Chemical and Biological Thermodynamics: Principles to Applications

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Lecture - 40
Differential Scanning Calorimetry (DSC)

Since previous lecture, we have started discussing the applications of calorimetry in chemical and biological systems of interest. We also recognized that even though calorimeters have been there since 18th century, but the recent development in technology has led to measurements of heats to a very small value. Therefore, it has become possible to address the systems of biological interest in which the heat changes are very very small.

We will now continue our discussion on differential scanning calorimetry. In this chapter as I just mentioned, differential scanning calorimetry is a very powerful technique and very important technique in elucidating the nature of transitions any transition. It need not be a transition in biological systems; for example, in proteins, it could be a solid melting transition vaporization. Transition calorimetry can be used to address these transitions in a quantitative manner, but in this lecture, let us focus our discussion on micro differential scanning calorimetry specifically applied to biological systems of interest and as I mentioned in the previous lecture that the calorimeters may vary differential scanning calorimeters may vary from company to company or from requirement based, but the principle of differential scanning calorimetry remain same.

Let us take a look at the first slide. The principle or differential scanning calorimetry we discussed in the previous lecture.

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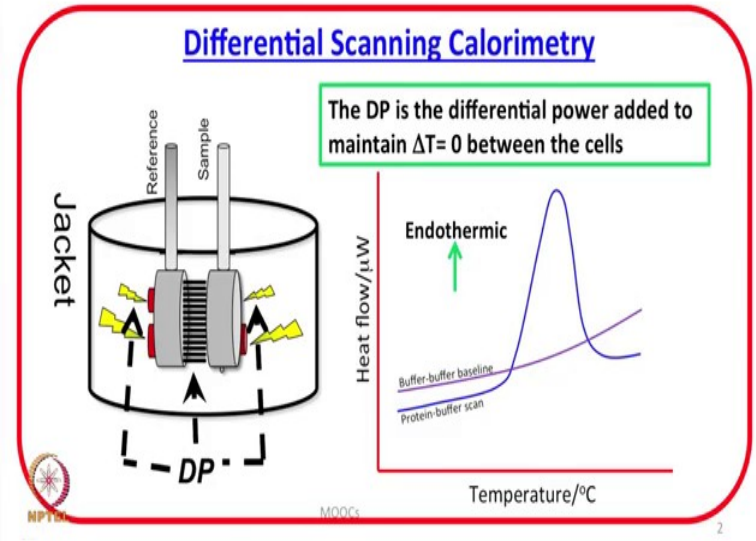

And what we discussed was the sample of interest is filled into the sample cell and the reference which if we are talking about the protein solutions. Usually the reference is buffered and that is filled in the reference cell now since the proteins nucleic acids are usually very expensive therefore, one cannot afford for a large amount to be used; therefore, the different companies or are making efforts to come up with the calorimeters with very small cell volume, the cell volumes which require only a few micrometers.

Today there are differential scanning calorimeters specifically suited for biological systems in which the cell volume can range from one m l to three hundred microliter and I am sure in future, we will have differential scanning calorimeters in which even smaller volume of the protein or nucleic acids sample may be required and then we talked that what is measured is the differential power which is added to maintain delta T 0 between the cells and we went on to discussing that when the protein sample is heated, we get a transition. We also highlighted the significance of carrying out a base line in buffer; buffer base line which should take care of the heat capacity effects due to solvent and also the geometric geometrical differences between the sample cell and the reference cell.

The design of the sample cell and the reference cell can make a big difference in one of the previous lectures, I showed that in differential scanning calorimetry usually the size or the shape of the cell is either lollipop type or it is capillary based. What kind of shape

should be preferred? If we are talking about the folding unfolding transitions in proteins and if we are interested in thermal unfolding of proteins; let us recognize that the loss of equilibrium thermodynamics can be applied to reversible systems and therefore, if we want to ensure the reversibility of the unfolding process reversibility of the unfolding process I mean when you unfold the protein and then you lower the temperature it should be able to fold back.

And therefore, if we want to ensure the reversibility then once the protein unfolds by means of temperature; it should not lead to any aggregation any form of aggregation and that is why you do not want the sample to be expose to a large surface area which should nucleate the formation of aggregation and in order to attenuate the onset of sample aggregation the best suitable design of the cell is of capillary type let us look at the slide.

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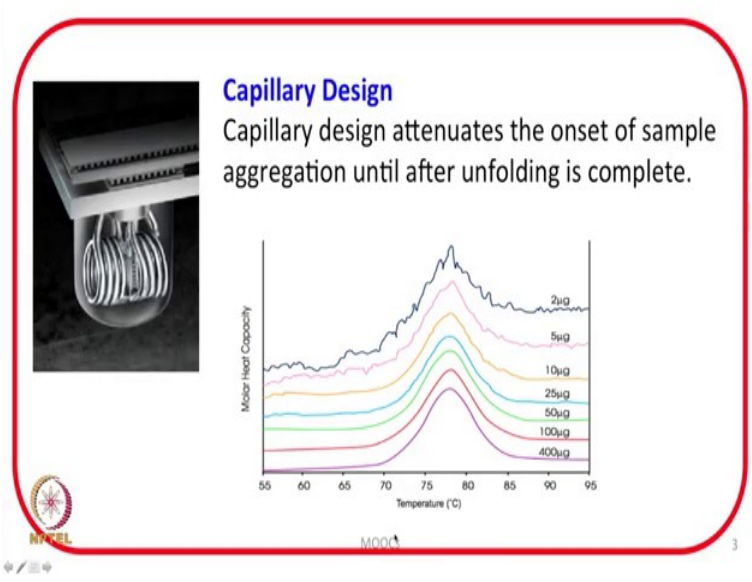

This is a design which is capillary design and you can see the cell will have one inlet and there will be another outlet; that means, you can sell fill the sample from one side and it can go through the capillary fill the cells and it can come out from other side.

So, this pair of sample and reference cell the capillary design is the most suitable for biological system and that is what is commented over here that the capillary design attenuates; the onset of sample aggregation until after unfolding is complete; I mention about the cost of proteins; the cost of nucleic acids sometimes can be very high and sometimes it may not be possible to buy commercial available proteins one may have to

isolate purify and eventually you may you may come up with very small amount of protein or nucleic acid and that is why it is very important to use very very small volume of the cell and this is one way; small volume of the cell and second is how low in concentration we can go what concentration do we require. So, that we can get a reasonable thermal unfolding transition.

Let us take a look at the slide; as we can see here that 400 typically; if 400 microgram of the sample gives a very nice unfolding curve, you start from here; you see a very nice shape of the unfolding curve is obtained and as you keep on reducing the amount of the sample even 2 microgram of the sample is good enough to at least give us an information on at least very accurately the transition temperature; the TM; the temperature where heat capacity is maximum; I will discuss more about this. So, this transition is very beautiful and you see the transition is fairly good even if one take 10 microgram of the sample five microgram some noise starts setting in even at 2 microgram although there is a noise, but at least it is giving an accurate information on the transition temperature.

And I am sure if very accurately; the area under the curve is measured then the calorimetry can enthalpy can also be very accurately determined. So, that is what I was talking about that today there has been so much development in the technology that we are able to measure the heat changes associated with very very small effects. Now let us talk in details about the protein unfolding why do we need to be study first of all protein unfolding when the protein is synthesized from ribosome. We have aminoacid sequence which you call as primary structure and then there is a formation of secondary structure then tertiary structure and then in some proteins it may lead to formation of quaternary structure we discussed about this in the previous lecture.

However when the protein folds a protein will fold because it is driven by certain intra molecular interactions and those interactions are hydrogen bonding ionic interactions hydrophobic interactions and few more interactions protein folding is a very fast process you need very fast techniques; however, an alternate method will be that you try to find out; what is hiding inside that what is holding the protein together; that means, you unfold the system and try to find out what was holding it together and that is worked differential scanning calorimetry is used for when it comes to study the protein folding unfolding; what you do in differential scanning calorimetry you heat the sample you heat

the protein and when you heat the protein the forces which are holding the protein conformation together start getting weakened and some point it will open.

And we have to device the mechanism; we have to design the experiment to find out what forces have been responsible for holding the protein conformation protein structure together and differential scanning calorimetry has played a large role in understanding the various forces which hold the protein together let us take a look at the slide.

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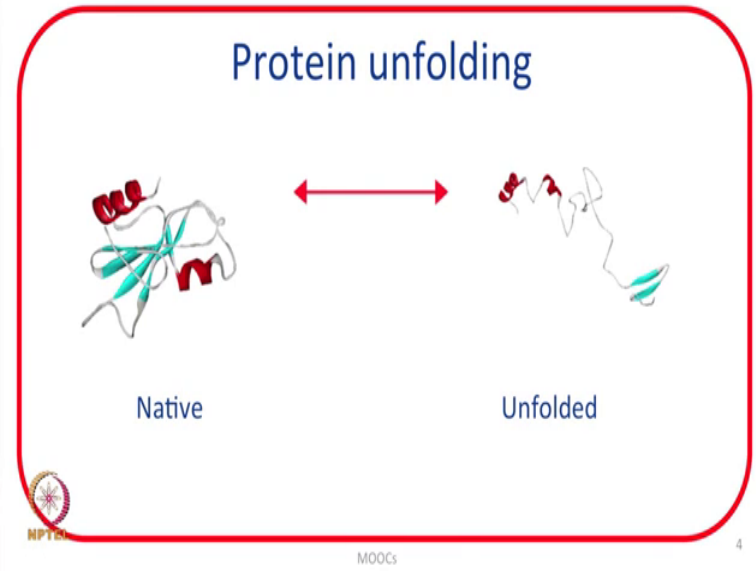

In this side, we are interested in understanding the thermal unfolding of the protein from native state to unfolded state and let us discuss how differential scanning calorimetry can be successfully applied to study this let us consider this ribbons as protein and this colored circles as the buffer molecules.

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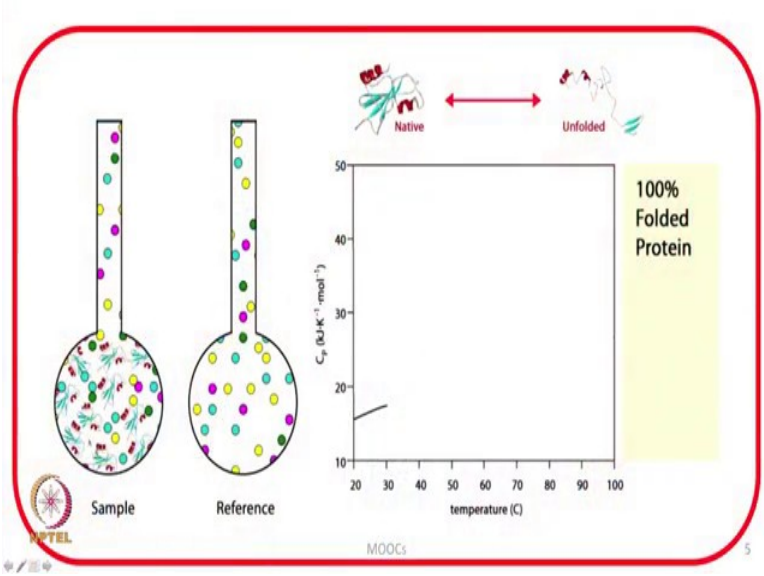

And that is why in the reference, you see only you have this colored circle which are the reference molecules which is buffer actually and we are interested in studying this unfolding native to unfolded. So, what you do in differential scanning calorimetry is start heating. You heat both sample and reference cell at a constant scan rate and what happens last time; you remember, we discussed that what you monitor is power and power can be converted to heat capacity and here you see you are plotting heat capacity as a function of temperature. So, when you start heating we start getting the change in power or heat capacity and then you see when the transition here the transition starts and it passes through a maxima this is the maxima where the heat capacity maxima is observed and beyond that if you heat it starts coming back and then the transition is complete hundred percent unfolded protein after the transition is O.

And you see the rub ribbons have disappeared; that means, the ribbons have been converted to unfolded conformation of the protein what we have obtained is a heat capacity versus temperature curve and we have to extract the thermodynamic information from this heat capacity versus temperature transition curve let us see how do we get that information first of all enthalpy of thermal unfolding we have thoroughly discussed the meaning of heat capacity and its connection with the change enthalpy heat capacity at constant pressure is defined by d H by d T at constant pressure you see here we are talking about the constant pressure.

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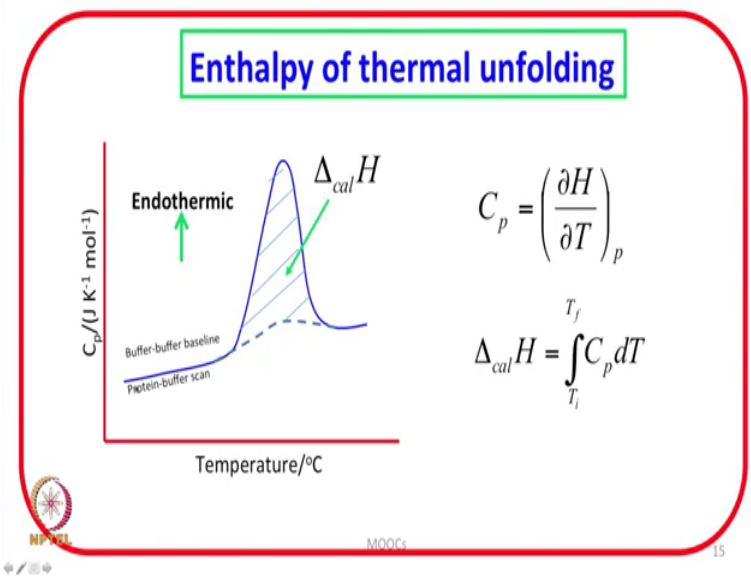

When an experiment is designed to be done on a differential scanning calorimeter; it is very important to de-gas the samples. The reason for de-gassing the samples is that you do not want any air bubbles in the sample because in differential scanning calorimetry you heat the sample and when you heat the sample and if there is air trapped; if there are bubbles in the sample when the heat; capacity will be largely effected because heat capacity of the liquid and heat capacity of air is very different and if the bubble come up you will get huge disturbance in your differential scanning calorimetry output profile.

And that is why the sample and reference cells of the differential scanning calorimeters are pressurized. You apply additional pressure; there is an internal mechanism of how to pressurize it depending upon where you get the calorimeter from, but the idea of putting the pressure on the sample and reference cell is that you do not want formation of bubbles that is one and that and the second is you are putting a constant pressure over there. So, let us look at the definition of C P is d H by d T at constant pressure which I can rearrange and write that delta H is equal to integration T i to T f C P d T. This also we have discussed many times.

And I will tell you why I am putting c a l as subscript over here what we have is we get this kind of; this is the transition curve and this is the I would say the excess heat capacity; why I can also call this as excess heat capacity because you are subtracting the protein buffers scan from the buffer buffer baseline. So, this is an outcome of this minus

this; that means, this heat capacity is the value of heat capacity over and above the buffer buffer baseline. So, therefore, if I choose the integration limit let us say I choose this as T i and I choose this as T f then the value of the integral can be area under the curve between C P versus or between C P and T or C P versus T within the limits T i to T f.

And that is what we shown over here if I choose this as T i and this end as T f then the area under this curve within this baseline and the overall heat capacity versus temperature curve between these 2 limits will be equal to delta H and c a l subscript. I am putting because this is calorimetrically determined value of heat capacity. There is no assumption in this what you are let us look at the slide what you are getting is it is an completely experimental curve with no assumptions made and the area is the calculated area with no assumptions made for this delta h is the true enthalpy of unfolding and let us label this as delta H c a l calorimetric enthalpy.

And I will soon discuss with you that why there is a slope associated in the beginning and why finally, also you see some slope and why this heat capacity variation and this heat capacity variation do not have the same slope why the slope differs why the heat capacity here is higher than the heat value of this capacity.

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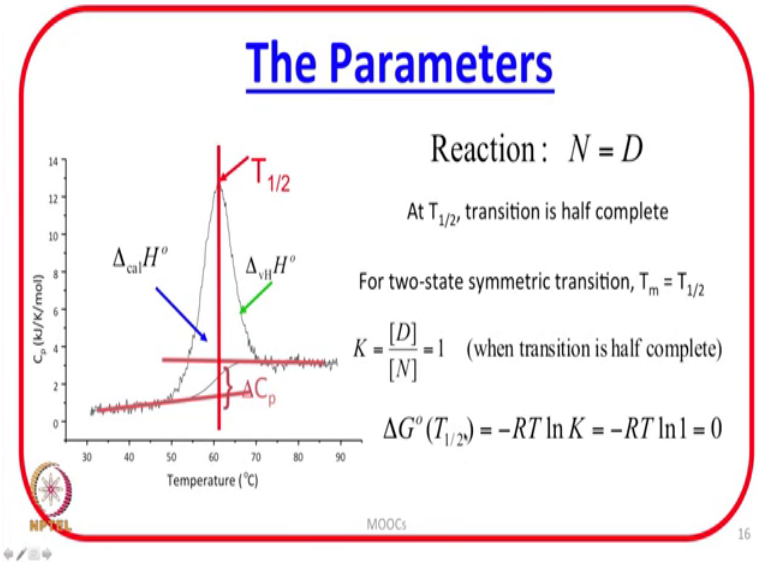

These all; we are going to discuss in the future lectures, but let us continue our discussion on what parameters are available from this complete differential scanning

calorimetric output. This is the output C P versus T and I just discussed with you that how we get calorimetry enthalpy this is area under the curve.

And if the transition is metric then the midpoint is either called T m or it is called T half why it is called T m and T half. I will discuss with you in details in one of the previous lectures; you remember I labeled this T m that is the temperature where heat capacity is maximum. Now we are talking about this reaction native to denatured reaction you are heating the protein from the native state and taking get towards the denatured state and then at T half that is at this temperature transition is half complete because if you consider this complete peak associated with the complete transition from native to denatured state then at a point where the area this total area is divided into 2 equal halves I repeat where the area is divided into 2 equal halves that temperature is T 1 half.

Let us remember this and that is what is commented over here that at T half the transition is half complete and if this transition is symmetric. Please note this; if this transition is symmetric then the T m that is the temperature where heat capacity is maximum will be same as T half that is what is commented over here for 2 state symmetric transition; T m is equal to T half. Now let us write down an equilibrium constant for this process and going to D.

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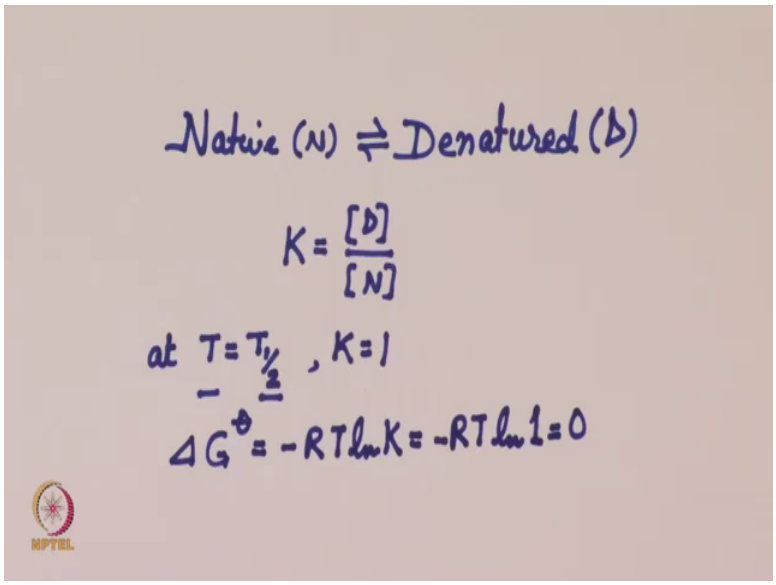

So, we are talking about this equilibrium native; if I write as N in equilibrium with denatured protein I will write this as D then K; I will write as the concentration of D divided by the concentration of N.

Now, at T equal to T half that is where reaction is half complete when the reaction is half complete then both this concentration will be same concentration of d will be N by 2. This will be N by 2 at T equal to T half K will be equal to 1 because both these concentrations will be same and delta G naught which is equal to minus R T log H will be equal to minus R T log 1; log 1 is always 0. We get the delta G naught is equal to 0. Let us take a look at the slide; this is what I was talking about that when transition is half complete the value of equilibrium constant for this 2 state transition is one and; obviously, the delta G naught will be equal to 0 and this information delta G naught at transition temperature T half.

Let us remember; we will call this as transition temperature if delta G naught at transition temperature is 0. We will use this information extensively in getting the temperature dependent thermodynamic quantities especially related to the folding unfolding process is in proteins and you see I am also writing here delta H Van't Hoff. We can get Van't Hoff enthalpy; how do we get Van't Hoff enthalpy?

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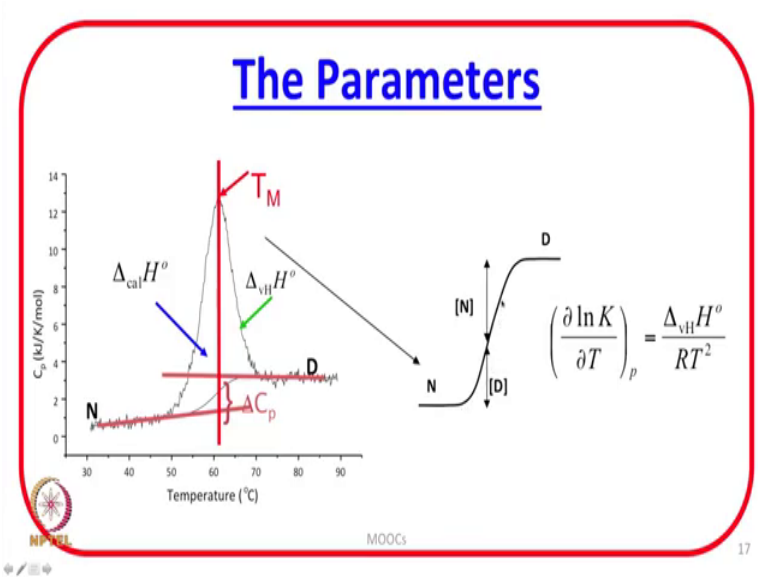

Van't Hoff enthalpy can be obtained if I can express equilibrium constant as a function of temperature and suppose if I integrate this differential scanning calorimetry curve you integrate the integrated plot will look like this right.

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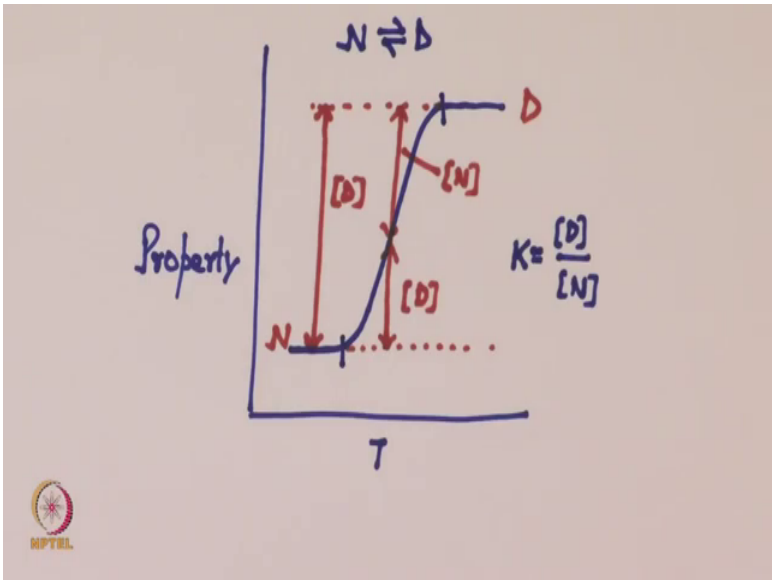

So, let me go back here; if the integrated plot let us say I will put as property the integrated one temperature and if the variation is like this then let us talk about these distances. If this is native state; this is denatured state, then this whole distance because here complete denatured protein will be complete denature denatured therefore, this whole distance I can write proportional to concentration of denatured state. And therefore, if I draw a line over here at this temperature then these distances can be approximated or will be proportional to this is concentration of denatured state this is this much protein has denatured and this much protein native is still. So, this one I can approximate by concentration of native state.

And that is what is seen in this slide that if I know D and if I know N then let us take a look at here; if I know D; if I know N then I can write K is equal to concentration of D over concentration of N; remember we are talking about N going to T and this K can be obtained at any temperature between this limit and this limit we can get temperature dependence of K. Let us go to slide and if I have the temperature dependence of K; I can get Van't Hoff enthalpy and this Van't Hoff enthalpy depends upon the shape that is why the arrow is pointing out on the shape of the DSC curve because the integrated form the

shape of the integrated form will depend upon the shape of the differential scanning calorimetric output and Van't Hoff enthalpy that is why which is obtained from the temperature dependence of equilibrium constant will depend upon the shape and hence will depend upon the shape of differential scanning calorimeter.

And difference in the heat capacity of denatured state and native state is delta C P change in the heat capacity delta C P can be calculated from C P denatured minus C P native. This delta C P is also very important and gives lot of information that we will discuss in future.

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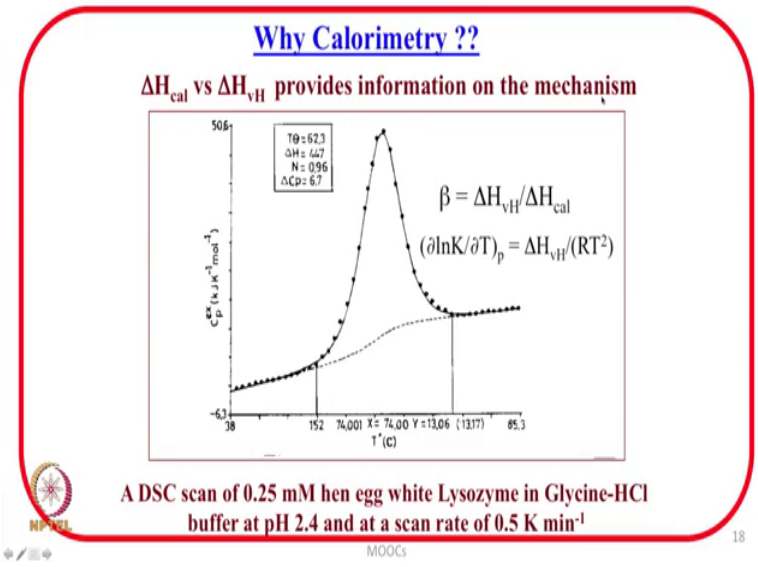

So the question is why the calorimetry calorimetric enthalpy and Van't Hoff enthalpy provides information on mechanism of folding unfolding transitions and from the differential scanning calorimetry curve we can get calorimetry enthalpy; we can get Van't Hoff enthalpy and the ratio of the 2 provides information on mechanism of the unfolding process.

So, you see; what we have started. Now appreciating that not only we are getting the numbers, but we have started connecting the information which is available with the mechanism. So, in this lecture; what we discussed is how to obtain this quantitative thermodynamic parameters associated with the protein unfolding that is transition temperature calorimetry enthalpy Van't Hoff enthalpy and now we have started appreciating that the calorimetry enthalpy and Van't Hoff enthalpy comparison can give

us information about the mechanism of the unfolding process and this we will discuss in details in the next lecture.

Thank you very much.