Physics of Biological Systems
Prof. Mithun Mitra
Department of Physics
Indian Institute of Technology, Bombay

Lecture – 50
Models of motor motion

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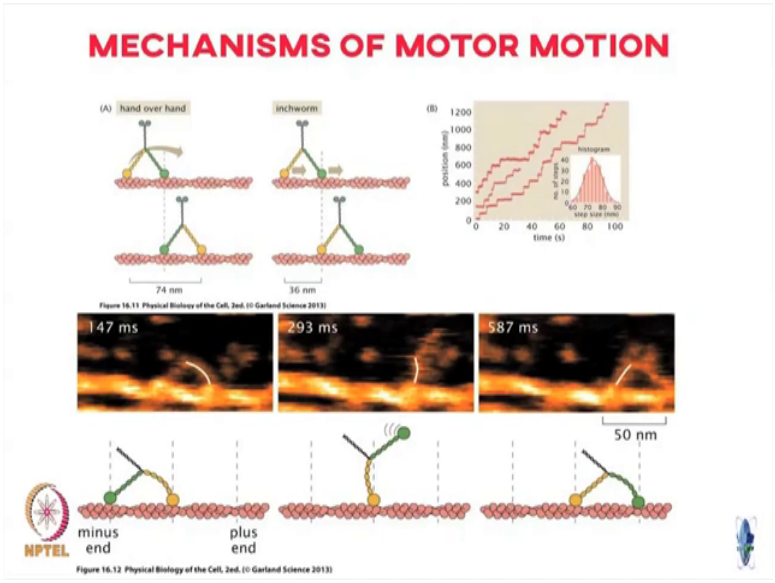

So, for example, I could think that let us say I have these two head domains of my motor that bind to this that bind to the filament right. Let me draw it a little bigger.

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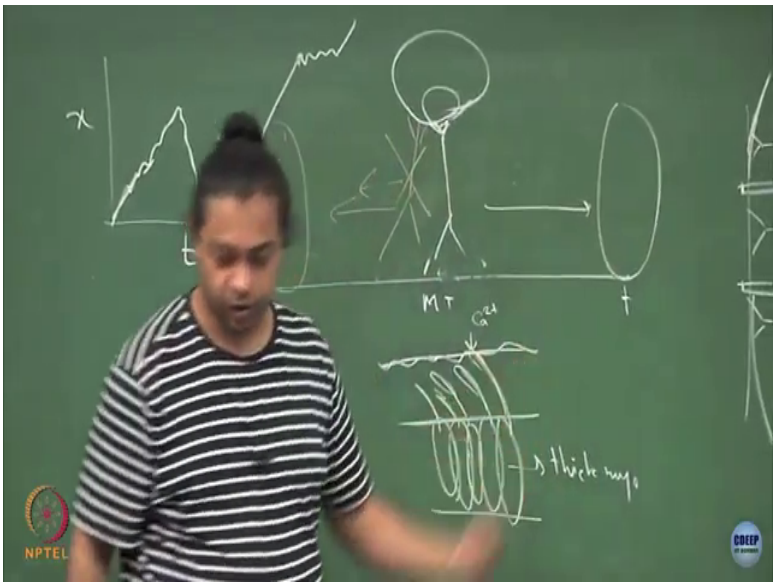

So, these are my two head domains and then here is a cargo binding domain. Now, how do these head domains move when it walks? So, you could think of two things. So, for example, here are my two head domains it could pivot on this leg and then turn this leg and move and then again pivot on this and move the other leg or it could move both legs. So, it moves this one forward a little bit and this one forward a little bit right. So, these are what is shown over here as this pivoting one is what is called the hand over hand. So, you press one hand and you pivot on that, the other one is called the sort of inchworm movement ok.

And, if you work out the numbers so, if you let us say you place a fluorescent tag on one of these heads ok. Then, if it was hand over hand it would have this leg would have un bound from here and then pivoted on this hinge and bound to somewhere over here and the distance fall. So, the distance in this head would cover in a single step would be of the order of somewhere around 74 nanometers where if it was doing this sort of inchworm movement

where both of these heads move simultaneously each head would move an order of 36 nanometers given this kinesin protein

So, you can now construct you can now do this single bead sort of experiments and you observe the position as the function of time, the position of this motor on the on the filament in the function of time.

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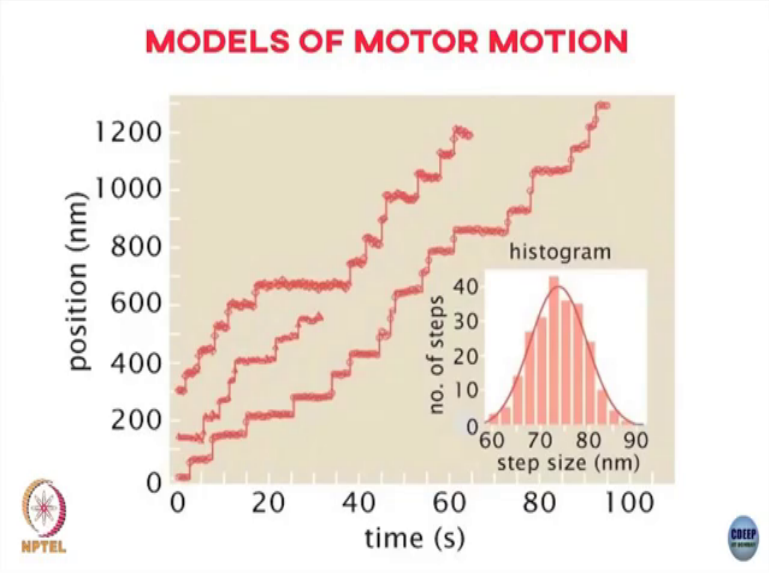

And, you can plot an histogram with I think you have the histogram, yeah. So, we can plot the position as the function of time and you can plot the histogram of step sizes that it takes. So, for example, here you can see that the histogram has a peak at around somewhere around 74 – 75 nanometers which what it is says is that the motion that this kinesin is doing is not this inchworm which would then have a peak around 36, but rather this sort of a hand over hand

motion where it and it has the peak at the right sort of this displacement of around 74 nanometers.

You can also explicitly actually see that in certain experiments. So, here is another experiment. So, this is the kinesin this is one head domain, this is another. So, this one stays bound it, the other one hinges around. So, the other one has now unbound in this middle panel this one stays bound and then this other one comes and attaches forward or somewhere over here. So, this directly shows this sort of a hand over hand movement. So, the and this is the schematic. So, this one stays bound, this one unbinds finds and again binds at the next position ok.

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Yes?

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What is the?

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What is meaning of assay? Assay means to sort of I do not know means to study at least colloquially the actual Greek Latin meaning is what it can have some rule, but technically and colloquially what I understand is some sort of a study. And, so, these sort of experiments can tell us how these motors. In fact, are moving and you can do this for different types of motors kinesin versus dynein and so on and see what sort of mechanisms are different from one motor versus the other, all right..

So, if I wanted to go ahead and build modules of these sort of things, these sort of translation of the motors kinesins, dyneins, myosins something like that walking on some sort of track microtubules or actins what sort of questions could I ask for example?

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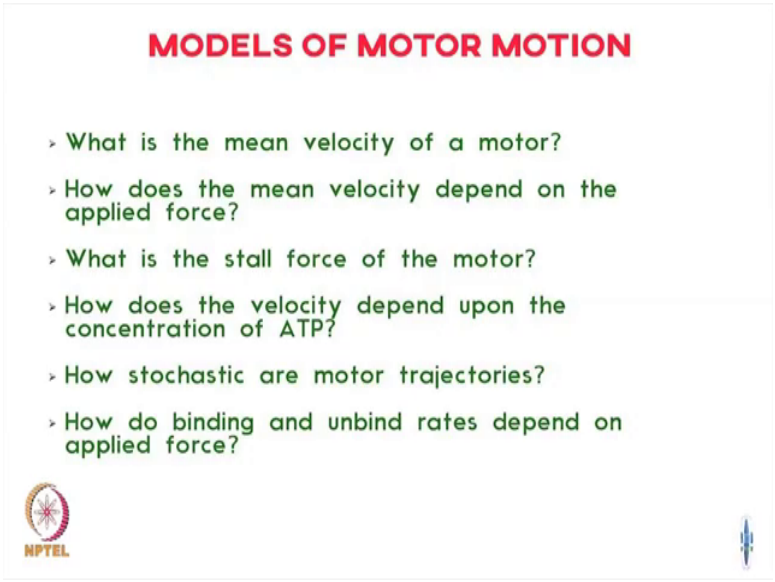

So, I could ask things like what is the mean velocity of a motor? I could ask how does this mean velocity depend on the force right this V as the function of F. How does that behave? I could ask what is this stall force of the motor?

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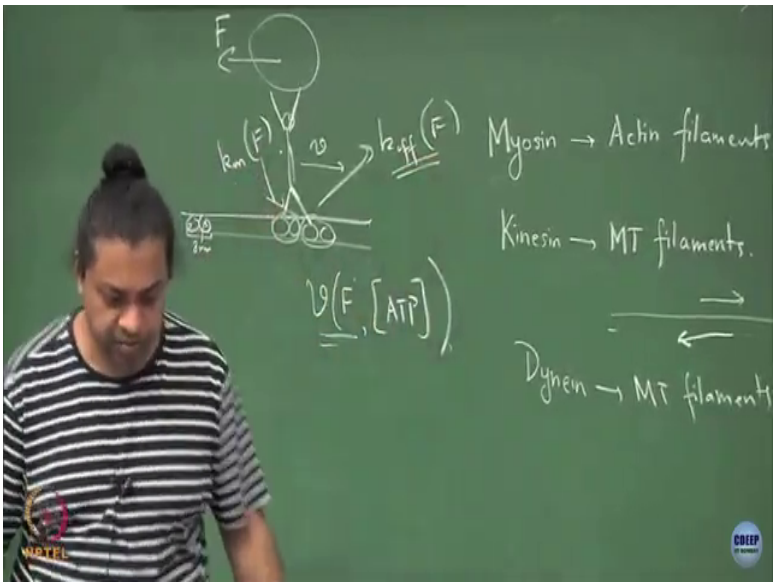

The stall force is again simply the force required to bring the motor to a stop. I could ask how does this velocity depend on the concentration of ATP right. So, I have said that this is the function of F, but in principle it is also a function of ATP concentration because these are active processes. If you do not have any ATP the motors would not move at all; if you would had very high ATP it will move with some velocity in between ATP in between ATP concentrations it would move with some other velocity. So, not only is it a function of force it is also a function of the ATP concentration because you have couple this chemical cycle of this ATP hydrolysis to the mechanical cycle of this motor stepping.

You could ask that how stochastic are these motor trajectories? For example, and just to show one here it is these are called stochastic events in that it is not that it takes a step at every fixed interval of time, it stays bound for some time and that time interval it stays bound can vary

from step to step. So, these are inherently stochastic processes. So, how do I model this sort of a process ok.

You could also ask that how do these binding and unbinding rates of the motors themselves depend on force. So, like I said these motors can bind with some rate, let us say k on and it unbinds with some rates let us say k off and theses forces these rates can themselves be functions of time right.

So, for example, if I was pulling on this motor and it was bound then I could imagine that may be the harder I pull may be it will be easier to unbind the motor right. So, this unbinding rate would then have a dependence on force and naively I would say that the higher the force the higher the unbinding rate ok, but how do these depend for different types of motors could be a valid question.

This is the difference saw some of these could be inputs of your model some of them could be outputs of your model, but in you want to answer sort of all of these sort of questions. So, I will not go into the models today, but let me show couple more things.

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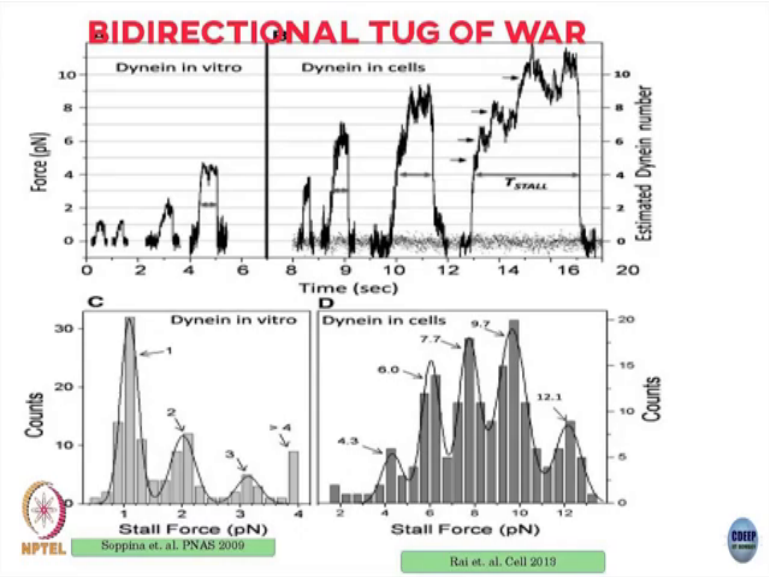

So, for example, this stall force that I was talking about. So, if you look at, so these are these are cargo trajectories. So, these are cargos carried by dyneins either in inside the test tube or inside cells and you can look at these trajectories and you can construct histograms that of the stall force the force that is required to stall the motion of the cargo completely ok.

And, you will see that these stall forces that peaks at let us say inside the cell it is peaks at around 1 pico Newton, 2 pico Newton, 3 pico Newton which you would then interpret by saying that this is a sort of cargo that was been carried by only one dynein motor ok. So, when you applied 1 pico Newton of force that dynein came to a stop, maybe this cargo was being carried by 2 dynein motors so, you need a 2 pico Newton's of force to sort of bring it to a stop and so on, this was carried by 3 (Refer Time: 07:39).

Inside the cells so, most cargo in the in vitros carried by one dynein single dynein motor whereas the inside cells most cargo would be carried by somewhere around 7 to 9 dynein motors because the peaks are somewhere around 7.7 or 9.7 pico Newton. So, per dynein motor this stall force is roughly around 1 pico Newton at least for this class of dyneins there are other classes where the numbers might change, but for this class it is around 1. So, depending on where you get the peak in your in the stall forces histogram you might be able to say how many motors were carrying a single cargo.

So, one of the questions that we I had asked was to say that well when I have these cargos which are being carried by multiple types of motors not only multiple motors. So, kinesins as well as dyneins how do I sort of how do I get sort of transport and one of the most common models or one of the most canonical models is one of the simplest ones, it says that this cargo motion is ultimately regulated by some sort of a tug-of-war ok.

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So, tug-of-war is a mechanism for bidirectional transport. So, you have these kinesin motors which exert some force in this direction, you have these dynein motors which exerts some force in this direction and the cargo will move in a resultant direction given by the balance of the forces between these two.

And, here the so, for example, the dyneins want to move this way. So, they exert; so, I do not have any optical trap or anything. So, these dyneins want to move this way. So, therefore, they exert a force on the kinesins, similarly the kinesins want to move this way therefore, they exert a force on the dyneins ok. So, the forces generated are internal and each motor use the force generated by the opposite type of motors ok.

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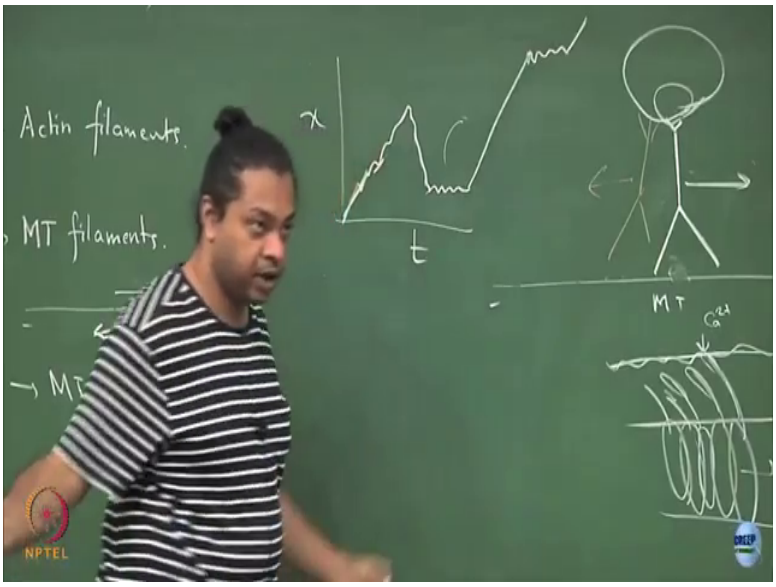

So, this is sort of canonical model, it says that there is a tug-of-war ah. If the forces sort of balance each other then you; so, if kinin let us say this is my cargo trajectory as a function of time let us say x is the function of time x being the position along the microtubule.

So, let us say if kinesins win then I will move along the plus end I will move along the plus end roughly if at some point some kinesins unbind and dyneins win then maybe I will you know start walking in the other direction, if the forces are balanced I could have no net motion and so on. So, we will see some sort of a stochastic trajectory like this.

So, it is a tug of war between these kine these oppositely directed motors kinesins and dyneins and what I will ultimately see it depends on the number of. So, therefore, the number of motors of each type that are bound at a given time. There is also a sort of. So, this is the (Refer Time: 10:31) is now roughly some 10 – 12 years old and has a lot of experimental

support, but on the other hand it has some experiments which says that this is not exactly right.

So, for example, if I said that I have this cargo which could be bound by both kinesins and dyneins and let us say somehow I manage to make all kinesins inactive. Then I should see according to this model this sort of a tug of war model I should see that all my cargo has now walked towards the minus end and everything is bunched up towards the minus end. Similarly, if I somehow manage to inactivate the dynein motors and I only had kinesins then everything would walk towards the plus end and I would see all sort of cargo bunched up at the plus end.

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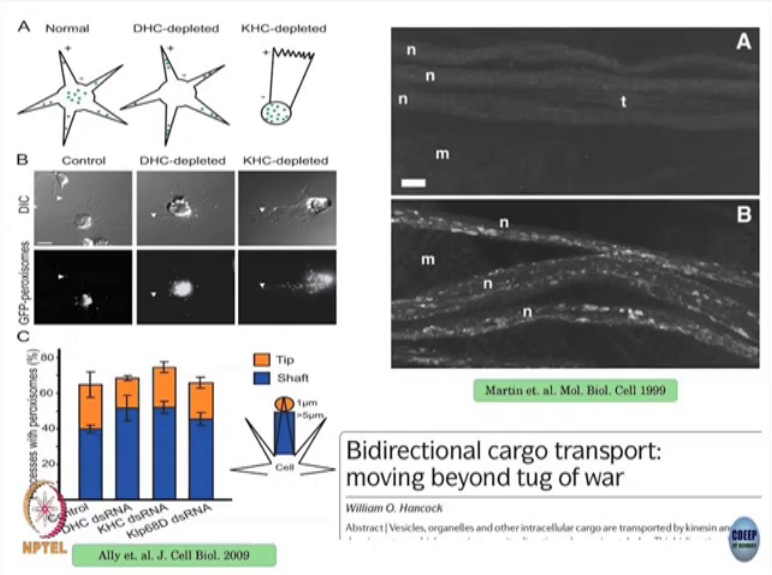

So, here is an experiment that sort of tries to test that. So, here is the particular cell, so this is a normal cell and you have some distribution of cargo. So, the minus ends of so, this is the cell body the minus ends of microtubules are over here the plus ends are over there along these

tips ok. And, in a normal cell you will have some distribution of cargo some here, some here and so on.

If you now sort of deplete dynein so, you make some sort of genetic modification, so that the dyneins do not get are not functional anymore. You will imagine that the kinesins been over in this tug of war sort of a scenario and all the cargo now gets bunched up at the plus end right. So, everything is now at the tip there is nothing at the center.

On the other hand, if you deplete kinesins which is this experiment you would sort of expect according to this tug of war picture that all the cargo are now in the cell body over here and there is nothing at the tips. This cartoon is somewhat different from this because in this modification the cell cells change the shape a little bit from this sort of shape to this sort of shape, but anyway you would expect all the cargo to be at the cell centre ok. So, that is the naïve expectation. You now do this experiment. So, this is the control experiment this is the experiment where there is no dyneins this experiment where there are no kinesins.

And, if you see that the distribution of cargo actually does not change much at all. So, if in this picture it might be a little difficult to make out. If you look at the histograms so, these the yellow parts of the histogram; the histogram are the cargos which are towards this tip and this blue are the cargos that are towards the shaft ok. So, this is the control cell this one is the cell where I think this is where dyneins where inactivated and this is the cell where kinesins were inactivated. So, there is some change in the numbers, but it is not like in this one everything has the blue has taken over or in this one the yellow has taken over.

To maintain a sort of similar concentration of cargo which end up in the tip versus which end up in the shaft irrespective of whether you have done these dyneins mutations or these kinesin mutations. In fact, there is other experiments which show that if you inactivate any one of these type of motors the cargo as a whole stops moving instead of going zooming across to the other end it sort of move stops moving at all which basically says that there is some sort of regulation of each of these motors by the other you will need both of these in order to see motion inside of cells ok.

So, there is some sort of regulation of these amongst these motors themselves and if you just inactivate one type of motor you can evens find that all motions inside the cell has stopped. So, again this is not very well understood how this regulation happens there are models, but it is not very clear as to what the answer is. So, these are all open questions just to how this sort of transport happens right.

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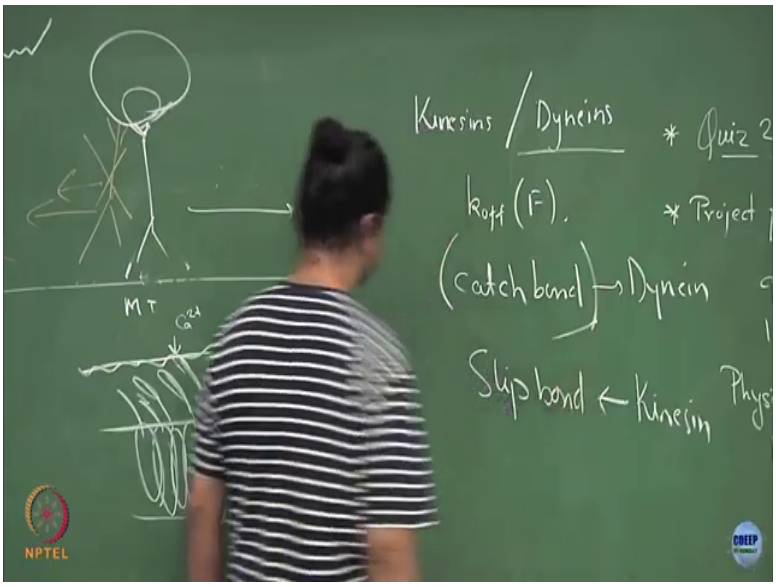

So, another sort of puzzle is what I was saying about dyneins and that dyneins are somewhat different from kinesin. Dyneins are somewhat more complicated motors. So, kinesins verses dyneins kinesins verses dyneins kinesins verses dyneins and to show that what you can do is that you can try to estimate the unbinding rate of dyneins or kinesins as a function of the force and here is what sort of experiments you would do.

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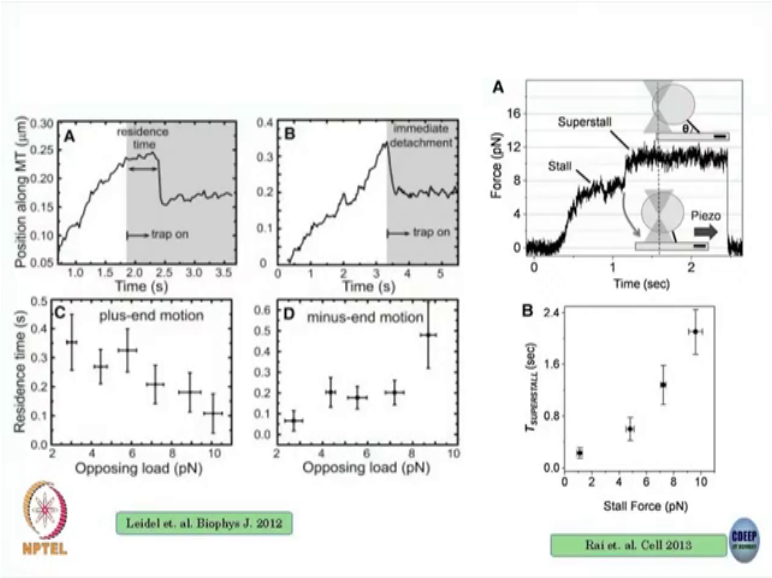

So, let us say again this is experimental trajectories. So, this is position along the axis as the function of time ok. So, what you do is that you let your cargo walk along you microtubule ok. Once it stalls you move so, these are moving in an optical trap sort of a setup. So, this is my optical trap. You move the optical trap a little bit, so that you can control the force that this cargo feels and then you observe how long does this motor stay bound under this force before it unbinds you measure this. So, this is when I turn my trap on like this you measure how long it stays bound before it falls off ok.

You do this many times and you can measure the average of this residence time as the function of the force that you apply ok. So, these are different experiments. So, we do this experiment many many times thousands of times and you get some sort of an average that depending on

the force that you are applying using this optical trap how long does this motor stay bound. And, you can do it for kinesins and dyneins.

So, for example, here is the curve for kinesins which says that the more load that load is an opposing force. So, like here the more force that you apply in the opposing direction the smaller my residence time which is sort of what I would expect that if I am pulling on this motor opposite to the direction that it wants to go in if you pull on this in the opposite direction I would expect that you know it comes off more often or it stays bound for a less amount of time ok. So, and that is what this kinesin shows that the more load I put the residence time sort of decreases.

On the other hand, dynein show very different behavior, it shows in fact, the opposite behaviour. So, the more I sort of pull on this the more time it is spend bound to the filament ok. So, the more the opposing force the more tightly it sort of stays bound to the filament. This is very counterintuitive most motors do not behave like this which is why this sort of an unbinding rate have or this sort of a bond with sort of strengthened under force under an opposing force there is a special name it is called a catch bond ok.

Whereas, these other bonds the like in kinesins; so, this is for dyneins this is for dyneins whereas, for kinesins the standard expectation value you know the unbinding rates sort of increases with force where the residence time decreases that sort of an example of a slip bond ok. So, from these sort of experiments by measuring the residence times, you can construct actually the unbinding rates.

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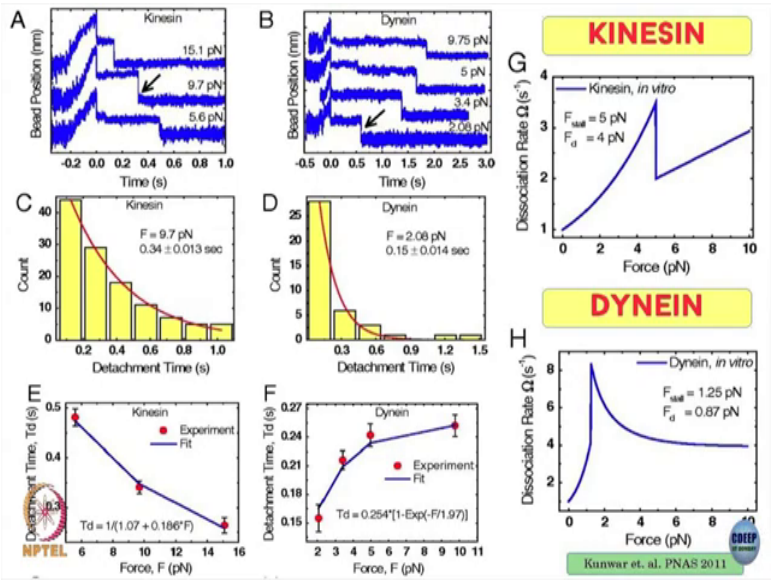

You can construct the unbinding rates and here is what let us forget about ah. So, this is basically that sort of a setup where you do it at different forces. Ultimately, what you get out of it is this dissociation rate the unbinding rate for dyneins and for kinesins. So, initially for both there is a regime where the dissociation rate increases with force, but then beyond the certain force which in fact, happens to be the stall force of these motors kinesin keeps on increasing again. There is a discontinuous dip, but it keeps on increasing whereas, dyneins actually decrease.

So, in this regime is where this catch bond comes into play and the more force you apply the smaller the disassociation rate or higher the residence time ok. So, when you are building models for these sort of motors a model that works for kinesin would be very different from a model that works for dynein because you have to take into the account this difference of the

nature not just the number, but the nature of these unbinding rates of these residence times. So, we will talk a little bit about some of these models next class.

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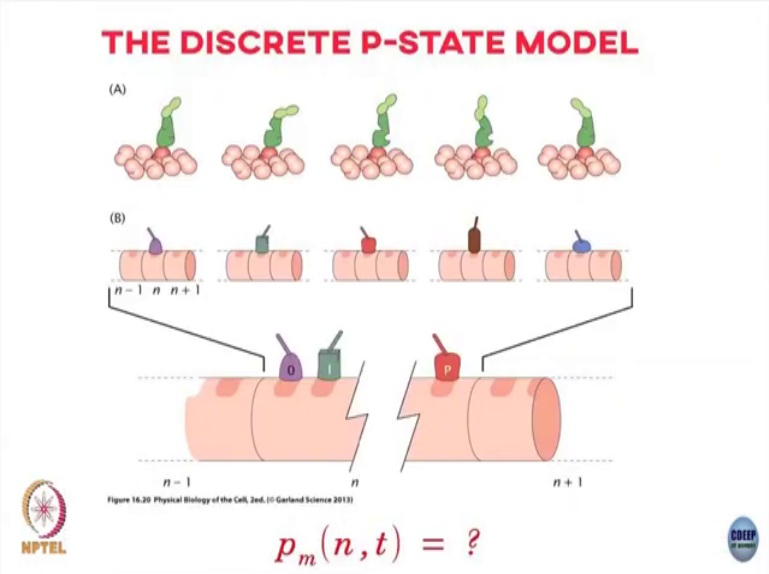

In a very generic sense let me say that what sort of models can I build what I can say is that these motors ah. So, one characterization of these motors is where they are on the track ok. So, if I label my tracks n minus 1 and n plus 1. So, this is the position along the track it is a lattice. So, it is a sort of lattice given by this alpha beta tubulin or this actin monomer sub unit ok.

So, there is a discrete random walk where these motors can hop from one unit of the lattice to another. So, you label the units of the lattice, then you could ask what is the probability of finding a motor in the nth box versus the n plus 1th box versus so on, but additionally you could have various internal states of these motors. You could have I do not know some n

number of steps or t number of steps in this case, it could be whether ATPs bound whether ATPs hydrolyze, if there are conformational states of the motor that it takes for example, this active versus inactive state of myosin all of these could be variables in the modeling.

And, then you could ask that what is the probability to find the m-th motor sorry to find the motor in the m-th state at position n at time t and you could write down master equations for this sort of a probability quantity ok. So, you could take into account the internal states of these motors by coupling the chemical cycle or whatever other conformational states that you will have, the positions of these motors along the track and the time itself and then write down evolution equations for these probabilities and maybe under certain approximation solve them as well ok. So, that is what we will try to do.

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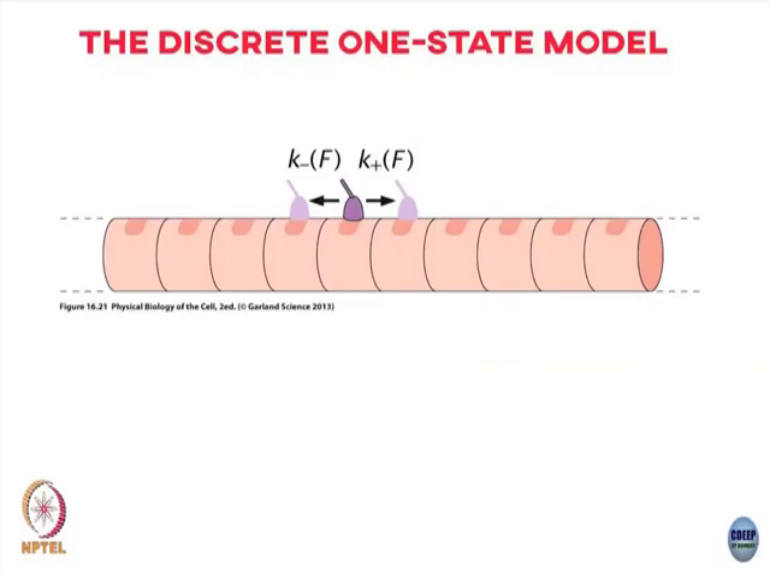

I just leave you with the zeroth order sort of model in this sense which is that I forget all about internal states. I just consider the position and this motor can hop forward with some rate k plus and hop backward with some rate k minus. And, like a standard random walk because these motors have directionality the two hopping rates will not be equal right it will be greater in one direction versus the other.

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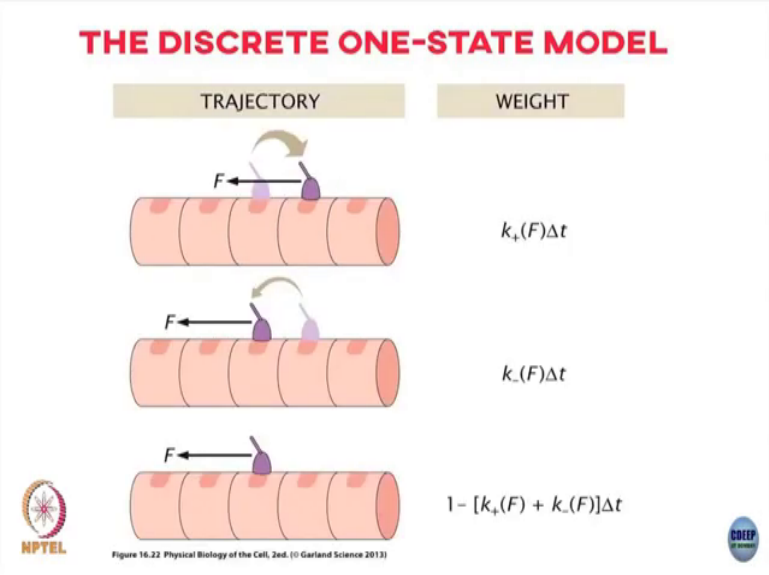

And, then you could write down what is the probability to find in position n at time t plus delta t by looking at all the possible hops ok.

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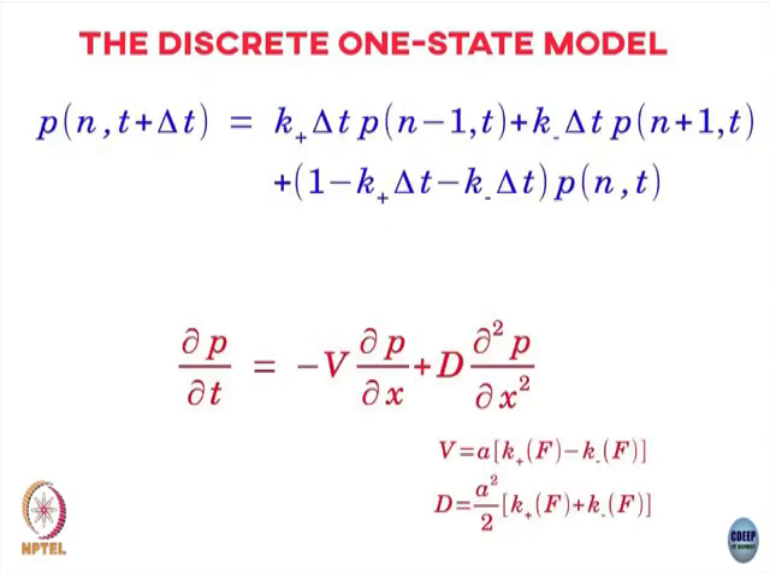

So, this is the hop from n minus 1 to n with a rate with a probability k plus delta t or hop from n plus 1 to n with a probability k minus delta t and some probability of staying at that same position. So, this is the discrete version. You can take the continuum limit of this to write down how this probabilities change in time and what that could simply give you the drip diffusion equation right.

So, if you took the continuum version you will just get some del p del t is minus V del p del x plus D del p d del x 2 where this velocity would be the difference of these two rates the forward and the backward, the diffusion constant would be the average of these rates and of course, the lattice constant of these two.

So, this in some sense the zeroth order, I have not considered any of the complexities of these real motors that I was talking about. What I will try to do over the next class is sort of bring in

some of these complexity that we described today and within this sort of a master equation framework and see how that changes these equations and how to study these equations.

So, I think I will stop here for today and continue on Friday.