Biomathematics

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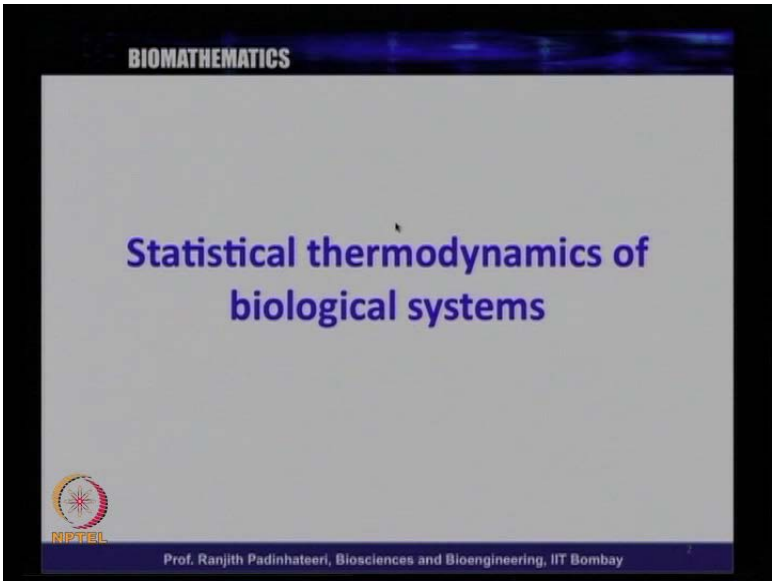
Lecture no. # 38

Thermodynamics of Protein Organization along DNA.

Hello, welcome to this lecture on biomathematics we have been discussing statistical thermodynamics of various biological systems. So, today we will continue discussing that. So, the module the section is basically the thermodynamics of biological systems. (Refer Slide Time: 00:33)



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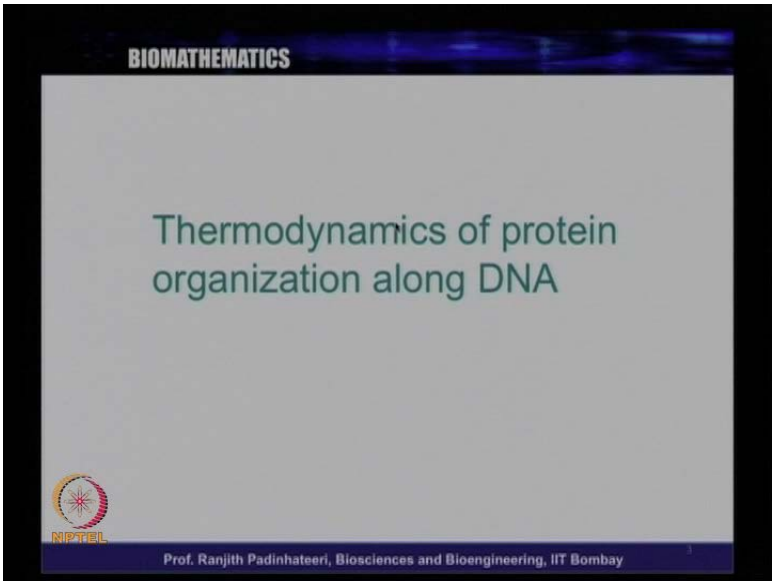

We will be in today's lecture specifically we will be discussing thermodynamics of protein organization along DNA. So, what do I mean by thermodynamics of protein organization along DNA, you all know that in cells, DNA is not just simple polymer there are many proteins that is binding and associating then with bound on to the DNA and many proteins will have to do various functions by by intro on to DNA. So what you want to understand is the simple how something system similar to you did not go in to the complexity that we seen in by see in biology, but we will take a simple system that we can think of as an in vitro system and we ask the question using thermodynamics can we say something about protein binding protein binding and dissociation.

So, one aspect of this is again a very wide the little large amount of worlds like very big topic that proteins bind in to DNA, but we will only ask a very simple question in this a very limited our purpose is very limited we will ask only one simple question. Imagine that you have a DNA of particular length and there are let say some number of binding size let say n binding size and there are some number of proteins available using thermodynamics can be predict how many proteins will be bound on to the DNA. If you have let say ten binding size and seven proteins are available, can we say all the seven will be bound or only five will be bound can we say can we predict something about this and the answer is yes, we can predict and thermodynamics will the statistical ideas that we discussed will tell you that how to predict this. So, let us let us think about it.

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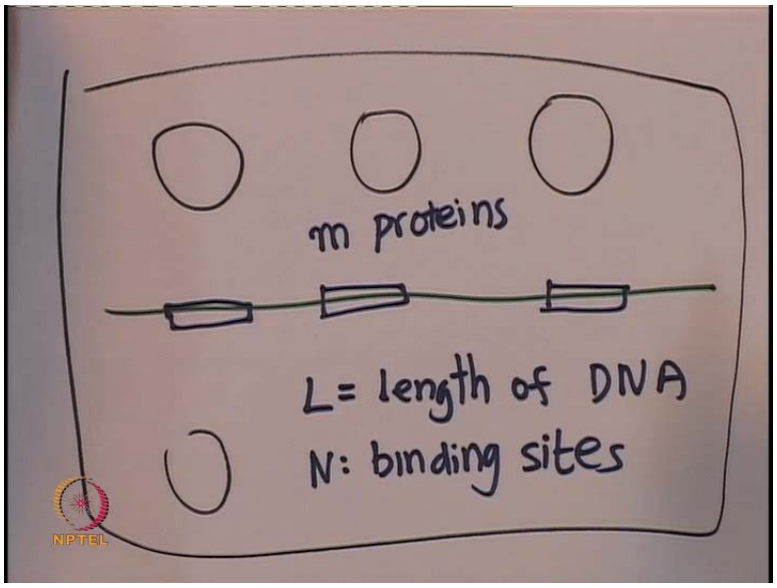

So, have a look at here what we are going to discusses. So, we have imagine that DNA as a line like this. So, we have a certain DNA of interest and we are thinking of in vitro situation let say somebody is doing an in vitro experiment were they take a DNA of particular length L. So, L is length of DNA.So, you take let say one K B to two K B or few m b DNA and imagine that there are certain binding sites along the DNA. So, let say there is a binding site here, there is a binding site here and there is a binding site here. The three binding sites and there are various proteins in the solution. So, this is the in vitro situation we are thinking about. So, there are, you have a DNA of length l and you have one to three here like. So, let say there are N binding sites.

So, this this proteins bind to some specific sites and if there are N of them here N is equal to three and then we have let say m protein. So, the here like circles I draw are proteins. So, let say m proteins in the solution. This is what somebody doing an experiment they take a DNA of length L and there are N binding sites and m proteins in the solution. Now the question is we are asking is how many proteins will be binding to this will all this three sites will be occupied or will only one be occupied or all that probably two will be occupied or what is the probability that each of this like its everything is some you can think of its probability or what is the equilibrium state at in equilibrium that is the what is the most likely state that is will it be already bound on this or this or like how many of them will be occupied.

So, the question we are asking is essentially what percentage of this sites what fraction of this sites will be occupied. So, we lets lets write if there are N binding sites and m proteins. In equilibrium, at at equilibrium, how many of the proteins will be bound on to the DNA, how many of the proteins will be bound on to the DNA.

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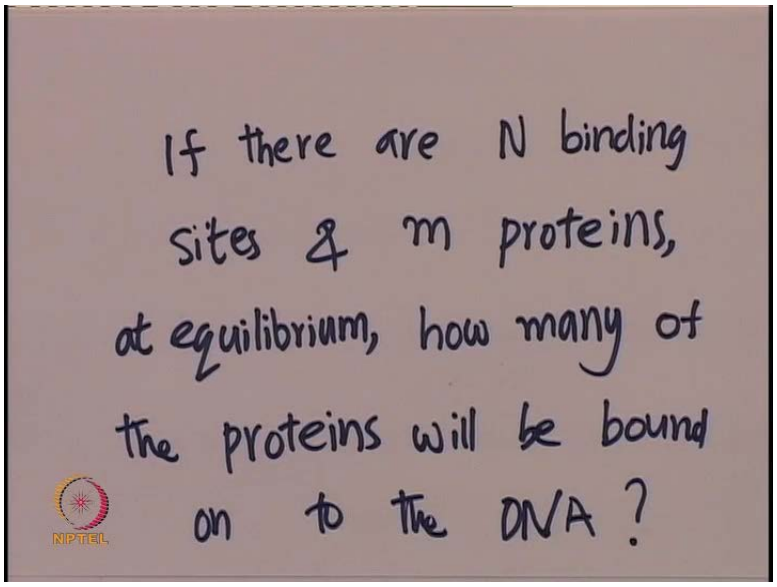

So, this is the question we are asking and we can at the end of this lecture there will be an answer this many what will be the the answer will be how much there will be a certain number like 20 percent, 30 percent, 50 percen,t 80 percent of the sites will be occupied by proteins we can we can answer this question. So, to look at this when we think we are discussing statistical thermodynamics. So, some ideas from statistical thermodynamics has to be used here.

So, we discuss that equilibrium is decided by the minimum of free energy you have to find the free energy and see look at where is the minimum of the free energy and that will be the equilibrium state there will be a stable equilibrium state. So, we want to calculate free energy of the system that is the way to go ahead. So, if we can calculate the free energy of a protein DNA system, we will be able to tell something about this this I am will be able to answer this question. So, let us think about the free energy of the system. So, before thinking of free energy what happen when DNA proteins bound to de bind to DNA. So, let us look at that a minute.

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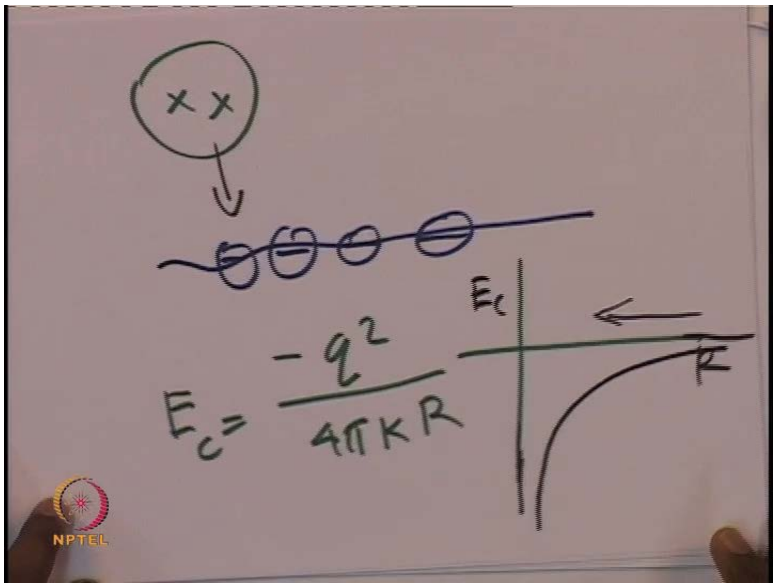

So, typically DNA is something like negatively charged and protein typically will be like positively charged in this case let say protein positively charged. So, when they bind there will be an attraction between this. So, if you think about this energy this let say this chain interaction energy the proteins will be. So, the let say using electrostatic attraction and the energy is q square by four pi some all the constant let me write K in to R. So, our R is the distance between the two charges. So, this is the coulomb energy. So, if we have the coulomb energy this will tell you that even the R is and there is a minus sign this is the plus charge and minus charge. So, there will be a minus sign there.

So, what does it tell this, tell that if you plot this Ec versus R coulomb energy versus R what you would get is that some curve like this? As R goes to very small as R goes to 0. So, this is 0 the energy will decrease decrease because as R goes to 0 this is go to minus infinity. So, the energy will be minimum the coulomb energy will be minimum, when they are when they bind binding means they come together. So, when this protein binds to the DNA R will decrease and the coulomb energy will be minimum. So, according to the coulomb energy you would they would one the proteins to bind. So, that the energy is minimum the in that sense you think of that all the proteins should bind. So, let us think about. So, there is some energy associated with binding and it involves coulomb energy plus some other interactions like sulfa interactions and. So, on and. So, forth. So, this will be little more complicated energy, but let say when one proteins binds to a DNA, the system gains an energy let say minus epsilon K_B T.

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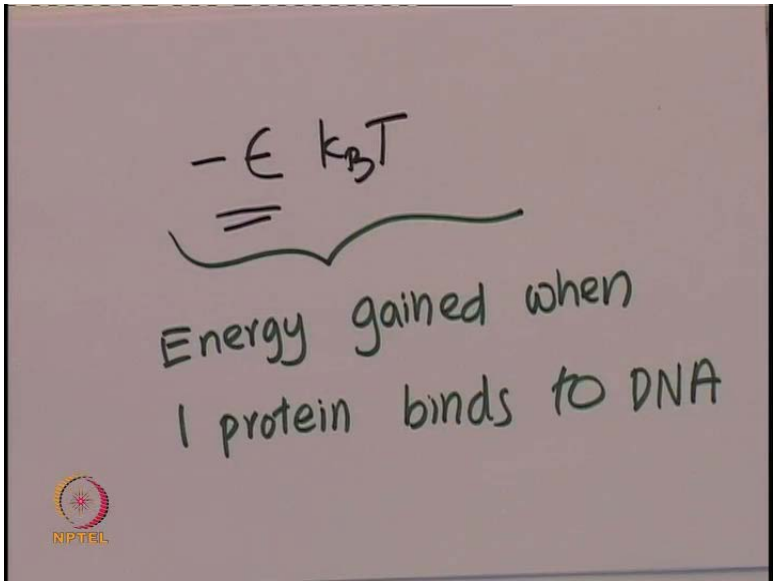

So, let say this is minus epsilon K B T. So, what is this. So, this is energy gained when one protein binds to DNA. So, this is certainly epsilon can be some number one two three four or one. five three. five. So, K_B T is the unit of energy. So, the K_B T is ten power minus 21 joule. So, certain amount of joule of energy will be gained. So, when a protein binds to DNA when a protein goes and binds certain amount of energy will be gained. So, this is the energy gain.

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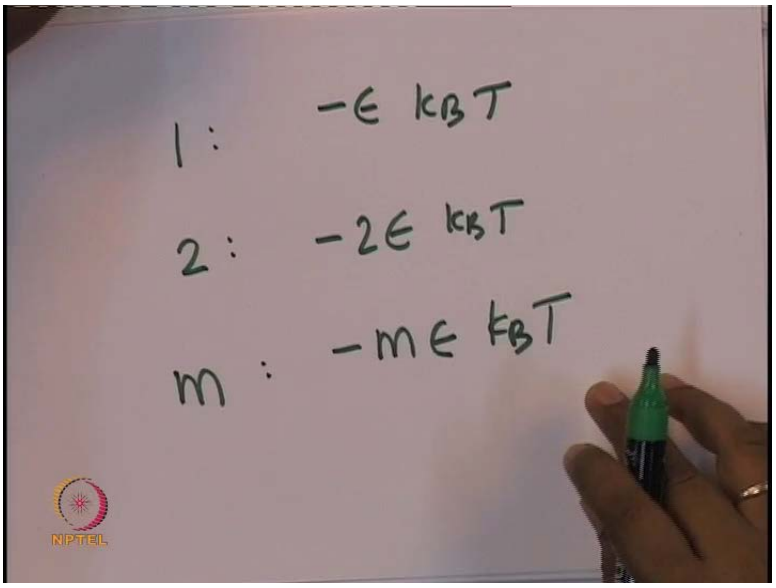

So, if there are two proteins bind the energy gain will be minus twoepsilon protein K_BT. So, let us think about it. So, when there are two proteins bind they, one protein minus epsilon K_B T is the energy gain, two proteins minus two epsilon K_B T. So, similarly three protein minus three epsilon K_B T there are m proteins the energy gained is minus m epsilon K_B T. So, this is the energy gain. So, let us let us what what are we writing here. So, let say write. So, if you plot. So, let us plot for a minute the energy gain.

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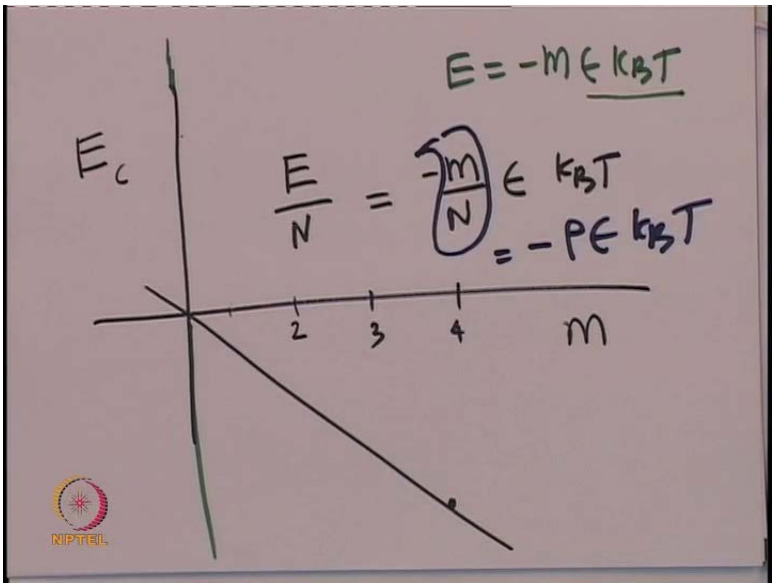

So, the the energy gain by protein binding versus number of proteins m. So, the more the if there is just one protein is just. So, this is this is zero. So, So, let say there are some certain energy gain and the the curve will be we said that the curve will look like minus energy is minus m K_B T minus m epsilon K_B T. So, epsilon is a number K_B T is a number so epsilon is the positive number K_B T is the positive number. So, this is like y is equal to minus some constants m times x. x is m here. So, this graph will look like this. So, something like this the graph will look some something like this. So, the what is that mean the more the number of the proteins bind the energy is less and less.

So, if there are m. So, this is m is equal to one, m is equal to two, m is equal to three, m is equal to four and. So, on and. So, forth. So, the more the proteins bind the energy is less. So, according to this energy concept the system would like more and more protein all the proteins to bind if there are four proteins if all the proteins binds the energy will be least. So, but what about, but we said that energy is not alone the thing that matters. what matters is energy minus temperature in to entropy some contradict called free energy that is what it matters. So, entropy also plays an important role in deciding what is the equilibrium of the system how many proteins will be binding. We have to also now calculate what will be the entropy. So, that is the thing that we are going to calculate entropy. So, before that lets think about this a minute here this energy is m epsilon K_B. So, let me divide this by N energy per there are N proteins energy per protein is minus m by N epsilon K_B T. So, m by N there are N binding sites out of that m is occupied.

So, what is this mean? This is basically the fraction of sites that is bound the m is the m by N will be the fraction of sites that is bound here m actually means the number of sites occupied number of proteins bound m is here basically the number of proteins bound. So, there are N binding sites m proteins bind. So, m by N is the fraction of proteins bound. So, let this fraction we call it a rho. So, if we can call this as density or fraction this. So, this will become minus rho epsilon K_B T just to remind that we can rewrite this in a simpler fashion and we will use this.

So, this rho is basically density; that means, how many proteins are bound out of there are N binding sites the fraction of proteins bound that is what it is simplify we are rewriting it in a simple manner. So, that we can use rho in a little easier manner. Now, we have to think about entropy. So, we said that energy is not enough if by knowing just knowing about energy wouldn't be sufficient. Energy only tells you some part of the story and the real thermodynamic equilibrium is decided by the free energy.

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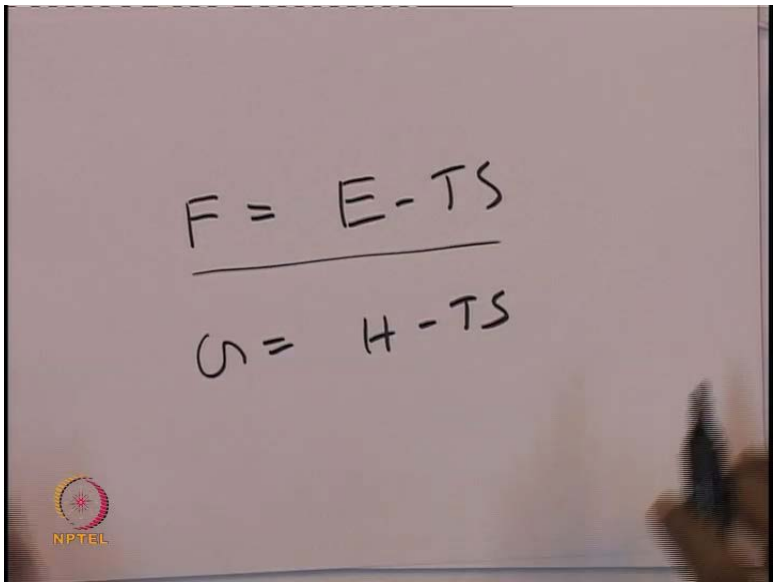

And the free energy as we said is F is E minus T S same as G Which Is H minus T S. H is also energy only. So, write in a different form. So, we will use this here, but does not matter exactly the idea is same you can subtract this part like, it you have to calculate entropy and this is then only you will get the free energy and free energy is what decides the equilibrium of the system. So, let us calculate what the entropy is. So, we said that entropy is nothing, but law is related to log of the number of possible arrangements. So, if you can count entropy or we just discussed how to be discussed how do we calculate entropy the way to calculate entropy is count all possible arrangements is just simple counting.

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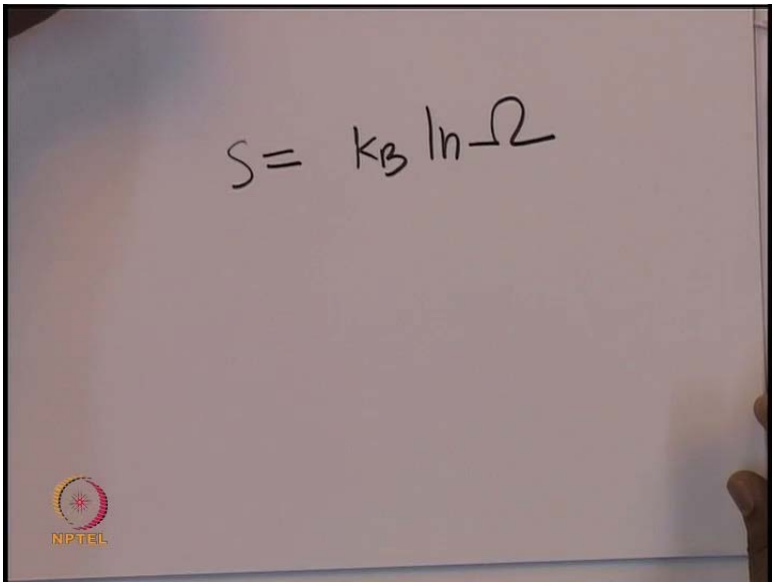

If there are five possible arrangements of proteins then entropy will be minus sorry K_B log five. So, we said that S entropy is K_B log omega which is the number of possible arrangements now let us think about what are these arrangements here. So, we have DNA here. So, let us think about this in a simple manner let us say there are three proroteins three binding sites and two proteins.

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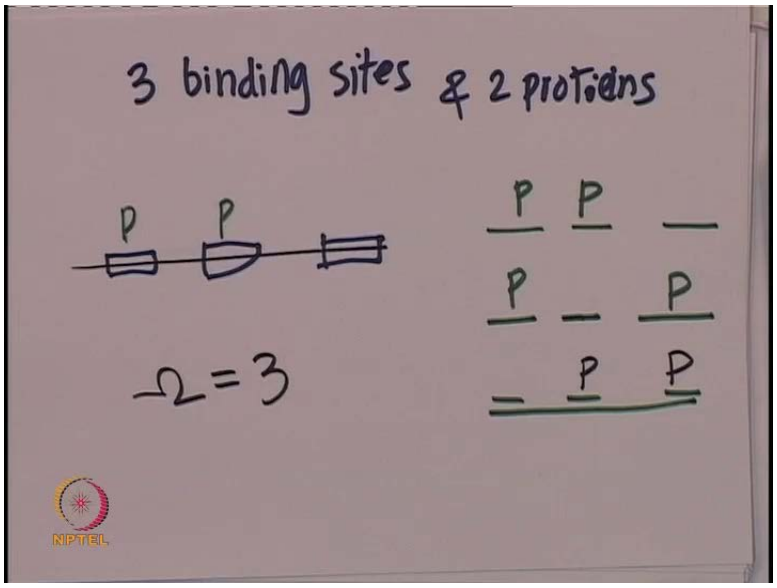

So, three binding sites and two proteins, how many of them will bind and. So, let say. So, we have this DNA and the binding sites are, let say this is one binding site, this is another binding site, this is another binding site. Now, what are the possible arrangements? So, let us think of this. So, you can have protein bound all the three proteins there are two proteins only. So, the proteins can bind here and here.

So, let say out of these three places two of them proteins bound, this is one arrangement. Another arrangement possible is this, is bound here this is bound here this is free, this is the third arrangement. This is another arrangement possible, out of this three binding sites two of them are bound by proteins. Sorry, another arrangement possible is out of this three arrangements the three binding sites out of this two of the this, two are bound this is one arrangement possible where the first site is not occupied. Is there any other arrangement possible.

So, we listed three arrangements here these two are bounds, the last one is free, here the middle one is free and the other two are occupied, here the first one is free and the other two are occupied. So, this is the, if we have three binding site and two proteins. There are three arrangements possible. So, omega number of arrangements here is three. Now think about little more four binding sites and two proteins.

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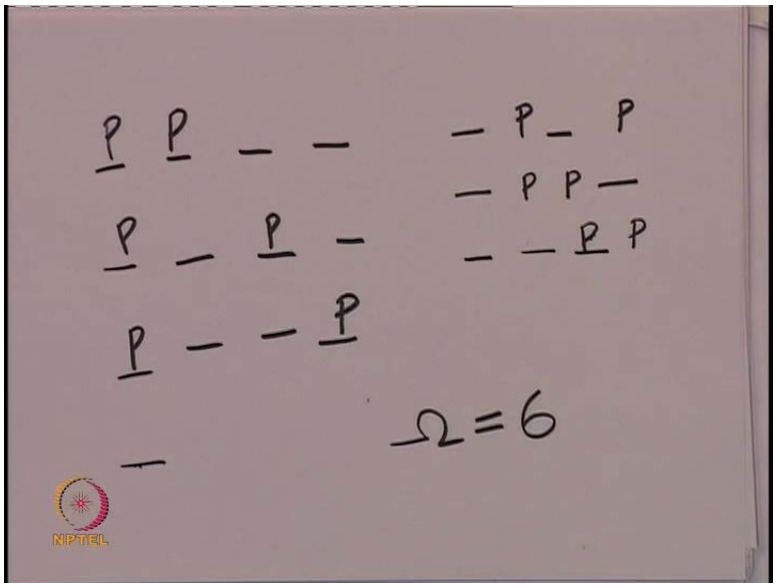

So, let us think of four binding sites. There are 4 binding sites and there are two proteins. So, then we can have this, we can have this, we can have an arrangement which is like this, we can have an arrangement which is. So, now, this here. So, this is one site we can have an arrangement this, this, this, this and we can have an arrangement this, and we can have an arrangement there are two arrangement in this particular manner possible and we already listed this arrangement P P arrangements you already listed. So, this is second, then we can have an arrangement which is this and this.

So, we now had one, two, three, four, five, six arrangements any other arrangement possible. So, the protein can bind she second site first here then here first these 2 sites are empty this and this are empty and occupied this two are this two are occupied this is impossible arrangement. Then here, this is the possible arrangement, this is the possible arrangement and this is the possible arrangement. So, I think only six arrangements are possible. So, here omega is six now think about there are four proteins and four binding sites there are four proteins and four binding sites is only one arrangement possible all binding then omega is zero omega is one and entropy K_B log omega is zero because log of one is zero.

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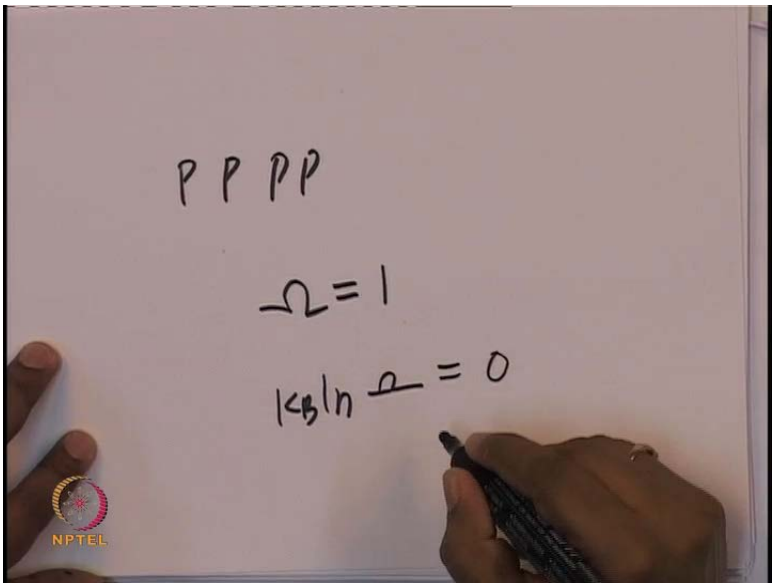

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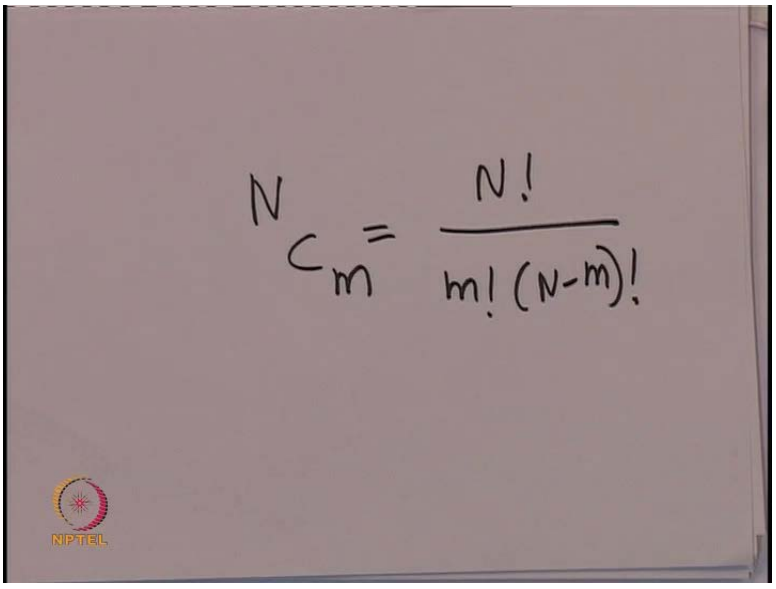

So, in this way we have to count. So, the question is, if there are N binding sites N out of them only m of them have to be chosen at a time, how many such arrangements are possible and the answer to that is Nc_m it is an combination R x you might have learn in school that in such cases there are Nc_m possibilities. So, Nc_m is N factorial divided by m factorial m minus N factorial. We studied during the probability this Nc_m. N factorial divided by m factorial by N minus 1.

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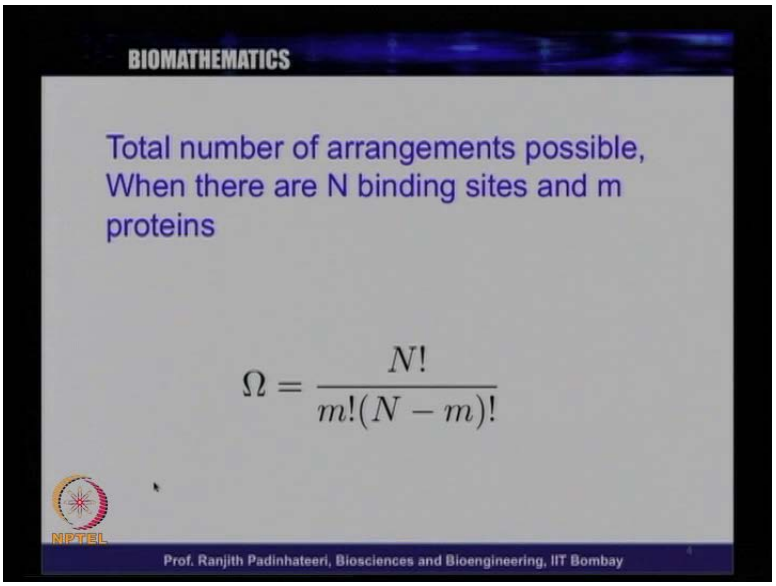

So, this many arrangements are possible. So, here is say that total number of arrangements possible when there are N binding sites and m proteins m proteins bound. So, there are m if you want m proteins to bind. So, m bound proteins that is what I mean here there are m bound proteins then this many arrangements are possible.

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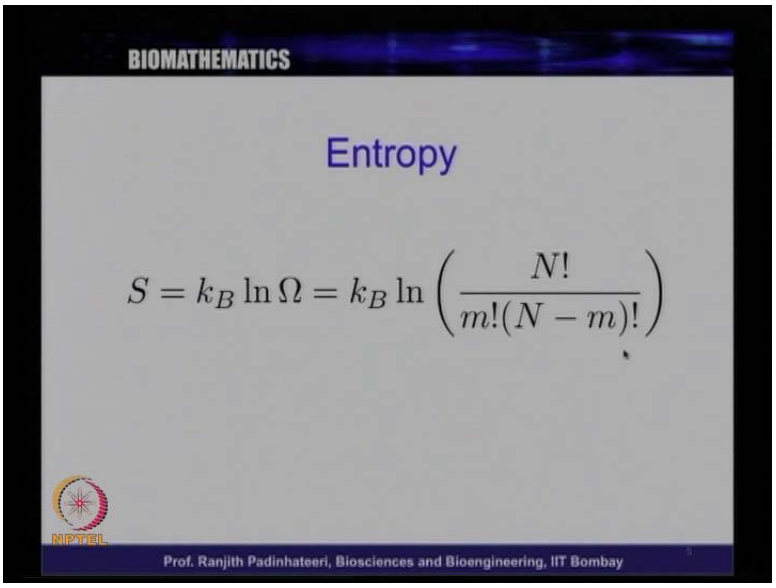

So, this is omega then the entropy has to be K_B log of omega. So, this is the entropy, S is equal to K_B log omega which is K_B log N factorial by m factorial into N minus m factorial. So, now, what is this can we say something something. So, this is our entropy if you know m, if we know look at here if we know N, if you know m, N minus m can be calculated and this number can be calculated, log of that can be calculated. So, the entropy can be calculated. So, for each N and m we can calculate entropy now you will. So, we get entropy, we get energy, when we can calculate the free energy. So, let us rewrite this in a simple manner for a simplicity. So, let say. So, there is. So, let us calculate this log of N factorial by m factorial by N minus one factorial.

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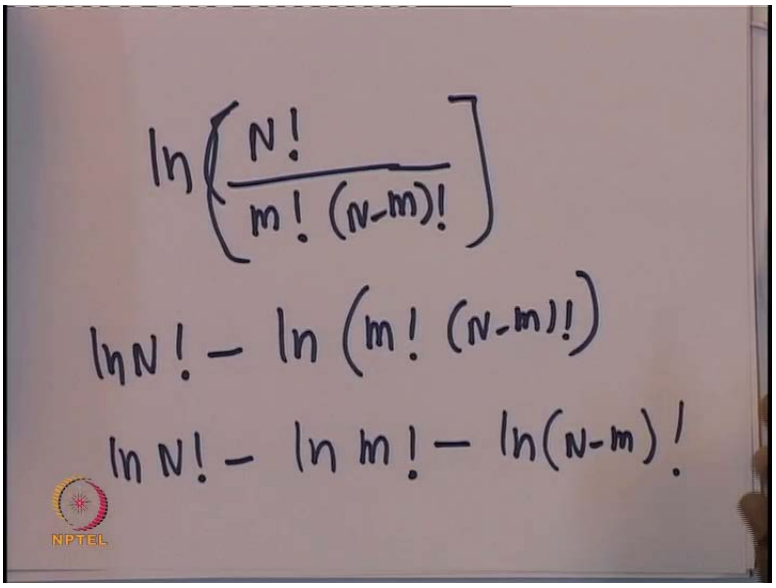

So, let us write here, log of N factorial by m factorial N minus m factorial. So, log a by b is log N factorial minus log of m factorial into N minus m factorial. log a by b is log a minus log b. Now this is nothing but log N factorial minus log of m factorial log a into log b is log a plus log b. So, log N minus m factorial.

So, you can rewrite this it tell out that there is a log N factorial can be can be easily calculated by if the N is very large there is an approximation. So, there is something called stirrlings formulae which say is that log N factorial is N log N minus N. So, log N factorial can be approximated, approximately equal to N log N minus N.

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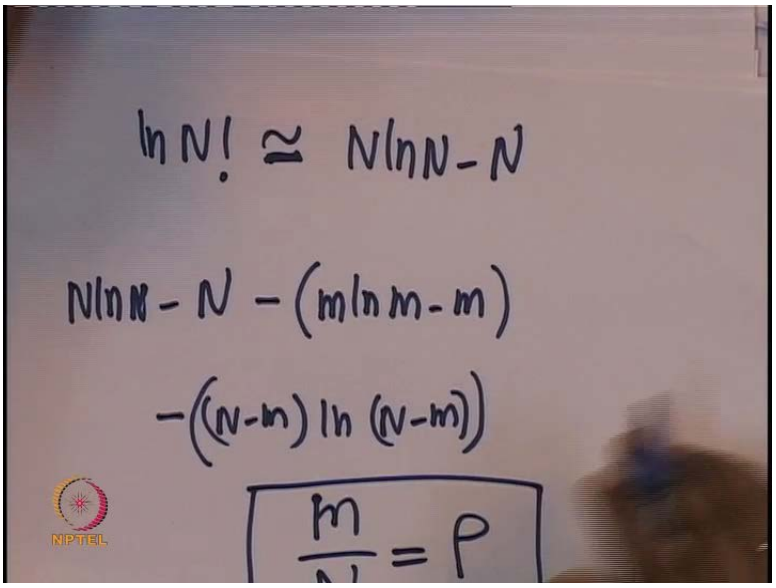

So, if you rewrite this, what would you get. Log this is N log N minus N log N minus N let us this term here is m log m minus m minus m log m minus m and here is N minus m log m minus m minus N minus m log N minus m.

Now, this is this. So, then you can see some terms canceling. So, there are few terms that will cancel from this, because there is an and we can rewrite this and we can use the definition that m by N is we define m by N is rho.

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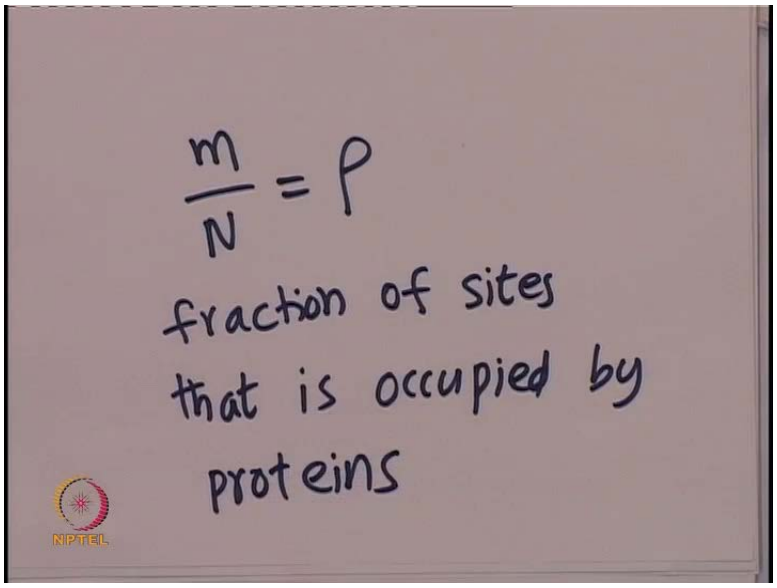

So, if we define m by N as rho. So, let say we define m by N is basically the fraction of the size that is bound by protein, this is fraction for del of sites, that is occupied by proteins. Fraction of sites there is occupied by proteins. So, that is m by N and if you use this definition s can be rewritten in this particular way S can be written as minus K_B N rho log rho plus one minus rho log one minus rho. So, we can rewrite it we can see that this is the entropy. So, we said that the energy is m epsilon K_B T which is minus N rho epsilon K_B T you can write in terms of rho here.

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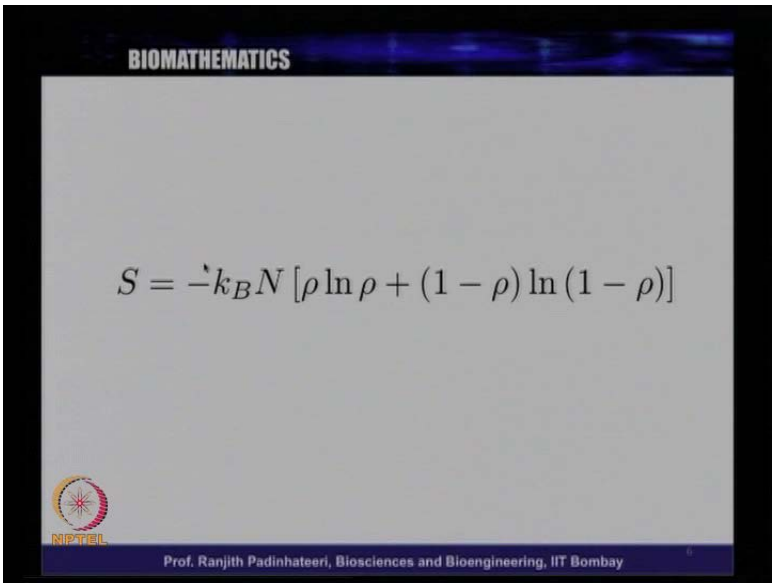

So, energy is there are m proteins bound each protein as a energy epsilon binding energy minus epsilon. So, minus m epsilon K_B T is energy and there are which is which can be written as m can be written as m rho and because we saw that m by N here if you look at here m by N m by N is rho. So, m is N rho. So, we can write this as minus N rho epsilon K_B T. So, this is energy and this is entropy.

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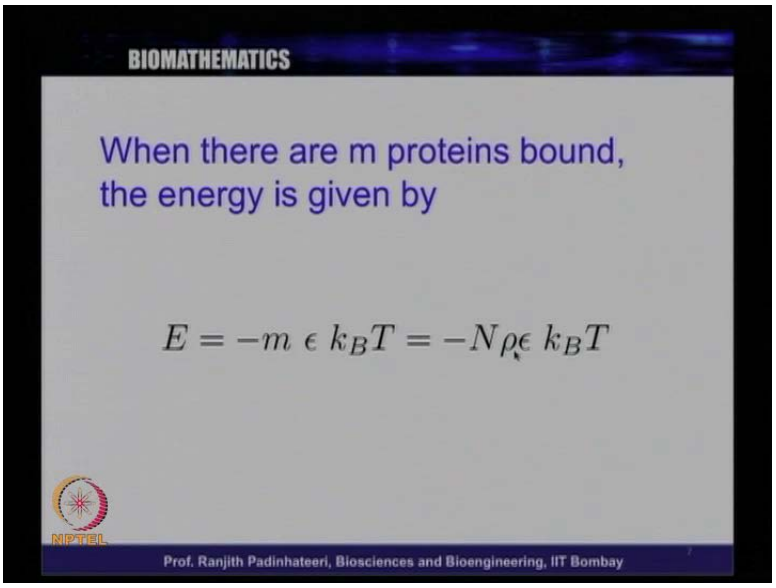

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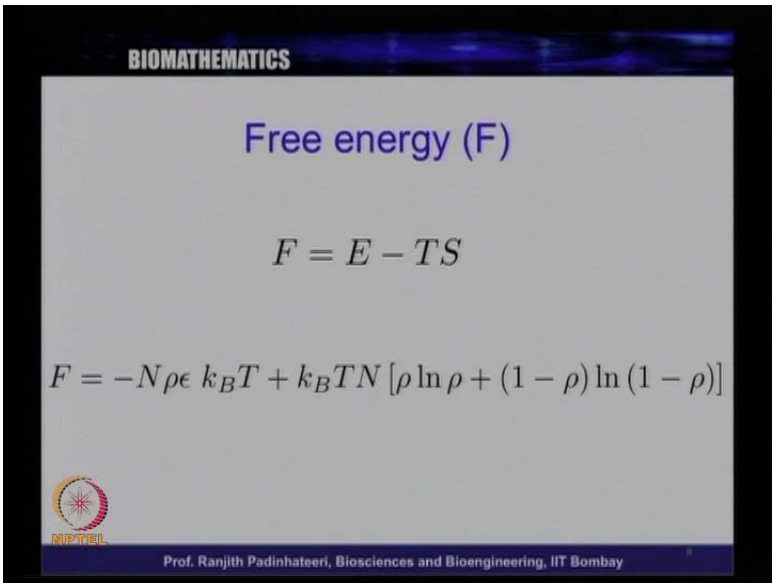

So, the free energy is E minus T S, which is this entropy part this is energy part minus plus plus entropy minus, minus will become plus. So, this is the total free energy. You want to find the minimum of what for once the question here to ask is that for what value of rho this will be minimum if you for what value of rho for what how if how many when what fraction of this bound for rho is basically m by N. So, m rho essentially to directly proportional to number of proteins bound. So, what value of rho this F will be

minimum. So, that is the question we have to ask and that will be the rho for which that will be the equilibrium value of rho.

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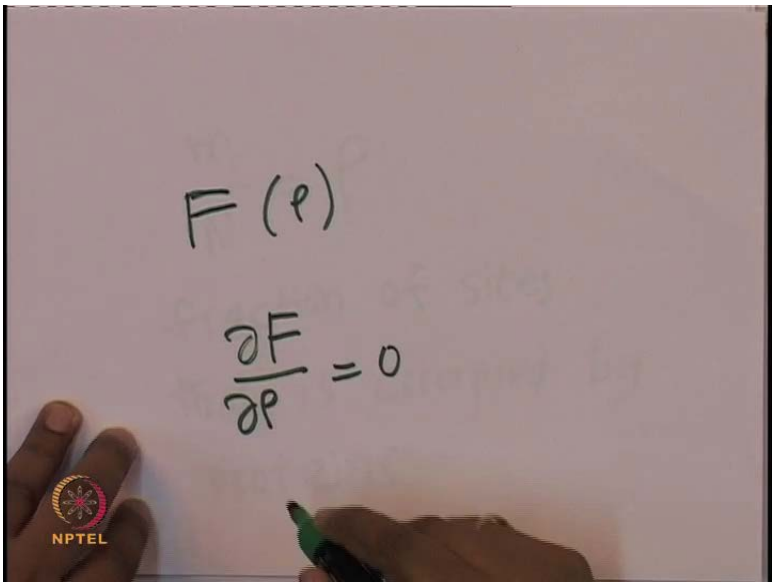

So, that is what we want to find out and we can imagine immediately imagine that if you have a function F which is a function of rho by calculating del F by del rho and equating to zero will give you the minimum of F, these learnt in calculus. So, that is what we want to do now. We will before doing it we will plot this function and see how does it look how does the free energy function looks like. So, we will plot this function and see if the free energy function. So, before plotting let us do some rearrangement. So, look at here. What we have here is free energy in terms of there is an N here, there is a K_B T here there is an N here; there is a K_B T here. So, I can divide throughout this by N and K_B T. So that, you can get F by N K_B T. So, I can divide throughout by N and K_B T.

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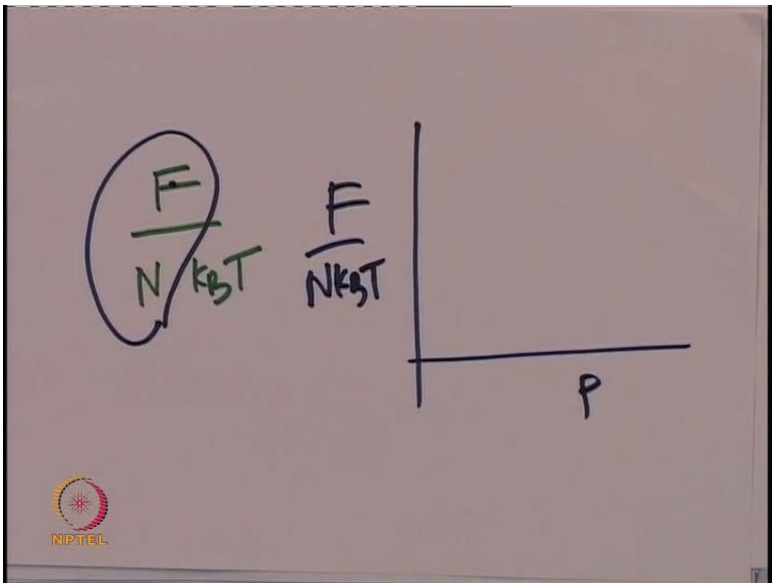

So, that I get F by N K_B T what is this mean, F by N means free energy per protein there are upper binding site let say F by N means and in unit K_B T means in units of K_B T will units of k ll units of K_B T. So, this is the dimension less number this will be energy this K_B T is also energy. So, this just for simplicity will make it some qualities just say essentially free energy only, but you will write in units of K_B T.

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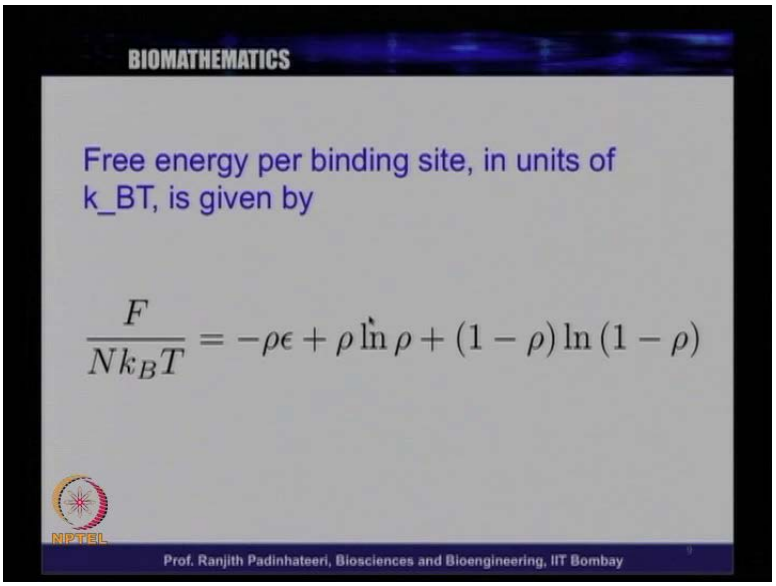

So, we will see see to plot it if we had F by N K_B T. So, we will plot F by N K_B T versus rho. So that is what we similarly. So, then if you write F by N K_B T what you get is minus rho epsilon plus rho log rho plus one minus rho log one minus rho. So, this is the free energy per binding site in units of K_B T that is what this is.

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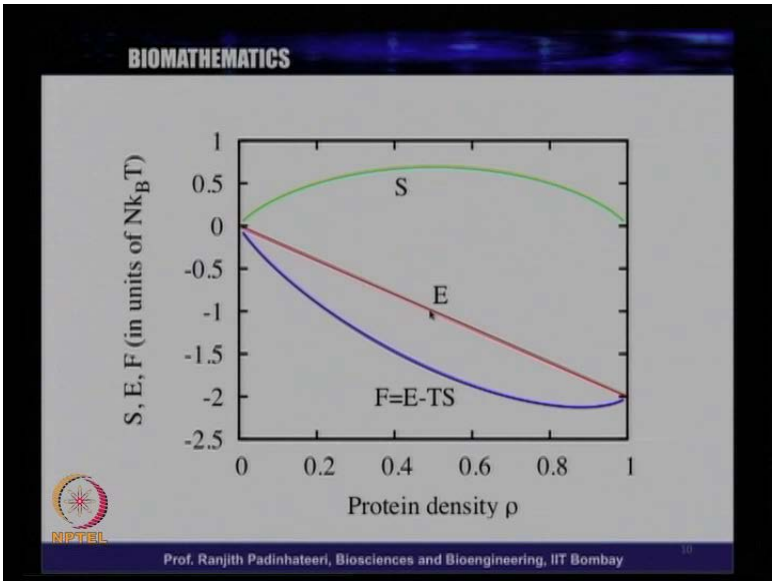

Now, if you plot this, what you get is this. So, look at here carefully. The red line is the energy, the green curve is entropy and the blue curve is the free energy which is this curve minus T times this curve. So, F minus T S is what this is. So, this is energy. So, this is minus we said the expression for energy we said this we discuss the energy expression which is sorry basically this one. Minus N rho K_B T and you plotting as a function of rho.

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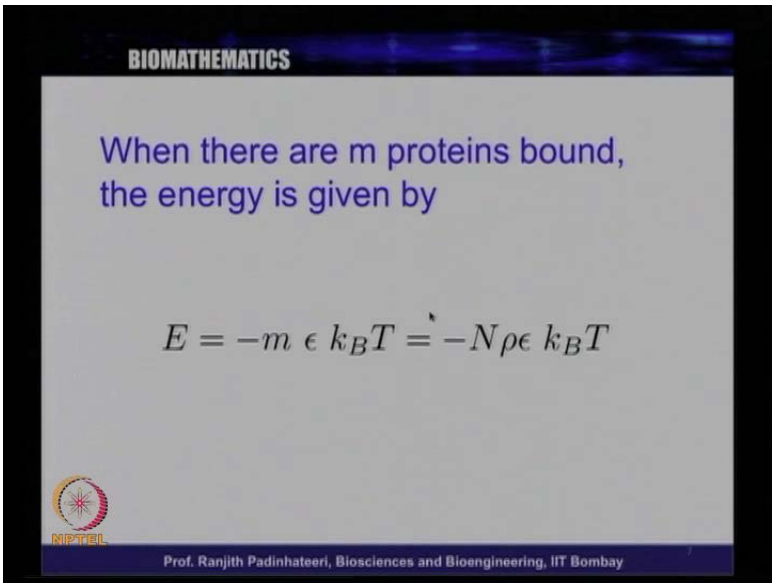

So, what you would get here is this and here this is entropy these are expression. So, we had some expressions. So, let's So, we have energy which is minus N rho K_B T and entropy which is rho log rho plus one minus rho log one minus rho.

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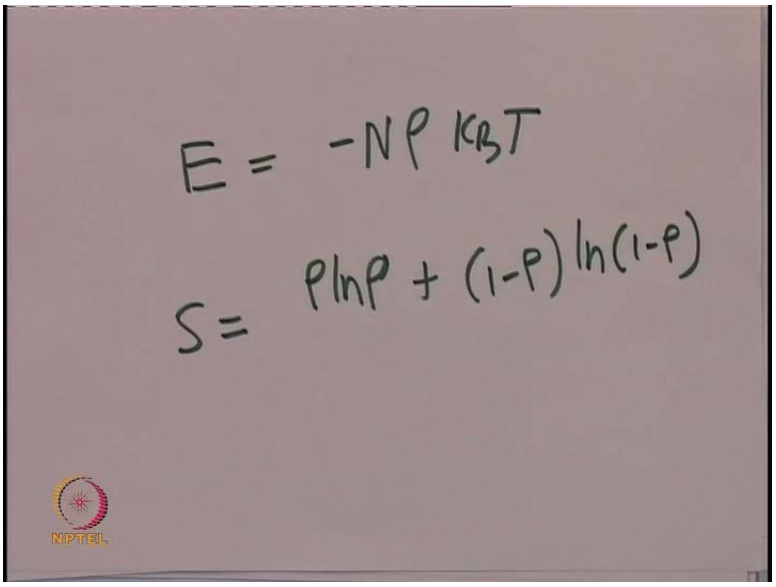

So, these are two this curves. So, if you look at here what thus it mean. This means then according to this energy is minimum when the protein density; that means, the fraction of bound protein is one; that means, every and everything is bound the protein density when the density is one, the free energy has its least value sorry the energy has its least value. Here the red curve is least, when this is one. On the other hand, entropy is maximum, somewhere in the middle roughly half of them like somewhere in the middle is entropy is maximum, here entropy is a function which is like looks like this, here is small value here is also small value somewhere in the middle is entropy is maximum.

So, entropy once rho is equal to zero point five or somewhere this while energy ones the rho equal to one and. So, entropy and energy compete and the real and the free energy will have a minimum somewhere in between. So, the minimum here is somewhere close to zero point nine somewhere here. So, this is the minimum of free energy. So, by just looking at the plot we know that energy is minimum here entropy is maximum here and the free energy is this.

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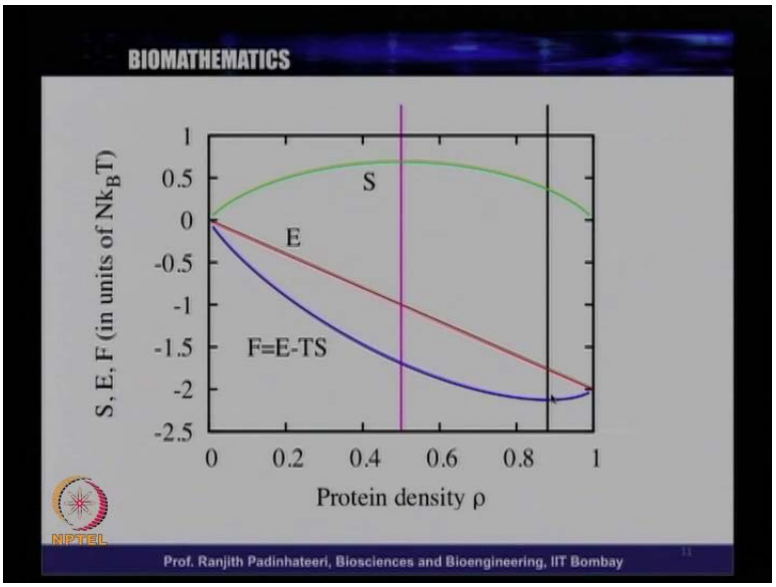

So, the. So, here for a particular value of epsilon is what is plotted here for a epsilon is equal to two is this plot is for and sorry epsilon is equal to two this plot is. So, this is the this vertical line represents the value of rho, for which for the pink line represents the value of rho, for which the entropy is maximum. The black line, the black vertical line represents the value of rho, for which the free energy is minimum. So, it turns out around

0.88 this is the value. So, and if this one is the value for energy is say for energy the E is least. Now you want to find out there are some general formulae for this minimum of free energy.

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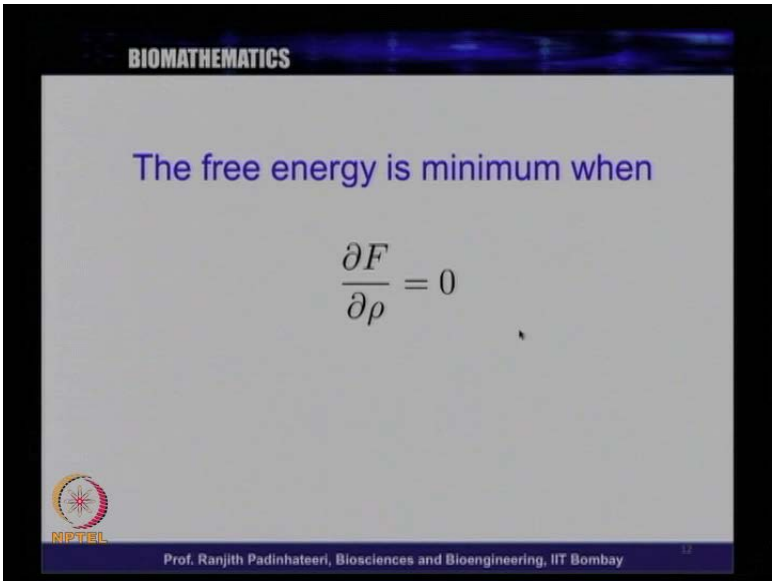

So, we said that the if we have an expression F of rho the free energy minimum can be calculated by calculating the derivative, del F by del rho is equal to zero and we said that the F. So, we will do this F by K_B T. So, let us call this F tilde let will do this F by N K_B T and the minimize this, this is also fine. So, which is basically minus rho epsilon plus rho log rho plus one minus rho log one minus rho. this is our f tilde. So, this is a new F.

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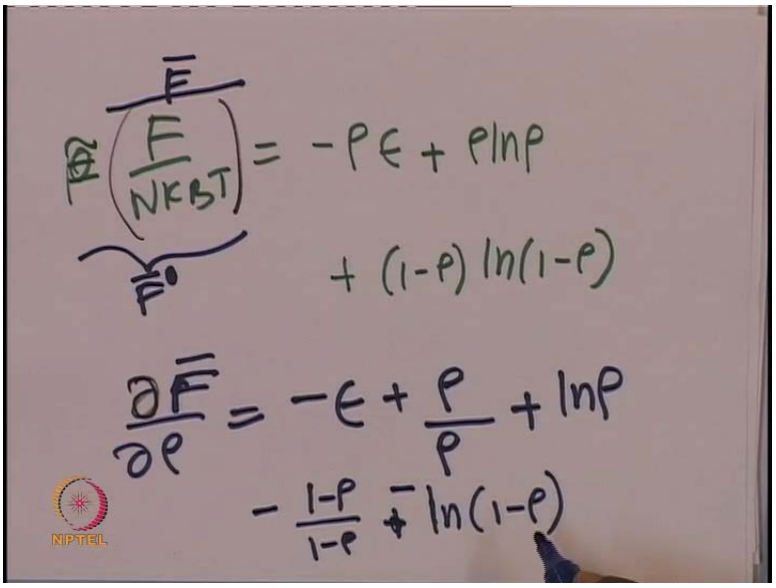

So, now if you want to calculate the minimum of this, you have to find derivative of this. So, let me call this quantity as F bar, this whole quantity as as F bar. So, this quantity as as F bar. So, I want to find out del F bar by del rho. So, the first derivative of this derivative of this will be epsilon and rho log rho will have a derivative, rho will be this log rho will have a derivative one over rho plus log rho, because derivative of rho will be one and there is a lo rho and similarly here you will have one minus rho by one minus rho plus log one minus rho. So, what is this and with there is a minus sign here because derivative of rho. So, there is a minus sign here. So, what is this if you calculate this what you will get is that minus epsilon.

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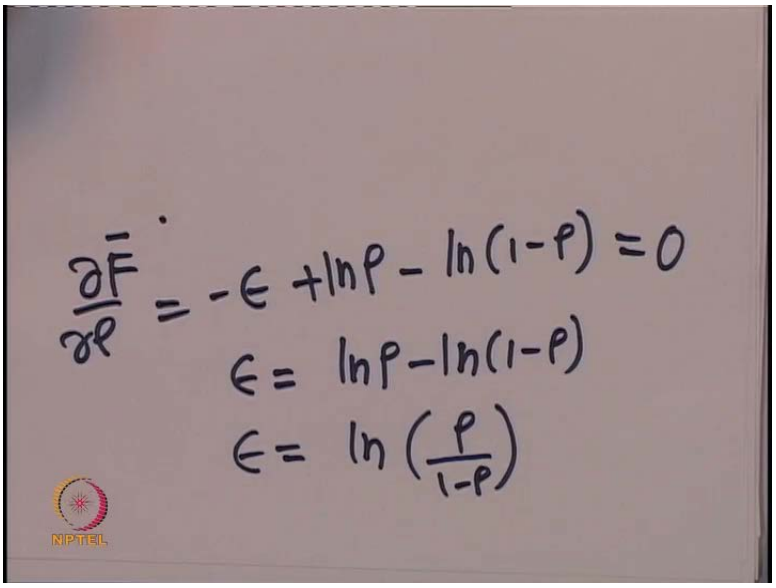

So, del F by del rho, del F bar by del rho here is minus epsilon epsilon and this is rho by rho is one and one minus rho by one minus rho and one there is an opposite sign this and this cancel one minus one will become zero. So, you have log rho minus log one minus rho. So, you have plus log rho minus log one minus rho and this as to be zero. This means that epsilon is equal to log rho minus log one minus rho, which means the epsilon, is equal to log of rho by one minus rho. So, there is a relation between energy and density, and you can invert this and write that e power epsilon is equal to rho log one minus rho.

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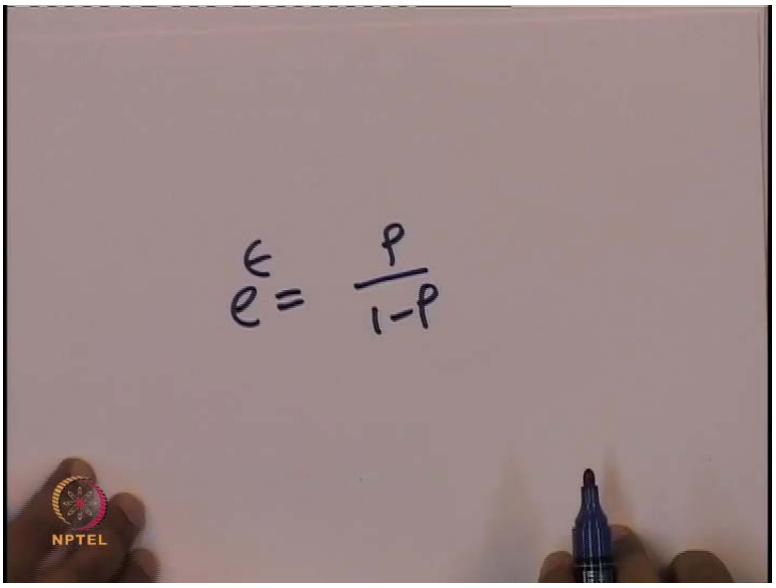

So, this would mean that e power epsilon. So, let us exponentiate this. e power epsilon is equal to rho by one minus rho. So, in other words this can be also rewritten as this particular way rho is equal to e power epsilon by one plus epsilon.

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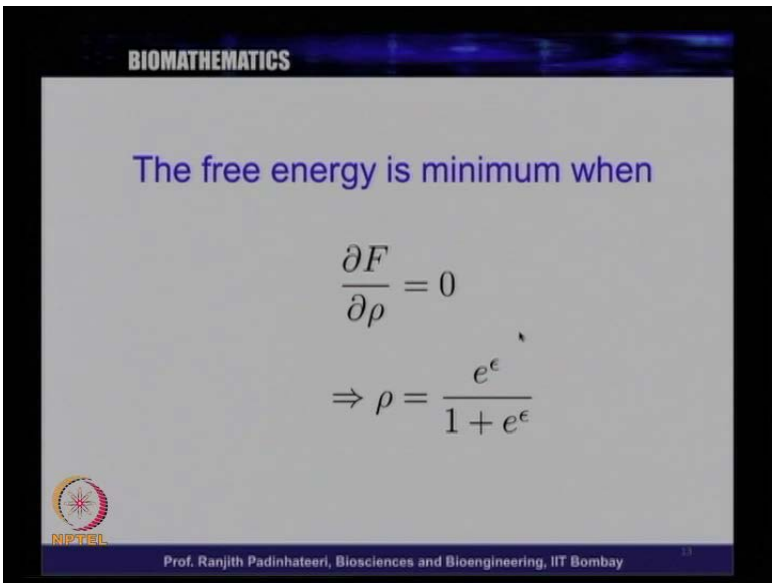

So, it turns out that if you know a value of epsilon, the rho for which del F by del rho will be zero is same as is this is given by this formula. So, when rho if epsilon let say if epsilon is zero; that means, there is no particular energy, binding energy. If epsilon is zero then e power zero plus one plus e power zero. So, when epsilon is let us look here

when epsilon is zero, this rho is basically e power epsilon by one plus epsilon one plus e power epsilon which is one e power zero is one by one plus one which is half.

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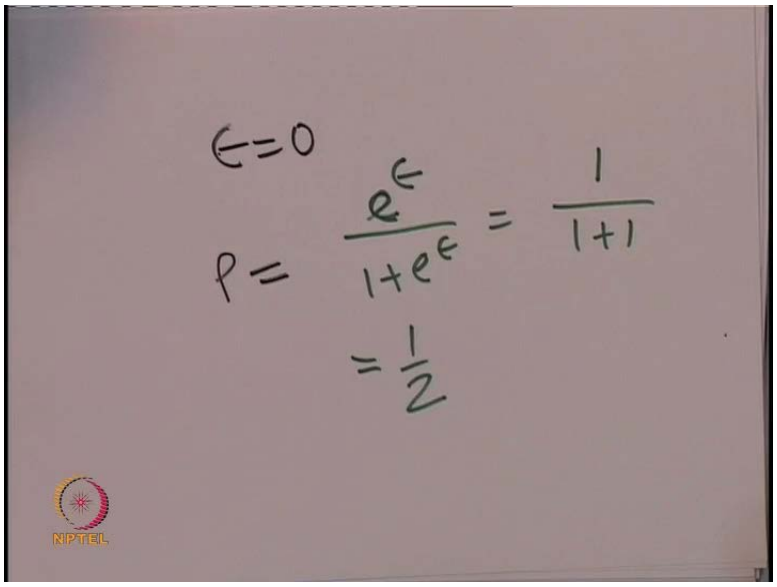

So, when there is no particular energy preference, only entropy then half of the sites only will be bound. There is no energy is 0, if energy binding energy is let say infinity; that means, all proteins are bound. They all would like to bind like if epsilon is infinity and the binding energy is minus epsilon K_B T then if epsilon is infinity. So, the. So, that the binding energy e is minus. So, we said that the binding energy is minus for one molecule is minus epsilon K_B T one protein.

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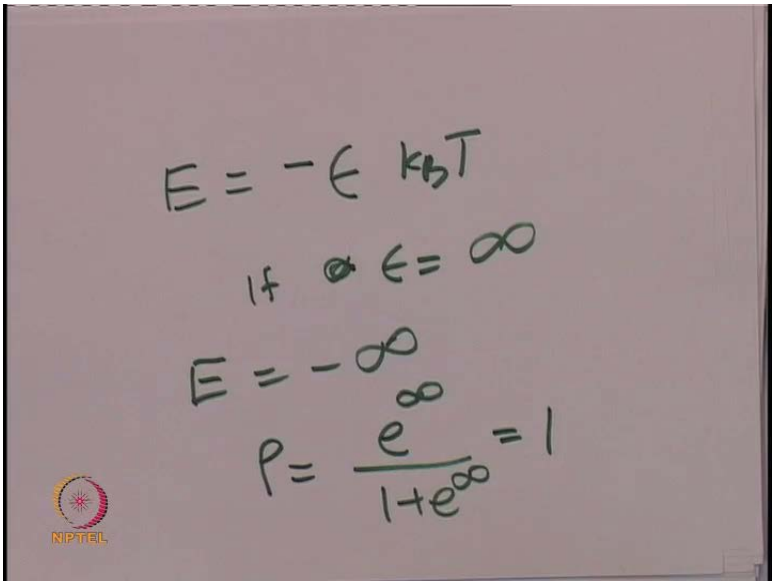

So, if epsilon is infinity, the binding energy is minus infinity K_B T so that means, by binding infinite energy will be gained. What is this mean that everything would love to bind, because this infinite energy gain. Then this energy will dominate over everything else. So, if you put here in this formula epsilon is equal to infinity, you will get, rho is equal to e power infinity by one plus e power infinity. e power infinity is infinity, which is. So, this will be large number and this will turn out to be one. So, the answer will turn out to be one and if epsilon is minus infinity; that means, if the binding energy is plus infinity; that means, it does want to bind at all; proteins do not want to bind at all.

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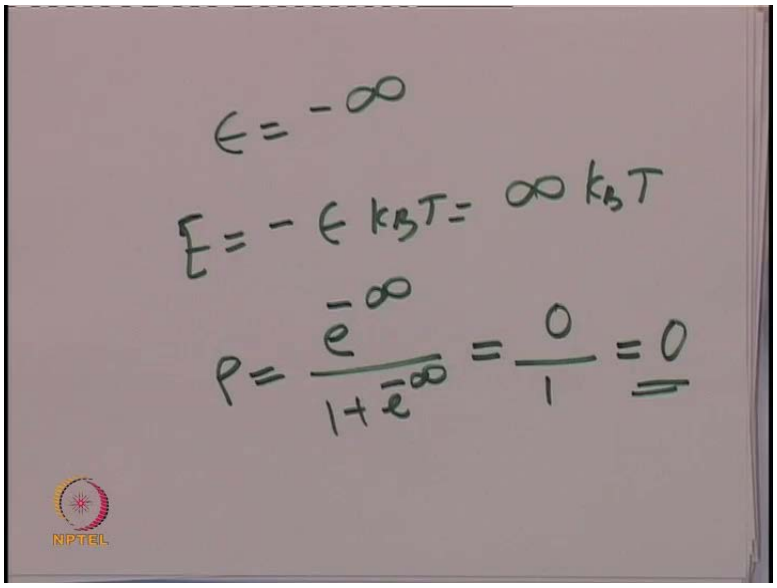

Then rho will turned out to be. So, let say there are another case another limit is epsilon is minus infinity. So, that the binding energy is minus epsilon K_B T is equal to infinite K_BT; that means, the binding energy is infinity; that means, does not want to bind at all. Then the rho will be e power minus infinity divided by one plus e power minus infinity and this will be this is zero by one plus zero which is zero.

So, if the binding energy is infinity, now no proteins will be bound. So, this will give us the correct the binding density the how many proteins. So, we can predict using ideas from thermodynamics, how many proteins will bound on the DNA. So, for epsilon is equal to two here is what plotted here, this will give you the protein density bound. So, the for epsilon the free energy minimum is around 0.88. So, 88 percent of will be bound and epsilon is equal two. So, this is one use of knowing thermodynamics we can predict what will happen in various biological context by this systems. So, this is the simple example in which example we discussed and the same ideas can be applied to little more complex situations and studied. We will now discuss another example which is very similar to this, is basically DNA melting. So, you all know that you increase the temperature DNA will melt. Double stranded DNA will melt and can we predict at a given temperature what will be the equilibrium state it will be melt or it will be completely melt or most of the melt completely they would have form single stranded DNA or is it the case there they all will be base paired. Turns out the thermodynamics our simple ideas that we learn in thermodynamics can predict this. So, we let us think of

a thought experiment. So, this is the very simple thought experiment where we can do all the calculation in paper and you can think of how to extend this to reality.

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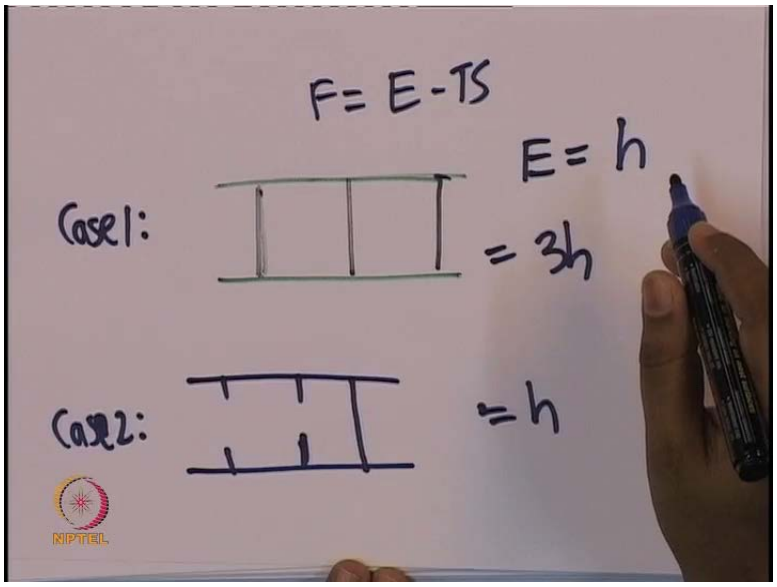

So, let us imagine that a three base pair double stranded DNA. So, let say you have a double stranded DNA, three base pair. So, now, you can imagine some situations like you can imagine that this is three of the all the three are base paired. You can imagine a situation where let say only two of them are two two contacts are two pair base pairs are broken. So, there is only one contact. So, we can imagine this kind of a situation where these are broken or you can imagine another, let us consider two cases. So, case one and case two. So, case 1 is all base pairing is intact like all this they are bonds and here there is only like one bond and to other base pairs are broken now we want to calculate the energy free energy for case 1 and case two and let us calculate the free energy for this case one and case two and let see what do we get. So, what is free energy free energy is energy minus E minus T S. So, this is our free energy F or G now what is E.

So, let us assume that E is equal to just like we did some value let say h. So, h is some value, which can be 100, 200 some some value like K_B T in units of which K_B T there will be some energy let let call this h. So, for each base pair the energy is h. h is basically energy per base pair. So, if there is a bond is there that will gain an energy h. So, this as an energy three h. This is an energy h case one because there is only one bond here. So, let us call this energy h energy h is the energy per base pair. There is one base pair that is

the energy gain of h you have to give. So, and if there are three base pair the energy is three h 3 h.

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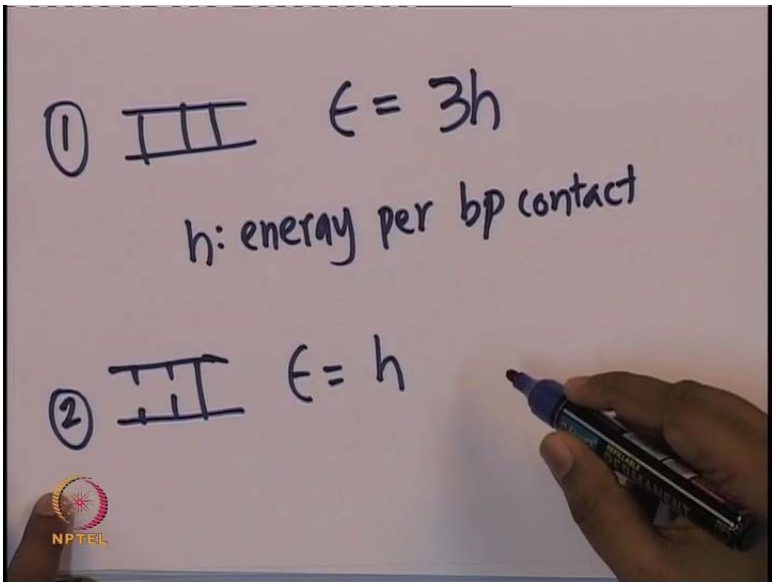

Now, what is entropy? So, let us think of these two cases again. So, we have two cases first case is all base pair intact and the second case is two base pair is broken and one is intact and this we found that this is an energy three h and this is an energy h because only h is basically energy per base pair base pair contact. If the contact is one base pair this one h.

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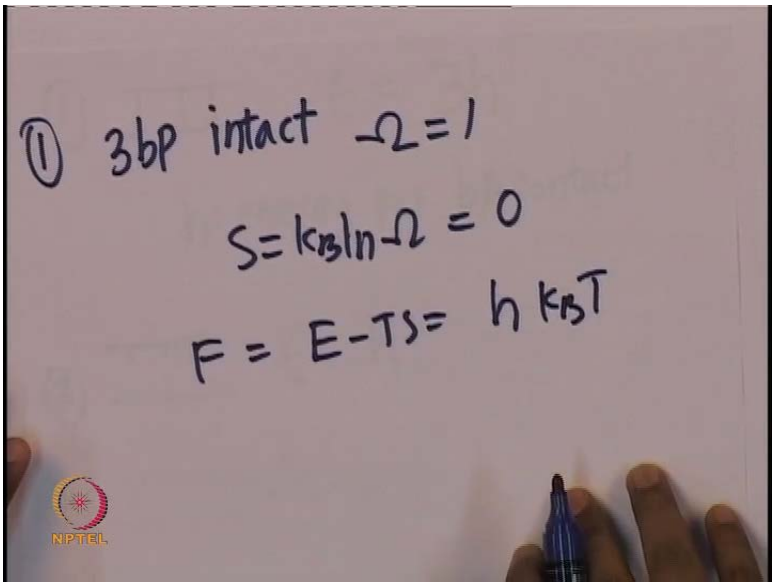

Now, we have to calculate entropy. So, what is the entropy for this case lets think about it entropy for case one. So, case one, three base pair intact. This is our case one. How many states are possible when the three base pairs intact there is only one state possible this is three base pair intact there is only one state possible? So, the omega is one. So, entropy is K_B log omega. So, this will be zero. So, free energy is E minus T S this is h minus zero this is just h. So, h K_B T is the free energy in units of this.

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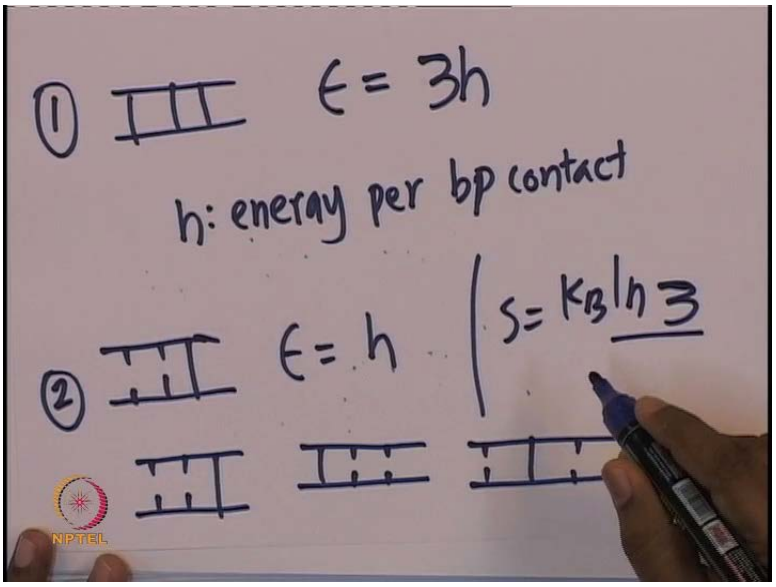

Now similarly we can calculate entropy for this the three cases possible for this. So, think about it such a state is possible you can have this and this to broken this is another state possible you can have this is broken and this is broken and the middle one is three states possible. So, the entropy for this is K_B log three. So, log three is roughly one. So, this is just K_B entropy is say K_B. So, the free energy for this case will be. So, for the case one the free energy will be just h K_B T case two will be sorry free energy is three h K_B T k because three bonds that is the energy and entropy is zero and the case two free energy is h K_B T minus just K_B log three which is K_B T.

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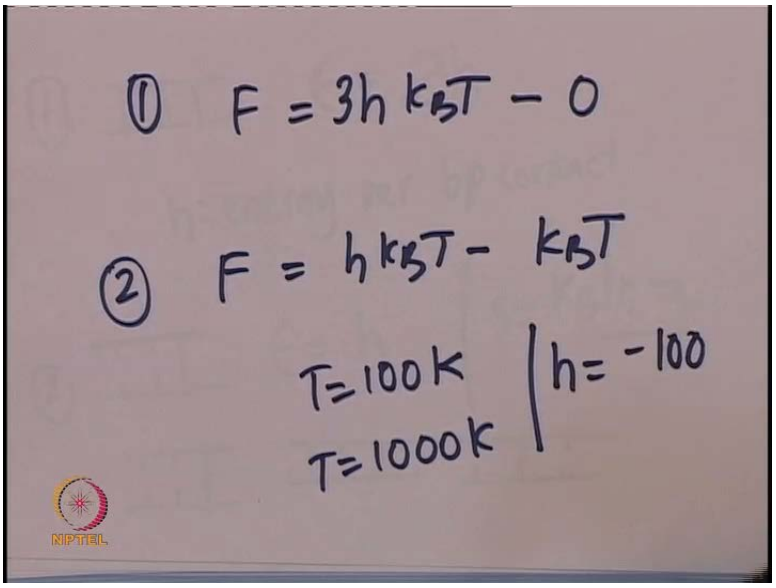

So, this will be the this will be the answer K_B log 3. So, now, you can calculate basically what will be the free energy now you do one this is an assignment for you vary temperature take T is equal to 100 kelvin and T is equal to 1000 kelvin and see which of this is minimum and take h is equal to minus 100 in this cases. So, for when T is equal to 100 K_B T. So, and T is equal to 1000 K_B T. This is an assignment for you to do see that which of them will be minimum and you will see that the state were the free energy is minimum, will be the state at higher temperature you will find that the state were all base pairs, most of the base pairs are broken will be the minimum energy state and that lower temperature all intact to the more number of base pairs will be intact. So, this is something which we expect and this we can see from our simple calculation.

So, the idea here is that we can go ahead and use this kind of an ideas for many cases so, but to summaries what we learnt today is, how do we think about calculating energy for two systems one is proteins bound and DNA ask the question at equilibrium how many proteins will be bound what fraction of proteins will be binding sites will be occupied can be answered by using thermodynamic similarly you can also as answer the question, what will be the state at given temperature such that the free energy is minimum. So, So, that will give us the whether the DNA will be half melt fully fraction of the site DNA will be base pairs will be broken when at a given temperature t can be also answered by this. So, with this I will stop today's lecture bye.