Chemical and Biological Thermodynamics: Principles to Applications

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Lecture - 56
Estimation of Binding Constants in Strong to Ultratight Protein-Ligand Interactions Using Differential Scanning Calorimetry

Every method which has a great advantage in studying something will also have some limitation. Somewhere, we have spent quite a few lectures on isothermal titration calorimetry and discussed a lot about its advantages that this technique is able to give us the thermodynamic signature in just one single experiment; however, the limitations of isothermal titration calorimetry are number 1 the solubility the solubility of several drugs in aqueous solutions is very poor and therefore, the choice of other solvents which can assist the solubilization of the drugs has to be done with care, we cannot use alcohol because the heats of dilutions of alcohol are enormously high therefore, alcohols should be avoided in isothermal titration calorimetry.

Similarly, if we use a certain percentage of DMSO or carbon tetrachloride or other things, we have to worry about the excess excessive heat of dilution; we have to worry about the effect of these kind of solvents on the conformation of proteins. The second limitation with isothermal titration calorimetry is that; we cannot very accurately determined the value of binding constants which are very very low and very very high.

Therefore, even though there are large advantages, many advantages of isothermal titration calorimetry, these are also associated with some disadvantages or some limitations not disadvantages; these are also associated with some limitations. Suppose, if the binding constant is of the order of 10 raise to the power 20, 10 raise to the 30, 10 raise to the power 40 or even higher, then can we use isothermal titration calorimetry? The answer is we can use isothermal titration calorimetry, but best thermodynamic parameter which is obtainable from such an experiments will be the enthalpy of binding.

It will be difficult to get the value of with binding constant with great accuracy and as we will discussed in this lecture that differential scanning calorimetry can be used to estimate the binding constant in strong to ultratight protein ligand interactions, but before that discussion, let us take a look at what is the range of binding constant that can be

determined by different methods. Let us take a look at the slide the recommended range of binding constants in isothermal titration calorimetry is 10 raise to the power 2 to 10 raise to the power 9 the binding constants.

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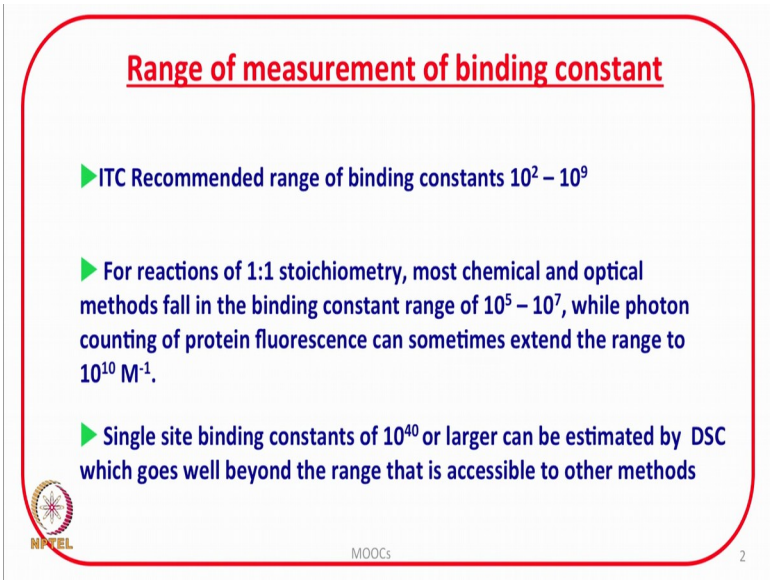

Which are less that 10 raise to the power and higher than 10 raise to the power 9 cannot be very accurately determined by isothermal titration calorimetry for reactions of 1 is to 1 stoichiometry, most chemical and optical methods fall in the binding constant range of 10 raise to the power 5 to 10 raise to the power 7 and whereas, if you use the fluorescence you can extend this range to 10 raise to the power 10, we have already discussed; how to use fluorescence in estimating the value of binding constant.

Single site binding constants of the order of 10 raise to the power 40 or larger can be estimated by DSC which goes well beyond the range that is accessible to other methods. So, in this lecture, we will be discussing the use of differential scanning calorimetry in estimating the values of binding constant and in one of the previous lectures.

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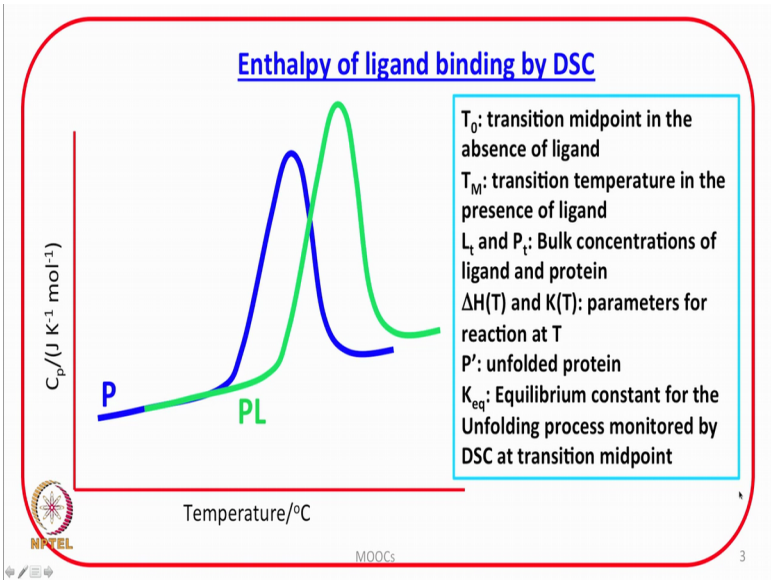

When I was discussing; how to get the enthalpy of ligand binding by DSC, we showed these kind of transition that is if protein is only in the native form, it will form; it will show this kind of thermal unfolding and if it is bound with the ligand fully saturated PL will appear at a higher temperature. Now let me define some thermodynamic parameters associated with these transitions let us call T 0 as the transition midpoint in the absence of ligand that is for the blue line T m, the transition temperature in the presence of ligand; that means, it is for the green line.

L T and P T the bulk concentrations of ligand and protein these are the total concentrations of ligand and protein taken in the study delta H T and K t, we will refer to parameters for the reaction at the general temperature T and prime, we will use for the unfolded state P prime means, we will be talking about the unfolded protein and wherever I use the subscript eq equilibrium for example, K eq, this will be equilibrium constant for the unfolding process monitored by DSC at transition midpoint. So, let us keep this in mind whenever I use eq, this will be corresponding to the process which is monitored by DSC at transition temperature.

Now when we do any reaction, when we study any reaction and later on want to express this reaction in a quantitative manner as we have discussed, while discussing the equilibrium constant that equilibrium constant should be expressed in the terms of activities and then we make certain assumptions.

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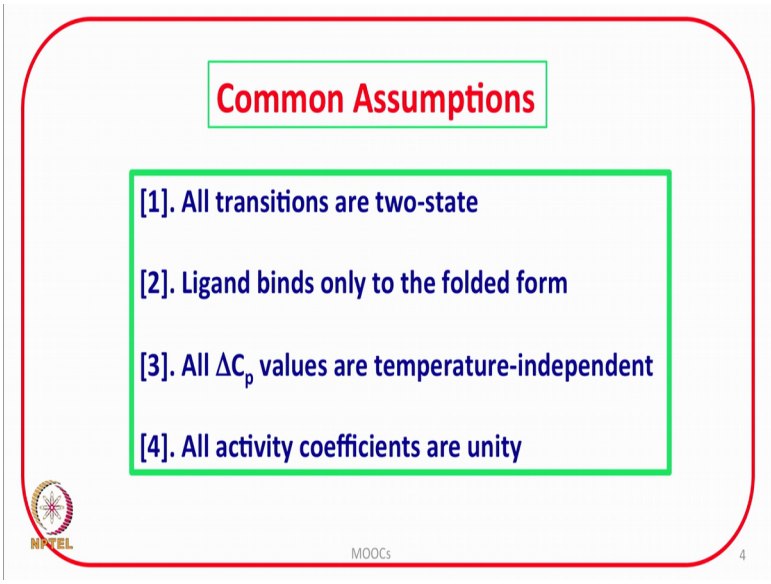

For further discussion, we will also make some assumptions and let us take a look at those assumptions. The common assumptions which will make here are one is that all the transitions, we will be assuming that these are 2 state, we will not assume that there is an intermediate state because that will make our treatment very very complex. Let us start with the simple treatment the second assumption that we will make is that a ligand binds only to the folded form in other words.

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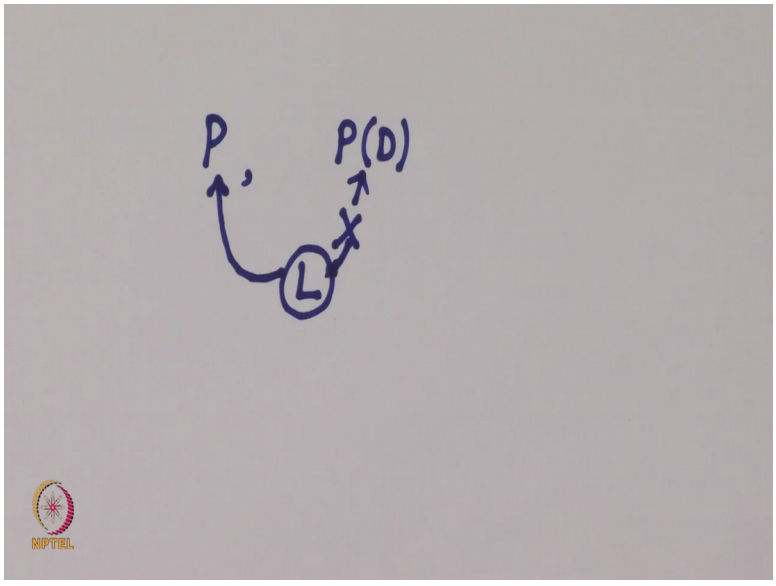

If I have P and if I have P denatured in the mixture and I add a ligand, ligand has only affinity with the native form and it does not bind with the denatured form that is assumption the third assumption is that all delta C, P values are temperature independent and the fourth assumption is all activity coefficients are unity we are making lot of assumptions here and as you will see we discuss the mathematical treatment that if we remove all the assumptions, then how the treatment becomes more and more complex. It can be done, but let us first begin with a simple process in which these assumptions are valid.

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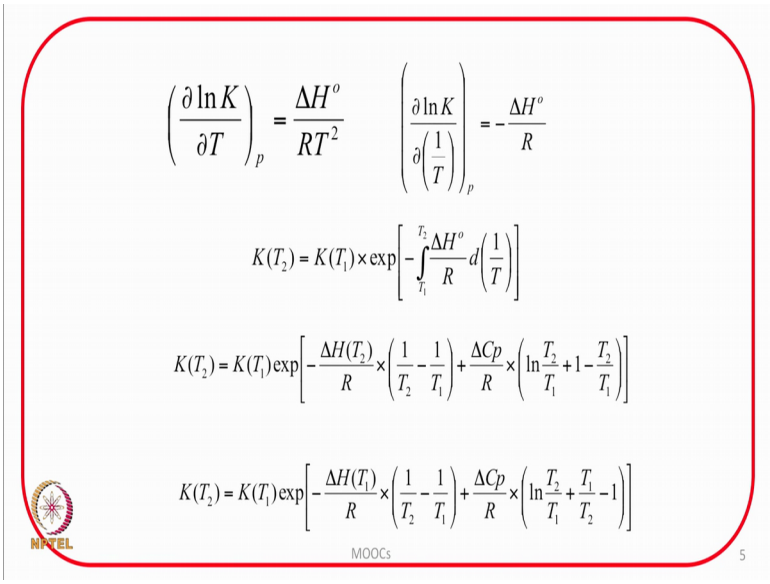

This is Vant Hoff equation, we have discussed many times Vant Hoff equation can also be manipulated and rewritten mathematical manipulation of this allows you to write in this form and one can go this further with this tap and express K T 2 is equal to K T 1 into explanation minus delta H naught by R D 1 by D from T 1 to T 2 and then it is possible to arrive at these kind of 2 equations where we express the temperature dependence of the values of K, I will just explain one of these and the other you can try yourself.

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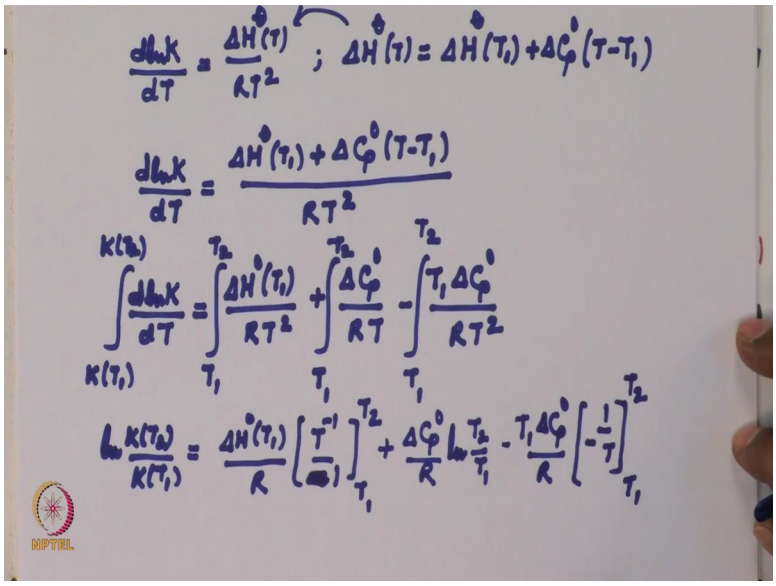

Let us start with the Vant Hoff equation which is D log K by D T of course, at constant pressure is delta H naught by R T square, actually it is a partial derivative at constant pressure, but for the sake of integration, I am just writing D log K by D T as delta H naught by R T square. Now delta H naught at any temperature, I can write as delta H naught at T 1, let us say if T 1 is my reference temperature plus delta C P of course, it will be naught into T minus T 1. This is by using Kirchhoff's law because this is at any general temperature.

Now I can substitute this over here. So, what I have is now D log K by D T is equal to delta H naught at T 1 plus delta C P naught into T minus T 1 over R T square. Let me go a step further D log K by D T is equal to delta H naught at T 1 over R T square plus delta C P naught over R T minus T 1 delta C P naught over R T square and I can integrate this K from K T 1 to K T 2 from T 1 to T 2 T 1 to 2 T 1 to T 2 and what we what do we get I get log K T 2 over K T 1 is equal to.

Integration of this is going to be delta H naught at T 1 which is constant by R and you have T minus 1 over minus 1 minus 1 from T 1 to T 2 plus delta C P naught over R log T 2 by T 1 minus T 1 delta C P naught by R again I have minus 1 by T from T 1 to T 2 and you have to now apply the limits and solve for the final equation. So, once you solve for this.

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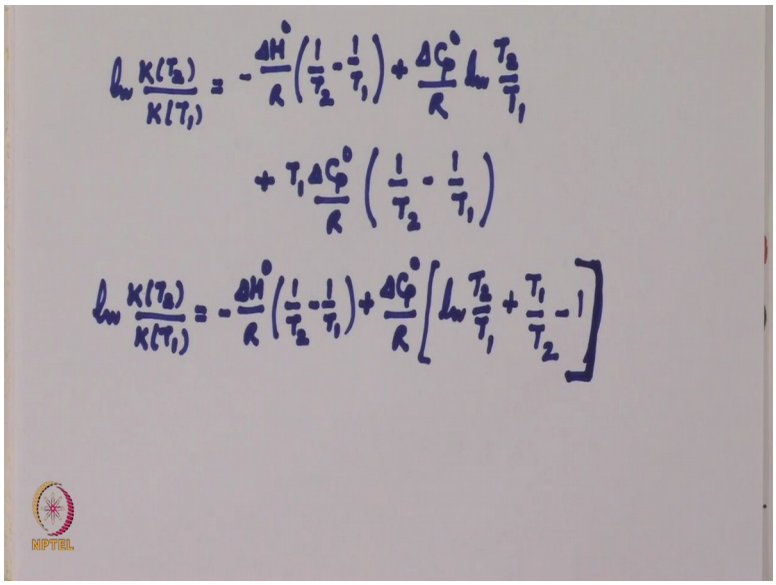

What you are going to get is log K T 2 by K T 1 is equal to minus delta H naught by R into 1 over T 2 minus 1 over T 1 plus delta C P naught by R log T 2 by T 1 and plus T 1 delta C P naught by R into 1 over T 2 minus 1 over T 1. So, what I have now is log K T 2 over K T 1 is equal to minus delta H naught by R 1 over T 2 minus 1 over T 1 plus delta C P naught by R and I have log T 2 by T 1 plus T 1 by T 2 minus 1.

Now, let us take a look at this slide. So, we are now at this expression we have derived this expression, I have removed log and brought an exponential factor. So, if you take the reference temperature as T 1, you come up with this equation and if you take the reference temperature with T 2, you can come up with this expression. Let us keep these 2 equations in mind which will be used thoroughly in the next discussion, we will discuss binding stoichiometry of 1 and if you understand the treatment associated with the binding stoichiometry of 1.

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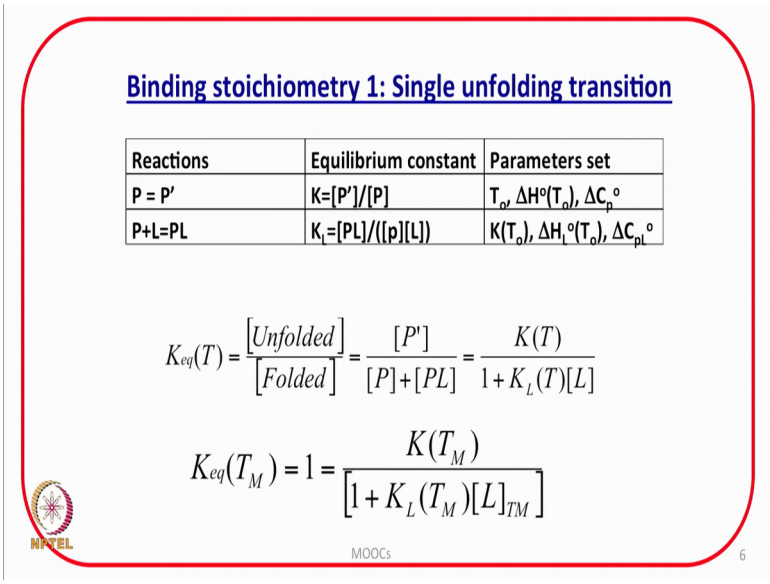

Then you can also deal with the further complexity that is when the binding stoichiometry is 2 or 0.5 or any other and also we will restrict to single unfolding transition to keep the discussion at relatively to begin with an easy discussion.

Let us take a look at this table, we are talking about protein unfolding to P prime as I said we will use prime for the unfolded state. So, when P goes to P prime, the associated equilibrium constant; obviously, is concentration of P prime divided by the concentration of P and the parameters set which are associated with the thermal unfolding are T 0 delta H at T 0 and delta C P T 0 as we initially discussed correspond to the transition temperature and when this protein is associated with the ligand then what we have is P plus L is equal to PL the equilibrium constant K L this is what you will be interested in K L is equal to concentration of PL divided by concentration of P into concentration of L and we can also you know get the parameters set the values of K at T 0 delta H L associated values at T 0 and delta C PL values under standard state conditions.

So, now let us start one by one with the equations.

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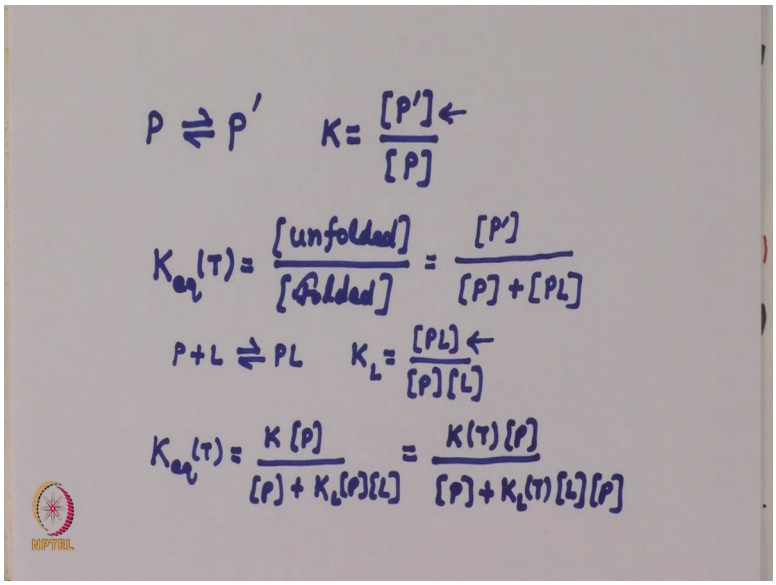

The first equation that we are talking about is P is in equilibrium with P prime, P prime is denatured state and associated value of K is equal to concentration of P prime over concentration of P and if now I have to write K equilibrium at temperature T for this, I can write the concentration of unfolded form divided the concentration of folded form what is the concentration of unfolded form is P prime is unfolded and the folded if you have P and PL both in the solution now you see P plus L when they are forming PL your K L is defined as concentration of PL over concentration of P into concentration of L.

So, now what I will do is I will choose PL from here P prime from here and substitute in this. So, what I have is K equilibrium at T is equal to P prime, P prime is equal to K into concentration of P divided by let me retain P plus PL is equal to K L into concentration of P into concentration of L. So, you remove P from here, I can write this as K. Now let me specify at a temperature T divided by P plus K L at temperature T into L into if I retain P into P and P-P can be cancelled. So, once you cancel this P everywhere, take a look at the slide, we come up with this equation that K equilibrium at any temperature T is equal to K at any temperature T divided by 1 plus K L at any temperature T into L that is a ligand concentration.

Now, if I express in the presence of ligand K equilibrium at T M; T M is the temperature where the transition is half complete and we have discussed several times that the value of equilibrium constant when the transition is half complete is equal to 1 and now what I

will do is instead of T you just write T M description is same. So, it becomes K at T M over 1 plus K L at T M into the concentration of ligand at the midpoint of transition. So, I hope that these 2 derivations are very very clear because if we understand for example, this one; let us write this down and work on this what we have derived is that K L K equilibrium at T M is equal to one and we have shown that this is equal to K at T M divided by one plus K L at T M into concentration of the ligand at T M.

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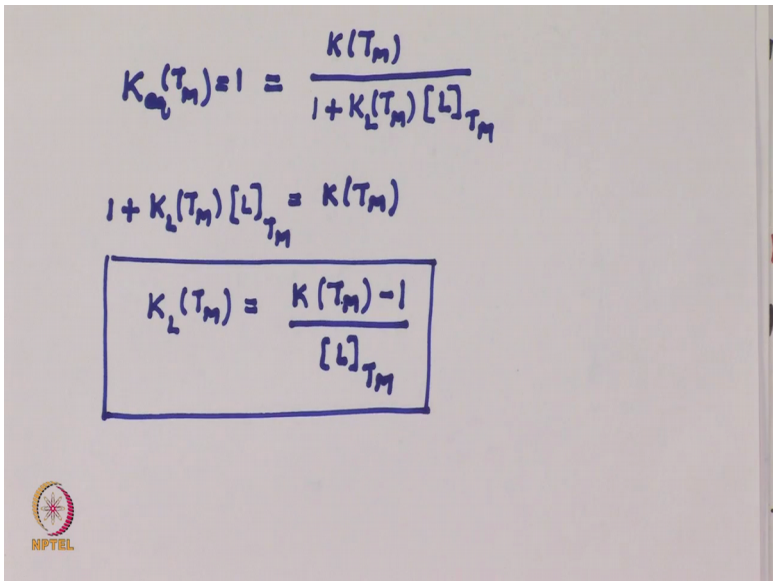

So, from this, I can write this equality 1 plus K L at T M into concentration of L at T M is equal to K at T M and from this, I can write K L because we are mostly interested in K L which is the binding constant associated with the ligand is equal to K at T M minus 1 divided by the concentration of L at T M this result you know connects with the K L and the concentration of ligand at T M.

Now, K L at T M or K at T M, these can be connected to enthalpies of reaction and the values of the equilibrium constants at any temperature by the equations that we derived earlier, let me show you back the equations which are required for discussion. I will have to go back by this slide, you see these 2 equations connect the value of K at any temperature with the value of K at an initial temperature and the corresponding values of enthalpies heat capacity changes and the temperatures. So, by making use of these equations, we will extend our discussion to derive further properties. So, what we have at present is this expression by the use of differential scanning calorimetry. We can easily

get the transition temperature calorimetric enthalpy and the change in the heat capacity associated with the unfolding with the absence of ligand, then we can design another experiment and run a DSC and in that experiment you first make a complex of protein with a ligand and then thermally unfolded, what you will get is the transition temperature here, we will get the calorimetric enthalpy and we will get the delta C P.

We have these 6 input parameters, 2 transition temperatures which are what are these transition temperature, in the absence of ligand transition temperature in the presence of ligand, we have 2 values of enthalpies of unfolding one enthalpy of unfolding in the absence of ligand and enthalpy of unfolding in presence of ligand and we have the corresponding values of delta C P. These will be the input parameters for the further discussion.

Now the other things which are required are the values of enthalpy of binding and heat capacity of binding; can the enthalpy of binding we determined by differential scanning calorimetry? Yes, enthalpy of binding can be determined by differential scanning calorimetry. We have discussed in one of the lectures that if you study the thermal unfolding in presence of ligand, in the absence of ligand and then the difference between 2 the enthalpies can be the value of delta H of ligand binding weighted by their fractions, we need to know what fraction of the protein is ligated to calculate the value of enthalpy of ligand binding by use of differential scanning calorimetry.

We will need to develop few more equations and go into details on how to get the value of binding constant associated with the ligand that is the main aim and then also discuss how to get the simulations; how to do the simulation, we will discuss the equations which are required for simulations come up with an expression for excess enthalpy derivative of which will give the heat capacity and then we will discuss; how the fitting of this theoretically generated curve with the experimental data points can help us in getting the value of the binding constant.

So, we still need to complete the discussion by deriving more more equations, what we have discussed in this lecture is first very first; the basic reaction that we are studying that is single binding reaction single unfolding transition. The associated equilibrium constant and parameter set and then we have derived some equations which connect, the values of equilibrium constant from one temperature to another temperature in terms of

enthalpies and the associated heat capacity, you will appreciate more about this discussion once we complete the whole discussion associated with this process. And that we will discuss in the next lecture.

Thank you very much.