Physics of Biological Systems
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Lecture – 26
Internal states of macromolecules

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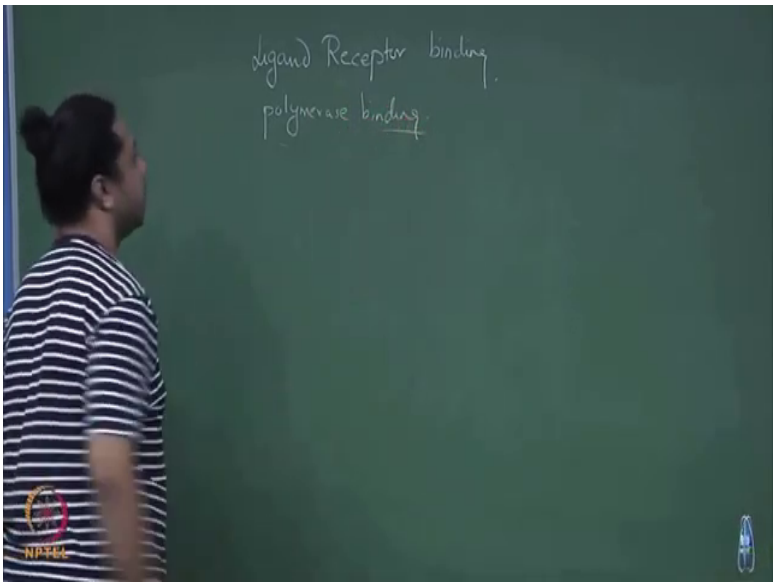

Right, so, what we will do is that we will continue along these lines today, but what I want to sort of focus on is this idea that if you think about a lot of biological macromolecules, they are characterized by internal states and we will see examples, but the most immediate example is for example, that of proteins. Proteins can exist in multiple conformational states depending on various factors and again we will see some of these factors and these are sort of discrete states.

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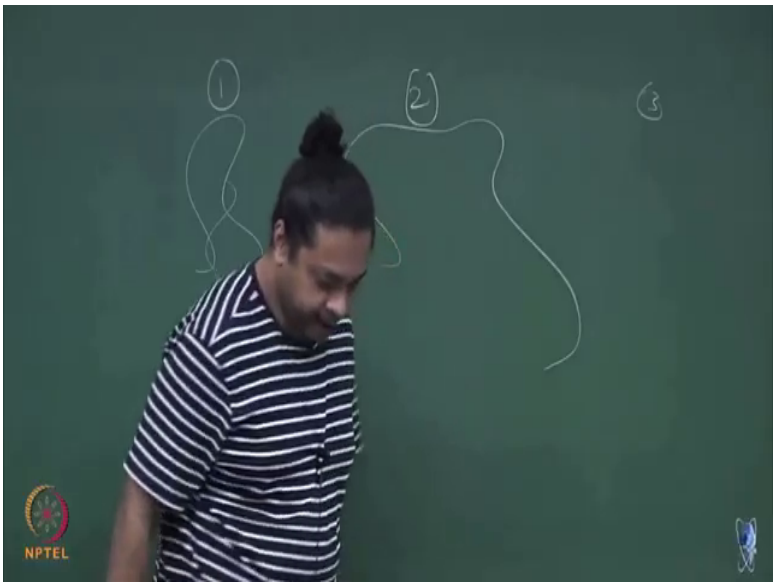

So, you can say that at some point maybe a folded protein looks like this and in another in another environment or in contact with another ligand a protein might looks that same protein might look like that whatever but, these are sort of discrete states. So, you can label these states this is conformation state 1, 2, 3 whatever.

And, you can then ask things like what are the what is the probability that when you look at this protein if you will find it in this state versus this state versus this state. So, this is something that is very common biologically that you have these internal states of macromolecules they could be proteins, it could be DNA it could be whatever.

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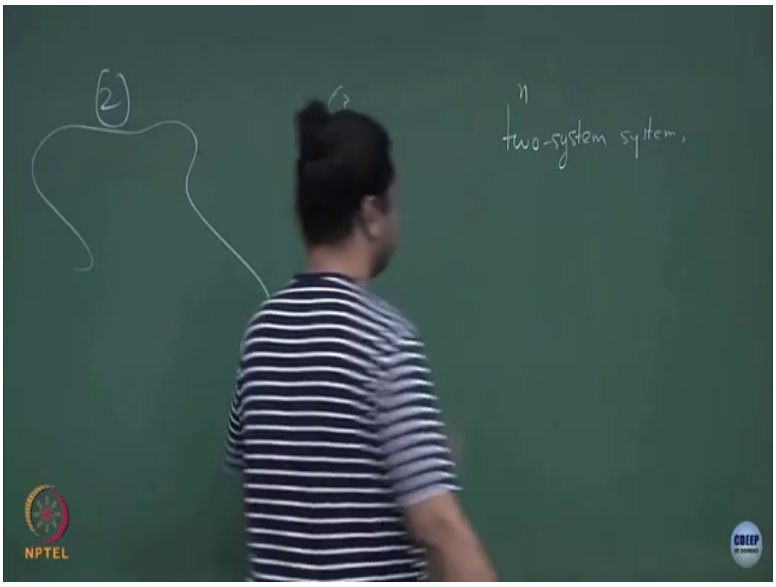

But, if you think about it from a statistical mechanics perspective this is the language that you can use to treat this is very similar to this sort of two state or two state systems that is very that you should know very well from statistical mechanics. Either two states need not be true n state systems, but discrete sort of set of states where you can count the number of states that are possible for you to access ok.

So, all the machinery of statistical mechanics, the simple machinery sort of two state systems without getting into continuous energy levels or things like that. This is very simple discrete state machinery. You can carry over very nicely into this biological well if you consider this if you consider this discrete states of macromolecules. So, that is what I will try to focus on today.

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The first example is that of ion channels and again as you know cell membranes often have these in-built ion channels in them to facilitate movement of ions into and out of the cell.

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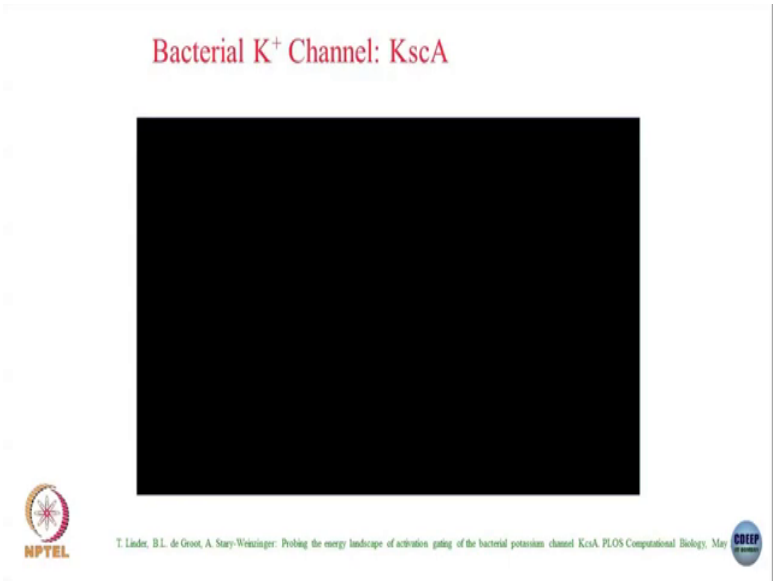

You can have specific ion channels for example, potassium ion channels which only allow potassium to pass through.

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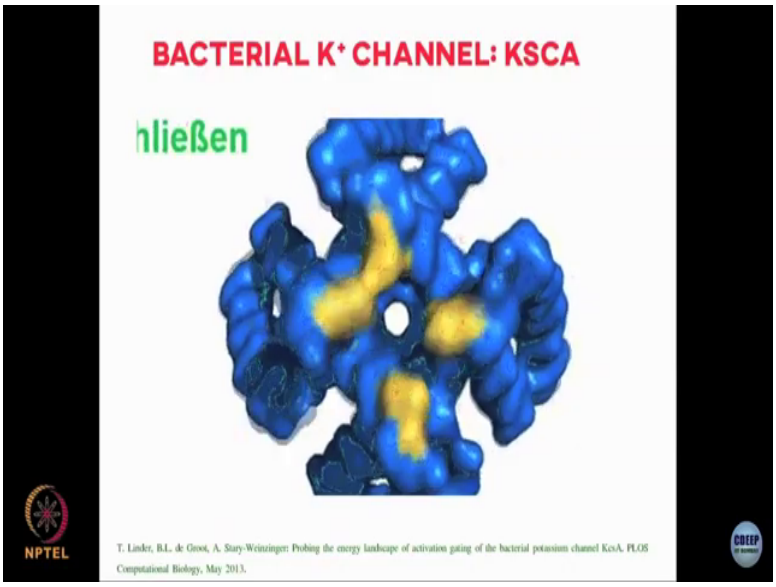

Sodium ion channels which only allow sodium to pass through and so on.

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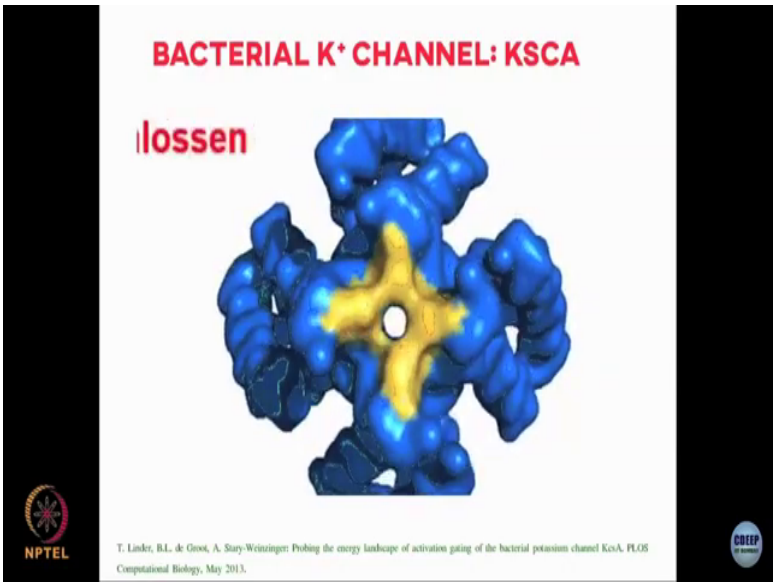

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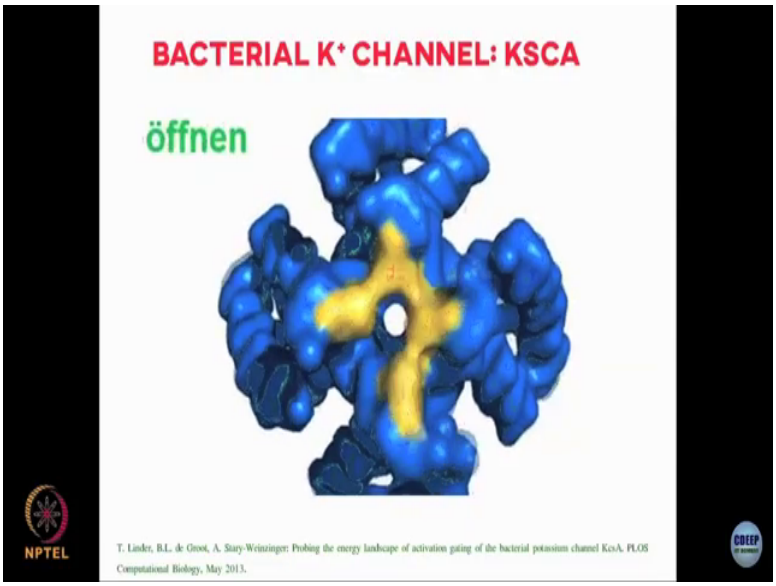

The ion channels will often exist can exist in two states – they can be open or they can be closed ok. So, it is not that this channel is always open and things are continuously passing through this channel might ordinarily be in a closed state and then no ions can get through here.

When required in response to a certain environmental or whatever internal changes in the cell. If the cell requires this sort of let us say potassium ions to come through, it will open this channel and then this ion will come flow through in inside or outside is the case maybe. So, again these are distinct. So, this ion channel is basically something like a transmembrane protein.

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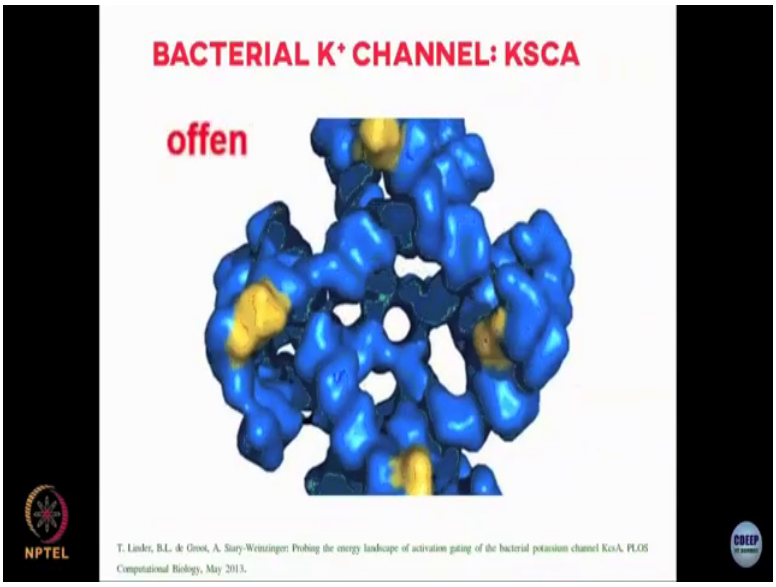

So, here is my cell right this is a transmembrane protein it is embedded in the membrane, it connects the outside of the cell to the inside and can exist in these distinct conformational states – the closed state like this where you cannot see the pore anymore or this open state where this sort of subunits shift up and this pore opens up and your ions can pass through there right.

So, this is an example of what I mean when I say that these macromolecules ion channel in this case can exist in these discrete states; in this case roughly you can see two states, open and closed.

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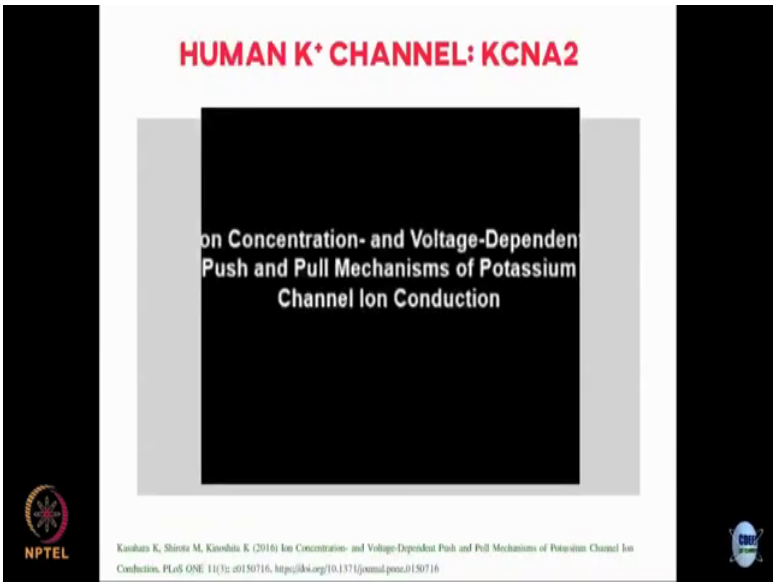

And, you will see this in all channels.

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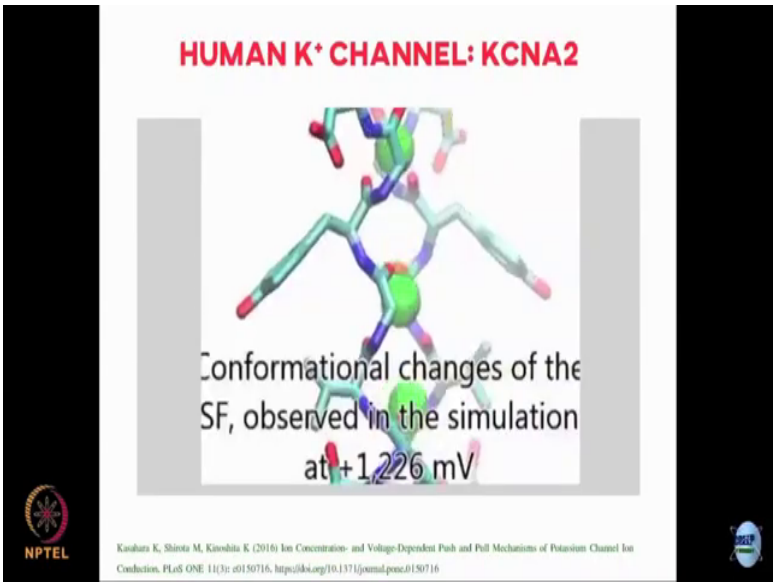

The mechanism by which it sort of opens and closes the channel will be different there are various routes that biology has evolved in order to pass in order to control when these channels will open and when this is with this will close.

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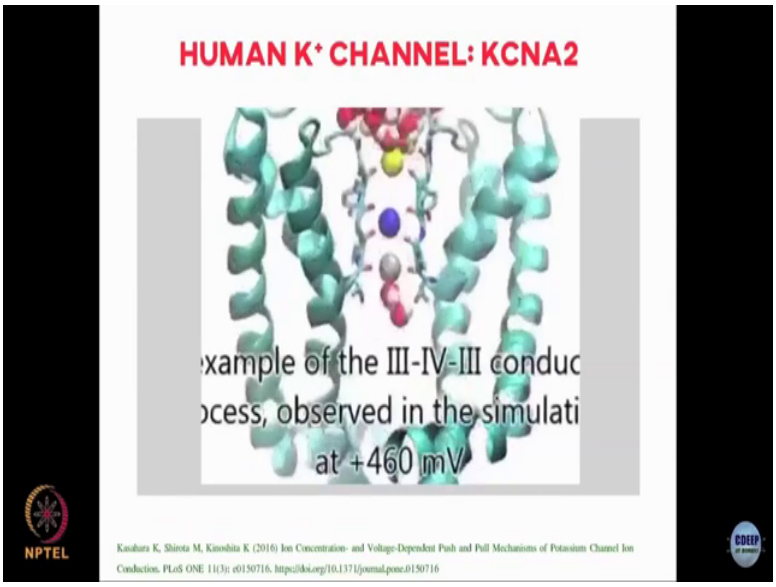

This for example, is a particular example of a potassium channel I think in bacterium not sure actually in which organism and this has a voltage this has a voltage dependent gate. So, by controlling the voltage difference between the inside and the outside you can control how often this ion channel will open our little close all right. So, let us look at this example first that of an ion channel.

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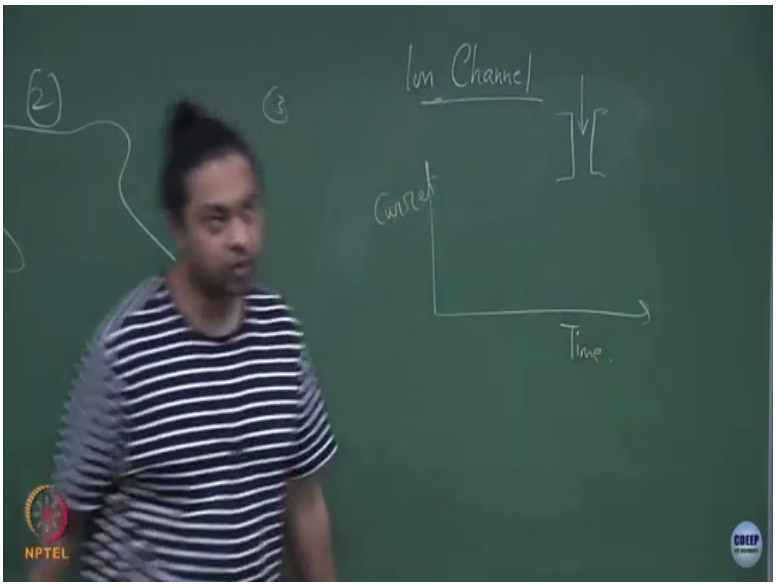

If you think about the flux of ions so, the current that is passing through this ion channel so, here I have an ion channel. Ions will small ions will pass through this depending on whether the channel is open or closed. If I think about how the flux of this flux of these small ions or the current will look as a function of time right how does that look?

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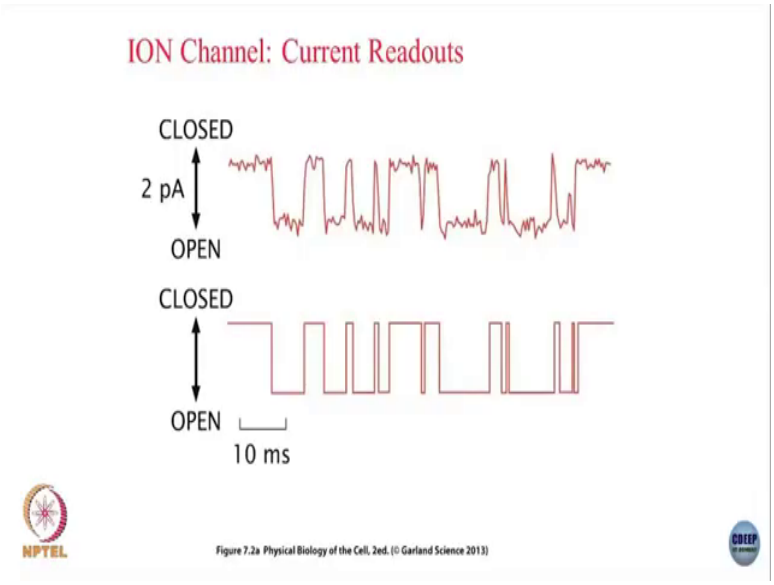

That looks something like this. Here is an actual trace of an ion channel reading ok. So, when the ion channel is closed that no current is passing through; when it is open you have some amount of current in this case around 2 pico amperes that is passing through the channel ok. So, that counts the sort of flux of these small ions that are passing through this channel.

And, what I want to say is that I want to represent this by in a gross sense in a two state system which means that the ion channel can be closed or it can be open which means that the sort of remove all these squiggles small quick squiggles and say that macroscopically the ion channel has only two states that are available to it. It is closed and in the closed state alcohol the current is 0 in this when it is open I will call the current is exactly 2 ampere. So, I will negate all sort of fluctuations around this ok.

You can see that in the real case that is not true. For example, somewhere over here you have a current something like 1 picoampere right. So, it is not an all or nothing system generally, but roughly that is what it looks like ok. You can sort of choose a threshold beyond which we will call if I could choose something like 1 picoampere or something and I say that if the current is less than that I will consider the channel to be effectively closed, if the current is more than that I will consider the channel to be effectively open right.

So, this is an this trace is an idealization of this, but you can just see by looking at it is not that bad of an idealization. It captures their sense of this stochastic opening and closing design channels. You can generate these sort of smoothening curves yourself.

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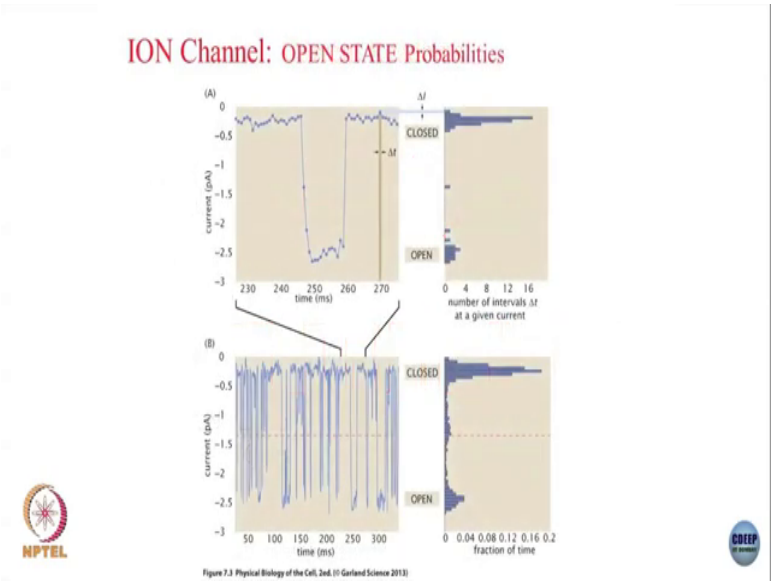

So, for example, here is a full trace of this current through an ion channel over a long period of time. What you can do is that you can construct a histogram of the various current value.

So, for example, over here is something this is zoomed in view of this part of this trees right. So, this is this dip over here.

And you can you can take some window of time delta t. You find out what is the current in that window and you construct a corresponding histogram. You do that over this whole sort of trees and you get this histogram of this current values as a fraction of and how long it spends with that particular current value and looking at this histogram you can sort of then decide that what is the current. So, this axis is sort of the value of the current right, this is the height or this is the fraction of time it spends with that current.

So, you look at this histogram and you can say that well roughly what threshold in should you choose in order to convert noisy data like this into smooth discrete two states or per data. So, for example, once you have constructed the histogram I can say that well this looks to be roughly the middle of this histogram this bimodal distribution that I obtained. So, we will cause call this is my threshold current. Anything that is less than this so, all these points while group into a closed ions, closed state of this ion channel all currents on this side and group into the open state of diameter.

So, that is sort of a practical prescription to how to go from this sort of real experimental data into this idealized two states sort of a data.

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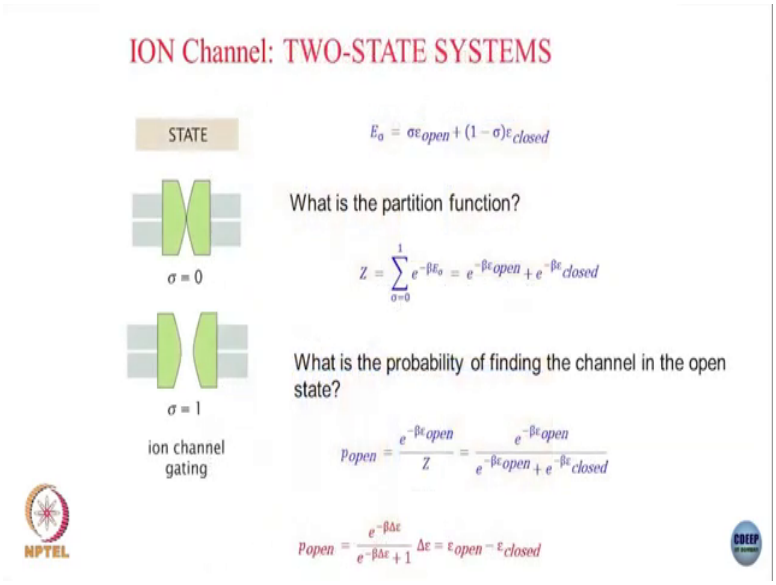

But, once you have done that again we can use this terminology of this two state system from stat maths so, for example, when the ion channel is closed I can say that my state variable which I represent by sigma that is I am going to call that as 0; if the ion channel is open I will call that the sigma is 1 right and when it is open let us say it has some energy when it is closed it is some energy epsilon open and epsilon closed and then once I have done that I can calculate what is the probability that I will find the ion channel in the open state right.

So, for example, what is the partition function the partition function is e to the power of minus beta times this energy right summed over all possible microstates; the microstates in this case are 2 because you have a 2 state system. So, it can either be closed or it can be open. So, this is my partition function. Once you have the partition function you can easily calculate what is

the probability of the channel being in the open state right. So, probability is therefore, what? e to the power of minus beta epsilon open divided by this partition function right.

Student: (Refer Time: 09:49).

Student: Sigma.

Sigma is a state variable. So, that is what I used to index my microstates of the system. So, generally in a gas you would say that its position momentum whatever right, in this case this internal variable which characterizes the state of my system is whether there ion channel exists in a closed state or in an open state. So, that I am calling by this variable sigma ok.

Student: (Refer Time: 10:16).

Student: (Refer Time: 10:18).

Sigma looks like probability, which one looks like probability, this one? Sorry, say the transform?

Student: I think its sigma (Refer Time: 10:28).

Epsilon.

Student: Yes sir.

This one?

Student: Yes.

This is you can consider this is the Hamiltonian of my system, this is the energy of my system right. So, think about it when the when sigma is 0, right the ion channel is closed, then this

term does not play a role, then what is the energy of my system? It is 1 minus 0 into epsilon close which means it is epsilon closed. Similarly, when sigma is 1 then this term does not play a role and the energy of my system is epsilon open it is just a convenient notation of writing that it is the energy that epsilon open if it is closed epsilon closed sorry epsilon open if it is in the open state e closed if it is in the closed state.

So, this then is the probability of finding my channel in the open state all right or if you want to write in terms of the delta the difference in this energies then it is this form the standard form that we did even last class for this roughly for this ligand receptor sort of a binding ok. Can you change this probability? So, this says that well I have some energy for this ion channel to be open some energy for when it is in the closed state.

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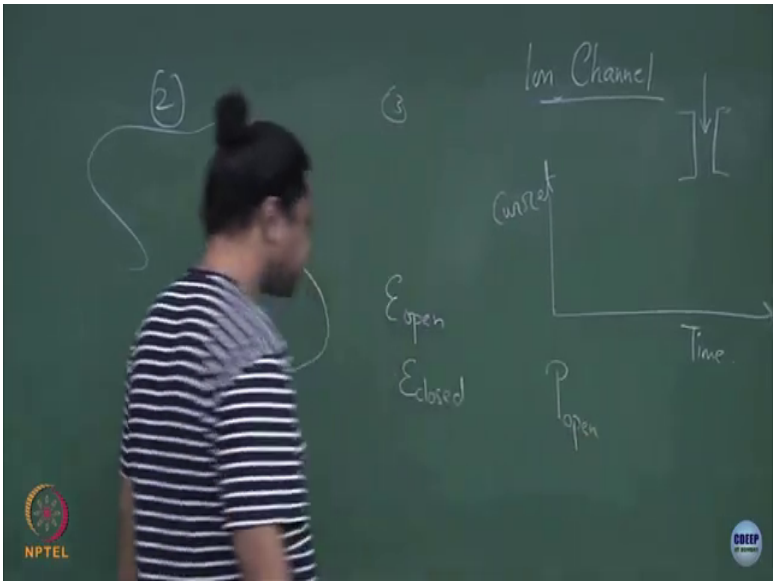

And, the difference between these energies tells me how often I will find this ion channel in the open state right. Can you can you shift around these probabilities and it turns out that you can do that experimentally.

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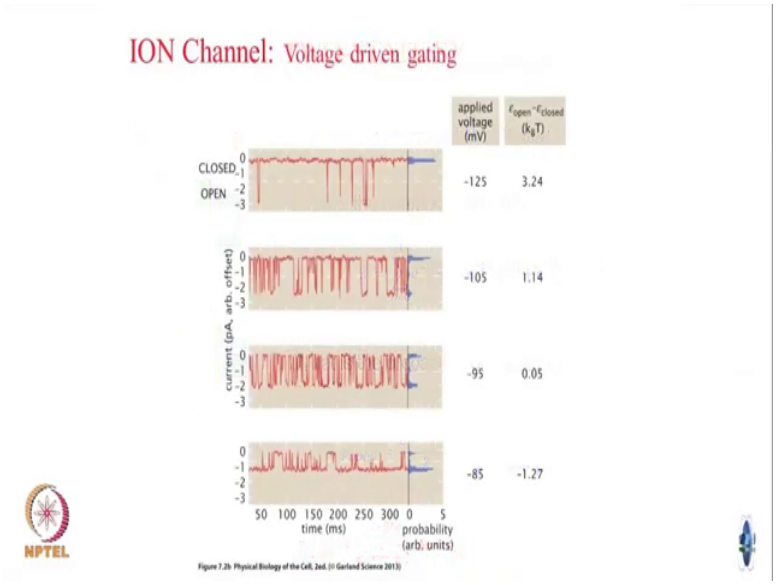

For example, here is voltage gated channel and you these are the traces of this current that is flowing through the ion channel as you apply different voltages across the membrane right. So, here is minus 125 minus 105 minus 95 minus 85. So, as you can see as you change the sort of voltage that you apply you change the probability of the open state as you go from top to bottom. As you go from top to bottom it becomes more and more likely that you will find your channel in the open state; here almost everything was closed here a lot of it is open all right.

So, in this sort of a language what you could say is that what you are changing is the difference in energies between the open and the closed state. So, you can estimate using that formula that you know P open is e to the power of minus beta delta epsilon by 1 plus e to the power of minus beta delta epsilon. You can. So, these delta this pre open becomes a function of this applied voltage therefore, this change in energy delta epsilon also becomes a function of the applied voltage and that is what you sort of estimate.

So, this rightmost column is a estimated from this formula that what is the effective difference between the open and the closed state energies as you apply more and more voltage ok. So, that is one way.

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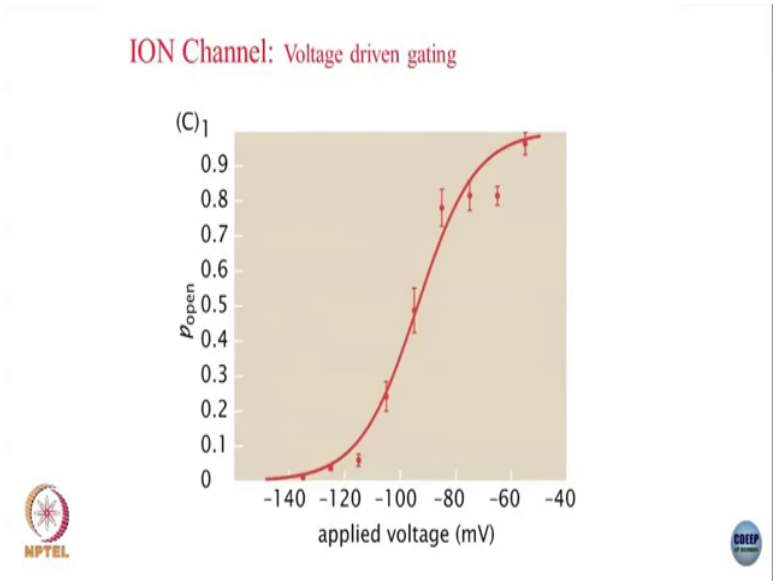

And, you can of course, plot this as well and again this looks like a sigmoid curve. So, this probability of being in the open state as a function of this applied voltage looks somewhat like

this. This sigmoid curve again which is something similar to what we saw in this ligand receptor binding case. This is one way. So, this is for voltage gated channels.

So, you can play around with how long what is the probability of finding channel in the open state. Another way is for example, if you have mechanosensitive channels you apply some tension to the membrane and again the tension can change the probability of how long will it be in the open state, how what is the probability of it will be in the closed state.

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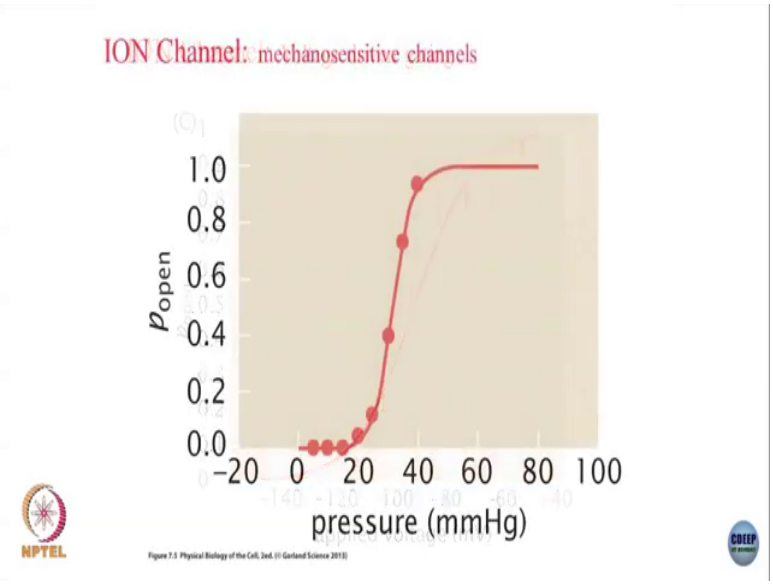

So, for example here is a sort of some data from a mechanosensitive channel and again it looks somewhat similar the more and more tension or pressure, that you apply you change the probability of it being in the open state.

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So, here for example, is a is a simulation of such a mechanosensitive channel.

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This is the Piezo 1 channel. So, here is your ion channel protein.

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As you have as you change the tension across the membrane your ion channel can open up and it can allow ions to pass through it. Yes?

Student: (Refer Time: 14:48).

Does it vary linearly?

Student: (Refer Time: 14:55).

Yes.

Student: (Refer Time: 15:05).

Not generally, I mean there might be channels where it does, but that depends more on the specific mechanism of the voltage gated, but generally I would say the answer is true.

Student: (Refer Time: 15:21).

Roughly, yes. So, I cannot say with certainty the curves that at least I have seen mostly look sigmoidal. It may be a very strong it the nature of the sigmoid might be weaker or stronger depending. So, if you are thinking in terms of for example the hill function.

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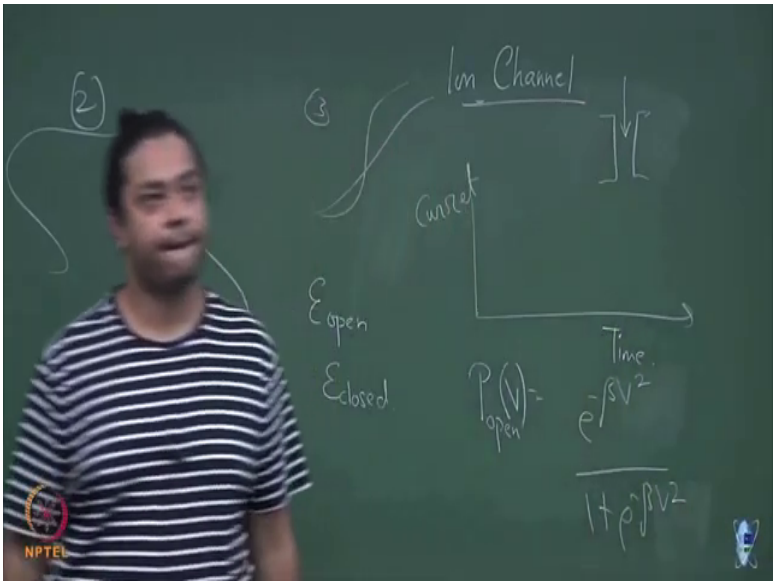

The degree of the steepness of this slope can differ, but generally as far as I have seen they all mostly look sigmoidal, but that is not a comprehensive answer, there may be channels where it does not. But, so, what you are saying is here that if I say that instead of delta epsilon I put V

right then this looks like a sigmoidal curve right. But, on the other hand I do not know what would happen if I put a V squared there?

Student: (Refer Time: 16:26) sinusoidal.

Student: There will be a sinusoidal (Refer Time: 16:31).

No. So, what am I saying is that if I put a function like this V squared what will that P open versus V look like. It will still look A, but this is no longer a linear dependence right. So, that is what I am trying to say that you will get curves which looked like this, but just because it looks like this you cannot say that this delta epsilon dependence on B is going to be necessarily linear.

Student: (Refer Time: 16:58).

But, as far as I know it will not be something so wacky that you will get something like this maybe your that is what I am trying to say.

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So, something like this is a hill function with a greater degree of n, but yeah. So, generally it can be non-linear, but it will not be completely of the charts all right. Let us go to the next slide huh.

So, this mechanosensitive channel. So, here you are what you do is that you change the tension across the membrane and that allows the channel to open and close and as the channel open and closes you get ions passing through ok. So, you can ask that well can we sort of estimate the sort of probability. So, here the probability was the function of voltage, for mechanosensitive channels it is going to be a function of for example, the tension that is applied across the membrane ok.

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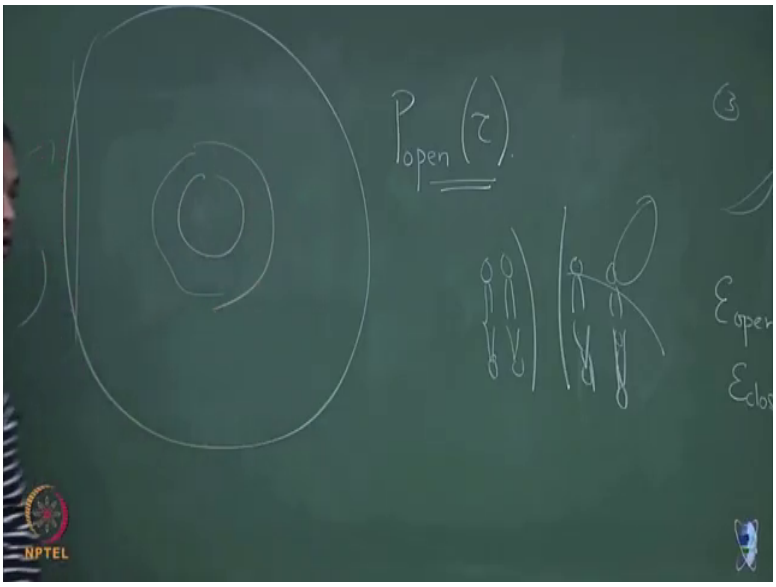

So, can I calculate the probability that the channel will be in the open state as a function of the tension that you apply across the channel. And, if this tension is applied again by different proteins, different proteins can come and bind. They can change the local curvature of the membrane, as you change the curvature you can change the tension that this location fields ok. Yes?

Student: Sir, (Refer Time: 18:24).

Yes.

Student: (Refer Time: 18:25).

Right. So, in this mechanosensitive channel so, you have this lipid bi-layer right which is my membrane something like this and then you have this channel which is embedded over here sorry, whatever you get the right. Now, let us say another protein comes and binds somewhere over here on this membrane ok. By binding it changes the local curvature of this membrane that will cause a tension of course, over here that will cause it.

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And, in fact, we will see an example of proteins which does that in a in a couple of slides ok

So, what I want to estimate then is this probability well is this probability that I will find this channel in the open state as a function of the tension that I apply. And, of course, you can do nice modelings taking into account whatever the various properties of the surface, but what I will show here which is from Philips is a very zero-th order sort of estimate.

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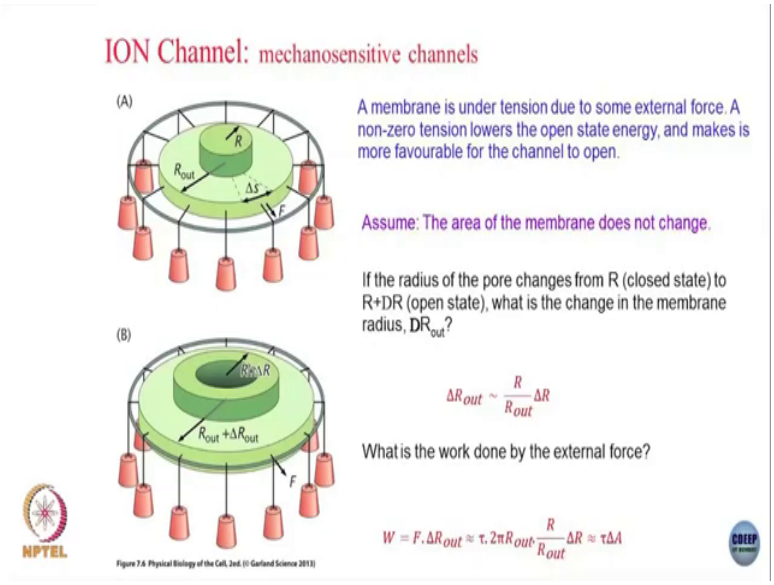

So, here is what Phillips. So, here is the sort of basic zero-th order model. You consider a membrane which is under tension due to some external force which could be due to this protein binding and so on. And, as you apply the tension you lowered this open state energy and it makes it more favorable for the channel to its open ok. So, that is my assumption it could be the reverse way I just assumed a channel where the more tension I apply the more favorable it becomes for this channel will become open.

And, so, my model this is that I had this channel. So, now, I am looking at it like from top. So, I had this ion channel which is closed which is embedded in this membrane this thing is my lipid bi-layer membrane, this is my ion channel ion channel which is now closed depending on how much pressure I apply let us say this channel has opened up. So, whatever things was here it has gone to somewhere over here ok. So, that is what it says that when it was closed it

has a radius of R, now it is gone to R plus delta R and similarly this outside membrane has also spread out a bit because of this applied tension.

If you assume that the membrane area is sort of conserved which means basically that whatever you do this lipid bi-layers will sort of rearrange themselves, but they will not pop out of existence or new things will not come in. So, if I assume that the membrane area is sort of conserved, then I can calculate roughly what is the change in the membrane radius when the radius of the pore goes from R to R plus delta R. This simply by using conservation of area. So, that is roughly of the order of R by this R out which is the membrane radius times this amount of change in this pore radius delta R right ok.

This is again a very zero-th order I have assume that this membrane is a circle and so on because it is found to get a hand waving estimate. Then you can calculate what is the work done by this external force the force which is sort of applying the tension and that you can calculate. So, the work done is the force times this change in the radius delta R out right. The force is the tension the tension is the force per unit length. So, it is tau into 2 pi R and then this change in this radius delta R out which I just write as this.

So, but what you get this 2 pi times R delta R is simply the change in radius area of the pore right which basically means that this work done is proportional to the tension that you apply and it is proportional to the change in area.

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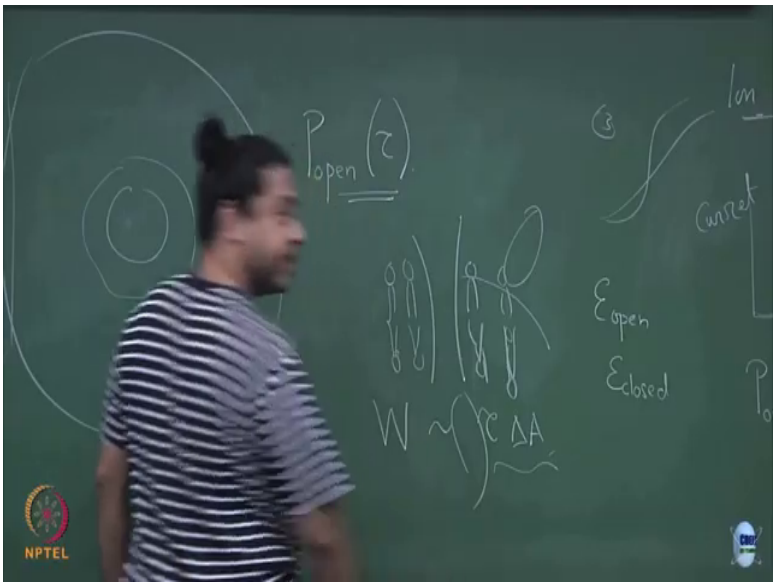

So, that is all that is that I want to sort of get out of this. There will be various other things over here depending on the properties of the membrane, I just for the moment I just in this sort of basic hand waving model I neglect all of this. All I say is that the more tension that you apply the more work that you are doing the more the change in area of this membrane protein, this transmembrane protein which is the ion channel the more work that you are doing.

So, I have an estimate of the work that I am doing as a function of the tension that I am applying.

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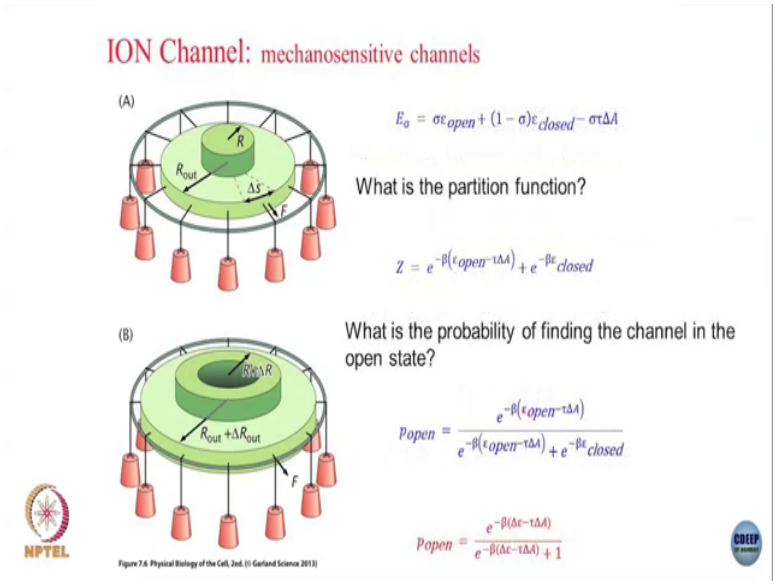

And, now, I can go back and do my same thing that I say that act zero tension I have some energies epsilon open and epsilon closed right and then as I change as I apply some tension I have to include this additional term which is tau times delta A and that will only come when the channel is open ok, not when the channel is closed. So, I now have this additional term, I had this thing earlier. I now have this additional term which models in this very crude way the effect of the tension.

Once I have that again I can do the same business of calculating the partition function and calculating the probability of finding the channel in the open state which is now going to be a function of your of the apply of the tension that you applied to this membrane. And, you can again now plot this of course, and will again look sigmoidal. If you apply a very large amount of tension you are almost guaranteed that the channel will be in the open state which means that your P open will go to 1; if we apply no tension it will come go to a low value which will

depend on this difference between del epsilon open an epsilon closed all right. So, and that is what you see.

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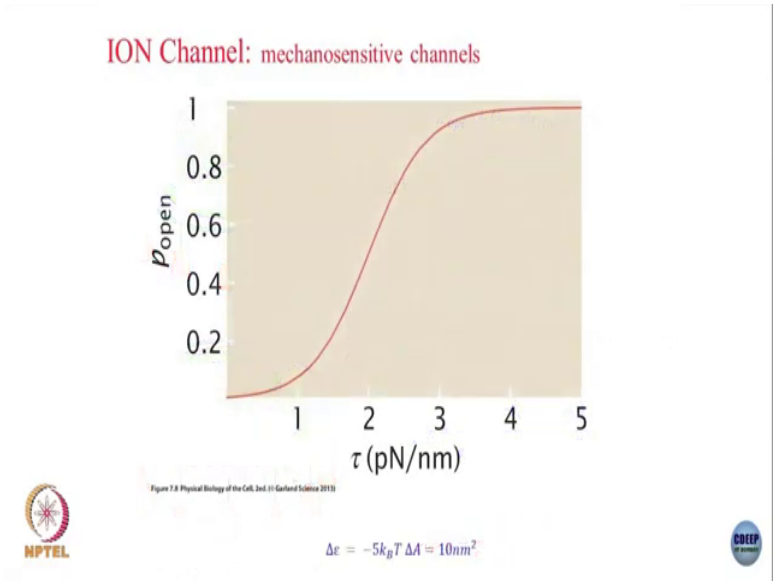


So, if you look at how the probability that you will find the channel in the open state as a function of this tau it looks again like the sigmoid curve. This is plotted using this formula with some values of delta I and delta tau delta A sorry typical values of delta I and delta A ok.

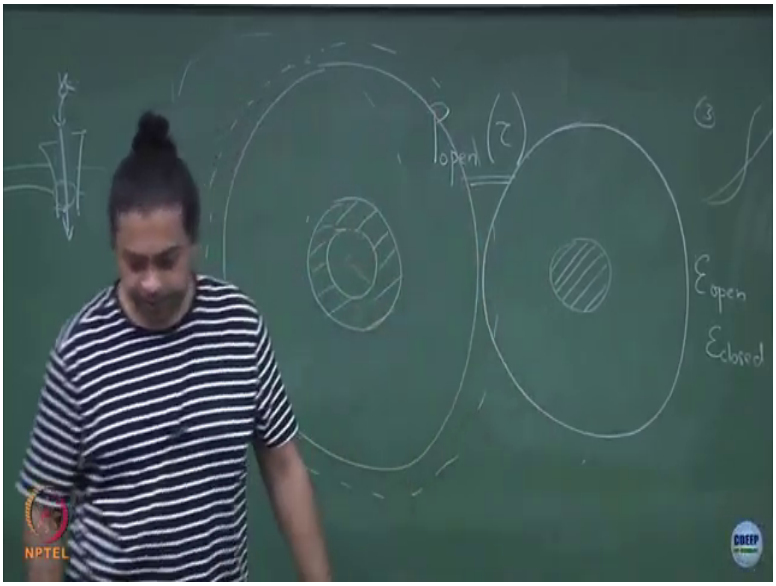
So, all of this has this sort of thresholding or sigmoidal behavior that acts beyond a certain whatever voltage or a certain force you will suddenly find it is more probable that you will your channel will be in the open state all right. So, this is one of the most simplest examples of these two state systems, the opening and closing of ion channels.

Student: Sir.

Yes.

Student: (Refer Time: 25:05).

(Refer Slide Time: 25:48)



So, what I am saying is let me draw a little better then surrounded by a circle all right. This is when my channel is in the closed state, then when my channel is in the open state the protein occupies somewhere over here and my overall membrane goes from what it was to something that is slightly larger. If you calculate the total surface area of this versus this by not this hole because this hole is no longer part, the remaining surface area this should be the same this should be conserved.