Chemical and Biological Thermodynamics: Principles to Applications

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Lecture – 49
Isothermal Titration Calorimetry (ITC) in Engineering Binding Affinity

Let us continue our discussion on the use of Isothermal Titration Calorimetry in Engineering Binding Affinity. In the previous lecture, we discussed in details about what are the various factors that can contribute to engineer, the binding affinity in a drug molecule, it could be improvements, addition or deletion of certain groups in an existing molecule or it could be a completely synthesis of a new molecule and what we discussed let us take a look at this slide.

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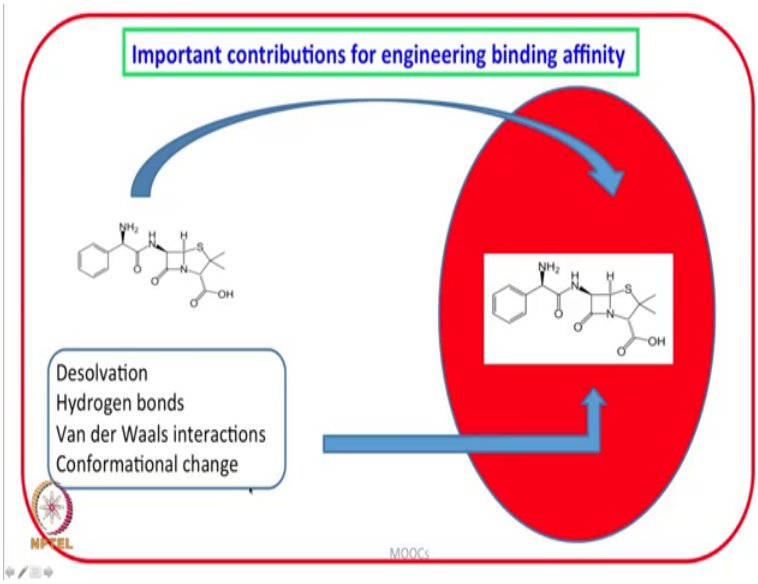

We talked in terms of this cavity. We also discussed that this molecule if it is to be placed in this cavity, then these are the various factors which we need to consider; Desolvation, Hydrogen bonding, Van der Waals interactions, Conformational change. So, when this molecule is being placed into the cavity, when it goes, it will interact with the constituents of the binding affinity. This interaction can be through hydrogen bonding, Van der Waal interaction hydrophobic interactions, but there is also an associated conformational change. Now, let us remember that hydrogen bonding will contribute negatively to delta H naught. Similarly, the other interactions like Van der Waal

interactions hydrophobic interactions will also contribute to delta H naught. Conformational change will mainly or will contribute in a big way to delta S naught because conformational change will lead to change in the order disorder of a system. When I talk about the desolvation, desolvation of a molecule means when the molecule is in aqueous environment, it is solvated whether it is water, then you call hydrated. If it is some other solvent, you say solvated, but since bio systems are mostly in aqueous solution, so we consider these water molecules.

So, the water molecules have to be removed from the immediate vicinity of the drug molecule and as well as from the binding side. When these water molecules are released, release of water molecules means you are generating entropy, but at the same time when the drug molecule goes and binds to the macro molecule, it is also possible that it will induce some conformational change in the macro molecule it may make because generally when a ligand binds a protein, it will make the protein little more strengthened, more compact because it is binding with the native state and this can also lead to change in entropy. The third possibility is that suppose I have an incoming. This is my binding side and I have an incoming drug molecule which has rotational degree of freedom when it is free in solution, but once it forms an hydrogen bond in the binding cavity or binds with whatever kind of interaction, its rotational motion is quenched. It is restricted. Initially it was able to freely rotate, but now when it is bound, it cannot further rotate. Therefore, there is a loss in entropy.

So, I have discussed at least three possible changes which can lead to change in entropy. Due to conformational change, one is where the drug binds to the macro molecule. It may either strengthen or maybe rarely weaken the conformational stability of a macro molecule and that will contribute to change in entropy. That is a conformational change. The other factor, the water molecules upon desolvation are released. That will add to the entropy and the third is if the confirmation of a molecule gets different types of restrictions upon binding, that will also contribute to change in entropy.

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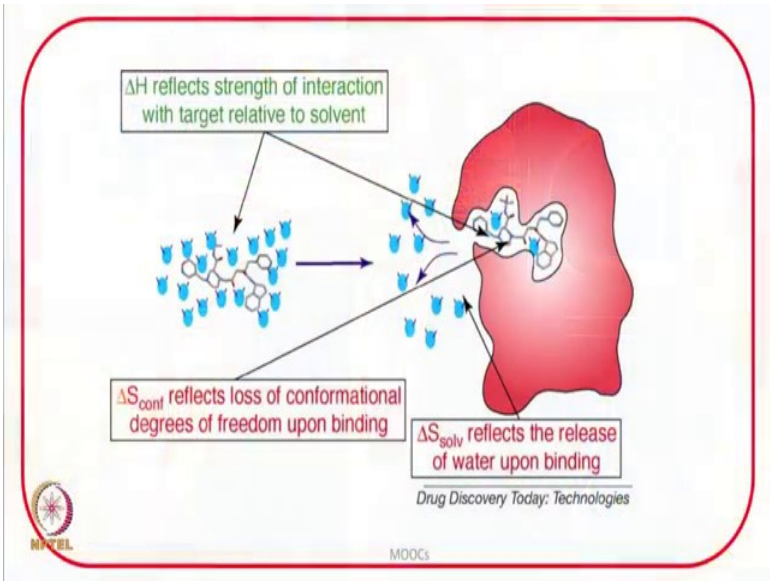

Let us take a look at the next slide. This is what I was talking about a molecule in solution will have water surrounding it and before this molecule goes and binds to the macro molecule, let us say this is the binding cavity. These water molecules will have to be removed and also, the water molecules which were earlier occupying the binding side have to be removed. Removal of water molecule requires energy. So, I can say that removal of water molecule which is called desolvation, it costs energy. There is a penalty of removal of water molecules, but at the same time if you see over here that the removal of water molecule generates entropy because the free water molecules are released. So, delta S conformational reflects loss of conformational degrees of freedom upon binding. That is another factor. I was saying that if there is for example, rotational degree of freedom in this molecule that will go bind and loose that degree of freedom and delta S solvation reflects the release of water upon binding. So, there are two phenomena that we need to consider over here. One is the removal of water molecule that is desolvation and energy is required, and the release of water molecules that is giving back in terms of increase in entropy.

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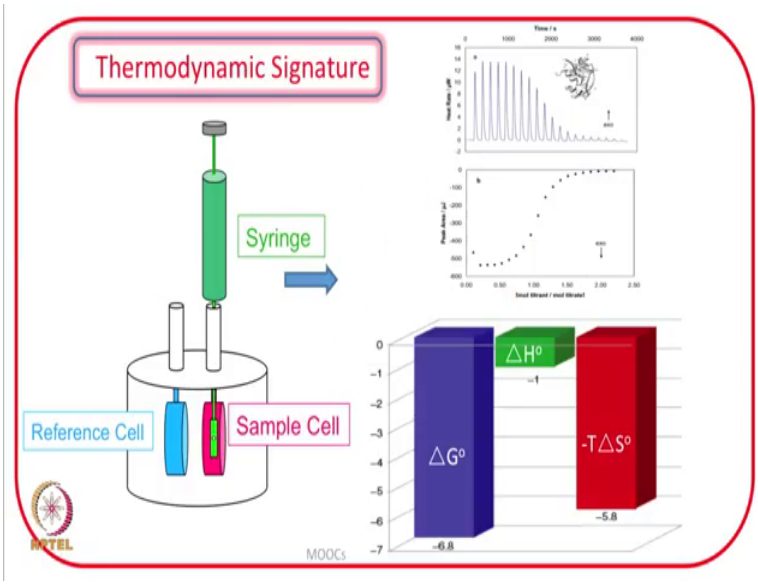

Now, once again if I highlight the slide on thermodynamic signature delta H naught and delta S naught are the two contributions to delta G naught. Overall affinity is reflected in terms of delta G naught which can be discussed either in terms of delta H naught or delta S naught or both. Let us go back to our discussion. We were talking about desolvation.

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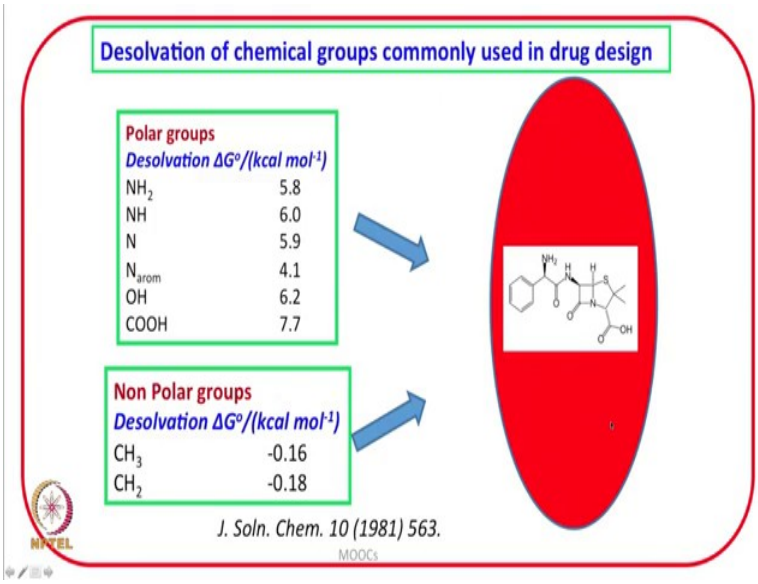

Now, let us discuss first a little bit about the desolvation and binding. We just discussed that the desolvation of a molecule water molecules have to be removed and therefore, it will cost. There is an enthalpy cost because energy is required to remove the water

molecule. So, this desolvated moiety molecule will go and fit into the binding cavity. When it goes and sits in the binding cavity, it will form new bonds and those new bonds will give energy because if this is exothermic interaction, then it will contribute to the negative value of delta H and at the same time, the conformational change, overall conformational change, desolvation change contributions to entropy have to be accounted for. However, if we concentrate for the time being on delta H naught, it is positive for desolvation and delta H naught is negative if the molecule after entering the binding site establishes polar pods and if the reward in terms of exothermicity is more than the endothermic desolvation process, that contributes to the negative value of delta G naught.

Let us take a look at the slide. This slide lists the values of desolvation in terms of kilocalorie per mole for various groups. These are the various groups, chemical groups which are commonly used in drug design desolvation delta G naught for NH2 is 5.8 kilocalorie per mole, NH is 6, N is 5.9. You see all these polar groups which are listed over here will interact with water and therefore, if water is to be removed from these polar group, it will cost in terms of delta G naught. You see it is all positive. That means, it is expensive. So, therefore, in order for this delta g naught to become negative when these molecules or these groups enter the cavity, they should make sufficient polar bonds, so that overall delta G naught can be turned back to negative.

Now, on the other hand if we look at the non-polar groups, the non+polar groups have a minimum delta G naught. Actually it is negative because we know that RA that the non-polar groups, hydrophobic groups, anyway they do not like water. They were like to go get out of water and that is why the desolvation free energy delta G naught for hydrophobic groups is negative. So, that means if we just consider these polar groups, when these polar groups a compound with sufficiently polar group is desolvated and put into the binding cavity, it must establish sufficient polar bond, so that the overall process can be changed to spontaneous process and non-polar groups anyway will go and bind into the cavity even if it does not establish new contacts because delta G naught, anyway delta G naught for desolvation is negative. The hydrophobic groups will anyway like to get out of water and you know this is the precise reason that the prevalent approaches have led to the synthesis of more hydrophobic compounds because people have been able to achieve good affinity by playing with the hydrophobicity of the molecule

whereas, the general trends in the literature suggest that it is the polar character, it is the solubility which has to be the main target.

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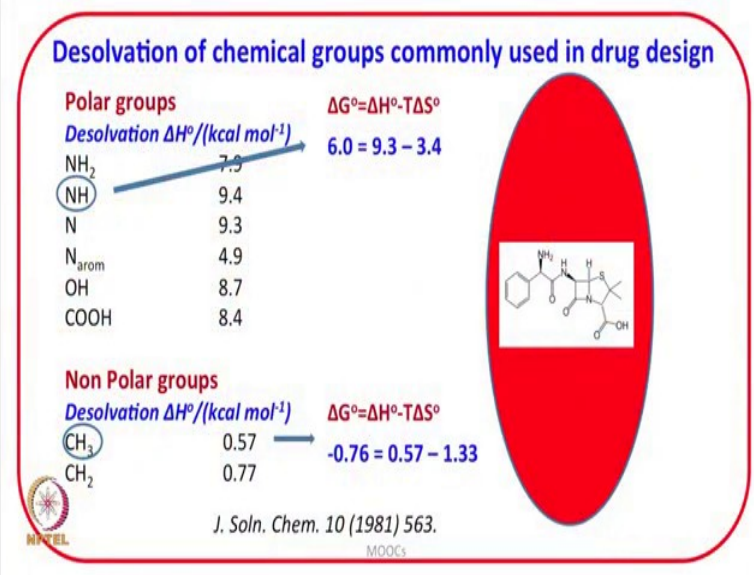

Let us continue this discussion. Now, let us talk about the enthalpic contribution of the same groups to delta G naught. If we look at here, delta G naught is equal to delta H naught minus T delta S naught and we in the previous table for NH, we noted that delta G naught was 6. Of course, it is positive, but you see delta H naught is 9.3 and delta S naught is 3.4. The desolvation of NH group is largely endothermic over here and you see this positive contribution is even more than the positive value of delta G naught and delta S naught is also positive. Actually minus T deltas notice negative delta S naught is also positive, but there is a huge enthalpic cost, there is a penalty call as a enthalpic penalty in engineering binding affinity because binding affinity which is reflected in terms of negative value of delta G naught should be assisted by the negative value of delta H naught. So, if delta H naught is positive, it is causing a penalty, an enthalpic penalty in engineering binding affinity and if we take a look at here, the desolvation of non-polar group.

Delta G naught anyway is small minus 0.76 and delta H naught you see 0.57 which is quite small and delta S naught is also very small positive and here again this justifies that since the desolvation of the non-polar groups is very small, it has been easier to improve

the binding affinity engineering by engineer binding affinity or in other words, make delta G naught more negative by working on the hydrophobic or non-polar groups.

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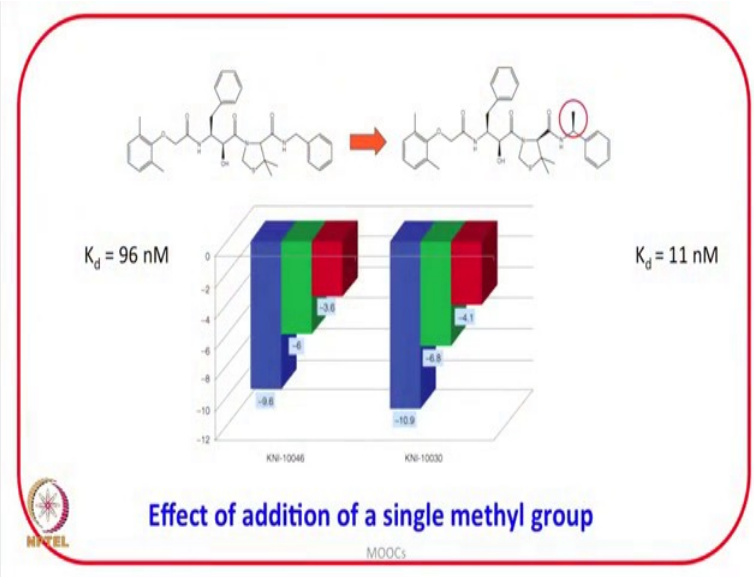

Let us take the example, next example for a better clarity. This is again an example with HIV 1 protease inhibitor KNI 10046 and its conversion to KNI 10030, the difference between these two molecules is only substitution of a methyl group. The binding of this molecule is associated with KD value of 96 nanomolar. One can write KA which is 1 over 96 nano molar in inverse and the binding of this is 11 nanomolar. The lower the value of KD, the higher is the binding affinity. That means, the substitution or addition of a methyl group has increased the affinity by almost a factor of 9. KD has gone down by a factor of 9 means KA has gone up approximately by a factor of 9, but let us examine what has gone here. Although the affinity has gone up by a factor of about 9, delta G naught accordingly has become more negative from 9 minus 9.6 to minus 10.9 and let us take a look at the constituents of this delta G naught delta H naught green delta H naught. There is a marginal increase from minus 6 to minus 6.8 and there is a marginal increase in entropy also. This is minus T delta S. Therefore, I am saying marginal increase in entropy from 3.6 to 4.1 k cal.

A good improvement in the affinity by almost an order of 9, a factor of 9 and which has been achieved by a little bit increase in enthalpy and a little bit increase in entropy and once again emphasizing that this is precisely the reason that since the hydrophobic

groups do not cost in terms of desolvation, the affinity can be easily improved by introducing some hydrophobic groups into the molecule and what you have seen here in this slide that the effect of addition of a single methyl group is increasing the affinity almost by a factor of 9.

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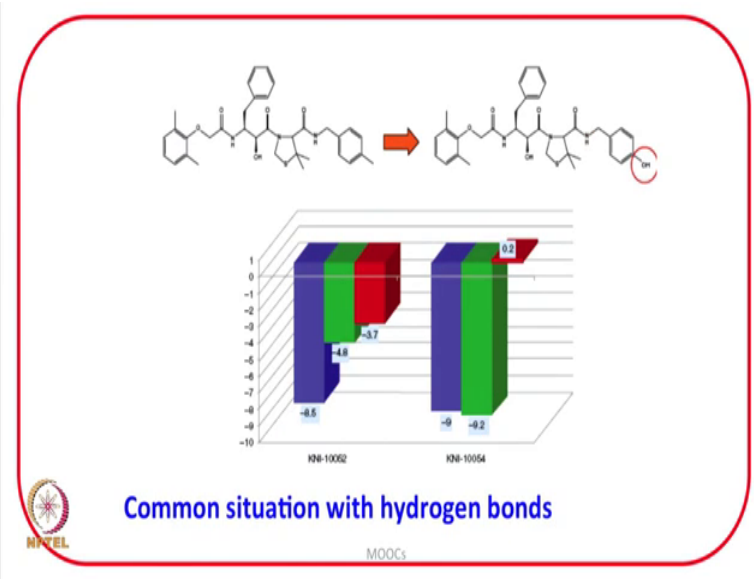

Now, let us take a look at what is the effect of addition of a polar group, common situation with hydrogen bonds. The difference between these two is only the methyl group has been replaced by hydroxyl group. Here hydroxyl group which is a polar group can establish hydrogen bonds when introduced into the binding cavity and here you see, the value has gone up delta G naught value has gone up from minus 8.5 to minus 9 which will correspond to binding affinity going up by a factor of 2 only if you calculate delta G naught negative changing from minus 8.5 to minus 9. If you convert this into binding affinity, it will turn out that it is only changing by a factor of 2 increasing by a factor of 2.

However if you see what has happened to enthalpy, a large change minus 4.8 kilo calorie per mole to minus 9.2 kilocalorie per mole, almost doubling the exothermic character of the binding and if you take a look at the entropy, the entropy minus 3.7 actually it is changing to 0.2. Although there is a large increase in the negative value of delta H, it is being compensated by delta S. A good increase in enthalpy exothermic content and a good decrease in entropy and this is what is exactly called enthalpy entropy

compensation, but nevertheless if we are interested in achieving more exothermicity in the binding, this example suggests that the way to go is to introduce more polar character into the molecule, but at the same time keep in mind that if we introduce more polar character into the molecule, if we introduce more polar groups into the molecule, even though in terms of interactions if it establishes hydrogen bonds or polar interactions, it will contribute to negative delta H. It will also cost in terms of desolvation.

Therefore, it is very important to keep in mind that the polar groups are not introduced at a wrong position. At a wrong position means suppose if the polar group is inside which does not establish a contact with the binding side, then the purpose is lost. Desolvation should be compensated by the intermolecular interactions or the interaction of these polar groups with the constituents of the binding side. Therefore, it becomes very important to introduce the polar groups at a right position, so that when this molecule goes and binds in the binding cavity, it is able to establish the polar interaction and contribute to the negative value of delta G naught and also, which is through contributing to the negative value of delta H naught.

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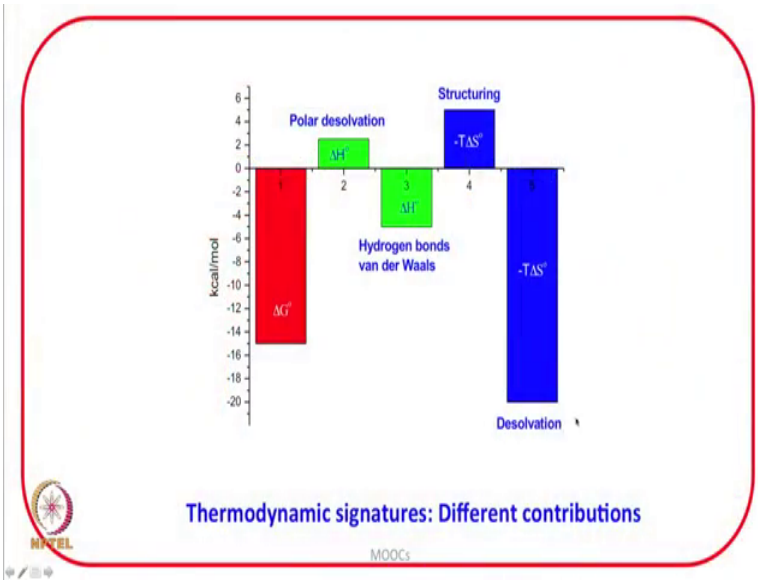

Now, let us take a look at the general guidelines which can emerge from this kind of discussion. This slide talks about thermodynamic signature and what are the different contributions. Of course, delta G naught has to be negative for the binding to take place. Delta G naught has to be negative, polar desolvation will contribute positive. It is costly,

it is expensive and that is why it is shown towards positive side. Polar desolvation puts a penalty hydrogen bonds and Van der Waals interactions will contribute negative. That is a favorable contribution, however as I just discussed that it must also be kept in mind that we do not introduce polar group at a wrong position which will also increase the positive value. So, hydrogen bonds Van der Waals interactions will contribute in a negative manner. Negative manner here is in good sense, that is it will contribute to more exothermicity structuring in the molecule will also impose a penalty and that is an entropic penalty because delta S naught is negative and desolvation although it is expensive in terms of enthalpy, but desolvation releases water molecules and therefore, it is contributing in making the delta G naught more negative. So, these various thermodynamic signatures help us in understanding that what kind of modifications can be done in a drug molecule, so that we can optimize affinity. We can achieve a better affinity of the molecule. These kind of data do give some general guidelines.

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Let us take a look at some of such guidelines which are, Enthalpic Hits should be preferred for optimization. This is what our discussion has led to that enthalpic hits means more exothermicity in the binding and that more exothermicity binding can be achieved by introducing polar groups which should establish strong hydrogen bond with the structured groups in the target. The next guideline which emerged from this kind of discussion is to eliminate buried polar groups that do not establish hydrogen bonds. Non-polar groups should fit tightly in the binding pocket cavities and the losses in

conformational degrees of freedom should be minimized. So, all these guidelines are either talking about delta H naught or talking about delta S naught and finally, it comes to getting a more negative value of delta G naught which will be obtained only if we have more exothermicity in the binding and the losses in degree of freedom or the confirmation losses in entropy due to conformational change that should be minimized.

So, what we have discussed in this lecture is that delta H naught and delta S naught are very important contributions to delta G naught which guide us towards rational drug design because if we are interested in getting more exothermicity in the binding, then addition of polar groups is more important. The drug molecule should have more polar groups and the more polar group should be at a position which should give back the energy, that is it will establish polar context with the binding side and lead to more exothermicity of the interaction and therefore, introducing the various groups whether a polar group should be introduced, but at what position it should be introduced, these kind of data give very good guidelines. That is why the thermodynamic signatures obtained over a large period of time gives us guidelines towards rational drug design and towards target oriented synthesis. We will take some more examples and make it more clearer in the future lectures.

Thank you very much.