Bio-Physical Chemistry
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Lecture – 13
Hydrophobic Effect (Contd.)

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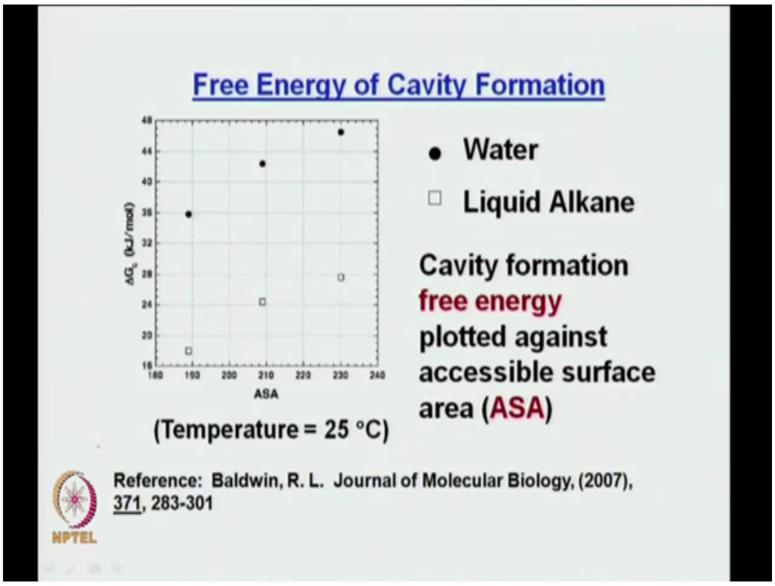

Free Energy of Cavity Formation

● Water

□ Liquid Alkane

Cavity formation free energy plotted against accessible surface area (ASA)

(Temperature = 25 °C)

Reference: Baldwin, R. L. Journal of Molecular Biology, (2007), 371, 283-301

So, we will carry on with the discussion on the Hydrophobic Effect and what we were I will just give a brief recap. So, you know last time we talked about this Free Energy of Cavity Formation and the reason we talked about cavity is we were looking at when we are looking at your putting a hydrophobic solute in water, you know how would you put it.

So, there are two ways right two ways means two steps, the first one is you actually form a cavity. So, when you form a cavity what happens is you take this solute you put it in water you are forming a cavity, that means you are moving the water molecules apart. And the next is after you form a cavity the solid molecules reorient in response to the solid molecule you have put in ok. So, this one was the free energy of cavity formation and said that your free energy of cavity formation is much higher in water than your liquid alkanes ok.

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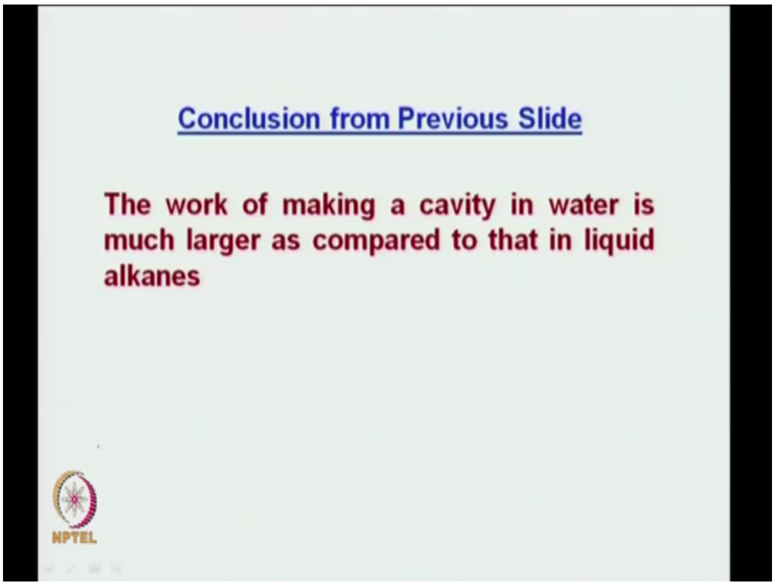

Conclusion from Previous Slide

The work of making a cavity in water is much larger as compared to that in liquid alkanes

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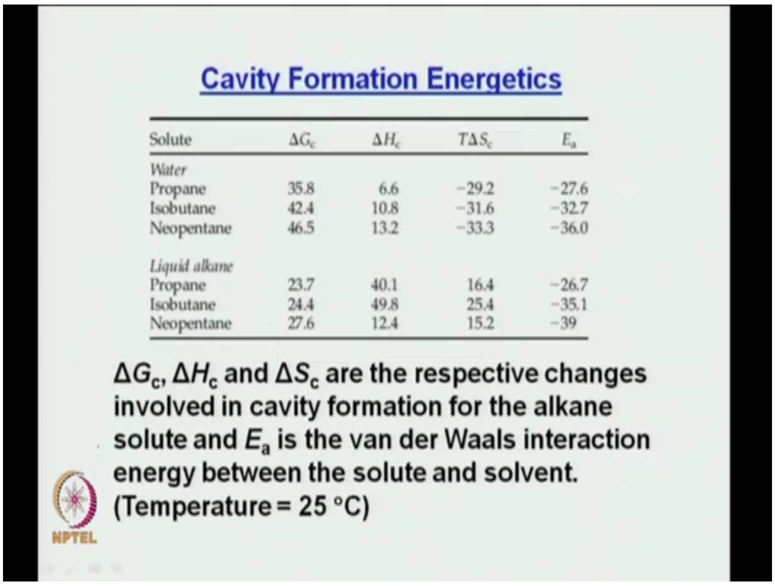

Cavity Formation Energetics

Solute	ΔG_c	ΔH_c	$T\Delta S_c$	E_a
Water				
Propane	35.8	6.6	−29.2	−27.6
Isobutane	42.4	10.8	−31.6	−32.7
Neopentane	46.5	13.2	−33.3	−36.0
Liquid alkane				
Propane	23.7	40.1	16.4	−26.7
Isobutane	24.4	49.8	25.4	−35.1
Neopentane	27.6	12.4	15.2	−39

ΔG_c, ΔH_c and ΔS_c are the respective changes involved in cavity formation for the alkane solute and E_a is the van der Waals interaction energy between the solute and solvent. (Temperature = 25 °C)

So, this is what the conclusion was and the cavity formation energetics showed a very unique thing, what it showed was that your energy of interaction which is your van der Waals Ea was typically similar whether you consider water or a neat alkane. But what was really the driving force behind you know the effects you see was this change in entropy which is negative in case of water, but positive in case of your alkanes.

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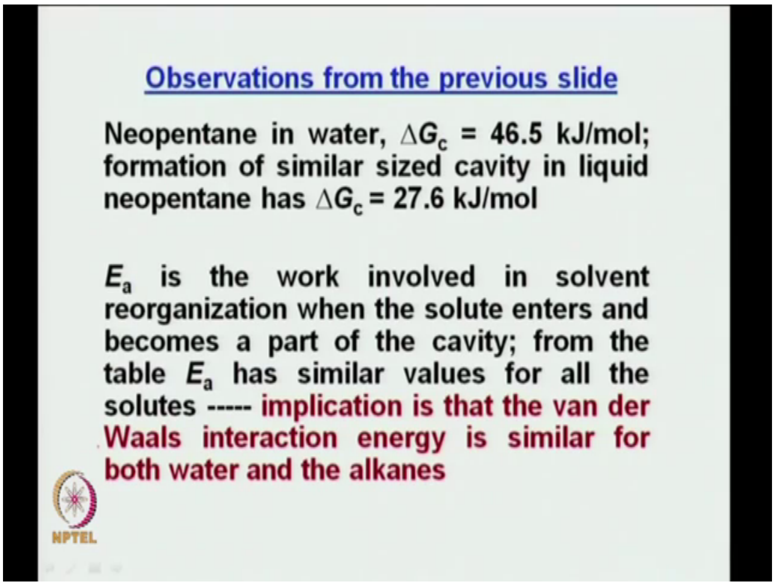

Observations from the previous slide

Neopentane in water, ΔG_c = 46.5 kJ/mol; formation of similar sized cavity in liquid neopentane has ΔG_c = 27.6 kJ/mol

E_a is the work involved in solvent reorganization when the solute enters and becomes a part of the cavity; from the table E_a has similar values for all the solutes ----- implication is that the van der Waals interaction energy is similar for both water and the alkanes

So, this was the observations and the fact that Ea you know was the same it just implied that the van der Waals interaction energy is similar for both water and the alkanes right.

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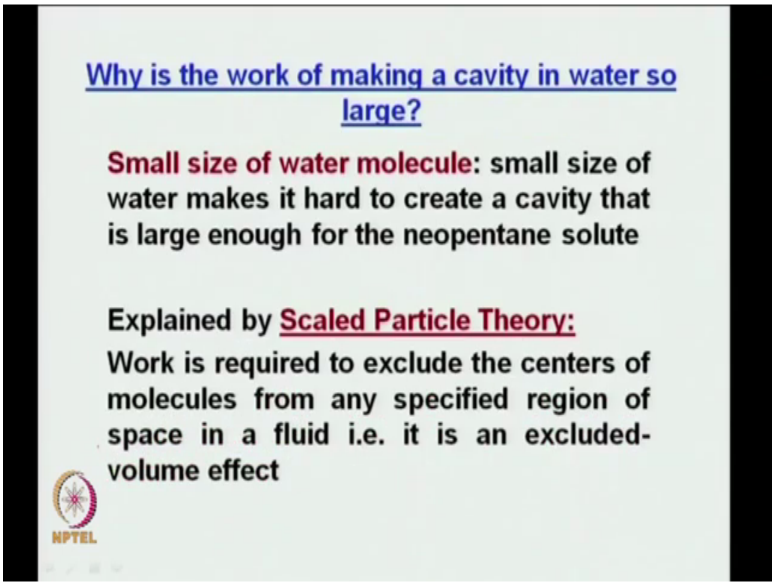

Why is the work of making a cavity in water so large?

Small size of water molecule: small size of water makes it hard to create a cavity that is large enough for the neopentane solute

Explained by **Scaled Particle Theory:**

Work is required to exclude the centers of molecules from any specified region of space in a fluid i.e. it is an excluded-volume effect

And then we were talking about why you know is the work of making a cavity in water so large. So, there were two reasons one was the small size of the water molecule we told something about you know scale particle theory, not that you have to worry about what scale scaled particle theory is.

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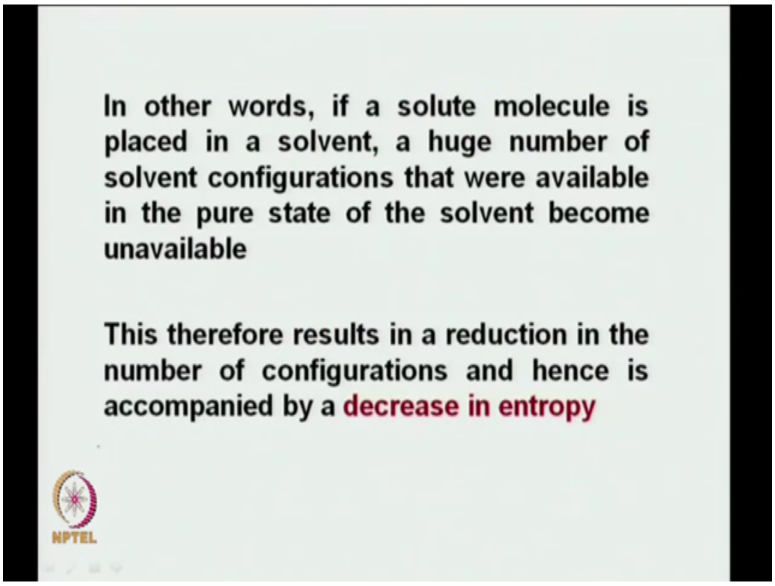

In other words, if a solute molecule is placed in a solvent, a huge number of solvent configurations that were available in the pure state of the solvent become unavailable

This therefore results in a reduction in the number of configurations and hence is accompanied by a decrease in entropy

But the essence is that when you put a solid molecule in water then whatever configurations water had before, because of the solid molecule the water the other water molecules cannot take that same place where the solid molecule is. That means, the solid molecule the hydrophobic solid molecule or this organic non-polar solid molecule is excluding the water molecules from the space it is occupying.

Hence the water molecules undergo a lot of reduction in configurations which is essential entropy and hence the entropy decreases that is why you see a decrease in entropy right.

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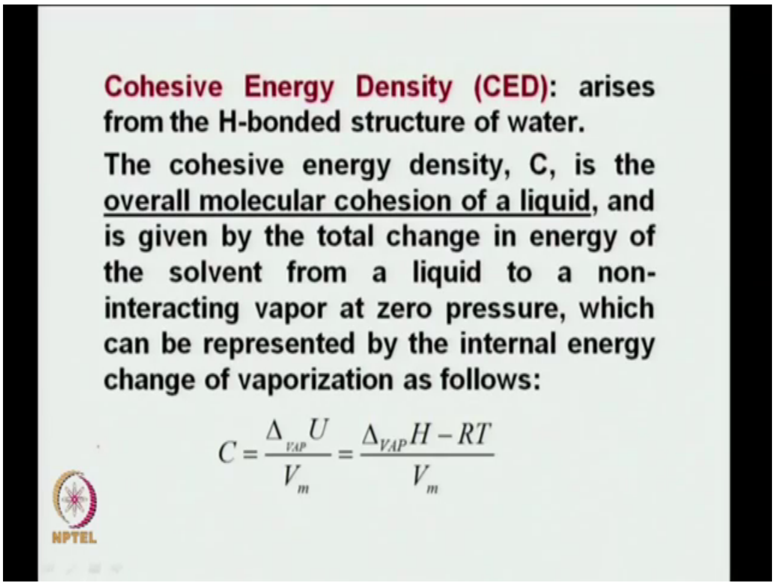

Cohesive Energy Density (CED): arises from the H-bonded structure of water.

The cohesive energy density, C, is the overall molecular cohesion of a liquid, and is given by the total change in energy of the solvent from a liquid to a non-interacting vapor at zero pressure, which can be represented by the internal energy change of vaporization as follows:

$$C = \frac{\Delta_{VAP} U}{V_m} = \frac{\Delta_{VAP} H - RT}{V_m}$$

There was also another thing which was more enthalpy which is the cohesive energy density and the cohesive energy density is kind of a cohesive force which keeps all the water molecules together and this cohesive energy density is by far the largest for water.

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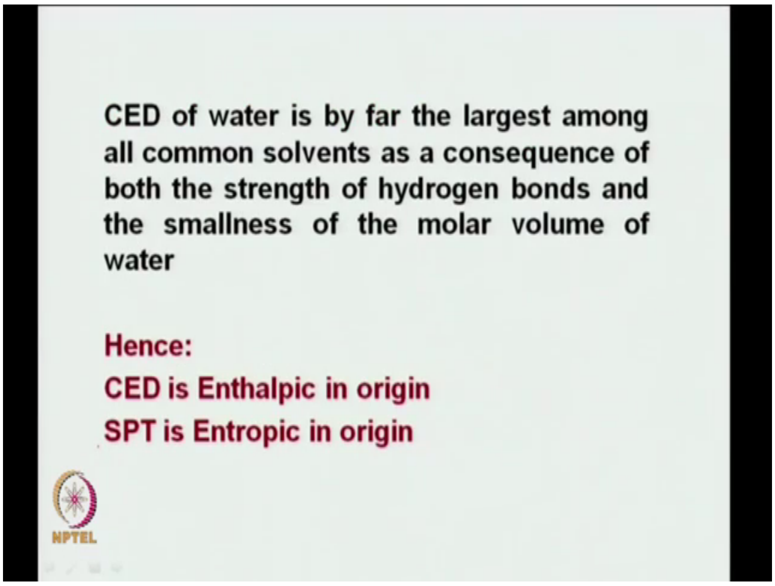

CED of water is by far the largest among all common solvents as a consequence of both the strength of hydrogen bonds and the smallness of the molar volume of water

Hence:

CED is Enthalpic in origin

SPT is Entropic in origin

Thus there are two barriers to putting in a non polar solid in water one is the cavity formation which was like this SPT the scaled particle theory entropic in origin and the other one is your enthalpic in origin which is your cohesive energy density right.

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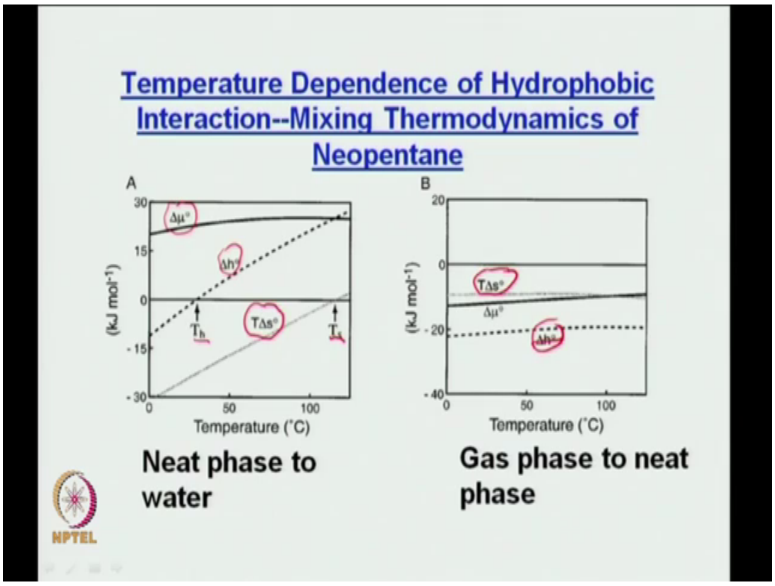

Temperature Dependence of Hydrophobic Interaction--Mixing Thermodynamics of Neopentane

Neat phase to water

Gas phase to neat phase

This is and this was the last one we had actually started to discuss. So, I will again you know talk about this a little more detail. So, let us look at these two panels now figure panels now. So, on the left you have something which is called a neat phase to water, you know this is what the panel is referring to. Now, what does it mean? What it means is again we are doing two experiments, the experiments are like this we are taking neo pentane we have a neo pentane neat liquid neo pentane right.

We are taking the neopent, a neopentane molecule out from neat liquid neopentane taking it out and putting it in water, that is what the neat phase to water means. That means, a neat neo pentane phase to a neopentane molecule being put in water that is at the neat phase to water and the right side since Gas phase to neat phase it means that you take a neopentane molecule, which is in the gas phase you take it and put it in.

Student: Liquid.

Liquid neopentane which is the neat phase again right. So, this is why it is a mixing thermodynamics; one is you are mixing in water the other one is you are mixing neopentane in neopentane. But from gas to neat state right now focusing on the left hand side panel let us look at this, you see on the top is your delta mu right this is delta mu 0. When you are looking at delta mu 0, what happens is you see how delta mu 0 moves as a function of temperature.

It kind of starts from here then goes on increasing and finally later you see there is a certain decrease in trend. But you do not see that that is where the curve ends. Look at what happens for delta h 0, for delta h 0 you see this is how the line goes, for delta h 0 starting from here it goes like this.

It has a huge slope right it is pretty steep, the same thing happens for delta h 0 right. This also starts from here and goes with the kind of a steep slope. But before going farther there are two more important things one is you look at these temperatures T h and Ts. What does this refer to? The delta h 0 line which is your enthalpy change; which is enthalpy change crosses the 0 energy at Th; that means, T h refers to a temperature where your change in enthalpy delta h is what? 0.

So now, you think about this if your delta h is 0 remember what delta g is or delta mu 0 is it is equal to delta h 0 minus T delta h 0. But at T h you have already said what at T h you have already said that your delta h 0 is equal to 0. Then what is your delta g 0 essentially equal to?

Student: (Refer Time: 06:17).

Minus T delta h 0, hence at this temperature which is see this T h is at a lower temperature right close to room temperature 25 to 30. That means, at the room temperature whatever change in free energy you see is not enthalpic in origin; what is it?

Student: (Refer Time: 06:32)

It is entropic in origin because a delta h 0 is equal to 0. Now, you slowly move over you see this delta mu 0 is slowly curving out, it slowly move over you go to a high temperature side. As the higher temperature side you see delta h 0 is far over the 0 line, but what happens to the T delta s curve? You see this T s this T delta s 0 curve right. Now, crosses the 0 line of energy at a certain point which is referred to as Ts. What does this T s mean therefore? So, T s means at this point your delta h 0 is equal to 0.

Student: 0.

That means, this change in entropy is 0. So, at the room temperature your change in free energy is primarily what? Entropic. When you go to high temperature this is high temperature this is close to 110 and 112 your delta s 0 is equal to 0. So, delta g is delta g 0 is equal to what?

Student: Delta

Delta h 0 that means at the higher temperature side your free energy change is primarily what?

Student: (Refer Time: 07:30).

Enthaphic.

Student: Enthaphic.

Right it is not entropic is it clear. So, this is the take home point of this figure or this diagram. Now, take the same thing and look at you know keeping the same thing in mind look at what do you have on the right hand side, the right side again is a gaseous neo pentane molecule when put in neat liquid neopentane.

What do you see? For one thing your delta mu 0 is negative right. That means, it is a favorable process right you are dissolving neo pentane gaseous in liquid neopentane; obviously, it is a favorable process. But more so look at the slopes of delta h 0 and T delta h 0, are those are those slopes as steep as you had in the left panel. See the slopes of delta h 0 in this case the slopes of delta h 0 and the slope of T delta h 0.

The slopes are very small; the slopes are very small. So one end on one hand where in water the slopes of these delta h 0 and delta s 0 are very steep. But the same thing when you take it in a neat phase neat neopentane the slopes are actually very small.

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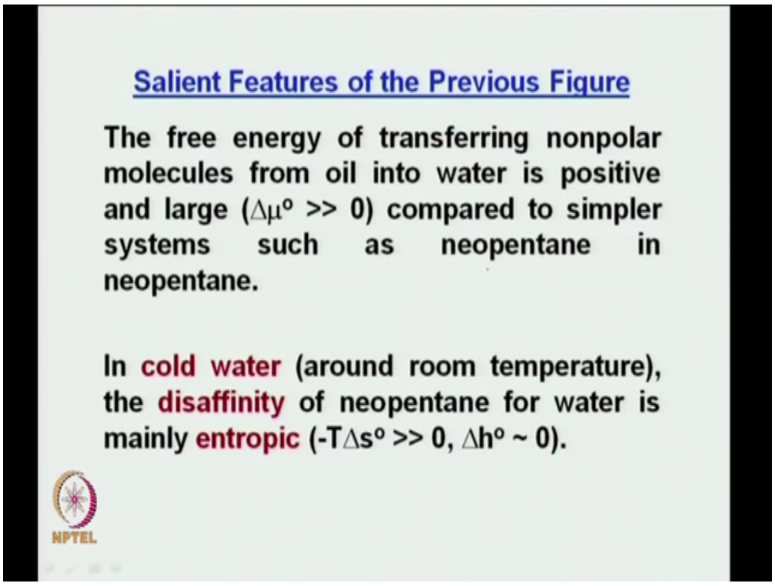

Salient Features of the Previous Figure

The free energy of transferring nonpolar molecules from oil into water is positive and large ($\Delta\mu^o >> 0$) compared to simpler systems such as neopentane in neopentane.

In cold water (around room temperature), the disaffinity of neopentane for water is mainly entropic ($-T\Delta s^o >> 0$, $\Delta h^o \sim 0$).

Now what do these slopes refer to? These slopes refer to a very important thing ok. But before I tell you what the slopes refer to just quickly go through the salient features of the previous figures. So, the free energy of transferring non polar molecules from oil into water is positive and large right, that is what your delta mu 0 was telling you; positive and large for the left hand side or the left figure.

Compared to simpler systems such as neopentane in neopentane right that is what we saw. In cold water around room temperature the disaffinity of neopentane for water is mainly entropic right that is what we saw, in cold water T h is equal to 0 delta h 0 is I mean sorry T h means delta h is 0 is equal to 0. At T h delta h 0 is equal to 0, that means delta g 0 is equal to minus T delta h 0. Hence in cold water your disaffinity is primarily entropic in origin.

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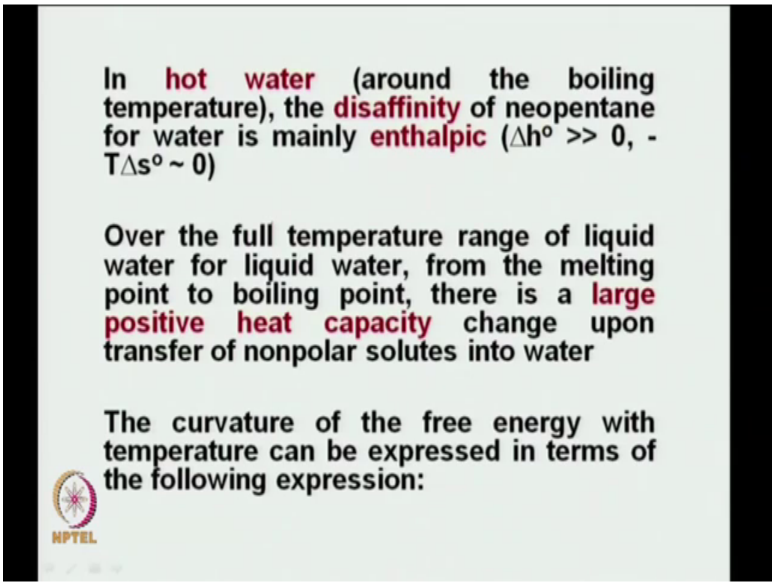

In hot water (around the boiling temperature), the disaffinity of neopentane for water is mainly enthalpic ($\Delta h^o >> 0$, $-T\Delta s^o \sim 0$)

Over the full temperature range of liquid water for liquid water, from the melting point to boiling point, there is a large positive heat capacity change upon transfer of nonpolar solutes into water

The curvature of the free energy with temperature can be expressed in terms of the following expression:

Now, you move up in hot water the disaffinity now is primarily enthalpic in origin, because the T s your delta h 0 is equal to 0. See you delta this is a Gibbs Helmholtz equation right, delta h minus T delta s delta g equal to. See how when you go from one end of the temperature spectrum to the other end of the temperature spectrum.

You know within this short range, how your dependence on the parameters changes and this is not what I am telling you these are actual experience where how people have done it and they have been actually be been able to figure this out right. So, over the full temperature range of liquid water and for liquid water rather from the melting point to boiling point there is a large positive heat capacity change and how do you know that? How do you know there is a large positive heat capacity change? We will come to that in the next slide.

And next point the curvature of the free energy with temperature can be expressed in terms of the following expressions.

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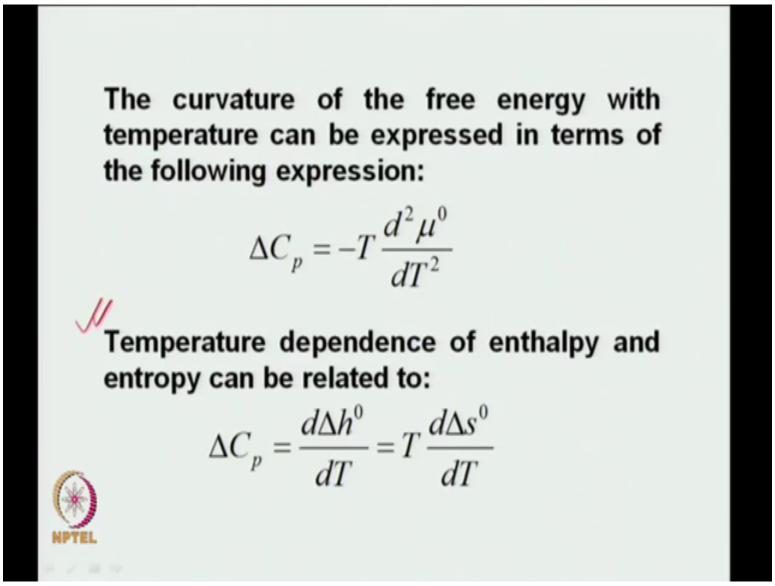

The curvature of the free energy with temperature can be expressed in terms of the following expression:

$$\Delta C_p = -T\frac{d^2\mu^0}{dT^2}$$

Temperature dependence of enthalpy and entropy can be related to:

$$\Delta C_p = \frac{d\Delta h^0}{dT} = T\frac{d\Delta s^0}{dT}$$

What is expression? The delta C p this can be derived is minus T d 2 mu 0 over dT square. So, it is a you know curvature is always given by the second derivative. If you would remember your quantum mechanics; if you remember your quantum mechanics remember your harmonic oscillator potential half kx squared and what was your k equal to? Your k was yes k was your force constant where it was equal to d 2 v over dx square. Where v is the potential and d 2 v over dx square is essentially what? The curvature of your curve of your well. The same thing is happening here.

So, here the change in heat capacity signifies or gives you an idea about the curvature of your free energy surface ok, which is in this case delta mu 0. Not only that look at this one look at so this is also very important this part, the temperature dependence of enthalpy and entropy can be related to delta C p. Where delta C p is equal to d of delta h 0 over dT you know that or is equal to T delta h 0 d of T right. Now if I am doing T delta h 0 over d of T, what is actually I am what is the actual thing I am finding?

Student: Slope.

It is just I am finding the slope remember the delta h 0 line. So, the slope of the delta h 0 line, so if you go back the slope of this delta h 0 line; the slope of this delta h 0 line is given by what delta C p. And because the slope is so steep which immediately tells you that the delta C p also is very high, because the slope is steep. Similarly on the right side on the right figure because the slope is so small, what does it mean? Your delta C p is small as simple as that right.

Now the same thing happens for the entropy curve for d del h 0 over d of T is equal to del of C p over T and hence here also the heat capacity figures in the slope of the change in entropy with temperature. So what it means is, if you would ever think about if you would ever think about putting a hydrophobic molecule like neopentane in water, you would always be getting the slopes and the reason you get the slopes is because you always undergo a huge change in what? Heat capacity.

So, that is another take home point. It is actually the heat capacity change which gives rise to the steep slopes, you see in case of neo pentane for water or neo pentane being dissolved in water. But, for the other case obviously the slopes are very small, that means delta C p or the change in heat capacity is small right.

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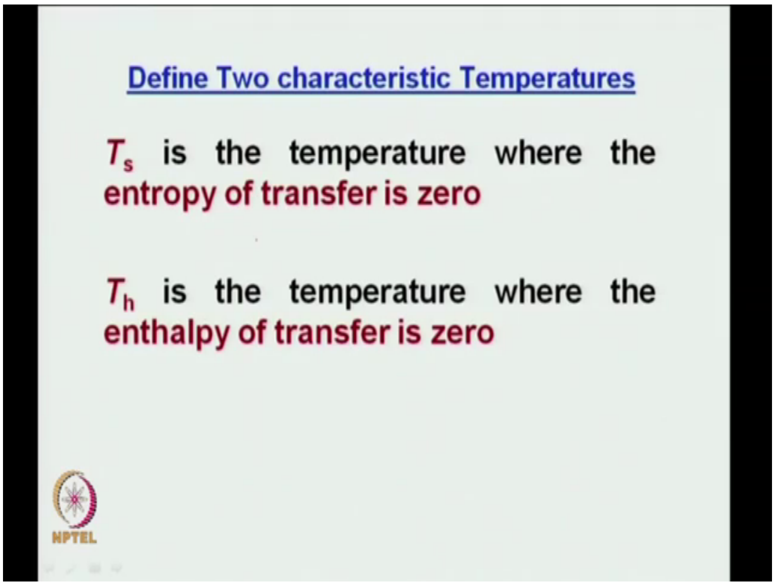

Define Two characteristic Temperatures

T_s is the temperature where the entropy of transfer is zero

T_h is the temperature where the enthalpy of transfer is zero

So, we have already defined these two characteristics temperatures one is T s, it is a temperature where the entropy of transfer is zero and T h is the temperature where enthalpy of transfer is zero.

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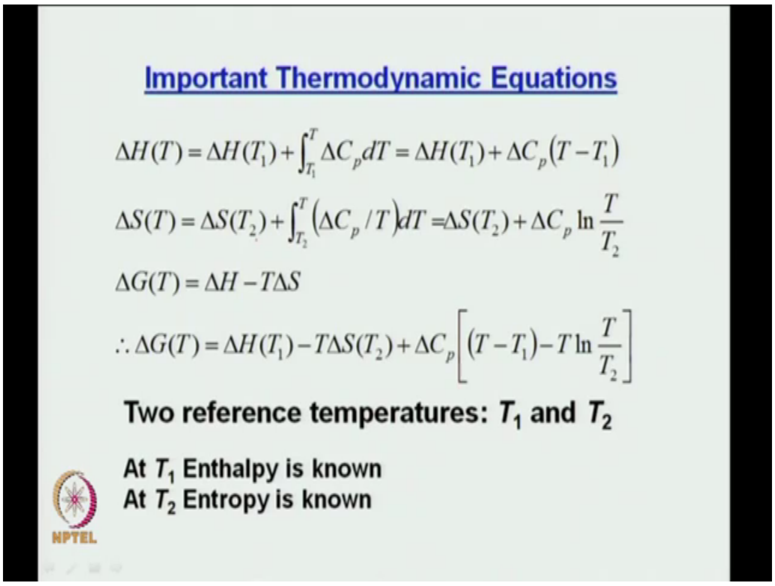

Important Thermodynamic Equations

$$\Delta H(T) = \Delta H(T_1) + \int_{T_1}^{T} \Delta C_p dT = \Delta H(T_1) + \Delta C_p (T - T_1)$$

$$\Delta S(T) = \Delta S(T_2) + \int_{T_2}^{T} (\Delta C_p / T) dT = \Delta S(T_2) + \Delta C_p \ln \frac{T}{T_2}$$

$$\Delta G(T) = \Delta H - T\Delta S$$

$$\therefore \Delta G(T) = \Delta H(T_1) - T\Delta S(T_2) + \Delta C_p \left[(T - T_1) - T \ln \frac{T}{T_2} \right]$$

Two reference temperatures: T_1 and T_2

At T_1 Enthalpy is known
At T_2 Entropy is known

Now, if you would be doing some you know quick thermodynamics.

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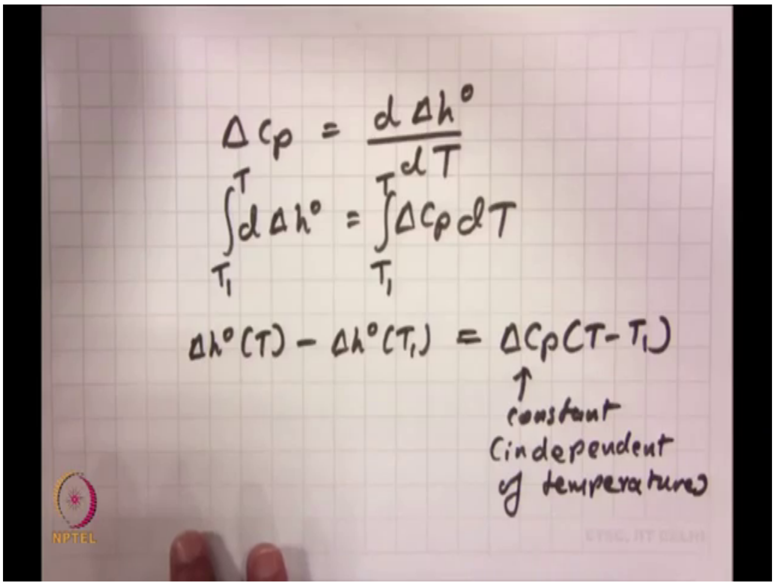

$$\Delta c_p = \frac{d\Delta h^\circ}{dT}$$

$$\int_{T_1}^{T} d\Delta h^\circ = \int_{T_1}^{T} \Delta c_p \, dT$$

$$\Delta h^\circ(T) - \Delta h^\circ(T_1) = \underset{\substack{\uparrow \\ \text{constant} \\ \text{(independent} \\ \text{of temperature)}}}{\Delta c_p}(T - T_1)$$

So, if you go back to this slide and then what I can write is I have delta C p is equal to d delta h 0 over d of T right, I have this. So, what I can now do is I can say that d delta h 0 is equal to del of C p d of T and then I integrate. I integrate between two temperatures say the lower temperature is T 1 and the higher temperature is a certain temperature T a; certain temperature T right.

And if I now expand this so what should I get I should be getting delta h 0 at a certain temperature T minus delta h 0 at a certain temperature T 1 is equal to; is equal to delta Cp, then I can write T minus T 1 what is the assumption here when I do this?

Student: (Refer Time: 14:58) T is (Refer Time: 14:59).

Right that means delta C p is.

Student: (Refer Time: 15:04).

Constant and it is as you said independent of temperature. And specially for protein folding the range of temperature between which we study actually the proteins folding unfolding, you know this assumption is not a bad assumption right.

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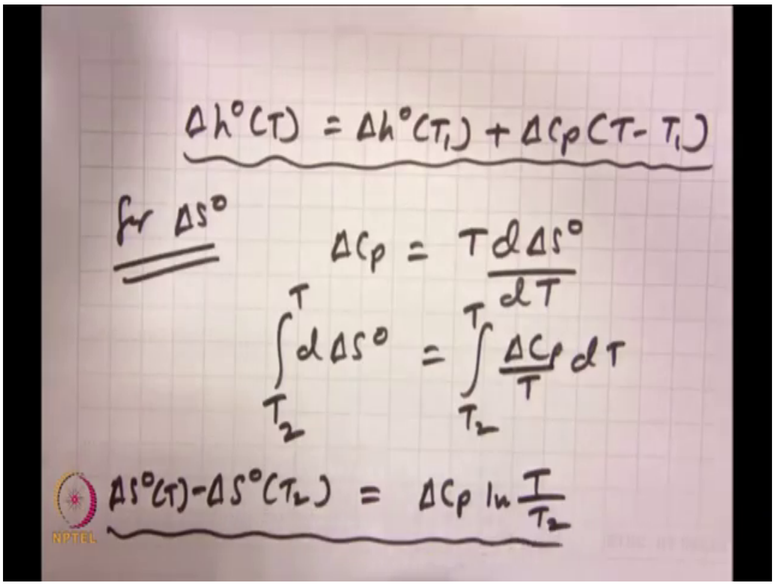

$$\underbrace{\Delta h^\circ(T) = \Delta h^\circ(T_1) + \Delta C_p(T - T_1)}$$

for ΔS°

$$\Delta C_p = T\frac{d\Delta S^\circ}{dT}$$

$$\int_{T_2}^{T} d\Delta S^\circ = \int_{T_2}^{T} \frac{\Delta C_p}{T}\, dT$$

$$\underbrace{\Delta S^\circ(T) - \Delta S^\circ(T_2) = \Delta C_p \ln\frac{T}{T_2}}$$

So, having said this we can reframe; this we can reframe this or we can rewrite this as delta h 0 T is equal to delta h 0 T 1 plus delta C p T minus T 1 ok. Now, same thing can be done with delta s. So, let us do it for delta s. So, what for delta s we will have is say for delta h 0, if I do it for delta h 0 I should be having delta C p is equal to T h 0 over d of T and this we know we do the same transformation. We say that d of delta s 0 is equal to delta C p by T.

Then d of T and we integrate again as before. Now, we integrate between two temperatures before we took T 1. Now, we take T 2 and we are going to T right and hence you can further write delta h 0 T minus delta s 0 T 2 is equal to delta C p. Now what I should I write here?

Student: Log.

Natural log.

Student: t (Refer Time: 17:07).

T over T 2 right. So, this is the other expression. So now, from this expression I can easily take delta s 0 T 2 on the other side and this get very similar to what I got for delta s 0 right. And now you can understand if I have the expression for delta s 0 in terms of delta C p. Similarly for delta s 0 in terms of delta C p, because you can see in both the cases delta C p plays a very prominent role right, because it figures then I can easily write.

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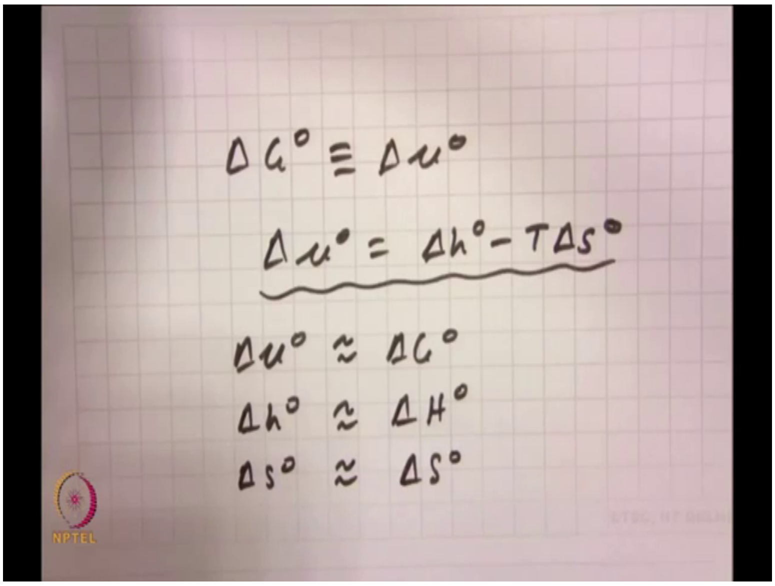

$$\Delta G^0 \equiv \Delta \mu^0$$

$$\underbrace{\Delta \mu^0 = \Delta h^0 - T\Delta s^0}$$

$$\Delta \mu^0 \approx \Delta G^0$$

$$\Delta h^0 \approx \Delta H^0$$

$$\Delta s^0 \approx \Delta S^0$$

Which obviously you will do delta g 0 which is equal to which is equivalent to delta mu 0. So, delta mu 0 is equal to delta s 0 minus T delta s 0. So, this you can easily figure out what it is.

But you will see it is given following the next slide when I goes to the slide, but I will do a small change in symbolism. So, when we talk about the next few slides what you will see is delta mu 0 is now being represented as delta g 0 delta s 0 is now being represented as delta all caps and delta s 0 is being represented as delta s 0 or delta s, ok. It is just a small change in symbolism that is it.

Right all these things you know. So, let us go forward and see what important thermodynamic equations we have. Now, look at these equations the first one we have derived the second one we have also have derived, the only thing again is there s was small because h naught where we are just taking h it is easily to put it is easy to put in the standard states it is not a big deal right and then you have delta G and you can see this is the final expression for delta G T right; this is the final expression for that.

So, there are two reference temperatures you know there is a reason why we took two reference temperatures T 1 and T 2. I did not take T 1 in both the cases to start with. Why did not I take that can someone tell me? Why did why did we take two reference temperatures, if we just did it what did we do?

Student: (Refer Time: 19:25).

When we had that figure what were the two temperatures where?

Student: (Refer Time: 19:29).

Changes going to 0 one was T h and one was?

Student: T s.

Ts; so that is why we have two different temperatures T 1 and T 2. So, when we will consider delta h 0 positive will consider T h, when we consider delta s 0 we will consider T s to be equal to T 2 and T h should be equal to T 1.

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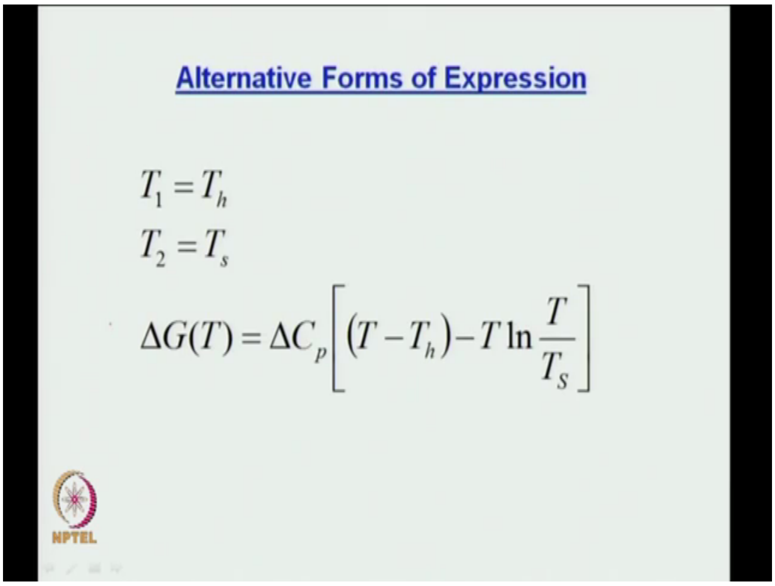

Alternative Forms of Expression

$$T_1 = T_h$$

$$T_2 = T_s$$

$$\Delta G(T) = \Delta C_p \left[(T - T_h) - T \ln \frac{T}{T_s} \right]$$

So, let us see how these you know come out. So, these are the alternative forms, the expression you have here is the most general form you do not know what T 1 and T 2 are, but you have just derived it.

You go to the next one now you see ok, let T 1 equal to T h and T 2 equals to T s these are most simple right. If T 1 is equal to T h, then what happens to delta H 0.

Student: (Refer Time: 20:08).

0 if T 2 is equal to T s what happens to delta H 0 0 right. So, when T 1 is equal to T h this delta H goes to, see when T 1 is equal to T h this delta H goes to the first delta h goes to 0.

Student: (Refer Time: 20:21).

When T 2 is equal to T s the delta h goes to 0.

Student: .0

So, what are you left with only the.

Student: Last term.

Last term and that is what you see out here. Where if made the small transformation the T 1 now is equal to T h and T 2 is now equal to T s that is what you have done. But this is just one and you know this is a very simple expression you have got.

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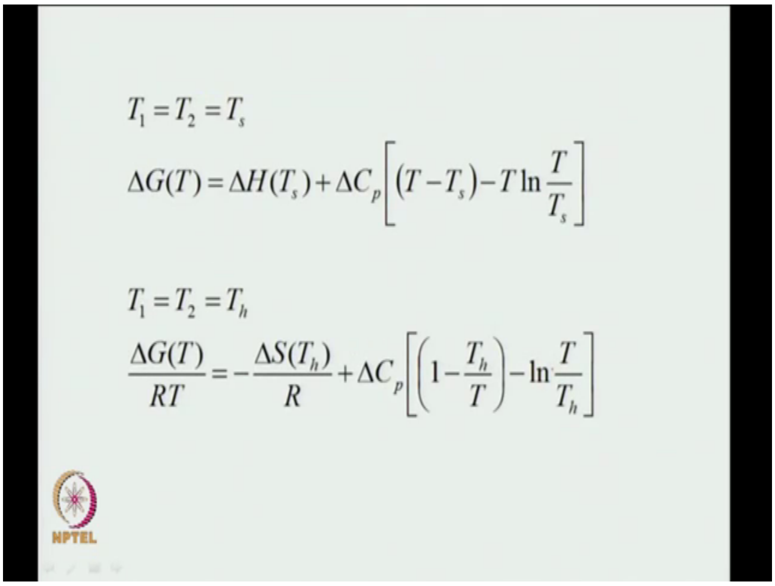

$$T_1 = T_2 = T_s$$

$$\Delta G(T) = \Delta H(T_s) + \Delta C_p\left[(T - T_s) - T\ln\frac{T}{T_s}\right]$$

$$T_1 = T_2 = T_h$$

$$\frac{\Delta G(T)}{RT} = -\frac{\Delta S(T_h)}{R} + \Delta C_p\left[\left(1 - \frac{T_h}{T}\right) - \ln\frac{T}{T_h}\right]$$

Now, I can do this I can say that let both T 1 equal to T 2 equal to T s or let both T 1 is equal to T 2 equal to T h and you know I can have this. These are just arbitrary reference temperatures it does not matter what I take and you see if we do that I know this is how it comes out. So, if T 1 is equal to T 2 is equal to Ts, then you immediately know at T s if delta S would be 0 and hence you see there is no delta S term the delta H stays.

Similar when T 1 is equal to T 2 is equal to T h you know at T h delta H is equal to 0. So, the delta H is equal to 0 term or delta H term drops out and you get this. So, depending upon you know depending upon what you want how you want to look at it you can use either of these expressions it does not matter

Because all these expressions are derived from the same general expression delta G is equal to delta H minus T delta S that is why we have denoted for ok. There is no you know no further significance it has to it, it is only tells you that under the given condition say T 1 is equal to T s T 2 is equal to T h and all these things then delta G can be expressed in these forms right.

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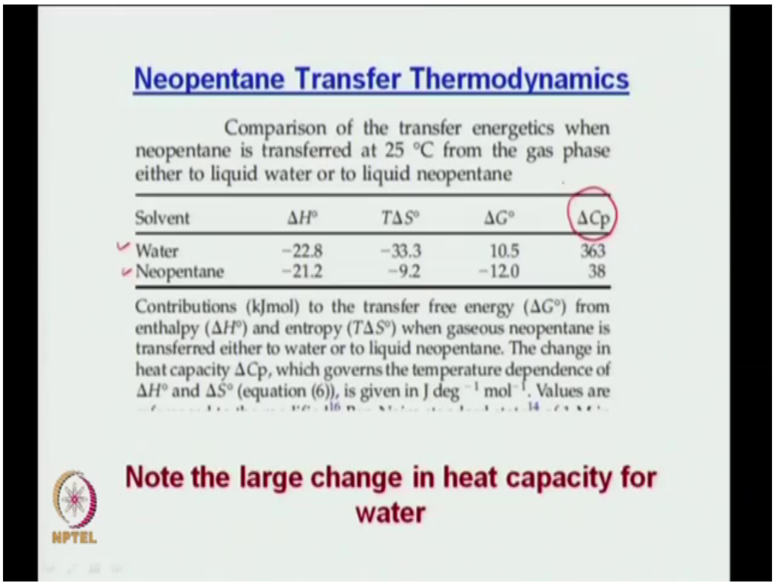

Neopentane Transfer Thermodynamics

Comparison of the transfer energetics when neopentane is transferred at 25 °C from the gas phase either to liquid water or to liquid neopentane

Solvent	$\Delta H°$	$T\Delta S°$	$\Delta G°$	ΔCp
Water	−22.8	−33.3	10.5	363
Neopentane	−21.2	−9.2	−12.0	38

Contributions (kJmol) to the transfer free energy ($\Delta G°$) from enthalpy ($\Delta H°$) and entropy ($T\Delta S°$) when gaseous neopentane is transferred either to water or to liquid neopentane. The change in heat capacity ΔCp, which governs the temperature dependence of $\Delta H°$ and $\Delta S°$ (equation (6)), is given in J deg^{-1} mol^{-1}. Values are

Note the large change in heat capacity for water

Now, in the next slide gives you some thermodynamic data, what it tells you is it gives us it gives you the transfer thermo dynamics for neopentane. And when I am talking about the transfer thermodynamics here what I mean is it is a little bit different. What this is you have neopentane in the gas phase and you do two things neopentane in the gas phase goes to neopentane in water; neopentane in the gas phase goes to neo pentane in neo pentane.

And that is why you see in the table you have water right and then you have also the neo pentane. So, the first one look at this the enthalpy the entropy, obviously is negative. But look at what is the most striking feature you see is you look at the last column.

Student: Yes.

Delta C p the delta C p for neopentane being dissolve in water is how much, it is so high 363 compared to that how much is the same for neopentane in neo pentane only 38. So now I can imagine why the slopes were so steep for neo pentane being dissolved in water, As compared to neopentane being dissolved in neopentane it is just because this delta C p is so high. So, this is a characteristic feature, a characteristic feature is if you would be having a non polar solute, the moment you dissolve the non polar solute in water you would be encountering what a huge.

Student: Change in (Refer Time: 23:32).

Change in heat capacity, why is this relevant? Why do you think this is relevant? This is relevant because when you start unfolding proteins, what happens? When you start unfolding proteins your hydrophobic groups start to getting exposed to water. If your hydrophobic groups are starting getting exposed to water the same thing is happening here right.

So, hydrophobic groups are essentially non polar, if they started getting exposed to water. That means, there would be a large change in the heat capacity and it is proven that proteins protein folding or unfolding always undergoes or it is always associated with a pretty decent change in heat capacity and this exactly falls out from whatever discussion we had up till now.

It just make sense right because if you are going to have it for a single non polar solute being dissolved in a water you think about protein having. So, many hydrophobic hours and when you are opening them up all of these would be adding up and hence you would be getting a huge change in heat capacity is it clear. So, this is where the relevance is. Now we will discuss about this protein thermodynamics more in subsequent classes, but I just thought we would you know try to connect this neo pentane dissolution with protein folding or unfolding.

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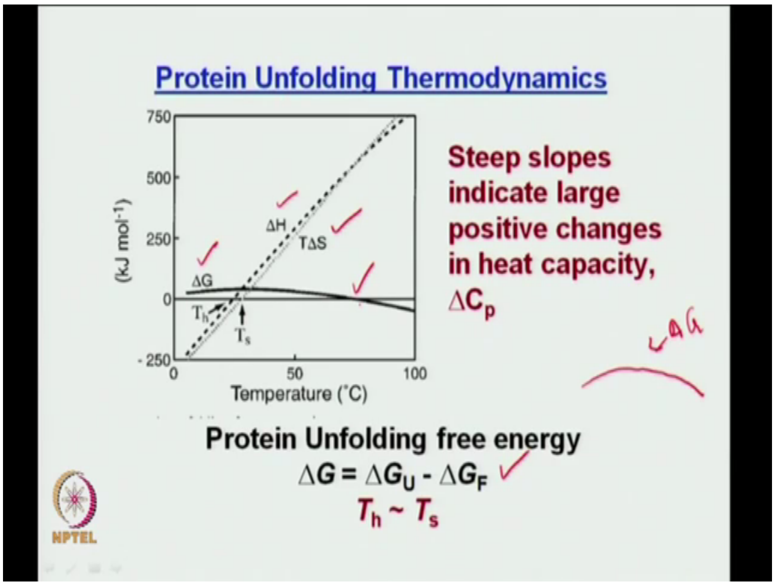

Protein Unfolding Thermodynamics

Steep slopes indicate large positive changes in heat capacity, ΔC_p

Protein Unfolding free energy

$\Delta G = \Delta G_U - \Delta G_F$

$T_h \sim T_s$

So, now this protein unfolding thermodynamics will not do much, but I will just tell you what the major difference is. Two things in the protein unfolding thermodynamics look at this delta H and T delta S change this still very steep.

This still very steep possibly more steep than what you had seen for neopentane in water, which means that obviously because this steep. That means, there is a large change in heat capacity right that is what we just discussed. So, as I said steep slopes indicates large positive changes in heat capacity. Now what we are looking at is it is a protein unfolding free energy. So, this see this is delta G this is delta G what we are looking at is this one, it is delta G is equal to delta G U minus delta G F what is U stand for?

Student: Un (Refer Time: 25:48).

Unfold state S stands from the folded state. We are calling it protein unfolding free energy because, when we are unfolding the protein that is the free energy that is associated with this unfolding; we are not we are not looking at the reverse ok. So now, you see what happens this delta G actually has a certain curvature right it is not a straight line, this delta G has a certain curvature associated with it.

Why do you think this curvature is shown as it is? That means, the curvature so delta G the curvature actually goes something like, this and come down comes down this is your delta G. Why do you think it goes to a curvature like this? What did they show a positive curvature first of all?

See we are still the what about the temperature? Temperature is still room temperature, at room temperature if you would take a protein would you expect it to unfold. At room temperature see temperature not very high, depending upon the protein say I am taking a pretty stable protein a stable a protein which is easily dissolve you know soluble in water and in maintain this structure at room temperature.

Then if you are going to try to unfold the protein at room temperature; obviously, the unfolding free energy would be positive, because it is thermodynamically not spontaneous you cannot unfold the protein just like that at that temperature, you have to apply some external agents say denaturing is not or whatever. But just based on temperature that process is not thermo dynamically spontaneous and that is why you see delta G goes to the positive, because there is unfavorable.

Now, there is one more thing ah this we will discuss later, but see it does not stay positive. That means, when you go to higher temperatures it goes to the negative and also when you go to lower temperature it goes to the negative right. Do you know what happens when delta G is equal to 0, what does that signify? Any ideas?

Student: Equilibrium.

Yes that means k is equal to delta G well let me say delta G 0 is equal to 0 I mean delta G 0 is equal to minus T l and k delta G is equal to 0, right. Now, this gives your equilibrium constant ok. What is the equilibrium constant between? It is between your protein unfolded state and the protein folded state right.

Remember this I will not talk about this more right now, but remember this when we talk about protein stability right. Actual protein thermodynamics this is very important, you know when you fold or unfold proteins or even DNA RNA and all those things there is a certain temperature known as T m right it is the midpoint temperature it is the thermal melting temperature right. Say at midpoint at the T m what happens?

Student: Half into half (Refer Time: 28:49).

Half and half, so k is equal to 1.

Student: Yes sir.

What happens to delta G 0 then?

Student: 0 (Refer Time: 28:56).

0 right.

Student: 0 (Refer Time: 28:57).

So, now you can understand why delta G would be crossing 0 at two places. If you are going to increase temperature, then obviously you know that at a certain high temperature it would unfold; that means, k would be equal to 1. So, that is why you see this higher place where the protein unfolds or DNA unfolds a.

In this case we are talking about a protein, but is not it surprising (Refer Time: 29:22) also start going to low temperature, the protein is also going to unfold. Do you know what this is known as? See one is if you are going to high temperature protein is going to unfold, it is called heat induce denaturation (Refer Time: 29:35) simply denaturation. But when you are going to also the cold side the protein is also unfolding, this is referred to as cold denaturation.

Student: Cold denaturation.

That means, you are denaturing your protein in the cold right,

Student: Yes.

But, anyway that is just the point I wanted to make. But you keep these things in mind we will discuss about this more later. Now, there is one more aspect what do you think is a major difference do not talk about del G, but what do you think is a major difference between the figure you see out here for a protein and the figure you saw for neo pentane in water? Ok. Let me go back to the figure.

Student: Difference in T h and T s.

Difference in T h and T s, so here you go, see this was is a neo pentane in the water. Look at the difference between T h and T s the gap. What is this? This is huge right.

Student: Yes.

Now, you do the same thing and you come to a protein, what happens? The T h and T s are very.

Student: (Refer Time: 30:33).

Close to each other. Now, why would this be; why would this be? Can you reason out? The reason is actually given to in the next slide, but I do not want to go to the next slide. Tell me what is a major difference between dissolving a neopentane in water and trying to unfold a protein in water is the same thing.

Neopentane if you would dissolve it in water, that means you are taking hydrophobic molecule and putting it in water. You are not folding you are protein and doing the same thing. You are exposing the hydrophobic groups to water essentially the same thing. What is the major difference between a protein and neo pentane apart from the fact that one is neo pentane the other one is a protein?

Student: When you (Refer Time: 31:20).

Many different interactions are present sure that is one many electrostatics and all those things are present which you do not have in neopentane. There is one more thing you know there is something we discussed in the very first class, delta G is equal to delta H minus T delta S.

Student: (Refer Time: 31:37).

What happens when you change?

Student: Entropy decreases (Refer Time: 31:39).

The entropy the entropy plays a major role right, because now you are making it more and more flexible right. But think about a neo pentane would you see any entropy change out there?

Student: No.

None right because neopentane take here it take there is the same thing, this neopentane does not undergo any folding and it just neopentane. So, that is why this T h and T s they come very

Student: Close to each other.

Close to each other, the gap is so small in case of a protein and there is one more thing is it clear. So, essentially that is what it says at the bottom T h is almost equal to what? T s right.

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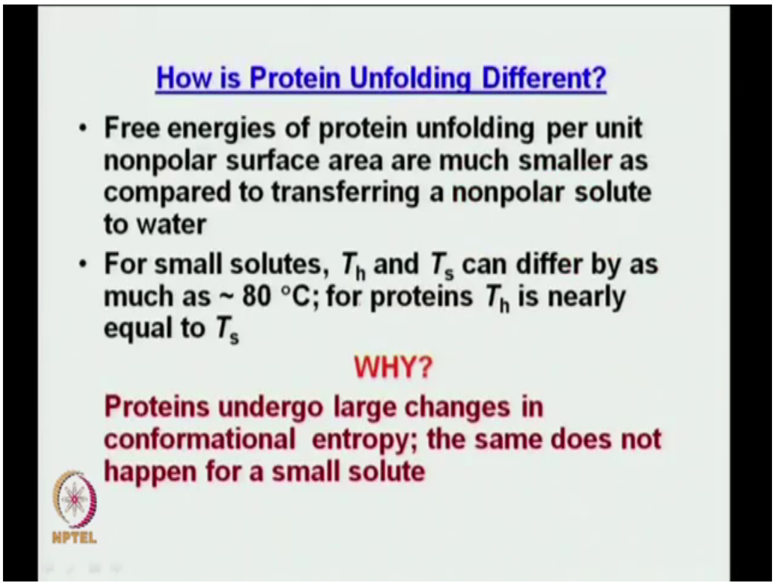

How is Protein Unfolding Different?

- Free energies of protein unfolding per unit nonpolar surface area are much smaller as compared to transferring a nonpolar solute to water
- For small solutes, T_h and T_s can differ by as much as ~ 80 °C; for proteins T_h is nearly equal to T_s

WHY?

Proteins undergo large changes in conformational entropy; the same does not happen for a small solute

So, that is what it says how is the protein unfolding different? So, free energies of protein unfolding per unit non polar service area are much smaller as compared to transferring a non polar solute to water. Number two for small solutes T h and T s can differ by as much as almost 80 degrees Celsius that is what you observe for neo pentane, that was your solute. But for proteins T h is nearly equal to T s even from this figure.

And the reason being proteins undergo large changes in confirmational entropy. The same obviously is not there for your solute neo pentane. Yes you are also right electrostatic interactions and all those things are also present, but delta S is the single most important factor, because you undergo such a large increase or decrease in delta S. When you do this protein folding unfolding process right.

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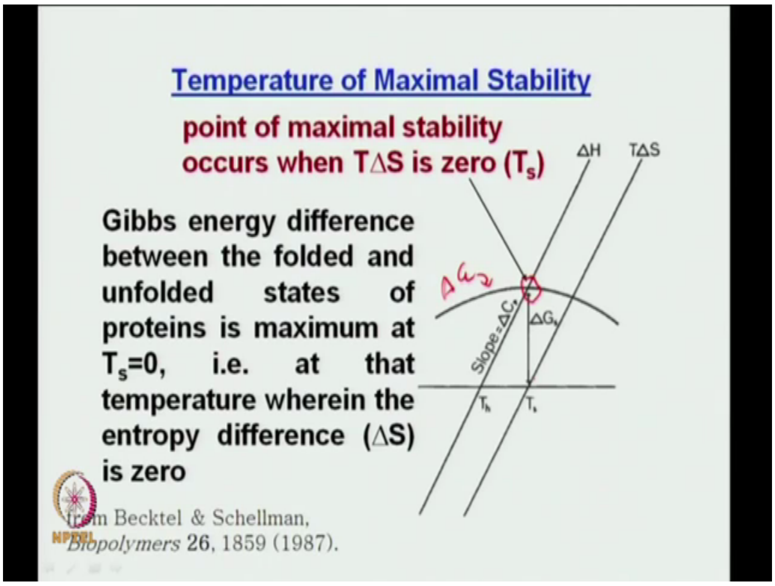

Temperature of Maximal Stability

point of maximal stability occurs when TΔS is zero (T_s)

Gibbs energy difference between the folded and unfolded states of proteins is maximum at T_s=0, i.e. at that temperature wherein the entropy difference (ΔS) is zero

from Becktel & Schellman, *Biopolymers* 26, 1859 (1987).

This is one more thing that I need to tell you which is now what I have done is we have zoomed into this delta G curve, we can see this curvature so this is essentially your delta G. So, this let me tell you so this is essentially your delta G this curve. See when you in this figure it was not very evident. But what I have done is I have just zoomed into this portion and you look what happens you see delta G undergoes a maximum. Where does it undergo maximum? It undergoes a maximum, so look at this point.

Student: Yes sir.

You look at this point so delta G is undergoing a maximum at this circle point and what did this circle point correspond to?

Student: T s.

It corresponds to Ts. Hey what does this tell you? This tells you where the entropy of transfer is 0; your delta G is actually at it is extremum, which in this case is maximum. Does it make sense?

Student: Yes sir.

Well thermo dynamically it does make sense right, why? Well it says here the Gibbs energy difference between the folded and unfold states of proteins is maximum at T s equal to 0. There is at that temperature where in the entropy difference is 0.

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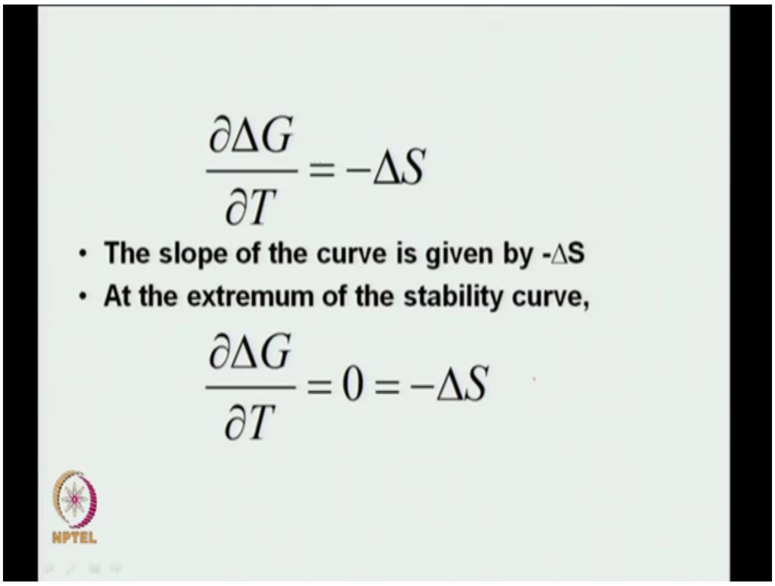

$$\frac{\partial \Delta G}{\partial T} = -\Delta S$$

- **The slope of the curve is given by -ΔS**
- **At the extremum of the stability curve,**

$$\frac{\partial \Delta G}{\partial T} = 0 = -\Delta S$$

How do that make sense? You know this d G is equal to what? V dp minus S dt if you had go back to your thermodynamics right. So, del of del G of del of G over del T is equal to what?

Student: Minus S.

Right and then del of del G over del T would be minus delta S right. So, essentially what you are looking at? You are looking at is the slope, so that means the slope of change in free energy with temperature gives you entropy ah with the proper sign. Hence, if it if this slope is going to the maximum or this delta G is going to the maximum, what will happen to the slope?

Student: 0.

It is 0 and that is why your delta s is 0 is not it because your dy over dx at any inflection point is going to be 0 it is extremum right.

The same thing is happening here the delta G is maxing out there and where does it become maximum? It becomes maximum where the entropy of transfer is essentially 0; that is what the link and that is what if you would remember I did not tell you at that time, you also saw it; you also saw it. If you would look at it again let us see here, you can see this T s look at this T s here and look at the delta G out here.

There also delta G is kind of maxed out, but we do not see what happens later. So, I think this is you know typically what I had to discuss about hydrophobic effect

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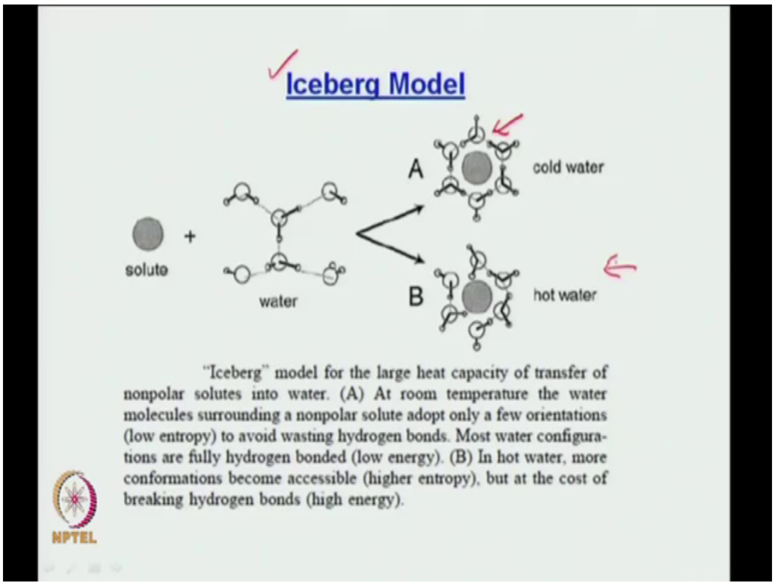

Iceberg Model

"Iceberg" model for the large heat capacity of transfer of nonpolar solutes into water. (A) At room temperature the water molecules surrounding a nonpolar solute adopt only a few orientations (low entropy) to avoid wasting hydrogen bonds. Most water configurations are fully hydrogen bonded (low energy). (B) In hot water, more conformations become accessible (higher entropy), but at the cost of breaking hydrogen bonds (high energy).

This last one point; you know how would you; how would you actually rationalize at the molecular level this large increase in heat capacity? How would you rationalize? So, the rationalization can be done by many models. One of the models which still stand and which kind of gives you a very logical interpretation of what is happening is what is referred to as the Iceberg model right.

What does iceberg model tell you look at this you have a solute which is your neopentane, you are trying to dissolve it in water right you have not yet dissolved it in water ok. I am looking at the left hand side right I am looking at the left hand side of the arrows, you can see water has it is own tetrahedral hydrogen bonding network you have not disrupted it right.

Now, the moment the solute is disrupted in water or put in water, what happens? You know the solute is going to occupy a certain place the solute is non polar. So, the water molecules will not waste their hydrogen bonds by trying to bond with solute molecule, instead what it will do or what will the water molecules do? The water molecules will actually bond to themselves right.

But they will bond to themselves such that they avoid they avoid the solute which is in the center and they form kind of a hydrogen bonded shell which is called the first solvation shell or solvation shells round the non polar solute. So, this is what you seeing out here. See you look at this you look at this here you can see the water molecules are hydrogen bonded to each other right.

Surrounding the solute molecule now this is at cold water means room temperature; you know 15 degrees 20 degrees 25 degrees whatever. So, in this case your water essentially is what? Ordered also energy is low, because the temperature is low right it is ordered. So, it is entropy is low and the reason again water is doing it because it does not want to waste it is hydrogen bonds; it wants to utilize it is hydrogen bonds to the maximum and it can do so by hydrogen bonding amongst themselves right.

Now, why is it called the Iceberg model? It is called the iceberg model, because you remember in ice what happens it is your anomalous expansion of water. When you go to ice in the solid form ice also has as a some sort of a tetrahedral order right. So, it is not exactly an ice, but it is kind of an iceberg model it resembles the kind of order you tend to have in ice.

Only that is the similarity that is why it is called an iceberg model. Do not think that the same kind of coordination you have in ice is going to be maintained here, it is not going to be like that ok. So, this is clear now think about this case high temperature. So, this is I have marking by the arrow it is a high temperature case. What happens to the high temperature? You are putting in energy right.

And if you are going to put in energy, what will happen? You will try to dissolve to the water molecules. Now, what will the water molecules do? So, water molecules will try to assume many different orientations, that means at cold water the water molecules were ordered, is not it. In cold water the water molecules were ordered round the non polar solute, you pump in energy what happens? It goes towards disorder more disorder because it has to respond to this change.

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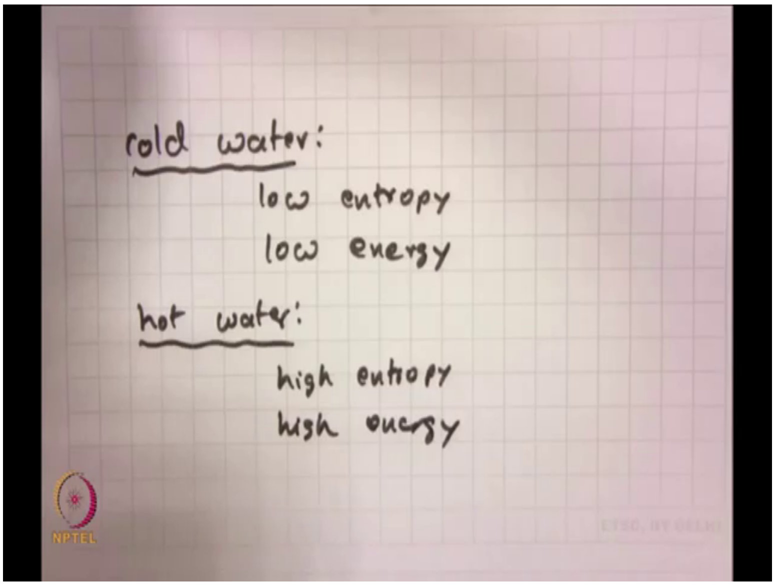

cold water:

low entropy

low energy

hot water:

high entropy

high energy

So, that means, when you are in cold water let me write it one I am saying too many words I guess, when we are in cold water; when we are in cold water; when we are in cold water we can say we are in a low entropy and low energy state. Now, when we go to a hot water, that means when we increase a temperature we go to a high.

Student: (Refer Time: 40:01).

Entropy and also it should be high energy or high enthalpy, is not it.

Why? Because when you pumping in energy and what does this energy is going this energy is going in baking the whatever hydrogen bonds you had formed. So, this energy is actually baking a hydrogen bonds and hence you have the system has actually more energy. So entropy obviously corresponds to delta S and you know that delta S is increasing entropy is increasing, also high energy means your enthalpy is increasing.

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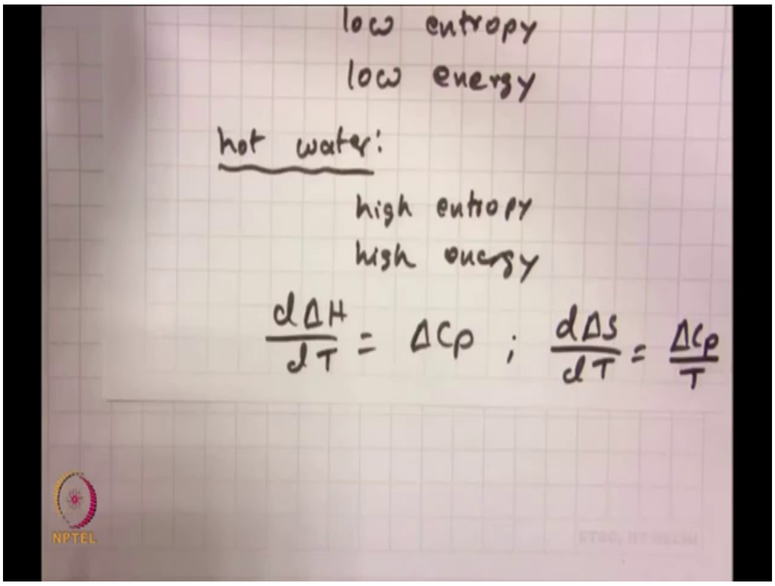

low entropy
low energy

hot water:

high entropy
high energy

$$\frac{d\Delta H}{dT} = \Delta C_p \; ; \; \frac{d\Delta S}{dT} = \frac{\Delta C_p}{T}$$

Now, what is the relation between delta H and delta Cp? Is a slope. That means we knew that d of delta H over d of T is equal to delta Cp, see if my delta H has to increase, then delta Cp?

Student: Increase.

Has to increase the same thing happens for what entropy. What was entropy? d delta S over dT is equal to del C p by T, right. So, d delta S over d of T is equal to del C p by T. So, both of these are increasing and both of these have a strict delta C p dependence and because it is increasing and because you are looking at the slope or the rate of change it just figures that a delta C p should also change be positive and change by a huge amount.

And hence this is the molecular interpretation behind why you see a large change in heat capacity, because your water molecules essentially go from an ordered state low energy state to a disorder state and high energy state, order to disorder delta s is positive. Low energy to high energy delta H is positive and hence delta C p has to be positive, that means delta C p has to increase.

So, this is the molecular nature that means at the molecular level what is probably happening in solution around your non you know non polar solute or even you know hydrophobic side chains of your protein, when you are actually unfolding it. So, if you would ever look at we look at it later as I keep saying, but if you would ever look at protein unfolding thermodynamics you will always see a huge change depending upon. What protein you have in heat capacity?

Student: Heat capacity.

That means, a change in C p or the delta C p would be high just because of this reason, is it clear how it is coming around you know think about it. It is actually very logical because both delta H and delta S actually depend up on C p vice versa, if delta H and delta S are increasing then delta C p has to.

Student: Increase.

Follow should increase; that is the only way how delta h and delta s can increase. So, this is typically how you know at the very basic level or the very molecular level molecular terms you can think about increase in heat capacity right.

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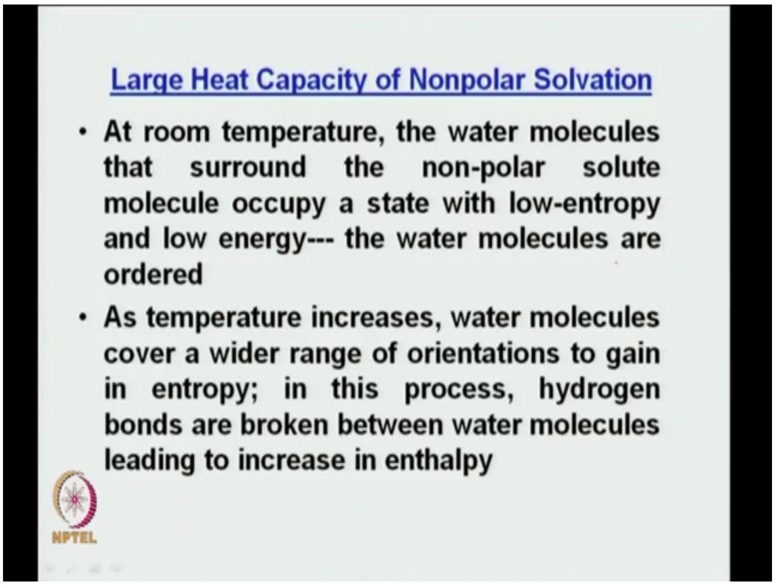

Large Heat Capacity of Nonpolar Solvation

- At room temperature, the water molecules that surround the non-polar solute molecule occupy a state with low-entropy and low energy--- the water molecules are ordered
- As temperature increases, water molecules cover a wider range of orientations to gain in entropy; in this process, hydrogen bonds are broken between water molecules leading to increase in enthalpy

So, at and it says at room temperature the water molecules that surround the non polar solute molecule occupy a state with low entropy and low energy the water molecules are ordered. As temperature increases water molecules covered a wide range of orientations, because it has to respond to the change in entropy getting disordered. In this process hydrogen bonds are broken between water molecules leading to increase in enthalpy, that is essential energy and hence you see a change in C p increase in C p ok.

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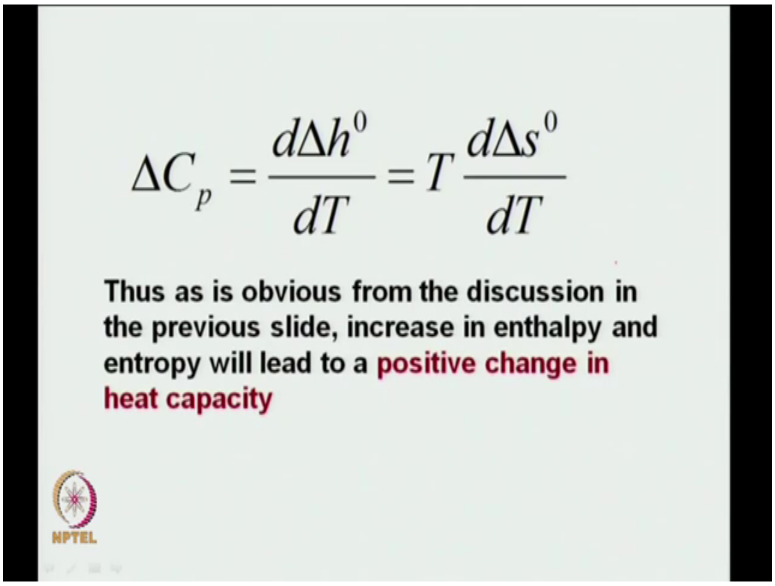

$$\Delta C_p = \frac{d\Delta h^0}{dT} = T\frac{d\Delta s^0}{dT}$$

Thus as is obvious from the discussion in the previous slide, increase in enthalpy and entropy will lead to a positive change in heat capacity

So, that is what we just said. So, that is as it is obvious from the discussion, in the previous slide increase in enthalpy and entropy will lead to a positive change in heat capacity right. So, you know given a situation given any problem, the idea is that you should be able to explain no without any equations at the molecular level physically.

What is happening you provide a physical picture and I hope you know this helps you do that and this is how you should be able to to relate thermodynamic see this is thermo dynamics right. All these things you have known you have read when you are doing your first law second laws and all those things and this is where you see they are being used this is actually a practical application of your thermodynamic principles.

Whatever you knew you are being you are applying out here. So, this is all what I thought I would be telling you about you know hydrophobic effect, it is actual application folding. I will take it up a little bit more when I do talk about protein thermodynamics. I just do not want to make the jump here right. Now, because I still have one more thing to cover in forces.

We had covered electrostatics right which included different interactions you know charge dipole; charge dipole mine dipoles, then you went to this you know hydrophobic effect right. But there was one thing also which is hydrogen bonding and hydrogen bonding is important, because you know your secondary structures alpha helix beta sheets we have seen before.

They are all hydrogen bonded right secondary structure is actually symbolize alpha helix by this intra helical hydrogen bonding or better sheet by the intra strand hydrogen bonging. So, hydrogen bonding has to be able to play a significant role in providing stability or governing the protein folding thermodynamics.

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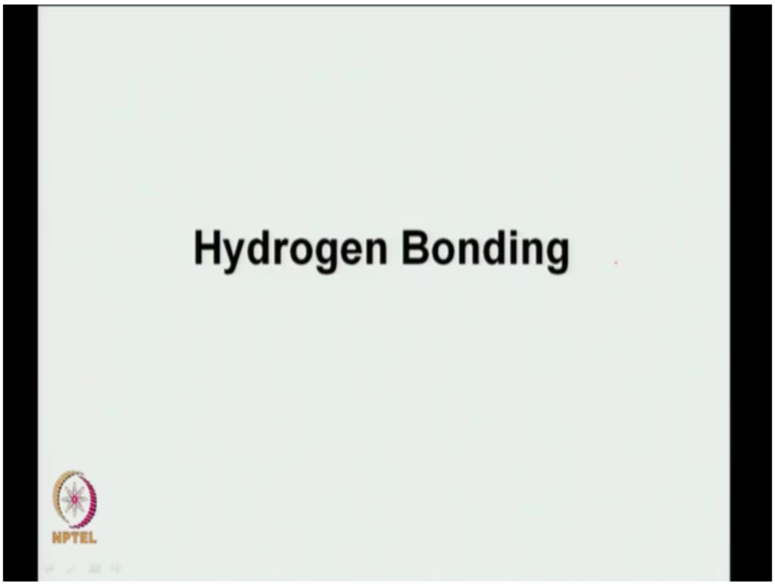

Hydrogen Bonding

So, the next topic is hydrogen bonding and how do we look at it.

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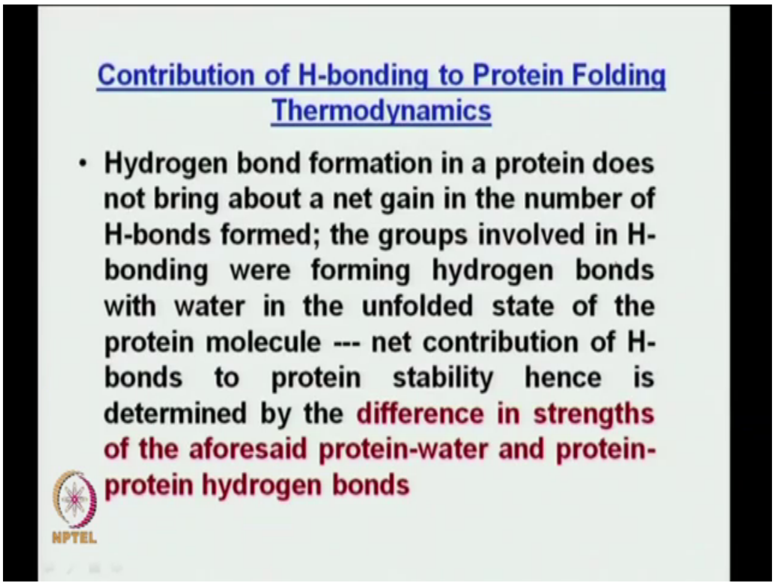

Contribution of H-bonding to Protein Folding Thermodynamics

- Hydrogen bond formation in a protein does not bring about a net gain in the number of H-bonds formed; the groups involved in H-bonding were forming hydrogen bonds with water in the unfolded state of the protein molecule --- net contribution of H-bonds to protein stability hence is determined by the difference in strengths of the aforesaid protein-water and protein-protein hydrogen bonds

So let us think about hydrogen bonding, it is contribution to protein folding thermodynamics. So, what it says is hydrogen bond formation in a protein does not bring about a net gain in the number of hydrogen bonds formed. Why does it say? So it says so because the groups involved in hydrogen bonding, were forming hydrogen bonds with water in the unfolded state.

So that means, when the protein was not folded, whatever your groups were forming hydrogen bonds because what are the groups which form hydrogen bonds. It is not a hydrophobic groups right is the Nh CO and all these things your salt bridges and all this. So, when they are open your water molecules you know they were they were polar. So, they could even easily hydrogen bond with water right. So, you have an you actually do an exchange in hydrogen bonds, initially when the protein was unfolded the polar groups NhCO these groups they were hydrogen bond with water no problem.

Now, when you fold the protein you know depending upon the orientation, depending upon the type of fold you have it will start hydrogen bonding forming hydrogen bonds within themselves inside the protein right. But so where does the stability come from? The stability comes the difference in strengths of the aforesaid protein water and protein protein hydrogen bonds.

Right that is what is going to be one of the factors which will be governing the change in energy or the gain in energy you have when you go from a folded protein to an unfolded protein that is one. See this have you think about it is, it is actually major issue right because even before they were forming this intra helical intra strand hydrogen bonds they are actually happy forming the hydrogen bonds with water. Only that the hydrophobic groups were probably not very happy sitting in water. So, they had to come inside next.

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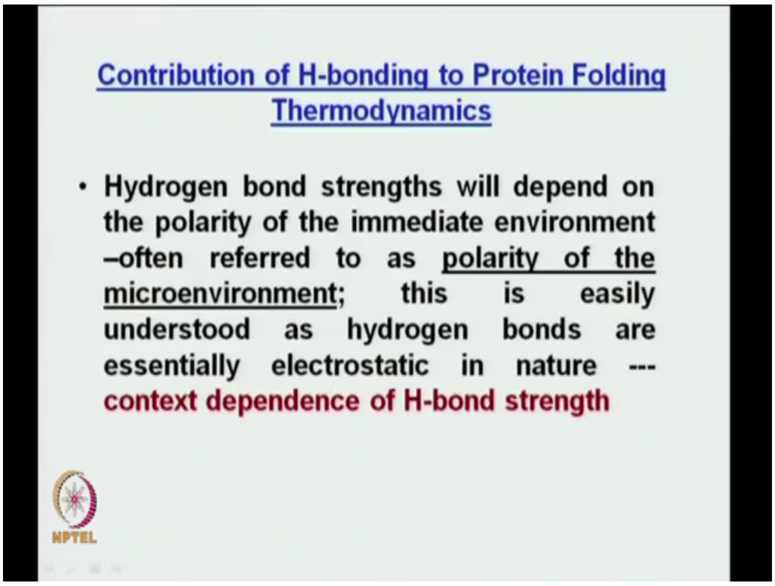

Contribution of H-bonding to Protein Folding Thermodynamics

- Hydrogen bond strengths will depend on the polarity of the immediate environment –often referred to as polarity of the microenvironment; this is easily understood as hydrogen bonds are essentially electrostatic in nature --- context dependence of H-bond strength

Now, hydrogen bond strength will not be universal everywhere or not be the same everywhere you know. Why do we say that we say this because the hydrogen bonds strengths will depend upon the polarity of the immediate environment. That means, you know you think about this.

Suppose you have a hydrogen bond in a in inside of a protein right, where we have a protein core (Refer Time: 47:43) hydrogen bond now inside the protein core its essentially nonpolar. Remember hydrogen bond is essentially electrostatic in nature right. If you consider electrostatics all of those had a term d the dielectric constant at the numerator remember.

Student: Yes.

The d so that means higher the dielectric constant, essentially that dielectric constant will be able to shield the interaction between the corresponding charges. But when you are inside a protein core what happens if d is anyway low? So that means, hydrogen bonds strength out there should be higher.

Student: Yes.

Because it is an if it is an essential electrostatic you know force you are not being able to shield those two from each other that much right. But if you have say surface hydrogen bonds so somewhere else, then those hydrogen bonds what will happen. Now, they can interact with water hence d will be higher and thus the bond strength essentially will be lower.

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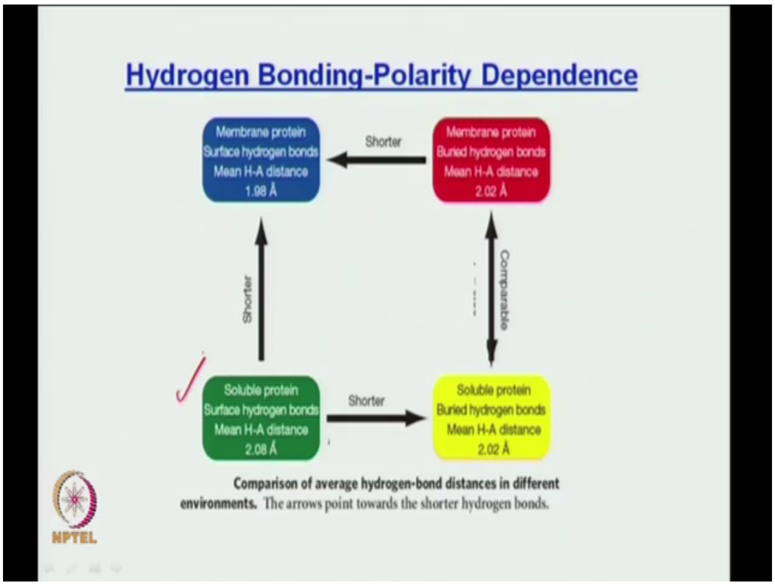

Hydrogen Bonding-Polarity Dependence

Membrane protein
Surface hydrogen bonds
Mean H-A distance
1.98 Å

Shorter

Membrane protein
Buried hydrogen bonds
Mean H-A distance
2.02 Å

Shorter

Comparable

Soluble protein
Surface hydrogen bonds
Mean H-A distance
2.08 Å

Shorter

Soluble protein
Buried hydrogen bonds
Mean H-A distance
2.02 Å

Comparison of average hydrogen-bond distances in different environments. The arrows point towards the shorter hydrogen bonds.

Now, this is H this is exactly what is being shown here you look at this. So if you take let us start from the soluble protein which is green one let us start from here. The soluble protein you have you are looking at surface hydrogen bonds, surface means on the surface of the protein you have this NhCO groups or this polar groups. They form hydrogen bonds in water and the average distance is the mean H A distance is 2.08 angstroms its a 2.01 angstroms.

You take the same thing you go to a soluble protein with buried hydrogen bonds. Now what you are doing is you looking at hydrogen bonds, but this time you are looking at the buried portion of the same protein. Now, what is happening to the bond length? It decreases, now it make sense right. If it is going to the buried portion there d will be lower, hence it will not be able to shield the corresponding charges and hence the bond will be stronger hence the strength of the bond is signified by a lower bond length. So, it is about 2.02 angstroms.

Now, you go to the membrane protein buried hydrogen bonds, you have taken the protein and you have taken a membrane protein and put it inside a membrane. See the buried hydrogen bonds are buried hydrogen bonds they are in the core when you take a membrane protein or a soluble protein.

The core is always hydrophobic; hence that is why it is logical that they were be similar. But look at this if you take the membrane protein and surface hydrogen bonds and compare the soluble protein surface hydrogen bonds, you see there is a remarkable difference. Why is this so?

This is because when we take the membrane protein and surface hydrogen bonds; remember the membrane proteins are sitting on your membrane right. The surface hydrogen bonds the way they point is the way they point is the surface hydrogen bonds actually points towards interior of the lipids and when they point to the interior of the lipid molecules. The lipid molecules is essentially what is hydrophobic?

Student: Hydrophobic.

And, if the lipid molecule is a hydrophobic again d is very low out there, because remember in a protein core you can have you can have what you can have polar amino acids. But in the lipid core you cannot have polar amino acid it is lipids are just lipids, you know hydrocarbon chains or hydrocarbon tails you cannot have anything else. And, hence that is why the d is lowest out there and that is why you see the bond length is also the lowest, because hydrogen bond is

Student: Strongest (Refer Time: 50:58).

The strongest out there ok. So, tomorrow what we are going to look at is; tomorrow we are going to look at is can you prove this can you actually prove this. This is called contextual dependence of hydrogen bonds. That means, you are looking at the hydrogen bond where is it placed and depending upon where it is placed. That means, it is immediate environment which is called the microenvironment, how would the hydrogen bond still differ can you actually prove it.

See it is easy to understand by proving in case of a protein it is not so easy and that is what we are going to discuss tomorrow. And that should be essentially last topic for our forces in protein folding and the reason I am telling you spend so much time is. If we do not understand these forces see these forces are in case of protein folding, but you think about the forces this forces are universal.

Whatever forces we are we are taking proteins as an examples but whatever forces we were talking about these forces. We know, and whatever you go these forces are not going to change their magnitude might be changing, but the nature of the forces are not going to change they are going to remain the same. And that is why knowing those a little more detail is actually I think very important ok.

So, tomorrow we will start with hydrogen bonds.