Chemical and Biological Thermodynamics: Principles to Applications

Prof. Nand Kishore
Department of Chemistry and Biochemistry
Indian Institute of Technology, Bombay

Lecture - 55
Identifying sites for Drug-Protein Interactions DSC of Protein-Ligand Complexes Enthalpy-Entropy Compensation

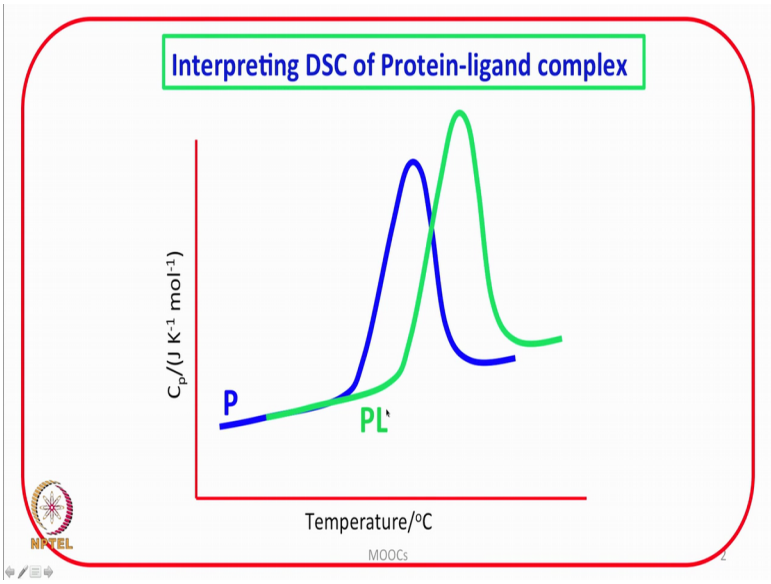
With the basic understanding of all the thermodynamic parameters and knowledge of working of calorimeters specifically, the calorimeters which are designed to study the reactions of biochemical interest, we have discussed at length, how these calorimeters can be applied to study protein ligand binding isothermal titration calorimetry as we discussed in earlier lectures is able to give thermodynamic signatures associated with the protein ligand binding in just one single experiment.

Then we also discussed how to design experiments to understand the nature of interactions which are responsible for the binding, identifying the nature of interactions is very very important as I have been repeatedly discussing that if we can understand the nature of interactions, then the nature of interactions can be connected with the functional groups on the drug molecules which are responsible for binding and once we identify the functional groups in connection with the binding affinity and the other thermodynamic signatures, then these give us guidelines for rational drug design.

The next question comes; can we use isothermal titration calorimetry in identifying the sites for drug protein interactions and how a combination of isothermal titration calorimetry and differential scanning calorimetry can give further information, not only about the thermodynamic signatures, but also about how the protein unfolds in the presence of ligands. When we talk about thermodynamic signatures and associate, the affinity with the standard reaction Gibbs energy change then automatically that standard reaction Gibbs energy change takes into account, the changes in enthalpy and changes in entropy; enthalpy entropy compensation phenomena is another aspect which has to be kept in rational drug design and in this lecture.

We will also discuss about enthalpy entropy compensation and its possible sources of origin both in protein ligand binding and in unfolding of proteins when studied by variety of methods.

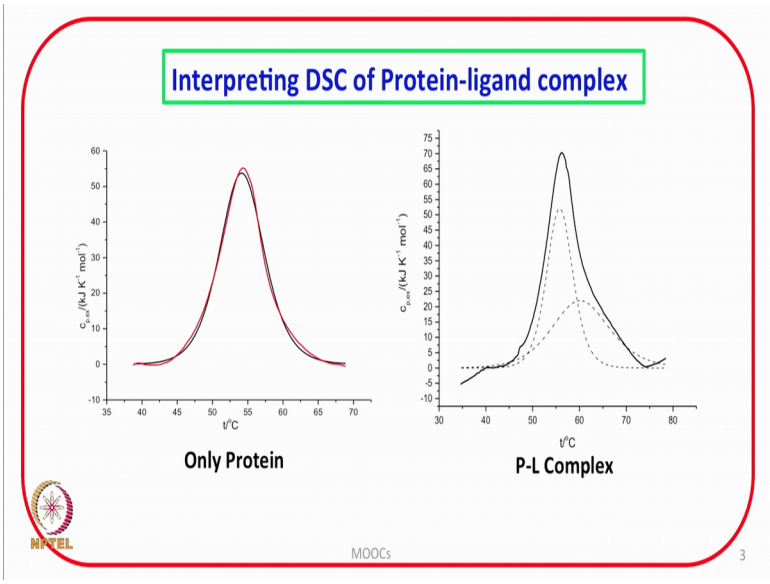
(Refer Slide Time: 03:55)



Now, let us take a look at how to interpret the DSC of protein ligand complex as discussed in one of the previous lectures, let us take a look at this slide, when we thermally unfold a protein P, then we can find out its transition temperature calorimetric enthalpy etcetera and when the ligand is bound to the protein and you unfold P L usually its unfolding will be at higher temperature and we will get the corresponding thermodynamic parameters associated with the unfolding of P L complex.

Now this kind of situation will arise when the protein is fully saturated with the ligand what I mean is P L showing only one peak. This kind of situation will arise when the protein is fully saturated with the ligand. Now let us take a look at if you thermally unfold only the protein.

(Refer Slide Time: 05:19)

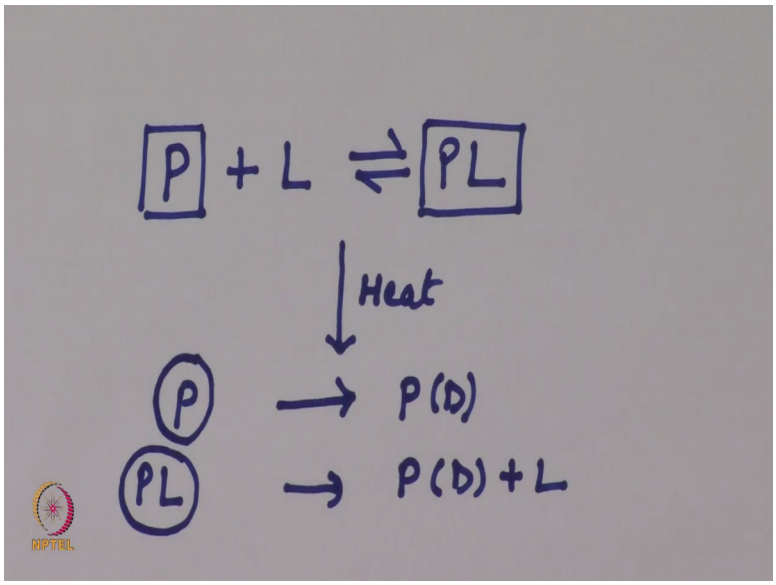


We will usually get a single endotherm 2 lines are shown here, one line shows the experimental data points and the second curve shows the fitting of a suitable model to the experimental data points.

Now, let us take a look at the next figure, it is possible that sometimes, we may get this kind of DSC profile for a protein ligand complex in the previous lecture. In the previous slide I showed only 1 endotherm for a P L complex, but here I am taking another example where P L complex may show like this kind of endotherm; the overall endotherm is the black solid line and we can clearly see that it is not symmetric endotherm especially, when we look at the right hand side the curve deconvolution shows that there are 2 endotherms embedded under this overall endotherm, these are these 2 endotherms are shown by the dotted lines. Now what could be the reason for these 2 endotherms.

Let us discuss that what is usually the meaning of one single endotherm or what is usually the meaning of 2 endotherms which are de-convoluted under an overall single endotherm when do we get the 2 endotherms one possibility is when the protein has 2 domains and both the domains are unfolding independently one after the another, you will get 2 endotherms, the other possibility is that in the protein ligand complex if there is unsaturation or in other words if all the binding sites are not saturated.

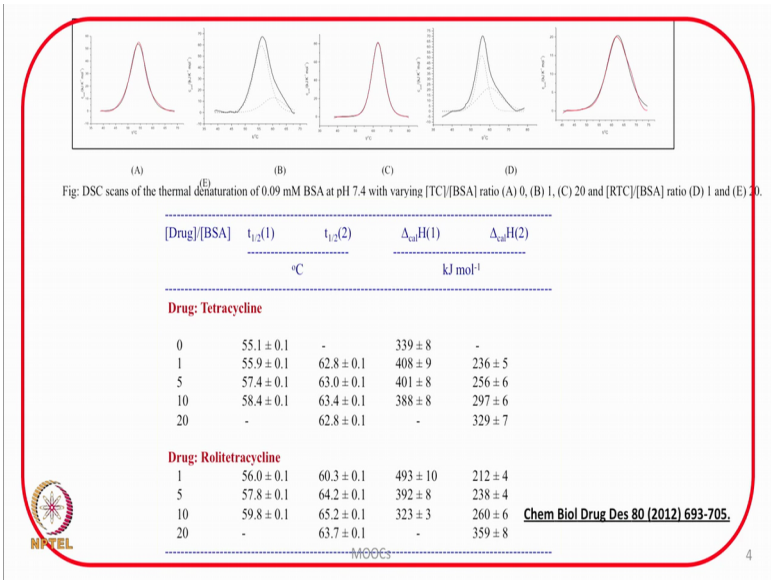
(Refer Slide Time: 08:11)



Then what will happen; let us take a look at this reaction if you study P plus L and this forms P L and if at equilibrium you have sufficient amount of P L and sufficient amount of P and then when you heat this you have these 2 reactions going on P going to P denatured and P L you are heating it is P denatured plus L and 1 will get a transition a corresponding to P and transition corresponding to P L and depending upon how close they are either it will be showing in the form of a single endotherm with a shoulder or there will be 2 well separated endotherms.

Now, let us go back to the slide and take a look at this DSC curve, one way of interpreting this kind of DSC curve is that at equilibrium, there are sufficient amounts of both P L and P which are showing up as 2 separate transition the one which is at higher temperature can be due to the P L complex unfolding and the one which is appearing at a lower temperature that is corresponding to the protein which still unlike it.

(Refer Slide Time: 10:10)



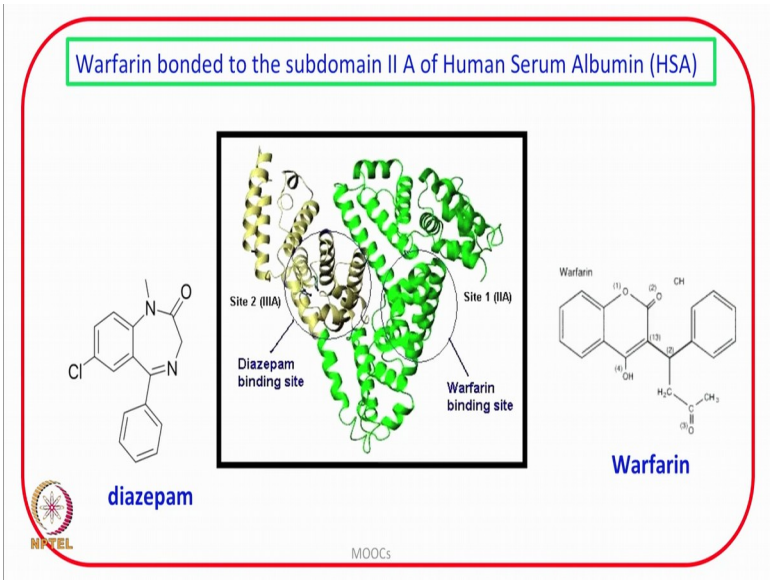
Now, let us take a look at this figure, this figure; these figures corresponds to DSC scans of thermal denaturation of bovine serum albumin at pH 7.4 in the varying molar ratios of tetracycline to BSA, this one is without any tetracycline, this is in the presence of tetracycline, we can clearly see that when you add tetracycline, we see that the overall peak is no more kind of symmetric peak, but there is a shoulder which could be due to the unfolding of the protein ligand complex and the other one can be the one which is still not ligated.

Then there are 2 more figure C and D shown over here and C and correspond again to the one which is without any rolitetracycline and this is in the presence of rolitetracycline and again we can see here that the one peak is now divided into 2 peaks; that means, again showing one of the interpretation to such a behavior can be that at equilibrium, we have both the P L and P and that is why it splits up into 2 endotherms and this table shows the corresponding data; that means, it is possible to get t half and delta H for each endotherm and then once you have the transition temperatures and enthalpies associated with each endotherms then more insides into the thermal unfolding of the protein can be discussed.

So, this is about that when a protein is ligated with the ligand and when you thermally unfolded it may show it one endotherm, it may show 2 endotherms and the situation can become more complicated if the protein has more domains and those domains are

unfolding independently and even complexity can further come, if the ligand binds to one particular domain and the other domain is unfolding in an independent manner. Now let us take a look at the next slide; human serum albumin HSA as we discussed earlier because of its structure.

(Refer Slide Time: 13:16)



Because of its properties can bind variety of ligands, one such ligand which binds to human serum albumin is fatty acid. Fatty acids can find variety of sites in human serum albumin or bovine serum albumin and show a significant amount of binding.

And these binding sites are seen in this picture FA1, FA2 and all other fatty acid biding sites possible are shown over here in addition to the fatty acid binding sites, there are well defined other drug binding sites on serum albumin and that is why, if we are interested in understanding the binding of a certain ligand with this transport protein serum albumin, we must work with the fatty acid free protein because if we work with the protein which already had has fatty acids then that will affect binding studies, if we work with the fatty acid free serum albumin, then we can design experiments to understand that at which binding site the incoming ligand is binding.

Let us take a look at the next slide, this is the structure of serum albumin and as we have earlier discussed, there is a warfarin binding site, then there is a diazepam binding site; several ligands binds to warfarin binding site and several ligand bind to diazepam binding site and it depends upon the molecular structure of the incoming ligand. For

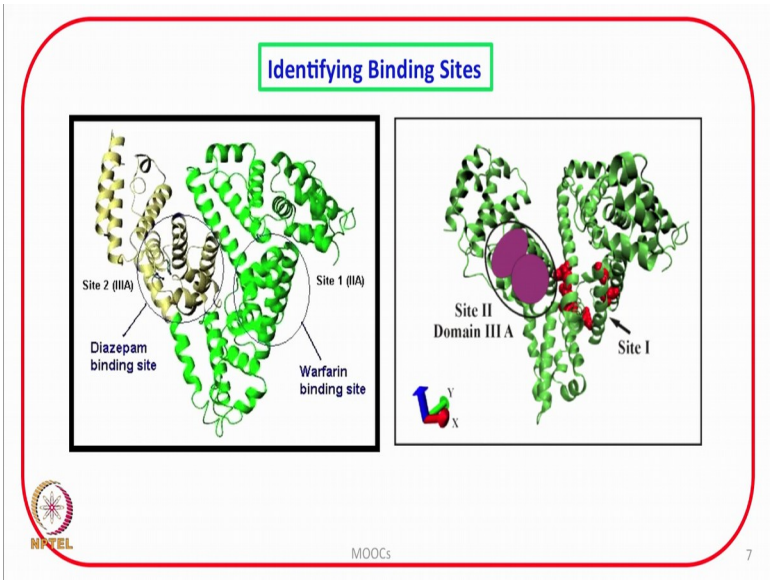
example, take a look at the molecular structure of warfarin and also of diazepam. Now how to design the experiments, we have well defined warfarin binding site, we have a well defined diazepam binding site and these 2 molecules warfarin and diazepam can be called site markers because it is well established that warfarin binds to sudlow site 1 and diazepam binds to sudlow site 2.

Now, how to design the experiments what one can do is in identification of the binding sites is that you make a complex of warfarin with serum albumin and study the binding of the drug. The main drug which we want to understand where it binds, we will already have in hand the binding constant and other thermodynamic signatures associated with the binding of warfarin with the protein. Now if the binding site is already occupied by warfarin and the incoming ligand does not bind at this site with an affinity which is much higher than warfarin, what in other words mean is that if the binding affinity of the incoming ligand is less than the binding affinity of warfarin for site 1, it will not be able to displace it therefore, it will not bind it will not show binding.

And once you remove warfarin from that side, we titrate with the free serum albumin, it will show binding at site 1. In other words, it shows a binding at site 1 in the absence of warfarin, but in the presence warfarin, it does not show binding that establishes that the incoming ligand is binding at site 1. Similarly another experiment can be designed by blocking site 2 with diazepam and then during these kind of experiments. So, this kind of combinatorial experiments help in identifying that at which site out of these 2 site 1 or site 2 the incoming ligand is binding.

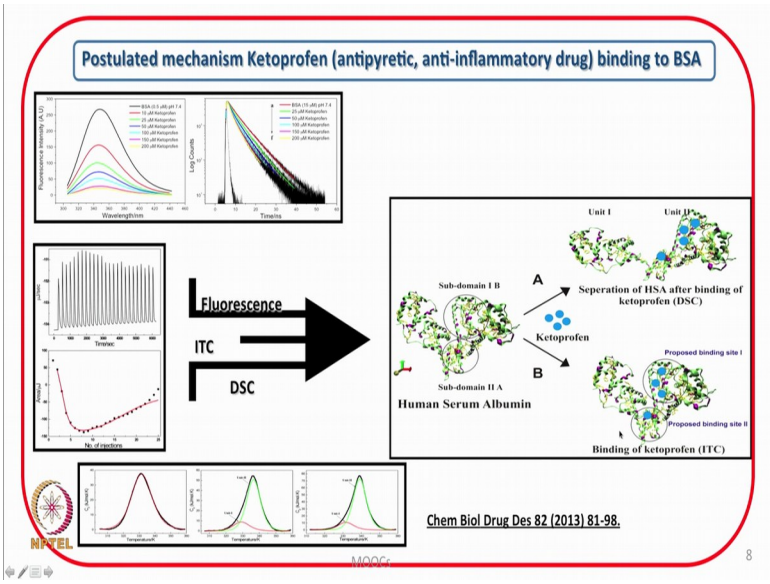
Therefore thermodynamic signatures which are obtained by the use of isothermal titration calorimetry can also help us in identifying the binding sites of the protein. So, when it comes to identifying the binding sites, for examples by using a similar approach.

(Refer Slide Time: 19:31)



It can be established whether the incoming ligand binds site 2 or site 1 and in order to arrive at this kind of result, as I just discussed in the previous slide that we need to do experiments with the site markers and see whether the binding signatures are affected in the absence and in the presence of the site markers, what else we can understand by applying a combination of isothermal titration calorimetry and differential scanning calorimetry.

(Refer Slide Time: 20:05)

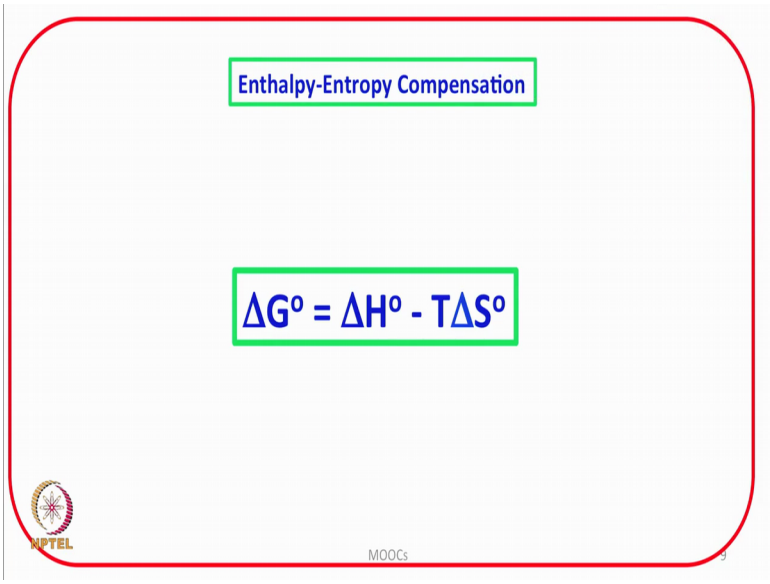


Isothermal titration calorimetry and differential scanning calorimetry will give you the thermodynamic signatures and will also give you the thermodynamic parameters associated with the unfolding of protein conformational characterization of the protein can be done by the use of fluorescence spectroscopy and also by the use of circular dichroism spectroscopy. So, therefore, if we study the binding and unfolding process by using a combination of isothermal titration calorimetry differential scanning calorimetry UV visible spectroscopy, fluorescence spectroscopy, circular dichroism spectroscopy, we can discuss the mechanism of binding as well as the thermal unfolding of protein in great details as seen in this slide.

Let us take a look at this slide, this slide shows the binding of Ketoprofen which is a antipyretic anti inflammatory drug to serum albumin by the use of isothermal titration calorimetry, the binding site of Ketoprofen on serum albumin can be identified; that means, we can address this step where the ligand is going and binding and then when you study the thermal unfolding of the protein of the bound protein and we can see that there are 2 well defined endotherms embedded under an overall endotherm in the presence of ketoprofen these results suggested that these 2 domains of serum albumin are unfolding independently where the bound domain is unfolding at the higher temperature and the unbound domain is unfolding at the lower temperature and that is why we are getting the 2 transitions within an overall broad transition.

For further details on the mechanism, I refer you to this research article, but what I want to highlight over here is that by combining the calorimetric and spectroscopic techniques.

(Refer Slide Time: 23:36)



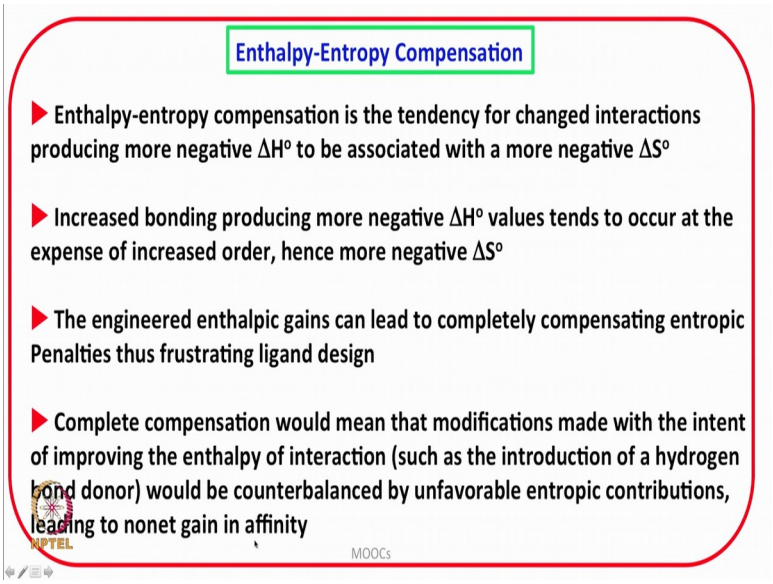
These kind of detailed mechanism can be postulated, now let me talk about the enthalpy entropy compensation whether we talk about the protein ligand binding or we talk about the thermal unfolding of protein, in both the cases, we are talking about the enthalpy changes, we are talking about the entropy changes, the enthalpy changes and entropy changes both can be used to further get the standard reaction gives free energy changes. Let us take a look at the slide, we have delta G naught is equal to delta H naught minus t delta S naught delta H naught, the negative value of delta H naught assist more in this spontaneity of the process and the positive value of delta S naught assist in the negative value of delta G naught or in other words towards the spontaneity of the process.

Now, it is a combination of delta H naught and delta S naught which will decide the overall effect of delta G naught in the rational drug design. Suppose if we are able to achieve the desired delta H naught, what I mean if the desired delta H naught should be more and more negative, if we get sufficiently negative value of delta H naught, but that is offset also by the negative value of delta S naught then overall the net effect on delta G naught will not be significant. In other words, if the enthalpic gain is being offset by the entropic loss then in pharmaceutical industries, it will lead to frustration towards get more affinity. So, therefore, towards the rational drug design, the efforts have to be that we achieve a better negative value of delta H naught and not much compromise on the loss on conformational degree of freedom not much loss on the entropic front.

Enthalpy entropy compensation has been a subject of discussion for a long time enthalpy entropy compensation has been observed in many phenomena like protein ligand binding and the protein unfolding along with some other processes, where there is a change in delta H naught and delta S naught, although there have also been reports in literature that in certain class of compounds enthalpy entropy compensation has not been observed, some authors have even pointed out or questioned the enthalpy entropy compensation and have hinted or pointed out that the compensation is occurring due to the errors in delta H and delta S naught and it is not a real phenomena.

Anyway, there are still papers appearing on the validity of enthalpy entropy compensation, but a straight look at this equation; delta G naught is equal to delta H naught minus t delta S naught does suggest that; in order to get more negative value of delta G naught, delta H naught has to be negative and delta S naught has to be positive and if there is an enthalpy entropy compensation, if there is a complete compensation leading to Nonet gate gain in delta G naught, then the efforts will can be frustrated. Let us take a look at the slide enthalpy entropy compensation is the tendency for changed interaction to produce more negative delta H naught which can be associated with more negative delta S naught usually it is observed that you gain in enthalpy, but you lose in entropy.

(Refer Slide Time: 28:07)

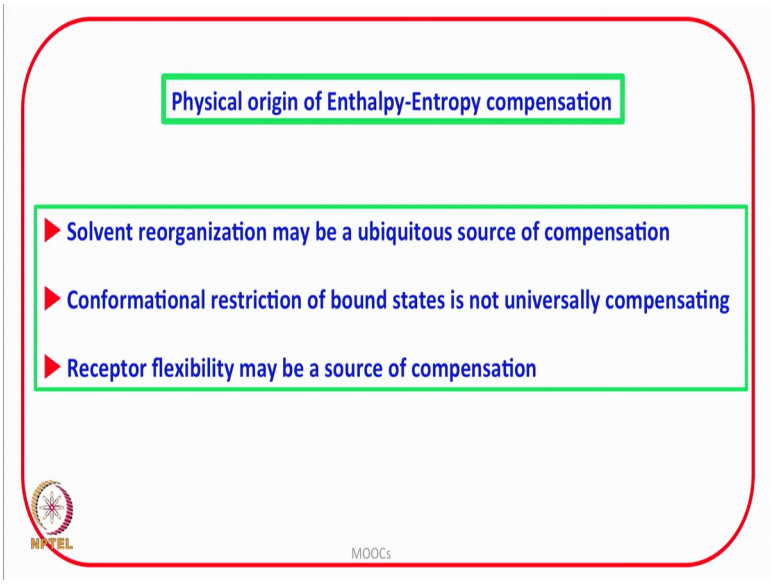


Let us take a look at the next comment increased bonding produces more delta H naught values and these tend to occur at the expense of increased order, hence more negative delta S naught. So, this is the reason for the compensation because if 2 molecules interact favorably, delta H naught will be negative, but also there is a loss in entropy.

Let us take a look at the next comment; the engineered enthalpic gains can lead to completely compensating entropic penalties thus frustrating ligand design and this is exactly the point that I was making; next comment complete compensation would mean that modifications made with the intent of improving the enthalpy of interaction such as the introduction of a hydrogen bond donor would be counterbalanced by unfavorable entropic contributions leading to nonet gain in affinity.

Now, let us take a look at physical origin of enthalpy entropy compensation based on literature review, the enthalpy entropy compensation in case of protein ligand binding, I have just discussed that negative value associated with the formation of favorable bond for polar bonds is also happening at the expense of the decrease in the disorder or increase in order the other reasons.

(Refer Slide Time: 30:19)



Which have been proposed in literature is one is solvent reorganization, may also be one of the sources for the compensation conformational restriction of bound states is not universally compensating and receptor flexibility may be a source of compensation in conclusion; enthalpy entropy compensation phenomena cannot be ignored because a

specially in pharmaceutical industries if we are not interested in improving the affinity then the contributions of delta H naught and delta S naught make a big impact in getting a more negative value of delta G naught.

So, in this lecture, what we have discussed is that how the use isothermal titration calorimetry and differential scanning calorimetry together can help us in not only describing the protein ligand binding in full details, but also in understanding how this process or what is the mechanism associated with the unfolding of the protein in the absence and presence of the ligand. We further discussed the significance of enthalpy change and entropy change in overall delta G naught and the phenomena of enthalpy entropy compensation which cannot be ignored.

We will further discuss that although thermodynamic signatures associated with the binding of a ligand with protein can be determined by isothermal titration calorimetry successfully, but when it comes to very high association or the associations which are associated with the very high values of the affinity or K, then differential scanning calorimetry becomes very important. And this we will discuss in the next lecture.

Thank you very much.