Physics of Biological Systems
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Lecture - 28
Transcription and translation

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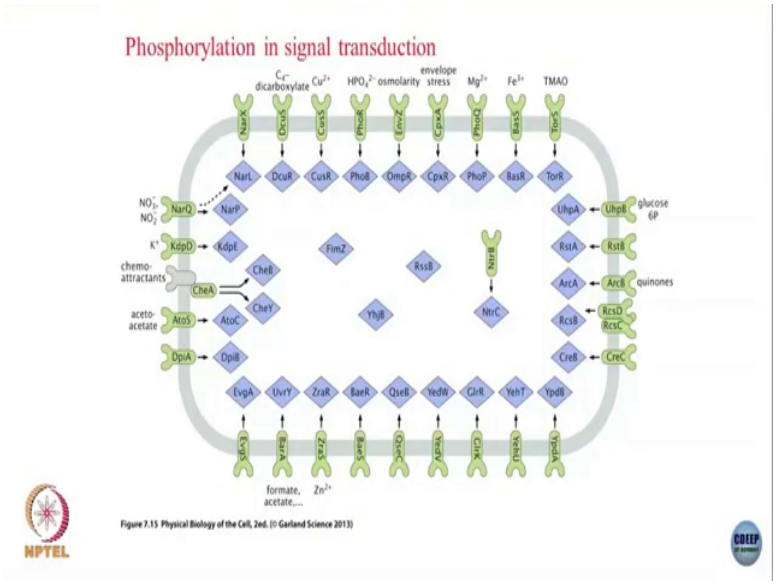

Alright, the third thing that I want to show you is to again deploy a similar approach in the case of hemoglobin.

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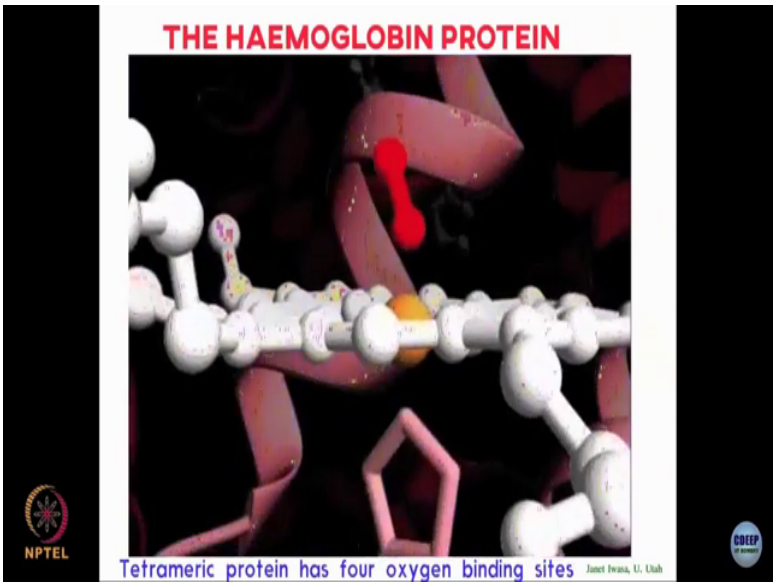

So, hemoglobin of course, you all know as a protein in your blood it is a tetrameric protein and it has 4 oxygen binding sites. So, this is one particular oxygen binding site.

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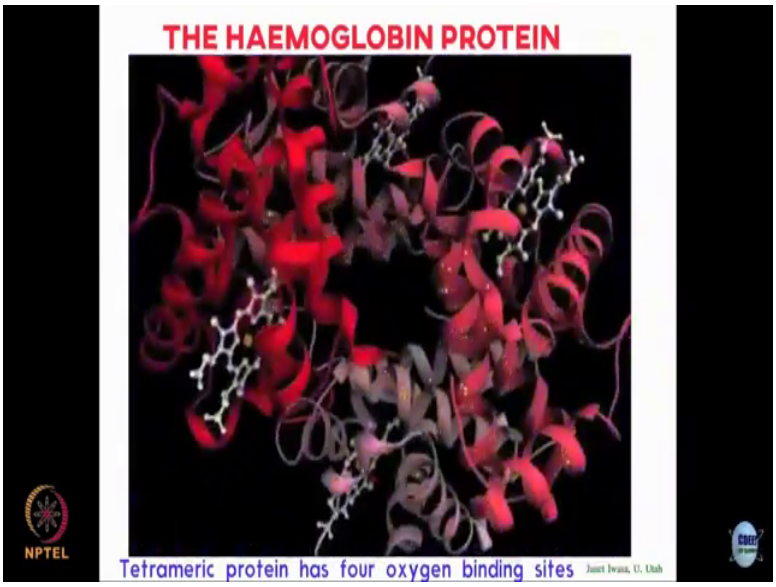

And, if once it zooms out hopefully you will see that this whole hemoglobin protein has 4 oxygen binding sites, ok. So, this whole tetrameric structure is one hemoglobin protein.

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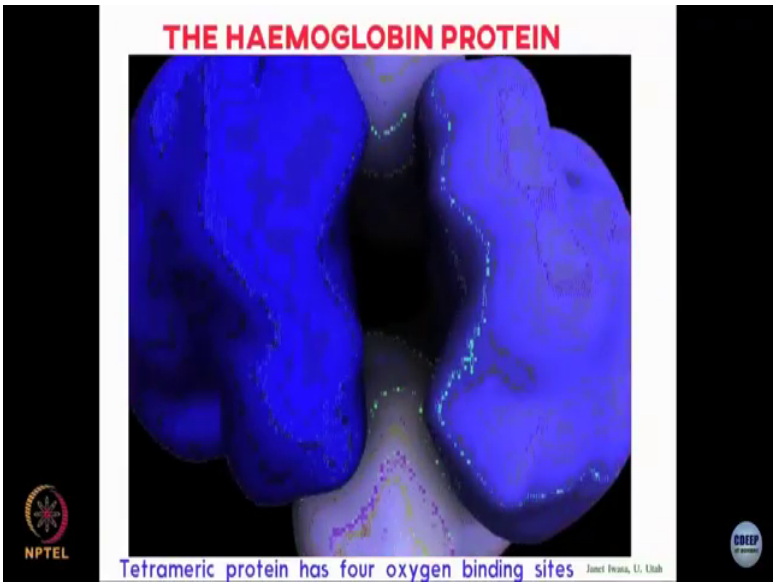

And, again you can ask similar questions at what is the probability that you have bound oxygen, that these sites on this hemoglobin are bound as a function of let us say oxygen concentration.

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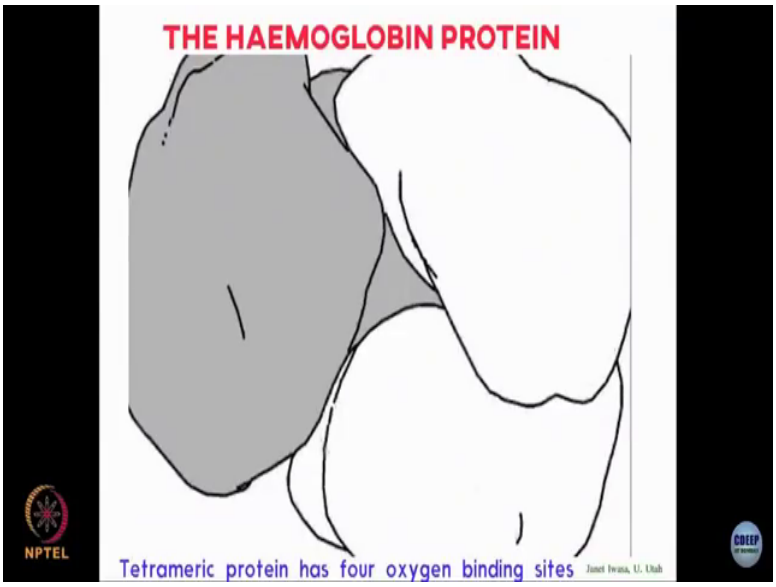

And, again we will use this sort of a discrete state formalism; counting before we do this hemoglobin we will do a sort of toy protein which is called the dimoglobin.

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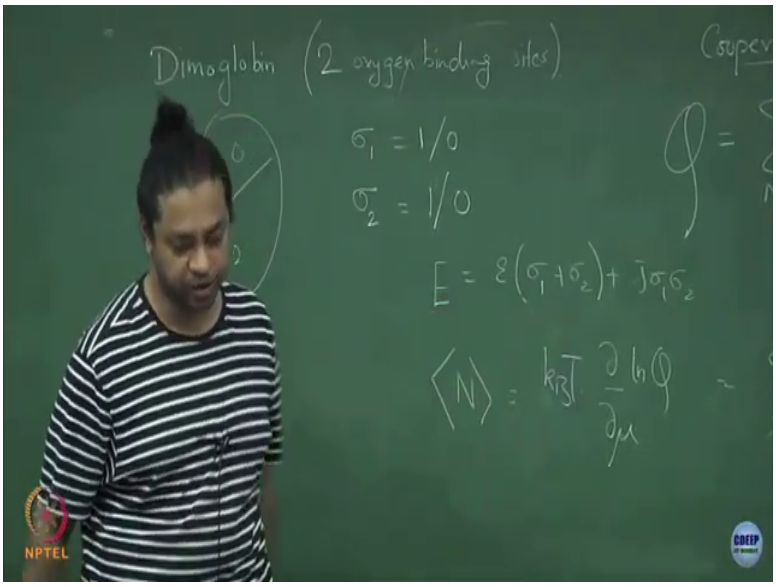

Where instead of having 4 oxygen binding sites, I will assume it has 2 oxygen binding sites. So, this dimoglobin is a sort of hypothetical protein with his 2 oxygen binding sites.

And, the basic idea that I want to show is that how this probability that an oxygen comes and binds to this dimoglobin or ultimately hemoglobin, change depending on whether these binding events are cooperative or not.

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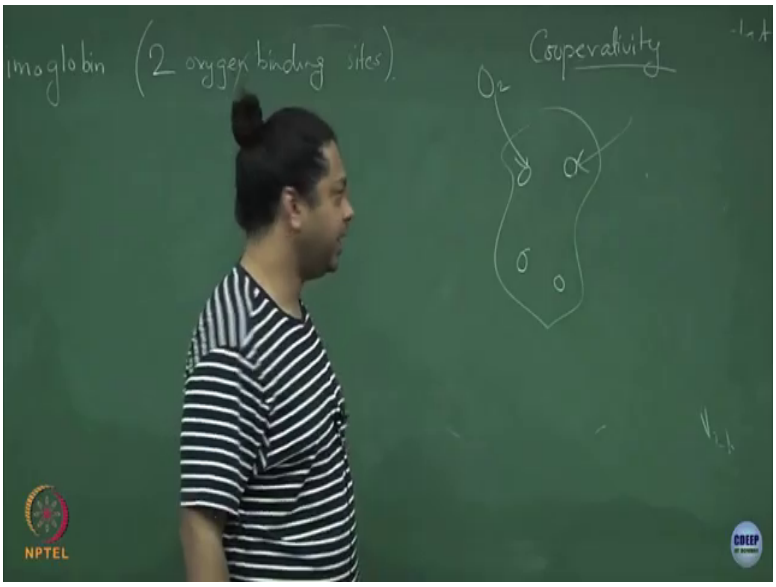

So, if I have this hemoglobin protein and I come and an oxygen comes and binds in one of these 4 centers; does it affect the probability for oxygen to come and bind to any of these other centers, right. And you could imagine that it does, because whenever you have some sort of a binding you will produce some sort of a structural change, the structural change might make it easier or more difficult for another ligand to come and bind, right.

So, in general what I want to sort of explore with this is this idea of cooperativity, ok. When you have this multi ligand binding the ligand in this case being oxygen then what role does cooperativity play in this binding process, ok. So, here is sorry, so, here is my dimoglobin protein so, this protein has sort of 2 binding sites 1 and 2, right. So, I will again use two state variables sigma 1 and sigma 2 to characterize the internal states, right. Sigma 1 is going to be

1, if there is oxygen in this side and 0 otherwise sigma 2 similarly, will be 1 if there is an oxygen bound to this site or 0 otherwise.

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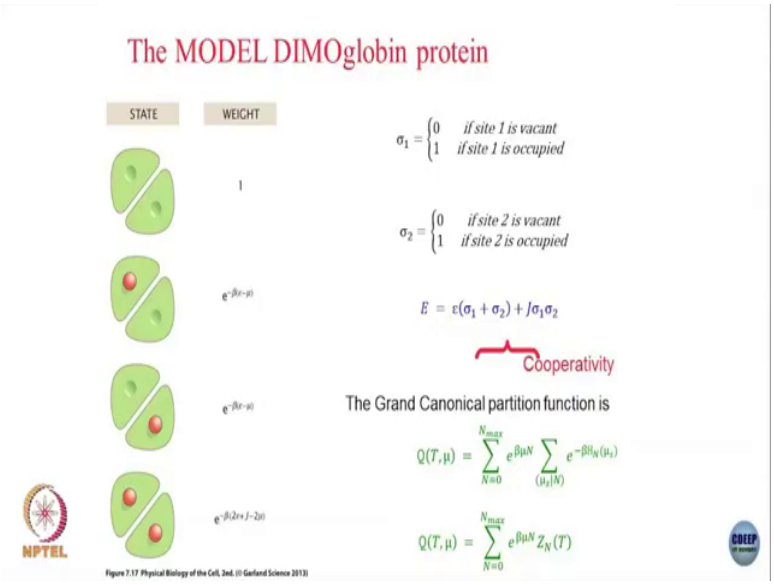

So, the machineries again extremely similar ok; so, here is the idea that I have this model dimoglobin protein; it has 2 oxygen binding sites and I label it using 2 state variables sigma 1 and sigma 2, right. But, I need to write down the energy function so, let me say that I write down an energy like this; that if I have a single oxygen that is bound either at site 1 or at site 2 that has some energy advantage of epsilon, ok.

On the other hand, if I have both oxygens bound then the cooperative sort of energy comes into the picture and I have this interaction term which is J times sigma 1 sigma 2. So, this will only come into play then both sigma 1 and sigma 2 are 1, right. So, therefore, both sides are occupied by an oxygen. If not then the energy is simply if you have only an oxygen here or

here the energy is simply going to be epsilon. So, this is an example of how to introduce cooperativity in a model like this; when you have multiple ligands that are binding.

So, again I can write down the of the Boltzmann factors for each of these states, I have again 4 states 2 into 2. So, here nothing is bound therefore, the energy is 0, the weight is one here it is e to the power of minus beta epsilon minus mu. So, let me just what is the grand canonical partition function by the way?

Student: Z to the power of.

Z to the power of.

Student: (Refer Time: 05:05) canonical.

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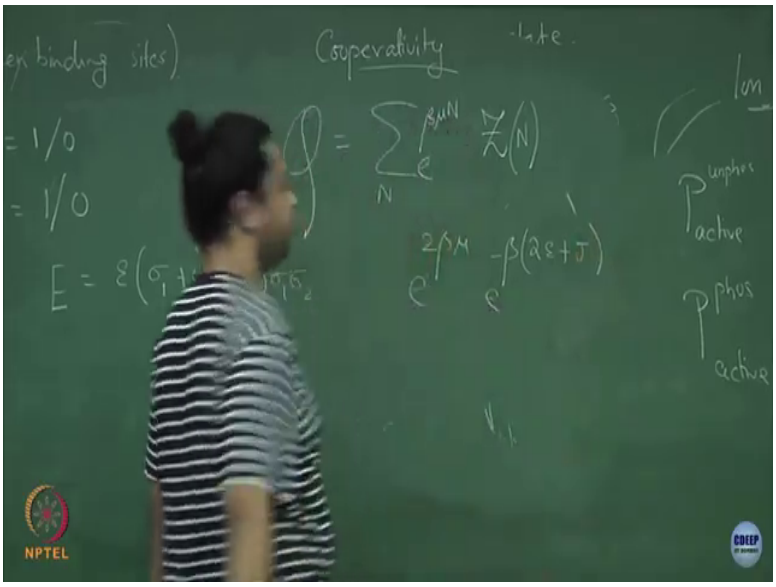

Into.

Student: Into the canonical.

Into, ok. So, let me write Z small Z to the power of N into the canonical partition function of and then.

Student: Summed over (Refer Time: 05:22).

Summed over all possible values in that, right; since there is too many Zs let me just write it as e to the power of beta mu N, right; e to the power of beta mu is the fugacity, mu is the chemical potential, alright. This you are all familiar with the grand partition function. So, here

is something so, where the number of oxygen particles that will bind is variable, right you can have 0, you can have 1, you could have 2. So, I will use this sort of a grand partition function formalism in order to calculate the probabilities.

So, when you have so, when you have just one binding then you will have an e to the power of beta mu into the picture, here and here e to the power of beta mu and the canonical partition function for that is simply e to the power of minus beta epsilon. Here when you have both bind when N is capital two then they will have e to the power of 2 beta mu and the energies we have written down forgotten energy is epsilon sigma1 plus sigma 2 plus 2 J is sigma1 sigma 2, ok. So, the energy of this is minus beta 2 epsilon; not 2 did I write 2 J or J J dot J 2 epsilon plus J, all right. Yes, J.

So, this is what this term is 2 epsilon plus J minus two mu, ok. So, that is the weight of that term. If we sum up all of this that gives you the grand partition function, right. So, the grand partition function is then e to the power of beta mu N then the ZN T.

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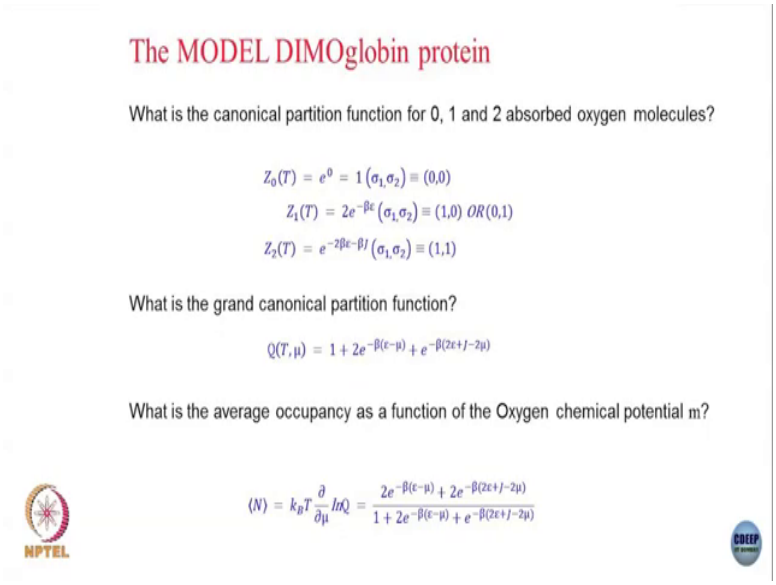

So, in this case we have worked out what is Z 0, what is Z 1 then what is Z 2 and therefore, you can write down what is the grand partition function? Ok. So, this is the total grand partition function. So, now, we can ask that well therefore, what is for example, so, now, that I have the grand partition function, I could ask what is the average occupancy of this dimoglobin molecule, right that is on an average how many oxygen molecules will be bound to this dimoglobin? How do I calculate the average number given the partition function grand partition function?

Student: (Refer Time: 07:52).

Yes, that is of course, true, but what is the short form formula?

Student: (Refer Time: 08:03).

That is in terms of the probabilities which is of course, correct, but if you know the partition function then del del.

Student: Log.

Log Q by del mu, right and something k B T, ok. So, you can calculate or if you want as shown an exercise you can just write down it is a summation since this is anyway a very small discrete system, you can explicitly count and write down what are the probabilities. So, you can write down what is the average number of oxygen molecules that are going to be bound?

The average of you can see and this you can write so, this is just this right, as a function of this oxygen chemical protein. So, what tells you how much oxygen is there? That is the chemical potential that signifies the strength of the how much oxygen is there in the environment and that will determine, how much oxygen, what is the average oxygen occupancy of this time of you? Ok, alright.

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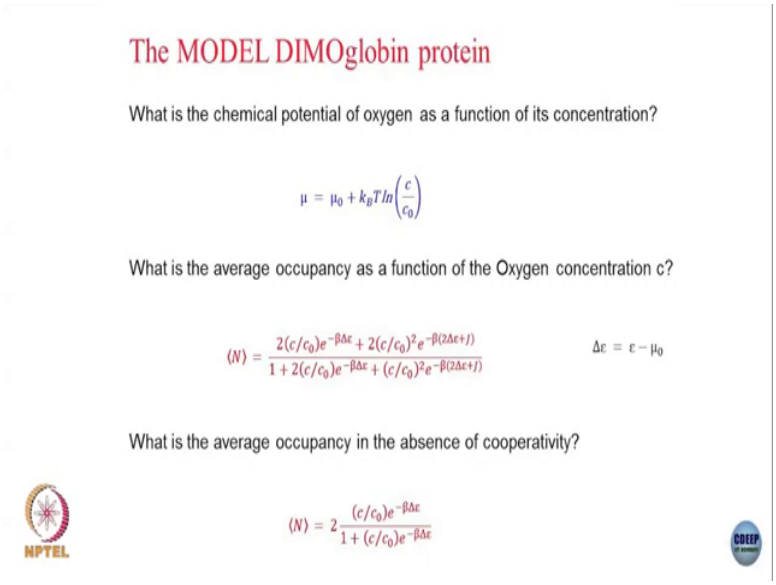

So, instead of using the chemical potential you can also express the chemical potential as a function of concentration, I have not really shown that I will show that next class so, we will derive how to write the chemical potential as a function of the concentration. And, that is simply written as mu is sum mu naught k B T log c by c naught. So, if you do not know this you can take this as a given I will derive this next class, ok. I will show how to get this; so, the chemical the larger the concentration the larger the chemical potential, ok.

So, now, we can therefore, so, you can if you rewrite this previous formula in terms of the concentration; you will get some answer. So, here is the average occupancy in terms of the oxygen concentration. And, you will see that what would you so, for example, what would you expect if there was no cooperativity? If J was equal to 0; you would expect to recover

back the ligand receptor formula that we got last class, right. Where we had a delta e basically difference between the two states; a single ligand binding to a single receptor, right.

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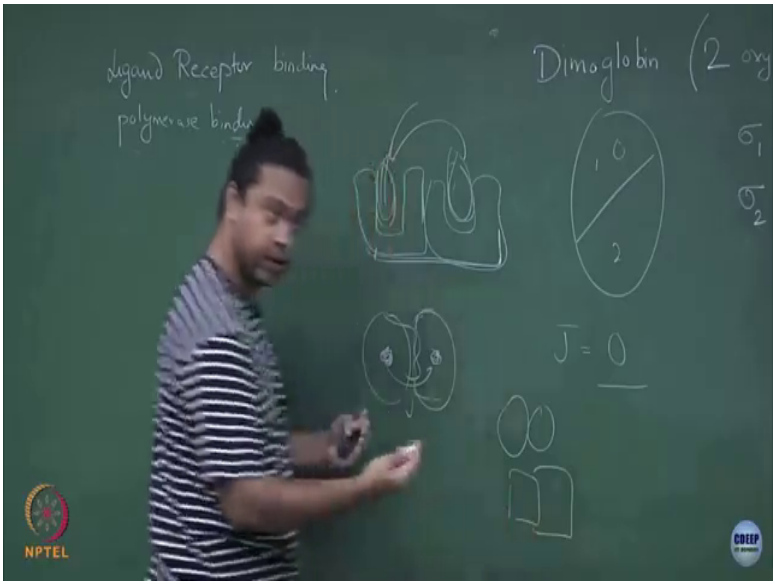

So, you would expect to recover twice that rather to be precise, because you now, have effectively what this would if it there was no cooperativity what it would function was that you could have two ligands coming and binding here one here and one here. And, these would be independent of each other therefore, if I consider this whole thing as a single protein, the average occupancy would simply be twice that.

But, because you have this cooperativity, because you have this interaction energy J that changes the sort of average occupancy so, this is just of course, two times whatever you had earlier. So, if you remember this was the formula we got earlier c y c naught e to the power of minus beta epsilon like this; in the absence of J you just get two times that.

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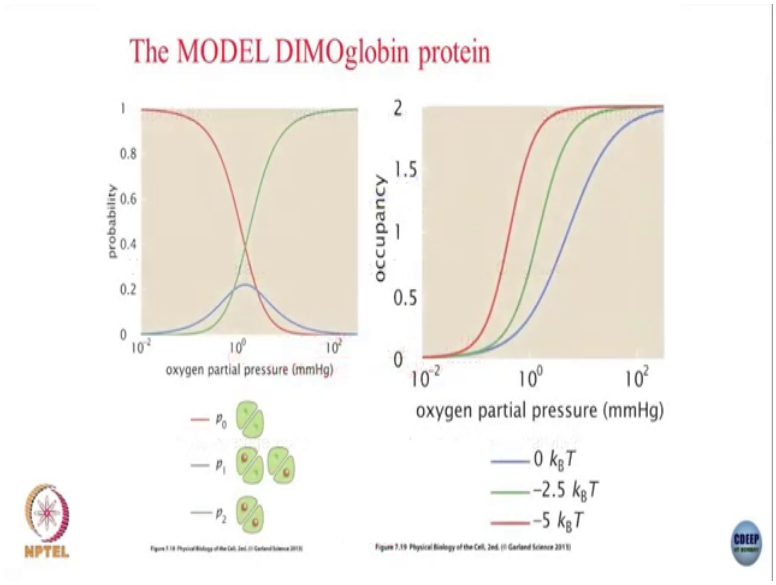

But in the presence of j so, in the presence of so, for example, this is the blue curve is the curve in the absence of J there is no interaction, it just behaves as too sort of independent receptors interacting with two ligands as you introduce more and more cooperativity the curve shifts more and more to the left, right which means, that you will get more oxygen occupancy at a lower concentration of oxygen; if you have cooperative binding. So, whenever you have one if that big it that makes it more likely that you recruit another oxygen to the second side, then the average occupancy will increase at a lower value of this oxygen concentration or oxygen partial pressure.

If you plot these probabilities so, this is the probability for example, that there is no oxygen bound which falls as you increase the oxygen concentration, the probability that there is only one oxygen bound sort of peak somewhere in the middle. And, then again falls and for higher values of the pressure its very likely; that you will have both sites that are bound by the where

oxygen is bound to both sides of this dimoglobin protein, ok. So, this is sort of quantification of how cooperativity effects this binding curves? This occupancy probabilities function of the concentration is this sort of clear, ok.

So, you can now, so, this is we did this for this; you can also do as model in a similar spirit which is a very famous model, but instead of introducing this interaction energy J remember our idea was that I have this protein, I have this protein which lets say has two subunits if a ligand comes and binds to one of these subunits; it maybe causes some conformational change which makes it more likely to recruit a second ligand here, which I am representing by this interaction an energy J, alternatively you could also say that there are this protein this dimeric protein could exist in one of two possible conformational states, right. One is this one is this circle state and maybe another is something like this, right.

And, you can reframe this problem in terms of this conformational states and that is actually a classic model in the literature which is called the MWC model or the Monod Wyman Changeux model.

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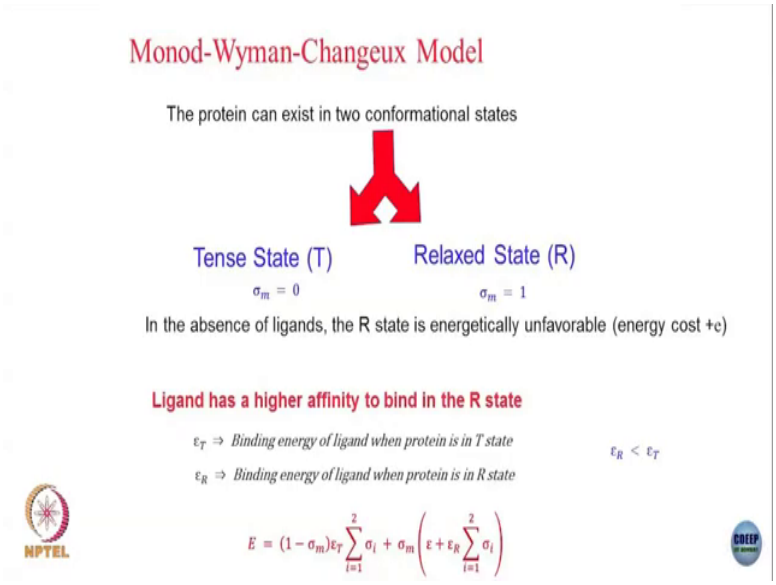

So, again the spirit is the same the sort of language that we use is slightly different so, what you say is that the protein can exist in two states, the tense state of the relaxed state, and again I have a state variable associated with that sigma m is 0 if it is in the tense state, its 1 if it is in the relaxed state, ok. And, let us say in the absence of ligands this R state is energetically unfavorable, ok. So, the tense state is lower in energy so, this is my R state, this is my T state and it has some energy cost of epsilon, ok.

On the other hand the ligand might prefer to oops, the ligand might prefer to bind to this relaxed state. So, the ligand has a higher affinity to bind in this R state, in this relaxed state. So, you can call this epsilon T as some binding energy when protein is in the tense state; epsilon R is the binding energy when the protein is in the R state and because this ligand is a higher affinity this epsilon R is smaller than epsilon T. It is more stable when it binds in the

relaxed state, ok. So, I am sort of reframing the problem using this sort of tense this conformational state id.

And, again you can write down this sort of an energy so, you have two sort of sites 1 and 2, each of them has a sigma i, in addition you have this conformational state variable sigma m which can be either 0 or one depending on whether it is this tense state or the relaxed state. So, for example, in the tense state this thing comes into the picture.

So, you have some epsilon T depending on how many ligands are bound; if both are bound then you have 2 epsilon T, if it is in the relaxed state then these terms come into the picture there is a conformational energy of epsilon plus depending on how many ligands are bound 1 epsilon R or 2 epsilon R, right. So, this is then my Hamiltonian for this system. And, again you can sort of list out what are the various states that are possible and what are the corresponding weights for these states.

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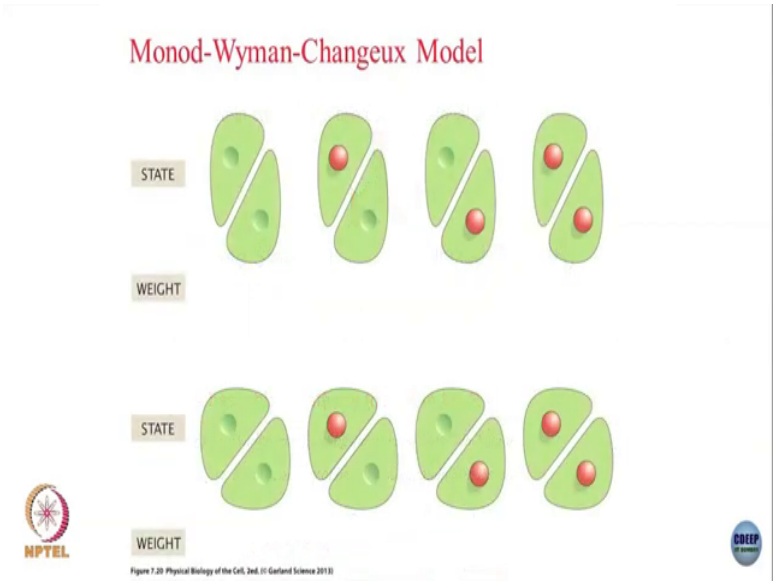

So, for example, if this is these are my tense states, these are my relaxed states I can calculate what is the energies of each of these, right. So, this is one of course, this is e tense minus again there is a e to the power of beta mu, this is the same as that and here it is two times epsilon T and 2 beta mu, alright. In these you have a structural energy for the relaxed state which is in addition to this ligand binding, which is epsilon so, here even when nothing is bound it is unfavored by N factor of e to the power of minus beta epsilon.

And, then depending on how many are bound you will have whatever epsilon R plus epsilon minus mu epsilon, right and here you will have 2 epsilon R plus epsilon minus 2.

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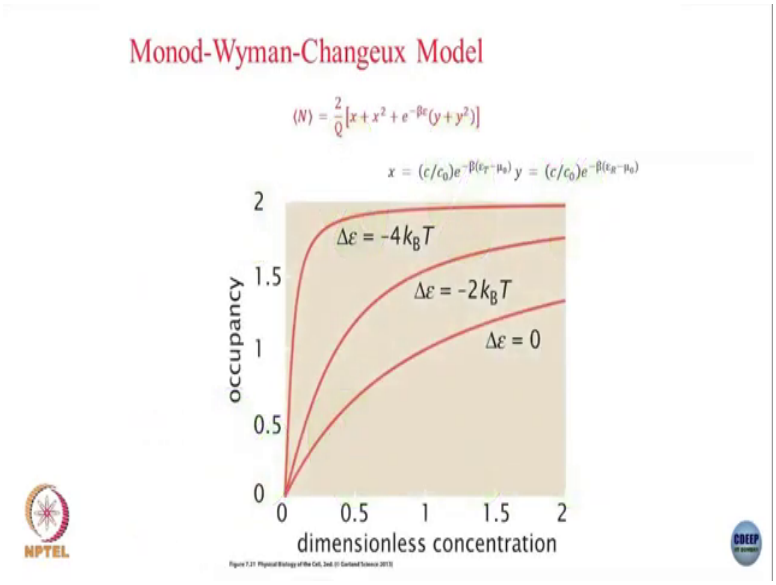

So, the once you have reached it out all of this you can simply write down the partition function and, you can calculate again what is the average occupancy and then depending on how favorable you make sort of tense state in comparison to the relaxed state; you will get different curves for this occupancy profile as a function of the concentration of the ligand (Refer Time: 06:57), ok. So, this is written in term of some energies of the tense state and the relaxed state and this difference in energy is between the tense and the relaxed complications, ok.

So, these are sort of two sort of ways you can think about this from an interaction energy perspective, that these ligands sort of interact where it and it has some sort of cooperativity energy J or you can think of this from this conformation state perspective that when a ligand comes and binds; its more likely to make it go transition into this different state, the relaxed state in this MWC sort of a model, ok.

So, now, that we have done this we can now, go forget about this dimoglobin and go on to the actual hemoglobin. It is exactly the same except that you now, have 4 possible ligands, right. Instead of having 2 states you now have 4 states and therefore, the terms get more complicated, but the spirit is exactly the same.

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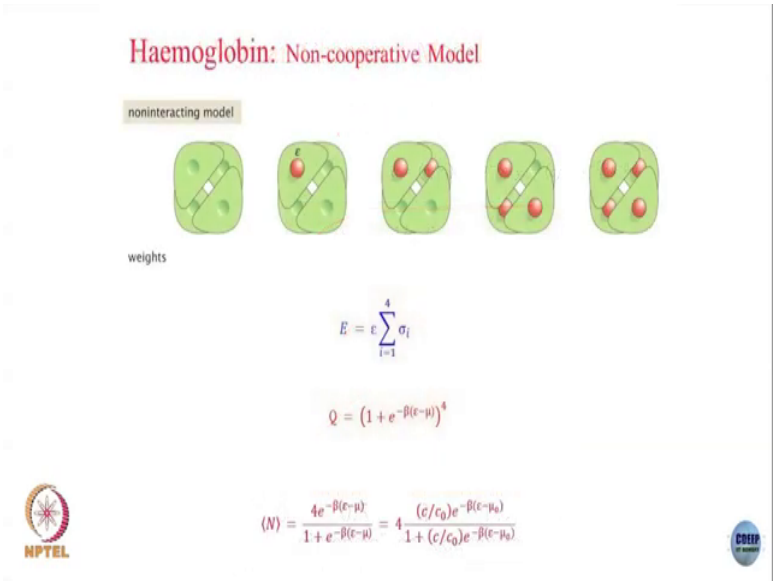

So, just to start off with I can do the non-interacting model; where I say that well I do not consult talk in terms of interaction energy so, I will talk in terms of this J; so, the first model that you can so, you can build in complexity layer by there. So, the 0th order is when I say that each of these 4 sites on the hemoglobin they are non-interacting when oxygen comes and binds here it does not affect the energy of each of these any of these others and then you can simply write down the weights there are 4 sites.

So, therefore, there are 4 variables sigma 1, sigma 2, sigma 3, sigma 4 each of them can be 1 or 0 depending on whether there is an oxygen bound or not. And, you can write down these states so, this there are 4 possible ways to get one oxygen bound, right on these 4 sites so, you have a factor of 4. So, we have 2 oxygen bounds you have 6 possibilities 4 c 2, to have 3 bound you have again 4 possibilities, 4 c 3 and to have all 4 bound there is a single possibility, ok.

So, you can write down again the partition function, once you write down the partition function as expected, because this is a non-interacting model is just the single partition single particle, single ligand partition function raised to the power of 4, right. Because these are not intractable and then you can calculate what is the average occupancy and that is just 4 times the single ligand occupancy. We will compare these results to what the actual binding data for hemoglobin shows. So, this is the single ligand occupancy; which is sorry this is the non-interacting model where the answer is simply 4 times the single ligand model.

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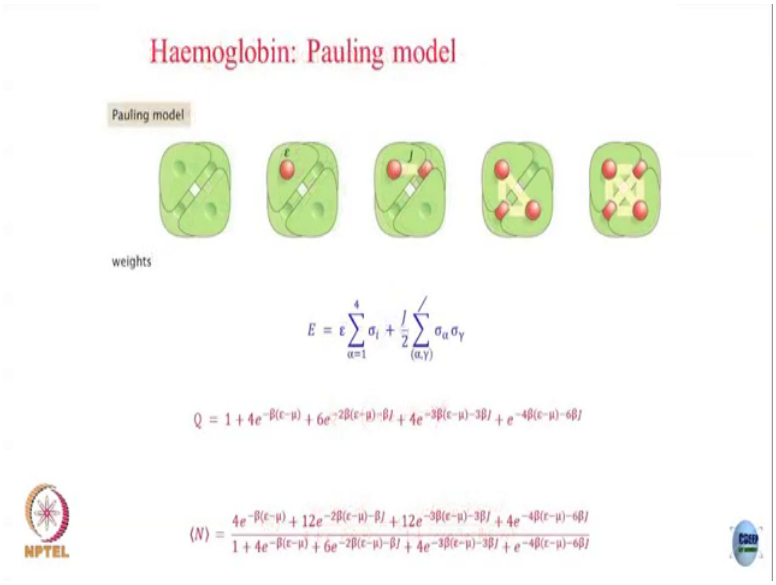

You can now, start to put in cooperativity. So, for example, the first level is what is called the Pauling model for hemoglobin, where you say that you have an interaction energy J when you have more than one ligands that are bound right. So, for example, in here there J does not kick into the picture here there are 3 bonds sorry, here there is 1 bond so, there is 1 J here for example, there are 3 bonds between this and this there is an interaction energy within this and this between this and this. So, there are three J terms and here there are how many? 6 J terms, right.

Student: (Refer Time: 20:19).

So, I write down my Hamiltonian in this form, that I have epsilon sum over i sigma i plus J by 2 sigma alpha sigma gamma; where this alpha gamma run over all pairs, right of this sites and J by 2 simply, because I am double counting I am counting 1 and 2 and 2 and 1 together. And, it

is a restricted sum in the sense that I cannot have alpha and gamma is the same, ok. So, this prime implies restricted somewhere alpha cannot be equal to gamma. So, this is then my Hamiltonian and then I can do whatever I can write down the weights, right.

So, the again there are 4 possibilities 6 for this 4 for this, one for this, this has 1 beta J term, this has 3 beta J terms, this has 6 beta J terms; depending on how many bonds you form with this interaction J. And, again this is therefore, my whole partition function and again I can calculate what is going to be the average occupancy, right what is the average N? Again you take, you can take a derivative with respect to mu of this log of this partition function, that will give you the average occupancy.

So, you get some complicated term it depends on what is your mu and what is your depends on, what is your epsilon, what is your J, but it is definitely not what we had earlier which was just 4 times the single ligand occupancy. It has changed, because you have introduced this sort of a cooperative binding in the model; that one ligand sort of makes it more favorable to recruit other ligands. So, this is one level of complexity.

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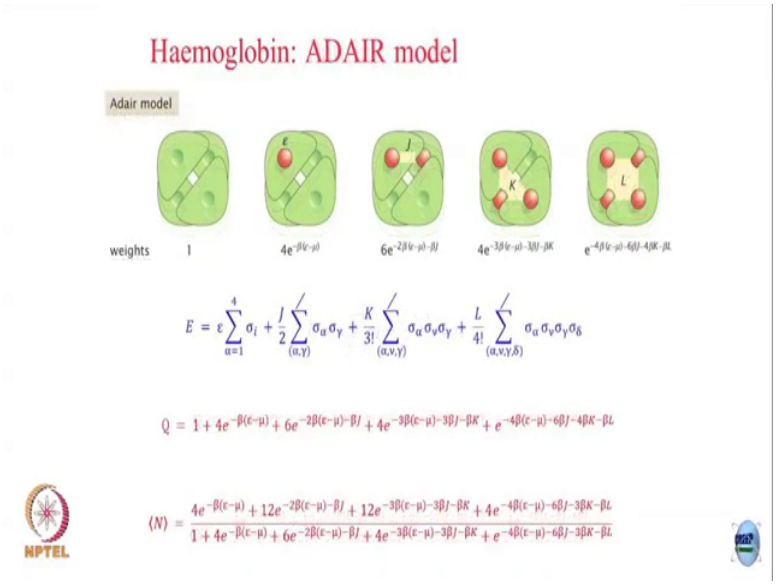

You can keep on building more complexity for example, in the next level you have what are called which is called the ADAIR model. And, there instead of just having pair wise interactions, you can have interactions between triplets or between quadruplets as well ok. So, let me just write down the Hamiltonian.

So, this and this are the same as I had in the Pauling model, right between these are between pairs then I have some interaction energy between triplets. If you have three things that are there then I have an additional favorable term which is of the strength kappa K and if I have all 4 that are bound I have an additional, even more favorable and that interaction term is there for example, ok. And, again you can write down what are the weights of these states and you can calculate what is the grand partition function. And, again there will be a function of now, not only J, but J K and L.

So, this is more parameters as opposed to this Pauling model, but it may or may not be better suited to fit the actual binding data for hemoglobin and again you can calculate, what is the average occupancy. So, now, that I have done this I just want to show some data for this hemoglobin and see that which of these actually fits the data whether it is a non-cooperative model or the Pauling model or the ADAIR model, ok.

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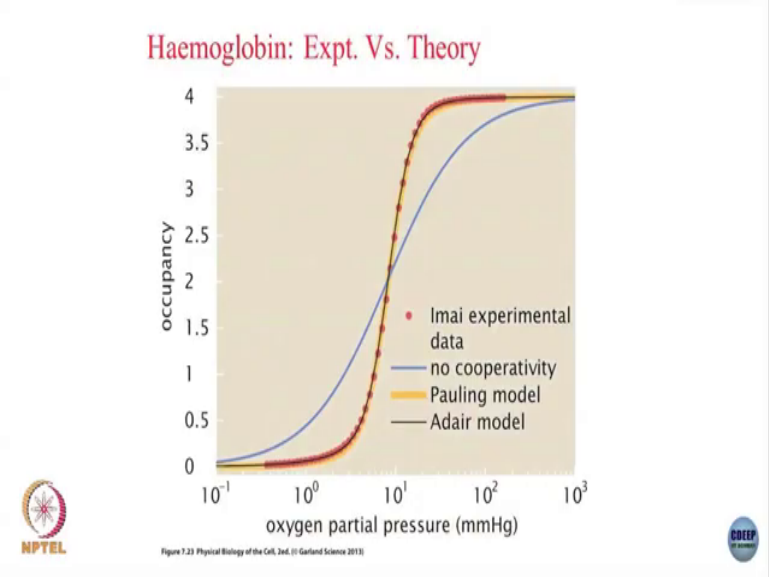

So, here for example, this dots are this experimental data for the average occupancy as a function of this oxygen concentration on this side; this blue line is the non-cooperative model so, it is just 4 times that of this single ligand, which as you can see does not fit the data very well.

On the other hand both the Pauling and the Adair models fit the data to a very high degree of accuracy, right. So, what this says is that these even if these 3 vertex or these 4 vertex terms

are present, they are not really playing a very important role; it is enough to consider that you have pair wise interactions between the ligands in order to explain the experimental binding curve for hemoglobin, ok. So, just this Pauling model is actually good enough to explain the binding curve of hemoglobin.

But, definitely you need some level of cooperativity; if you do not have any cooperative binding you cannot reproduce this behavior, ok. Different ligands and receptors will be fitted by different models there might be ligands, where a non-cooperative model might be the correct model to use, there might be ligands where this Pauling model does not work well, but the Adair model on the other hand will be the correct model to use. So, just because this works for the Pauling works for hemoglobin and the non-cooperative does not is no guarantee that you know; in general the non-cooperative model it is a wrong model.

There might be ligands, receptors and ligands whether data is actually better fitted by the non-cooperative model. And, you can sort of also think about what the effect of this cooperativity is by looking at these probabilities of finding these different, the probability that there is no oxygen versus the probability that there is 1 oxygen versus 2 and so, on; probability that there is 4 oxygen; how do these change in the presence and in the absence of this cooperative binding?

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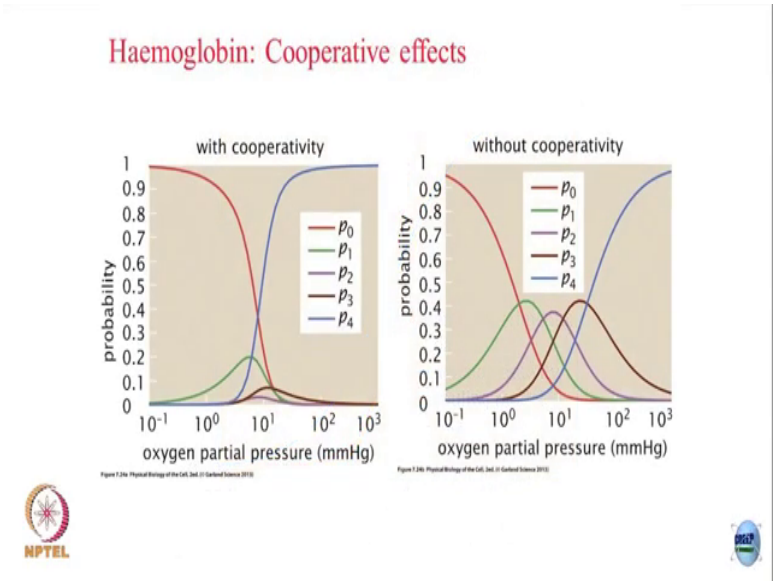

So, this is that curve this is just this formula in the I think this is formula in the Pauling model for certain choice of this J and so on. So, if you do not have, if you did not have cooperativity then this is your p 0, it falls, but once it falls first p 1 rises so, you will have some region where it is likely to have only 1 oxygen bound some region by region, I mean some concentration of oxygen where is more likely to have 2 oxygens bound somewhere it is more likely to have 3 oxygens bound and then you get to this 4. Whereas, once you introduce cooperativity its more like either you have nothing bound or you have all 4 that are bound, the other ones this 0, 1 and sorry this 1, 2 and 3 they are suppressed to a much larger extent in relation to this non-cooperative model.

The so, what its saying is that the moment is sort of recruit one, because it makes it more and more likely that you will recruit more. You very quickly switch from this no oxygen bound to this all 4 oxygen bound without these terms ever becoming a dominant factor; as opposed to

this non-cooperative model with each of this as a regime where they will play a dominant role. So, again the statistical mechanics is fairly simple; it is just a matter of writing down what are the various states and you know, the states are reasonably discrete you know 2, 4, 8 whatever you can actually its countably few.

So, you can write down the corresponding states and weights for each of these states and therefore, get what are these sort of occupancy probabilities for different ligands, different proteins, different modifications, science, channels and so on and so forth. So, you can take this whole machinery of this calculating the partition function and from there calculating probabilities and apply it across a variety of protein systems ligand receptor sort of systems, ok.

So, I think I will stop here today, I will continue.