation condensation model? The one in the middle. So, here the scenario is like this that you start again from an unfolded state you go to the statement between where you might form something which is well developed or you might form something which is not so well developed; that means, loose right and then everything falls in place together ok.

So, essentially you can see out here what happens is that you form this loose model, all the then the secondary structure defines the development of the tertiary structure because the loose model has shades of secondary structure the loose nucleation condensation I mean the nucleus and then everything falls in place without formation of intermediate.

But, just thing about it that if there would be certain stabilizing interactions by you know in sense of certain interactions between amino acids which are present out there then the middle one for the nucleation condensation model might become an intermediate right in that case what would happen is that intermediate would stay.

And then you would move on to the final structure, in that case you might be having an intermediate you might be. But, here we are what we are talking about is the nucleation condensation where which does not go through an intermediate you know that is what we are trying to focus on.

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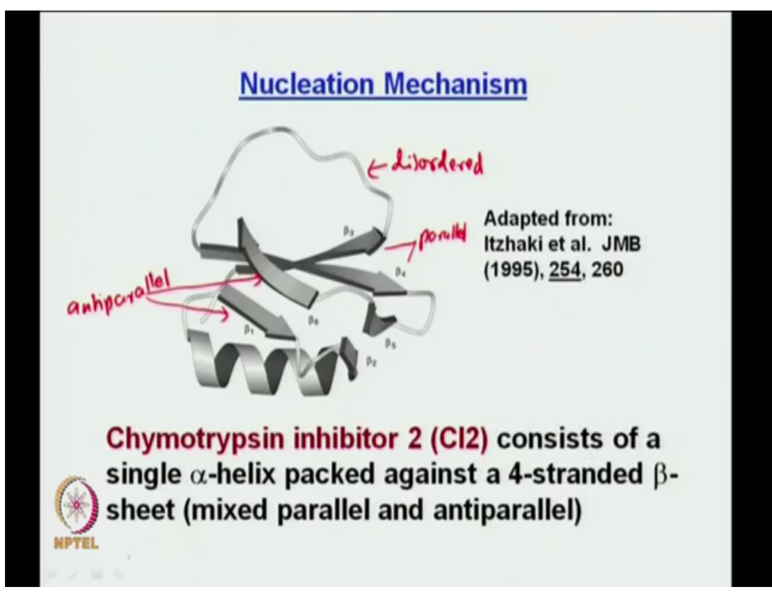

So, then we talked a little bit about the nucleation mechanism and this was or this is one of the best examples of a nucleation condensation model with chymotrypsin inhibitor 2 as I said it was worked on by the research group of Alan Fresht in Cambridge and it is a small protein, but still it is unique because it is shades of different you know secondary structural element right. Alpha-helix beta sheet mixed a random call well developed random call and all this ok.

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Transition State

- Transition State (TS) is a structure that is at the energy maximum along a reaction coordinate
- On a 3D surface, TS is conventionally defined as a Saddle Point i.e. at a maximum of energy along a reaction coordinate and at a minimum of energy perpendicular to it

Now, this was the last topic we were actually discussing the other day, the transition state. So, you know, can we apply transition state to protein folding? We will come to that again later, but let us first look at what a transition state really is I mean you know this, but you know for the sake of convenience or for the sake of continuity let us look at it again.

So, transition state is a structure that is at the energy maximum any given reaction coordinate; remember, there is not a reaction coordinate there can be many reaction coordinates right you know a couple of bonds might break not exactly one bond.

So, in that case then you can take one bond to be the reaction coordinate, you can take other bond to be the reaction coordinates. So, hence reaction coordinate, it is not just one it can be multiple right.

But, for chemical or for molecules where you have only a few bonds breaking then the reaction coordinate essentially those few bonds are same, but for a protein when you know again we said that essentially there is no covalent bond right it is all very loose interactions in that case your reaction coordinates is really multidimensional because you can follow you can follow any trajectory or you can follow reaction coordinate ok. So, that is why it is becomes very complicated.

Now, how would you conventionally defined a transition state and this you know. On a 3D surface transition state is defined as a saddle point. Now, what do you mean by saddle point? I will show you the figure in the next slide. So, saddle point means you think about horse saddle.

A horse saddle look something like this right. So, the horse saddle is looks something this so that means the saddle is kind of a pit if you are coming from this side ok, but if you are moving in orthogonal direction then it is not a pit it is a high energy right. So, that is what it means.

So, that means, you are giving along if you are moving along a reaction coordinate where a bond is breaking then you are going like this and you are coming down and if you are coming from an orthogonal reaction coordinate then what happens is it goes like this; it is like a mountain pass.

A mountain pass is like this where you can easily pass over, but then if you look at it from the down what happens is it is a barrier for you. So, that is what essentially a saddle point is. Now, that is precisely what is being told to you in the figure.

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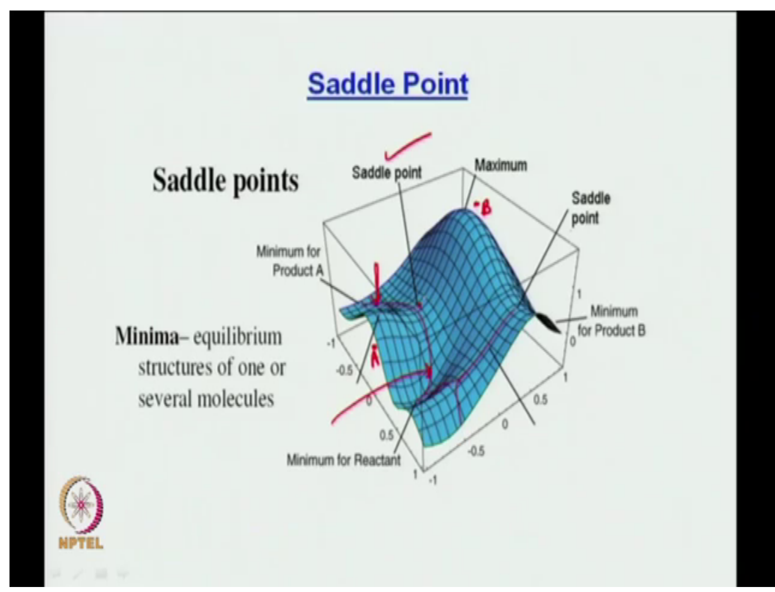

Now, look at this very carefully. So, this is what is saddle point is right this is your saddle point you see where it is. This is where it is and if you start from here and you come here, does not it look like a horse t saddle if you join these two points? It looks like a horse saddle right. So, then if this is one coordinate and if you are now moving along the perpendicular direction you think about this you are moving you follow the red curve you follow this red curve ok.

Now, you follow this red curve you see this red curve being drawn out here see what happens to the red curve the red curve is now perpendicular to the one where you are looking at in the prospective of these two points I have drawn. So, is this red curve is essentially not a minimum right it goes to a maxima at the saddle point. Is it clear or not?

Look at this red curve see this red curve where the arrow is again right. So, this is essentially the red curve I am talking about you look at this red curve wherever it starts and wherever it ends it actually goes from maximum. That is the transition state for that reaction coordinate, is it not?

But, if you look at the same point see if you look at the same point which is saddle point from the prospective of joining these two points say this is A, this is B and if you try to join these two points the saddle the this point is no longer a maxima this point is essentially is what?

Student: (Refer Time: 07:41).

A minimum, but this is in a direction orthogonal to the one you are considering before. So, that is essentially what your saddle point is ok. So, that is how a transition state is conventionally defined as a saddle point.

It is a maximum in one direction, but it is a minimum in a direction which is orthogonal to it ok. So, this is what your transition state is in terms of a saddle point ok. It is called saddle point because it looks like a horse saddle that is why it is called a saddle point precisely that is what it is ok.

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- In chemical reactions, only a few bonds are being made or broken in the TS and since these bonds are strong, one needs to apply quantum theory
- For protein however, TS involves the making and breaking of many weak bonds that can be described by classical statistical mechanics and Newton's equations
- Hence TS for proteins can be very wide and long

Ref: Fersht A. *Current Biology* (1994), 5, 79-84

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Now, this is what we have discussed before in chemical reactions only a few bonds are being made or broken in the transition state and since these bonds are strong, one needs to apply quantum theory ok.

So, in a chemical bond you would be having fully developed covalent bonds one covalent bond would snaps a another covalent bond would form or a couple of covalent bond would snap, it does not matter in which order it is and because this covalent bonds are obviously, strong bonds then you would have to apply quantum theory ok.

Now, this is essentially what? Eyring's theory is right. Eyring's theory is depend upon what? How a bond vibrates because if a bond has to be broken and the bond has to be formed before

a bond breaks it has to vibrate right and so, that is why it is equivalent to the frequency of vibration ok.

So, that means, the rate at which a transition state decomposes into your final products should be equal to what the frequency of a vibration times a certain energy factor which is your exponential term we will come to the equation again and then we will try to look at it.

But, when we move over to a protein the transition state in this case is a little hard to imagine, again think about this. There is no covalent bond been broken. Is there any covalent bond been broken in a protein? Because your covalent bond is essentially a peptide bonds and whatever amino acids you have, nothing is changing.

What is essentially changing? What is changing is your different energetics in terms of what? May be a Van der Waals, may be hydrogen bonding, may be salt bridges, but these are not covalent bonds per se right.

So, then if we the way we defined the transition state was developed on the basis which Eyring you know based it on was the frequency of breaking of this bonds essentially the vibrational frequency, then I have do not have any bonds to break ok. So, how do I bring this into a picture ok?

So, obviously, then because these bonds are loose because these bonds are loose then what will happen is your transition state will not be a very sharp peek why? Because in case of a chemical in case of a molecule broken your bonds are very strong and hence your transition state is very sharp.

But, for a protein because the bonds are weak and because you know in a protein at a any given time you can have many different confirmations then even at the transition state you can have a very diffused transition state; that means, one which is very long or very wide ok. So, that is what he says.

For protein, however, transition state involves the making and breaking of many weak bonds that can be described by classical statistical and mechanics and Newton's equations. I do not need quantum theory for this. Now, what is the result? Hence transition state for proteins can be very wide and long it is not shortly pit. So, then how do I envision it or how do I depict a transition state for a protein? So, this is how I can think about it.

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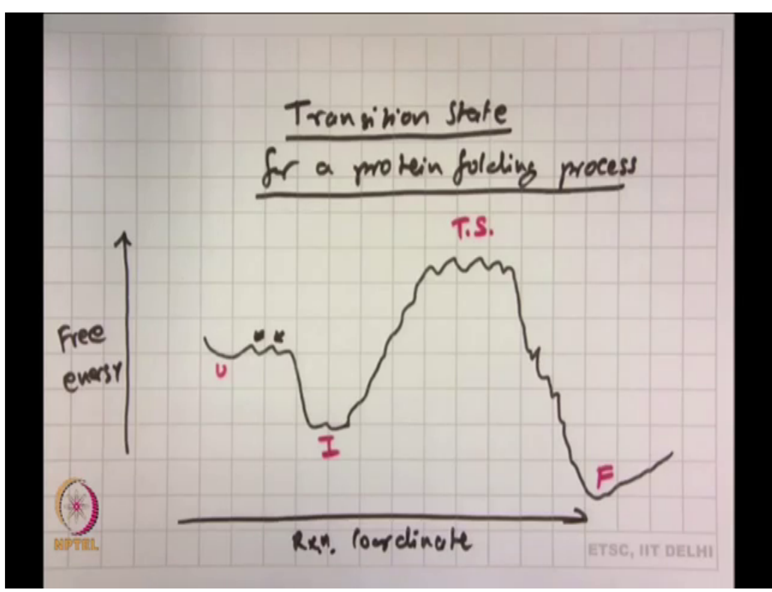

So, let us consider the transition state for a protein folding reaction or for a protein folding process ok. Now, this drawing is exactly not to skill, but you know let us try to do it.

So, suppose I am going from the unfold side to the folded side. Now, let me draw it first then I will explain it to you then you can draw it by yourself it is very simple actually ok. So, this says your free energy and this is your reaction coordinate this is your reaction coordinate.

So, because I am going from an unfold state to the folded state what I can say is this is U for me, this is I am saying one intermediate I and this is my folded state F and obviously, this is then my transition state T S ok. What do you see here? This is not something you have you know encountered before, I can tell you that.

If you would be drawing a transition state let me is this energy diagram for a bond or for a molecule undergoing breakage of bonds then what you would do is you would go from the reactant side to the product side you would just draw a smooth curve right and just go over and the transition state would be a very sharp.

But, the problem is here. You start from an unfolded state we know that a protein does not exist in one confirmation especially in the unfolded state. It would be having many confirmations, good. So, that means, obviously, your unfolded state is already pretty broad to start with right. Now, not only this your confirmations can your confirmations are dynamic in the sense that, it is just not one set of confirmations, these confirmations are always interconverting about each other.

So, what will happen? Because they are interconverting about each other, some confirmations can be of lower energy or higher energy than the other confirmations. If that is the case what you will see is in the free energy plot you will see fluctuations right. It will not be a smooth curve you will see fluctuations is it not.

So, this is what you see you see you see this fluctuations these are your fluctuations ok. So, these are your fluctuations. Why are these fluctuations? Because you are considering a dynamic process where even when it is making the transition, it is still undergoing fluctuations from one confirmation to the other ok.

See, you start from the unfolded state you know you come to the intermediate. Now, because it is an intermediate before you move over to the transition state and then the folded protein. So, because in a intermediate; that means, you already have progressed towards the folding of a protein right towards the protein getting folded.

If that is so, then the intermediate should be having a less defused value than the unfolded state, is it not? Because, now, a lot of confirmations are already locked because everything is decrease in entropy. So, in that case what will happen is the intermediate is a little less defuse or little less shallow or little less broad than unfolded state is it not?.

Now, again you can see wriggles on this. See even if it is making the transition it still undergoing random dynamic transitions between its different confirmations. Now, you look at the transition state. This is what I was saying. You are breaking weak bonds, these are not covalent bonds you are breaking weak bonds.

You can be making and breaking many weak bonds right. In that case you will not be having a shortly defined transition state. Instead what will you have? You will have a transition state which is very broad right very wide and with again this energy fluctuations.

So, this is our transition state for a protein folding reaction if you do consider that the transition state is applicable for the protein folding reaction is different from that of a molecule undergoing actual session of covalent bonds ok. So, in this case the transition state is wide, long and also it has these fluctuations which are from interconversions between different confirmers right.

And, then obviously, once you go to the transition state and because you are applying transition state everything else is valid your whatever assumptions you took equilibrium has to maintained, Boltzmann statistics, all the things are valid and then after that it slides over to the folded state ok.

Now, remember this small fluctuations where have you have seen this fluctuations before? You seen this fluctuations if you remember, when we are talking about the protein folding in an energy in a funnel landscape? In a funnel landscape you had many fluctuations, is it not? So, this is essentially the same that final landscape was what?

It was a 3-dimensional landscape; that means, no matter what confirmation you start with you are looking at a 3D plot each and everyone comes down in a parallel sequence. Here you are reducing the dimensionality, you are trying to just present it simplify it. But, still these small fluctuations are always be there ok.

So, this is how you would define or he would try to model the transition state picture of a protein model in the sense means in terms of a picture and this is vastly different from that of a molecule undergoing bond session right.

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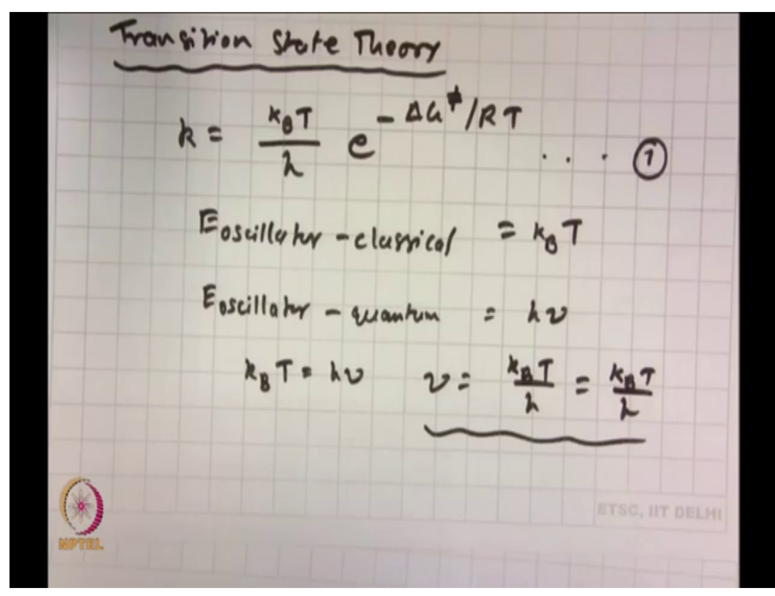

Now, let us look at the model. And this you know in terms of equations let us go to transition state theory. See, why do we care about transition state theory? Now, why do where do you

learn transition state theory? You do not learn transition state theory when you are doing thermodynamics. Where do you learn transition state theory?

Student: Kinetics (Refer Time: 17:35).

We do kinetics right and we have already done thermodynamics in protein folding, now we have to look at kinetics and the best way for us to look at the kinetics is to somehow use the transition state theory to our advantage when we are describing a protein undergoing folding unfolding does not matter, whichever it is ok.

Now, from the definition of Eyring, what do you know is your rate constant k is equal to now this k B T, where k B is the Boltzmann constant times temperature over h e to the power minus delta G of the transition state this is by R T, say this is 1 ok. Now, this is what you know. Then delta G can be broken into be delta h and delta s and all these things.

Now, what is k B T by h? Can someone tell me what is k B T by h or k T by h, what is that?

Student: Frequency.

It is frequency and it is frequency it and it is very easy to remember. Why? It is like this k T by h, how can you derive it? Very simply. So, remember from a classical energy oscillator from a classical energy oscillator; that means E oscillator which is classical what is that equal to?

Student: (Refer Time: 18:56).

k T right? It is a thermal fluctuation; E oscillator concentrate is equal to k B T. So, this is equal to k B T. Now, you are trying to bring an equivalence. Now, what is E? Oscillator if you have kind of developed from a quantum theory I can write as h nu right ok. So, then if you equate these two; that means, k B T is equal to h nu or nu is equal to k B T by h or is equal to k B T by h. So, that is why this k T by h is your frequency vibrational frequency right.

So, this frequency is the vibration or the frequency of vibration in which the bond breaks and hence the rate at which the transition state is breaking down is equivalent the vibrational frequency that is essentially what it is ok. So, that was your Eyring's theory ok. Now, we modify it a little bit we keep everything the same, but when we go to for protein folding process what we say that this is what we will do.

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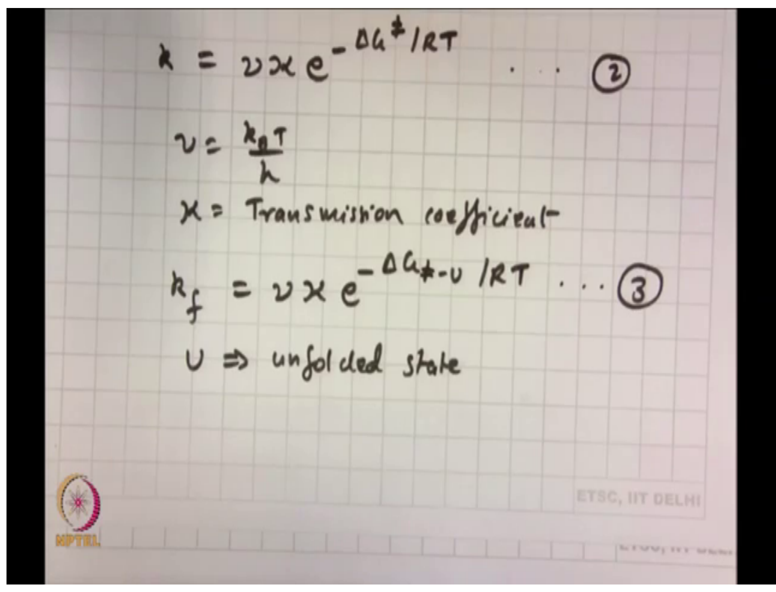

We will say that our rate constant k is equal to our rate constant k is equal to nu say nu where nu is equal to k B T over h showed before times a certain coefficient known as a transmission coefficient kappa a certain coefficient known as a transmission coefficient kappa times e to the power minus delta G dagger over RT; let this be 2, where kappa is referred to as the transmission coefficient.

So, have you heard about transmission coefficient before? So, what transmission coefficient tells you is transmission coefficient essentially transmitting right; transmitting in the sense; that means, you are starting from the unfolded state you are going towards the folded state.

And, the transmission coefficient tells you that if I have this many molecules on the way to the folded state, how many of those molecules actually go over to the folded state that is essential your transmission coefficient right that is what it says.

Now, this is again this is the equation we use for rate constant when we are talking about a protein folding reaction or a protein unfolding reaction, it does not matter which direction you are looking at it.

So, now, if you keep this in mind based on this what I can write is suppose I am looking at a protein folding ok; that means, the protein has started from the unfolded state it is going to the folded state and it is a two state there is no intermediate you know that is our assumption.

If that is what we take, then I can write that for a protein folding I can write k f is equal to right nu, then the transmission coefficient kappa e to the power minus delta G. Now, you are going from the unfolded state to the folded state, this delta G would be what? The difference between the free energy of the transition state and the free energy of what, the unfolded state?

Student: (Refer Time: 22:21).

So, then I can write. So, I will change this like this what I will write is?

Student: Minus U.

Minus U by R T, where U is the unfolded state where U is the unfolded state where U refers to the unfolded state and this is equation 3 for us.

So, then you can understand if I am going to come from the opposite direction; that means, if I am so this is what folding if I am going to have k U what will this delta G become? It will be this hash sign minus f right because now we will going from the folded side to the unfolded side.

So, your starting point is the folded in the case of an unfolding reaction. But, this is the folding reaction, so, your starting points is what? Your unfolded state ok. So, you just make that change.

Now, we also discussed this briefly. How would you know how would you know which part or which amino acid is involved to what extent in the actual kinetics of the protein? To put it simply, a protein has many amino acids. How would you know which one is kinetically important?

How would you; how would you which how would you know if we are going to destroy this one or if you are going mutate this one then something bad is going happen to the protein or something good is going happen to the protein; that means, there would be some changes in the protein.

Student: Active site.

Student: Active site.

Active site, ok. Now, that is active site for what you what you do for any enzyme where you have substrate catalysts and all these things? But, how would you prove it; how would you prove it?

Student: (Refer Time: 24:19).

How would you prove this difference? Remember, it is a two-state reaction. The only thing I have is a folded state and unfolded state. I have nothing in between I cannot see anything in between.

So, the only way I look, the only handle I have is my transition state because that is only state that is probably kinetically accessible to us in terms of what? The transition state theory this equation right because what is it?

Your k is governed by the nu times kappa times e to the power minus delta G, where delta G is what? The difference between the transition state and the free energy difference the transition state and your starting point whether it is the folded or the unfolded state it does not matter.

So, this is where it is unique. Unique in the sense that, see if there is intermediate it makes it easy for us because at least we can isolate the intermediate we can look at it and then make some judgment, but here we do not have anything accessible to us. So, then in that case, you will always have to fall back on the kinetics which is the rate constant and hence the transition state.

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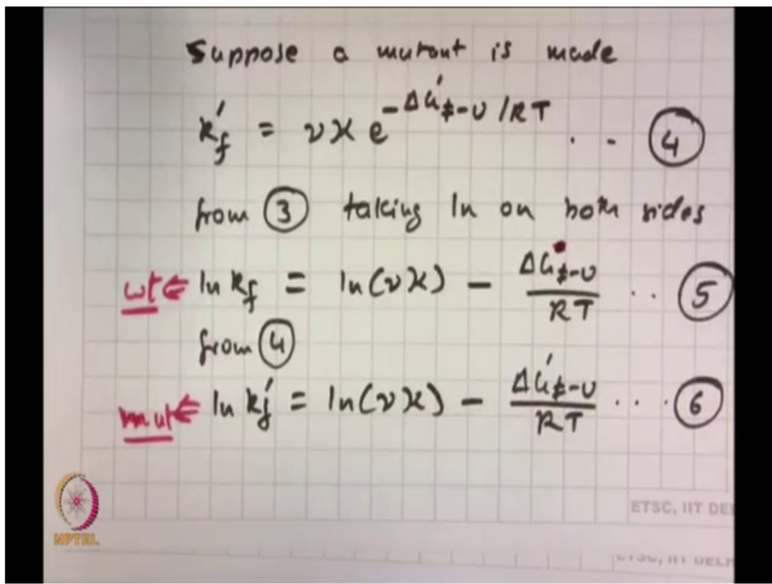

That means, if you are going to make a mutation and if you are going to make a mutation say suppose you make a mutation, suppose you make a mutation suppose a mutant is made now suppose a mutant is made and then what you say is the rate constant folding k f and because it is a mutant I label it by a prime because now it is a mutant.

So, k f prime then would be equal to nu kappa these things are not going to change ok, but this one is going to change possibly delta G right u and this is prime over RT and this would be equation number 4. Simple, right? I have changed the mutation I have done a mutant right. If there is a change in delta G it will be getting reflected in this and let us see where we are going from here ok.

Now, what do we make use of these equations? Say, for 3 from 3 taking natural log on both sides we can write natural log k of f is equal to nu times kappa minus delta G U over R T. So,

this would be say 5, simple right? Then we can also what we can also do is from 4 what we have is natural log k f prime is equal to nu kappa minus sorry.

So, let me do this. So, please cancel this. This is not delta G prime right this is just delta g this is not the mutant this is not the mutant. This is the mutant this is 6 ok. So, this is not the mutant this is of the what do you call that protein which not a which is found in the native state?

Student: Wild type.

Wild type, right? I mean no mutation. So, this for the wild type. So, let me write it. So, this is for the wild type. This is for the wild type and this is for the mutant good. So, that means, say a mutant is affecting in the transition state in some way or the other. If you would be able to measure the kinetics and hence get the rate constant you would be able to figure out the difference.

Now, what difference are you going to figure out ok? So, the difference is this is what leads us to a very important feature which is used regularly in protein folding to try to look at effects in the transition state you know what amino acid is affecting to what extent.

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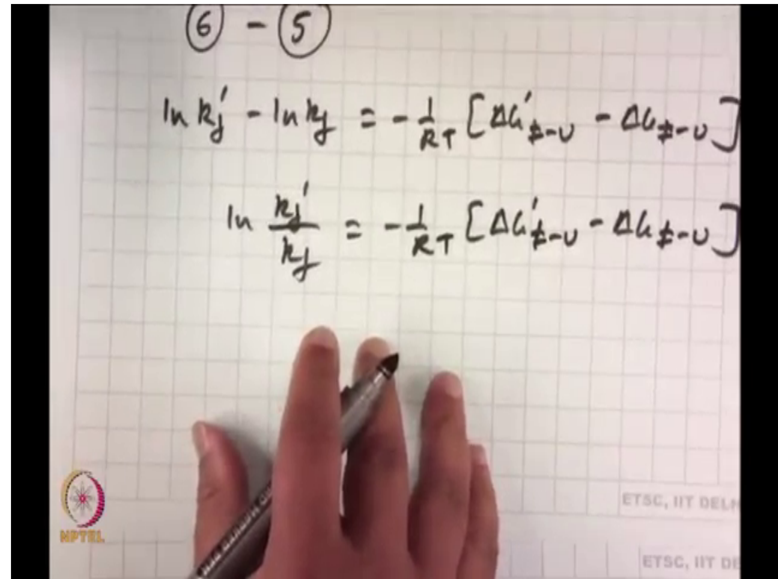

So, what now I do is I do 6 minus 5 I do 6 minus 5. If I do that then I have natural log k f prime minus natural log k of f is equal to minus 1 by R T delta G prime U minus delta G U ok. So, then I can rearrange it and write it like this I can write ln k f prime by k of f is equal to minus 1 by RT del G prime U minus delta G U.

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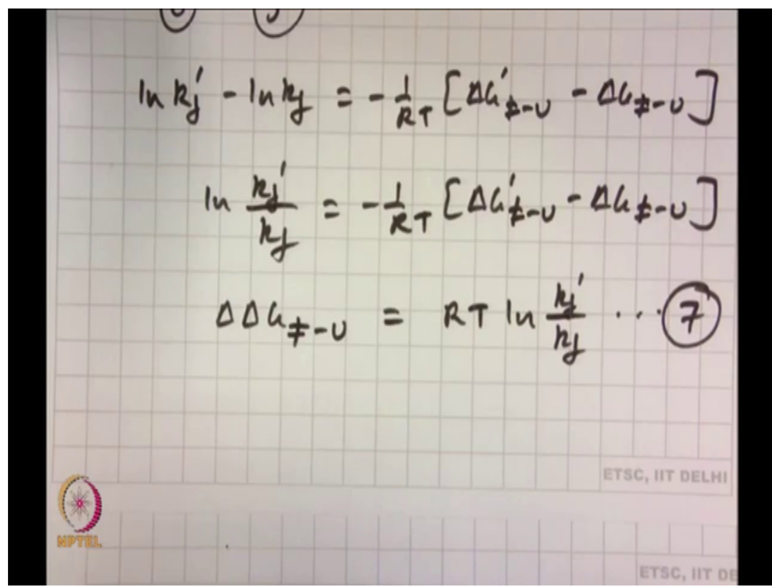

Finally, I will introduce this term delta delta G U is equal to RT natural log k f prime over k of f, let this make this equation number 7. What is the significance of delta delta G? What is the significance we have encountered this before. The significance of delta delta G is it is the difference between two delta Gs right and what are the two delta Gs in this case? One delta G is coming from the wild type the other delta G is coming from the?

Student: Mutant.

Mutant right. So, we can think about two very extreme circumstances: one is that there is no effect that means, both these are equal that is equal to 0; the other thing is because we would be taking a fraction the other would be 1, that means, both of these are equally affected in some way or the other. Now, let see what we mean by that.

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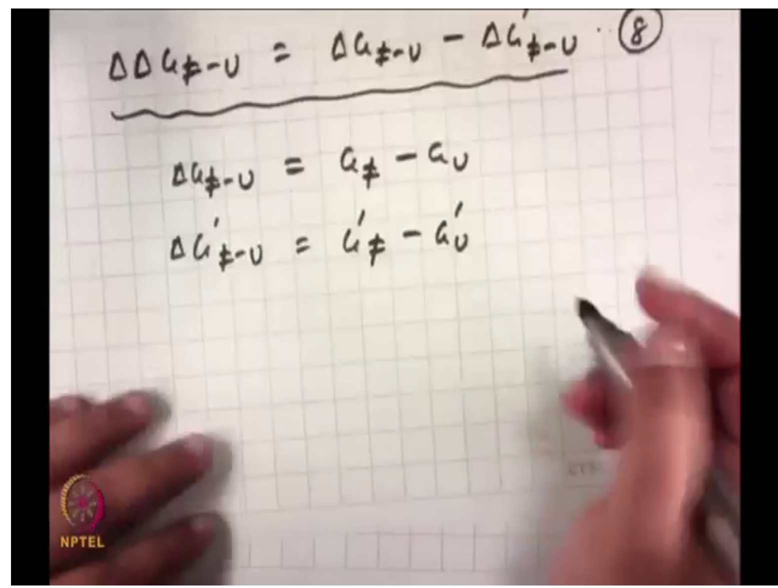

So, the way we have written delta delta G out here is I can rewrite it as or the way the convention I did was this is equal to delta G of the wild type minus delta G of the mutant. Now, this is the convention I have put in ok.

So, now, see where I am going. This delta G u is essentially what? Is essentially G that is the free energy of the transition state right minus the free energy of the unfolded state, is it not? That is what we know it by.

Similarly, the delta G prime u is the free energy of the transition state of the mutant minus the free energy of the unfolded state of the mutant right ok.

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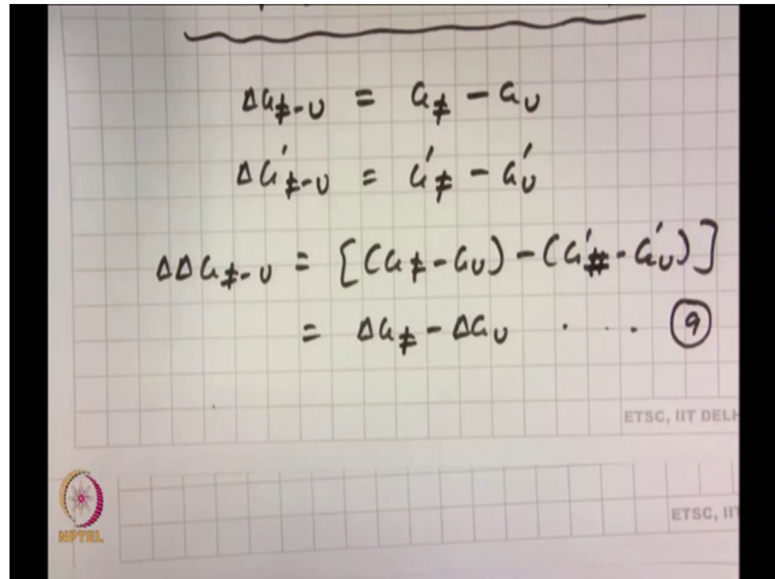

Now, if I label this say as equation 8, if I label this as equation 8 then using these two in equation 8 what we have is delta delta G U is equal to what I can write is then G minus G U minus G prime of the transition state this is the transition state minus G U prime of the mutant ok. Now, can you tell me what this simplifies to? Let me give you a hint.

Now, because you have this transition state and the unfolded state why do not you group this one and this one and why do not you group this one and this one. So, what does this tell you? So, this is equal to delta G minus delta G U is it not? Is it not? Right.

So, see and this is something you know very well that means, you just look at the you just look at the I mean the energy pathway the unfolded the transition state and all these things and then you come up with this ok. Now, keep this in mind because we would need this very soon.

Now, let us look at a few diagrams of protein folding before we move on to the actual place where we use this equation we have just derived ok.

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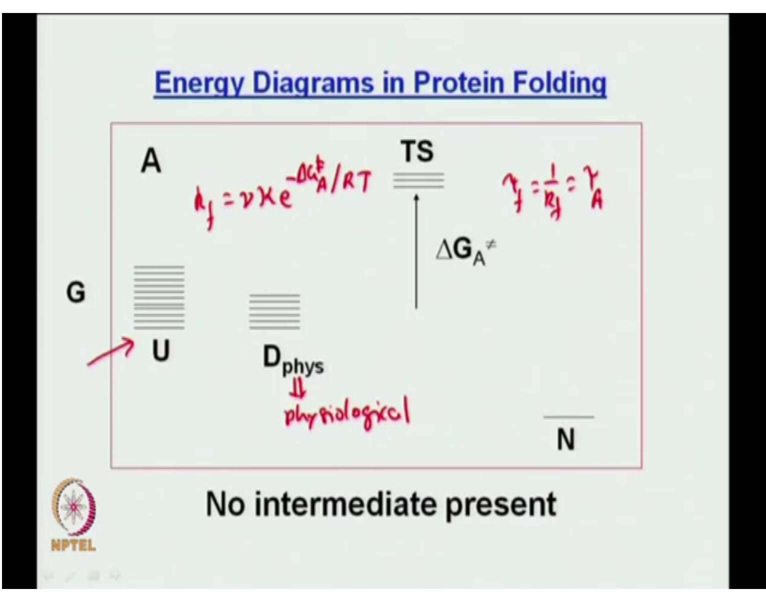

So, let us consider some energy diagram. So, this energy diagram goes like this. So, there is a certain reaction coordinate on the x-axis, on the y-axis it is your free energy. Now, try to understand this figure. You look at the unfolded state you look at the unfolded state U. Now, in the unfolded state you will see we have drawn many lines. What do those lines refer to?

Student: Ensemble of (Refer Time: 34:18).

Ensemble of confirmations right and you know in the unfolded state it is going to be maximum disorder, it would be having maximum flexibility. So, that means, it would be having the maximum number of confirmations available. So, you can see in terms of all the other states

present you have the maximum number of lines in the unfolded state ok. So, that is the starting point.

Now, what you say is ok, the next point is D which is your D natured state, but under physiological conditions. So, this D phys phys means physiological. So, under physiological conditions what might happen is that it might having a little more compaction, it might not be as unfolded as it was right. So, that why see the numbers of lines has decreased. So, then it was become compact.

Now, from here you go to the transition state; that means, you are going from the left unfolded state to the final native state the transition state has only three lines. So, because the transition state after transition state you are going to move over to the native state what will happen the transition state would be having lesser number of confirmation states right and then finally, the native state is just one state, just one unique state ok. So, this is for the sake of argument that is what we are going by.

Now, if you go by this, there is no intermediate present as written at the bottom of this slide. So, if I would write if I would write my k ok; that means, this is what k folding or unfolding, what should I write? I am going from the left to the right.

Student: k. (Refer Time: 35:56). I should be writing k?

Student: Folding.

Folding, right? So, that means, I can write k folding is equal to there was nu kappa e to the power minus delta G and I can write A by R of T, is it not? So, this is one case ok we will be considering the series of cases. So, then if this is k f if this is k f that is what we were talking about then I can write tau which is tau f is equal to 1 by k f and I say it is equal to tau A. Why? Tau A because this is case A for me and tau f is essentially 1 by k f; that means, the inverse of that right ok.

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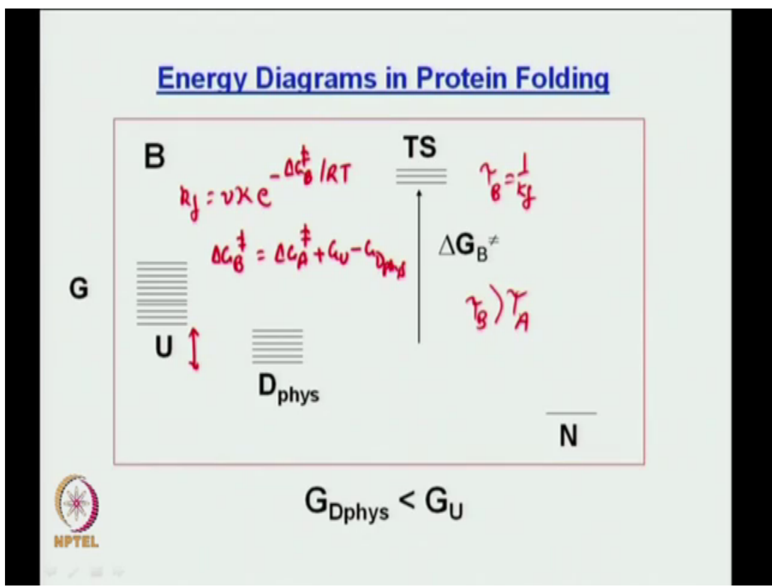

Then, what is the next? What is the another figure? Now, let us look at this. Here again there is no intermediate, but the small change is this, in the previous slide; in the previous slide the unfolded state and the D denatured state under physiological conditions what is the same energy only there was the compaction. But, in this case what you are saying is that when it goes to the physiologically relevant state still denatured, then there are some stabilizing interactions coming up.

If there are stabilizing interactions coming up what will happen? The free energy would be lowered right and hence this D phys physiological here has the lowered free energy than the U ok. So, your transition state is not affected, transition state remains the same.

Then, what will happen is you will be having new barrier which is delta G b in this case hash and then you will go to n right. So, here what I can write is k f is equal to again nu kappa e the

power minus delta G B by RT ok, but what is delta G B equal to for me here? Can I write this?

So, delta G B where should I write let us see if I can squeeze it here. Delta G B is equal to delta G A which was there before in the previous one, now tell me what should I write?

Student: Plus.

Plus, can I write this? G U minus G can I write this or not? See this is by how much in a stabilized and what is this? This is essentially G U minus ?

Student: (Refer Time: 38:36)

G of D physiological right. So, then what has happened to the barrier? The barrier has become larger. See, if the barrier becomes larger and this is tau B is equal to 1 by k f in this case, then I can write here I can write here tau B would be greater or lesser than tau A?

Student: (Refer Time: 39:01).

Tau it is the folding time, would it be greater or lesser than tau A?

Student: (Refer Time: 39:01). Is small.

Student: Greater Than greater than.

It is greater than tau A, right? that means, tau A. So, the case A you would be folding faster than B, why? Because your energy barrier has increased. Why? Because your physiologically

denatured state which you are considering is stabilized has compared to the unfolded state and it has come down in terms of the free energy, clear or not? Is it clear ok?

See this is very similar to what probably you guys have done in terms of have you done Hammond's postulate and all these things in physical organic chemistry by any chance? ok.

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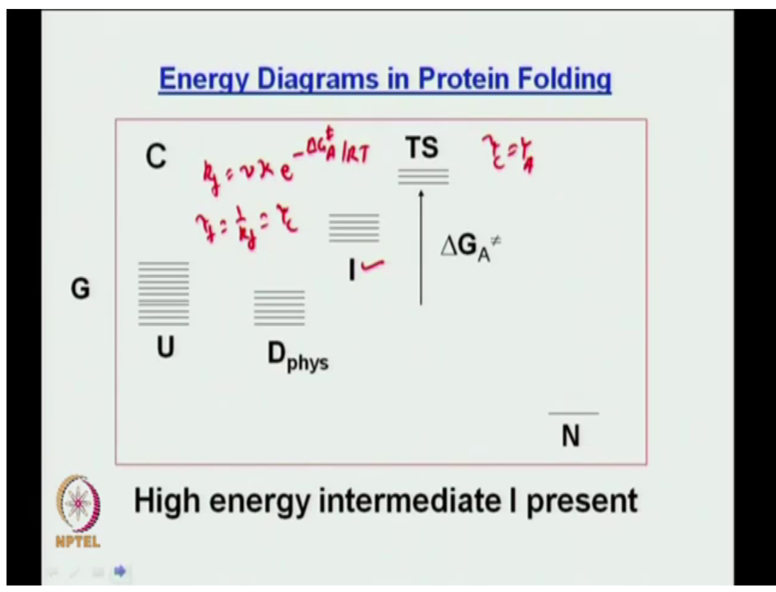

Now, let us consider this. Here we are now invoking an intermediate the presence of an intermediate. Now, what does the presence of intermediate do for you? In this case it just gives raise to another state an intermediate which is I here. So, this is the intermediate, but here this intermediate is high energy intermediate. So, the intermediate essentially does not what? Effective kinetics. So, in this case your k f is still what? It is still delta G A that hashed sign.

So, here k f is equal to nu kappa minus delta G A by RT where tau f is equal to 1 by k f this is say tau C and then I can write tau C would be equal to tau A because it is the same thing. So, if tau C is equal to tau A that means, both of these are of lower value than tau B ok.

Now, can you tell me what is the last so, there would be another alternative to this what were what would that be?

Student: Intermediates.

So, the intermediates come down lower right ok.

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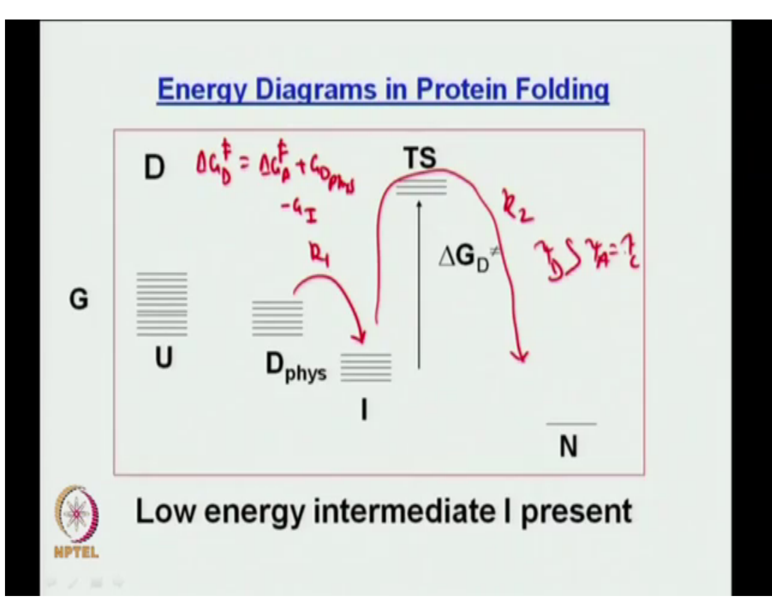

So, then so, this is what exactly happens now you can see the intermediate has lowered down. Now, if the intermediate is lowered down, this process has two steps. What are the two steps? High energy intermediate did not matter to your kinetics, but the low energy intermediate would matter. Why? Because you would be having two phases. What are the two phases? This one would come here

Student: Yes

Say, that is k 1 and this one would go all the way from here to here to the native state and that would be say k 2 ok.

So, which one is more defining in terms your rate? k 2 because that is

Student: (Refer Time: 41:56).

A much bigger barrier. So, in this case; so in this case what is delta G D equal to? What is this equal to? Tell me now. You start with this, then what should I write?

See, when it was remember the second case no intermediate it was G of U minus G D. So, in this case U and D are the same essentially, but I am talking about this D and I now. So, I can write plus G D physiological, then what is the next one?

Student: Minus g.

Minus G of I, is it not?

So, that means, this delta G D double dagger is essentially or hash is essentially from the intermediate to the transaction state it is no longer from D because this intermediate is not high energy this is the low energy intermediate. Now, you can immediately realize what will

happen to the tau D? Tau D would definitely be less than tau A rather more than tau A and tau C.

So, that means, I can write tau D see if this is D then tau D would be greater than tau A and tau A was equal to tau C. The simple reason being your intermediate has stabilized now and hence again your energy barrier has gone up ok.

So, these are four very simplified instances, but covers the most of protein folding in terms of the basic premise. The first two were no intermediates, the second two is started considering intermediates right. Now, this is just one intermediate, you might be having a series of intermediates then accordingly that will vary ok. But, what is the take home points from here, how do we look at this?

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Energy Diagrams in Protein Folding

- Rate of folding drops when D_{phys} is at a lower energy than that of U
- Also any intermediate between D_{phys} and transition state that is of lower energy than that of D_{phys} will slow down the rate of folding
- High energy intermediates do not slow down the folding rate provided they do not lead to states that are higher in energy than the transition state

The rate of folding drops when D physiological is at lower energy than that of U, that we saw good. Now, any intermediate between the D physiological and transition state that is of lower energy than that of D physiological will slow down the rate of folding, that is what we just saw ok.

High energy intermediates do not essentially slow down right because they are not affecting your kinetics. So, for our case we do not even bother about the high energy intermediates, good.

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Diagrams in Protein Folding: Consequences

- Accumulation of folding intermediates is unfavorable
- Stable elements of structure in D_{phys} slow down the rate of folding since they lower the energy levels of D_{phys}
- Thus nonnative interactions such as extensive hydrophobic interactions in D_{phys} will slow down folding
- Formation of stable elements of native structures in D_{phys} is also unfavorable

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Now, what are the consequences? Now, think about this. The consequences if you would be having intermediates and if those intermediates would be of low energy, what would happen?

Your folding rate would be affected and that would be lowered as compared to that case where you do not have any intermediates. So, this is what you mean by a fast folder.

Essentially chymotrypsin inhibitor 2 was the fast folder why because it did not have an intermediate. It was?

Student: (Refer Time: 45:05)

What? It was lacking intermediates. Hence it could fold faster than say another chymotrypsin inhibitor 2 hypothetical which would be having an intermediate right. That is what it is trying to say ok.

Now, stable elements of structure in the physiological denatured state slow down the rate of folding since they lower down the energy levels of D phys that is what we have also seen. This is just being rephrased. Thus nonnative interactions such as expensive hydrophobic interactions in the physiological denatured state would do what? Would affect your?

Student: Folding rate.

Folding rate by slowing it down, as compared to the case where you would not be having any such stabilizing interactions present.

See here I am not talking about intermediates, I am only talking about what? The cases A and B, where there was no intermediate I only had the D phys. In case the D phys was almost the same as U or was the same as U and the in second case which is case B, D physiological was of lower energy than U, good. So, formation of stable elements of native structures in the D physiological is also unfavorable.

Now, see how do you rationalize this? How do you rationalize this? So, let us look at this real quick ok. Now, suppose I am considering this chymotrypsin inhibitor 2 and remember, if you

remember the structure of the chymotrypsin inhibitor 2 I had a helix is there along with the other beta strands.

Now, suppose you do something what you do is, you put in certain amino acids like say you are redesigning protein. Your protein is some certain amino acids which stabilizes the helix, in what sense? What it does is if it is stabilizing the helix, it is stabilizing the native state because it is stabilizing the helix and it is also stabilizing the transition state. So, this is what I mean.

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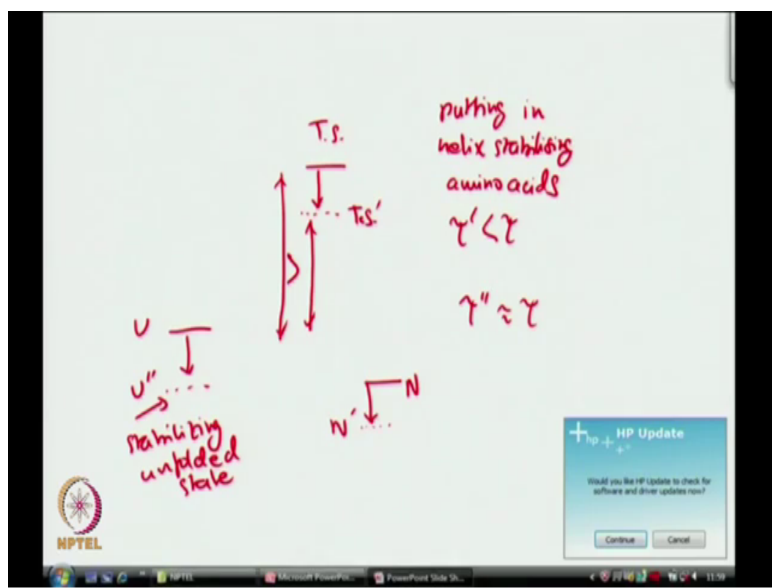

Now, I will be draw it very simply. So, this is your unfolded state ok. This is your transition state and this is your native state right. Now, I have gotten rid all those different lines right, very simple. Now, what is happening is you are putting in helix stabilizing amino acids. If you are putting in helix stabilizing amino acids, what will happen?

Now, I am saying is in the first case the transition state is affected so is the native state. So, native state is being stabilized, but the unfolded state is left unaffected; remember, the unfolded state is left unaffected ok.

So, if the transition state comes here and the native state comes here say so, this is say T S prime and this is N prime ok. Now, tell me under this new modification which you have introduced what happens to the folding rate or what happens to the folding time? Is it lesser? Is it folding faster or is it folding slower?

Student: Faster.

It is folding faster. You can understand right it is folding faster because the barrier the effective barrier has decreased. Why? Your unfolded state was not affected ok. So, that means, this barrier is lower than this barrier; that means, this one is greater than this right. So, this barrier is greater than that barrier and hence in this case what is happening is your tau prime

Student: Less

Is less than tau where prime is belonging to the mutant, good no problem.

Now, think about the other consequence. The other consequence is now until this point we had said that your unfolded state is not getting affected, right. Now, you are put here now you are putting in helix stabilizing agents to such an extent to such an extent that it is also effecting the unfolded state and it is also stabilizing the unfolded state.

So, do you understand now what is happening? Initially when you did it you introduce the mutations in such a way or introduce the amino acids in such a way that it was only affecting the native state and it was affecting the transition state ok.

This is just an assumption please, just let us just go by it right. Because you get the native state from the transition state then transition state follows the native state essentially or native state follows the transition state, it follows the energetic consequences and hence both of them are stabilized. But, the unfolded state stays where it is and because the unfolded state stays where it is barrier gets decreased.

Now, the movement you make some other mutations which also stabilize your unfolded state what happens? If now your stabilize your unfolded state this will also come down. So, this is U double prime which is your second mutant; second mutant is that means, you are also stabilizing unfolded ok. Now, tell me qualitatively what would happen to tau? Would it change or would it not change? Do not worry about the absolute value, just tell me.

Student: (Refer Time: 50:27).

It would not changed right it would not change why? Because you see not only you have stabilized transition state you have also stabilized your U and hence the energy barrier as effectively what remains the same? Is it not, ok? So, that means, in this case for. So, I can say that tau prime would almost be equal to tau and start double prime would almost be equal to tau. It is clear or not? Good.

Why did we go through this discussion you know try to understand this. In the previous slide, we had in the previous slide now let us go to that previous slide, look at the last sentence. What we said is formation of stable elements of native structures in the physiological denatured state is also unfavorable. Now, why did I say that? See if you are stabilizing something we should not be saying it is unfavorable right, we should always go to what is there.

But, the problem is when you stabilize the unfolded state, you are not doing anything. Are you doing anything? Because you just saw when you stabilize the unfolded state because that is where you are going.

If it is just the mutant which is not affecting the unfolded state no problem, but if your mutant if your mutant is stabilizing the unfolded state there is no change in the free energy barrier; if there is no change in the free energy barrier the protein does not change anything. It is taking the same time to go over to the; to go I mean to go over the barrier.

So, you know think about it logically why would evaluation go for that? Because you are putting in more energy stabilization at the cost of what? Nothing. See, you would not do that, is it not? This is the crux of the matter. I would not try to randomly stabilize my protein because finally it of no consequence to me.

But, listen when I am doing this energy stabilization, I am actually doing some modification. I am using this extra stabilizing interactions but finally, I went in with this extra stabilizing interactions and I came out with nothing. It was of no consequence to me in terms of the protein folding scenario; that means, the protein folding rate.

Did you get the point? See, you would not do any random mutations right or in other words you would just not try to stabilize it as much as possible because these things can happen and finally, you would not get anything out of it ok.

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Window of Stability

There exists a window of stability for a local structural element

- If too weak, it will destabilize the folded state
- If too stable, it will overstabilize the unfolded state

So, then to rephrase it there is the window of stability remember we are talking about this local structural elements. If you remember we started from the nucleation condensation mechanism, where a nucleation condensation we had said that to form the nucleus you would be having adjacent local elements which would come and stabilize and then form the nucleus ok. This we are talking about the local structural elements.

So, it says that there exists a window of stability for a local structural element. Now, what do I mean by that? The window is like this. If too weak, it will destabilize the folded state. I would not want that also, see you are trying to maintain a balance.

See, if the local interactions are too weak then even in my folded state it would be weak, no matter how my tertiary interactions are and hence it would not be that stable. So, that is not called for that is not something you would want to do ok.

Also if too stable, what will happen it will over stabilize the unfolded state then also it is of no consequence to me. One is you under stabilize; that means, you destabilize the folded state that is the problem ok. The next is you do not try to over compensate by over stabilizing, then what you doing? You stabilize the unfolded state, then again you had having no change you are wasting you are wasting your good stuff.

So, then what is the window? The window is always between these two, is it not? And this is your window of stability for your local structural elements. You would not go one way or the other, you would always try to remain within these two points.

Keeping this energetic consequences in mind, is it clear? So, this is what is extremely important if you would try to design a protein if you would try to mutate a protein or engineer something, you would have to make sure that these things are not happening.

One is obviously, you are not going to destabilize the protein because the folded state will destabilize then the whole thing falls true falls apart, it is very unstable for you to see and the other one is you do this mutations, but finally, when I do the kinetics you do not see anything why? Because you have also tweak the unfolded state because you are over careful; being careful is good being, over careful is bad right that is the point.

Student: Yes sir.

Now, it is like this I will tell you a very may be it just happens to me. See, in my office there are many papers right many many different papers I lose track off, but if you ask me to find one file I will find it ok. You give me say 5 or 10 minutes or may be little more than that.

Now, suppose I get the paper and I put it somewhere else and I have forget where I have put it, I was being extra conscious now.

So, what has happened no matter how much time you give if I do not recall where I put it, I will never be able to find it, but I was being over compensatory. I was I was being over compensatory for the fact that I have so much stuff on my table I would put it in some other place where it would be safe and secure it was really safe and secure because I have forget about it ok.

This is exactly what you are seeing out here, is it not? I mean it is very simple at least from my side to understand because I am doing it every day ok.

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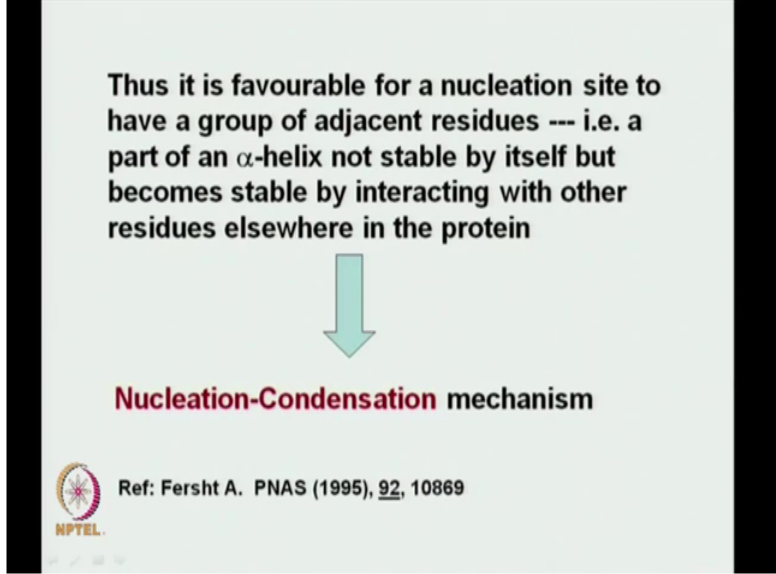

So, yeah I will just let me finish this part I will start with a next one next class. So, thus it is favorable for a nucleation site to have a group of adjacent residues that is, a part of an helix not stable by itself, but becomes stable by interacting with other residues elsewhere in the protein ok. So, that is stable by itself right.

And, this is your classic nucleation condensation mechanism. Now, hopefully you will understand where this nucleation and condensation term comes in. This is where it comes from.

You would not stabilize it too much, you would also not destabilize it too much that would it would never form the nucleus because if you destabilize it the local elements interactions would be low, it would never form the nucleus. Your protein would never go or even if it does go it will be having a very low stability right.

So, the next class we will start with something know as phi value analysis; phi value means how do you actually prove the transition state; that means, how do you actually prove to what extant a mutant is changing your energetics ok, that is what we would prove next class.