BioInformatics: Algorithms and Applications
Prof. M. Michael Gromiha
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture - 20a
Protein Stability I

In this lecture, I will mainly focus on protein stability one of the applications of using different bioinformatics tools as well as a parameters to understand the structure of a protein, as well as function of a protein in terms of protein stability. In the previous class, we discussed about predicting the 3D structure of a protein from its amino acid sequence because the amino acid sequence contains information regarding the 3D structure. So, you can utilize the information which you can obtained from the sequence to predict the 3D structures I will discuss about various methods to predict the 3D structures.

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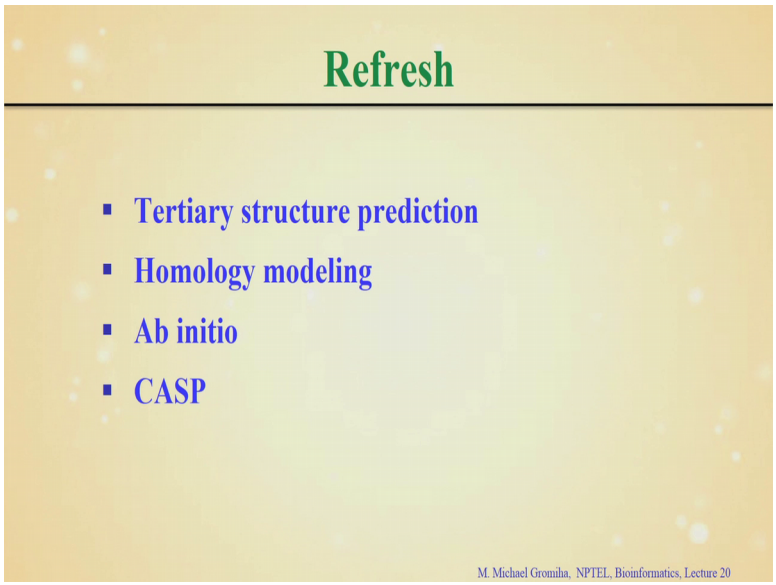

And the one of the major ones is the homology modelling. Homology modelling is based on?

Student: Homologous sequences.

Right that if 2 sequences share.

Student: High.

High identity or homology and then these sequences have similar 3D structures, right. So, if the sequence identity is more than 80 percent or 90 percent, right, you can obtain a reliably good model, right which can be similar to the structures which could be obtained by experiments. So, what are the various steps used in the homology modelling?

Student: Starting.

Starting from the template.

Student: Template.

And do the alignment; alignment corrections.

Student: Backbone.

And backbone generation loop modelling side chain orientations and the optimization right finally, we validate the structure right using the energy or the stereochemical properties. So, if a sequence cannot have in-detectable identity with any of the structures deposited in PDB in this case the knowledge based approach will fail and homology modelling may not give a reliable results.

So, you can use the fold recognition right based on the probability of the residues which can be in contact or the physiochemical properties or depending on the gaps you can able to identify the folding type even if it is not possible then you can try to use the ab initio methods you can start with scratch and this is based on the physical energetic calculations. So, you can get the structures for any protein sequences. CASP is the competition for the structural prediction algorithms right you can submit and then will evaluate based on known 3D structures.

When you go with the protein stability right what is the protein stability means? right.

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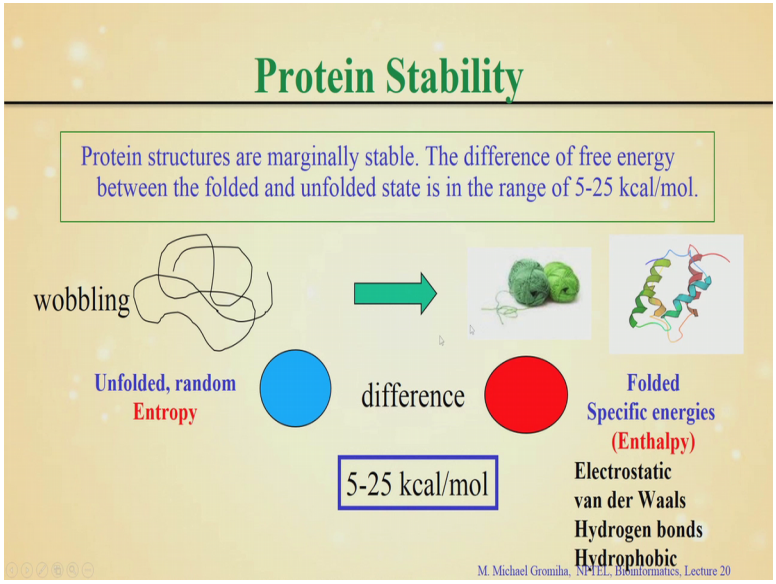

When the protein synthesized in random state and go to the folder state it fold from the random coil confirmation to stable 3D structures. So, these three structures are also important right to perform a function for example, you have a thread with loosely a loose thread then it is wobbling on the air. So, it can go anywhere right you can see the kite. So, it will go wobbling anywhere. So, in this case, it is difficult to perform a function. So, even if you have the kind of thread then may be it is stable right you have some specific conformation likewise in our day to day life.

Right, if you are stable you are healthy, then we are able to perform a function right it is very important for the proteins to have any stable conformation. So, if we look into this unfolded state that is a kind of wobbling conformation. So, the major factor which are influenced by this conformation, it is entropy depends upon the how many degrees of freedom this protein they have in the unfolded state, then we go to the folded state right this 3D structure is maintained by different types of interactions specifically you can say that electrostatic interactions van der Waals interactions hydrogen bonds hydrophobic interactions disulphide bonds and so on.

The electrostatic, van der Waals and these are non-covalent interactions under consideration the disulphide bond this kind of the bond between the 2 sulphur atoms consist. So, if we look into this contribution in the folded state and unfolded state the contribution is very high in terms of all the types of interactions and if you look into the

difference between the fold and unfolded state this is very less right and the difference is about 5 to 25 kilo cal per mole. So, why this marginal stability is important because in the living systems right, certain proteins should be available in right in specific quantities, if you produce more and more proteins and if that is not able to degrade, then the amount will be very high. So, it is easier to degrade any protein at any point of time. Second aspect is it is also important to change the conformation when a specific protein is interacting with any ligands or other biological molecule. So, in this case they have to maintain a small difference in stability between unfolded state as well as in the folded state and the difference is normally in the range of 5 to 25 kilo cal per mole. So, now, we have discussed about different terms one we discuss about the enthalpy this is the total energy of the system determined by different types of interactions. On the other hand if you discuss about the entropy, this is similarly the a unfolded state the system and these 2 terms can be related using free energy right that will give you the between the folded unfolded state that is 5 to 25 kilo cal per mole, then we discuss what is entropy or what is enthalpy what is the free energy. You talk about enthalpy right what is the definition for enthalpy.

Student: The youth of (Refer Time: 05:56).

Right, you might have studied in school, classes, right; its amount of heat used or released in a system at the constant pressure.

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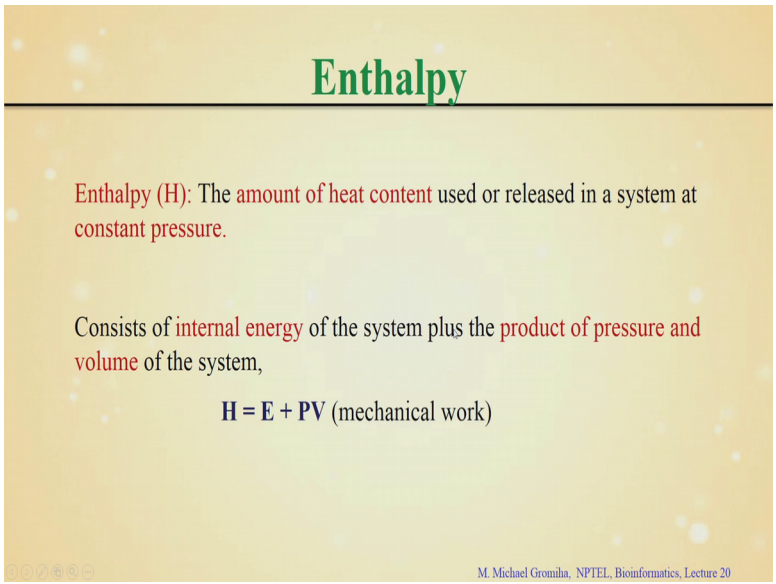

So, if you see the enthalpy it is consist of 2 terms one is the internal energy of the system plus the product of pressure and volume that is a mechanical one.

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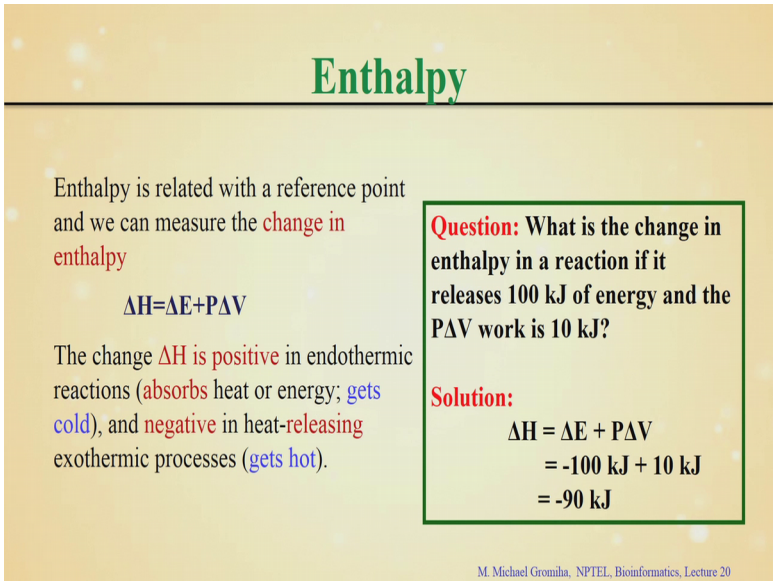

So, if you see the enthalpy it can be obtained related with any reference points right not for the any specific point right. So, in this case we can see the change in enthalpy not the absolute enthalpy you can change the enthalpy that is delta H, this can see with respect to any reference point this can be related as delta E plus P into delta V right because its change in volume and change in internal energy of the system. If delta H is positive then what does it mean it is endothermic reactions it absorbs heat because of this absorbance of heats it gets cold the environment gets cold. So, now, it is negative there is the heat releasing process this you can see exothermic process in this case the environment gets hot.

So, I have a question. So, what is the change in enthalpy in any reaction if you releases hundred kilo joule of energy and the P delta V work is ten kilo joule here what is the solution you can say delta H this is equal delta E plus P into E delta E what is the sign for delta E because it is releases. So, this is minus hundred plus ten kilo joule that is equal to minus 90 kilo joule right this we can get the enthalpy for any system.

So, if you look into the internal energy. So, internal energy represents all the energy contained in the material. For examples, it is kinetic energy or you can see inter intra

molecule interactions like bond energy electrostatic energies van der Waals force and so on.

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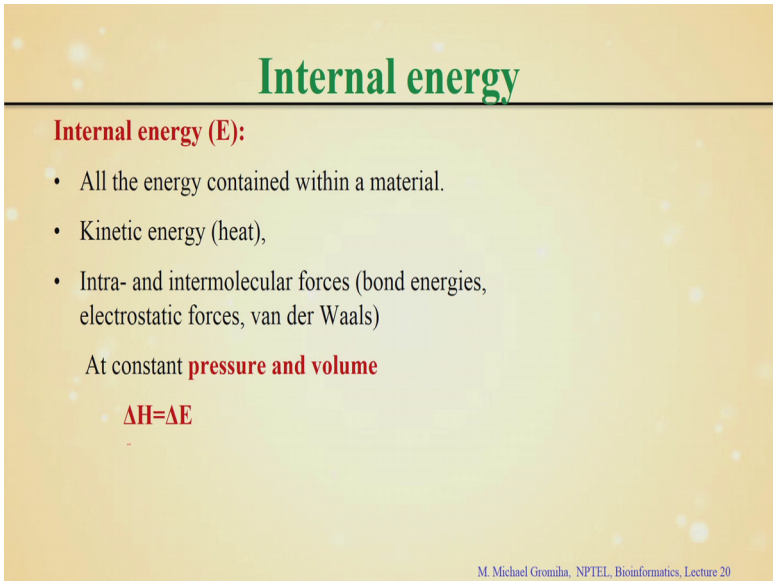

So, constant pressure and volume, delta V equal to 0 in this case you can see delta H equal to delta E right this is internal energy of the system right.

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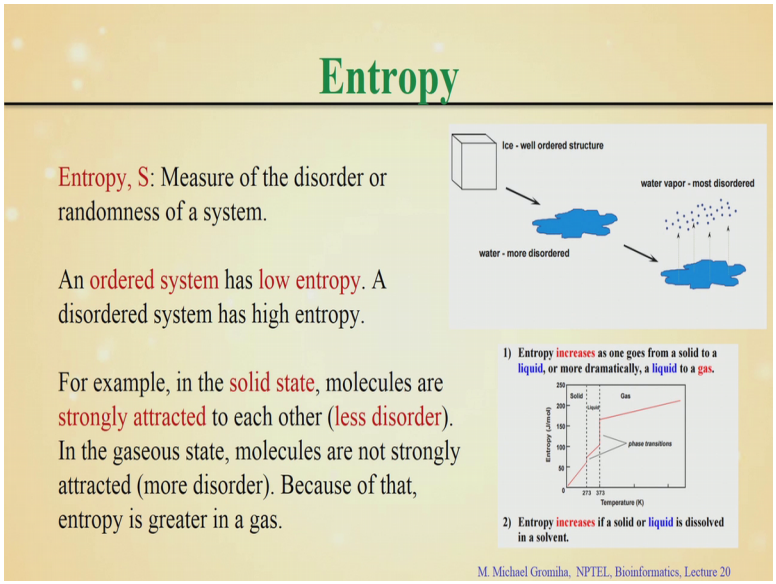

You can represent delta H as the internal energy of the system; that means different types of interactions when you go to entropy simply entropy means the disorderness or the

randomness in the system. So, we comparing the ordered system and the disorder system which will have high entropy.

Student: Disorder system.

Disorder system right the orders system as low entropy and the disorder system as less entropy for example, if you have a solid state this ice right you can see them go into water either into vapour. So, the case of ice; what is this more random or less disorder with less disorder it is more order. So, in this case, the entropy is.

Student: Less.

Less, right and compared to the water then its increases in water and you can see water vapour you can see its mostly disordered and also you can see the difference from the solid to liquid and liquid to gaseous state. It is very highly disordered from the liquid to gas state.

So, now if we have the enthalpy and the entropy and also along with this temperature, that you can explain the free energy that is called Gibbs free energy.

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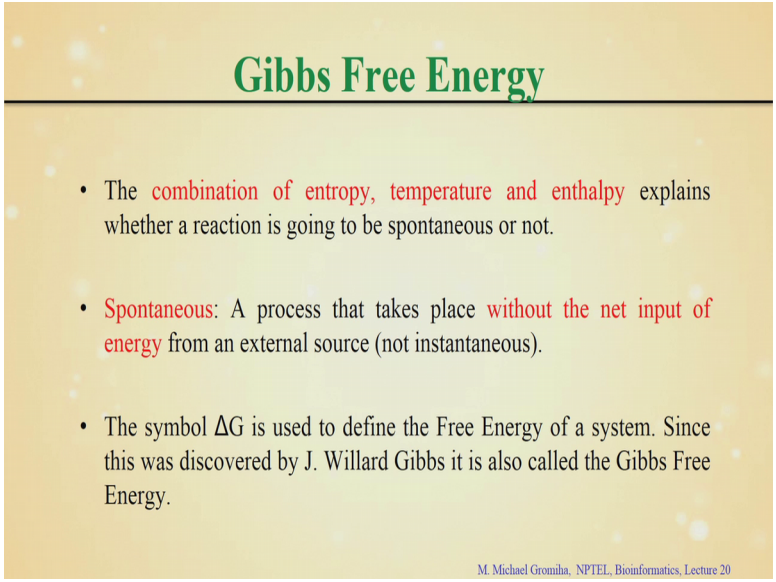

So, this free energy is spontaneous if there is a net input of energy. So, in this case right this spontaneous one. So, you have the Gibbs free energy we can use a simple delta G to different Gibbs free energy because we use the word Gibbs because firstly discovered by

the J W Gibbs. This is reason why we call this as Gibbs free energy. So, we can combine the enthalpy entropy, right.

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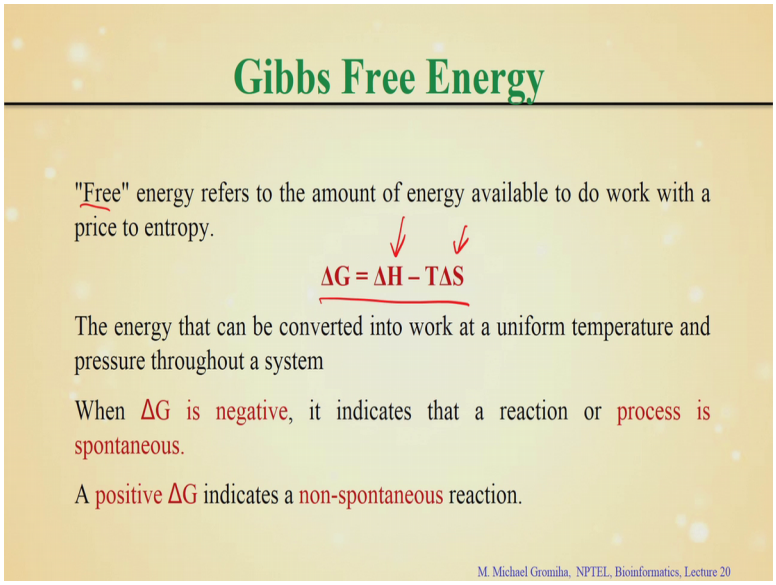

And temperature using the equation delta G equal to delta H minus T delta S why we call this free energy because it is a energy available to work with the expense of the entropy. So, this is why we call this is the entropy of the system. So, this is the enthalpy of the system. So, you can see delta G is given as delta H minus T in delta S if delta is negative in this case the process is spontaneous which can do work, we do not need any extra energy required to carry out the work if it is positive then we need non-spontaneous we need to give energy to do the work. This is the reason if you use this delta G usually it is negative. So, in this case it can do the work spontaneously, how to interpret this delta H and T delta S to get the value of delta G. So, if you see the different types of interactions.

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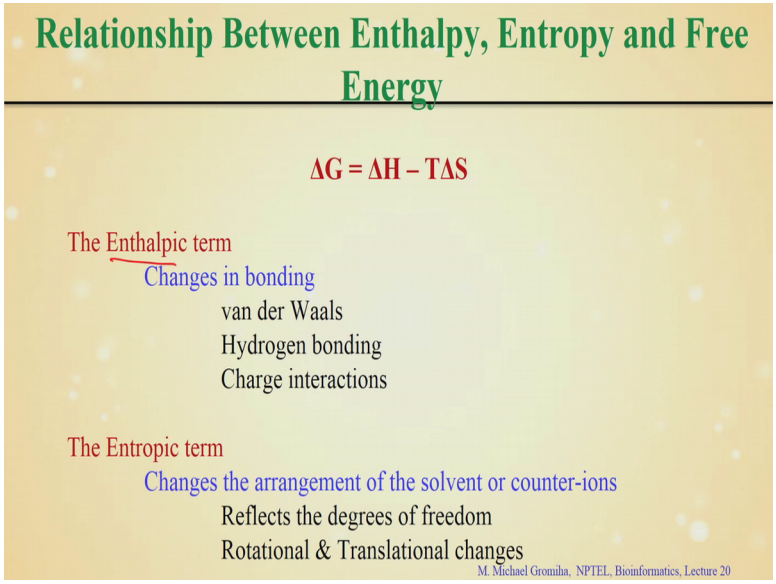

You can see delta H this enthalpy is a mainly the changes in bonding energy you can see different types of non-covalent interactions like van der Waals energy hydrophobic energy, hydrogen bonds and other charge interactions and look into the entropic term you can see this is the arrangement of the solvents in counter ions how they exchanges for example, the rotational degrees of freedom translational changes and so on. This will account for the entropy of the system.

So, we have enthalpy and you have entropy you can calculate free energy right that is the general definition for the free energy, if we talk about the proteins when you discuss in terms of the stability in proteins how you obtain this free energy how to obtain this delta G and how this delta G is related with the contributions of different interactions, you can get the experimental data using different experiments.

On one hand on the second hand you can see these experimental delta G can be related with the other interactions like electrostatic van der Waals and hydrogen bonding interactions. We will see how we obtain this information one hand delta G, you can obtain from the experiments, right.

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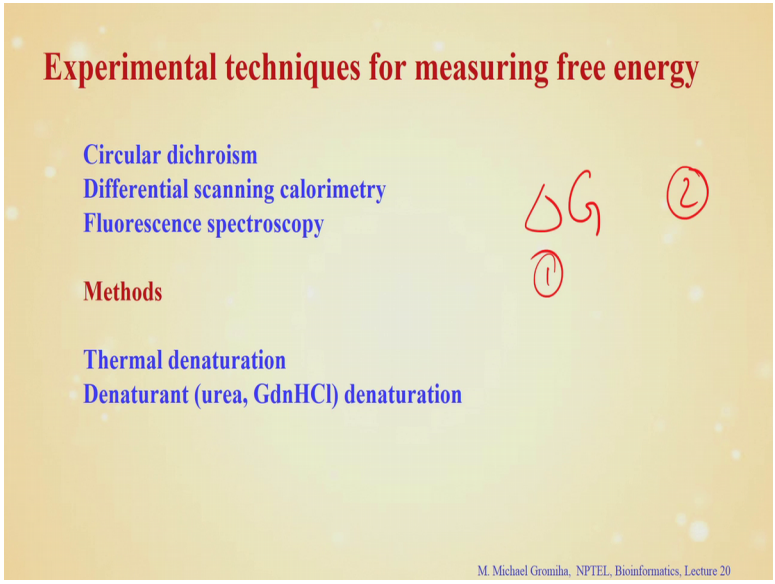

For example circular dichroism or differential scanning calorimetry or fluorescence spectroscopy and so on, right. There are 2 different ways either we denature with thermal by giving heat or we can denature using the denaturant like the urea or guanidine hydrochloride and so on. So, experimentally you can get this is one side. So, on the other hand, you can see get the delta G, you can calculate delta G from protein 3D structures various interactions and you can relate how far this can be related with each other. So, let us see first how we get the experimental free energy using any of these techniques right here.

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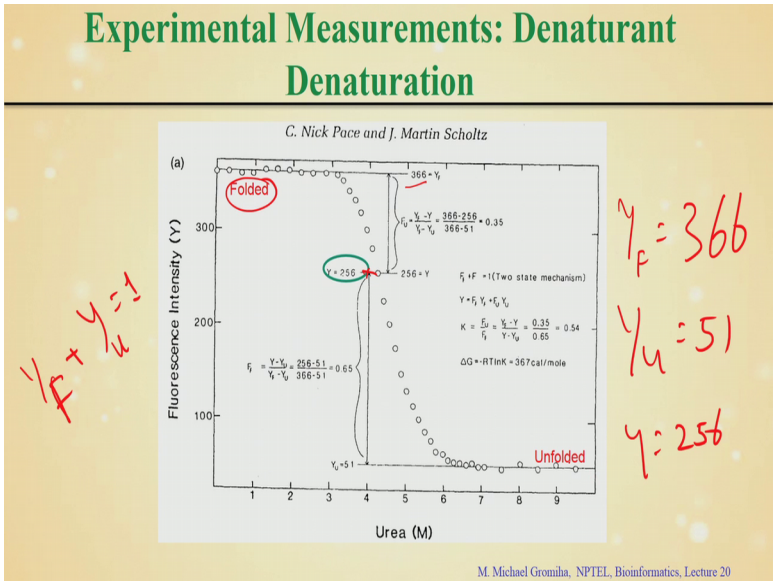

You can see the data for the fluorescence spectroscopy so and urea denaturation. So, we add urea there you can see the different concentrations of urea. So, here one to nine and you can see how we get the fluorescent intensity in these spots right in the left hand left most side. So, if you and see, here this is the folded state right and slowly if you add the denaturant it comes to slowly denature and if you see the denaturation; this side and finally, in this stage, you can see is completely unfolded.

So, in the folded state if you see the intensity is 366 right. This is the called y f this equal to 366 and unfolded state you can see this is y u equal to 51, then using this graph for different concentration and the fluorescence intensity values it is possible to derive the value for delta G at any point for example, if we take this point. So, in this case y equal to 256, right 256, then what is the contribution for the folded state and what is the contribution from the unfolded state.

If we take any point then if you add up the contribution for a folded why folder plus the contribution for unfolded this equal to one right here in this case a fully folded fully unfolded and if we take any point; that is the fraction.

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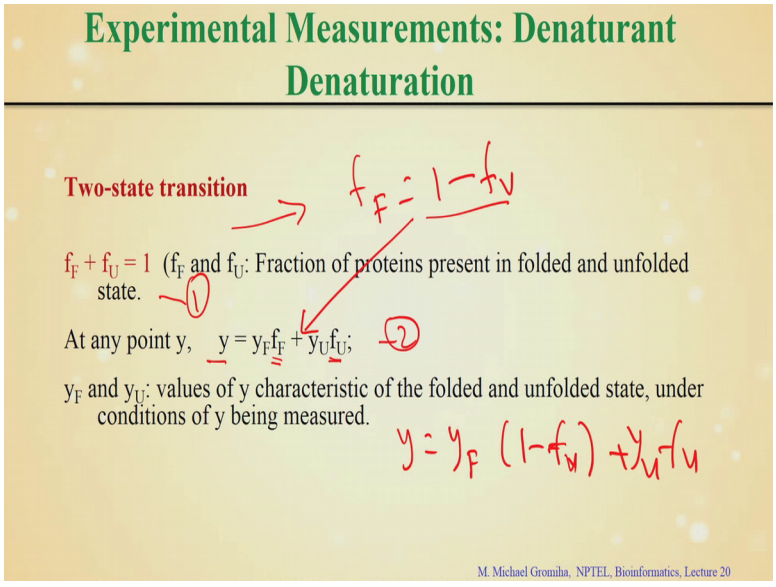

In this case, you can give the value as any point y in the fraction y you can give as this is the fraction for the folded state and here is a fraction for the unfolded state y equal to y f into fF plus yu into fU. Now you compare this equation the equation number one and

equation number 2 you can derive the values now for fU and fF; for example, here fF plus fU equal to 1.

So, fF equal to from this equation right, fF equal to one minus fU now you substitute this value right in this equation.

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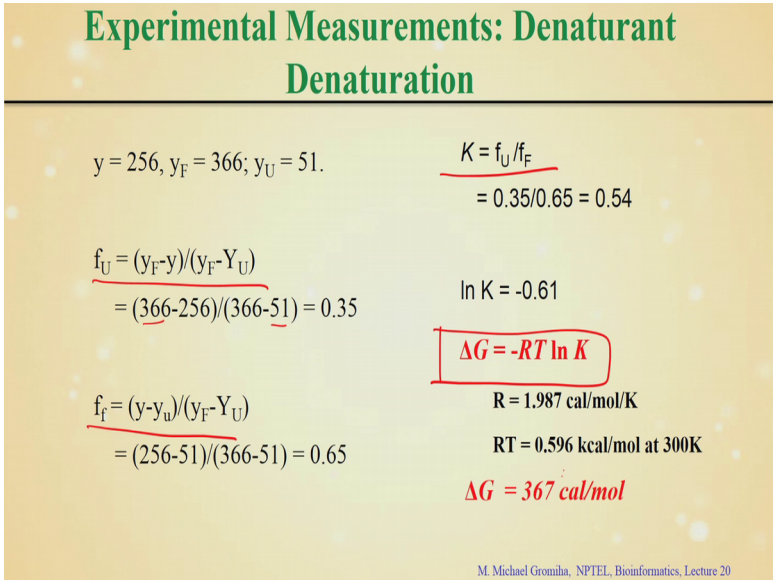

You can calculate the value of fU right, yF minus y divided by yF minus YU likewise you can get the fF as y minus YU divided by yF minus YU right for if you take this number right fF equal to minus fu. So, here this will become y equal to yF into one minus fU plus yu into fU right, then you combine together right the fU and fF and you solve this equation you will get fU as this equation now you have the values yF equal to 366, right here you can see the 366 and here y unfolded equal to 51 and you take any point for example we take this point right this point.

So, in this case y equal to 56, substitute this values in this equation then f u equal to 0.35. This is equal to yF is 366 unfolding is 51 and the mid-point you take 256, then the fF. This is given as y minus YU divided by yF minus YU right you give the values of yu yF right we will get that value 0.65. So, we have the unfolded state and the folded state fractions we can convert this information into equilibrium constant that is k.

k is given as fU minus ff, here fU you calculate 0.35 fF we calculate as 0.65 right, we take the ratio, then it is 0.54, then we take the logarithmic of this one right this is equal to

minus 0.61 from that we can calculate delta G. So, this is minus RT; logarithmic of this equilibrium constant. So, RT equal to 0.596 kilo cal per mole or 300 degree kelvin ln k is minus 0.61. So, substitute is value here and delta G, you can get 367 kilo cal per mole. So, if you do the experiments at different concentration of urea you get the fluorescent intensity values this will give you the intensity at the folded state and the unfolded state and that the different stages from the folded to unfolded state.

From the intensity values of the folded state unfolded state and at any point you can derive the equilibrium constant and from the equilibrium constant you can calculate the free energy right experimentaly you can get right for any protein right using the fluorescence spectroscopy likewise you can do it with the circular dichroism as well as differential scanning calorimetry. So, this is based on denaturant denaturation likewise you can use thermal denaturation. So, what is thermal denaturation?

Student: (Refer Time: 16:55).

They heat, right.

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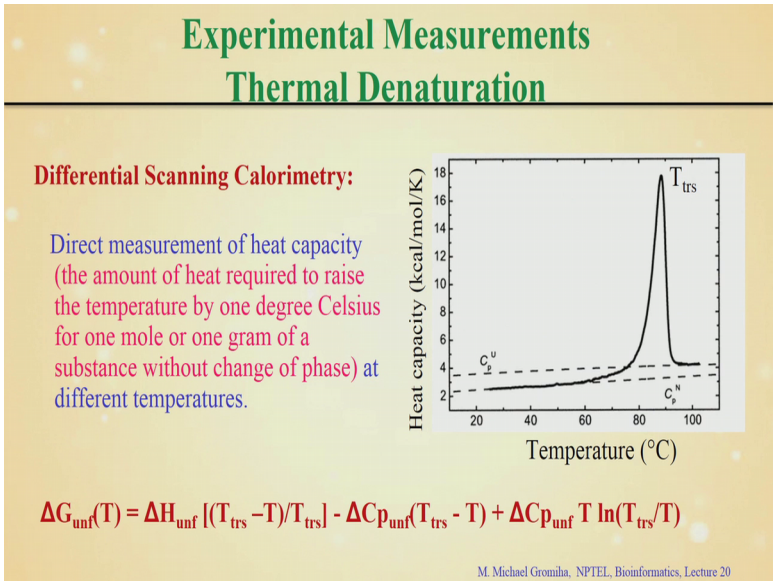

So, here you can see with respect to temperature versus heat capacity. So, in this case, you can directly measure this transition temperature it is the amount of heat required to raise the temperature by 1 degree Celsius with respect to the 1 gram of substance without any change in the state right with no change in the phace you can calculate. So, you see

the increase in temperature and finally, you get the transition temperature right where the protein started to melt and you have delta Cp because here the Cp at unfolded state and Cp at the native state and get the difference right get the difference this will give you the delta Cp using the delta Cp and the transition temperature you can calculate the unfolded delta G at any temperature.

T is any temperature and you can get the other information from this graph and you can get the delta G using thermal denaturation. So, you have the data of delta G with the denaturant denaturation and thermal denaturation right these are the experimental data now the question is how we interpret this data using the contribution from different interaction energies. So, how to do this?

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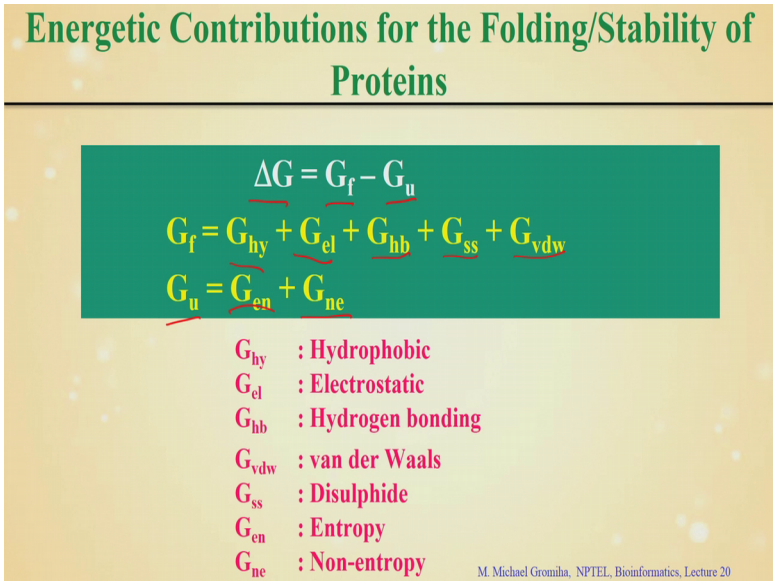

So, as we discussed earlier what is delta G.

Student: Free energy.

It is a free energy difference between.

Student: Folded.

Folded state unfolded state. We can see the folded state and you can see the unfolded state. So, what are the contributions of the folded state is really enthalpy of the system. So, different interactions; what are the interactions?

Student: Hydrophobic.

Ghy; hydrophobic free energy.

Student: (Refer Time: 18:27).

El electrostatic free energy.

Student: And (Refer Time: 18:28).

And hb is hydrogen bonding free energy disulphide bonding free energy and you can see van der Waals free energy these are all due to this folded state. If you take the unfolded states what is the major contribution the entropy right. We can see the entropy contribution and if you compare the folded and unfolded state unfolded state is not completely random there may be some interactions or present which are available in the folded state this is what we call as non-entropic term.

For example, if you have some sort of hydrogen bonds which are present in the unfolded state this is for this we use non-entropic term now we see how we estimate all the interactions put on protein 3D structures right we already we explain various parameters what the various parameters we discussed using 3D structures hydrophobic surrounding hydrophobicity different types of interactions and the solvent accessibility and the contacts right we discuss all these aspects.

So, we use some of this information to estimate different interactions which contribute to the folded state of the protein. So, let us just discuss about hydrophobic free energy can be directly measure the hydrophobic free energy we cannot measure right this kind of imaginary force right. So, we can get the partition coefficient right. So, relative solubility of either the water plus organic solvents. You can use the octonol you can ethanol right you can use the concentration to get the partition right. So, you can see this non-polar to aqueous and the different concentration.

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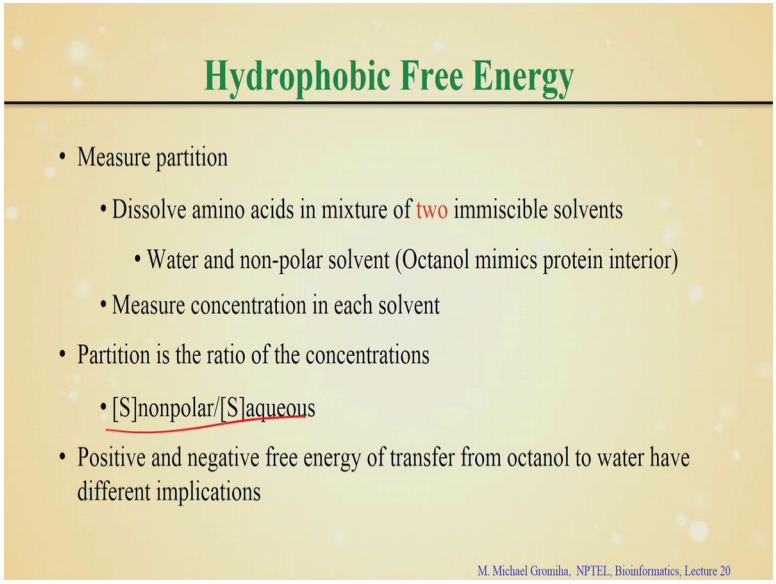

And you can see the free energy. Positive gives for the hydrophobic and the negative gives this non-polar amino acid.

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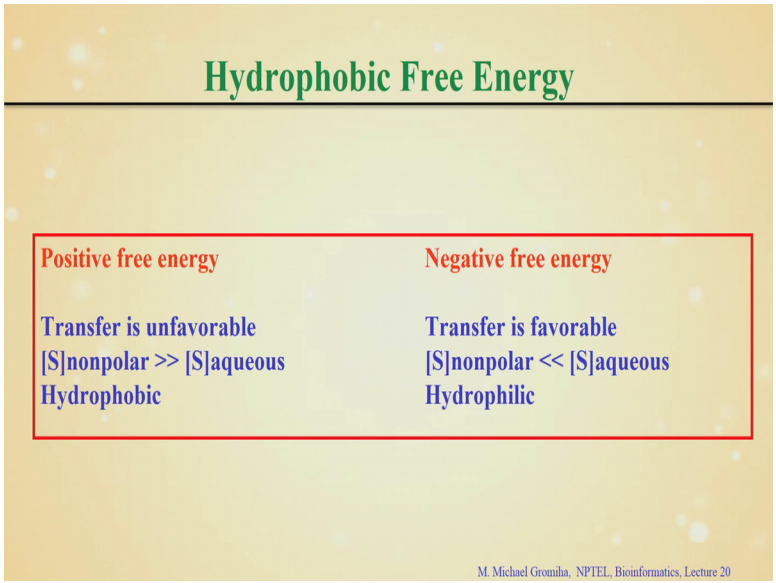

So, if you see the positive free energy. So, transfer is unfavourable in this the case the solubility of non-polar is more. So, this use the hydrophobic residues and negative for energy in this case it is less for the non-polar and using hydrophilic residues.

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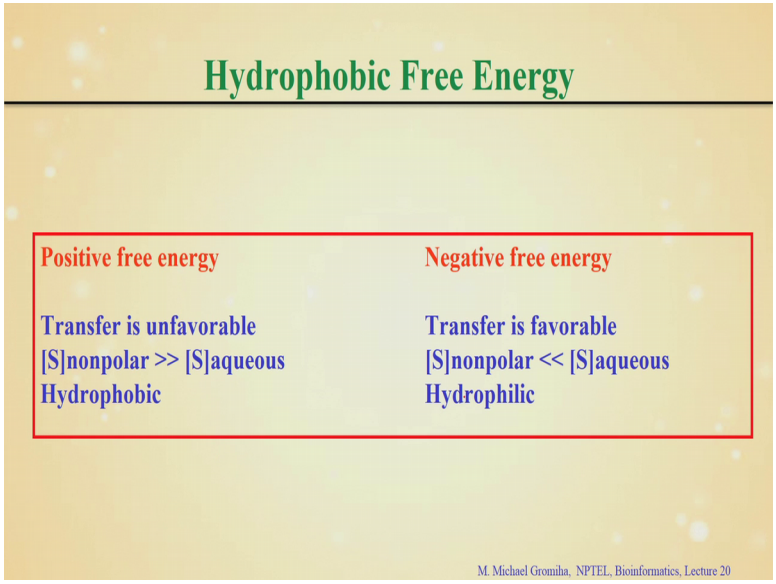

So, now if we have this amino acid residues, we have the values, but for the proteins we cannot directly measure the hydrophobic free energy, but if you look into these parameters that we discussed in the previous classes about solvent accessibility and hydrophobicity; what is the correlation?

Student: (Refer Time: 20:44).

Negative correlation; it is inversely relater right, if it is highly accessible, the less hydrophobic and if it is less accessible this is buried they are highly hydrophobic, right.

You can use this information right, hydrophobicity we cannot measure directly, but accessibility can we calculate, yes, if you have the structures, then you got different methods what are the methods to compute solvent accessibility?

Student: Excess and (Refer Time: 21:07).

Access and Naccess right.

Student: (Refer Time: 21:09).

We have the getarea right we have several methods we can calculate. So, then we convert this solvent accessibility to calculate the hydrophobic free energy right I will show you how to do that.

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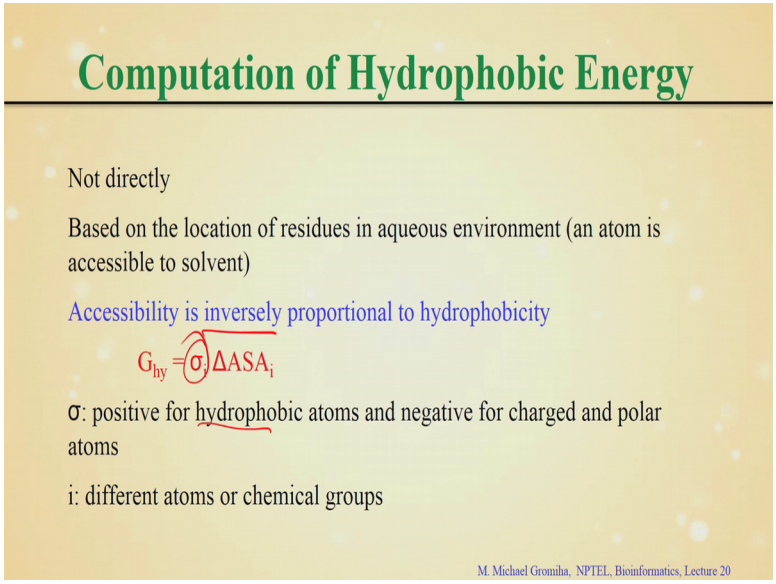

So, we know that accessibility is inversely proportional to hydrophobicity. So, we can write G hy hydrophobic free energy which is proportional to delta ASA of i. So, this proportionality we put this sigma I this is atomic salvation parameters how different groups of atoms like carbon or hydrogen or nitrogen or the oxygen behaves in the environment of this protein environment.

So, you can see different chemical groups and if you see this is positive for the hydrophobic atoms and negative for the charged in polar atoms right because of the inversely proportional between the hydrophobicity as well as the accessible surface area. So, now, how to get these numbers right if you have this atomic salvation parameters.

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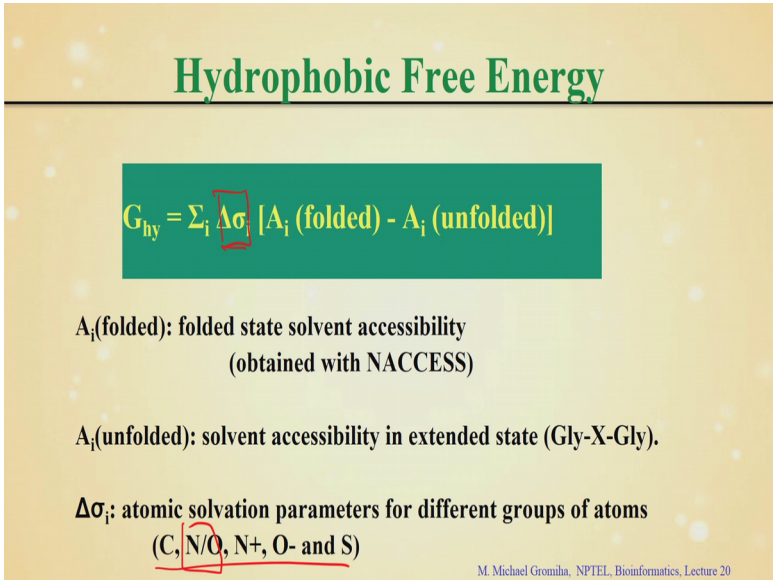

And then we can calculate free energy right, for example, if you have the atomic salvation parameters for the different types of atoms, here I use 5 different types of atoms right what are the different atoms in protein structures?

Student: Cho and (Refer Time: 22:19).

C, nitrogen, oxygen.

Student: Sulphur (Refer Time: 22:22).

Sulphur and the nitrogen, we have 2 different types one is a neutral and other is positive charge oxygen, we have 2 types neutral and negative charge. So, here the neutral nitrogen and oxygen we combine together right N and O and N plus O minus and S, but we can use different classifications right, we can use nitrogen oxygen separately and the carbon main chain carbon and the side chain carbon separately right depending upon the connectivities depending upon the amino acids we can use several classifications right for simplicity here we use 5 different classes if this is known and then we can calculate the hydrophobic free energy this is the method initially proposed by Eisenberg in the 1984, right they published a paper in nature.

So, here Ai folded is the folded state accessibility A unfolded is unfolded state accessibility. So, can we get unfolded state accessibility, yes, because we if you have the Gly x Gly conformation and we have calculate different systems and see the average or

see the maximum values we can get unfolded state can we get the Ai folded state accessibility.

Student: Yes sir.

You can get if it is each atom. So, which algorithm can be used DSSP.

Student: No.

No, we cannot use DSSP because it gives you a residue wise, NACCESS we can use right or getarea you can use for each atoms. So, we have the value in the folded states and you get the value of all unfolded state and if this is known delta sigma i you can calculate the hydrophobic free energy. It is very simple. Now the question is how to get this delta sigma i, right.

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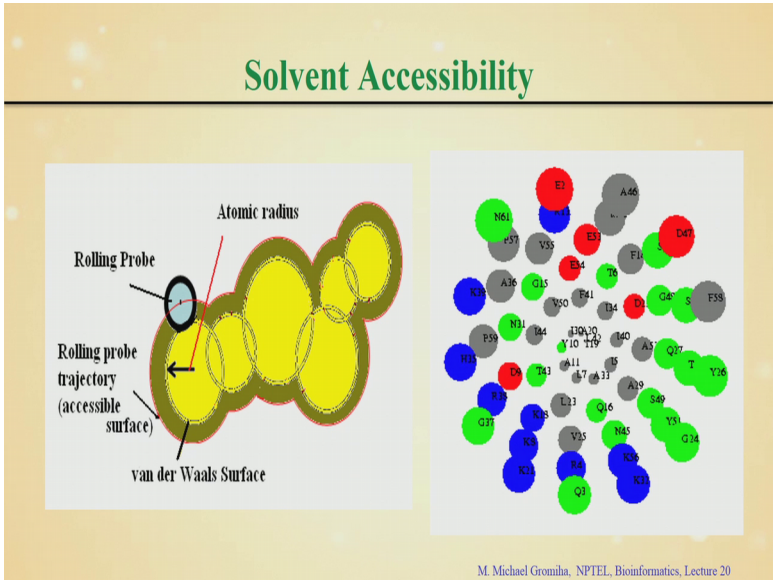

I show you the accessibility, the accessibility criteria we know by rolling water molecule you can see how far this accessible to a solvent each atom accessibility to solvent right. Here, this is a figure you are familiar with this figure now, right.

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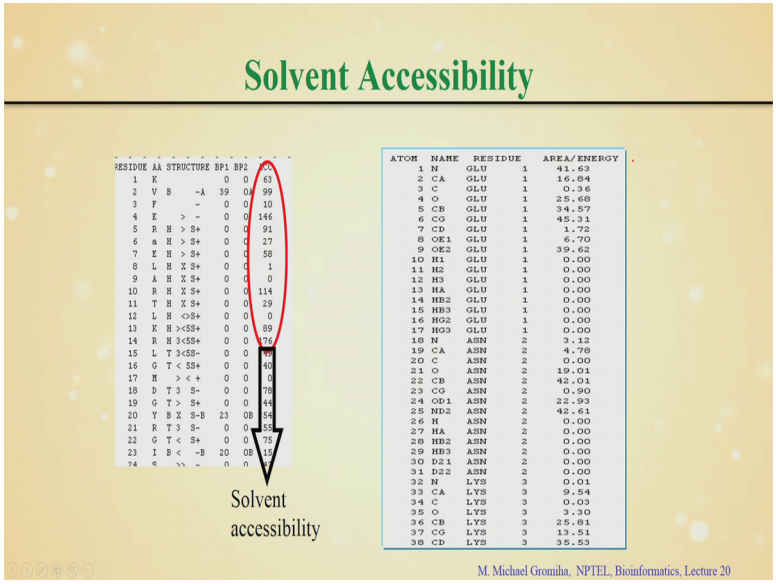

You can see this residues are in the interior and these residues are at the surface and if you run the program you can get the values right either the residues accessibility or the atomic accessibility for calculate this hydrophobic free energy which data is required atom wise or residue wise.

Student: Atom wise.

Atom wise. So, you can see different atoms you can use these values to calculate the hydrophobic free energy.

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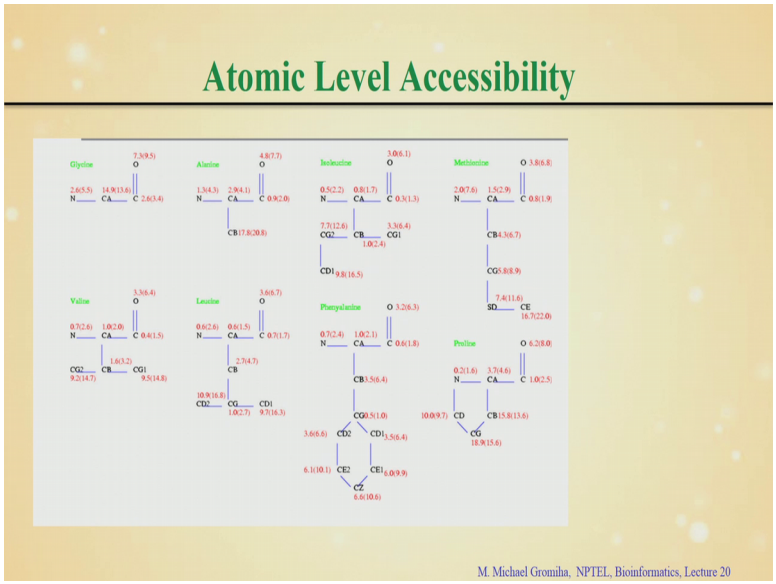

So, here; this is a atom level accessibility. So, we can calculate for each residues and all atoms. So, this for the glycine alanine for all the 20 residues we can have the extended state accessibility right you can have the values, right.

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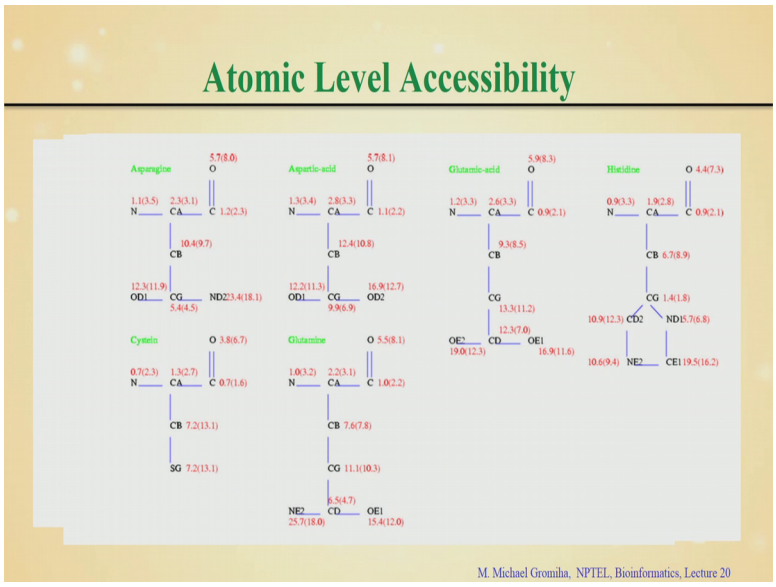

These are the 20 different amino acids we have the data for all the atoms this is fine this is for the extended state, right.

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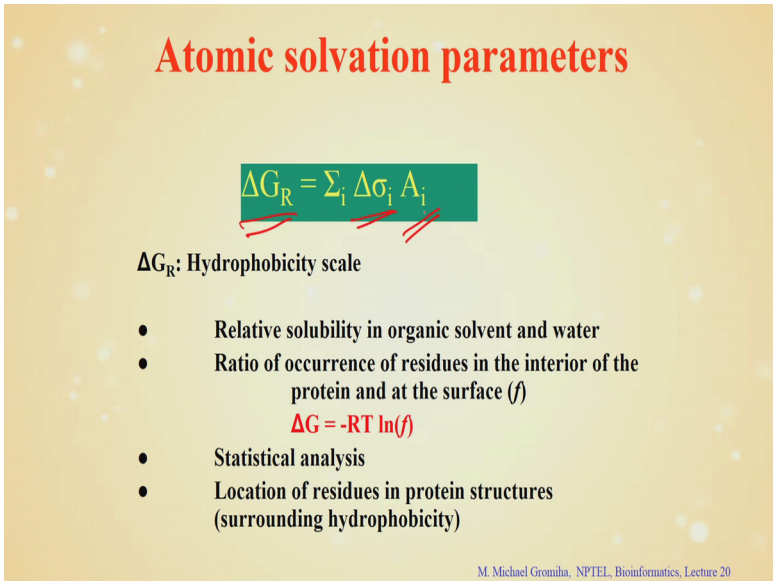

Now, we have the folded state then you can calculate, but the problem is how we get the delta sigma I we need to calculate then how to get this delta sigma i. So, we relate delta

sigma i with respect to accessibility and the hydrophobicity. So, if the delta gr represents the hydrophobicity scale for example, you can take any hydrophobic indices right we discussed various kind of hydrophobic indices either you have obtained from experiments thermodynamic transfer experiments or you can get from the computational methods for example.

Student: Surrounding.

Surrounding hydrophobicity right you can use any value for delta GR. So, how many different amino acid residues?

Student: 20.

20 residues so far each amino acid residue; for example, alanine you can derive an equation delta sigma i and the ai also we know for each the atoms we know if you take alanine.

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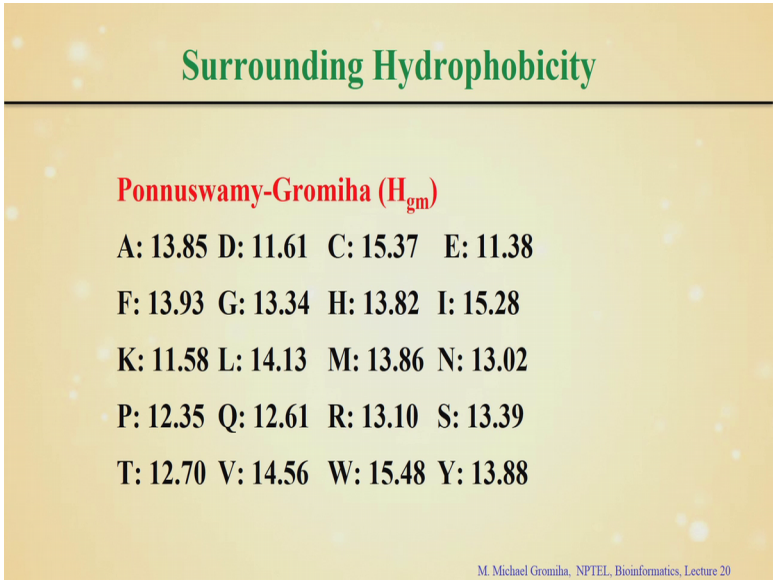

You can use these hydrophobicity value what is the value for alanine.

Student: 13.85.

13.85; this 13.85, this is the left hand side, we relate with this atomic solvation parameters, how many atoms in alanine.

Student: (Refer Time: 25:58).

Right, 5, 5 atoms right; 4 main chain atoms plus 1; 1.

Student: Side.

Side chain atom.

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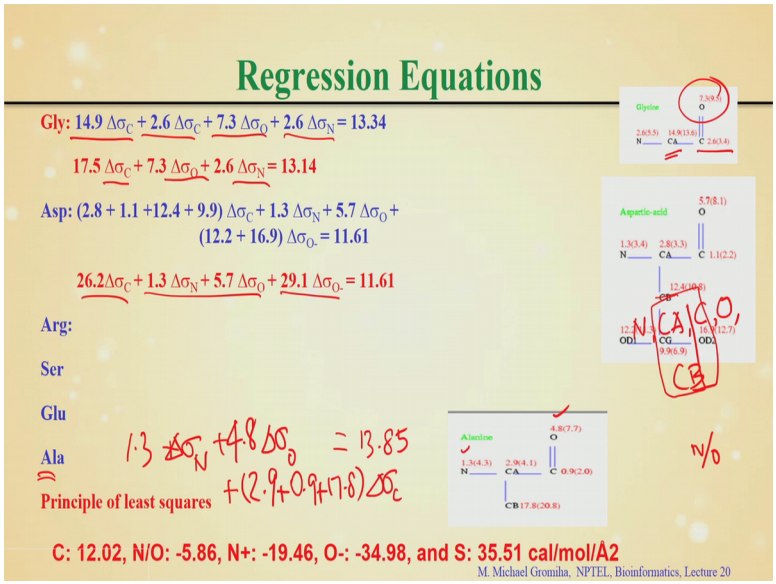

So, if you make you know different groups right for example, if you see this alanine right alanine. So, we can see that this is the data for alanine right y axis that is equal to 13.85 x axis this side right left hand side you can see that is a combination of different sigma i. So, different atoms in leucine alanine this is N C alpha C O and C beta, but here this come to C right here and you can see N and O together.

So, in this case you can see this N is 1.3, right, this 1.3 delta sigma N there is a O here, there is 0.9 plus 0.9 delta sigma O plus for the C, C right. So, what is the C totally, this is 2.9 and 2.9; oh sorry, this is not 0.9, this is O is 4.8, right, O is here. This is 4.8, C is here, you can this is 2.9 plus 0.9 plus 17.8. This is a delta sigma C right you can write this equation.

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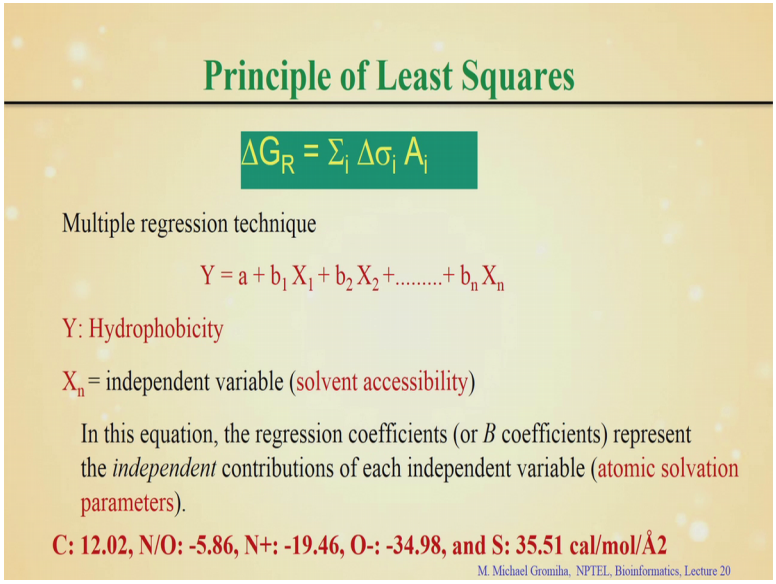

You can use this equation right y equal to a plus b 1 x 1 plus b 2 x 2 plus up to bn xn here if you see y is the hydrophobicity right here this is Y is hydrophobicity and delta sigma is the solvent accessibility this is the parameters we need to identify this one right this is the b 1 b 2 all these things right and ai is the solvent accessibility.

So, you need to calculate the coefficients b. So, for the alanine we get this one right for the glycine this is a glycine. So, we see this is the C, this is 14.9; 14.9 into delta sigma C and 2.6, this also C right 2.6 into C and O is 7.3, right.

7.3 is here. So, 7.3 O and N is 2.6; 2.6 sigma N, what is the value for the glycine. So, we get the values here glycine equal to 13.34. So, in this case it is equated to 13.34; likewise you can do for all are 20 different amino acid residues. Here we take the glycine for you have the data only for the delta sigma C and delta sigma NRO, so only 2 variables. Now you will get the equation likewise alanine you got the 2 variables if you go with the aspartic acid, how many groups you have.

Student: (Refer Time: 28:55).

Right you can see the C.

Student: (Refer Time: 28:59).

And N and O, you can combine together and there is O minus; what is O minus?

Student: negative charge (Refer Time: 29:04).

The negative charge; you can see the aspartic acid right. So, here if you see right you can see this od 1 and the od 2, this is 12.2, right and 16.9, right, you can add the add up these 2. So, it will be 29.1. So, you get 20 differential equations. So, 20 equations how many variables.

Student: 5.

5 variables right. So, now, we can see this y axis. So, you can see the; this is the hydrophobicity values. What is the dimensional of this matrix?

Student: 20 into.

20 into.

Student: 5, 1.

Right 20 into 1. So, you have the 20 different values.

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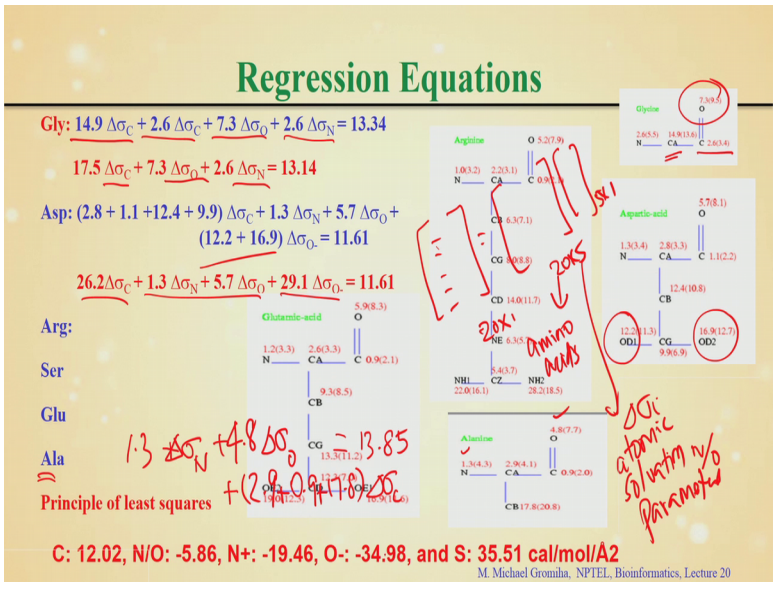

For example alanine 13.35 a Aspartic acid, we have 20 different amino acids right. So, this you can write in terms of these are the; what is this dimension?

Student: 20 into 5.

20 into 5 right because this is a 20 amino acids and 5.

Student: 5.

Atomic solvation parameters right this is 20 amino acids and this 5 for the.

Student: I (Refer Time: 30:14).

Delta sigma I atomic solvation parameters what is the dimensional of this matrix.

Student: 5.

5 into one right because this is for the 5 delta sigma i plus 1. So, in this case right if you have 5 equations and 5 variables.

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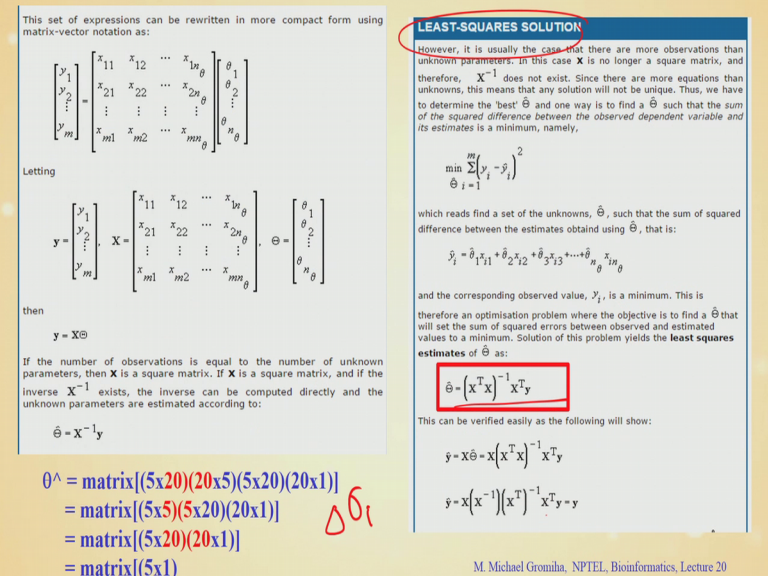

Then easily, you can solve it using principle of least squares right this is the equation this is the hydrophobicity and this is the 5 different types of atomic solvation parameters this is the matrix which have these values, but here our case, we have 20 equations and we have only 5 variables in this case we use the principle of least squares to see that identify the numbers right the vibrations.

So, that the deviation is the minimum. So, you can see the deviation is the minimum of this y minus y bar the whole square if you do like this you can calculate the atomic solvation parameters using this equation x transpose in the x right this inverse.

And the x transpose into y if you do like this finally, you end up with the matrix 5 star 5 into 1. So, because its 5 into 20 this matrix and inverse this 20 into 5 and you can do the transpose there is again 5 into 20 right and this transpose 20 to 1, then you get solve this matrices finally, you get the matrix 5 into 1. So, here you can get the delta sigma i parameters.

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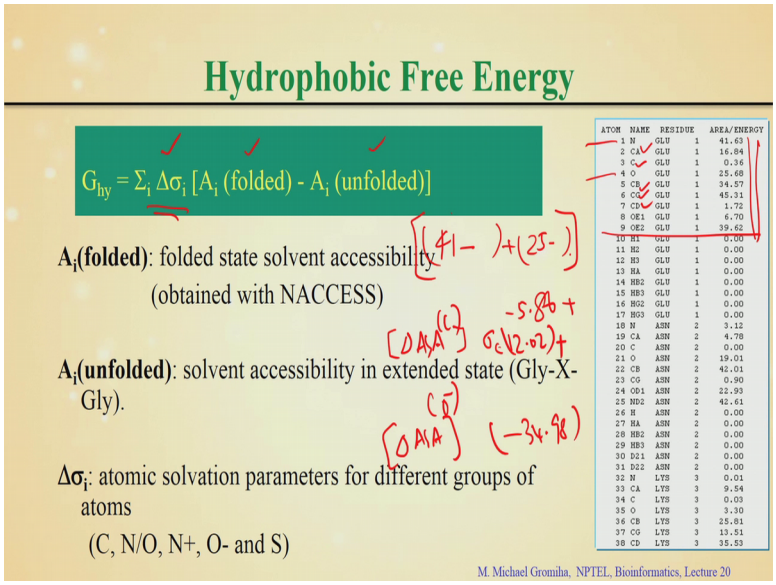

So, if we do this we can get the values for all these 5 parameters and I show the number this is the numbers you obtain for the 5 groups for example, carbon 12.02 and nitrogen oxygen minus 5.86 N plus minus 9.46, O minus 34.98 and sulphur 13.1, it makes sense because if you see the hydrophobic groups mainly carbon and sulphur you can see the positive values right for the transferred into the protein environment and for the polar residues and the charged residues that is minus and if you compare the polar and the charged one charged one you have more negative right, then the polar ones; that means, these numbers are meaningful and they are reliable right, you can calculate the energy.

So, when you get the delta sigma I right now we can easily calculate Ghy because we know this we know the Ai folded right we know the A unfolded. So, now, I gave a data right for example, is for your particular protein you run Naccess and then you can get the folded state ASA. So, example if we take the glutamic acids right residue number one.

So, here you have the values of ASA for different atoms for the same glutamic acid you can get the unfolded state accessibility right I will show it here right. So, glutamic acid you can right it is here.

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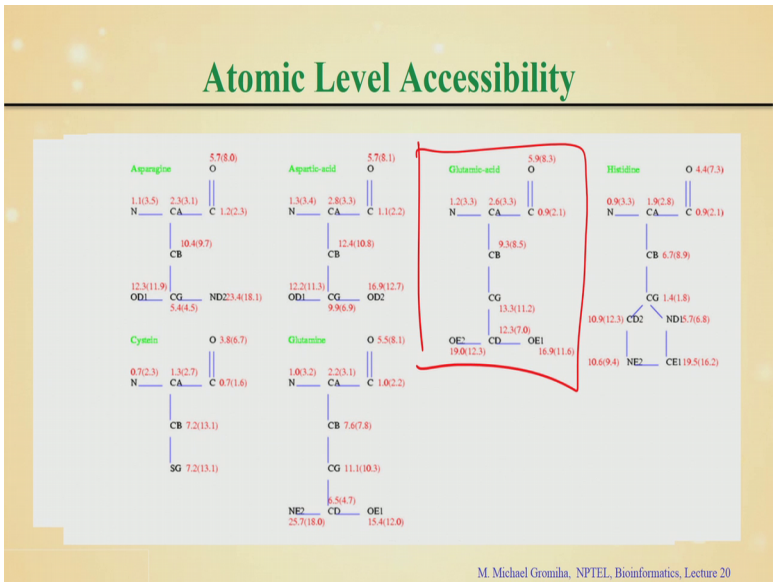

So, you can see the glutamic acid we have the unfolded sate ASA. So, the unfolded state ASA we have folded state ASA we have delta sigma I we have. So, you can calculate the hydrophobic free energy. So, how to calculate the hydrophobic free energy right delta h? For example, if you take this one for this we take the N and O we can join together, so in this case forty one minus sixth forty one minus unfolded state we have to get from this a figure and then again for the O 25 minus you can see the value from this one multiplied by what is the value for N and O minus 5 point 8 6, then plus go with all the carbons 1 2 3 4 5; 5 carbons, you get the difference in accessible surface area and multiplied by delta sigma of or the sigma of C what is the value of sigma of C this equal to 12.02 right this 12.02 plus you can go for this y one and y 2 . So, in this case the value is minus 34.98 right minus 34.98.

And you can see the delta ASA of C of O minus. So, we need the data from the folded states and for all the 20 residues, you have the data in the unfolded state and for each atom type you get the difference and multiplied with the atomic salvation parameters. So, this is a folded state unfolded state multiply then we get the values. So, do it for all the

residues and add up everything together then you will get the value for the G hy right for all the atoms in a particular protein.

So, easily you can calculate so if we have the 3D structures you can calculate the hydrophobic free energy even you can use the PDBparam; that is the server which calculates more than fifty structure based parameters you can get the hydrophobic free energy right for any protein of known structure right it is easy now you can calculate the hydrophobic free energy.