Cellular Biophysics
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Part: 02
Macromolecular Crowding

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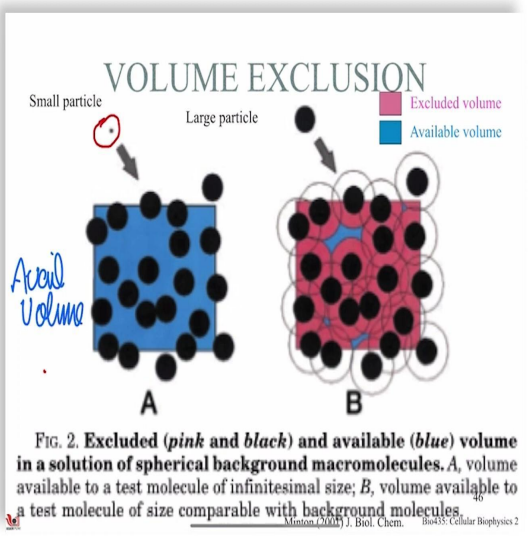

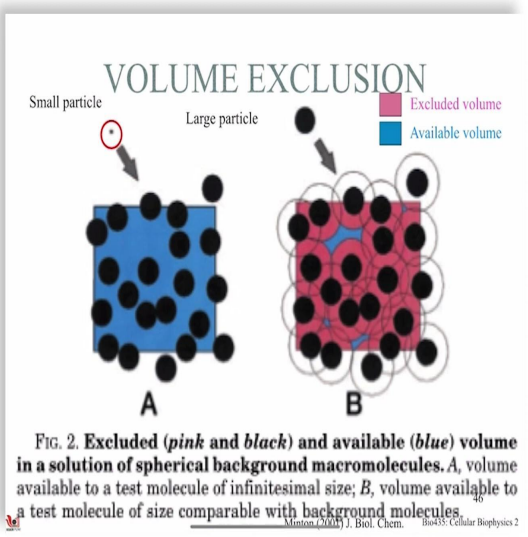

In its simplest form volume exclusion states the following that you either have a small particle that you are trying to insert into a volume, which contains an available volume. This is the blue part. In which case, the black molecules that already filled the region are having inter molecular spaces, and that is where your tiny molecule is likely to fit in. Now, on the other hand, if the excluded volume, by the molecules themselves are a certain radius beyond their own sizes, like in this case, the molecule that can be added needs to be smaller, and a

comparable sized molecule in that sense, becomes almost impossible to add. So, in a way, you have a size selection.

You see, that was the question we asked, how do you get size sieving? Well, tiny molecules can go in large molecules, they experience an excluded volume between sizes of molecular sizes and their interaction radii and prevent things from getting in, and this is a paper by Minton from 2001.

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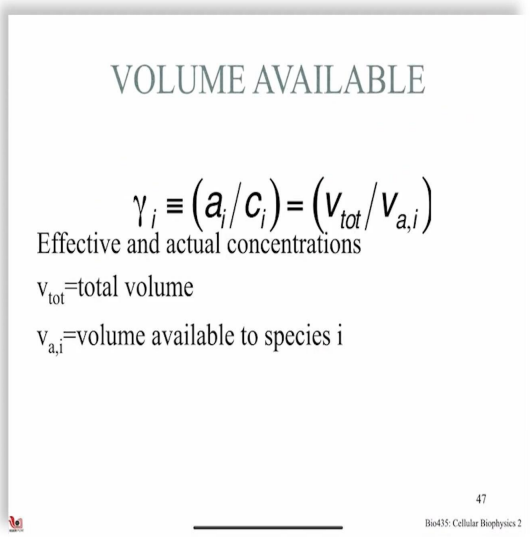

So, the volume available by definition then becomes the volume available to species i. And its ratio to the total volume and this is in some senses, telling us in some senses the volume available, whether the as the molecules volume increases that you need to add, this number goes on to one potentially, and tells you about the so called possibility of adding something in.

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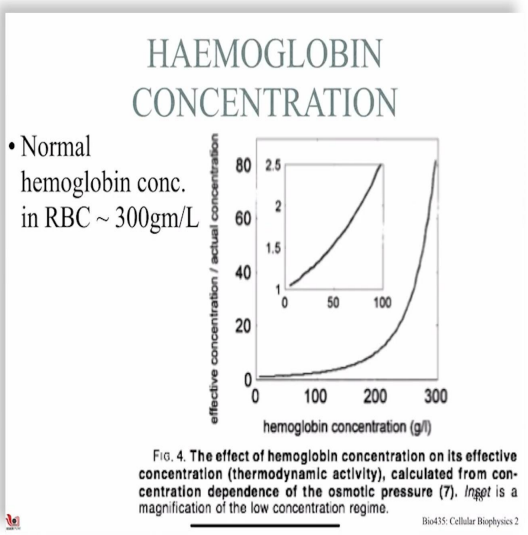

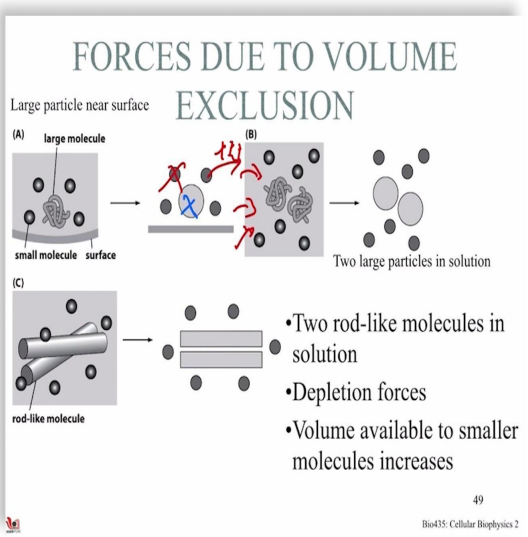

So, coming back again to hemoglobin concentration and the osmotic pressure, this graph essentially demonstrates increasing effective concentration and the role of hemoglobin on osmotic pressure. But what does volume exclusion actually do? So it turns out that you can actually exert forces. And this is quite interesting because so far we have talked about entropic forces, we have talked about electrostatic forces in the passing with the Lennard Jones potential, we have talked about forces that come out of mechanical properties, these are very strange set of forces the exclusion volume excluding forces almost magical. So, the idea is the following. If you have a large molecule or a small molecule in a structure, we can simplify them as big blob, big circle and small circle.

Then, in a dilute system, they do not really interact with each other, it is not much of an effect that we can observe. But if we now increase the small molecules in a large amount, then what we appear to find is that the large molecules assuming that there is more than just one of them in the system are more likely to aggregate together. The second example, demonstrates that two rod-like solutions molecules in a solution are more likely to come together due to so called the depletion forces.

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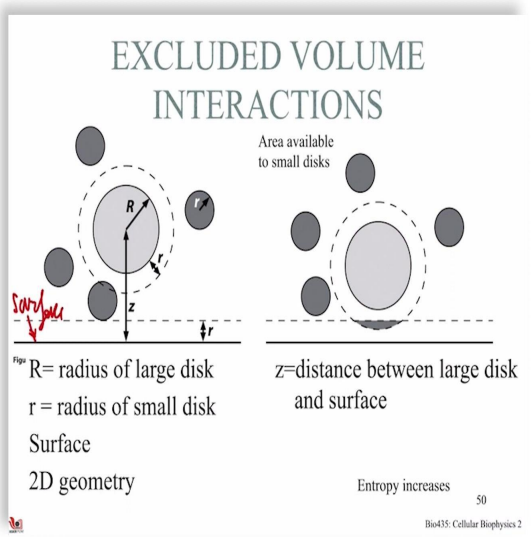

And, these excluded volume interactions can be treated geometrically to arrive at an exact answer about the extent to which they act and when do they act. So, we consider R capital to be the radius of the large disk, r small with the radius of the small disk in a two dimensional geometry and z as the distance during the large disk and the surface being this solid line here. And in such a case and I asked this rhetorical question, where does this volume exclusion arises, is finally nothing but entropy.

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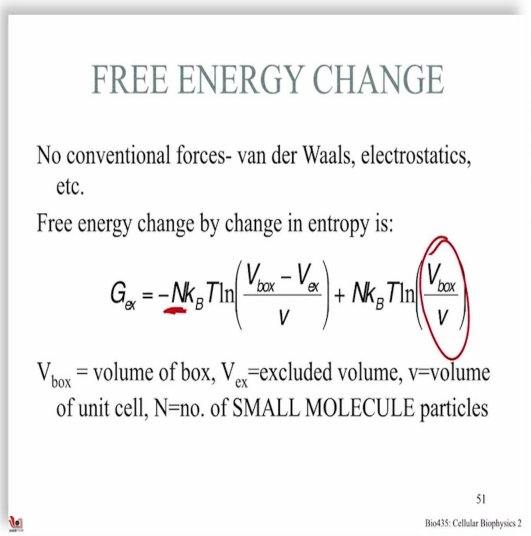

So, we can go back and ask if there are no conventional forces, like we were saying earlier. Van der Waals electrostatic Et cetera, the free energy by entropy change alone due to excluded volume G excluded is given by this expression, which looks a little complex.

$$G_{ex} = -Nk_BT\ln\left(\frac{V_{box} - V_{ex}}{V}\right) + NK_BT\ln\left(\frac{V_{box}}{V}\right)$$

So, let us break it down. The first part is the negative term with the number of small molecule particles N capital times kB T, which is your familiar scaling factor times the natural log of the box volume, the volume we are studying minus the excluded volume that is lost due to the presence of small molecule particles and it is plus the original box volume relative to the volume of a unit cell number of units you means omega in the brackets on the right hand side.

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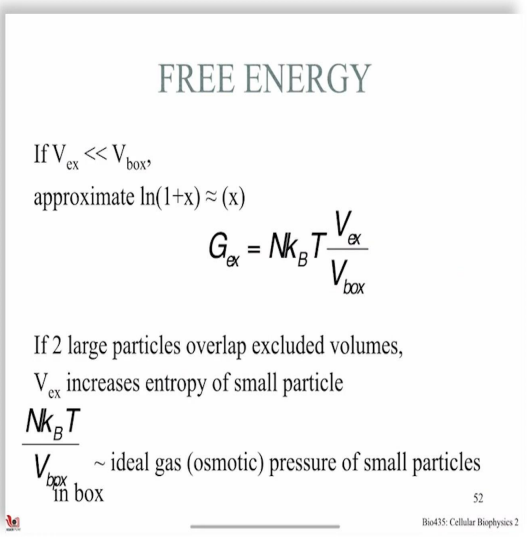

So, given this, when the excluded volume is much smaller than the box volume, we can approximate ln(1+x) to approximately x and we end up so that ln in the brackets V_{ex} by V box becomes this 1 minus V_{ex} by V box becomes this. So, the G excluded(G_{ex}) become this.

$$G_{ex} = Nk_BT\frac{V_{ex}}{V_{box}}$$

If two large particles overlap, the excluded volume increases the entropy of the small particle N times k_BT upon V_{box} is the approximate ideal gas osmotic pressure small particles in a box.

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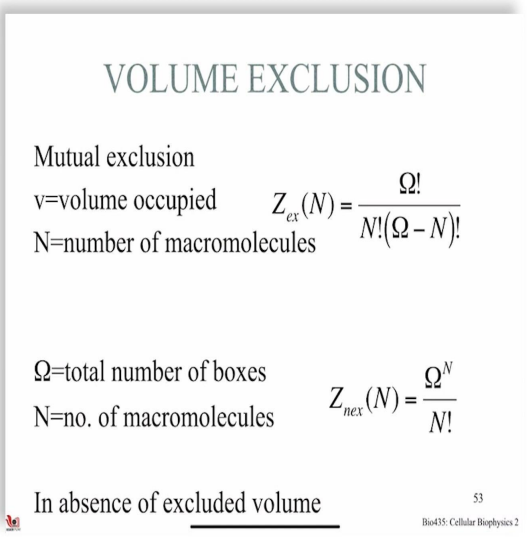

So, volume exclusion works in some senses by changing the number of possible combinations by which we can arrange things. So, the Z term the Zustandssumme of excluding volume interactions then particles included is,

$$Z_{ex}(N) = \frac{\Omega!}{N!(\Omega - N)!}$$

Which of course, can simplify to.

$$Z_{nex}(N) = \frac{\Omega^N}{N!}$$

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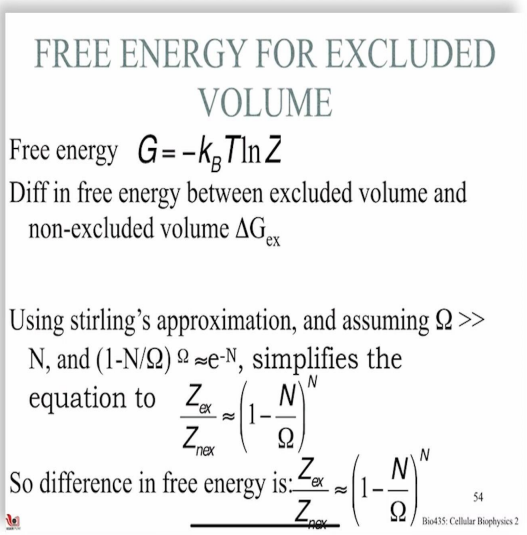

The fee energy for excluded volume then becomes,

$$G = -k_B T ln(Z)$$

And using Sterling's approximation and assuming the Ω the number of site lattice sites is much greater than N, which means, again the dilute limit, which is a little ironic because we are considering crowding but the arithmetic works $(1-N/\Omega)^{\Omega}$ is approximately e^{-N} and that simplifies the whole expression of the excluded volume Zustandssumme and the difference in free energies to be approximately $(1-N/\Omega)^N$.

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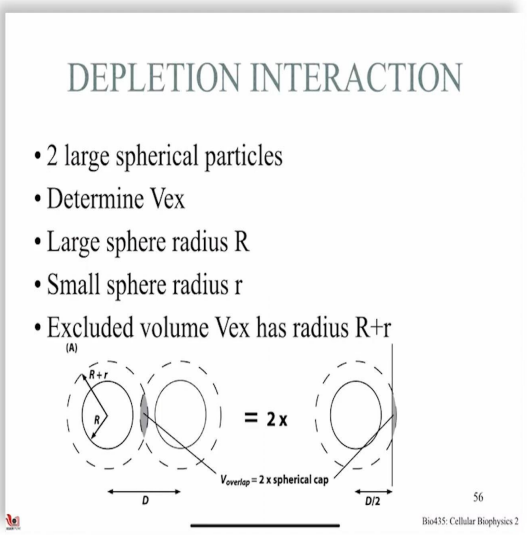

The second part which I mentioned earlier, namely depletion interactions. Concerning two large spherical particles are coming together we determined something like an excluded volume and this can be based on two large spheres of the same radius and a small sphere of radius small r excluding volume has the radius R capital plus r small(R+r). So, I think this small sphere radius should actually read the exclusion volume radius here.

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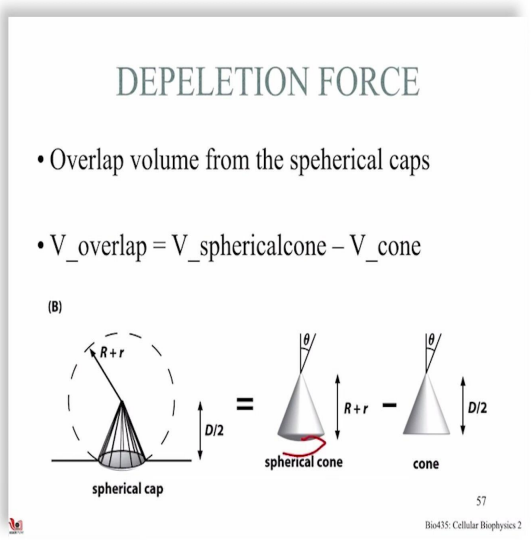

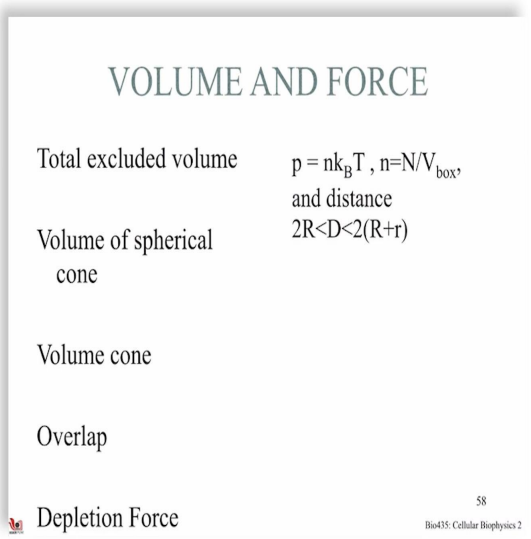

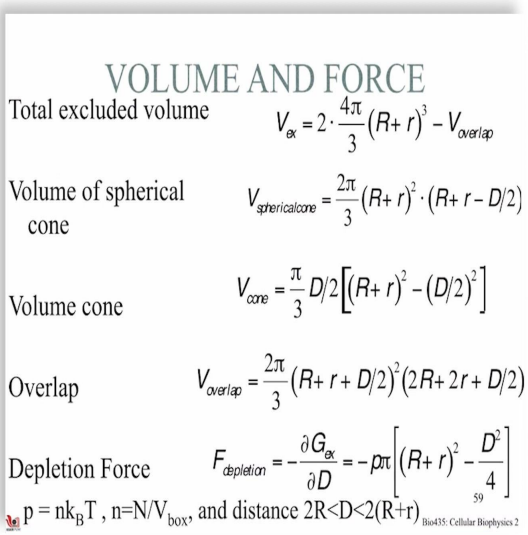

The overlap volume from the spherical caps is the cone minus volume of the cone spherical cone minus the volume of the cone this little part here and so, volume and depletion. So, the depletion interactions can be got at by combining volumes and forces to tell us that the total excluded volume is V excluded in geometric terms,

$$V_{ex} = 2 \cdot \frac{4\pi}{3}(R + r)^3 - v_{overlap}$$

And the spherical cone, then, in fact,

$$V_{sphericalcone} = \frac{2\pi}{3}(R + r)^2 \cdot \left(R + r - \frac{D}{2}\right)$$

And D, if you remember was the distance between the two particles, volume cone and is sort of simplifies and then the overlap simplifies and there is a bit of arithmetic I urge you to look at this if you want to verify the answer, but the depletion force then comes down to

$$F_{depletion} = -\frac{\partial G_{ex}}{\partial D} = -p\pi\left[(R + r)^2 - \frac{D^2}{4}\right]$$

And so, p which is n times k_BT, in n which is N/V_{box} and distance is 2R and distance is D which is greater than 2R, which is the hard shell radius meaning to say the sum of the radii of the two objects that the distance cannot be less than that particles can intersect. But in this arithmetic that also cannot exceed R + r, it becomes an interesting program.

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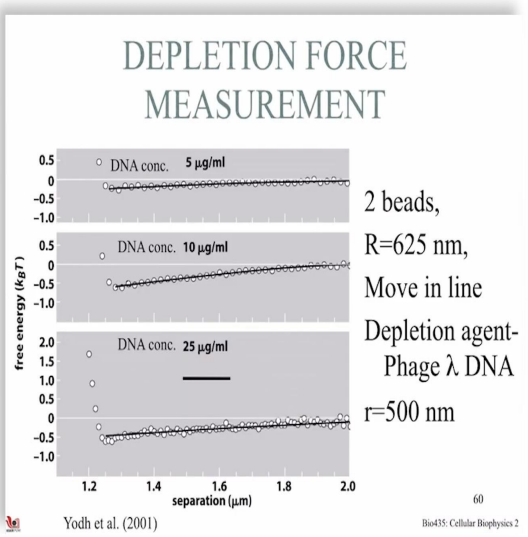

So, depletion measurements like this, were made and experiments to find out whether this is real by calculating the free energy for increasing concentrations of DNA from top to bottom, from 5 micrograms per ml to 25 micrograms per ml, showing that the interaction between two beads results in lower and lower free energies of interaction of the beads. So, the beads were the ones that were being measured DNA was the crowdant and the interaction was followed.

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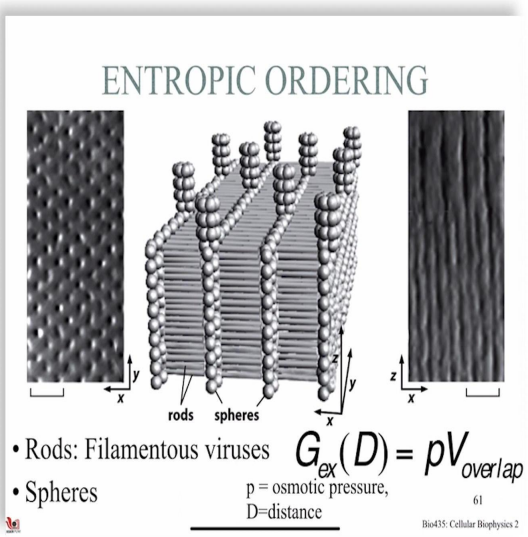

Entropic ordering, which was another topic I wanted to maybe just briefly mention is a further aspect of this, which is that we always talk about entropy as a way to create this order. But it turns out that entropy ordering also counter intuitively drives indeed the process of

order formation at high concentrations. And this is the free energy of it is given by p times $V_{overlap}$, where spheres are ordered in a system containing filament rods. And these filament rods in this particular case that were added were viral filaments, something like tobacco mosaic virus.

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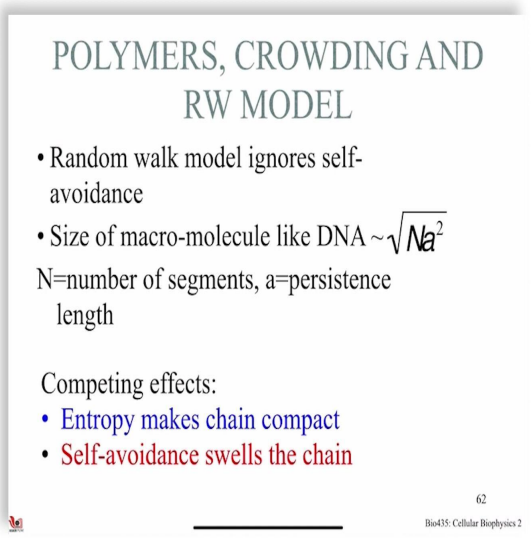

So, we have talked about volume exclusion, depletion interaction. And now we are going to talk about how polymers are affected by crowding and the random walk model and the random walk model, which we talked about last semester. If you remember, allows for polymers to cross over onto themselves, it ignores in that sense self-avoidance. If N is the number of segments and a is the persistence length, then for the size of macromolecules is characterized and the root mean square, end to end distance is characterized scales at least, $\sqrt{Na^2}$. But there are competing effects that we know entropy, which will force the chain to become more compact and self-avoidance.

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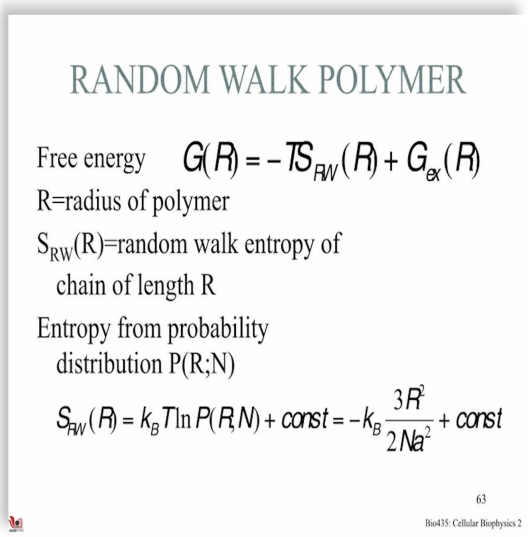

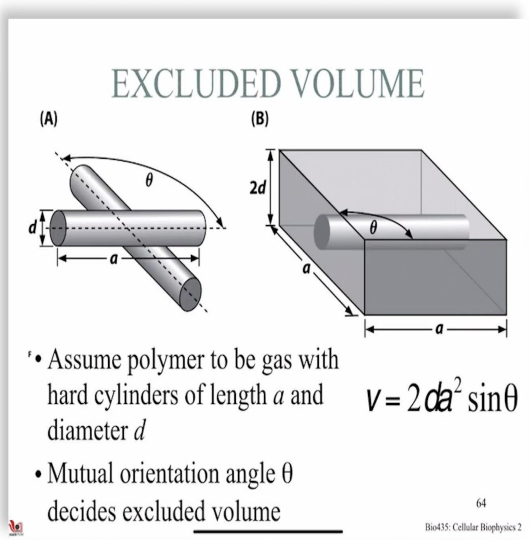

Which will actually force it to become larger and swell it. So, if we consider these things, then we probably need to revisit our random walk model for a more realistic scenario and that involves, so called self-avoiding random walk, which considers the excluded volume interactions and is based on simple geometric arguments of these rods, where the angle between 2 rods isθ. And, a is the persistence length of the minimum rod length, the mutual orientation angle defines the excluded volume which is given by

$$v = 2da^2 sin\theta$$

from geometry.

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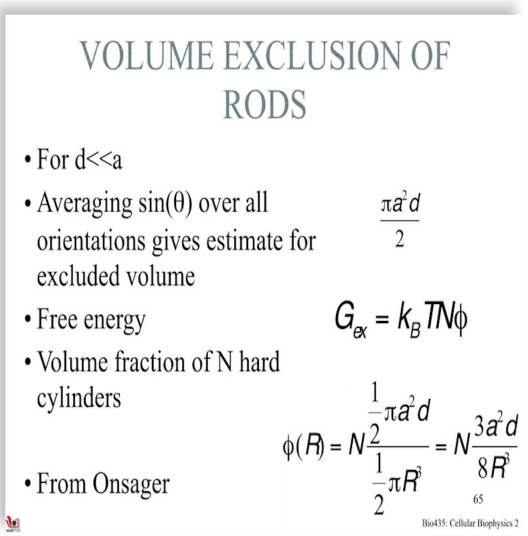

And for d is much less than a, the averaging of $sin\theta$ overall orientations gives an estimate of the exclude volume $\pi a^2 \frac{d}{2}$, the free energy of G_{ex} is $k_B T N \phi$. And for N hard cylinder hard, meaning they can pass through each other. From Onsager approach, you can get a closed form solution for the packing density as a function of R being N times this sort of complex looking geometric expression, which simplifies to,

$$\phi(R) = N\frac{3a^2 d}{8R^3}$$

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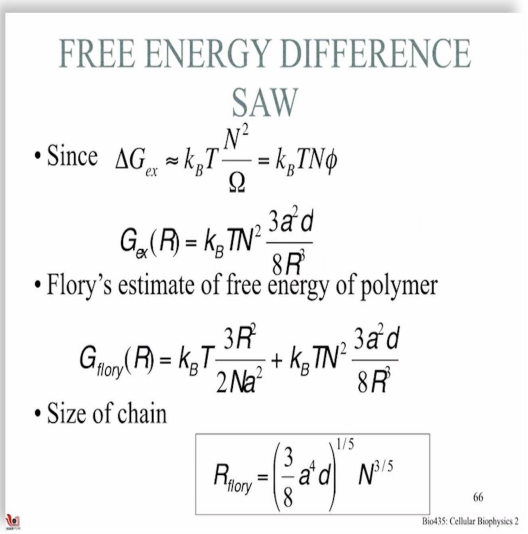

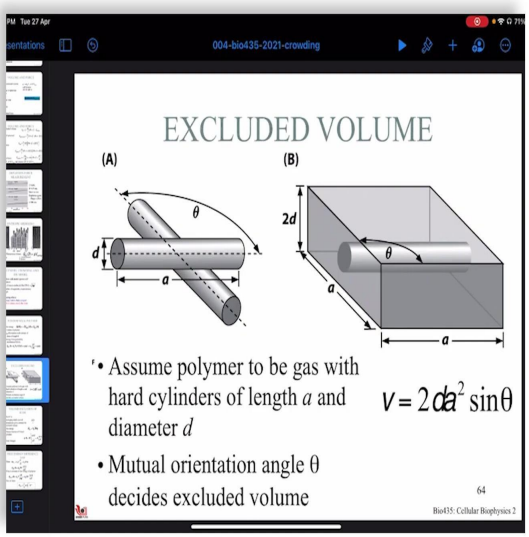

And, the free energy difference between the random walk model and the self-avoiding walk is such that the radius of the flory radius, unlike the characteristic random walk radius, scales as a function of $N^{\frac{3}{5}}$, N being the number of segments with the pre-factor that is determined by a and d. So, just let us remind ourselves what were a and d? a was our single rod length minimal rod length, which we call the persistence length and d is the diameter the thickness of this. So, in a way of parameter that we have not considered so far in the random walk formalisms that we had been looking at.

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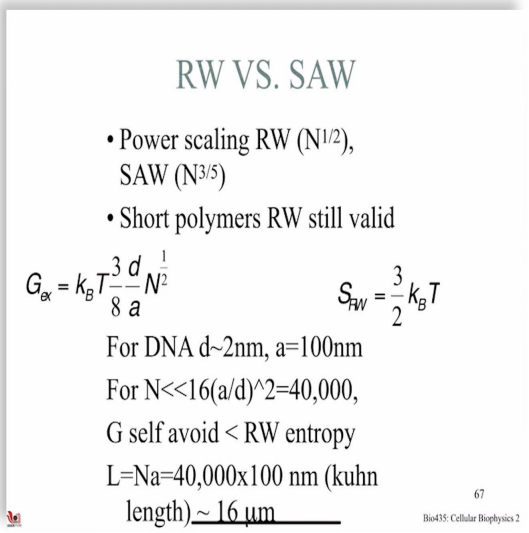

So, random walk versus self-avoiding random walk means that while the random walk scales as $N^{1/2}$ or under root of N, self-avoiding walks scales as the fifth power and the third root of

N, for short polymers random walk is still valid. For long polymers this begins to break down.

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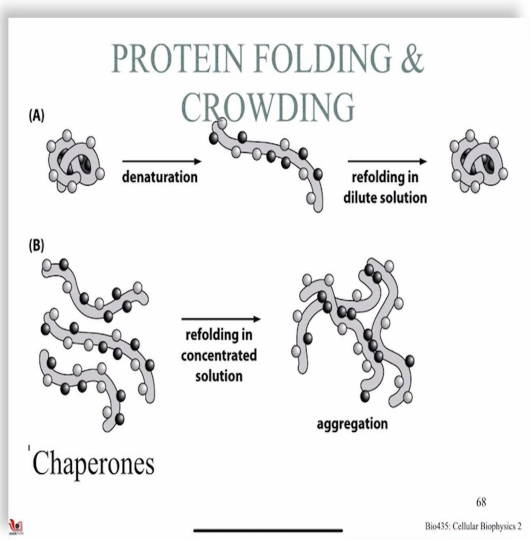

And, this becomes relevant when we start looking at some of the more complex phenomena, like protein folding in the presence of crowding were denaturation and refolding of protein in a dilute solution does not result in the same processes, that it results in a concentrated solution where aggregation can result. And this becomes relevant when we then indeed talk about protein folding. And this was the last figure in McGuffee and Elcock and I encourage you to look at it, I might have a chance to discuss it in one of the live sessions. It is not that important, just suffice to say that when proteins are in vivo, and they are folding, it is not at all surprising that they are in a concentrated solution.

So, what prevents aggregation? And the answer to that question is basically chaperones. Or you could argue in a sort of biologist's perspective of why are they, why do chaperones exist well? Because at concentrated solutions, proteins and their folding tend to aggregate and keeping them apart, allowing for folding in a manner that is consistent with the structure that one would hope to achieve is important.

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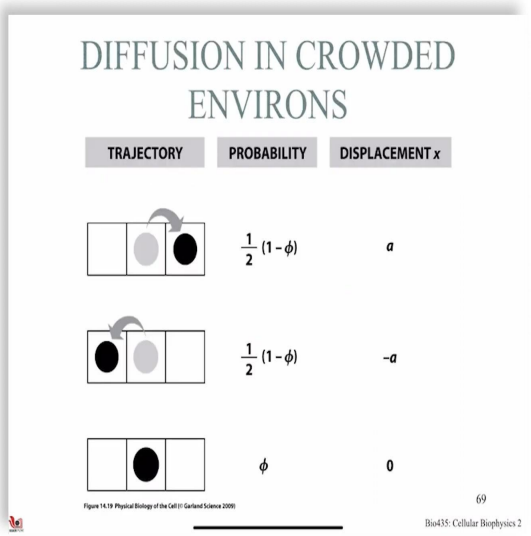

The last bit, I want to talk about diffusion in crowded environments. And again, like in the railway station and all, railway, train entry analogy of the Bombay local. If a particular already occupies a space, then the probability of hopping left or right is now scaled by the volume fraction occupied by the crowdant. And in other words, if that volume fraction is let us say half, then you are multiplying 0.5 by 0.5. And this is what makes things a little more interesting.

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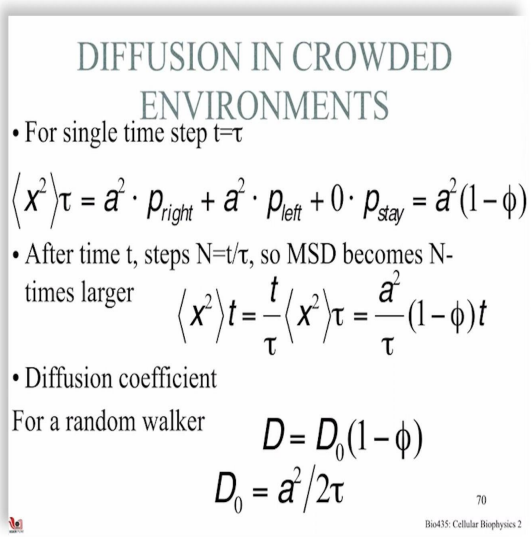

And, therefore diffusion crowded environments then becomes

$$\langle X^2 \rangle \tau = a^2 p_{right} + a^2 p_{left} + 0 p_{stay} = a^2(1-\phi)$$

, which effectively means that after N steps, N which is t by τ and MSD N times larger becomes N times larger. And the diffusion coefficient for a random walker becomes D, D_0, which is your so called dilute limit or no crowdant condition, multiplied by (1-ϕ), or $\frac{a^2}{2}\tau$, which is our definition $\frac{a^2}{2}\tau$ as we have used. The mean square displacement fact then becomes a^2/τ, which is t upon 2 or half t sorry 2 d t times (1-ϕ), my mistake.

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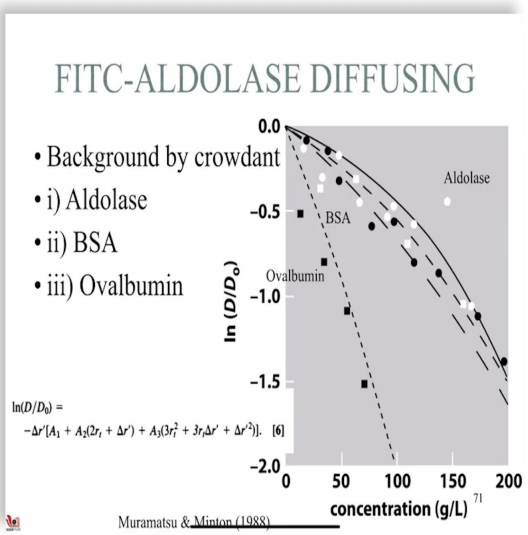

So, proof, is there any proof for all this? Well, if you now look at the natural log of the diffusion coefficient, measured the effective diffusion controlled by the optimal diffusion coefficient, the ideal diffusion coefficient, then you find that they follow a trend that fits this expression. This is a slightly scary looking one with a bunch of constants. But it effectively implies that as the concentration increases, the diffusion coefficient goes down, we talked, when he talked about diffusion earlier, we emphasize the fact that it is a constant, and it is a physical reality and you can measure it and it is true.

But when you have concentrations of crowdants, which now begin to resemble what we see inside the cell, things get very strange. So, this is another way of saying that we have to keep in mind, whenever we make these measurements, inside cells, a lot more is going on. And this is really proof of that the expression was fit by Muramatsu and Minton in 1988. And this is what the equation looks like.

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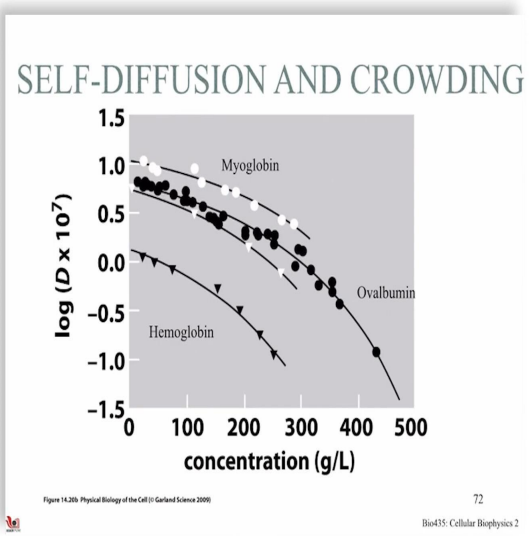

In fact, self-diffusion and crowding tells you that, in addition to other things crowding, you can also have self-diffusive. In other words, self-crowding, higher concentrations of the molecule that you are looking at, result in lower diffusion coefficient of the material itself.

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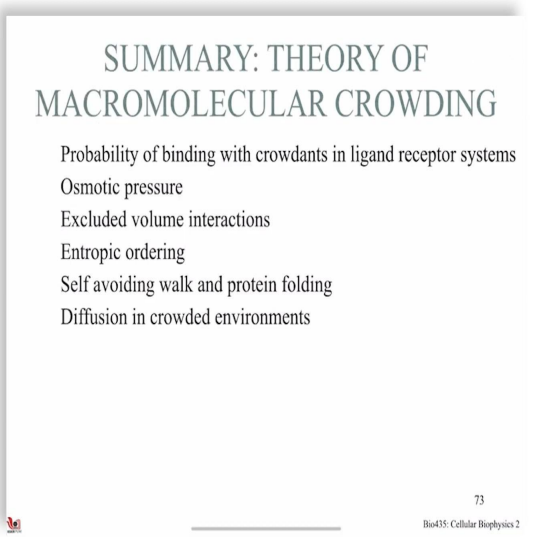

So, in summary, we have talked a bit about the role of binding probability under the influence of crowdants in the ligand receptor system, we talked about two kinds of situations that one where the crowdant and the ligand are of the same size versus one where there is a offset or ratio between the crowdant size and the ligand size. And we got two expressions for probability, I would like you to go back and look at them. We then talked about how osmotic pressure depends on crowding, we delve a little bit into excluded volume interactions.

Entropic ordering due to viral rods, for which there is some experimental evidence the theory is a bit trickier, self-avoiding walks which gives you a different scaling of polymers, if you consider volume exclusion in random of polymer.

And, we talked very briefly about how it might influence protein folding in the need for chaperones. And finally, we ended with diffusion in a crowded environment.

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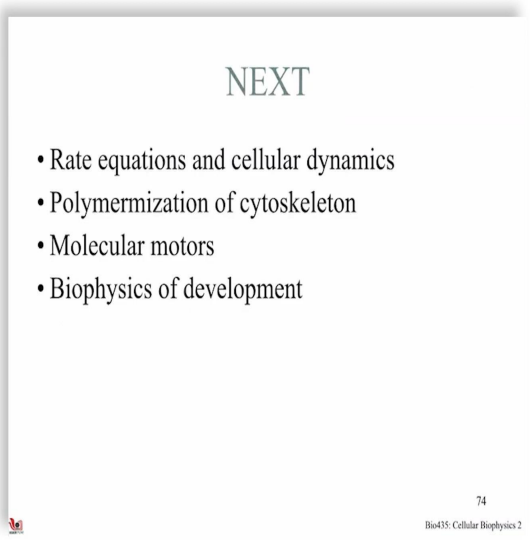

So, for the next set of lectures, I am going to talk to you about rate equations and cellular dynamics and how they relate to cytoskeleton in polymerization, molecular motors and the biophysics of development and that will complete this course. Thank you very much.