Physics of Biological Systems
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Lecture - 40
Examples and Implications of Depletion Interactions

So, in the same spirit, I can do this other thing which is this 2 spheres now and again. So, let me just sketch the calculation. Is this is clear ok. So, let me do this case; of when I have this two large spheres.

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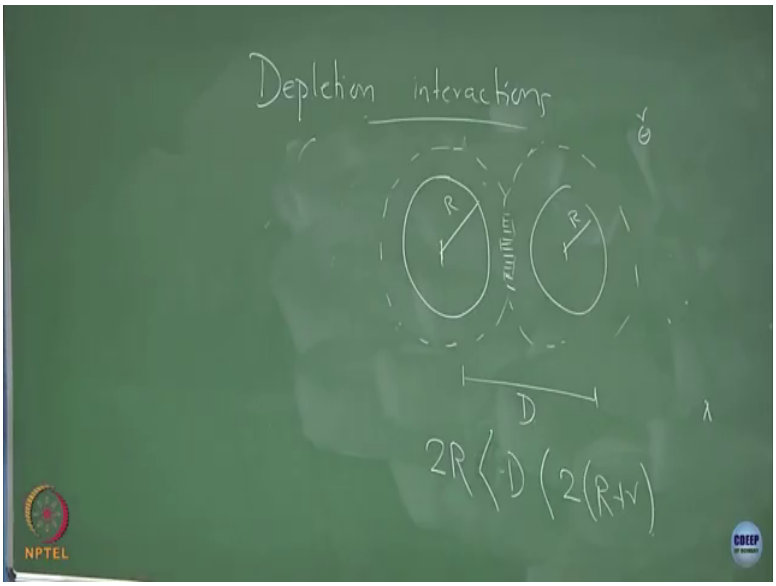

So, I have these two large spheres with their own excluded zones, and if I have these approach close to one another, there will be a common region overlap volume which I need to calculate. So, let us say that, these spheres are of radius R on both of these, the small particles are of radius small r and let us say that, the centre to centre distance between these two spheres let

me write that as capital D. So, in what range of capital D will there be a force? So, there will be a force if D is of course, has to be larger than 2 R, when the 2 spheres are touching and smaller than 2 R plus r.

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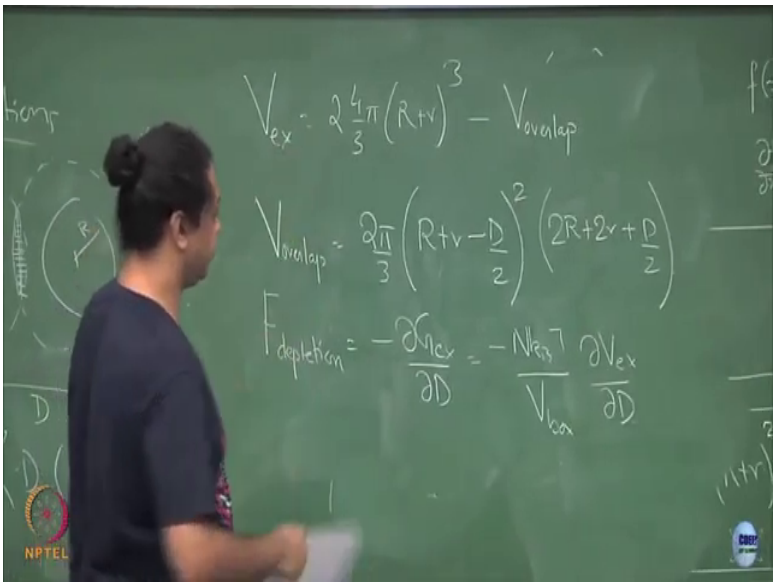

And what will be the excluded volume? The volume that is excluded by these two large spheres is simply the four third pi the excluded volume of each of these spheres, R plus r whole cube. There are 2 such spheres. So, 2 into four third pi R plus r whole cubed minus whatever is the overlap volume; minus whatever is the overlap volume.

If the spheres are far apart, then there is no overlap volume and this is simply whatever is extremely, if they are close then you will have some nonzero value (Refer Time: 02:18); and the overlap volume is what the overlap so, if I draw it like this the overlap volume is simply 2

times the volume of the spherical cap right and you can use the standard things for the spherical cap. So, I will not derive the spherical cap volume that is a standard geometric ok.

So, you can calculate this overlap volume goes as 2 pi by 3 R plus r minus D by 2 whole square into 2 R plus 2 times small r plus D by 2. So, this if you just look up if you derive the volume of the spherical cap and its 2 times that volume you should be able to. Then remember that, what I want to calculate is this force due to depletion, which I had written is minus del G excluded volume; del d in this case the separation of the spheres and G was just V excluded by V box.

So, this was minus N kB T by V of the box whatever box that you have, del V excluded by del D, and the V excluded is just minus of V overlap. So, this would just become V overlap by density ok. And then, if you just put in this del, del D of this overlap volume.

So, there is a D over here, what you will get is this expression, if you simplify a little bit and again n is, small n is nothing but this N by V box is the density of these Crowder's again. And again, you will see that there is an attractive interaction simply because of entropy, smaller Crowder's want to maximize this space, and that is going to lead to an attractive interaction of this form.

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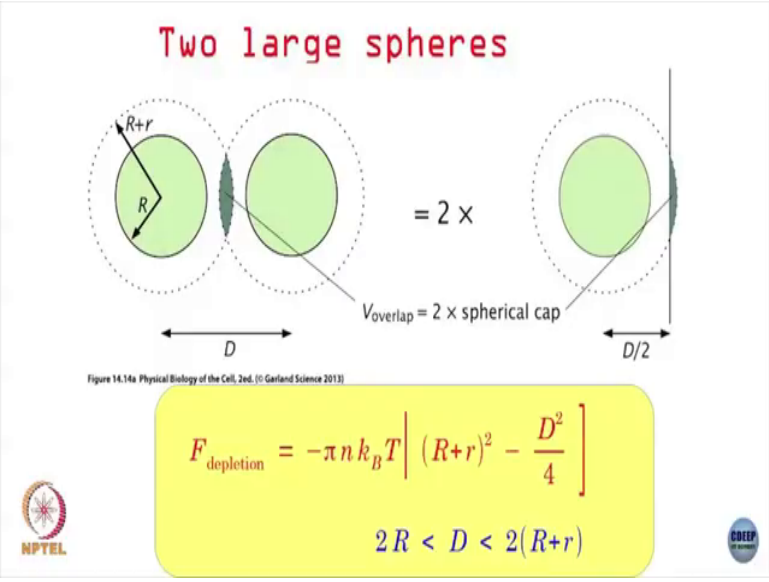

See, another way of saying Samarth is that, if I have many many Crowder's, the increase in entropy because of the Crowder's is of order n whereas, the decrease in entropy because of this bigger particles is of order 1 right, whatever it is it is of order 1. Given that, you have a thermodynamically large number of Crowder's, the system will always try to go in the direction that maximizes the entropy of the Crowder's themselves ok.

This is why even if I did not take into account explicitly the large particle entropy over there in that original calculation; this conclusion still holds true. If this if the number of these large particles becomes comparable to the number of this Crowder's, then of course, this calculation will no longer good, but that is an implicit assumption that you have simply because of volume restrictions you have a few of these large particles and many many of these smaller Crowder's.

So, you can put in some numbers actually, typical numbers of protein sizes and so on, this to get an estimate of what ranges this force is lying. So, if I put let us say that, this small bigger particles are a micron in size and these smaller particles are of some nanometers.

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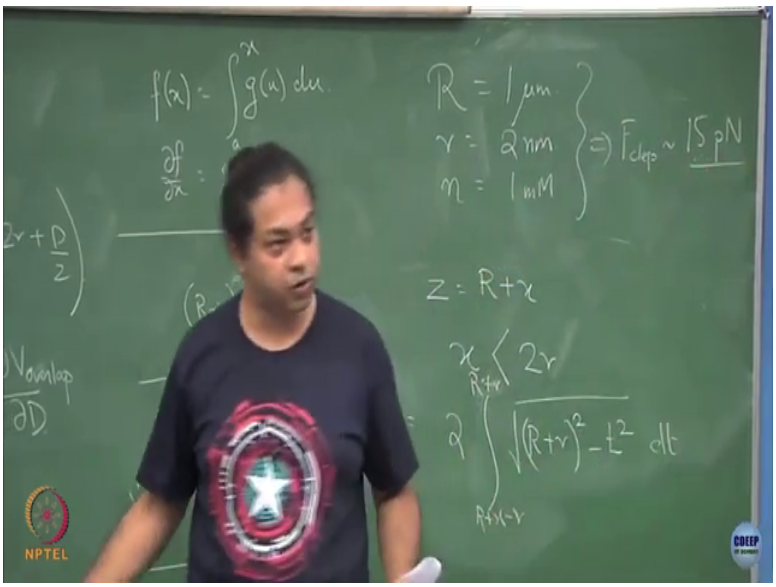

And, I have some concentration of these smaller particles this is again let us say milli molars, if you put it together in this sort of a formula, what this will give you is that this depletion force. If I put in exactly this numbers, it comes out something like 15 pico meters, which is an appreciable force in the cellular context. So, for realistic densities and realistic sort of sizes, you get an appreciable amount of depletion force on these larger particles. So, its not so small that it will not have any effect. In fact, its large enough that in many cases one definitely must take this sort of depletion forces into account ok.

So, what sort of cases would one see a depletion force like this. So, here are some ok. So firstly, before they do that here are experiments experimental results.

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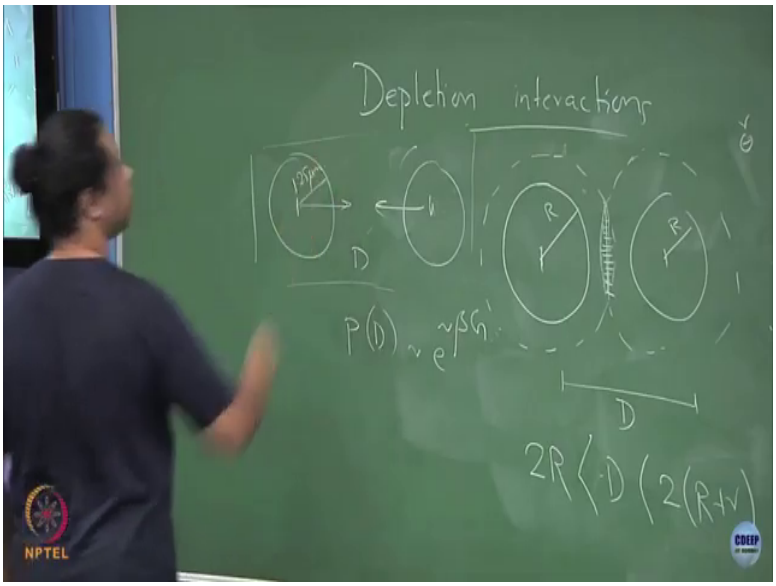

So, this is a setup where I have two large particles, which have been constrained to move only along one axis. So, the use of optical traps and you vary the concentration of these smaller Crowder's. So, this is 5 microgram per million ml, 10 microgram, 25 microgram. So, these are experiments and you can calculate the free energy. How do you calculate the free energy? You do this experiment many times you so, basically the only variable I have here is this D, the separation between 2 spheres because of constrain them to move along the line.

You can calculate the probability distribution of how at what separation do you see these large particles P of D, and that is going to be something like e to the power of minus beta G. So, you observe this probability distribution experimentally, from there you can infer what is going

to be this free energy and you can then plot this free energy as a function of the separation of the spheres and this red line, if I am not mistaken is the result of this calculation; the simple sort of calculation 2 spheres; the food depletion force between 2 spheres and the spheres were 1.25, this large spheres were of radius 1.25 microns.

And you see that this sort of decreases until you reach this 1.25 microns. Beyond that, it will shoot up because you cannot interpenetrate the 2 spheres and I you cannot see over here that maybe if you look at this the value of the minima, the height of the minima, in that sense that sort of increases as you increase your Crowder concentration which is basically this N by V box. So, that is also consistent with what is here ok. So, you can directly do experiments and sort of verify this order very simple calculation, but nevertheless it captures most of the essential business.

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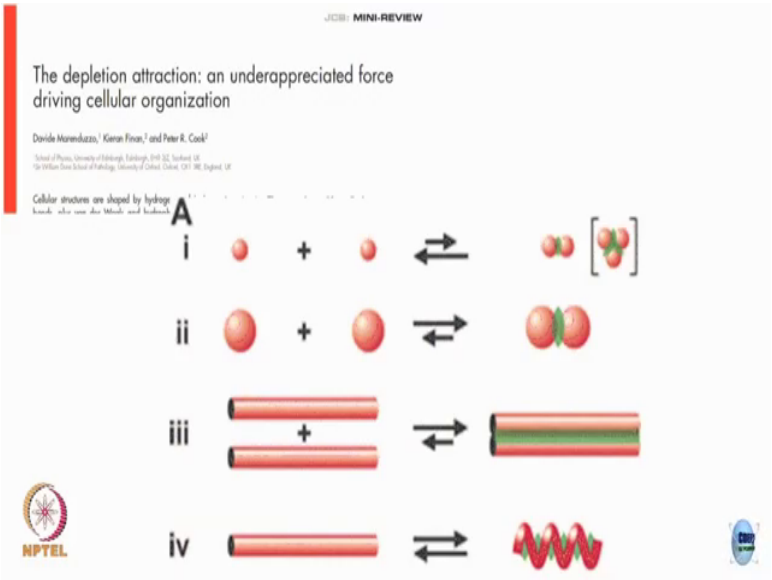

So, why would it be useful? So, this is actually a very nice review paper by Morenduzzo et al in 2006 it was taken It argues perhaps a little ambitiously that a lot of cellular organization is actually driven by this depletion attraction. But, what is definitely true is regardless of how important it is definitely true that in many cases you need to take into account it may not be the driving force, but it definitely be an important component in order to understand these structures. So, if we have small if you have spheres they are going to be driven together in the presence of Crowder's.

If you have a long stiff macromolecules, they are going to be oriented because of Crowder's. In fact, if you have a somewhat flexible polymer, you can show that it can drive a sort of helix formation again simply because the presence of Crowder's themselves.

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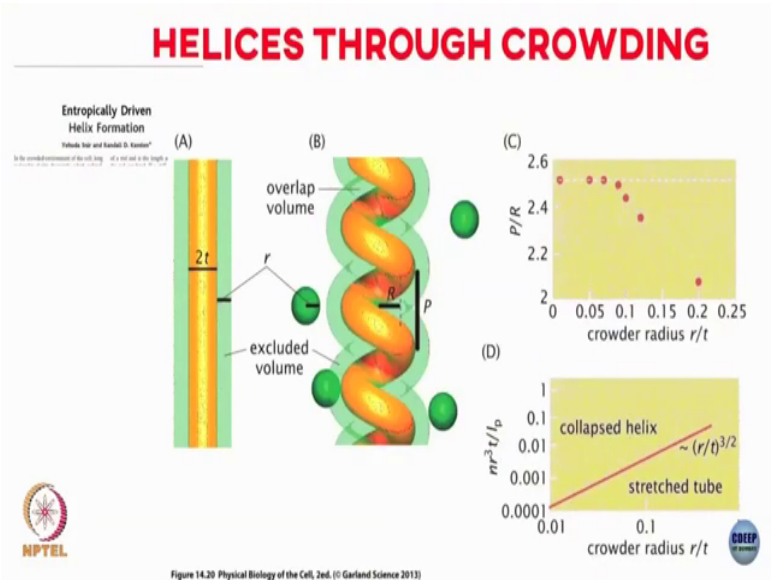

And that is actually a follower paper by Andrew Cameron. You simply argue that you have a cylinder, which has some radius t in the presence of many many small Crowder's of sizes r ok. Now, this has some overlap volume of course, if you keep it in the straight conformation. If you drop it, if you configure it into a helix. Because of this overlap volumes, the available entropy to this smaller particles is going to increase and therefore, there is going to be some force driving it towards this helical state ok.

And if you do this calculation, you can show that the pitch and the radius of the helix that you form will depend on the Crowder radius. So, this is a non dimensionalized Crowder radius r by this t the radius is cylinder and you can show that for very small Crowder's, for tiny Crowder's, you approach a pitch to radius ratio of around 2.5 roughly approximately. And, if you look at a alpha helixes of proteins, the pitch to radius ratio is around 2 ok. Its not evidence of anything, its not saying that alpha helixes in proteins are driven by the surfer crowding.

But, its simply saying this could be one of the factors that drive alpha helix formation or in general, any helix formation helixes are the extremely common in biology. We saw those helical tales of bacterium's and so on, which is was the protein DNA of course, is a helix proteins of these alpha helixes.

So, it turns out simply by this force due to this depletion interaction. You can drive a rod like object into forming a helix. They even go further and say that well, its not that this rod I can fold it any way I like; this rod might have some bending rigidity, I might have to pay some energy cost if I wanted to bend this rod and we have seen in polymers that this bending rigidity is characterized by this persistence layer right. The length scale over which this tangent correlations will decay.

And so, you can plot which of these structures will be the free energy minimum depending on this ratio of this persistence length versus the Crowder's radius, and in some cases you will the helix structure will be favored, in some cases this tube structure will be favored. If your is if

your persistence in length is very large, your rod is very stiff, it costs a lot of energy to bend it then of course, you would like to be in this stretch tube.

Because, this is inverse lp on the other hand if its very floppy, if you can bend it very easily, you are more likely to go and fall into this helix conformation. So, this is also an interesting calculation to look at actually have. We can calculate the pitch and radius is a function of crowded size.

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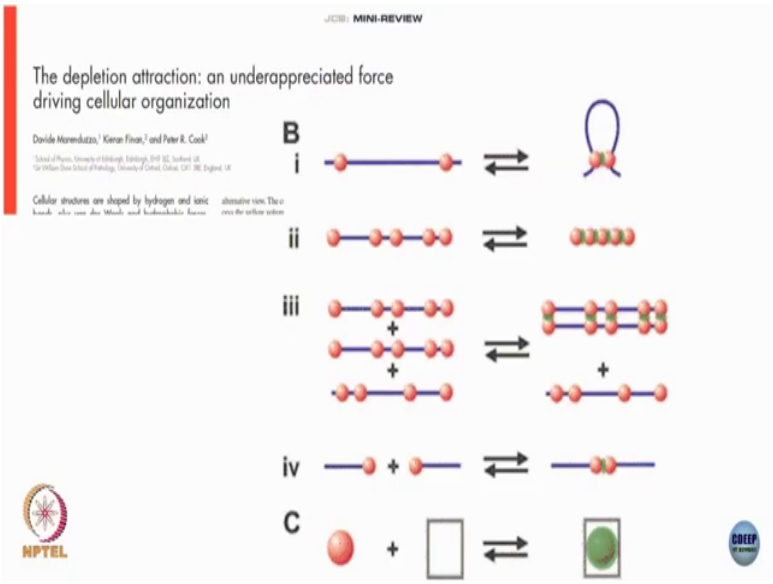

They go even further and they say that well this can also be behind again this is simply conjectures but, its interesting conjectures nonetheless. For example, we saw that when chromosomes organize, one of the motives; one of the common motives for this formation of these large loops right.

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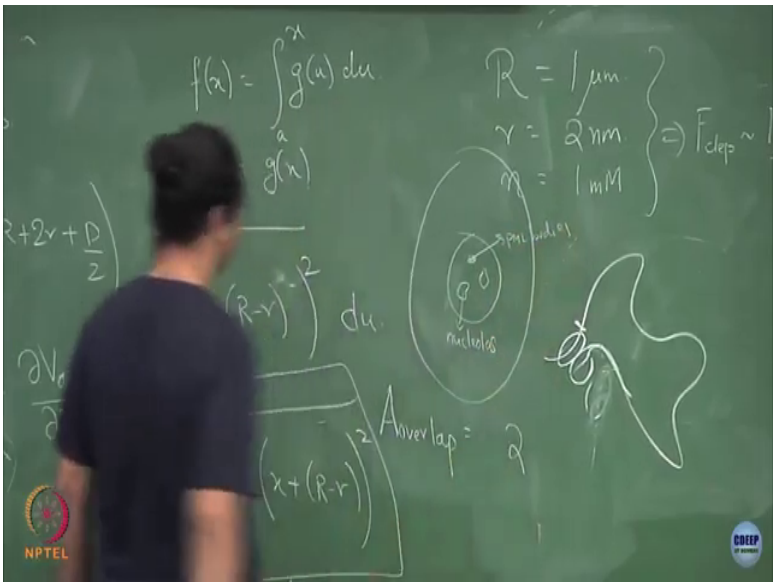

And they argue that, when these jeans need to become active and so on so, if I have a stretch of DNA; if I have some stretch of DNA something like this, you might have promoter complexes or large protein complexes coming and sitting upstream of the genes start side. And similarly, another promoter complex coming and sitting here and then, if these are sufficiently large you would have some sort of depletion interaction between these two which would drive the formation of a loop so, which would bring this coil close together and that could initiate transcription or repress transcription.

And for certain other scenarios as well in the context of chromatins such as heterochromatin and so on, I am not going into the details. But, the idea is that, if you calculate the magnitude of these depletion interactions, they are large enough that they might be able to drive some of the features that we discussed during this chromosome pattern lecture.

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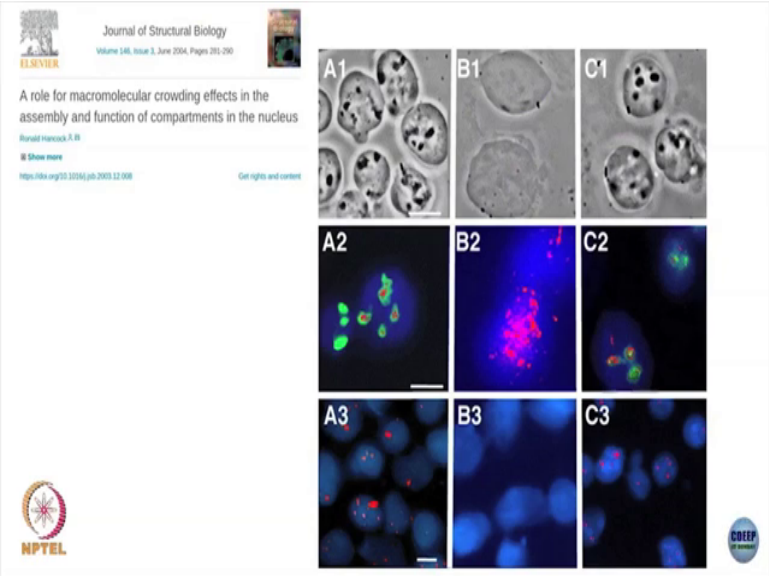

There is another paper for example. So, these are disparate papers looking at disparate biological systems, all arguing for a common conclusion that this crowding forces need to be taken into account in a diverse in various diverse scenarios. So, this is the paper that looks at compartments inside the nucleus; if you have a cell and so, I have my cell and inside the cell, I have my nucleus and inside the nucleus, we will often find large aggregates for example, some are called nucleoli or nucleolus if its singular; then there are other objects which are called PML bodies and so on.

So, this is a set of experiments where they actually take this nucleus. So, this is these are nuclei; inside this nuclei, you will see these compartments which are the nucleoli and the PML bodies. In this case, what they do is that, by lowering the salt concentration they increase the

volume of the nucleus and then by introducing various Crowder's, they decrease again the volume. So, when they increase the volume, you change this N by V box basically.

Once you change this N by V box, what this says that, you disrupt the formation of these aggregates inside. So, these aggregates this greens are the nucleoli and these reds are these PML bodies in this case. So, when you have increased the volume of the nucleoli of the nucleus sorry not the nucleolus; so, when you increase the volume of the nucleolus, you change this concentration of Crowder's and that is sufficient to actually disrupt the formation of the that is sufficient to disrupt these macromolecular assemblies the nucleolus and the PML bodies.

In this, you will see that these green spots have disappeared, in this the red spots have disappeared. If you bring the nucleus back to its original size, these are gain formed back. So, what they are arguing is that these macromolecules are assemblies that we see inside the nucleus; a major driving force behind their organization is simply this crowding effects due to various small particles that are present inside the nucleus absorption.

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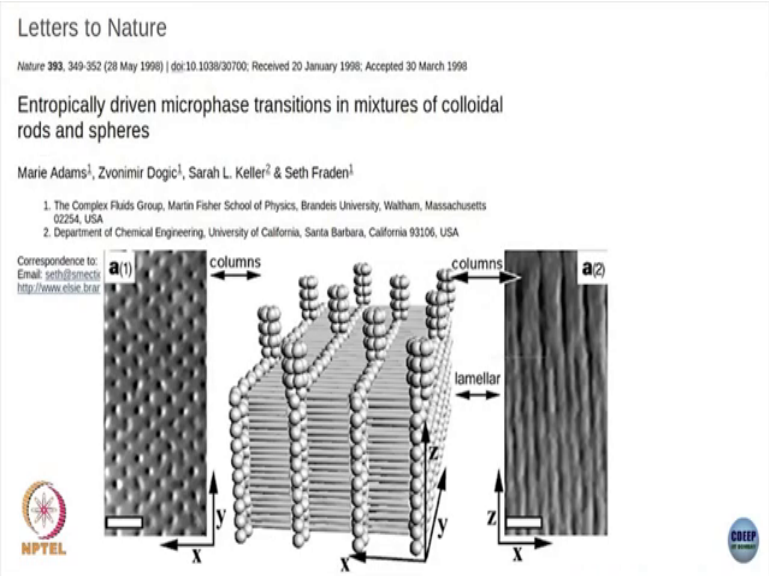

Its this depletion interaction that causes the formation of these bodies. This is again a different paper, this is somewhat in vitro. So, we use the mixtures of rods and spheres; the rods in this case being actually filamentous viruses. And, they show that this repletion interaction can lead to very nice micro phase separations, where you have these rod like viruses in disperse with these spheres and then again viruses a lamellar structure self organized; they have done nothing to organize it this way its a self organized structure arising purely out again out of this depletion interactions.

So, there is a variety of proof experimental proof that shows that, this depletion interaction is actually something that is the magnitude of the depletion interactions 10s of pico Newton's is actually large enough that you need to take into account, when you are talking about self

assembly your macromolecules are assembly in the cellular context. Its something that is not often done but, its something that is important enough that needs to be taken it ok.

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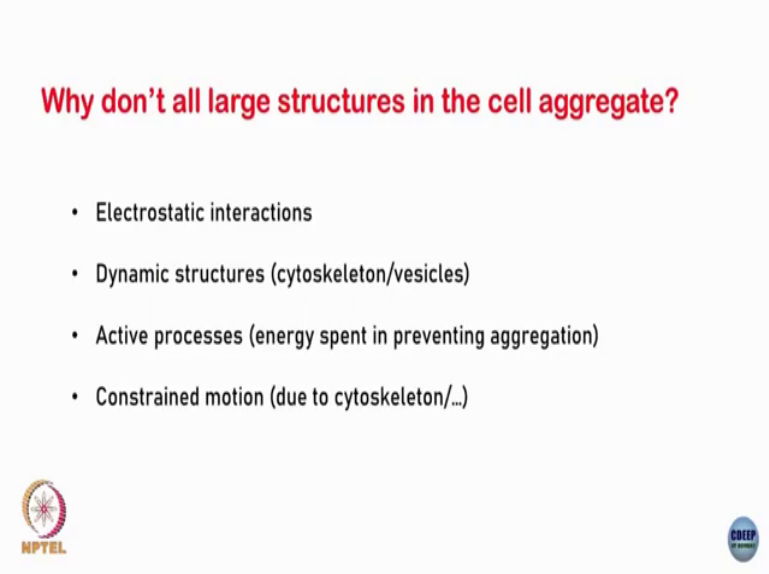

So, you might ask that well given that, you know the cell is very crowded you have millions and billions of objects, why do not I see everything there is clumping up together? If I have argued that these are large enough forces, what prevents aggregation? So, what prevents aggregation? One thing is that of course, we have done a very ideal calculation, I have not when I wrote down the free energy.

I simply wrote down this minus ts part; minus ts part; in principle there is of course, an e path as well most importantly electrostatic interactions right. Often these proteins are charged and so on. So, you will have electrostatic interest. So, we will have this energetic part in the free energy and that will change the equilibrium from what we have argued.

Secondly, this there are dynamic structures. So, cytoskeletons in vesicles. So, its not the level of crowding is not uniform throughout the cycle of the cell. You can have aggregates that are disrupted simply because, the structures the underlying structures are dynamic. Then, there are a lot of active processes the cell actively spends energy in trying to prevent aggregation.

Further, there is constrained motion due to the cytoskeleton and so on. So, for example: if you think about this very dense active assembly is that we saw in the first few slides; maybe there are large proteins which would want to aggregate, but they are prevented because of this filament is active that is present in the background. So, there are a variety of reasons why not everything is clumped together, but that does not mean that you should neglect these forces that arise (Refer Time: 19:09).

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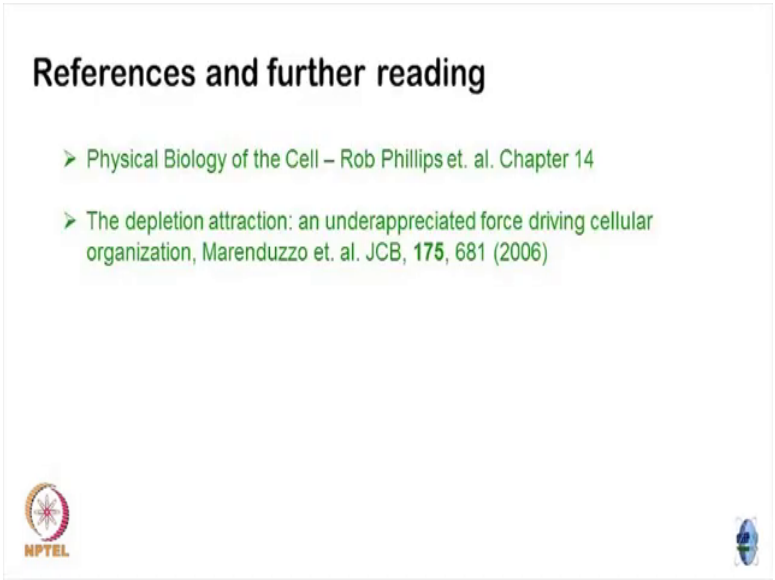

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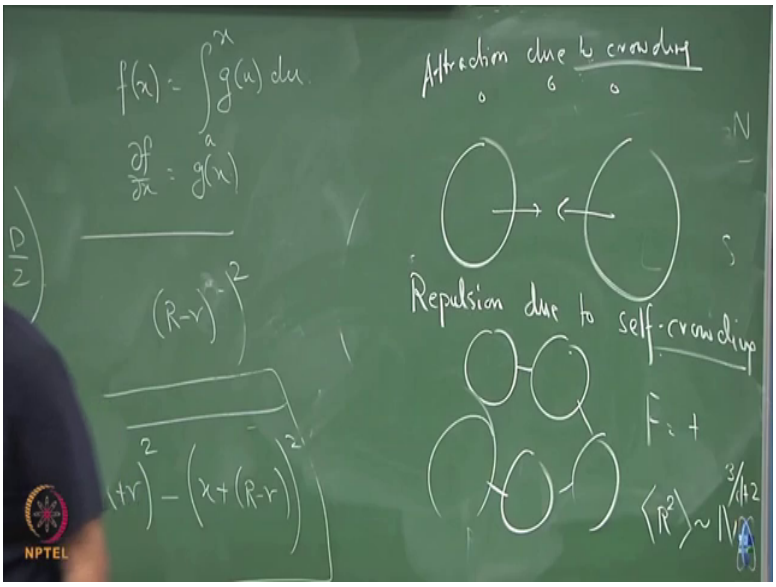

Let me stop. You can also show, I do not have time for that. So, I will just give it as an assignment maybe you can also show that; so, this case was a case where I said that, I have these large spheres and if I put them in a background of these small small Crowder's, then they are going to drive assembly; they are going to feel an attractive force towards each other ok.

So, this is an attraction, this is an attraction, attractive interaction due to crowding. But, if you do the opposite limit, somewhat related towards some what you are asking that if I have now many many of let us forget about the smaller spheres themselves. If I have many many of these large objects right, which have their own excluded volumes then, this is going to lead to a this is going to lead to an effective repulsion.

Again so, again due to crowding, but due to self crowding in some sets ok. So, if many many of these large objects then effectively because they cannot occupy the same volume if you do

this similar sort of calculation. You will see that there is a force, but that force is now positive which means its a repulsive force. And so, if you do this calculation and maybe if you do this if you link these large objects, then what you have got is now self avoiding polymer right.

And you can calculate this entropy, this free energy or this entropy due to this self avoiding beads and hence calculate so, and hence calculate how this R square is going to go as a function of the number of these Crowder's now, the Crowder's then being the monomers themselves in this case. And you can show that for self avoiding behaves its not half, but its whatever 3 by the dimensionality plus 2. So, I might do it on Tuesday, or I can give it in an assignment ok. So, let me stop here for the time being.