**Bio-Physical Chemistry**
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**Lecture - 33**
**Protein Folding Kinetics: Rapid mixing and Relaxation Techniques (Contd.)**

Let us continue with the discussion on these Rapid Mixing Techniques. So, yesterday we talked at some length about the continuous flow technique, right and then we started with this microfluidic, right.

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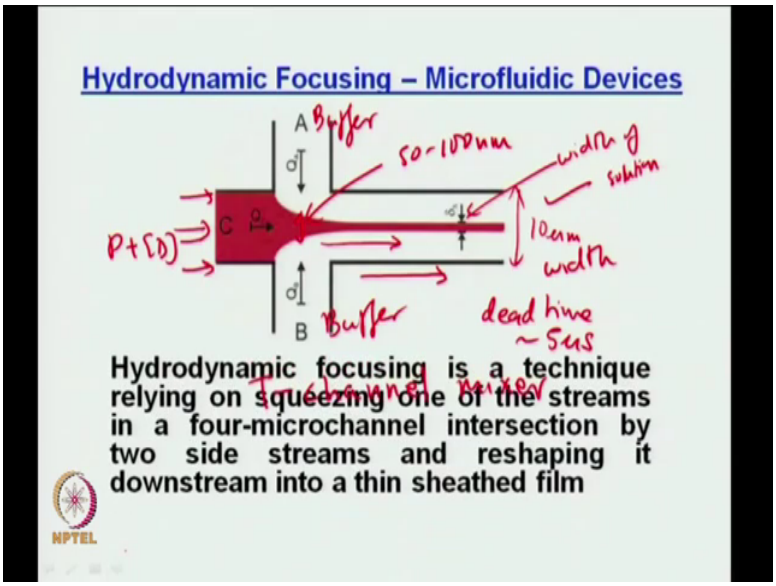


So, this was what we were discussing at the time. So, that means, in the ever increasing efforts of trying to make these a lot faster in terms of decreasing a dead time. So, one of the latest things that people are looking into is microfluidic mixing. So, as the microfluidic name suggests it is micron sized channels through which your fluid will flow, right and that is this is

the only one aspect. There are many other things that are related of this. That is why micro fluidics nowadays is a very important field if you would ever look up, I mean look that up rather.

So, what you are trying to do here is in any sort of mixing, in any sort of mixing what you have is you have diffusion of two solutions, right. So, if you would ever try to decrease the length scale over which diffusion occurs, then automatically what will happen is, what will happen? The time it takes to diffuse and mix would decrease, right because would it will make the length scale smaller. So, it would happen at a faster rate and hence your dead time will be smaller because your mixing is faster.

So, the same principle is applied here. How is it applied here? The way it is applied here is as we were discussing yesterday, you have essentially like a it is kind of a T-channel mixer, right. It is kind a like a T-channel mixer, ok. So, you know why it is called a T-channel mixer? It is called a T-channel mixer because if you if you covered this or if you covered this it kind of looks like a T, right.

Now, what you are doing is to the centre channel you are passing in your protein and denaturant, right. Now, through the side channels what you are doing is you are passing your buffer. Now, because your protein channel; your protein solution is going like this and you are passing a buffer solutions at orthogonal, so what will happen is because you are passing the buffer solutions from both sides it will try to squeeze the protein channel flow, right.

So, if you try to squeeze it, so this is what happens you can see, this is what this arrow is telling you. This is the length scale over which your mixing is happening. Now, this length scale is typically of the order of, I will tell you this length scale is typically of the order of some nano meters you know, so 50 to 100 nano meters. It is that small. So, this is this is the length scale over which your mixing is happening. And because the length scale is really short that means, your mixing is occurring typically quite fast, ok, decrease your dead time.

So, what happens is after that you can see it kind of channels down and then you are looking at it as a function of its flow along the micron size channel. The channel dimension or the

channel width is 10 micron, as it says it is 10 micron this is the width of the channel, ok. This is a width of the channel, it is 10 micron. The channel depth is also about 10 micron, ok; the channel depth is also about 10 micron. So, if this is the channel width now look at the width of this flow. So, this is, ok. So, this is the width of solution. Can you see that the width of solution is far lesser than the channel width?

So, the channel width is 10 micron, you think about the width of the solution is much much lesser than that, right. And what it just tells you is the bottom line is your mixing is occuring very fast, over a very small length scale. So, the dead time as I was telling you as someone pointed out, the mixing, the dead time in this case it is written in a later slide, the dead time is about 5 microseconds.

So, we started from stopped flow method with a dead time was 1 millisecond. We went to a continuous flow with the dead time was what? 45 to 50 microseconds. Now, we are also in a continuous flow about microfluidic continuous flow with the dead time is 5 microsecond, right. So, based on this rapid mixing technique this is the best that we have been able to do thus far, right.

So, just to elaborate on these points this was about hydrodynamic focusing which we discussed last time.

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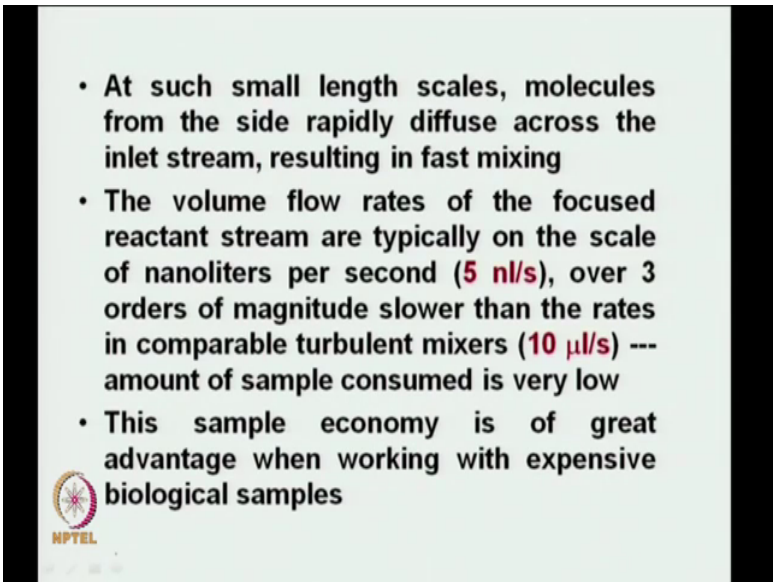


So, here at such small length scales molecules from the side rapidly diffuse across the inlet stream resulting in very fast mixing, right, ok. The volume flow rates of the focus reactant stream are typically on the scale of nano litres per second, right. So, 5 nano litres per second. Now, this if you compare with what you will be using in continuous flow, it is about 10 micro litres per second.

So, you can see the orders of magnitude improvement you have in terms of sample consumption too. In one case it is 5 nano litre per second, in the other case it is 10 micro litre per second, because you know in continuous flow there is a disadvantage. The advantage disadvantage is you have to flow your solution continuously, right as opposed to stopped flow where the flow is stopped, ok. And this sample economy is of great advantage when working with expensive biological samples, right that is straightforward.

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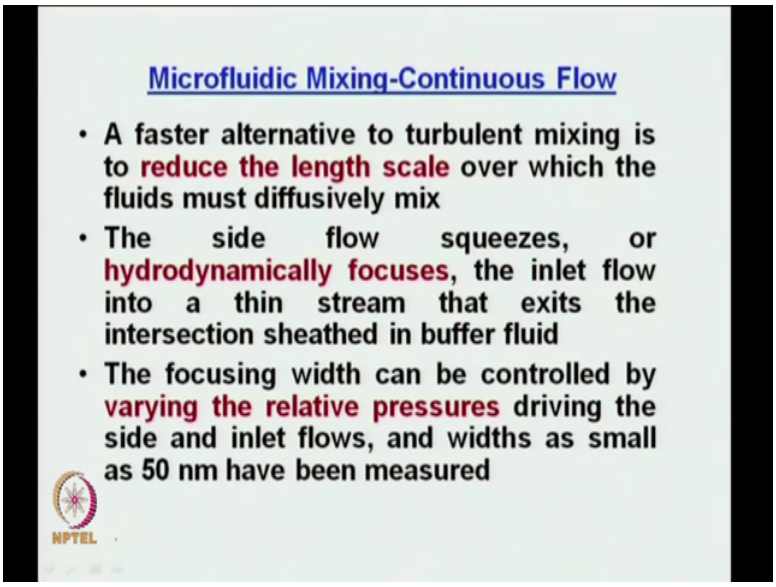


So, again a faster alternate to turbulent mixing is to reduce the length scale that we mentioned before. The side flow squeezes or hydrodynamically focuses the inlet flow into a thin stream that exists rather that exits the intersection sheathed in buffer fluid you know I will tell you what that means. And the focusing width can be controlled by varying the relative pressures of either side flow and the inlet flow.

Now, what is the side flow? What is the inlet flow? That you have seen, but it will be clear in the next diagram.

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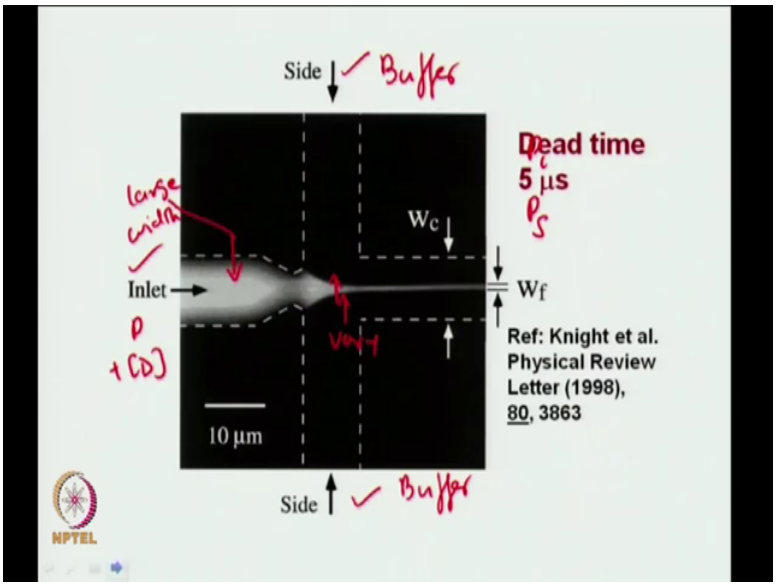


Now, can you see this diagram? So, what are you seeing is again these are the two sides, side one, side two and this is your inlet. So, what you what do you have in inlet? The inlet is again your protein plus.

Student: Denaturant.

Denaturant, right. The side ones you have.

Student: Buffer.

Buffer. So, here also you have buffer, ok. Now, you can see what is happening in here is this similar to what you saw in the previous figure. Here the protein plus denaturant inlet that width of the stream is pretty large, right. So, here the width is pretty large, right.

So, in this case if you can say it is a large width. But the moment it comes here; but the moment it comes here what happens? You can see you are having the buffer from coming from both sides, it squeezes that means, it hydrodynamically focusing and the width has decreased, right and then it flows. And after that it is typically like a continuous flow. That means, along with the length of the flow channel you can convert it to time and then get the respective time points essentially, that is what it is. After that it is essentially just like a continuous flow, right. It is a continuous flow in that sense, ok.

So, now what is W c and w F? It will be clear W c is essentially the length of the channel and W f you can see is the length of the focused beam or the width of the focus beam. So, again W c is the width of the channel and W f is a width of the focused beam. Now, you can realize one thing, what you can do is how would you vary; how would you vary; how would you vary the width of this focused stream; how would you vary this? That means, how would you vary the width of this focused stream? Tell me how would you do it?

Student: (Refer Time: 08:03) predominant pressure.

That means you would try to play with the pressure of the inlet rather the side channels.

Student: Yes.

The inlet remains the same, but you start playing with the pressure of the side channels. See the more pressure will be having the more it will be squeezed, the less pressure will be having the less hydrodynamic focusing you are going to have. So, then you would there would be a ratio, there would be an optimum ratio of the pressure of the side channel and the pressure of the inlet channel where you get your maximum optimized, maximally optimized conditions, right you just cannot increase your pressure forever.

So, let us consider two extremes, right. Now, think about this. Let us consider two extremes. Suppose, if this if the pressure of the inlet channel see if P i is the pressure of the inlet channel, right and P s is the pressure of the side channels, ok. So, P i is the pressure of the inlet channel P s is the pressure of the side channels. Do not worry about writing it its there in the next slide, but I need this figure for explanation.

Now, see what will happen if pi is much greater than P s? What is going to happen you tell me? Would it be focused?

Student: (Refer Time: 09:13).

P i is much greater than P s, it would not be focused, right because this squeezing force is not that high. Instead what might happen is if P i is much greater than P s then some of the protein plus denaturant can actually move into the side channels is not it because the pressure is much higher. Is not it clear or not? If P i is much higher than P s then what might happen is obviously, you will not be having a focused stream, but secondly, some of your protein plus denaturant can actually move into the side channels because the pressure is not higher, right. And those side channel pressures cannot withstand the pressure of your inlet channel, ok.

Now, this is one extreme, right; this is one extreme. Now, think about the other extreme. What is the other extreme? The other extreme is P s is much greater than P i, what will happen?

Student: (Refer Time: 10:01) p two is (Refer Time: 10:01).

Ha?

Student: (Refer Time: 10:02) squeezed.

It will be?

Student: squeezed.

No, it will be squeezed. But will it be squeezed I mean how far can you go for squeezing. Squeeze, squeeze, squeeze, after that what will happen? Tell me the extreme.

Student: (Refer Time: 10:14) Stopped flow.

Right. What will happen is if the protein is flowing like this it is squeezed, squeezed, squeezed. And after that what will happen? It essentially stops the protein flow, right. So, because now the side channel pressure is very high. So, it will flow into the channel which has a protein in the denatured into that means, it will stop the flow of the protein plus the denaturant solution. So, essentially that is called pinching off. That means, you pinch off the protein plus the denaturant flow.

So, again two extremes one is your P s is very low as compared to P i and the other one is your P s is very high as compared to P i, ok. So, this is what is actually told to you in the next slide. Are you clear about these two situations? What is going to happen? In one case, the P i flows into the corresponding channels without any hydrodynamic focusing.

In the other case, where P s is much greater than P i these two essentially cut off the protein flow like a scissor pinch it off and they can actually flow into the inlet channel where you have the protein denaturant. Again, it is of no use to you.

So, these are two extremes. So, as I said the dead time is 5 microsecond it is taken from this reference knight et al. So, they were the first people who actually you know devised this microfluidic apparatus and showed the application of this.

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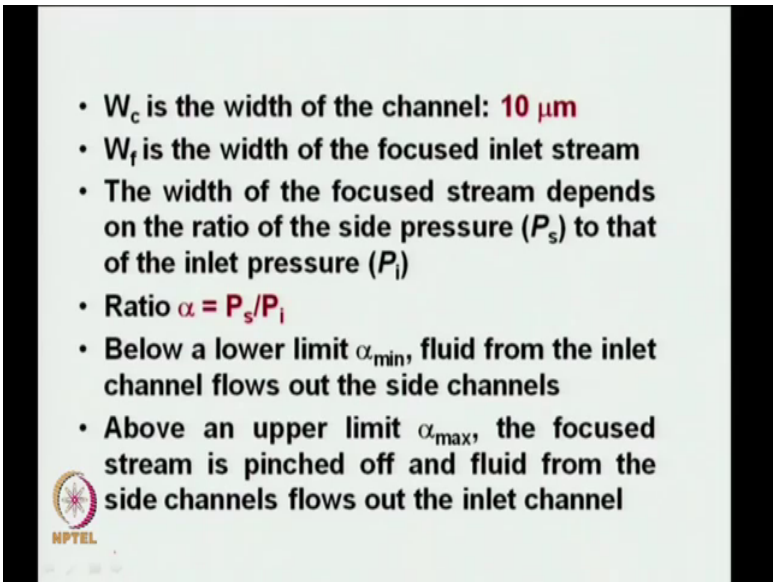


So, again W c is the width of the channel which I was saying 10 micron. W f is the width of the focused inlet stream, right. The width of the focused stream, this is what we just discussed depends on the ratio of the side pressure to that of the inlet pressure, right. Let the ratio be alpha P s by P i. So, below a lower limit that is alpha minimum, fluid from the inlet channel flows out into the side channels, is not it. This alpha min. What is alpha min? Alpha minimum is the minimum that means, P s is much lower than P i that is what we said. The inlet stream would flow into the side channels, right.

Then, the next point is above an upper limit which is alpha max what will happen the focus stream is pinched off and fluid from the side channels flow out into the inlet channel. Just a reverse of what you have for alpha min that is what it is the picture is just reversed. But you understand, right why it is going to happen. So, you can see; so you can see no matter how

much you try to squeeze there has to be an optimum condition where all the flows are essentially the same steady flows and need not pinching off sections of your protein flow.

So, let us look at a picture a series of images where you would understand how this variation of alpha affects the width of the inlet flow stream, ok. So, this word is, ok.

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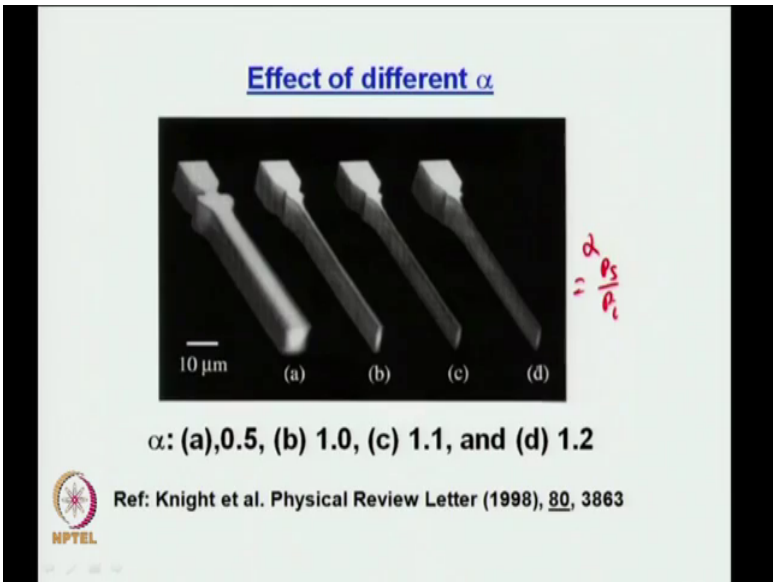


So, here these are for like a, b, c and d, for 4 different values of alpha. Remember, what was alpha? Alpha was equal to P s over P i, right, ok.

See, at what a is for alpha is equal to 0.5 that means, both have equivalent pressures, right. So, the stream is thick, ok. Now, you go to 1, what happens? The stream gets narrowed, is not it. You go to 1.1, this stream is further narrowed. You go to 1.2, then also the stream is narrowed, but this is a very fine sheath, very fine stream. Remember, this is a very fine stream.

What it tells you is a possibly 1.2 is, the maximum you can go to, if you go beyond 1.2 what will happen is you will.

Student: Pinch it off.

Pinch it off, ok. So, let me tell you how these images were taken. These images these are actually fluorescent images. So, you are taking with fluorescence microscopes. So, what you do is you put a fluorescent dye which is an indicator in these channels, right by some way or the other, right. And then you monitor the fluorescence of the dye using your microscope that means, you are doing an imaging, right and then you take these images and put it on your paper which you publish. That is how the deal because I have taken it from that paper, ok.

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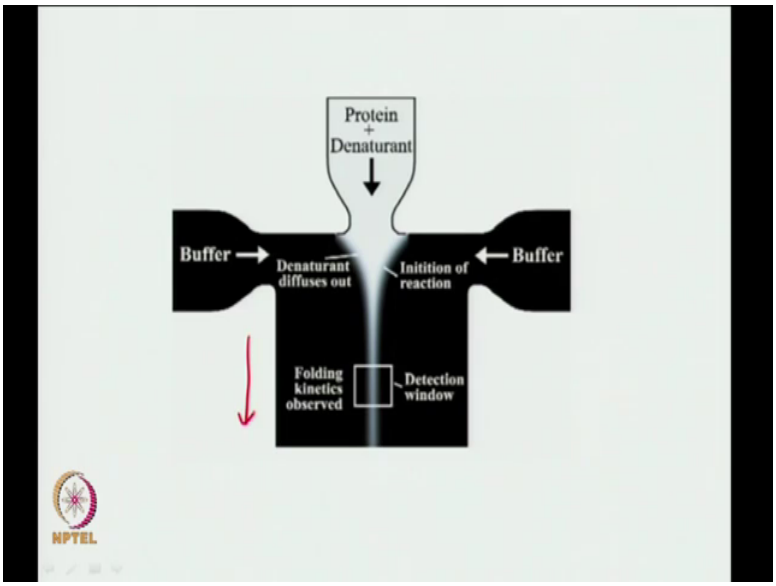

So, this was about your microfluidic mixing. So, this is really state of the art. So, finally, this sums it up. So, this what it says is you can see it is a protein plus denaturant coming here, this is the buffer, this is the buffer. Now, remember because the buffer is coming here and the buffer is coming from here the protein has very high denaturant. Now, the buffer is mixing, so the denaturant actually now gets diluted. So, that means, the denaturant is diffusing out, right.

And after that what you are having is the protein now faces a very low denaturant concentration, so it starts refolding, and then you monitor the refolding along this flow access and this is essentially a continuous flow. The only difference is the way you mix it and the dead time you get, ok.

So, this, this is you know typically the best in terms of a continuous flow technique for people have been able to achieve, right, a dead time of 4 to 5 microseconds you know that is done good. Because you realize you can put in a lot more pressure. For example, you can go for high P s you can go for high P i, does not matter. But what will happen is your force pressure is force per unit area.

So, if the channels are very narrow which is 10 micron then the pressure is very high, then it might distort your channels, right. So, you cannot go for infinitely high pressure that is why it is always necessary for you to go for an optimum where the flows are proper and you can monitor them at ease without any other artefacts or hiccups, clear.

So, this was all about rapid mixing technique. These techniques, I am repeatedly telling you are being used nowadays very widely, more so micro fluidics not only in protein folding, but in many other cases and in many other problems, right. So, microfluidics has its own importance nowadays, right.

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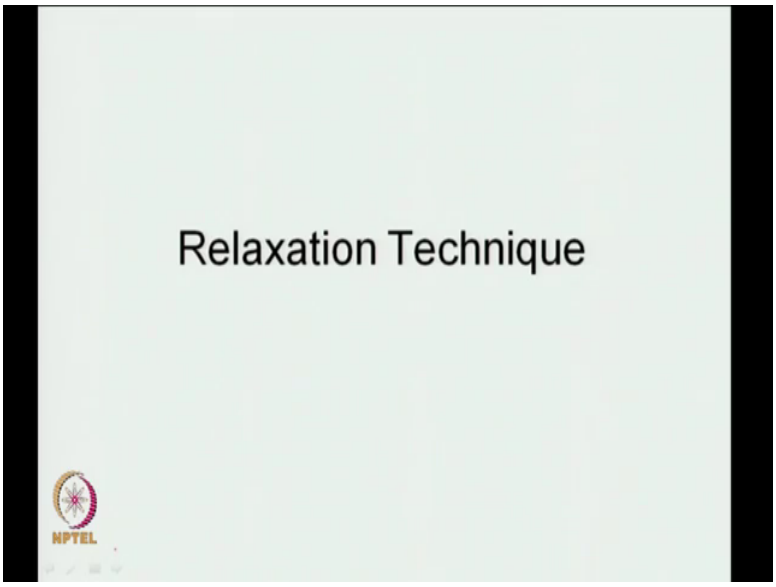


So, then next let us go to another technique it is called a relaxation technique. So, all these were mixing techniques, right. Now, relaxation technique means you will allow your system to relax, but it does not mean that you start relaxing too, right. It is a relaxation technique. Not for you, for the protein.

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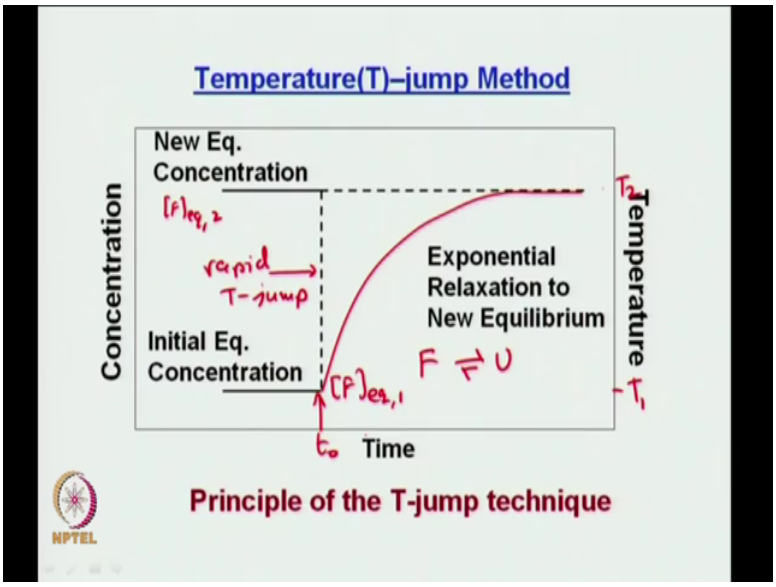


So, this is what is it is known as. It is a temperature jump technique, ok. What do you do? Ok. This is the principle, right, follow me properly.

Suppose you have a native have a folded protein, right. Now, what happens when you increase the temperature on the folded protein? The protein gets unfolded that is denaturant. Now, so essentially what will happen is if you would do a temperature jump that means, a protein will move towards the unfolded state. Agreed, good.

Now, we have to do kinetics that means, we have to follow this unfolding as a function of time. So, like in rapid mixing what we did was we separated the mixing and the protein unfolding that means; mixing was too fast as compared to the protein folding. Why? Because if the mixing was slow then the protein would also be folding within that time and you would

not be getting anything. So, that means, you have to decouple these two processes you have to mix it as fast as possible, that is the concept of dead time, right.

So, similarly out here you can understand what will happen is you have to make the temperature jump very fast. You make a very fast temperature jump, but the protein does not respond to the temperature by that time because the protein responds us slowly. It is like rapid mixing, instead of rapid mixing you do a rapid temperature jump that means, you increase the temperature jump say if increase the temperature from 10 to 20 degree Celsius in one flash. I will tell you within a few nanoseconds and so.

Now, the protein being protein would find it hard to respond at that time instantly, but it would take time to respond to it and it would slowly respond to this new temperature change or new temperature, this temperature change. And this response to this temperature change is referred to as relaxation. That means, a protein relaxes to the new temperature change. Now, essentially what does it relax too? Ok.

Now, we can understand one thing it is like your in quantum mechanics you have heard of Born-oppenheimer approximation, right. So, Born-oppenheimer what happens? You excite electrons move in a flash, right that is why your transition is always vertical. The nuclei follow it essentially, right because nuclear much heavier. That is where you can decouple those two processes that was Born-oppenheimer. So, this is essentially the same, right. You are making the temperature jump really fast, so that the protein cannot respond at that faster time scale and then you monitor the protein responding as a function of time. This is relaxation kinetics, ok.

Now, what are you doing? Suppose, you have this initial temperature T 1, ok, suppose you have this initial temperature, so this initial temperature is T 1 and this final temperature is T 2, ok. Now, tell me guys what happens when you increase your temperature from T 1 to T 2? At T 1 when there was no temperature change your system was at equilibrium, so that means, at T 1 your equilibrium k 1 or K equilibrium 1.

Now, increase a temperature what happens? The temperature is increased within a very short time to T 2. So, along with T 2 what has changed? Your k has also changed, that means it is no longer k 1, it is k 2 because you know equilibrium constant depend on what temperature k 2, right. Now, because equilibrium constant has changed, now remember say if you are talking about a two state scenario, if you are talking about a two state scenario.

So, what we will be having is we will be having something like F in equilibrium with U, is not it. We have a folding we have a folded protein where increasing the temperature we are going to the unfold state, right, we start from folded protein. So, if F is an equilibrium with U at T 1 it had an equilibrium k 1. At T 2 it has an equilibrium k 2, right.

Now, if the protein is unfolding with this temperature jump what happens to the concentration of the folded states in both the cases? In the first case the concentration of folded state is a concentration of F 1, in the second case the concentration of folded state is F 2. Which one is lower or higher?

Student: F 1.

F 1 is higher than F 2, is not it. Because by doing a temperature jump you actually decrease the concentration of the folded state, ok. Now, good. If that is the case; if that is a situation this is how it goes, right. So, here you are having the concentration of the folded state which is F. So, let us let this be concentration of folded state at equilibrium T 1 that is where what your initial temperature is and you do the temperature jump, ok.

Now, let this be see this is the final, this is the final temperature T 2 that means, your folded state has to come to equilibrium at this new concentration because the equilibrium constant has changed, because your equilibrium constant has changed, right. That means, this F equilibrium 2 is different than F equilibrium 1, is not it. They have to be different because you have done an unfolding, ok.

Now, think about this. This is this dotted line is a rapid T-jump, this dotted line is a rapid temperature jump that is why it sees almost vertical, right it is a rapid temperature jump, ok. Now, see initially you were T 1, please follow this initially you were T 1, you did the temperature jump. Now, you are T 2, in terms of temperature, but the protein has not been able to respond to this temperature change. It is still at its concentration what? F equilibrium 1.

But remember since you have changed the temperature T 2 the protein has to respond to that. So, what will the protein now do? The protein will finally, the folded state or the protein will finally, respond in such a way that the folded state concentration goes to F equilibrium 2. So, this progress of the protein as a function of time because of this temperature change is referred to as relaxation kinetics.

That means, it is relaxing to what? It is relaxing to your new equilibrium situation. Is it clear? What is it relaxing to? It is relaxing to its new equilibrium situation which is at temperature T 2, because the T 2 then after making the temperature jump you are having an equilibrium situation. Guys, is it clear or not? Is it clear how the technique works? At least the principle behind the technique.

So, then what you are monitoring is you are monitoring the change in the concentration on the folded state because of the temperature jump between two equilibrium situations, one is at T 1, the other one is at T 2. So, to sum it up, to sum it up this is what happens. When you have it at T 1 you make the temperature jump to T 2, but the protein initially the protein before the temperature jump was at equilibrium. No problem.

The moment to make a temperature jump to T 2 the protein has not been able to respond, so it finds itself in a non-equilibrium situation is not it. It is no longer in equilibrium because equilibrium has changed to k 2. So, what the protein will now do is it will respond to this new k 2 and it will change accordingly relax accordingly and then that relaxation is your relaxation kinetics, ok. That is essentially what the temperature jump technique is

There are a couple of ways in doing the temperature jump. I will tell you one way. Hopefully will get it done in this class. But before doing that let us look at some kinetics. Let us see what I have in the next slide. Yeah.

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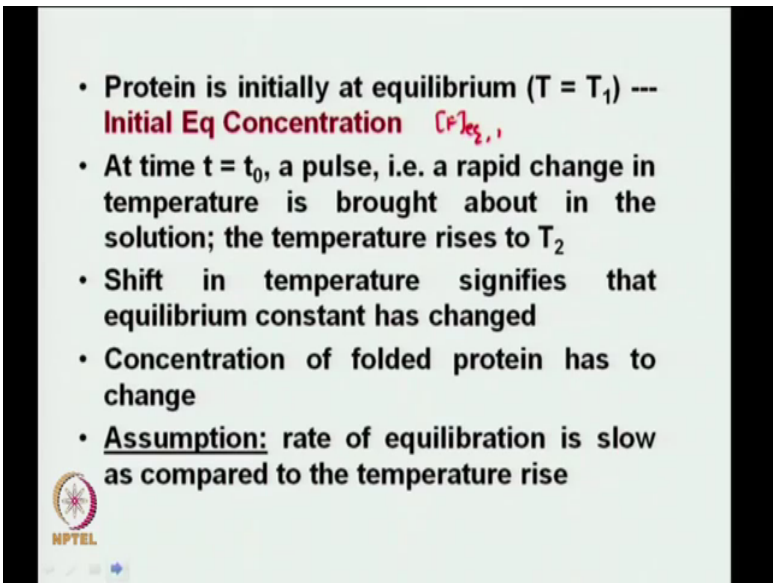


So, this is what I was trying to tell you. So, protein is initial at equilibrium T is equal to T 1. This is the initial equilibrium concentration, right. So, which I said was F equilibrium 1. So, I can write this is F equilibrium 1, ok.

Now, at time t is equal to t 0. So, what do I mean at time t is equal to t 0? Let me go back real quick. So, let this be t 0. So, this is my time axis. Let this be t 0. At t 0 remember, what does t 0 refer to? t 0 refers to 0 time that means, t 0 refers to initial time, that is 0 time essentially from where you start to look at it.

Do you remember the first data point of your continuous flow stopped flow it is essentially like that? Ok. So, at time t is equal to 0, t 0 a pulse there is a rapid change in temperature is brought about in the solution the temperature rise to t 2 good.

Now, shift in temperature signifies that equilibrium constant has changed, right from k 1 to k 2. Now, concentration of folded protein has to change too, because it has to respond to the equilibrium, new equilibrium. Now, what do you do is you assume that the rate of equilibration is slow as compared to the temperature rise that is what I said that means, your temperature rise is rapid and the protein relaxes after the temperature is risen. Both of these cannot happen together, then it is a problem. These two processes have to be decoupled that is why a temperature rise has to be very fast, like your mixing was very fast, ok.

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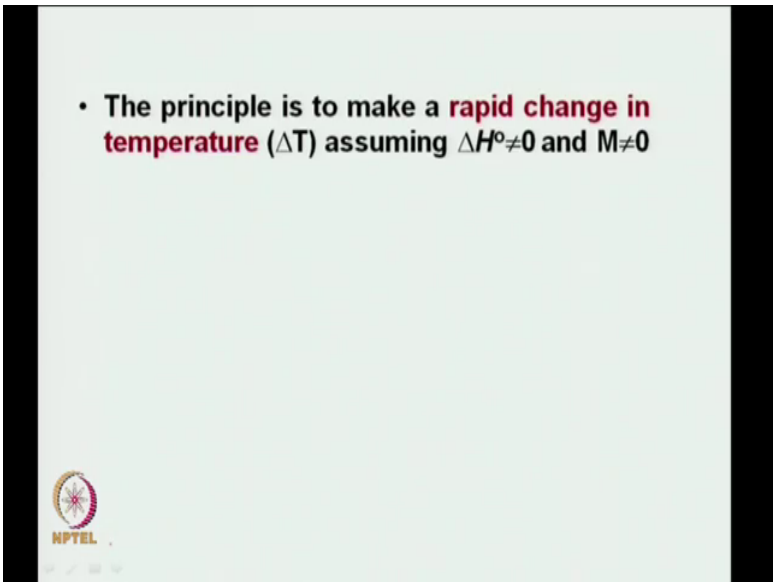

So, I will come back to this later, but let us look at some thermodynamics, kinetics and thermodynamics.

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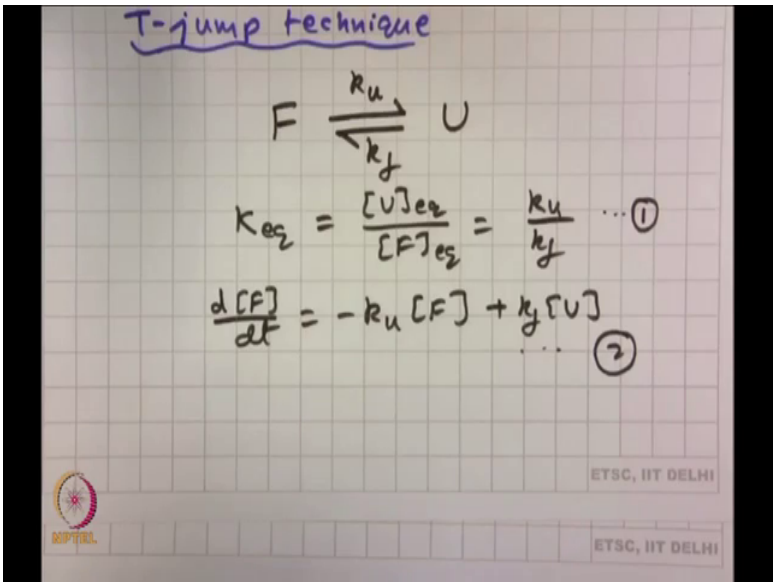


So, let us look at the T-jump technique. So, this is what you are following. You are following an equilibrium between the folded state and the unfolded state. So, let this forward one be k unfolding, the backward rate constant be k f, ok.

Now, k equilibrium is equal to concentration of U equilibrium by concentration of F equilibrium is equal to k u over k of f, ok. This we know. So, let this be equation number 1, ok.

Now, suppose I am doing a kinetics. So, for example, suppose I am trying to follow the change in the concentration of F with time, ok. So, then d F by dt based on kinetics would be

what? Minus k u into concentration F plus k f into concentration of U, is not it, ok. So, let this be number 2, ok. We are trying to get an (Refer Time: 27:31) of the relaxation time. We will see what it comes to; this something you know.

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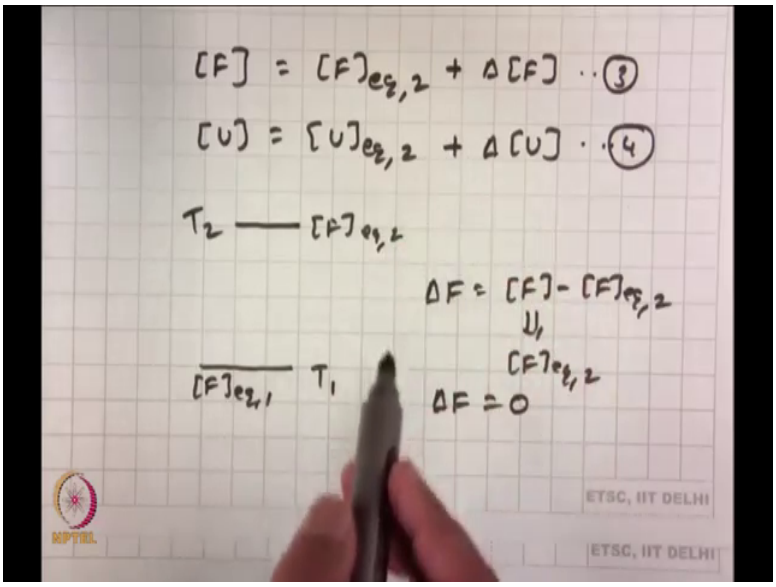


Now, let us do this. What I say is let the concentration of F be equal to F equilibrium at temperature T 2 plus delta F, I will tell you what that means, and concentration of U is equal to U equilibrium at temperature T temperature T 2 plus delta U, ok. What does this mean? So, let this be 3, let this be 4. What does it mean? You know this is what I was trying to tell you what I was trying to tell you was this was your F equilibrium 1 at temperature T 1. Then you made the temperature jump, you jumped to F equilibrium 2, this is a temperature T 2 that is what you jumped to, right that is what he should be going to, he jump to T 2, ok.

Now, initially what F equilibrium 1? The final concentration you have to go to is F equilibrium at 2, where K equilibrium is defined by concentration of U equilibrium over concentration of F equilibrium, ok. You are going to follow something as a function of time. What you are following as a function of time is the concentration of the folded state that is what how we have set it up its d F over d of t. You could have easily written d over d of t does not matter, ok. So, that means, again you are you are following the concentration of the folded state as a function of time.

So, once you have made the temperature M to T 2, right. This guy F equilibrium 1 has to respond, so that it has to go to finally, what? F equilibrium 2, right. So, that means, for him to go from F equilibrium 1 to F equilibrium 2 it will take time, that difference that difference between these two essentially is your delta F, you understand it is your delta F. So, what is the delta F? Delta F delta F is the difference. And look at this, what is delta F from here? What is delta F from here? Delta F is equal to concentration of F minus concentration of F equilibrium 2, right.

Now, think about this, what is your final concentration of F you are going for? See from 3, delta F is equal to concentration of F minus concentration of F equilibrium 2, is not it, from 3, ok. Now, what is the final concentration of F you are going for? Concentration of F, what is the final concentration you are going for?

Student: F equilibrium.

F equilibrium 2. So, finally, that means, finally, this should be F equilibrium 2 after the thing is over. That means, what should be the final value of delta F be? 0. So, now you understand what the difference of delta F is or what does delta F signify. The delta F signifies how far your system is away from your F equilibrium 2 because initially when you made the temperature jump the protein never respond to the temperature jump. It was always lying where at F equilibrium 1. So, the difference between F equilibrium 1 and F equilibrium 2 is essentially what your delta F.

So, with time what are you following where with time what you are following is you are following this delta F essentially changing as a function of time. And when is the change going to stop? The change is going to stop when delta F is equal to 0, that means, when concentration of F is equal to concentration of a F equilibrium 2 because beyond that it will not change anymore. Is this clear or not guys? Is this clear? Right.

So, this is exactly how it happens, right. Please go through it. It is a very interesting technique it is a very state of the our technique and the concept is also very interesting. But it is not that hard to understand, if you just think about it very clearly, ok.

So, again essentially you are looking at a delta F to start with. So, what was the initial value of delta F, can you tell me? What was the initial delta F, the maximum? What is the maximum delta F?

Student: F equilibrium 1 minus.

Very good. So, maximum delta is F equilibrium 1 minus F equilibrium 2. The minimum is 0, right. And what you are doing is you are looking at this difference delta F coming to 0 as a function of time that is a relaxation, precisely that is what it is [FL]. That is why this delta F has your meaning, ok, good. And because we are looking at these at the higher temperature T 2 we always say F equilibrium 2 or U equilibrium 2 because that is what it is finally relaxing to, right, ok.

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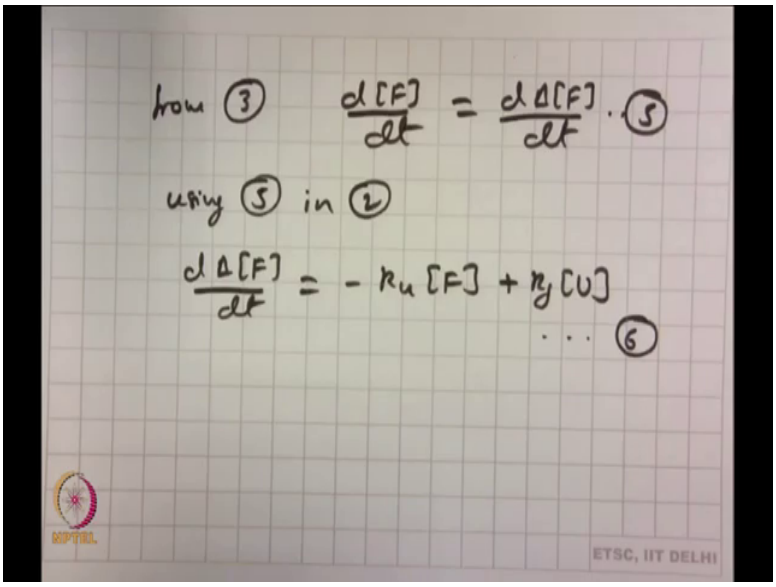


Now, going by 3, going by 3, see from 3 we can write can you write this? d of F over d of t, d of F over d of t is equal to can you write this? From 3 remember, d of F over d of t, F equilibrium 2 it is a constant, is not it. It is an equilibrium value, it is a constant. It will not change with a function of time because an equilibrium value. So, it is essentially 0, d F over d of t or d F equilibrium 2 over d of t. So, what are you left with this d of del F over d of t. So, then you can see what happens.

If you look at your equation 1. So, let me write this using 5 not 1, in 2 using equation 5 in 2 I can write d of del F over d of t is equal to minus k u concentration of F plus k f concentration of U, ok. Let this be equation 6. You follow this, right, clear, ok.

Now, the next step is this. What do you think the next step will be now? Remember, I have to get everything in terms of delta F. I cannot have F because that is what I say said. What we

said is we are looking at the difference relaxing is a function of time finally; the difference would be 0, this delta F. So, what, so what further simplification can I do now?

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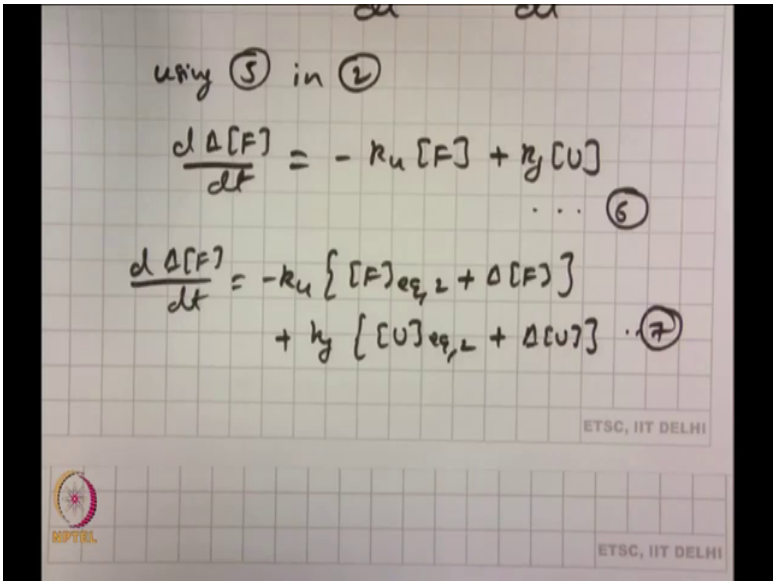


So, can I not do this? Can I not write d delta F over d of t is equal to minus k u? I already have expressions for F and U from 3 and 4, so what I can write is F equilibrium 2 plus delta F plus k f U equilibrium 2 plus delta U. This is equation number 7. Clear. I just split it up. Just expanded, using 3 and 4, equations 3 and 4, ok.

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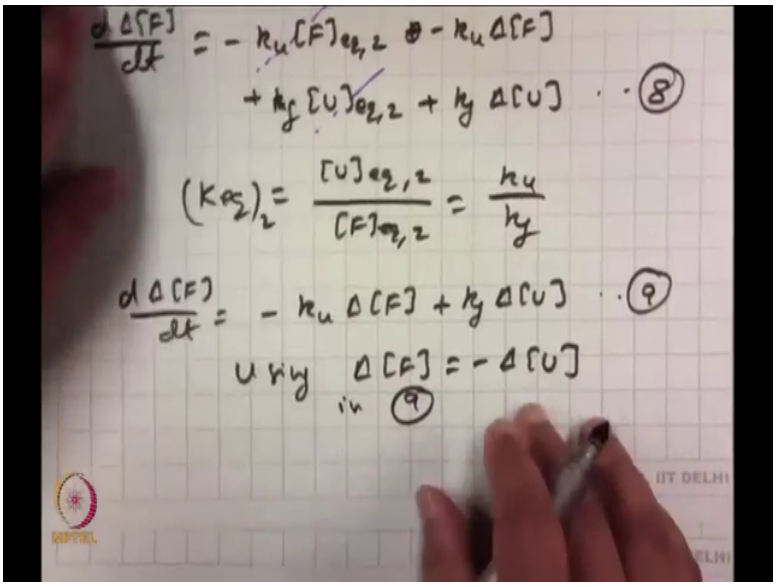


Now, let us fully expand it d delta F over d of t is equal to minus k u F equilibrium at the second temperature T 2, sorry minus k u delta F plus k F U equilibrium 2, plus k f delta U, ok. Let this be equation number 8. Can you tell me what further simplification I can do? At least give me an idea, ok. Let me give you a hint. What is K equilibrium equal to? What is K equilibrium equal to?

Student: U equilibrium.

Concentration of U equilibrium. Now, this K equilibrium as it temperature T 2. So, it would be 2 over 2. So, this would be what? k u over k of F, ok. So, does this help you cancel out two terms? So, you look at equation number 8, which are the two terms that cancel out? This one. So, that means, this one cancels out, right. And which one? This one cancels out. So, you

are done, right. So, that is done. So, what are you left with this T of delta F over d of t is equal to minus k u delta F plus k f delta U, right. Let this be 9, ok.

Now, what further approximation? See, I have to have everything in terms of delta F because I have doing that deed of delta F over d of t. Can I write this using delta F is equal to minus delta U? Can I write this based on stoichiometry? See whatever change you have in U, would be the negative of F or whatever change you have in F it would be negative of U. So, that is what we are using now. So, use this in 9 and see what you get.

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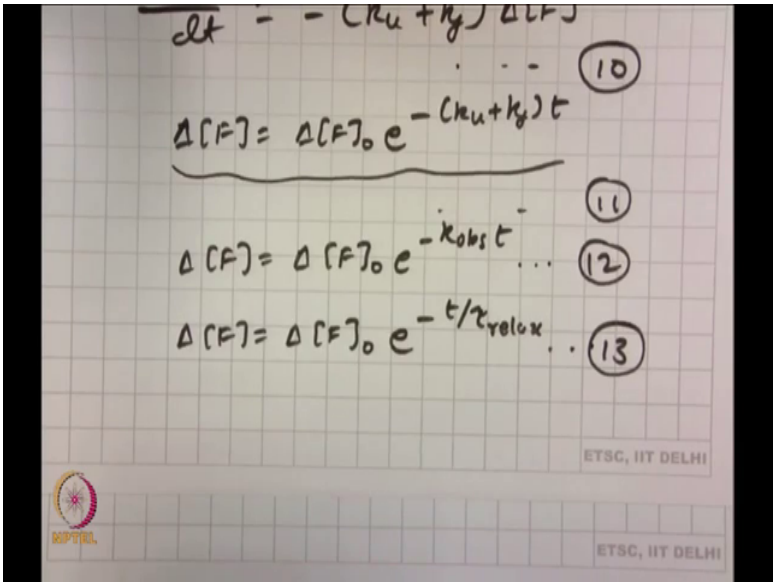


So, what do you have this then d of delta F over d of t is equal to minus [FL] k u plus k f times delta F, is not it. Remember delta U is equal to minus delta F or delta F is equal to minus delta U, right. So, see you started from you know a seemingly not so simple equation is [FL],

you have so many other parameters. But finally, have come down to a very simple thing it is a variation of just one parameter, what is that?

Student: Delta f.

Delta F. That is what we started with. You know that is what your premise was, that is what a basic principle was of this relaxation technique. So, then if I integrate I can write delta F is equal to delta F naught e to the power minus k u plus k f times t. This is equation number 11, ok. Or I can write delta F is equal to delta F naught e to the power minus k observed times t. So, this is say 12, ok. I can also write this as delta F is equal to delta F naught e to the power minus T by tau relaxation. Equation number 13, ok. So, these are 3 different ways I can write the equation. What is delta F naught? Delta F naught is the difference at difference at time t is equal to.

Student: 0.

0, that means, that is the time where you just make the jump, the protein has not responded, is not it, ok.

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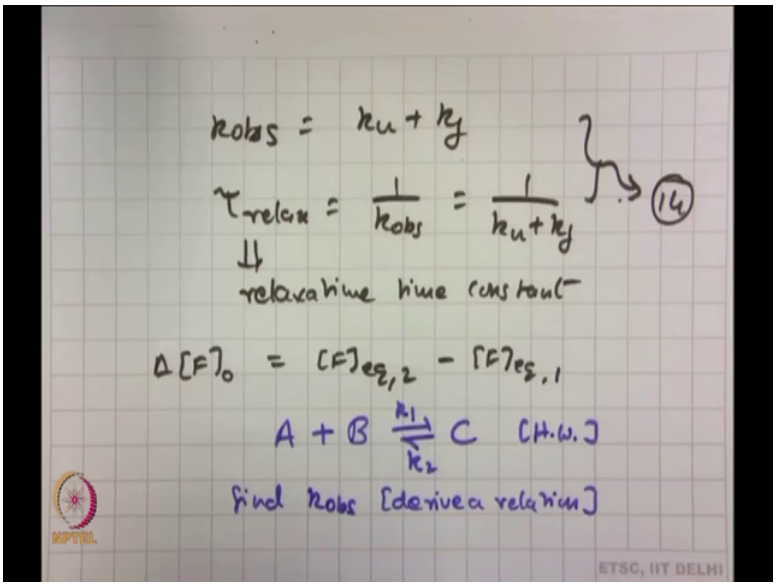


So, therefore, k observed k observed is equal to k u plus k f and tau relaxation is equal to 1 by k observed is equal to 1 by k u plus k f. And let this be 14. So, let these two combine the equation 14.

So, now, you can observe why it is called relaxation. So, this is called your relaxation time constant. So, this is called your relaxation time constant. So, you can understand this relaxation time constant or the relaxation rate constant which is k observed is not either k u or k f, what is it? It is a combination of k u and k f. You remember your chevron plot, what did you have? We had k u out this side; we have k f out that side, right depending upon which side you are. But then tell me if from here I am getting only k u and k f together, then how would I get them individually? I need k u, I need k f. But from this what am I getting? I am getting only.

Student: K equilibrium.

Right. I use K equilibrium because see I would always do a thermal denaturation. So, whatever the temperature T 2 in this case say it is 50 degrees, I would be having a point which is 50 degrees out there. I would know exactly what my K equilibrium there is. So, again k q equilibrium is equal to k u by k f. I have two equations, two unknowns I can easily find these out and then I get my chevron plot really. This is what is done, ok. This is what is done and is based on what we just saw, right, ok.

So, now tell me guys this we have discussed, but I just want to know whether you realize what is delta F naught? What can you write delta F naught to be? You told me.

Student: (Refer Time: 42:11).

Yeah. So, I can write delta F naught to be one that is the maximum difference, is not it. So, that is a time t is equal to 0. The protein is not responded, it is still at equilibrium 1. Where does it have to go to? It has to go to concentration of F and equilibrium 2 and then with function of time this difference in decreases and that is your relaxation, ok.

So, this is your T-jump relaxation technique, right. This is principally what happens. This is a kinetics which is connected with that. What I want you to do is, what I want to do is as a homework is if you have an equation like this going to see, right. Let this be k 1, let this be k 2. It is a homework problem, ok. Find k observed, that means derive a relation for it or expression I mean for k observed, in terms of k u k f and something else. You will soon see what is happening, ok.

Just let me give you a hint with regards to this. Look at tau relaxation. What is tau? What is the unit of tau it is time? So, it would be either second minutes whatever, ok. If tau is time then what is k observed? What is the unit of that?

Student: Inverse.

It should be inverse of time. Now, what is k observed equal to? k u plus k f, because k observed is equal to k u plus k f and because you are adding these both of these should be having the same dimensions that means, they should also be inverse of time. Now, when is k only inverse of time when you are essentially having a first order reaction and this is a two state, A going to B and B coming back to A.

So, that means, k u is also inverse of second, k f is also inverse of second. But guys look at the one I just gave you. When you go from A plus B to C, when you go from A plus B to C then the previous reaction was unimolecular both ways, this reaction is not unimolecular both ways. It is unimolecular in the reverse way, but bimolecule in the.

Student: (Refer Time: 44:48).

Forward. But remember your tau observed will always be inverse of time, will always be time that means, your k observed will always be inverse of time. So, this is the hint. You must have to make sure that your dimensions are maintained, right. So, derive it and we will see what happens. So, this is a little more complicated situation. I will tell you what people have used this for. People have used this to look at the hydrolysis of water the rate constant H plus OH minus going to H 2 O, D plus OD minus is going to D 2 O that is what people use this thing for because it is very fast, ok.

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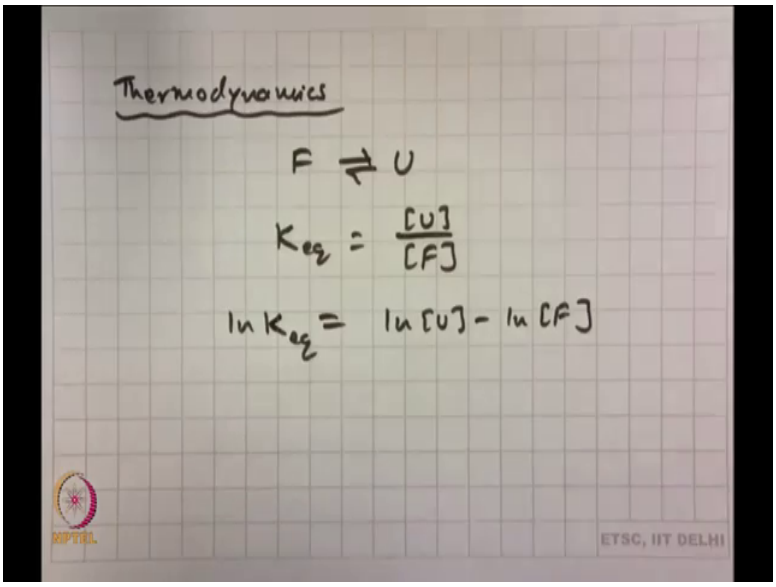


So, this was the kinetics of your temperature method. Let us quickly look at some thermodynamics or the T-jump. So, here again you are having F going to U and then K equilibrium is equal to concentration of U over concentration of F, right. So, then I can write natural log of K, I am removing the equilibrium part, K is K equilibrium is equal to let me write K equilibrium is equal to, ok.

Now, see I am doing a temperature jump that means I am doing a change in temperature, ok.

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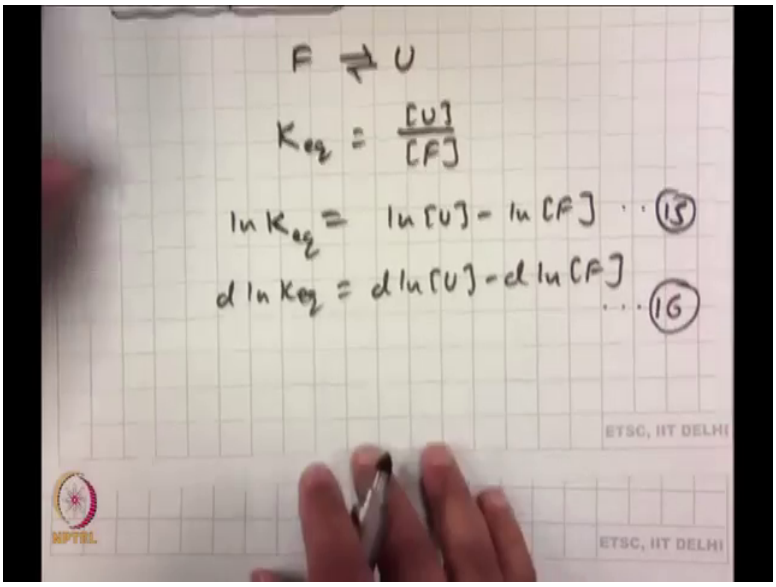


So, let me just do a differential of this equation. So, what I can write is then d ln K equilibrium is equal to d ln U minus d ln F, ok. Just differentiating throughout. So, let this what was the last 14. So, let this be 15. Let this be 16, ok.

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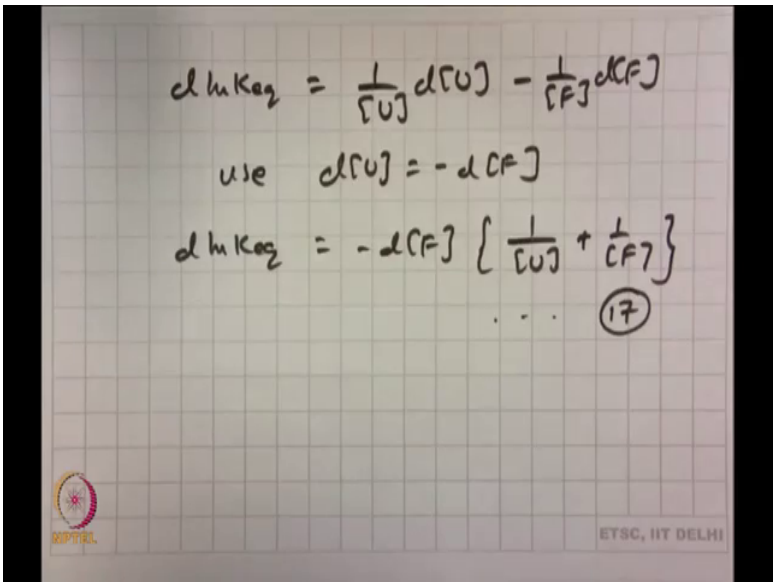


Now, what I can write is, from this I can write d ln K equilibrium is equal to 1 by U d U minus 1 by F d F. Are you guys with this? 1 by U d U is essentially d ln U, 1 by F d F is essentially.

Student: d ln F

d ln F, good. Now, use d U equal to what? Minus d F that is what we saw. So, then K equilibrium is equal to what will happen? d of F 1 by U plus 1 by F, ok. So, let this be equation number 17. Let this be equation number 17, right, ok.

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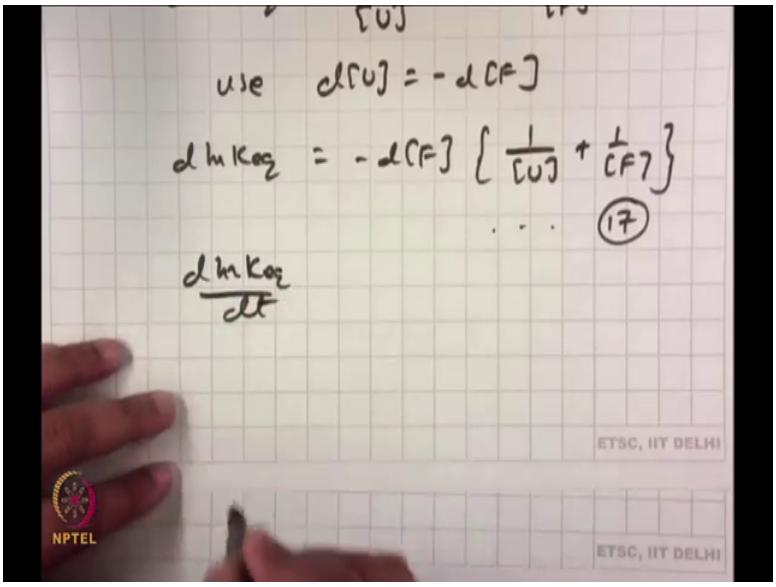


Now, d ln K equilibrium by d of t sorry, it is not temperature d, sorry let me write it again. Let me write in the next page actually, hold on.

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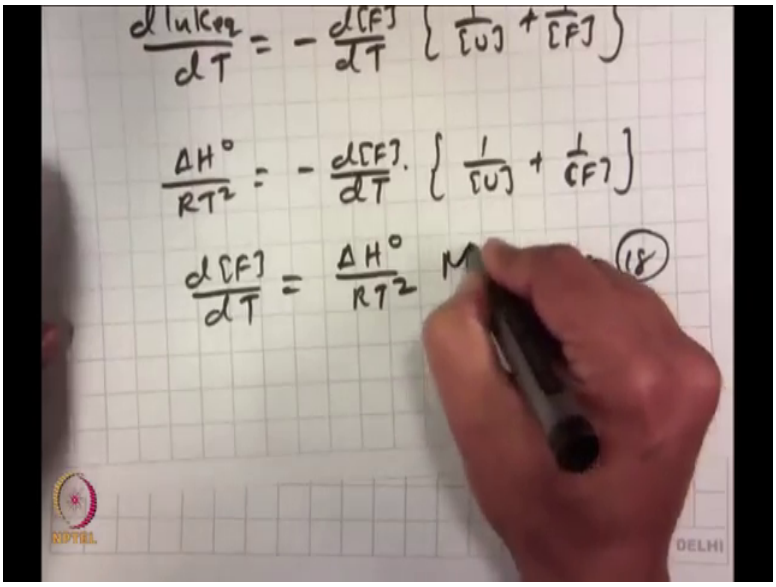


d by d of T is equal to d of T 1 by F, ok. Now, what is d ln k over d of T? Does someone remember from thermodynamics?

Student: Random equation.

Random equation. Yes, good start. What is expression?

Student: Delta H by RT square.

Good. It is equal to minus T of F over d of T, times 1 by U plus 1 by F or I can write d of F over d of T is equal to is equal to delta H naught by RT squared, delta H naught by RT squared. Then a constant M let this be equation 18 where, so this is a constant M. What is M?

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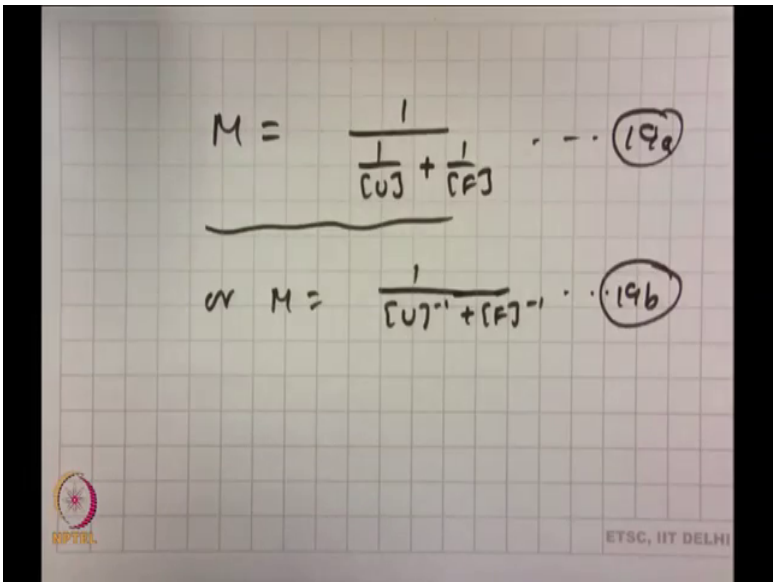


M is equal to, M is equal to. What is M equal to?

Student: (Refer Time: 49:55).

1 by is not this, that is what M is. Say M is 1 by the inverse of U and F or I can write or M is equal to 1 by U inverse plus F inverse. So, this is 19 a, this is 19 b, ok. So, having known what M is or what the expression for M is let us go forward, then you know what I can write is. Then, this is what I can write.

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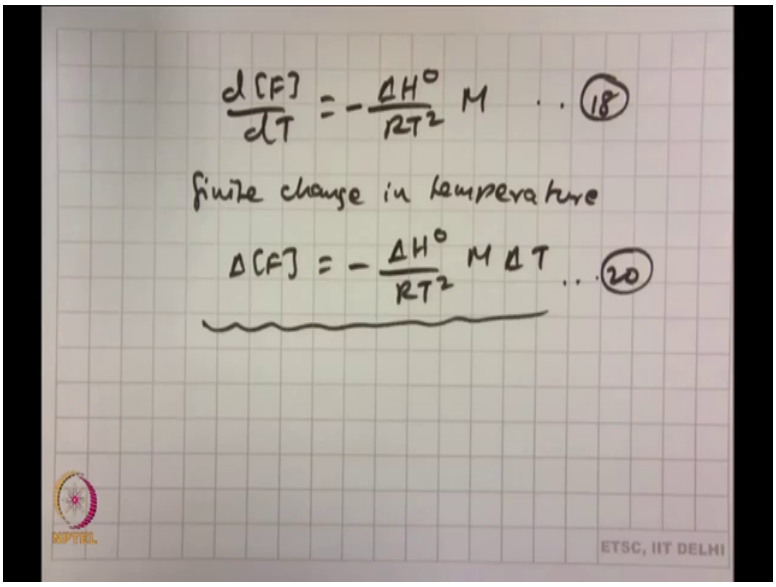


So, this is what we had d of F over d of T is equal to delta H 0 by RT squared M. So, this was equation 18. Now, what I do is I do it for a finite change in temperature. Say for a finite change in temperature I have delta F is equal to; I missed a minus, is not it.

Student: Yes.

Right. So, here it would be minus, here it would be minus. Delta F is equal to minus delta H naught by RT squared M delta T. And this would be equation number 20, ok, ok. So, keep this in mind. So, this is purely based on thermodynamic concentrations, ok. On the left hand side of the equation you have delta F, it is related to the change in temperature delta T. By what? By delta H naught and M, any of the RT squared.

Now, what does it mean to you? What it means to you is look at this. There are two very important parameters delta H is not an M, right. If delta H naught is equal to 0 that means, there is no enthalpy change. What will happen to delta F?

Student: 0.

That will also be 0. So, what does it tell you? What it tells you is if you have to observe a temperature jump in which you have a difference in concentrations that means, you have to have a relaxation. What do you need to have? There has to be difference in delta H or there has to be a difference in enthalpy.

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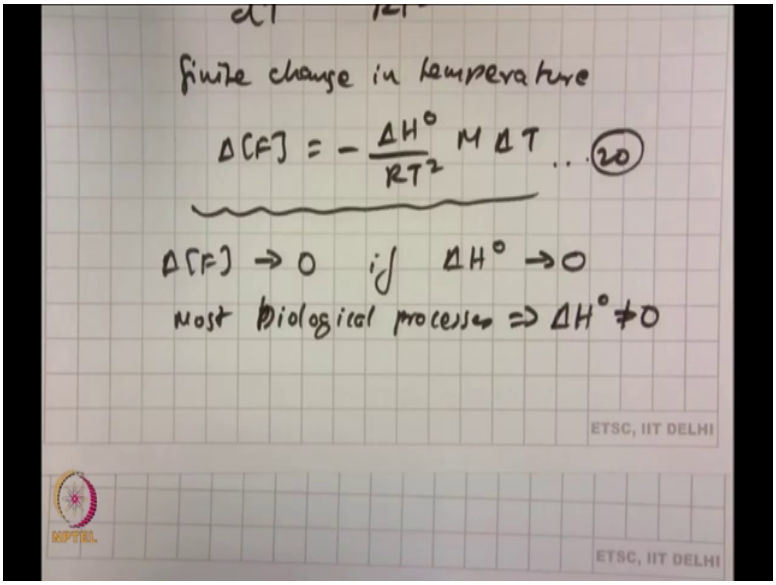


That means, delta F will tend to 0, if delta H naught tends to 0, but most biological process is for most biological process is delta H naught is not equal to 0, so which saves us, ok. In case

of what is defined in r. That is one. What is the other one? One is delta H naught, what is the other one? M, right.

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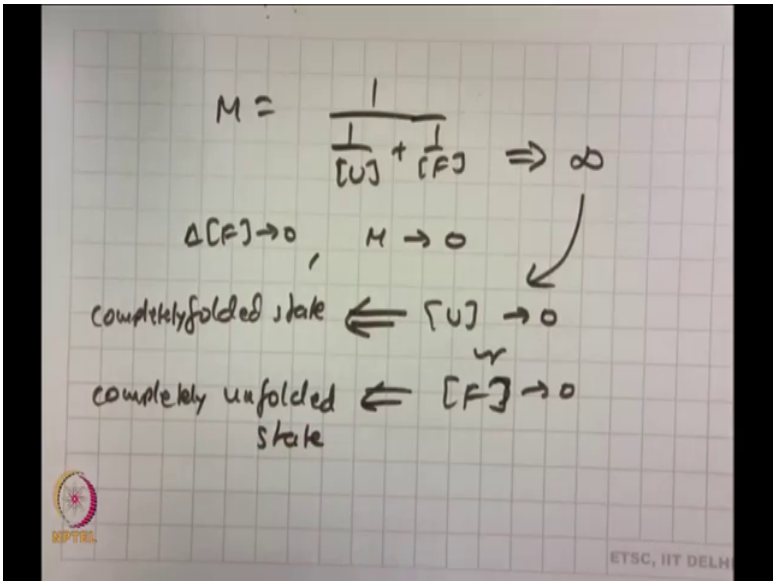


Remember, what M was, remember what M was. M is was equal to 1 by F, ok. For delta F tend to 0, M will also tend to 0. If delta H naught is not 0 which we just said, then for delta F to be 0, M should also tend to 0, ok. Now, under what conditions can M tend to 0 tell me? M is equal to 1 by something. Under what conditions can it tend to 0? Tell me two different conditions. See, what is 1, 1 by what is 0?

Student: Infinity.

Infinity, ok. That means, the denominator has to be infinity. How can you have infinity?

Student: u equal to zero.

Either U equal to 0.

Student: Or (Refer Time: 53:52).

Or either F equal to 0, is not it, ok. If U is equal to 0 and F is equal to 0 what does that signify? When do you have U is equal to 0? When you are completely folded. When do you have F equal to 0?

Student: (Refer Time: 54:07).

When you completely unfolded. So, what does it say? What it tells you is if you are in the completely folded position and a completely unfolded position, do not even worry about using this temperature jump technique. Why? Because you are not going to see anything because of this. So, then there is a range, there is a permissible range or there is an acceptable range over which you would do your temperature jump, ok. You would do your temperature jump that is what it means. That is I will discuss in the next class. But at least do you can you appreciate the significance of M here, ok.

For M to be 0, this has to go to infinity this has to go to infinity. And the only way it can go to infinity is either U goes to 0 or F goes to 0. If U goes to 0 that is your completely folded, completely folded state. If F gets to 0 that is completely unfolded state, ok.

So, tomorrow I will start from here. Almost done with the techniques. There is a little more thing that I will show you where people actually apply this temperature jump technique that means in over what range. So, you can understand, right. The only place you can you can the only place you can apply this temperature jump is various appreciable populations of both folded and unfolded, not at the extremes, right.

And if you remember something, if you remember something when we talked about these transitions what did we say that, the K equilibrium is best obtained where or the delta g is best obtained where? Where you have appreciable populations of both. So, see these actually

makes sense, right. It brings you back or it takes it takes you back to something we are discussed before. So, then you will see where this would be most applicable in which part of the thermal transition, ok.