Bio-Physical Chemistry

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Lecture - 29
Protein Folding: Mechanisms and Kinetics
(Contd.)

So, we will start where we left of the last day we were discussing the implication of kinetics right on in Protein Folding. So, the topic we are going to start off today is known as phi value analysis. Now you will see it is an exquisite way of trying to determine how the transition state is affected because the only way the transition state can be probed is by kinetics right. This in protein folding at least it is very hard otherwise to figure out what is happening in the transition state.

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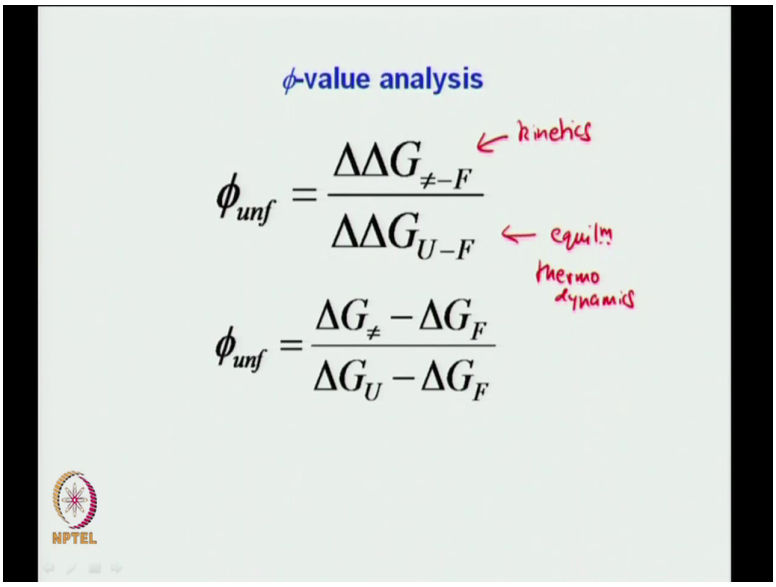

So, what is this phi value analysis? So, going in more detail let us look at this formula first. So, what it says is phi unfolding; that means, you are unfolding a protein you are starting from the folded state you are going to the unfolded state. What is it equal to?

It is equal to delta delta G dagger minus F; that means, because you are going from the folded state to the unfolded state right it is phi unfolding right hence the difference between the transition state and the folded state is the one which is your energetic barrier.

And you are considering a delta delta why it is a delta delta? Remember it is a delta delta because if you are taking just one protein which is the wild type then it is delta G dagger minus F. But now you are comparing two proteins what are the two proteins in one protein is a wild

type; that means, you have not made any changes in amino acid sequence in the other protein what you have done is you have made a mutation.

And hence the difference between these two delta G's referred to as delta delta G ok. So, the top one is the one which is related to the transition state as you can understand what is the bottom one? The bottom one is the one which is related to what? Tell me in the simplest term, it is related to

Student: (Refer Time: 02:44).

An equilibrium term. So, it is essentially the difference between the unfolded and the folded state between the two proteins one being the wild type the other one being the mutant.

And to make it little more clear for you this is how it goes so; that means, phi unfolding is equal to the change in the barrier minus the change in the folded state which is in the top one. The numerator and the denominator essentially is delta G U minus delta G F ok. Again this one comes from equilibrium thermodynamics and this one we get from kinetics ok.

See the bottom one we have always refer to before we can always have equilibrium free energy changes for proteins remember all those transitions we talked about before right that is easy to for us to have.

But for having the same information with respect to the transition state we got to go through kinetics. Now why we are going through all these or why was this idea developed? Now this is why it was developed remember we started this when we talked about, when we well before actually coming to this the topic we started on is or was rather the Nucleation Condensation Model was not it; it was a nucleation condensation model.

And if you would try to figure out, if you would try to figure out which amino acid which amino acid is being involved in the transition state towards the final structure formation. This is something you would be doing. Again what it means is what you are trying to figure out

now is if the protein has 150 amino acids right not all amino acid would equally effect the folding of the protein.

Instead what you would do is say a protein forms a hydrophobic core right which is the interior of the protein. And you would know for the protein to be stable the hydrophobic core the interactions have to be pretty well developed if that is.

So, then what it will also tell you is that those amino acids in the hydrophobic core would be one of the key components for the protein to remain folded right if that is. So, what you would do is you would try to target one of those amino acids replace it with another mutant and see to what extent it is effecting your folding or unfolding that is essentially what you will be doing ok.

And that is essentially what you are trying to figure out and to end this part of the discussion. This tells you the residue level it is like a residue level interrogation of how a protein folds; that means, you pick amino acid by amino acid you change that amino acid with another amino acid. I will tell you under what conditions or what are the approximations you can do or what changes you can do.

If you would change this amino acid with another one then you would absolutely be able to figure out to what extent this amino acid was being involved in the folding unfolding pathway. And this way you can pick out as lot of amino acids that is essentially what you want to do, because getting residue level information in proteins specially for a two state folding protein, but there are only two states folded and unfolded is very hard, is it clear?

So, again we have to get residue level information. Now let me just take you back to something you would know remember if you would try to figure out whether that hydrogen is involved in a certain reaction right, whether that bond involving the hydrogen is involving the certain reaction what would you do?

Student: (Refer Time: 06:51).

You replace it by deuterium remember this primary kinetic isotope effect secondary kinetic isotope effect. What are you trying to do? You are trying to figure out where that bond is actually involved in the reaction and then to what extent is it involved right. Exactly the same thing you are doing here right. Instead of a bond what are you replacing? You are replacing an amino acid that is essentially what it is in a nutshell ok.

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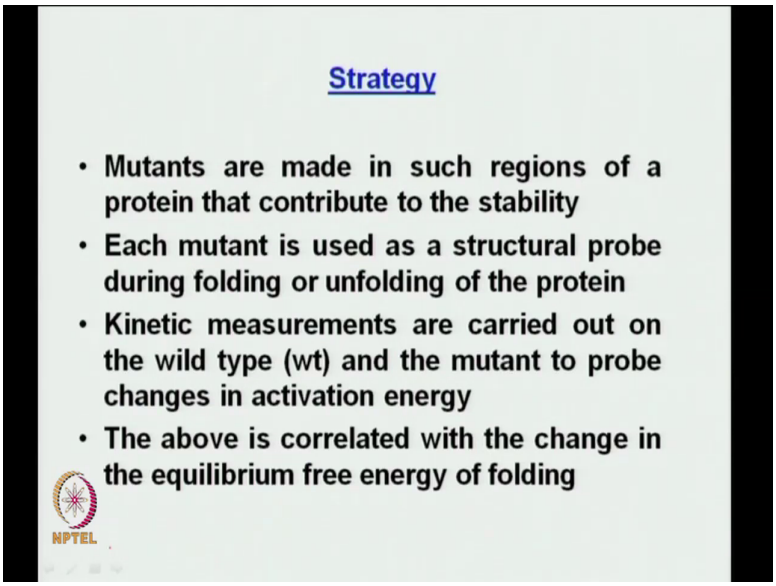

Then what is your strategy? Your strategy is this; the first one is you have to make mutants in such regions of the protein that contribute to the stability. Now it make sense right, if you take a certain mutant which has very little effect on the stability of the protein you are not going to get much information because you know finally, the final structure of the protein or the native structure of the protein is not exactly determined by that amino acid.

Hence the change whatever you would be getting would be either very small or insignificant. So, you would have to by logical choice, look at a certain region of the protein which is inherently involved or intrinsically involved or very importantly involved in terms of it is interactions. And with its neighbors and all these things. So, you pick up the hydrophobic core you know that is one of the things which we always do ok.

Then now each mutant is used as a structural probe during folding or unfolding of the protein that is what I said; that means, if you would be doing the kinetics I will show you later. You can look at you can look at the folding arm or the unfolding arm and you would be able to figure out to what extent this amino acid is affecting this arm or that arm ok. Now wait for that one, now what you would do is you would do kinetic measurements right.

You would do kinetic measurements on both the wild type and the mutant. So, remember if you go back to the phi F the phi unfolding rather. So, phi unfolding was equal to what delta delta G hash minus F right so; that means, if you would do kinetic measurements on the two mutants what you would you get? You would get this numerator the denominator is coming from what? Equilibrium measurements. For that you do not do kinetics because that is essentially equilibrium and hence you compare those.

So, then that is what the last statement says the above is correlated with the change in the equilibrium free energy of unfolding because I am talking about phi unfolding. Parallelly I can also talk about the reverse process that is phi folding. Here I am starting from the unfolded state going to the sorry the folded state going to the unfolded state I can also start from the unfolded state and go to the folded state either way no problem at all ok.

So, let us look at a free energy diagram right and let us try to keep it very simple we are talking about a two state protein right.

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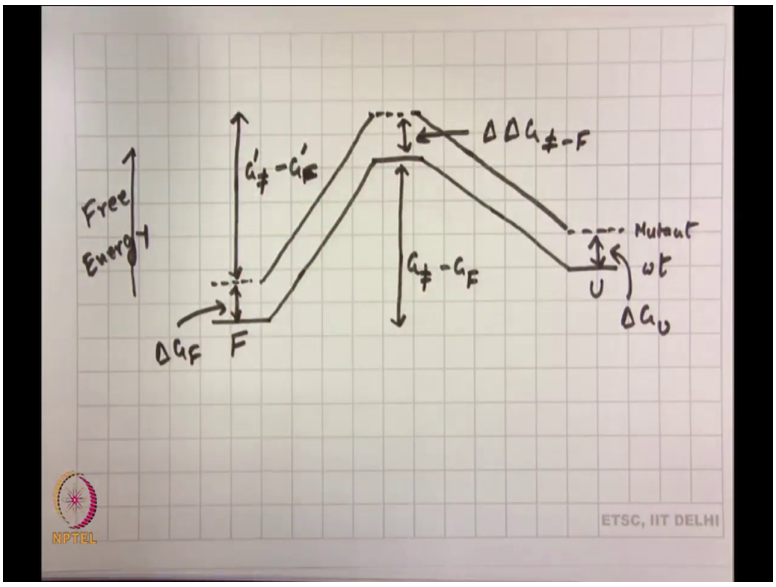

So, let this be the wild type say this is the folded state because you are talking about unfolding. You start from the folded go to the unfolded. Say, this is the transition state right and say this is your unfolded state ok. So, wherever you have these solid lines this one belongs to the wild type; that means, you have not done any changes in the protein whatsoever.

It is how it is found in nature that is it. See, if I just join these say this one.

Student: Sir..

Right. So, this is the transition state of the wild type. Now what you do is you do a certain mutation right. And say right something like this happens now this is just a profile of what changes we are going to look at.

So all the dotted lines are your mutant, all the dotted lines are your mutant. So, here also I will be having a similar thing ok. This is my free energy, this is my free energy in the y axis and the x axis. I have a certain reaction coordinate it does not matter what it is ok. Now what is this one? So, from here to here from here to here what is this one if this is a transition state right. So, this is the transition state for the wild type this is the transition state for the?

Student: Mutant.

Mutant so, then this one I can write as G dagger minus G F ok good, now if this is the mutant for me right. So, the this dotted line is the mutant. So, then for the dotted line I can write this is what is G prime, the prime refers to the mutant minus G this is F is this clear. So, one is the free energy difference between the transition state and the folded state of the protein in the wild type which is G hash minus G F the other one is for the mutant which you see on the left hand side ok.

So, now because you see the folded state has also moved and the unfolded state has also moved, with respect to the wild type for the mutant this one, I can say this is delta G F and this one I can say it is delta G U right. Can you tell me what this one would be now? This difference, between these two what should I write. See this one is

Student: (Refer Time: 13:51).

Delta delta G is not it right because I am going from the folded state to the unfolded state. I am looking at unfolding.

See, the idea of this figure is just look at what this figure is trying to tell you or portrait to you, is essentially these things. What are the thermodynamic parameters that are involved in this actual transition? So; that means, if the mutant is changing my folded and unfolded state then the change for the folded state would be given by delta G F, the change for the unfolded state

is given by delta G U. G hash minus G F is the t s barrier G hash minus G F with the primes is the same barrier for the mutant.

So, if I take a difference between these two barriers I will be getting delta delta G; that means, the difference in barriers of the two transition states which is delta delta G hash F. Is it clear what we are talking about now? So, this is what we are going to talk about throughout in the rest of the class.

So, now, you can understand one thing instead of going from the folded to the unfolded state if you would go the other way around what would happen this delta G U and delta G F would remain the same no problem, but what would change is instead of this minus F. What should we write?

Student: Minus U.

Minus U.

Student: (Refer Time: 15:15).

That is the only small change that would happen clear. So, if from left to right I am talking about phi unfolding then from right to left I should be talking about phi.

Student: Folding.

Folding and what will happen in my phi unfolding would be that, whatever wherever I have F it would be replaced by.

Student: U.

U ok. I will come to that again later. But anyway is this diagram ok for you at least to start with ok. Now now let us actually talk about this phi unfolding this phi value see if it is a ratio;

if it is a ratio typically what would we think it would it would lie between 0 to 1 right we are talking about a ratio.

Now, that is what it is essentially. So, then the two extreme cases are one is phi unfolding 0 the other one is phi unfolding 1 right. So, let us look at those cases in a stepwise manner.

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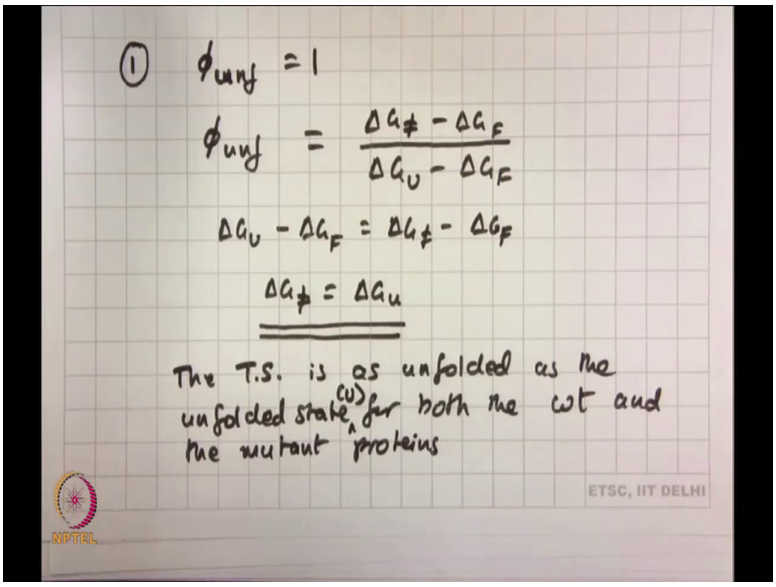

See, suppose I have phi unfolding equal to 1 if phi, if I have phi unfolding equal to 1. Now from before which is at the first slide what was phi unfolding equal to? Phi unfolding was equal to if you look at the second equation it was delta G hash minus delta G F right.

Then what was the next one delta G U minus del G F because I am going from the folded to the unfolded state U is my final F is my initial right. Now if phi unfolding is equal to 1 say if this is equal to 1; that means, what can I write. So,now phi unfolding is equal to 1 for me? So,

I can write delta G U minus delta G F is equal to delta G hash minus delta G F is not it? Very simple because phi unfolding is equal to 1 if that is the case, what am I left with delta G hash is equal to what?

Student: Delta G U.

Delta G U; now try to understand the significance of this. What are you seeing? What are you seeing is if my phi unfolding equal to is equal to 1, then whatever change I have in the transition state is equal to the change in free energy of the unfolded state. Now that is what you would expect right phi unfolding is equal to 1 that is what it means.

To put it in words what it means is whatever the structure I am looking at the protein is as unfolded in the transition state as it is in the unfolded state for both the wild type and the mutant.

Do you understand this or not? That means, there is a 1 is to 1 correspondence between what the change in free energy of the unfolded state and then change in free energy of

Student: Transition.

Ohe transition state for both the mutants; that means, this I do for the wild type and then I do it for the mutant, is it clear?

So; that means, I can say based on this the TS which is the transition state is as unfolded as the unfolded state for both the wild type and the mutant proteins.

Student: By the transition state.

Ha.

Student: By the transition state.

Say, that again I did not understand. So, what we are saying is that the transition state is as unfolded as the unfolded state. So, let me write this unfolded state which is U for both the wild type and the mutant proteins; that means, whatever change in unfolding free energy you have in the unfolded state would be exactly reflected in the transition state. Is it clear? Good. Now if this is the case what is the other case? The other case would be phi unfolding equal to.

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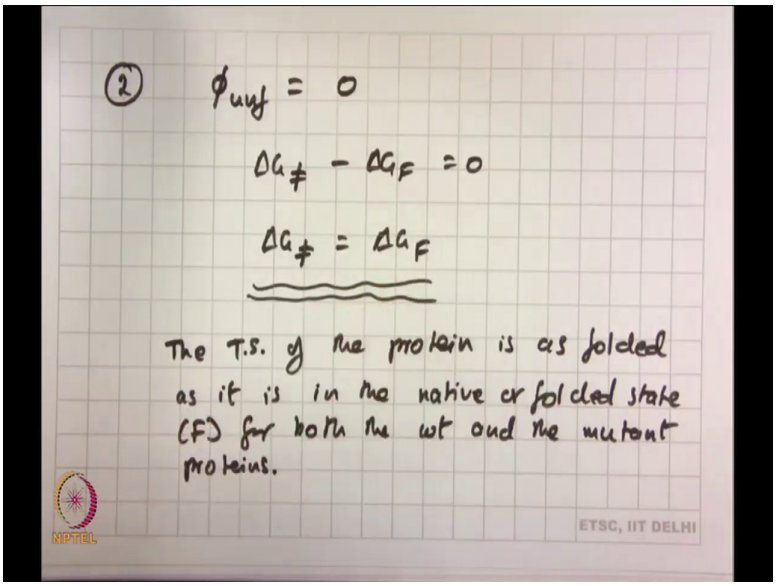

Student: 0.

0. If phi unfolding is equal to 0 what is it mean? If phi unfolding is equal to 0 then based on this equation; based on this equation if phi unfolding is equal to 0 the numerator is 0 then I can

write delta G hash minus delta G F is equal to 0 or delta G is equal to delta G F. Now what is the difference between phi unfolding equal to 1?

The phi unfolding equal to 1 says whatever change I have in the unfolded state gets absolutely reflected in the transition state. What is this one say? What this one says is these two are not related; that means, if it is a two state situation we are not talking about the unfolded state then we are talking about which state the folded state because it is two state scenario, it is always the two state situation.

So, then what we can write here is the transition state of the protein is as folded the transition state of the protein is as folded as it is in the native or folded state F for both the wild type and the mutant proteins ok.

Tell me is the difference between these two clear to you if phi unfolding is equal to 0. Then you can see immediately what happens is my delta G there is the change is transition state is equal to what the change of the folded state right. So, the now it is no longer related to the unfolded state because whatever change in the transition state you are having that is not getting reflected in the folded in unfolded state where it is getting reflected it is getting reflected in the.

Student: Folded state.

Folded state right and that is why this is clear where del G hash is equal to del G F ok. So, this is one way of looking at it; that means, when you are moving from the folded state to the unfolded state clear ok.

Now, because we are doing this let us do the other alternative; that means, let us talk about a folding scenario.

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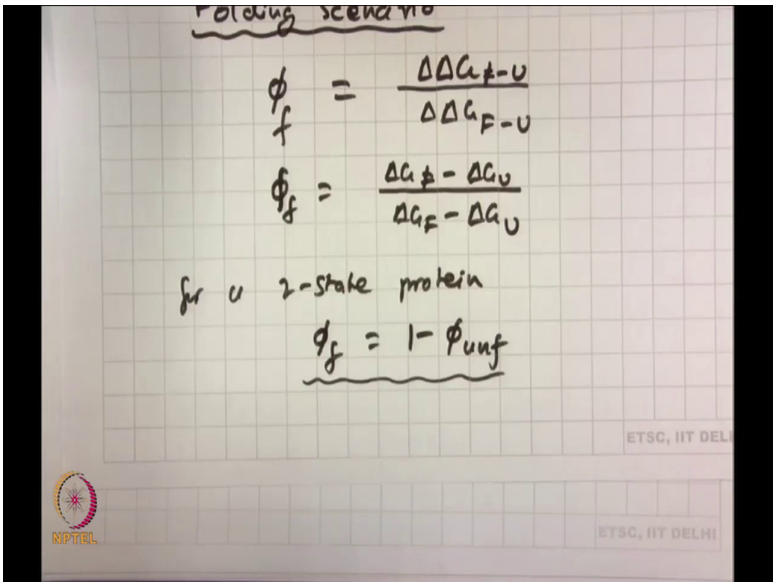

If you are talking about a folding scenario; that means, you are moving from the unfolded to the folded state then I will be talking about phi folding instead of one folding as simple as that right. So, in this case my phi folding would be equal to what now tell me? What should I write in the numerator? Delta G and I told you I am going from where?

Student: Transition.

From the unfolded to the.

Student: Folded.

Folded. So, this should be.

Student: U minus (Refer Time: 23:50).

Hash minus.

Student: U.

U good, F gets replaced by U you look at your previous equation that was for unfolding now you look at this equation this is for folding which you are going from the unfolded state. So, the energy barrier is in between what? The unfolded state and the transition state.

Delta delta G the initially it was U minus F this one would be?

Student: F minus U.

F minus U good. So, if you write it again phi F would be equal to then delta G minus delta G U over delta G F minus delta G U right this is your folding scenario. And if you take up any paper nowadays people mostly talk about the phi folding ok. For a two state situation for a two state protein; that means, only denatured and the folded state of the native state.

Phi F is equal to 1 minus phi unfolding this relation holds. So, phi F or phi folding is equal to 1 minus phi unfolding see it makes perfect sense you think about this if phi unfolding is equal to 0 where did you what did you find?

Student: (Refer Time: 25:20).

You found delta G hash was equal to delta G F and based on this equation what would phi folding be?

1, if phi folding is 1 what would you get you would also get delta G hash is equal to delta G F is not it? You look at this ok.

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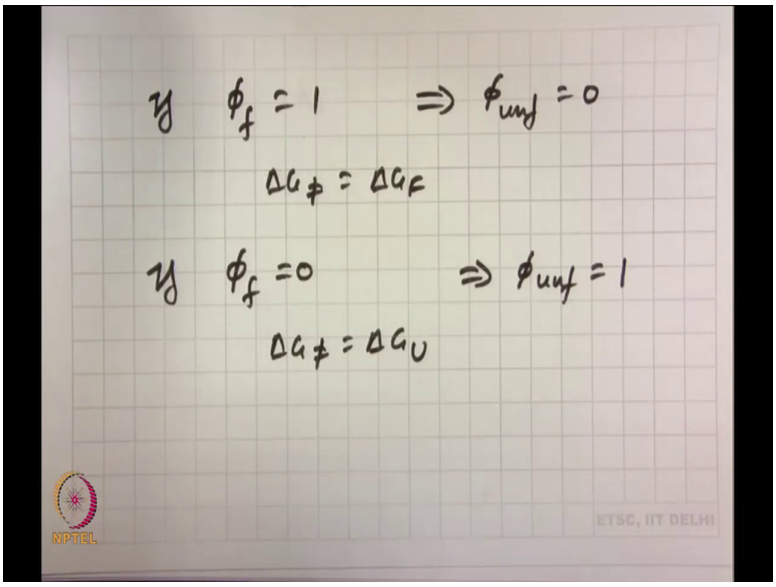

So, from this folding scenario what are the two cases? So; that means, if phi folding is equal to 1 if phi folding is equal to 1, I will not do the math anymore. If phi folding is equal to 1 what would you get tell me, from here that the delta G U cancels out. So, what am I left with?

Student: (Refer Time: 25:57).

Delta G hash would be equal to.

Student: Delta G F.

Delta G F and this was equivalent to what; phi unfolding equal to.

Student: 0.

0 that is what we found right. If phi folding is equal to 0 what would that imply from here?

Student: Delta G.

Delta G hash would be equal to delta G.

Student: U.

U good; delta G hash would be equal to delta G U and this is something where we saw where phi unfolding is equal to.

Student: 1.

1, clear. So, these can be used interchangeably no problem at all.

Student: (Refer Time: 26:46).

Provided?

Student: 2 states.

2 state absolutely and there are also certain cautions or assumptions you have to maintain, when you do these mutations ok. It just it is just not straight forward when you do this mutations because your changing a lot of parameters out there ok. So, let us look at these assumptions and then I will give you a practical example. What is assumptions of the method? See mutation should not alter pathway of folding now this is logical right.

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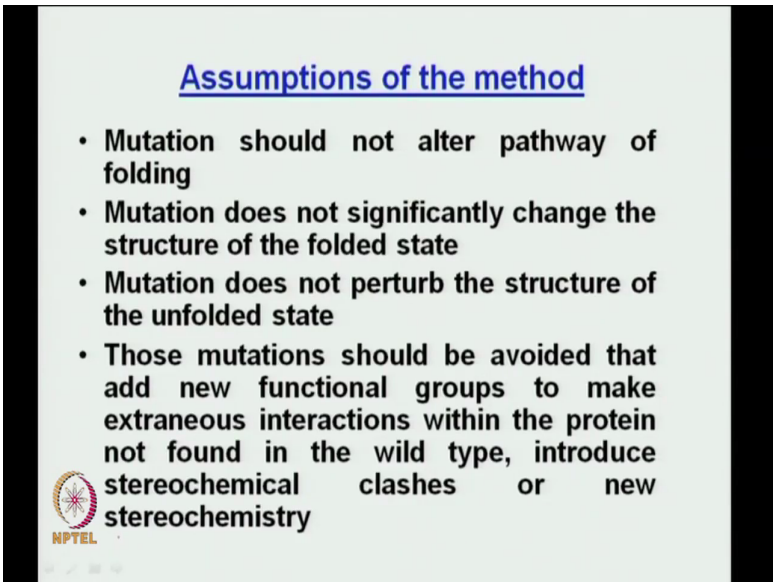

If I have certain amino acid a 1 and I change it to a 2 and the way that protein was folding initially the pathway changes then, we cannot compare anymore because now we are comparing two different pathways it is not a fair comparison right. So, one of the main assumptions is that this one is not change.

So, you can understand one thing this imposes a huge restriction the restriction is you just cannot change any amino acid arbitrarily with any other amino acid it has to be a logical charges I will tell you what those charges are ok.

That means the total energetic change would not be that huge otherwise there might be a drastic change in the protein folding pathway as such mutation does not significantly change the structure of the folded state right. Now this is also logical if my folded state structure is

fully changing then possibly I am comparing two different structures in the transition states and again that is not a fair comparison ok.

Same, mutation does not perturb the structure of the unfolded state too much ok. So, remember one thing unfolded state anywhere does not have too much structures to start with not like the folded state as such, but this does not mean that if you are doing mutations it would not affect the free energy of the unfolded state it would it would so, that is something you also have to keep in mind.

And last, but not the least there also some other ones, but we will not be talking about those mutations should be avoided which add new functional groups to make extraneous interactions within the protein not found in the wild type; that means, you cannot make a mutation because say suppose there was an amino acid to start with, you replace that amino acid and the new amino acid say from the salt bridge. So, then there is a huge change the movement you have this extraneous interaction also new hydrogen bonding coming in you cannot use this.

Because then again you have to take care of all those interaction energies how would you do that transition state comparison has to be done with minimal structural change and minimum change in what the type of the folded structure and all those things also it should not introduce stereochemical clashes or a totally a new stereochemical disposition otherwise it might destabilize or change a folded state or unfolded state to a huge extant.

So, these are the assumptions right. So, now, you can understand how hard it is to do a phi value analysis because the simple thing is when you would pick out an amino acid, that amino acid will be interacting with a lot many other amino acids depending upon which portion you pick out right and no matter how cautious you are there would be certain difference and changes.

That is why it is very hard for you to find a phi folding which is equal to 1 why? Because intrinsically you would always be making some unwanted changes no matter how carefully you have been and this you are talking about a change which is that of very important part of

the protein in terms of the stability of the protein that is why it is very hard to find very hard, but you will be finding high phi values ok.

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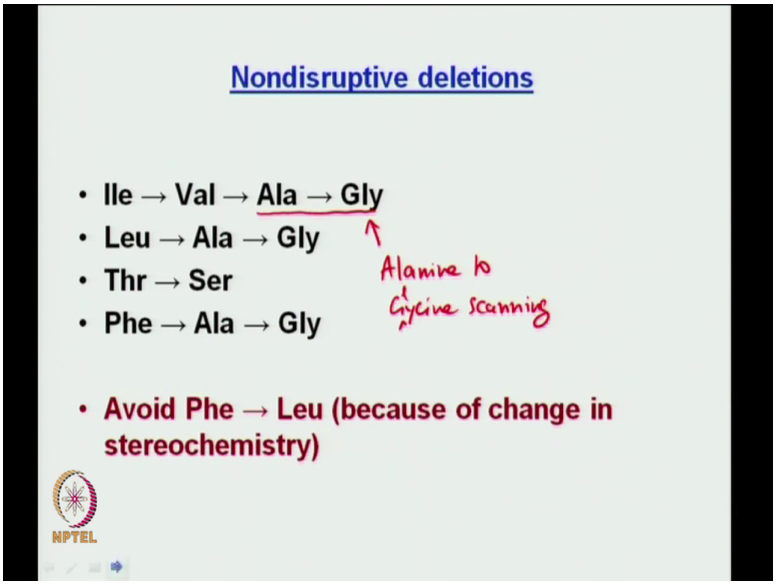

Which are phi F which are closed to 1. So, then what are the. So, these changes are referred to as non disruptive deletions a logical say a word because you are not try to disrupt the structure.

Or you are trying to keeping you are trying to keep the disruption to the minimum. So, what are the changes? The first one you can go from isoleucine to valine to alanine to glycine right even in this, you would be seeing that there is a pretty much change in the size, but still if you talk about this delta G cavity and all these things they can be taken care of and people have done that.

The next one is leucine to alanine to glynin to glycine the 3rd one is threonine to serine the 4 th one is phenylalanine to alanine to glycine see phenylalanine to alanine is actually drastic change, but people still have been able to do that with a certain amount of confidence ok.

Now I would just ask you to focus on this mutation alanine to glycine, if you would ever you know look up these phi value analysis or mutation analysis then what people do is the people do something know as based on this alanine to sorry glycine scanning.

See what does it mean? See it is not easy to do mutations in all proteins, but alanine to glycine is still a mild mutation. So, typically think about helices if a helices are pretty well packed you cannot just change an amino acid arbitrarily, but if you are trying to find out if you are trying to find out to what extent that part is involved in the transition state or not what you do is you take alanine mutate to glycine that is what this say by alanine to glycine scanning; that means, you scan along the amino acid change where you replace alanine by glycine and look at the corresponding changes in your phi values ok.

This is something which has been routinely used specially for proteins where it is very hard to do mutations ok. Now since we are talking about non disruptive deletions like, let us look at a disruptive deletion see avoid phenylalanine to leucine because this brings about a huge change in stereochemistry.

So, this is something you will have to avoid ok, based on whatever assumptions we have taken right. So, just keep this in mind I cannot go through lot of examples, but at least I will show you one or two. So, that I can put forward the idea or make it little more clear right.

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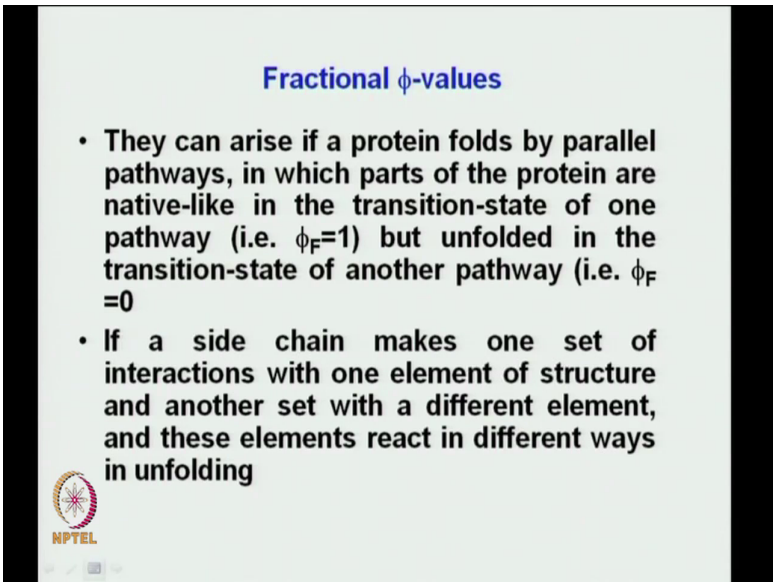

Now, you must be thinking about this if we have been talking about phi F is equal to 0 and phi F is equal to 1 what about the values in between that is the fractional phi values. Now these are very difficult to interpret. Fractional phi values can be you know interpret it in many different ways, example they can arise if a protein folds by parallel pathways in which parts of the protein are native like in the transition state of one pathway, but unfolded in the transition state of another pathway.

So, remember. So, now, it is not you are not talking about one pathway you are talking about parallel pathways when you are looking at, when you are doing this kinetics you are looking at an average response. So, in one parallel pathway it might be fully found in the other pathway it might not be found at all, but when you look at it what do you look at you look at an average of these two; that means, you look at a fractional value which is in between.

In other words in other words remember we talked about parallel pathways when we were discussing protein folding funnels right. So, this many times many or often times people take this fractional phi value as an evidence of parallel pathway or existing in your protein folding unfolding scenario ok.

This is another case if a side chain makes one set of interactions with one element of structure. And another set with the different element and these elements react in different ways during unfolding ok.

Now, I cannot give you examples of both of these, but at least I will try to tell you something relevant to the first one ok, but just keep this in mind fractional phi values that is why a very difficult to interpret and they make the analysis a lot more complicated so; that means, you have to do a series of phi value changes; that means, the series of mutations to figure out is it exactly a parallel pathway or is it just a one pathway I am having some other interactions coming in.

For the protein discussion we started with which was chymotrypsin inhibitor two what they have found is by doing a series of mutations that it is not a parallel pathway, but it is essentially one pathway so; that means, just by doing one experiment of phi value you cannot say whether it is a parallel pathway or a non parallel pathway that is essentially what I am trying to say ok.

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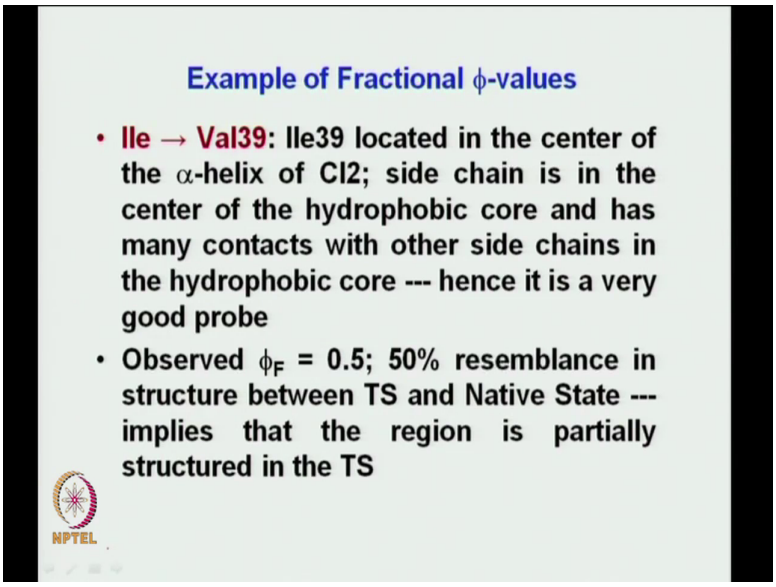

So, look at a quick example of a fractional phi value. So, this what it is in chymotrypsin inhibitor two there is an isoleucine at 39 position which is the part of the helix and you replacing it by valine right. So, this is look at it in the center of the alpha helix of chymotrypsin inhibitor two the side chain is in the center of the hydrophobic core and has many contacts with other side chains in the hydrophobic core.

So, initially you understand if you have to pick out a certain amino acid which is very essential for the stability of the protein you would picking out an amino acid like this which has an important place in the structure of the amino acid in terms of the interactions it makes.

Now on doing this what it was found was there is was a phi value of phi F of 0.5 see what does it mean? If you think about this phi F of 1 it meant was that the delta G hash was equal to delta G F right.

Now, if it is 0.5 what does it mean? That means, whatever you have in the native state half of it is being developed in the transition state that is what 0.5 means right. So, whatever we have in the native state half of it is developed in the transition state; that means, there is a half resemblance at least a 50 percent resemblance now then how can you interpret this half now think about this how can you interpret this half? There are two ways you can do it.

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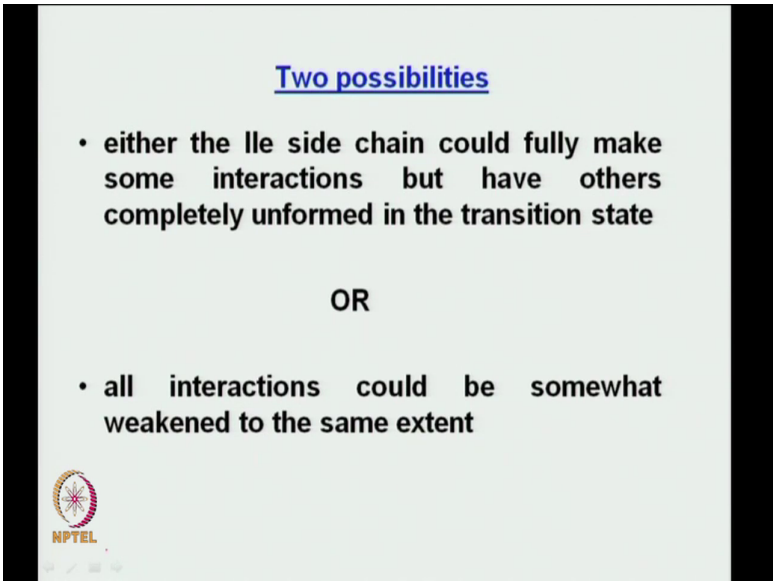

One is either the isoleucine side chain fully makes could fully make or fully make some interactions, but have others completely unformed in the transition state. So, what does it mean what it tells you is I have done this mutation alright now say I have 100 protein

molecules in the 100 protein molecules 50 of the molecules have already found that isoleucine that part in the transition state.

But 50 of the molecules have not reached that transition state even, but when I am looking at what am I looking at I am looking at an average so; that means, when I take the average 1 is 1, 1 is 0, I get what?

Student: 0.5.

0.5. Now what is the other interpretation this is what you can also say what you can say is I would not go by that instead I would say is all the interactions have developed, but they have developed only to the extent of 50 percent as compared to the native state; that means, all of those are developed, but only at the level of 50 percent, do you understand the difference.

In one case the first round what you had was 50 percent are fully developed 50 percent no development in the transition state. Now in the second case what you are saying is see I can also have a phi value of 0.5 which is midway by having all of those developed, but not to the full extent of one, but to the extent of midway which is 0.5. So, if you are doing only one midway analysis how would you figure this out? It is very hard.

Because, you already have two possibilities right. So, then you will have to go on doing a series of mutations, but anyway the idea is not, but the idea is to try to try to understand that having a phi value of 0.5 or having an intermediate phi value just complicates your data analysis right you have to do further experiments on that specific protein on that specific or doing some specific mutations ok.

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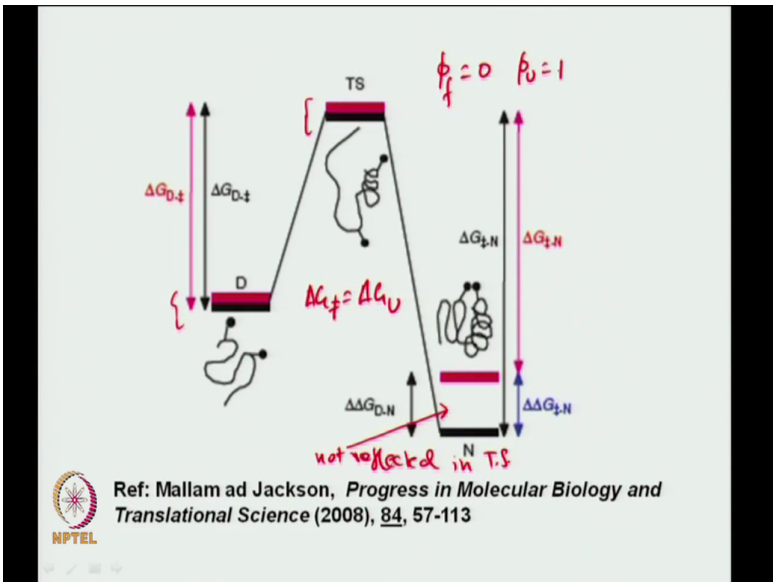

Now, let us look at this. So, this is now I was telling you I will show you a free energy example. I am asking about phi folding; that means, I am going from the native state to the denatured state tell me what should the value of phi folding be in this case? Remember if phi folding was equal to 1 what was the criterion the delta G hash would be equal to what?

Student: Delta G F.

The del G F do you have it here.

Student: (Refer Time: 40:47).

You do not have it you see. This is essentially what is this? This is essentially delta delta G right you see a change in the native state do you see a corresponding change here there is absolutely no change instead if you look at this here if you look at this and if you look at this these are very similar; that means.

I can say in this case that my delta G hash is equal to delta G U is not it I can say delta G hash is equal to delta G U why? Because this change whatever change you have here this change not reflected in the transition state so; that means, what should be my phi F equal to.

Student: 0.

0 or phi U is equal to?

Student: 1.

1 clear this is how your transition state would look in terms of the changes in free energy. So, then now you can realize.

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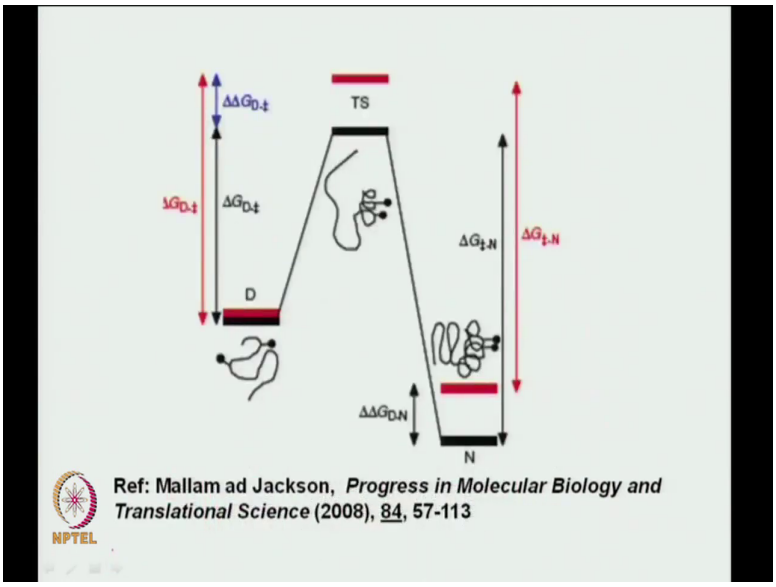

If this is phi F is equal to 0 how would phi F equal to 1 look would it look like this the same amount by which my native state is perturbed my transition state is also perturbed by the some extent see what does it mean? It means is it means is if I am doing a mutation and if my native state is getting perturbed or if my transition state is getting perturbed very similar to the native state; that means, that portion is being formed in the transition state before going to the native state.

I am repeating suppose you have a certain portion of the structure, say hydrophobic core formed in the native state. You know that is the native state, you do a mutation you saw that the free energy change that is the certain free energy change in the native state there is exactly a similar free energy change in the transition state.

So, what does that mean? That means, that hydrophobic core which was formed in the native state was actually being formed in the transition state because both are being affected equally right.

So, that is why we said remember when we were talking about this phi unfolding equal to 0 or phi folding equal to 1 what we said was it was as folded in the transition state as it was folded in the native state as folded; that means, to the similar extent.

Now, if you try to rationalize phi unfolding or rather phi folding is equal to 0 what would you say if it is phi folding equal to 0 what you would say is that which was the previous one this native state whatever difference you have, it was not reflecting in the transition state then what would you say. What you would say is that whatever structure structural. Region I am looking at by mutation that is not formed in the transition state, but is found in the native state.

That means that portion in the transition state is still essentially as unstructured as what the unfolded state. Guys is it clear or not? I am trying to go at length really slow to try to you know emphasize how important this measurement is it is not without flows. Let me tell you guys it is not without flows, but this is the closest thing you have this is the closest thing you have to what a single residue structural analysis and that is why it is.

So, important in the field of protein folding specially when you are trying to reason out, why an amino acid is there or why did evolution actually try to figure out that sequence of amino acids in the hydrophobic core. That is always what a final goal is. Try to reason out what evolution did clear ok. So, next day what I will do is if I will start now I will not be able to finish.

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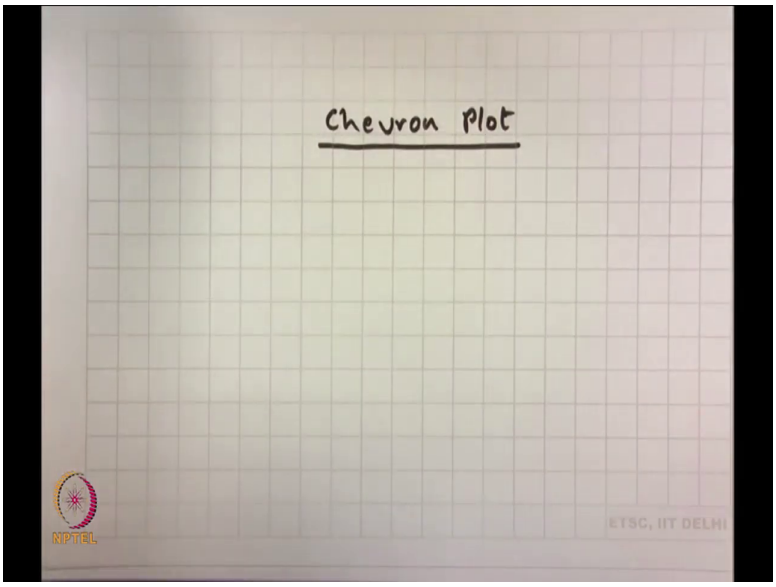

The next day what I will start with I will start with something known as Chevron Plot Chevron plot and a Chevron plot you will realized why it is. So, called there is a v in the middle and if you would look at this; if you would look at this slide.

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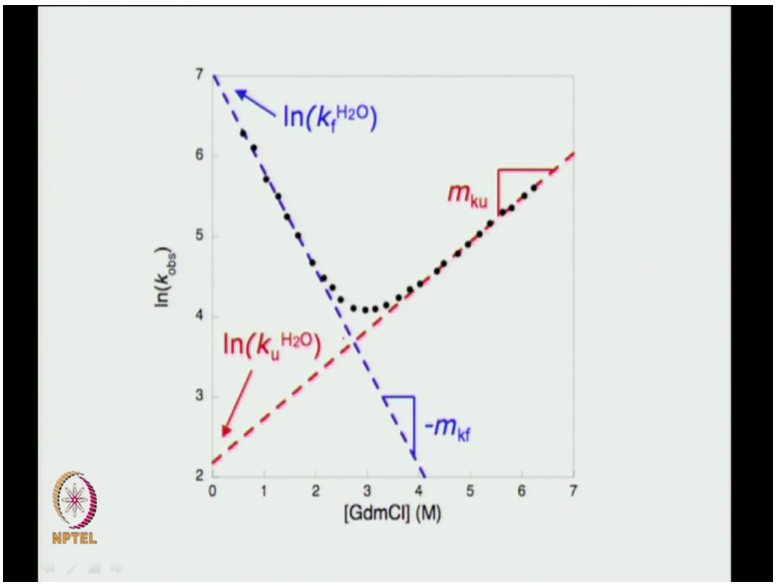

If you would look at this slide I am looking at the slide again you will see on the left hand side of the slide, on the left hand side of the slide there is a blue line, on the right hand side is a red line these points are all experimental points.

You look at the concentration of the denaturant which is the concentration of guanidinium hydrochloride which is the denaturant at the bottom. So, when guanidinium hydrochloride is low your protein is in what folded state when the guanidinium hydrochloride is high your protein is in what the unfolded state. So, now, you can look at to the lower end of this which is this region to the lower end this should be reflecting what rate constant for folding because you are in the lower end of the denaturant.

If you go to the higher end of the denaturant what would it reflect the rate constant for the unfolding situation because your protein is already unfolded right and see what will happen is

this is the beauty of the Chevron Plot remember when the protein is 50 percent unfolded; that means, when you are get t m or c m rather we will talk about c m what happens to k equilibrium?

Student: (Refer Time: 46:42).

1.

Student: 1.

Ok. Now if you are talking about folding or unfolding does not matter. Say you are talking about unfolding right then what will k equilibrium be equal to 1 right; that means, that k u by k f or k f by k u should essentially be equal to 1; that means, they should be essentially comparable.

And see you can easily figure out by doing a Chevron plot when they intersect the point at the intersect means that k u and k f have the same value and that is what your c m this is how you can also figure out what my c m is and see whether it matches with your equilibrium studies ok.

So, next class we will discuss this at length and this would be the last in terms of what should I say, in terms of the fundamental aspect of you know of protein folding or how to look at or how to study protein folding because we have looked at thermodynamics.

This is the kinetic part and once you are done with Chevron plot for if you do with, we would actually start looking at different ways of doing experiments; that means, instrumentation how do we do a kinetic analysis; that means, c d the probe florescence and all these things that is rest of the course would be focused on that. Ok [noise]