Physics of Biological Systems
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Lecture – 38
Diffusion in Crowded Environments

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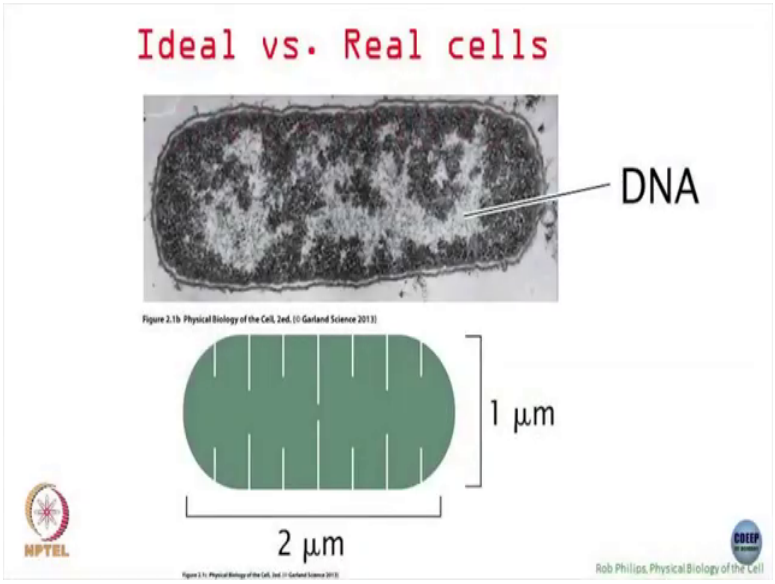

So, mostly when we have been doing this models we have been, although in the initial part when we did numbers and scales, we said that the cell is a very crowded environment and we calculated some of the numbers; numbers of proteins and so on. We have mostly been disregarding that, while doing the actual calculations right.

So, what I will try to do today is at least in some cases try to show that what are, what would be the effect of if we actually took into account that the cell lives in this extremely crowded environment. What sort of things would change? For example, when we have been doing

diffusion, through random walks we said that you know molecules take a step whatever and they trace out a trajectory like bacteria or when we are talking about macromolecules like polymers and so on.

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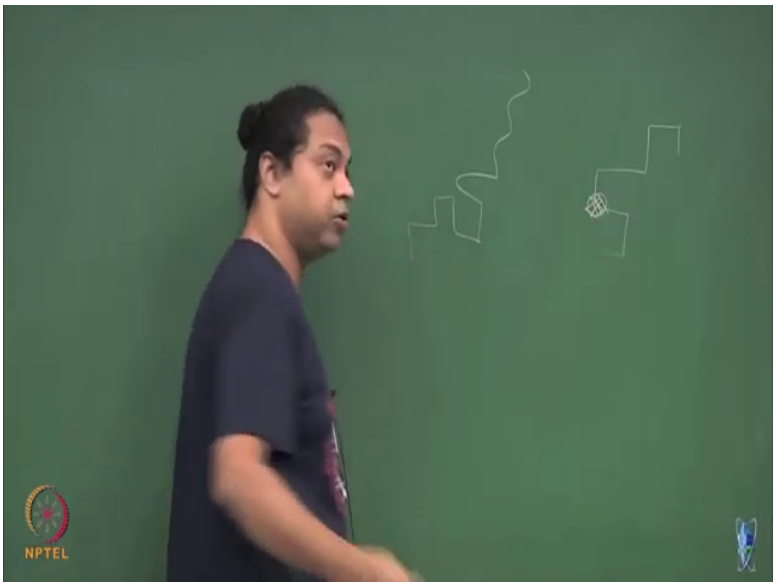

Again we said that sometime we can talk about macromolecules is freely jointed polymers where they can take steps anywhere they want, but in reality often these sites might be blocked by things that are occupied by other proteins or other organelles within the cell. And that will have some effect on the statistical properties that we have been calculating. So, that is what we will try to see.

Before, I start just to remind ourselves again this, so this is again some slides from the first lecture or second lecture. The inside of the cell for example, the E coli looks something like this; it is extremely crowded the white things were the DNA, the black things were everything

else, there is not lot of free space inside the cell right. And in fact, we have calculated the numbers basically so.

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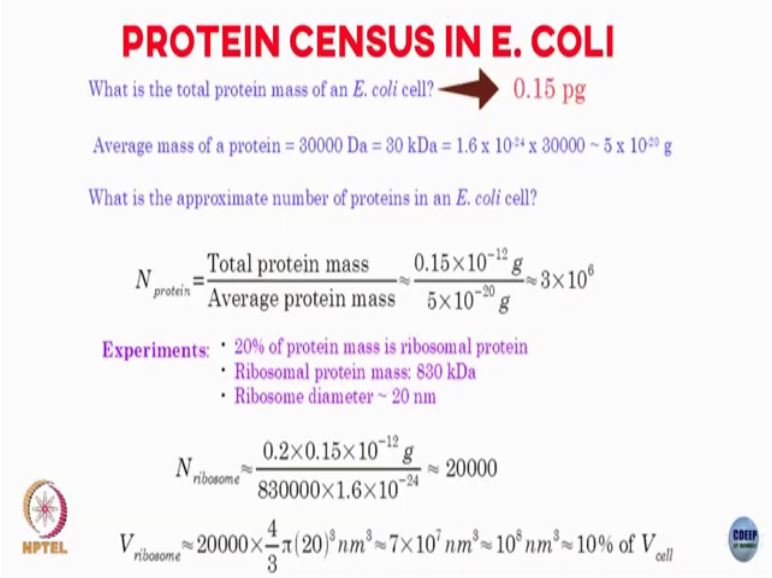

For example, we said that the number of proteins sort of estimate of the order of magnitude estimate of the number of proteins gave us some 3 million proteins or so of which there were some 20000 ribosome's and these take up a large volume of the cell right. Simply one type of protein the ribosome took about 10 percent of the cellular body.

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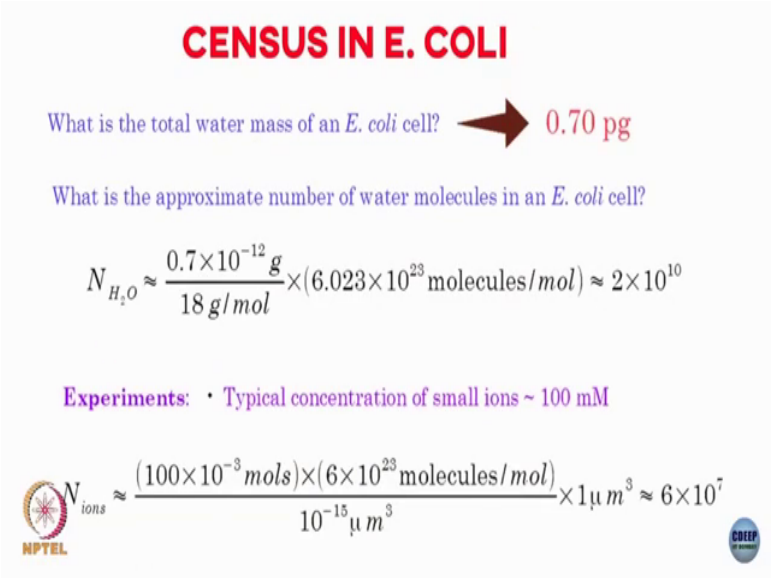

And this was just proteins, so if you looked at other things smaller molecules like water there was some 10 to the power of 10 molecules of water there was some 60 million molecule, small ions that were floating around in the cell. And whenever, object is doing a random worker diffusion or whatever it is going to come in contact with these obstacles.

In fact, if you we had also calculated, typically given some protein of border of nanometers what would be the interpreting separation? And that turned out to be off border of the protein size itself right. So, these were extremely; extremely dense objects. So, that is what we will try to see that in this sort of a dense background where, you have millions of molecules or billions in the case of water, what sort of an effect will that have on physical properties on dynamics and so on.

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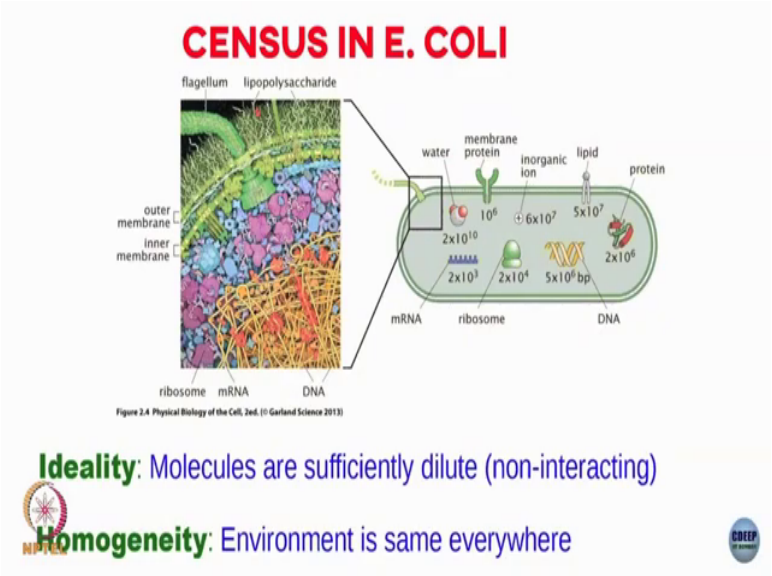

So, this was that slide ended up with which listed all numbers for all these are other things. Even the membrane in particular is also very crowded things. So, proteins which were diffusing on the membrane are also going to see a lot of other proteins in its path.

So, these were sort of the two assumptions that we had at the back of our mind when we have been doing. One is the systems are ideal, in the sense, that they are sufficiently dilute that we can treated by a non-interacting approximation, that this molecule does not see any when it is doing a random walk it does not see any of the other molecule; other molecules are so far away. That on an average it does not really see that, which is why we treat it using a non-interacting transformation.

And secondly, that the environment is sort of homogeneous; everywhere looks roughly the same and both of these are not true. I will try to show you that the inside of the cell is

definitely not homogeneous. The molecules which are travelling or doing random walk inside the cell definitely, meet other molecules has they do.

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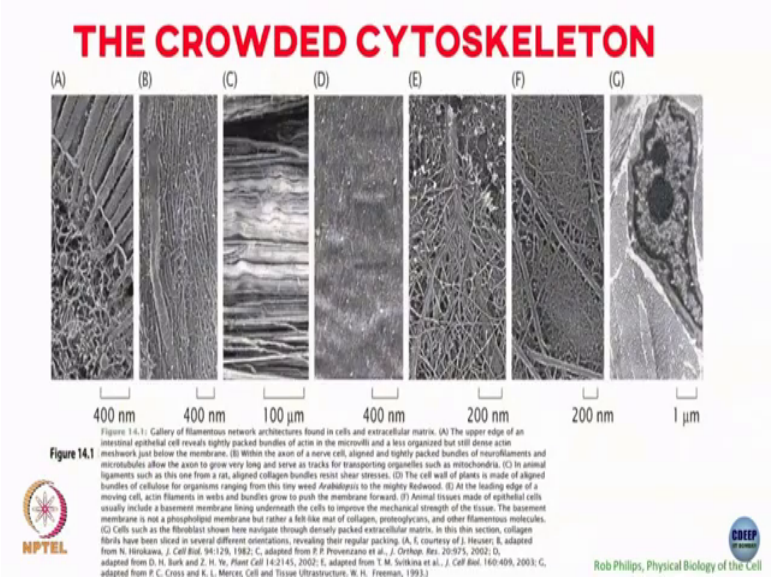

So, just to give you a sense of the density this is some pictures of the cytoskeleton which means, actins microtubules, intermediate filaments in different cells.

So, for example, if I can read the this is an epithelial cell which has these bundles of actin and then there is a filament of mesh of actins inside the membrane ,this one if I remember is I think the axons somewhere no b. This one is the axon of a neuronal cell and again this has this long filamentous assemblies along which your motors like kinesins and dyneins they travel along this filamentous assembly they transport cargo from one end to other to another.

Then c is I think some collagen fibers in the that case. So, these are align collagen bundles and so on. So, these are extremely dense objects they are definitely not homogeneous you have this bundler bundle like structures that you often see inside a cells. And so if you are looking for transport in this sort of a medium you need to take into account the background against this against which this sort of a transport is happening. And these span a range of scales from hundreds of nanometer upon onto the microns, depending on what sort of cells and what sort of structures we are looking.

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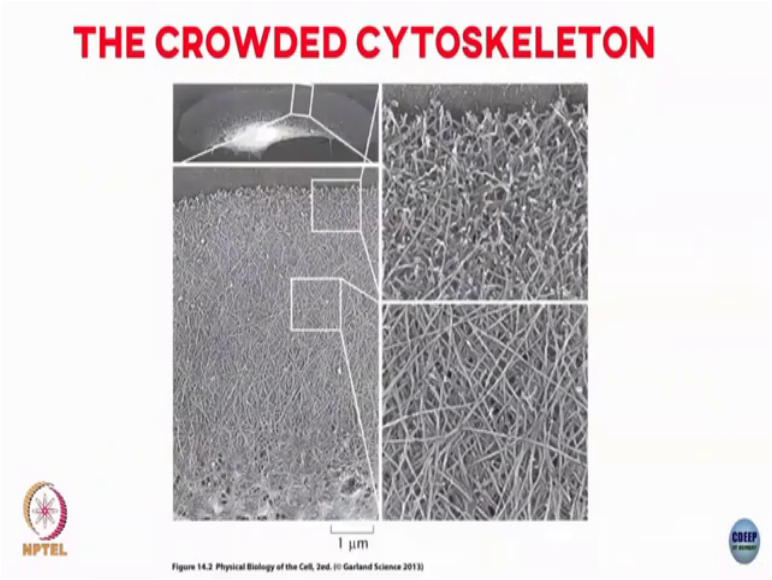

This is another picture of this cytoskeleton and again this is just inside the cell membrane you have this very very dense bunch of actin filaments that are crisscrossing and everywhere. So, they are spread round and its against this backdrop the things are performing diffusion or motion.

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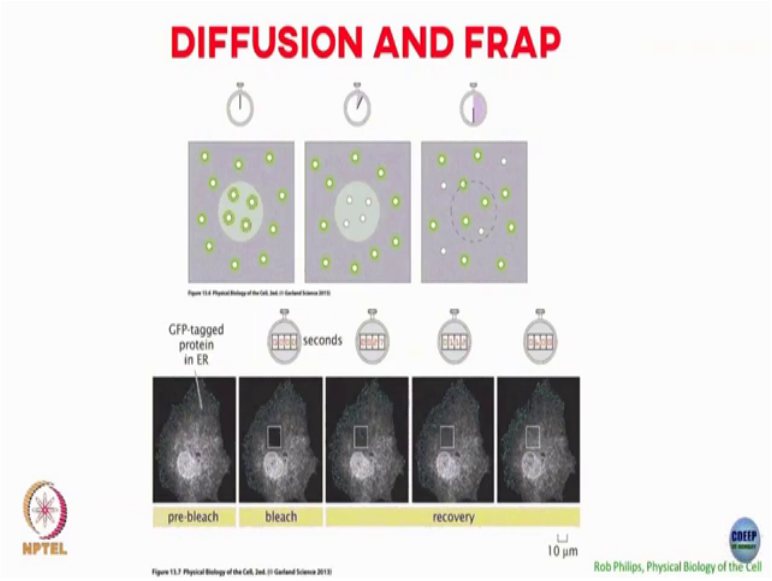

So, remember that. So, let us look at, let us start off with a simple case; let us look at diffusion and remember how we had quantified this diffusion coefficient was to say that through these frap experiment.

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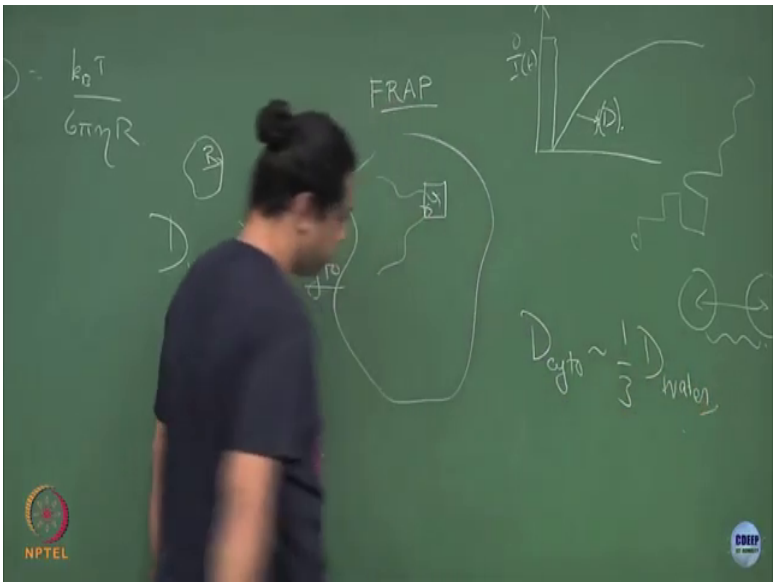

The fluorescence recovery after photo bleaching where we took a cell you bleach out an area which means that you kill all the fluorophores in this region. So, that it becomes dark and then, so this region you bleach out this region and then as object sort of diffuse back into this region from outside you will slowly see that the there is a recovery of fluorescence.

So, if you plotted the fluorescent intensity of this bleached region as a function of time it was some intensity when you bleach at it drops to 0 and then its slowly recover back to its background intensity. And by studying the kinetics of this recovery curve, so this dependent on the diffusion coefficient. So, this was the function of the diffusion coefficient and then by fitting whatever theoretical analysis you did to the actual recovery curves you can get an estimate of the diffusion coefficient itself right.

Now, if I look, if I take a same if I take one molecule; if I take some molecule I do not care what I take some molecule and I found out its diffusion coefficient in water let us say.

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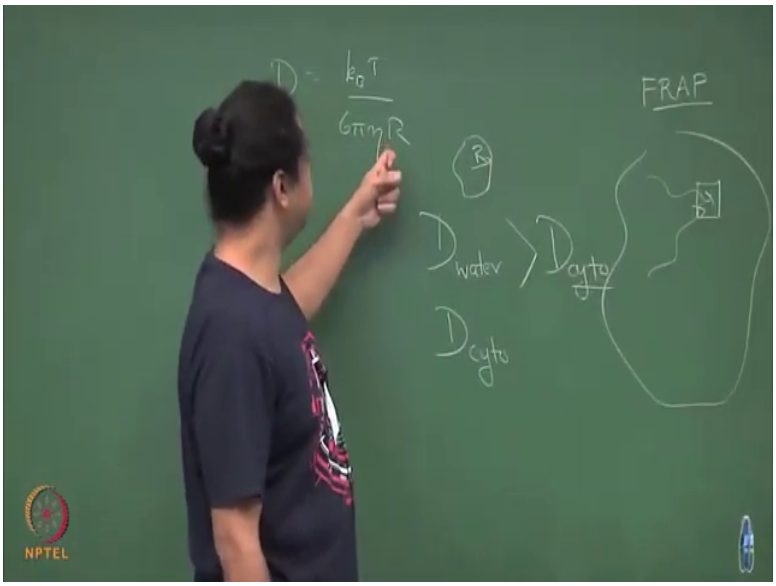

So, I find out the diffusion coefficient in water versus the diffusion coefficient inside the cell. So, let us call it d cytoplasm which one would be higher naively, water would be higher.

So, I can say that right; what else can I say. So, let us say that the size is whatever r typical size r. And if I roughly assume that the cytoplasm is three times is viscous as water, how much would this cytoplasmic diffusion coefficient would I expect it to decrease? Yes do you remember was what k B T by 6 pi eta R? Right.

So, I would expect the ratios of these diffusion coefficients would be simply the inverse ratios of this viscosity and therefore, I might expect that the d cytoplasm is roughly. So, the

cytoplasmic diffusion coefficient is roughly one third of the water diffusion coefficient, all right.

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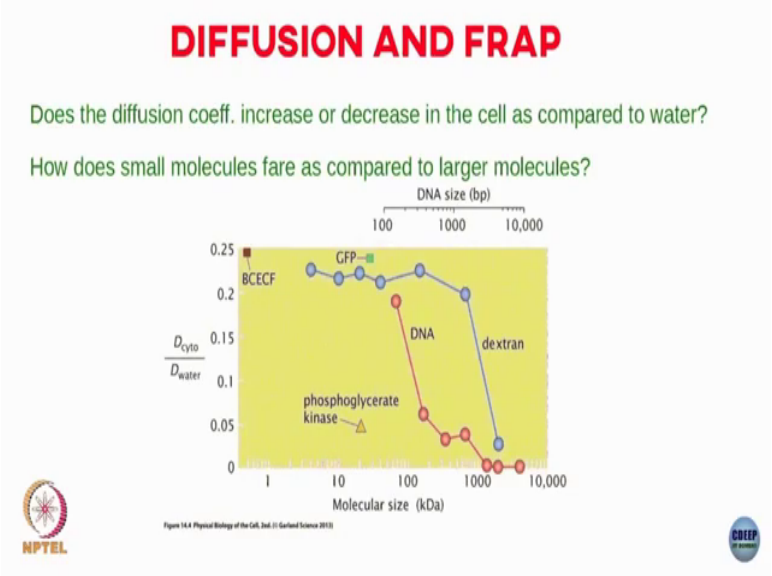

So, we can try to see whether that sort of matches up. So, we can see how this diffusion coefficient behaves inside the cell as compared to water. So, we have said that it will decrease and naively I would say that it will decrease by this amount, it would be one third the water value. Can I say something about how small molecules would fare relative to large molecules?

So, for example, if I took a molecules which is small versus the molecule which is very big in which case would I sort of roughly expect this sort of relation to hold true? So, in for which molecule would if the cell is extremely crowded if this is diffusing in a very crowded environment, both of these in which case would I expect the environment to have more of an effect? For the larger ones, right.

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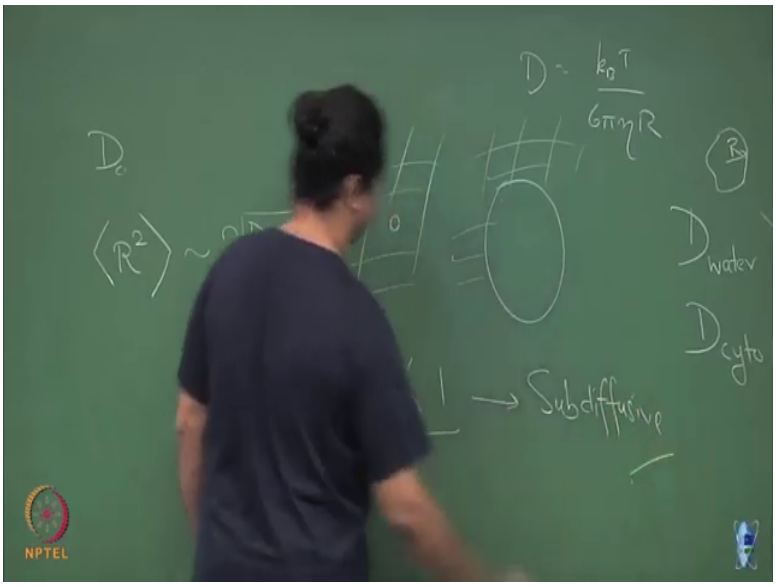

So, if I am going to see deviations from something like this I would expect the deviation to show up more, so large molecules as compared to small molecules. So, now, we can just look at some data, so here are diffusion the ratio of the cytoplasmic diffusion coefficient to the diffusion coefficient in water for a variety of different molecules these over here is small molecule is the green fluorescent protein GFP.

These are molecules these are polymers basically dextran or DNA which you can change the molecular weight depending on how long a DNA molecule you have or how big of a dextran molecule have. And what I see is that for these smaller molecules, so when this molecular weight is sort of small roughly the ratio is around Let us say 0.25 which is close enough to 0.33, its somewhat smaller than that, but at least its close enough to 0.33 ok.

So, most of the decreases sort of accounted by the change in viscosity, but if you go to larger and larger molecules this sort of relation breaks down, you can be smaller by almost two orders of magnitude either right and that effect is due to crowding.

So, when you have these other substances that are crowded though out this and its trying to (Refer Time: 11:40) this big molecule is trying to perform diffusion in the background of these smaller molecules you will see more of an effect than would be predicted by simple stokes Einstein relation and that is what you see for these sort of curves. This one for this very large DNA; very large DNA it almost drops to 0 does not really diffuse it, you would you could see ok.

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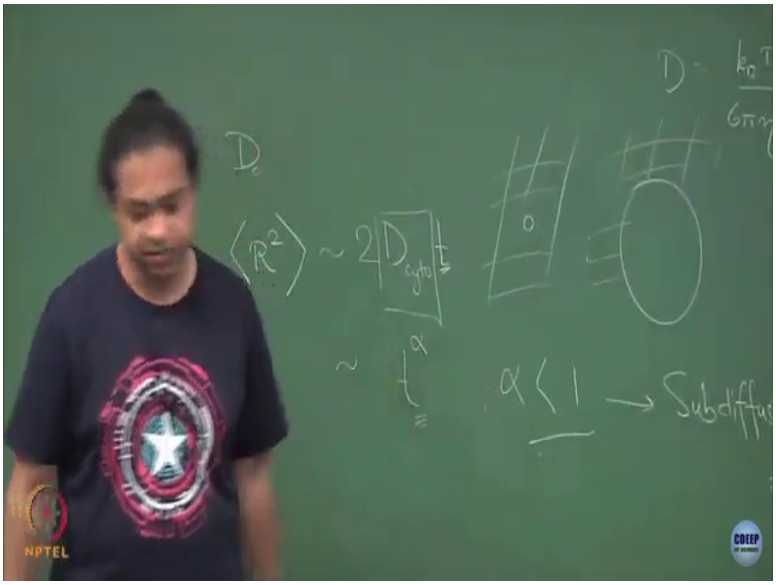

So, let us see let us see that I have a diffusion coefficient inside the cytoplasm let us say I let us say I am plotting this the variance of distance that has been traveled inside the cell and I get

something 2D cyto t and this is this D cytoplasm that has been plotted. What sort of other effects could you see how else could I modify this relation, any idea?

Student: Time.

Time. So, in this I could also say that this exponent instead of being t to the power of 1 becomes some t to the power of alpha where alpha is less than 1 right and that would be the class of walks which I could broadly call a sub diffusive walks.

So, we are talking mostly of this I will assume that this linear relationship with time holds true and I will try to quantify how this cytoplasmic, how this cytoplasmic diffusion coefficient changes, but you should be aware that. In fact, the more common outcome is actually with this alpha itself changes the walks are no longer purely diffusive mostly inside cells you see sub diffusive works with this time does not really grow as t to the power of 1.

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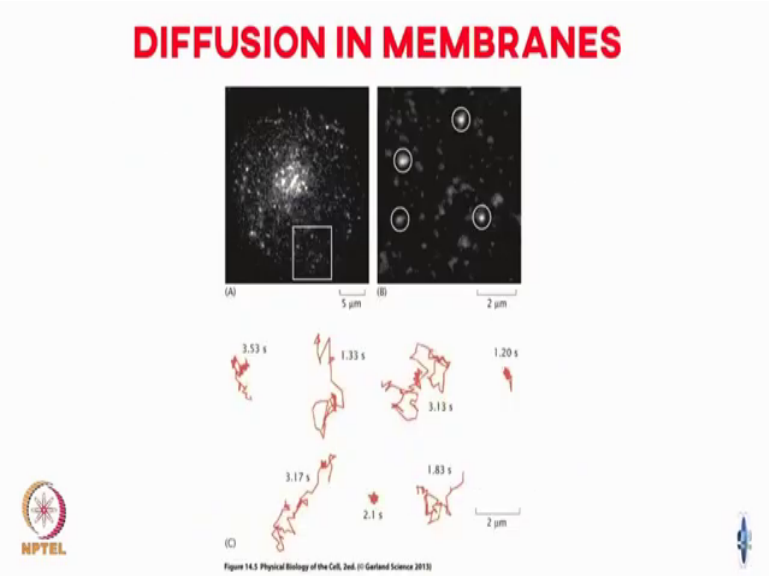

In fact, if you want to see that. So, for example, this is movie of a protein which is performing a 2 dimensional random walk on the cell membrane ok. So, I have this cell membrane and there is a protein which is performing the diffusive walk on this cell membrane.

So, these are actual particles which are performing diffusion and you can analyze their trajectories and if you see that the trajectory is actually look fairly heterogeneous there are some trajectories which just by looking at it without quantifying it whatever it without just by looking at it I would say well this one looks something like I would expect my random walk to look and I can then calculate the diffusion coefficient

On the other hand there are trajectories which do not really move at all. So, this one for example, this one for example, these just stay stuck there. So, it reflects the heterogeneity of the environment in which this protein is performing the random walk and in cases like this

often the more appropriate description is to use instead of trying to think in the context of the diffusion you try to think of it in the context of this sub diffusive walks.

Now, actually trying to calculate this powers and so on is a complicated business and I will not try I will not mention into that rather what I will do is mostly I will just sketches zeroth order calculation as it walk and just see how well that does.

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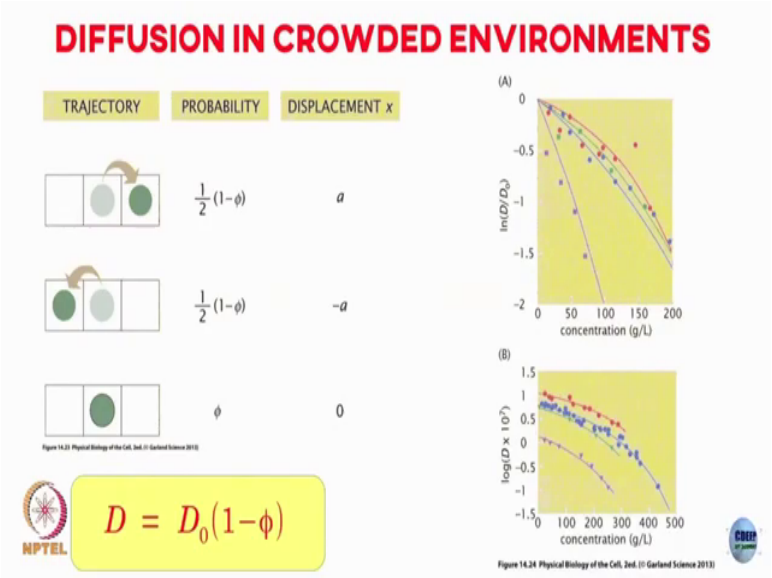

So, let us say. So, I will go back to this sort of a random walk picture and again I will try to think of it as a one dimensional random walk.

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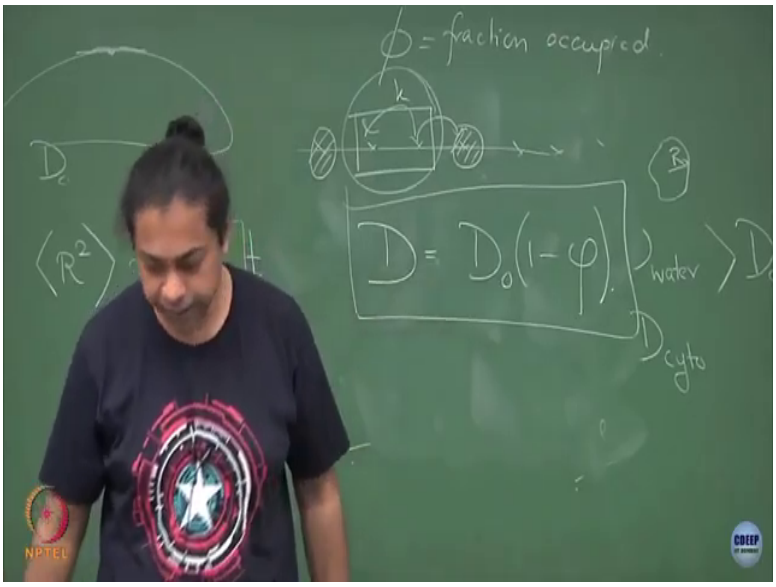

So, I have this lattice in which this; I have this lattice in which this protein or whatever object is doing a one d random walks. So, it can hop to this side or that side except now I have the caveat that some of these sites are occupied. So, some sites are occupied by some other objects and the volume and the fraction of sites that are occupied is phi.

So, I call as phi as this fraction of sites that are occupied then on an average if I am trying to hop to the right or to the right let us say with equal probability. The probability that I will this move is going to be allowed and the move remember a move is going to be allowed only if this site is free if its already occupied by something, then that is not going to be allowed. So, the probability is like half into 1 minus phi to the right and half into 1 minus phi to the left and a probability phi is just is there simply because it cannot hop and every time it does this it either hops by plus a minus a or c.

If we simply think of this sort of a random walk where this density of crowders is a sort of captured in this volume fraction of this fraction of sites occupied phi. So, if I consider this sort of a walk and I did the usual business of writing down the master equation and then taking the continue limit. What would the diffusion coefficient come out to be? Relative to the empty site related to D naught; D naught into 1 minus phi right.

So, this vary zeroth order calculation sort of tells me how this diffusion coefficient is going to vary as a function of this number of Crowder's. Its zeroth order because the picture is very simple its zeroth order for example, an easy thing I could do if I wanted to sort of incorporated this size dependence that we saw in the experiments is to say that well the object that is diffusing actually occupies multiple sites, its a big molecule and its diffusing in the background of smaller molecules.

So, in order for it to hop it has to have a consecutive whatever sites it occupies just to have that many sites consecutive briefly only then it can hop. And then I can come up with an improved version of this model that we will take into account the size of this object that is trying hop, but I am I am not really trying to going to that I can put it in the assignment.

But I will let us see this see this is my zeroth order of approximation and then I can see how this d naught into one minus phi and then I can see how well this does for actual real data. So, these are different molecules for which this diffusion coefficient has been calculated as a function of this concentration of Crowder's and for small enough concentration I guess you could say its falling roughly linearly and then it becomes non-linear.

But I on the other hand I do not expect this formula to work very well, but it captures I guess to certain extend this initial fall of this diffusion coefficient as a function of Crowder's. If you want to explain this better this curve better and people have done various improvements to this zeroth order model in taking into account more and more physically relevant factors you can come up with certain models which will give you a better and better fit to these data ok.

So, this is just one very simple way in which crowding this crowding inside the cell is going to affect the quantities that we are calculating in particular in this case the diffusion coefficient. Moving on, so if I want to this is very interesting at least interesting to me manifestation of this crowding and that is called the depletion interaction.

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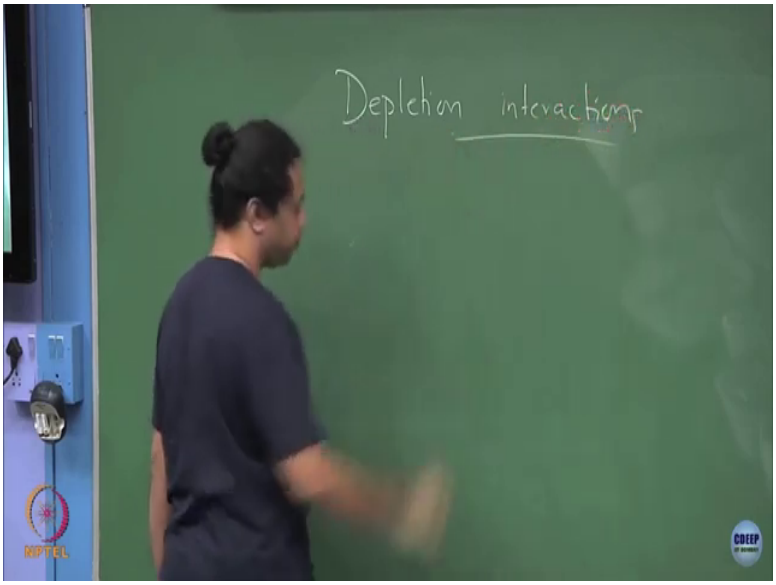

So, let us look at this manifestation of this macromolecular crowding which I will call depletion interactions and the origin is very simple with the manifestations are pretty nice.