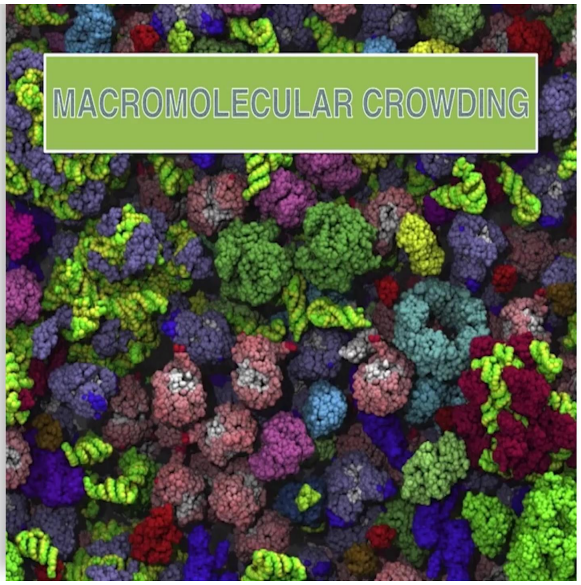
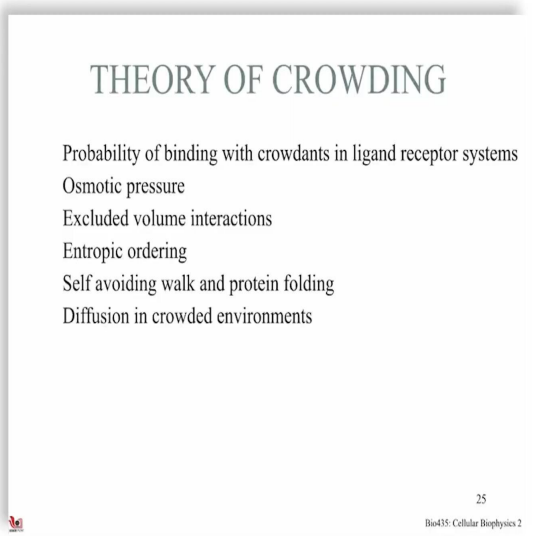
Cellular Biophysics
Professor Doctor Chaitanya Athale
Department of Biology
Indian Institute of Science Education and Research, Pune
Part: 01
Macromolecular Crowding

(Refer Slide Time: 0:16)



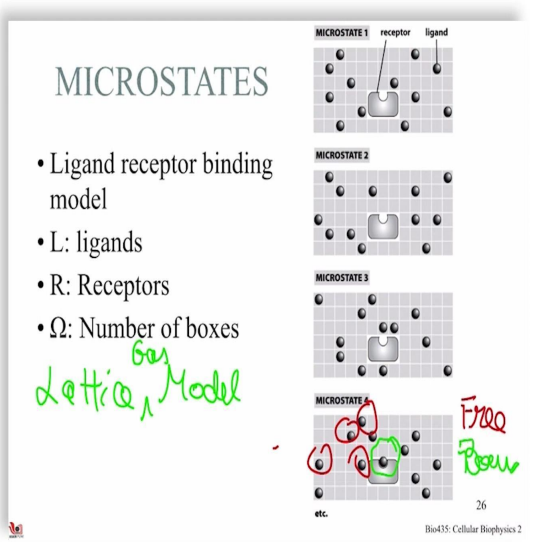
Hi, welcome back. We spoke last time, about macromolecular crowding. And I introduced to you some experimental data that provides us evidence that macromolecular crowding exists, that it affects rate constants. And that it adds up back to the theory that we had discussed in last week's general analysis. This picture again, from there to remind you that macromolecular crowding is kind of important and it is interesting to study it. But once we go from experiments and descriptions and numbers that we quantitative measurements that we obtained from experiments, then it is obvious that we need theory to make more sense of it.

(Refer Slide Time: 1:07)



And so for today, I am going to cover a whole bunch of topics, which I am going to do in two parts. I am going to start with discussing a solution for the probability binding with crowdants in ligand receptor systems, move on to osmotic pressure and the role of crowding in it, excluded volume interactions, entropic ordering, self-avoiding walks and protein folding, diffusion in crowded environments. So, we are going to talk about the first 3 parts first and then the second after that.

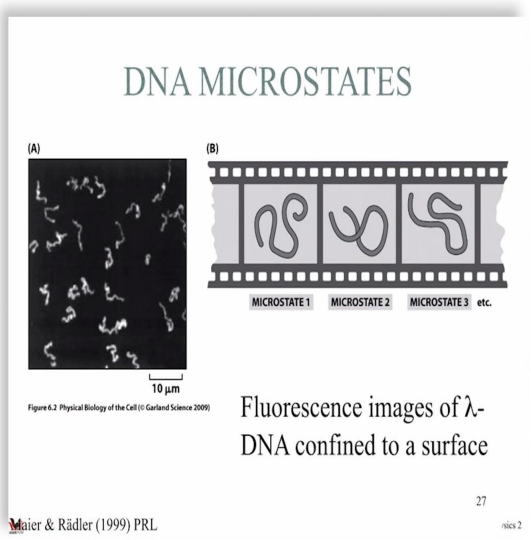
(Refer Slide Time: 1:43)

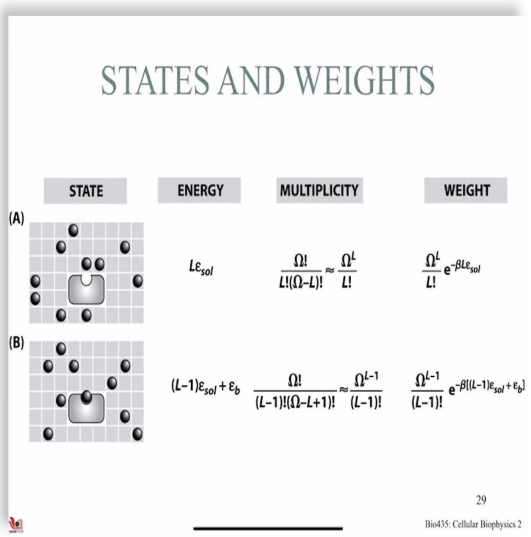


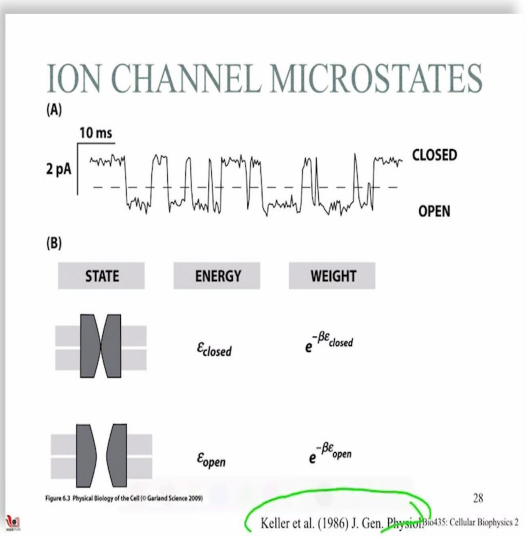
So, of course, we, as I had alluded to yesterday, very nicely can go back to the formalism that we had discussed last semester, in using the statistical mechanics engine for understanding statistical distributions multiplicities of probability of being bound or not bound and then

adding in the additional component of crowding. So, what we are looking at here is ligand receptor binding with L as the number of ligands, R is the number of receptors and Ω as the number of boxes, the lattice, the lattice model that we are used to form earlier, lattice gas model. And, indeed, what you observe here is, of course, that one of these is the bound state. While all these are free, free ligands and bound.

(Refer Slide Time: 3:00)

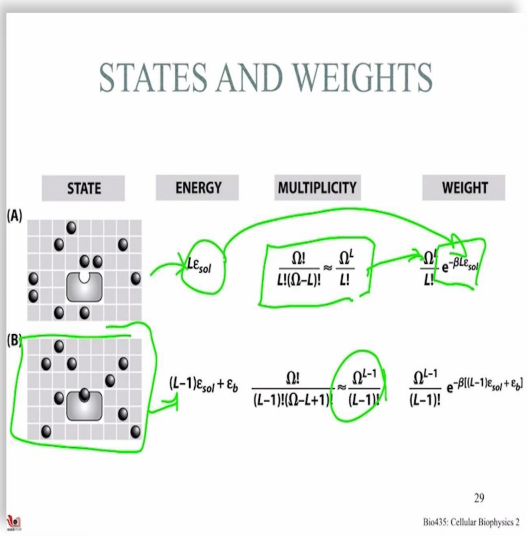






And knowing this we had last time, use this idea that you have multiple microstates, you have some over average. And if you want to know how likely one state is as a representation of all you need to go through the statistics of it and put together probability. And this is also true, not just of the DNA that I just showed you earlier, but also of the ion channel microstates, were closed and open can be considered to be the two states and the dynamics into the intermediate between them. This paper from Keller is a very nice elucidation to show of this problem for those of you who are neurobiology, fascinated by neurobiology, you might want to go back and look at this one. Because it essentially describes the biophysics of the biophysical basis of fundamental neurobiological phenomenon, this is electrical induction plans.

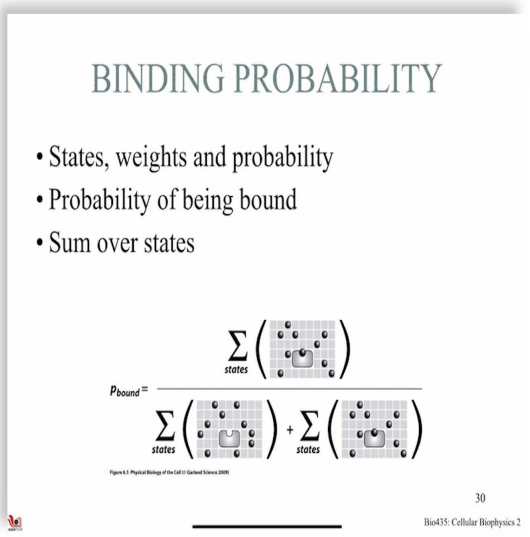
(Refer Slide Time: 3:53)



But anyway, we come back to our states and weights for binding and if you remember, we said that the energy associated with the soluble phase of the ligand is ε_{sol}, multiplied by L gives us the total energy of that state and then L-1 one molecule being bound into $\boldsymbol{\varepsilon}_{sol} + \varepsilon_b$ for the bound state, that is to say the whole system, that is what we are really trying to describe that goes into these equations respectively. And the multiplicity then and this is again your based on your combinations, preventing over counting meaning to say two arrangements whether things are the same power are removed.

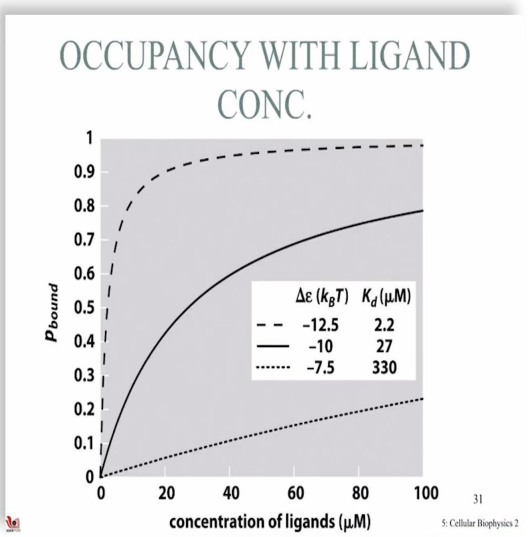
Therefore, Ω that is the number of boxes upon L! upon (Ω-L)!, which simplifies to Ω^L upon L! gives us how many various ways of doing arranging it in this way are possible. And, this then for the ligand bound state is given by $\Omega^{(L-1)}/(L-1)!$. The weights therefore become the Boltzmann's probability that is $e^{1/kBT}$ into the energy. And in the pre-factor comes the multiplicity that we usually then called Z Zustandssumme, all right some overall states. So, essentially, this is back to our old construction, so there is nothing new here.

(Refer Slide Time: 5:37)



And, if we want to look at the probability, then it is just simply the bound states sum divided by both bound and unbound states. I mean, this is kind of obvious that this binding probability, then we as we discussed last time, is a true probability goes from zero to one and has very nice properties of summation and so on.

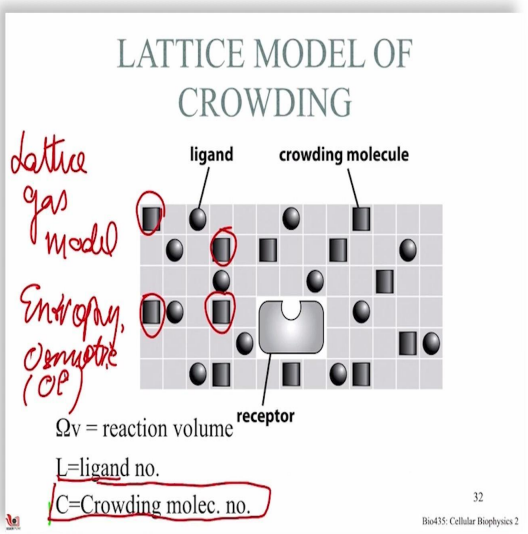
(Refer Slide Time: 5:55)



Now, when we look at the binding kinetics with ligand concentration, we see a curve that looks like this, where as the difference between the bound and unbound state increases, we get a more dramatic probability of binding with the $\Delta\varepsilon$ that is the difference between the two states being the key determinant of the likelihood of a reaction and this is expressed in bulk kinetics in biochemical terms as the dissociation constant which is lower. And you remember that in your ligand receptor binding or two molecules associating with each other, let us say two proteins associated with each other or a DNA and a protein that the or chemoattractant and a receptor or drug and target molecule, that the lower the K_d, the more stronger binding and this again in the light of drug discovery and we are in the middle of a medical emergency and all the science should point towards more development of antivirals.

But, if you are trying to build a better antivirus, you are a lower k_d for instance. And, that is related to the difference in energy free energy between the free inbound state and you are aware of this.

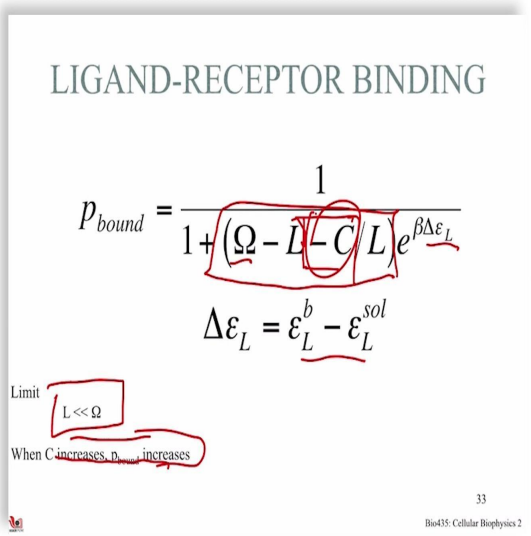
(Refer Slide Time: 7:31)



But, what happens when in this lattice model of ligand receptor binding, we now add some item called C, which is the number of crowding molecules. And, these in our lattice gas model occupy lattice sites like this here, preventing the ligand from occupying them, this is like back to our local train, when the train is already full, you are not going to be able to enter or if it is half full, chances you are standing are higher chances that you even move around much or in some senses your degrees of mobility are reduced and this is coming to if you remember our old discussions about entropy, osmotic pressure in the osmotic engine thought experiment and a lot of other paradigms that we have been discussing repeatedly in this course.

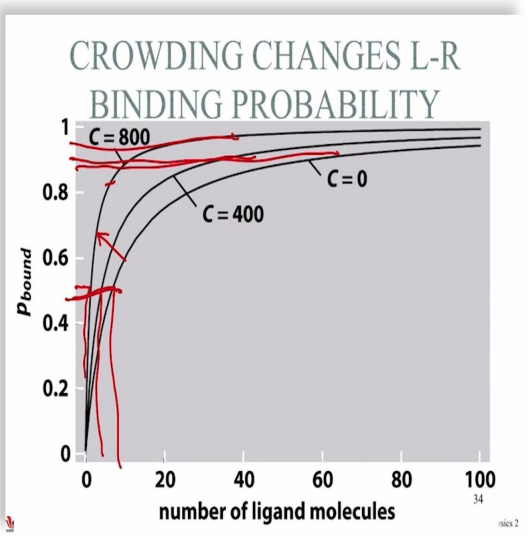
So, in that sense, that is not very surprising that you should expect that the presence of a crowding molecule should change something in your system.

(Refer Slide Time: 8:50)



In fact, what it does is that it changes the ligand receptor binding probability to an expression that now includes this minus sign here, which is the concentration of the crowding. So, number of crowding molecules in the lattice with Ω sites and your $\Delta\varepsilon_L$ the energy difference of bound to free ligand while it remains the same, it is multiplied by a pre-factor that is reduced by C. As always, we are talking about the dilute limit L is much less than omega and when C increases and this is the important thing, when C increases, we expect from the theory that P bound will also increase. In other words, increasing the crowdants concentration increases the probability of binding. So, just think about this equation for a bit, look at it. Some of you enjoy Python plot it and we will go and see what people have done both theoretically and experimentally.

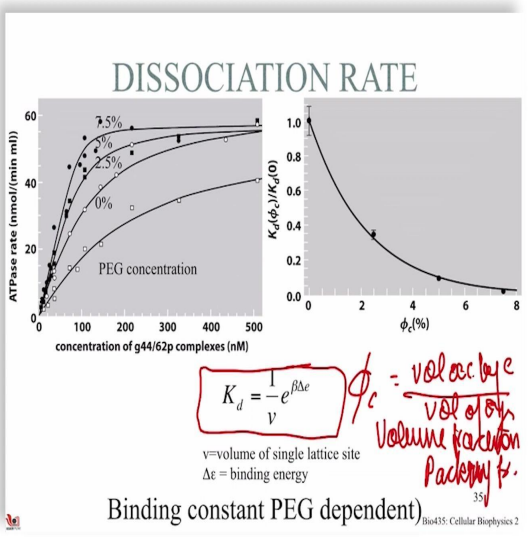
(Refer Slide Time: 10:05)



Turns out that when you plug this into an arithmetic, I mean, you just graph the equation essentially p_{bound} as a function of number of ligand molecules, you will find that with increasing ligand molecules, your curve becomes sharper and sharper. And, please remember I mentioned to you something like your half maximum or whatever you want to call it decay constant, how fast the curve goes from zero to saturation. Well, you see this here visibly and I just want to point it out to you, at this 0.9, 0.45 is it is half max, V_{max} half, that is somewhere here at 10. So, this one is at 10. This one goes up over here. So, 0.92, so 0.45. So, this one goes at 5 probably, and this one saturates also at a higher value. And it is half maximum 0.95 is point something like slightly higher and it is even smaller to 1.

In other words, the half maximum value of saturation of bound state decreases further and further as a function of increasing crowdants consistent with our theory.

(Refer Slide Time: 11:29)



What about experiments? Well, when you measure the ATPase rate with increasing by concentration as we had shown earlier, in terms of the T4 bacteriophage virus, DNA clamp loader protein g4462p, then with increasing crowded concentration from 0 to 7.5 percent weight by volume of polyethylene glycol, you see that the rate or the velocity of reaction, which is a readout, in some senses surrogate for the probability of being bound reaction not happen if the substrate enzyme are not bound also increases successively in a qualitatively identical manner.

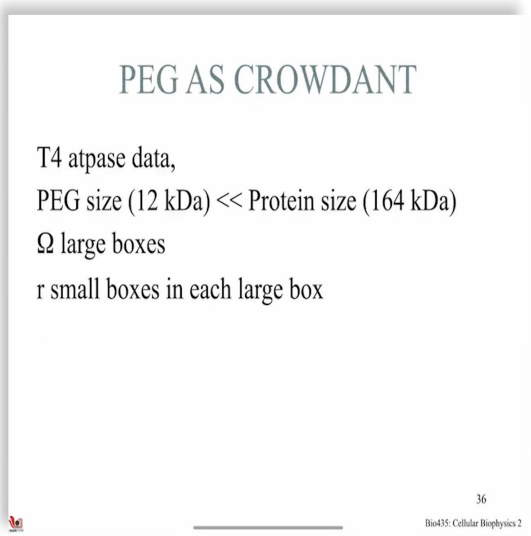
And that is really striking in these fit curves, these lines are indeed based on the model, in addition by doing a little bit of arithmetic, you can show that the K_d the dissociation constant, which is something that we always want to measure in binding or unbinding reaction, scales as,

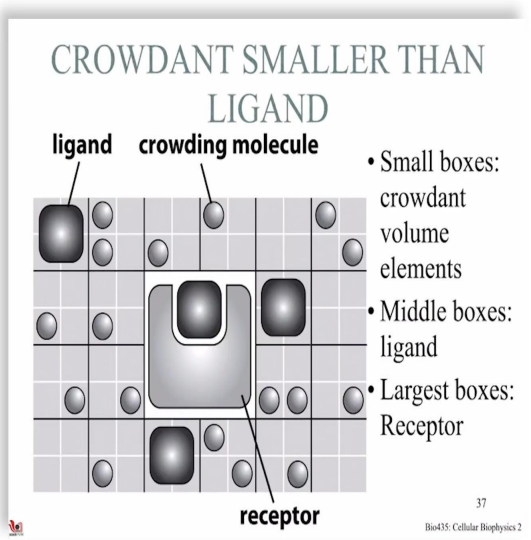
$$K_d = \frac{1}{v} e^{\beta\Delta e}$$

a function of 1 by v, where v is the volume of a single lattice site, which is the characteristic size of the molecule let us say the current into $e^{\beta\Delta\varepsilon}$. And Δε is again the difference between the bound and free states of energies β being 1 by kB T, your scaling constant.

So, this also implies that K_d with a crowdant in place upon K_d without a crowdant here, meaning the ratio of the two decreases further and further with φ_c, what is φ_c? Is nothing but volume occupied by c upon total volume of the system. This is also then referred to as the volume fraction. In literature of packing and so on it is also called packing fraction.

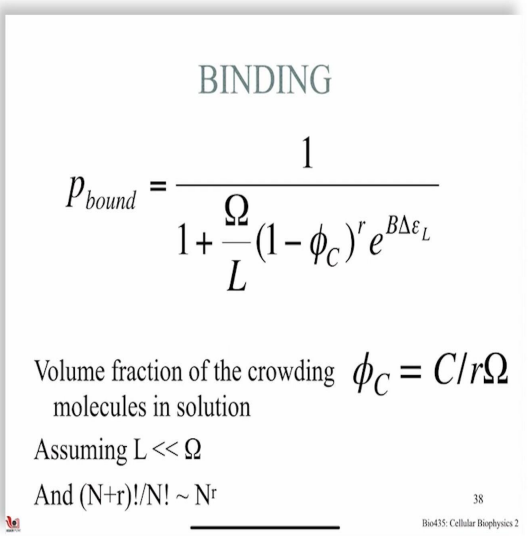
(Refer Slide Time: 13:52)





Now, this also means that binding constant can be K_d PEG dependent, again, T4 atpase data, PEG size of 12 kDa, protein sizes much greater 164 kDa, omega large boxes, r small boxes in each box. So, now, we have a model that is a bit more precise, where our crowding molecules relative sizes, we said from this should be about a 10th one tenth of the size of the protein based on the Dalton mass alone. And so, we say for simplicity over here it is some half for one forth. I am sorry, we can make it much smaller actually. For our calculation purposes, this is what we take small boxes of crowdant and middle boxes are ligand and the largest boxes are receptors.

(Refer Slide Time: 14:41)

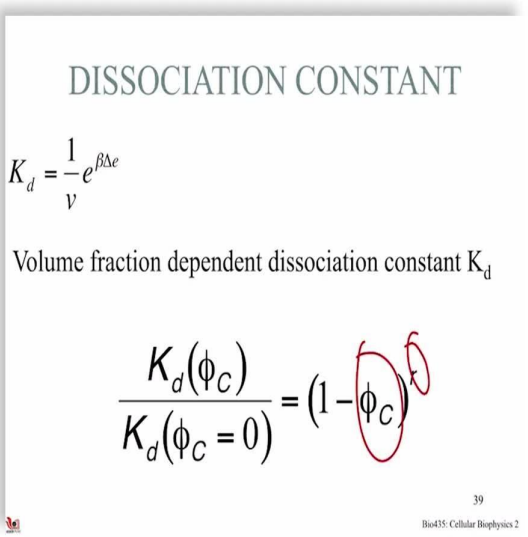


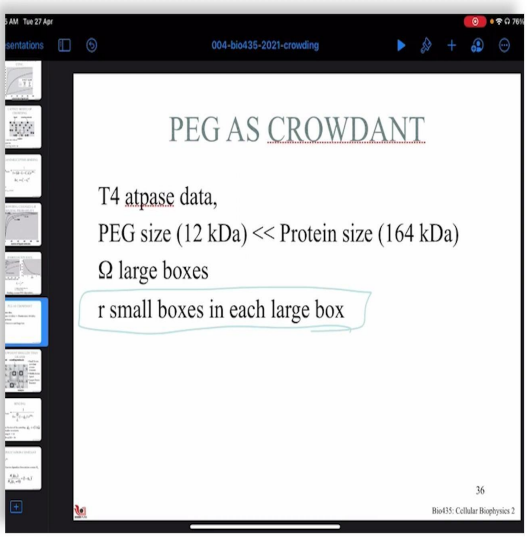
Then in such a case, considering the relative size asymmetry also, we have the probability of binding now becoming

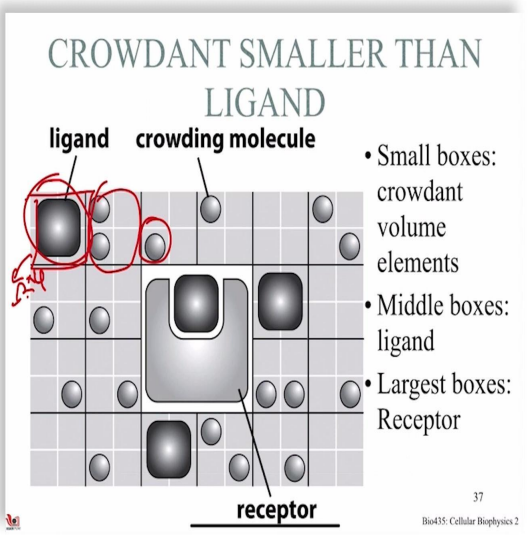
$$p_{bound} = \frac{1}{1+\frac{\Omega}{L}(1-\phi_c)^r e^{B\Delta\varepsilon_L}},$$

φ_c is the volume fraction of crowding, which since we are talking in terms of number of boxes, just simply C boxes upon r times Ω boxes that total number of boxes of the characteristic size of the smallest molecule are right. This is again assuming L is much less than Ω and (N+r)!/N! is approximately equal to N^r this is the simplification that we get.

(Refer Slide Time: 15:30)

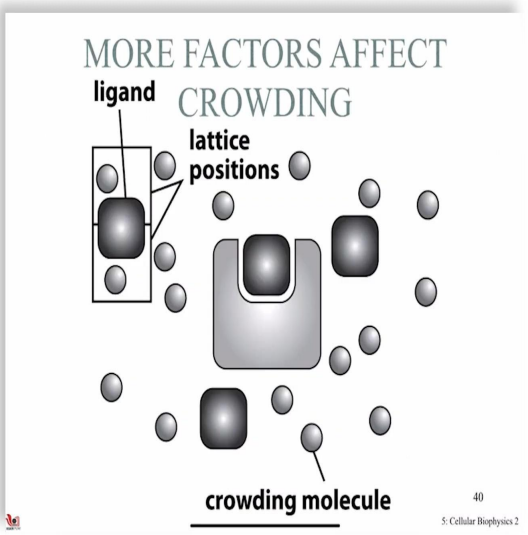


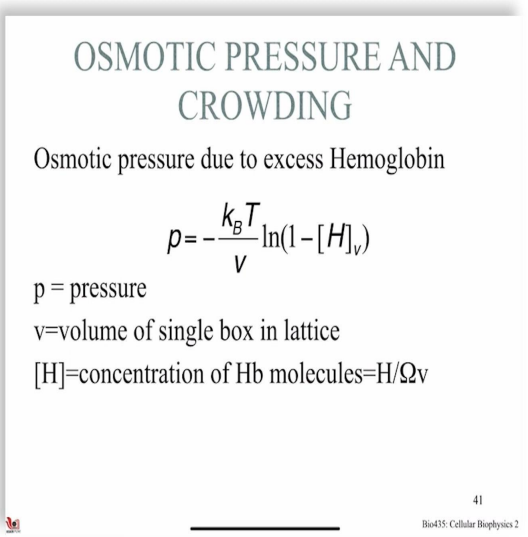




It turns out that K_d also changes with the volume fraction in the sense that $K_d(\varphi c)/K_d(\varphi c=0)$ where there is no crowdant is equal then to $(1-\varphi_c)^r$ in other words, the ratio of the dissociation constant scales with the volume fraction of crowdant to the power r which is our relative size of the molecules, where r small boxes in each large box are indicative of the number of smallest elements of the system. So, it is if you could say it is the multiplication factor telling us the relative size ratio between the crowding molecule and the largest ligand molecule because the largest ligand molecule is determining the Ω and so, knowing this factor that is 4, 2, 1 we are basically multiplying it by 4 in this concrete example. So, I hope that is kind of clear.

(Refer Slide Time: 16:41)



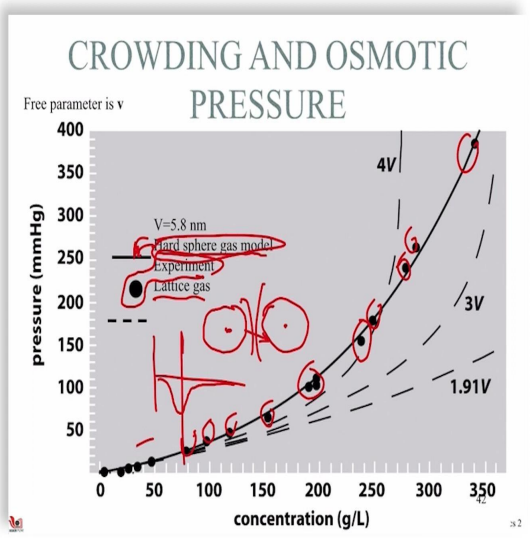


And so, we in fact have can say that in addition to relative sizes crowdant presents other factors also may affect crowding, which may relate two lattice positions and half positions meaning to say the ligand could be half between two boxes, the receptor could be between multiple boxes and junction points, but by and large, we find that the error in this methodology is minimal compared to these kinds of errors it refines the answer it is true. So, to move on to the next topic in the factor of crowding, we will talk now about osmotic pressure and how crowding affects it.

So, the osmotic pressure due to excess hemoglobin has been found by experimental optimization and merging with theory to be p, which is the pressure is minus $k_B T$ upon V volume of a single box in a lattice multiplied by the natural log of 1 minus the concentration

in this case of hemoglobin molecules in a given volume. Now, each concentration is basically the number of molecules time Ω into V in the denominator, V being the smallest volume of whatever the smallest element of the system is. This is saying that as excess hemoglobin is increased.

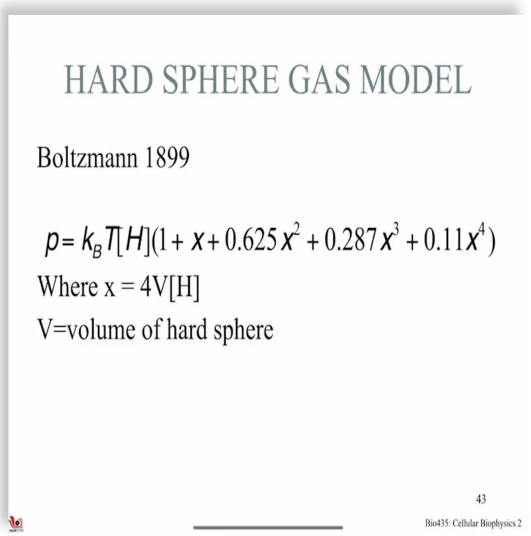
(Refer Slide Time: 18:15)



We will see that the pressure of the osmotic pressure of the system will increase. And the dots are lattice gas model that is the one that we have been working on so far. That is to say things do not intersect, they occupy a fixed position they do not move around. They have a discrete nature in a way our lattice gas model. The hard sphere gas model, on the other hand, which is the straight line, it seems to fit experimental data, which are the circles here. The best where increasing hemoglobin concentration increases the osmotic pressure in what looks like a quadratic curve upwards. With the hard sphere gas model, fitting the data best. The lattice gas model, it appears and the scaling factor here is just a factor of V, you remember our assumption about the size of the volume of the molecule.

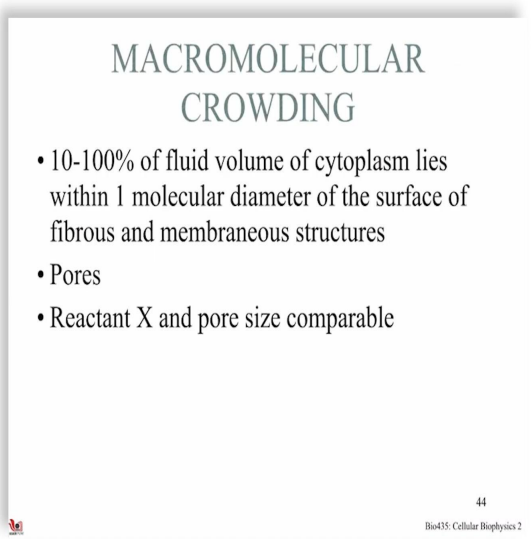
It comes in the range so for we sort of comes close to what we are looking at, but it is not the same shape. It does not fit as elegantly as the hard sphere gas model. Now, it is for gas model, just to remind ourselves is that two molecules are treated as spheres. They cannot pass through each other. They have volume interactions, that is to say, they at some point they repel each other at a critical distance they are able to stand close to each other and this is a continuous model as opposed to our discrete lattice model. So, this is sort of coming back to what I said earlier, which is that these models can be improved upon.

(Refer Slide Time: 20:21)



The exact equation of the effect of hemoglobin on osmotic pressures a little more complex and it comes from scaling of almost like a Taylor series of factor x, which is nothing but 4 times the volume into the concentration of hemoglobin where that volume is the volume of the hard sphere factor that we are assuming and multiplied by k_BT. So, in that sense, this hard sphere gas model allows us to predict fairly reasonably well the effect of crowding on osmotic pressure.

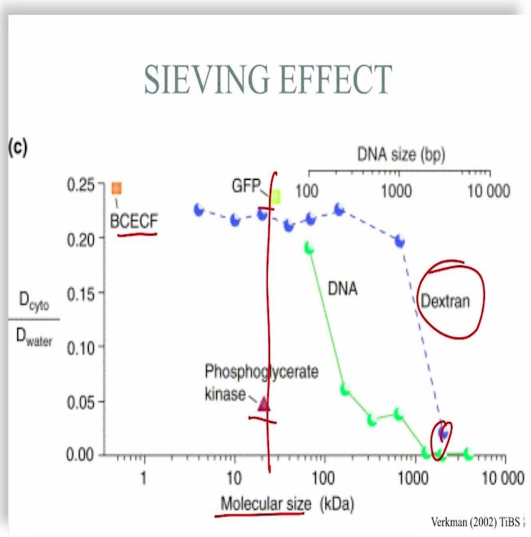
(Refer Slide Time: 21:09)



So, I mean, we talked about macromolecular crowding, we talked about the spacing and we want to know maybe a little bit more about the saline. So, there tend to be 100 percent of the

fluid volume lies within 1 molecule of diameter the surface of fibrous membranous structures and we have pores and reactant X and pore sizes are comparable.

(Refer Slide Time: 21:29)



And just to remind you the, the sieving effect that we saw might be explained by this kind of an idea, which is that as the size of the external molecule was increased on the x axis, the relative offset in the cytoplasmic division question measured upon the pure water diffusion question the ratios of speed changes such that it dramatically dips, in other words, the numerator reduces dramatically to go from point 0.2 to 0.05 in the case of dextran at greater than 1000 kilo dextran size. This does not necessarily apply as much to DNA because it scales differently because you could say it is a polymer and one can put a couple of proteins here, endogenous proteins to look at where this graph stands.

But as I said earlier, also proteins well, the answer depends. The sieving depends on exactly the size of the molecule.

(Refer Slide Time: 22:34)

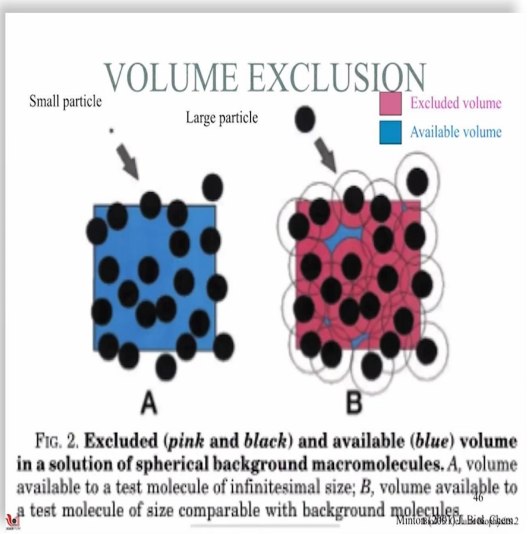


FIG. 2. **Excluded (*pink* and *black*) and available (*blue*) volume in a solution of spherical background macromolecules.** *A*, volume available to a test molecule of infinitesimal size; *B*, volume available to a test molecule of size comparable with background molecules.

And so, I am going to move to the effect that we think might explain this kind of sieving effect, which is volume exclusion.