Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 09
Membrane Proteins and Transport
Lecture - 44
Membrane Transport - II

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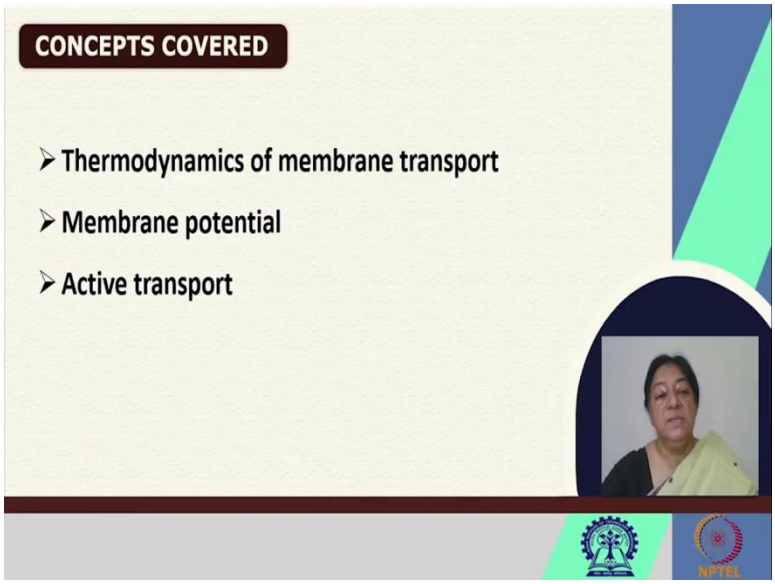

We continue a discussion on membrane transport. What we have been looking at previously is the membrane proteins, integral proteins, peripheral proteins and how they are able to conduct the transport of material from one side to the other. We have looked at passive transport. In this lecture, we will be looking at active transport and understand what we mean by a membrane potential in addition to the thermodynamics of membrane transport.

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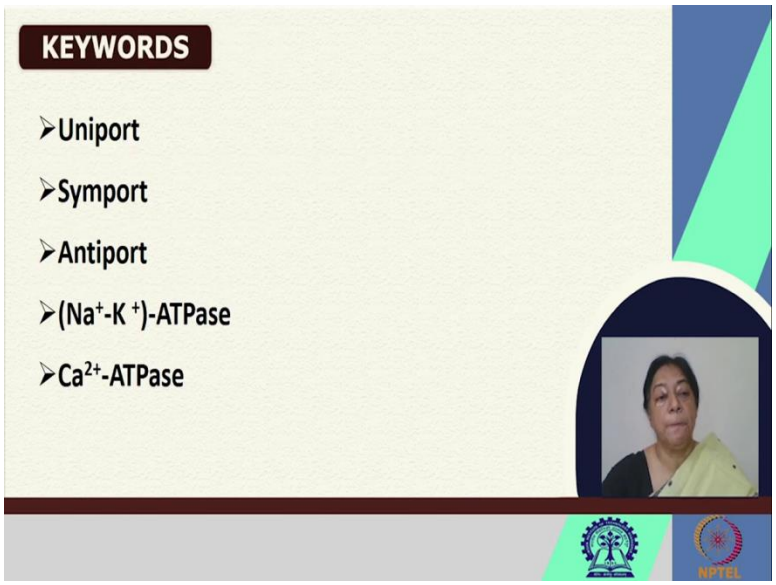

There are several terminologies that we will come across; a uniport, symport, and antiport, which we had previously looked at in the earlier lecture. Then we will look at ATPase type proteins and how they can transfer sodium, potassium, and calcium ions across the membrane.

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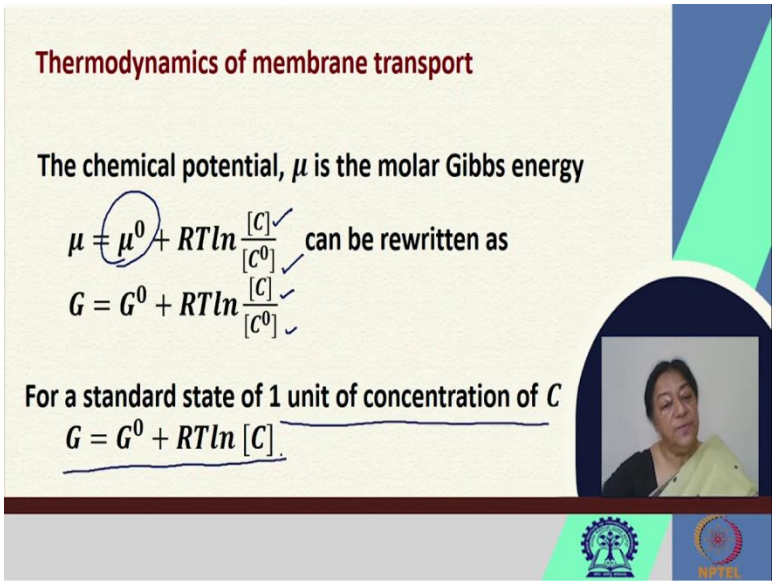

When we look at the thermodynamics of membrane transport, we have the chemical potential. The chemical potential is the molar Gibbs energy and the transfer is always from a higher chemical potential to a lower chemical potential. In this case we are talking about the concentration of the ions, as they are transported across the membrane.

If we look at the standard expression for the chemical potential, where we have the standard chemical potential given by μ^0 and we have the different concentrations; the concentration C and the standard state of concentration. In this case, which would be 1 unit of concentration of C,

making this $G = G^0 + RT\ln [C]/[C^0]$. Considering a 1 unit of concentration, we can reduce this to $G = G^0 + RT\ln [C]$.

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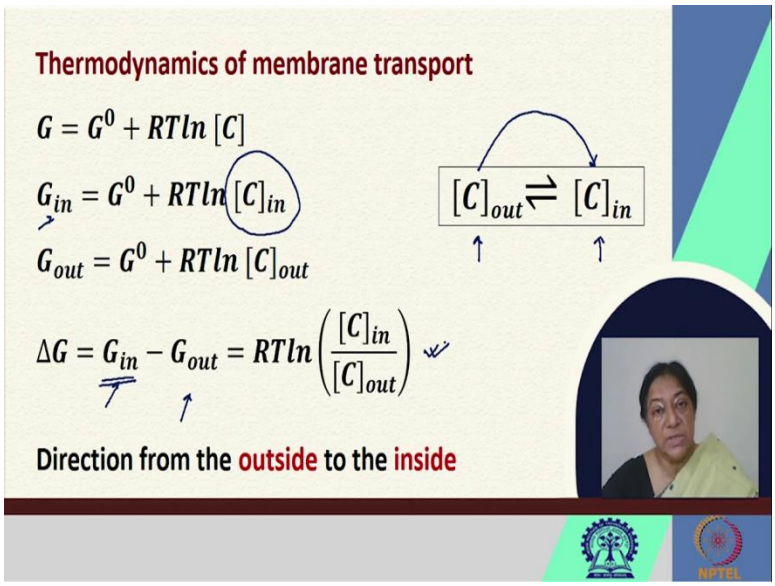

If we look at the transfer now, from a certain concentration outside the cell to a specific concentration inside the cell, we have the expression from the previous slide $G = G^0 + RT\ln [C]$. This means that when we are looking at the concentration inside the cell, we will have a corresponding G associated with the inside of the cell. Similarly, we will have one associated with the concentration of the ion outside the cell.

If we now want to look at the ΔG of this transport, we will have G_{in} minus G_{out} because we are going from outside to inside, which means the final process is the transport of the ion of a concentration C; C_{out} to C_{in}.

This gives our expression related to the ΔG associated with the transfer and we realize that if this transfer has to be spontaneous, then this value ΔG has to be negative. So the direction here is from outside to the inside.

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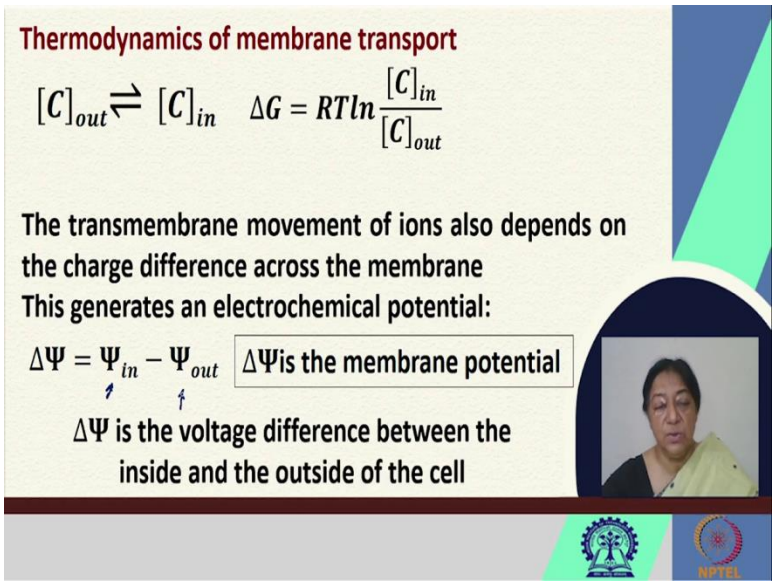

If we go further into the thermodynamics of this understanding, we have a ΔG that we found out is $RTln\ [C]_{in}/[C]_{out}$ and the transmembrane movement of ions will therefore, depend upon the charge across the membrane because we are transporting these ions across the membrane.

In addition to the chemical potential, we are going to have an electrochemical potential that is defined in a similar manner, where we have an electrochemical potential given by the Ψ_{in} - Ψ_{out}. We have a specific transfer of the ions, where $\Delta\Psi$ is the membrane potential. This is the voltage difference between the inside and the outside of the cell.

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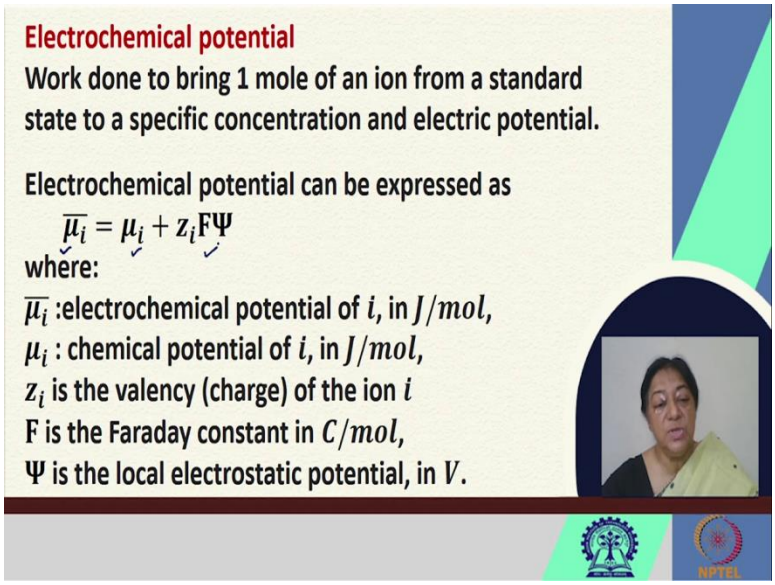

If we consider a similar argument for the electrochemical potential like we looked at for the chemical potential, then we have the work done to bring one mole of an ion from a standard state to a specific concentration and electrical potential. So, in this case we have an electrical potential

that will be defined as $\bar{\mu}_i = \mu_i + z_i F\Psi$, where we have the electrochemical potential of i, the chemical potential of i, and the valence the charge of the ion that is being transported, F the Faraday constant and Ψ the local electrostatic potential.

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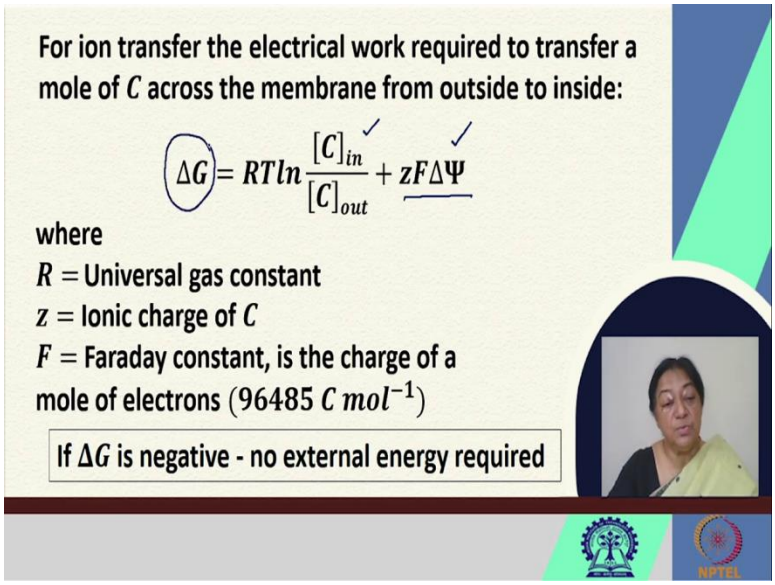

If we were to do a similar exercise for the electrochemical potential like we did for the chemical potential, the ion and the electrical work associated with this transfer would be given by $zF\Delta\Psi$. In addition to this what we have is, we have the chemical potential associated with the transfer and we have the electrochemical potential associated with this.

So we have [refer to slide] our RT, we have the ionic charge, we have the F and now we realize that if ΔG is negative, then the process would be a spontaneous process. So, we have a $\Delta\Psi$ associated with the electrochemical potential, we have a change in concentration of the inside and the outside of the cell and we realize that if this ΔG happens to be negative, then there is no external energy required for this specific transfer to occur.

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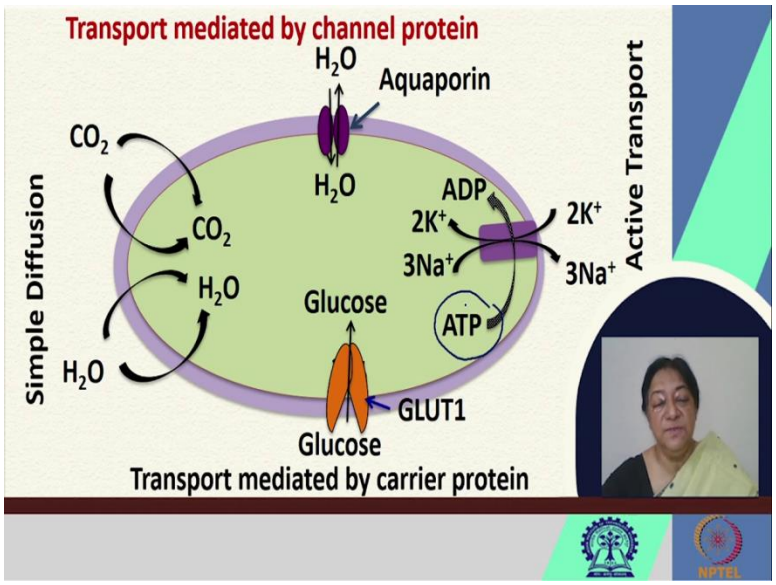

When we look at the different processes that occur, we have simple diffusions where we have the passive diffusion of some gases and water across the membrane. We can have specific transport that is mediated by a channel protein and we learned how the channel protein may be triggered to open for the transfer of material across the membrane.

We can have a specific carrier protein, that is going to be associated with conformational changes due to the transfer and active transport that we have, to require our ATP for the process to work. So, when we look at this active transport where we have the ATP associated with the transfer, there are several types that can actually occur where this process is called active transport.

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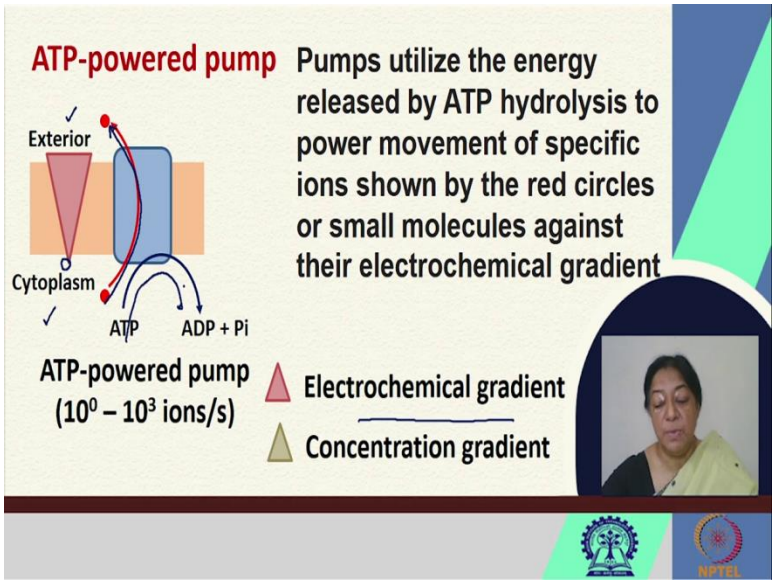

So, what we have is we have an ATP powered pump. In this case, we have the pumps that utilize the energy that is released by ATP hydrolysis and what this does, is this powers the movement of the ions across the membrane. Now, this transport could be with the electrochemical gradient or against the electrochemical gradient by the ATP hydrolysis.

This [refer to slide] is the exterior, this is the cytoplasmic part and the narrower end of the arrow indicates a lower electrochemical potential. So, when we have a transport of the ion from a lower electrochemical potential to a higher electrochemical potential, we realize that this process is not favored. What happens is we have the ATP hydrolysis that assists this pump in the transport of the ions, shown by the red circle here, across the membrane.

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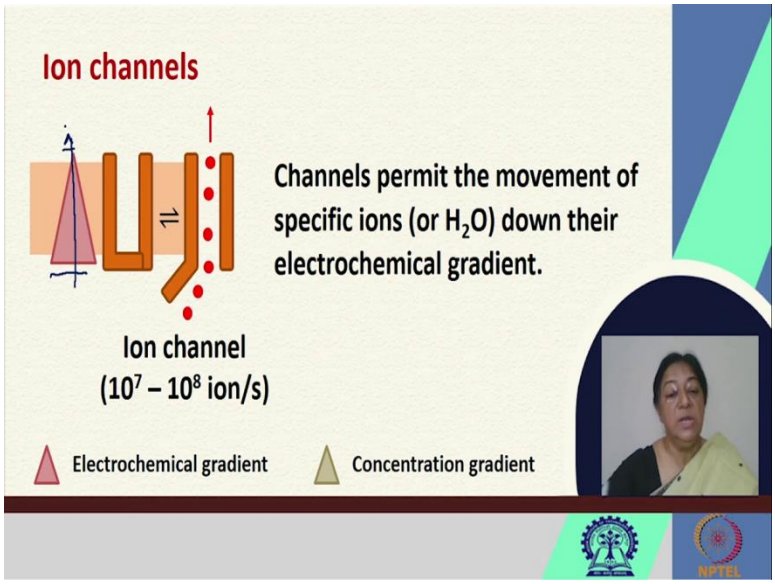

Similarly we can have these ion channels. The channels would permit the movement of specific ions or water, down their electrochemical gradient. So here we would have a diffusion, where we are going from a higher electrochemical gradient to a smaller electrochemical potential.

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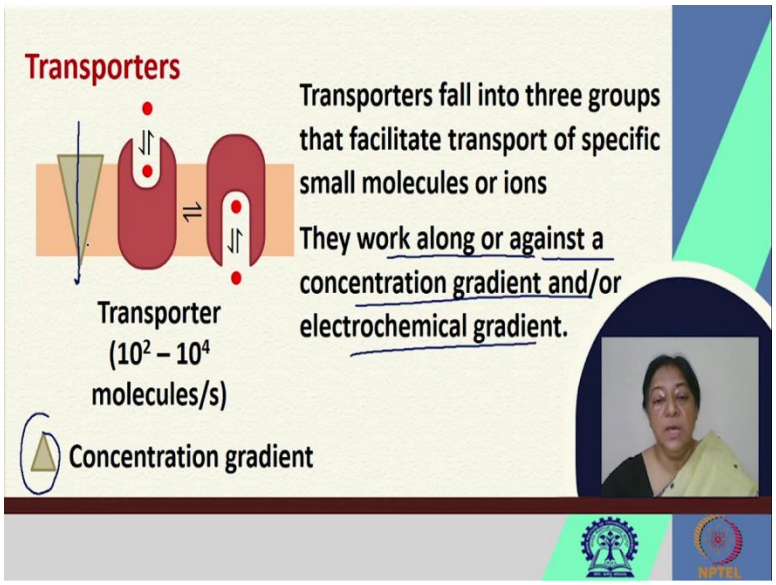

When we have the transporters, they have 3 groups of transporters that facilitate the transport of specific small molecules or ions and they can work along or against a concentration gradient and/or electrochemical gradient. So, when we are looking at the specific concentration gradient and we expect it to go in this [refer to slide] direction, if it were a diffusion across the membrane that would be facilitated.

However, it is also possible to have these work in a different fashion, where we would have ATP triggered movement that will make it transport against the specific potential; whether it is the concentration potential or the electrochemical potential.

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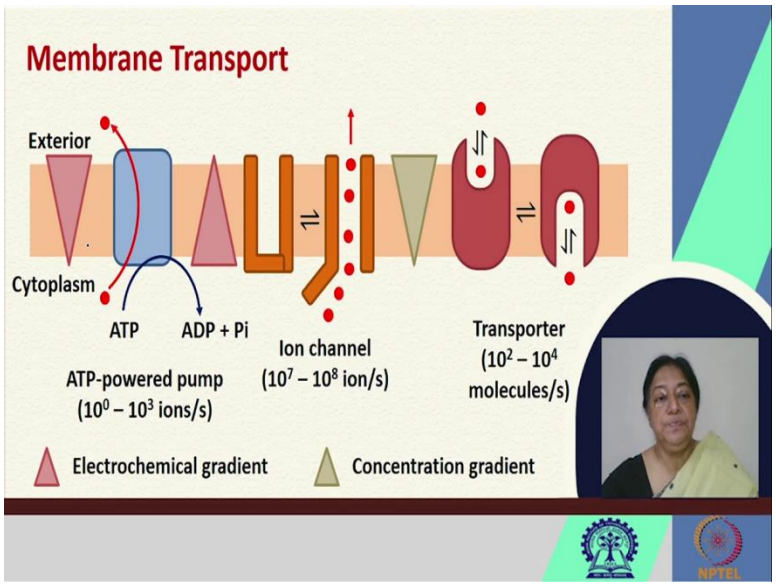

So the membrane transport in general, would have an ATP powered pump that would allow the process to occur against a concentration gradient or against an electrochemical gradient. Similarly, there would be an ion channel or the regular transporter of ions.

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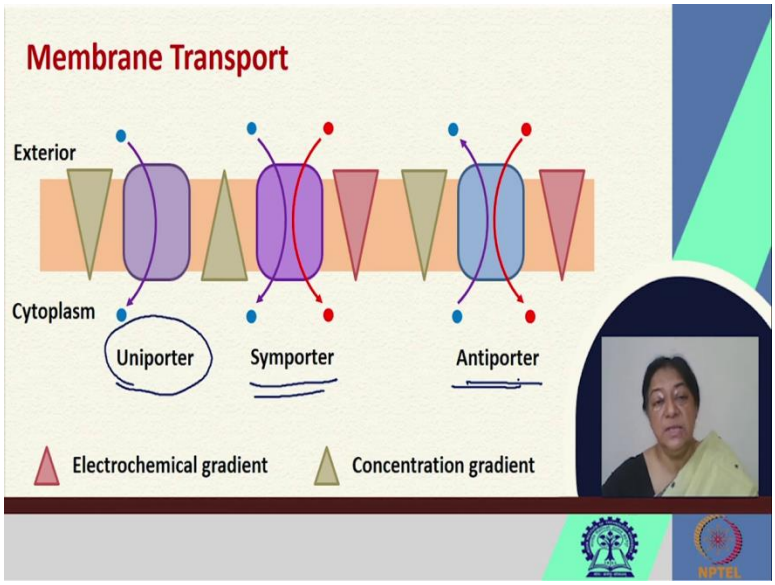

When we look at these processes now, we can have in the transport types, we can have a uniport, we can have a symport or we can have an antiport. And depending on whether they work towards the favorable potential or against a potential, we would have them assisted by the ATP hydrolysis.

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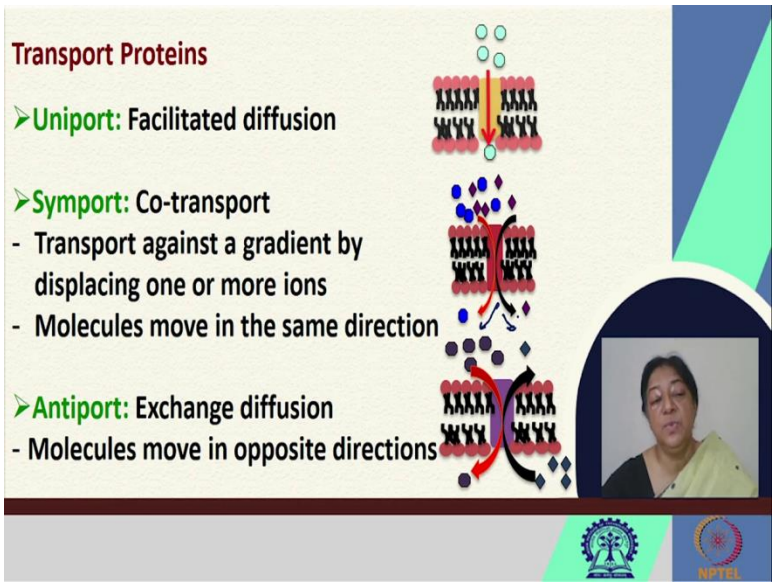

So when we look at these transport proteins, we have the uniport that is facilitated diffusion, that allows the diffusion to occur in the specific direction. In symport, the core transport is against a gradient, that occurs by displacement of one or more ions and the molecules move in the same direction and this could be against a concentration gradient or a potential gradient, where ATP hydrolysis would be used to assist this transfer.

The antiport allows the exchange diffusion, where the molecules move in opposite directions. A specific set of ions or molecules come into the cell and a set that goes out of the cell. So, these are the different types of transport processes that could occur with a uniport, a symport or an antiport.

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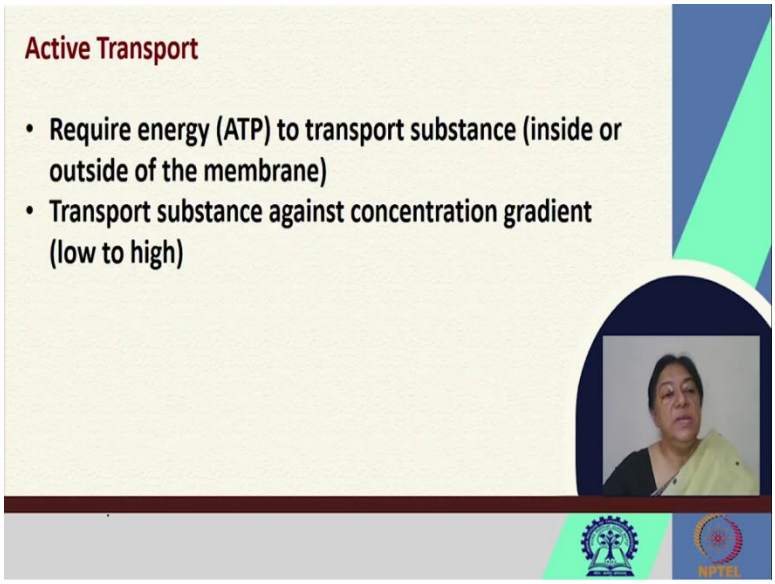

In the active transport that we are looking at, we realize that there is ATP required to transport the substance inside or outside of the membrane and this transport occurs against a concentration gradient where we are going from a lower concentration to a higher concentration.

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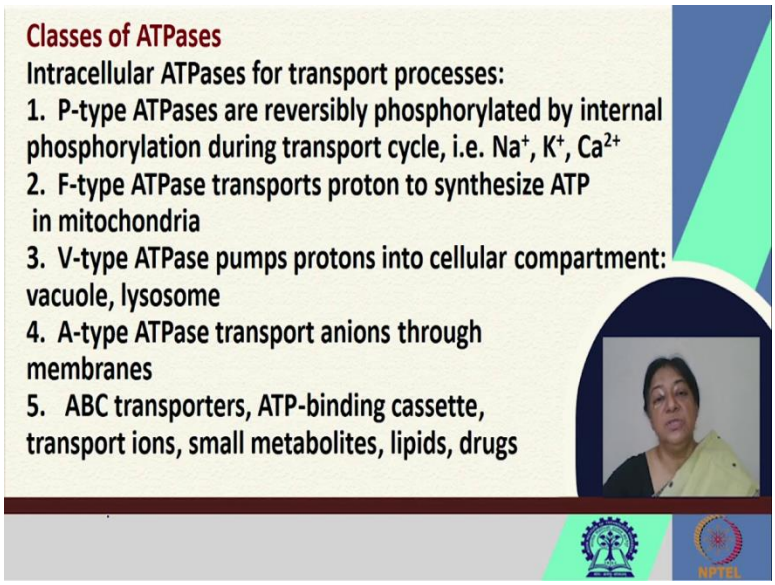

There are different types of ATPase associated with the transport processes, depending upon the types of ions they transfer. For example, a P-type ATPase is reversibly phosphorylated by internal phosphorylation during the transport cycle of say potassium, sodium or calcium. The F-type, they transport protons in mitochondria.

The V-type ATPase pumps protons into the cellular compartment. Then we have the A-type that transports anions and the ABC transporter types that are ATP binding cassettes as they are called, that transport ions and small molecules, lipids and drugs across the membranes.

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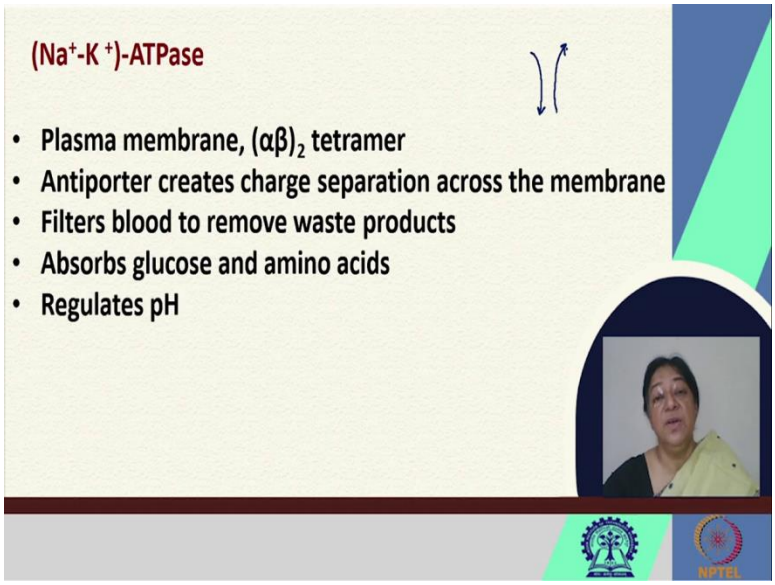

If we look at the sodium-potassium ATPase, we see that it is a plasma membrane, an $(\alpha\beta)_2$ forming a tetrameric protein. It is an antiporter, meaning that the transfer is in one direction for

some ions and in the opposite direction for the other ions and this creates a charge separation across the membrane.

So, there is an electrochemical potential associated with this transfer and this is a very important process because it involves the filtering of blood to remove the waste products. It absorbs glucose and amino acids, regulates pH and this [refer to slide] is what the structure looks like.

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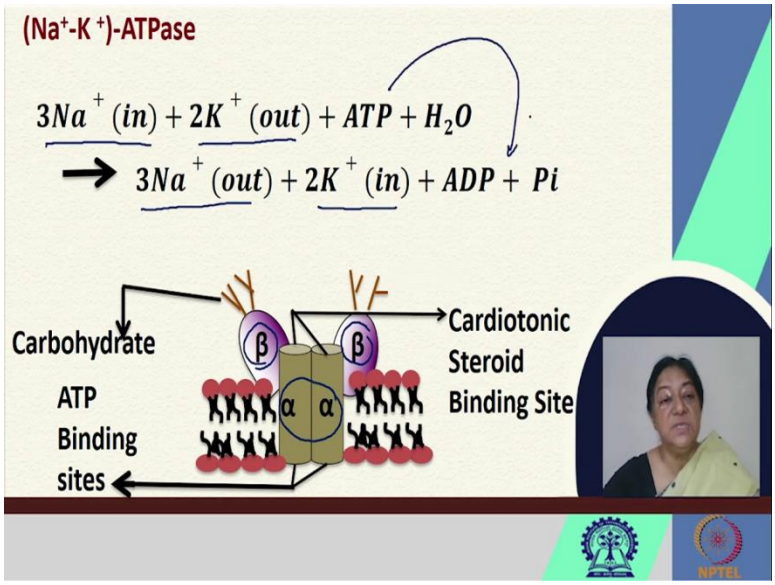

We have the two α units and the two β units. We have carbohydrates attached to these units and specific ATP binding sites for the processes to occur. This is the overall reaction: $3Na^+$ (in) + $2K^+$ (out) + ATP + H_2O → $3Na^+$ (out) + $2K^+$ (in) + ADP + Pi

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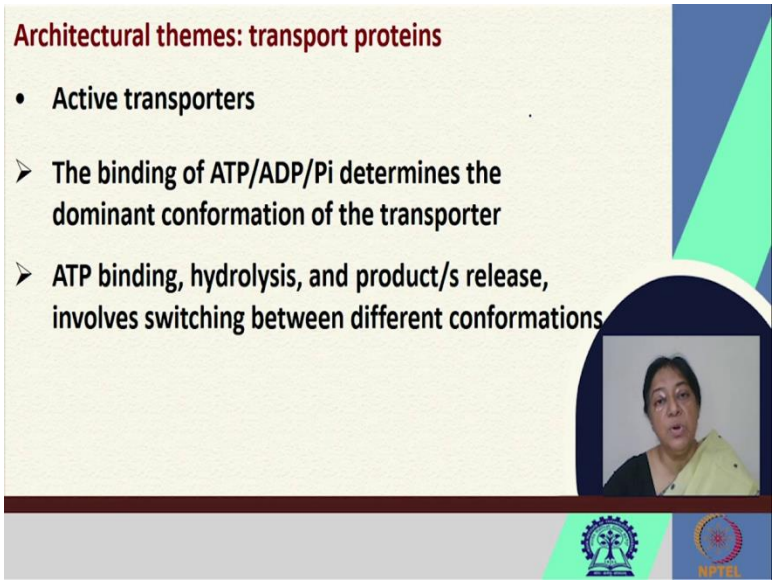

So when we look at these active transporters in general, we have the binding of the ATP, the ADP or the Pi that determines the dominant confirmation of the transporter, depending upon how or when it is going to transport the ions. So the ATP binding process, the hydrolysis process and the product release, involves a switching between the different conformations which is extremely important in the transport process.

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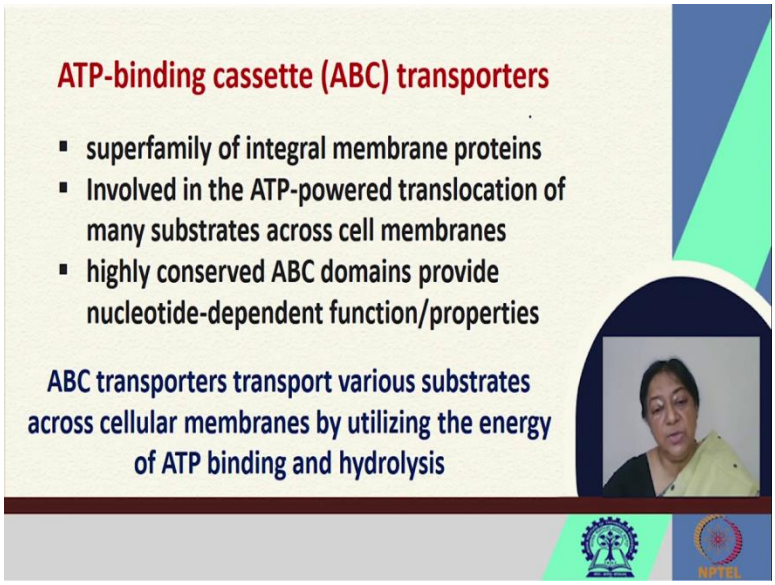

The specific types of proteins known as ATP binding cassette transporters, are a super family of integral membrane proteins that are involved in the ATP-powered translocation of many substances across the cell membranes and there are specific ABC domains that provide nucleotide dependent functional properties, where the binding of the ATP will result in a conformational change that is going to facilitate the transfer of the specific substrate, specific ion across the membrane.

So, the ABC transporters transport various substrates across the cellular membranes and they do it by the hydrolysis of ATP; once ATP is bound to the conserved ABC domain.

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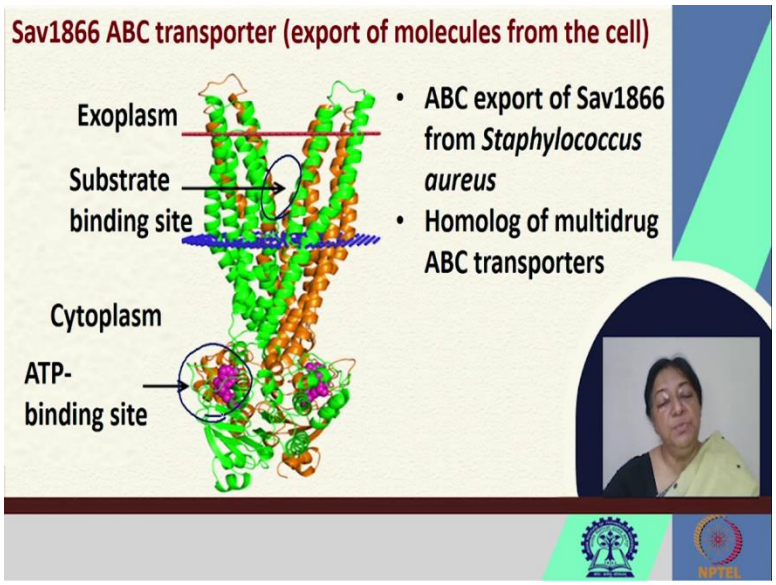

For example, if we look at this [refer to slide] specific ABC transporter, we see there is a substrate binding site and in addition there is an ATP binding site. The binding of the ATP to the specific site will open up or will create a conformational change in the protein, that will assist in the substrate binding. This is a specific type of ABC transporter, just to give you an example of how the process occurs.

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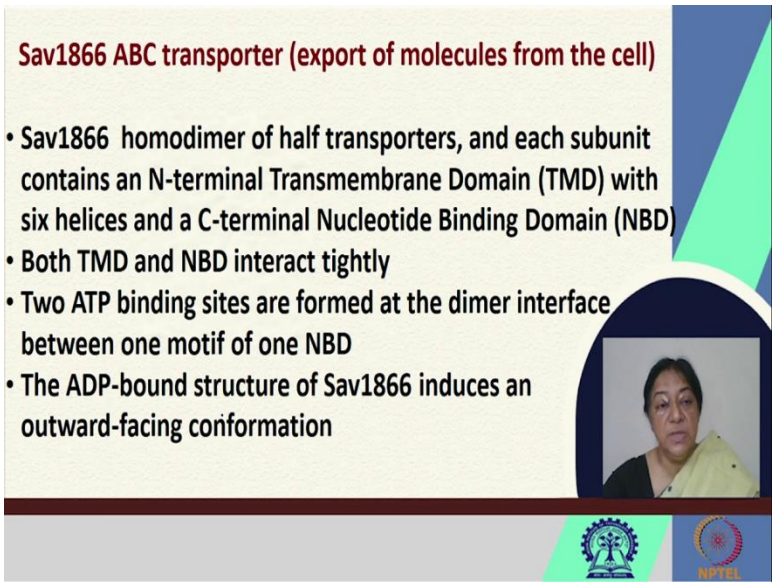

This specific protein is a homodimer of half transporters and there are two different types of domains here. One is the N-terminal transmembrane domain that has 6 helices and there is a C-terminal nucleotide binding domain, that is involved in the ATP binding.

They interact very tightly and there are two ATP binding sites that are formed at the dimer interface between one motif of one NBD. And then what happens is, there is an outward-facing conformation that allows the substrate to bind.

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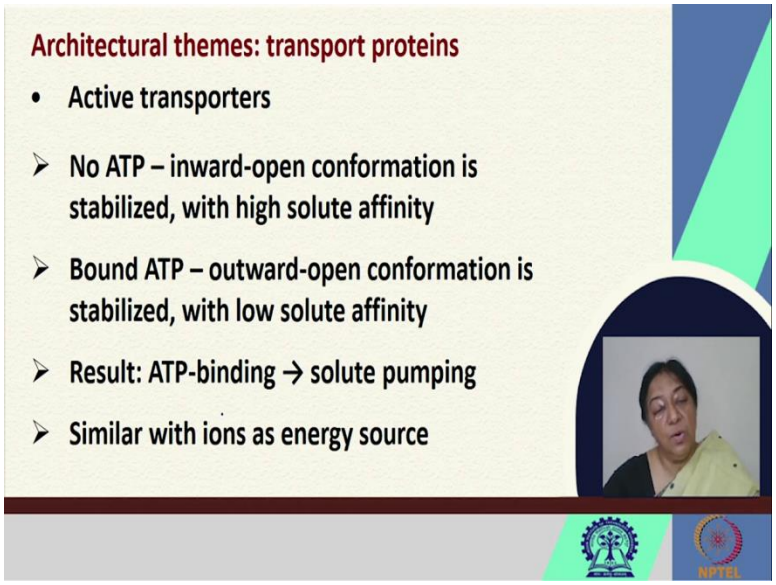

So when we look at these transport proteins, we understand that they are very active transporters and if there is no ATP, it has an inward open conformation this is stabilized with high solute affinity. However, when there is bound ATP, there is an outward open conformation that is stabilized with low solute affinity and this outward open conformation will then bind the substrate or the ion that has to be transferred within the cell. So we have ATP binding, we have solute pumping and this can be similar with several different ions as also as the energy source.

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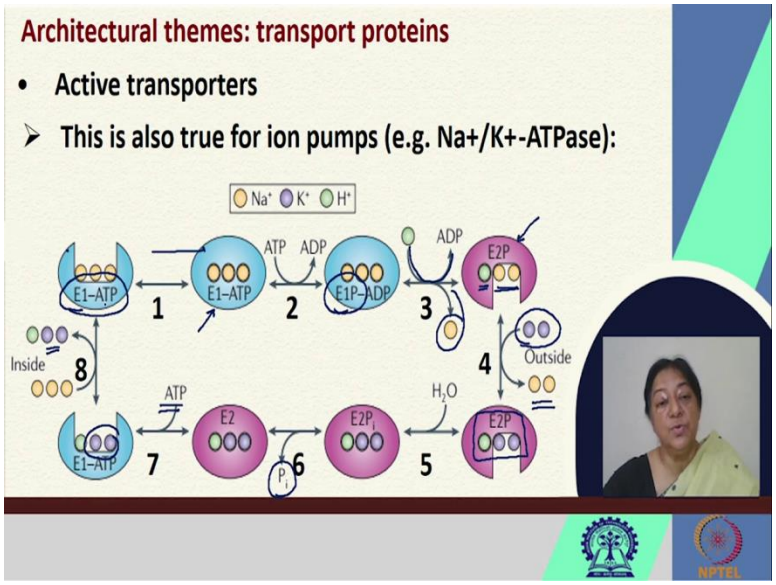

If we look at an example of the ion pumps where we look at sodium and potassium in a very brief schematic, we see the specific E1 ATP type. Once ATP is bound, we see there is a conformational change. We have now a closed structure to our complex. Then when we have the hydrolysis of ATP to ADP, then this is phosphorylated.

Next we have a proton come into the picture [refer to slide], where we have the loss of ADP and after the loss of a sodium ion we have two sodium ions attached with the proton. But now we see a conformational change to the E2 P-type. Now what happens is from the outside we have 2 potassium come into the picture, where 2 sodium ions are left out and we have this bound form as hydrolysis now occurs.

Then we have the loss of the Pi. We have an ATP assisted set, where we have a conformational change in step 7, resulting in the bound form of the two potassium ions now leaving the cell. So in a sense what has happened is, we have the transport of the sodium and the potassium ions like in the expression that we saw previously.

Now in this process what happens is, the ion pump we see works as an active transporter with the assistance of ATP, that allows this transfer or the transport to occur. So we have the transport of the sodium and the potassium ions.

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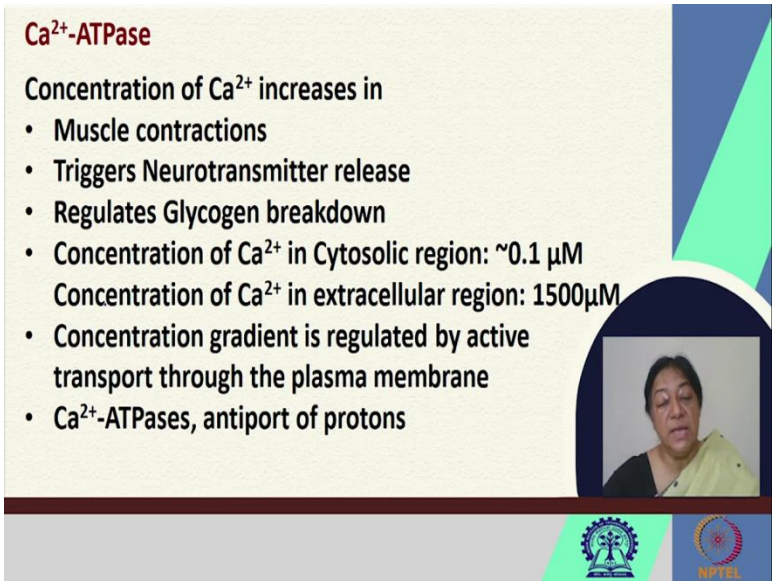

Similarly, when we have a Ca^{2+} ATPase this also works in a similar fashion and is important for muscle contractions, triggers neurotransmitter release and regulates glycogen breakdown; and the concentrations of calcium in the cytosolic and extracellular regions are different and there is a concentration gradient that is regulated by the active transport across the plasma membrane. This is an antiport of protons with the calcium ion.

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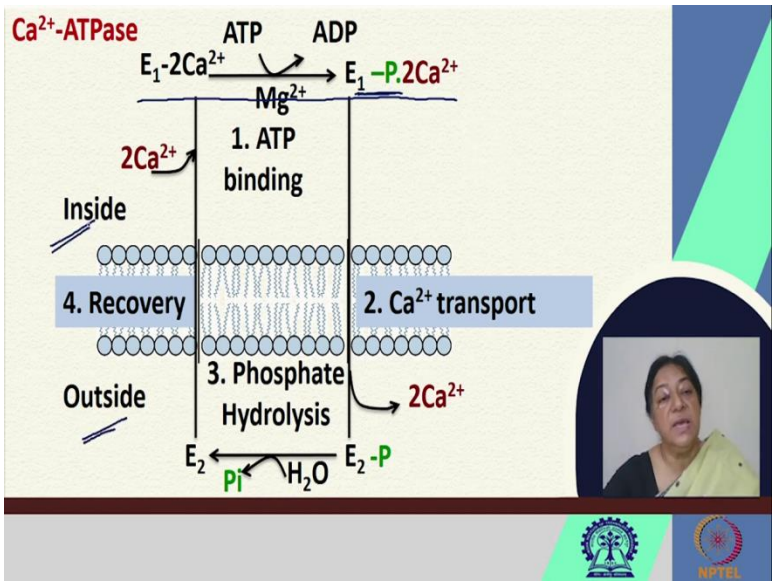

So, we have a similar set up here where we have ATP binding. This ATP binding as we can see creates a variation in the phosphorylation of the structure and when the ATP is bound, the main idea that we have to get from this is that the ATP bound conformation allows a conformational change in the protein, that allows the substrate to bind. There is calcium transport, then there is phosphate hydrolysis, followed by the recovery.

Thus we have the transport of the calcium from the inside to the outside of the cell.

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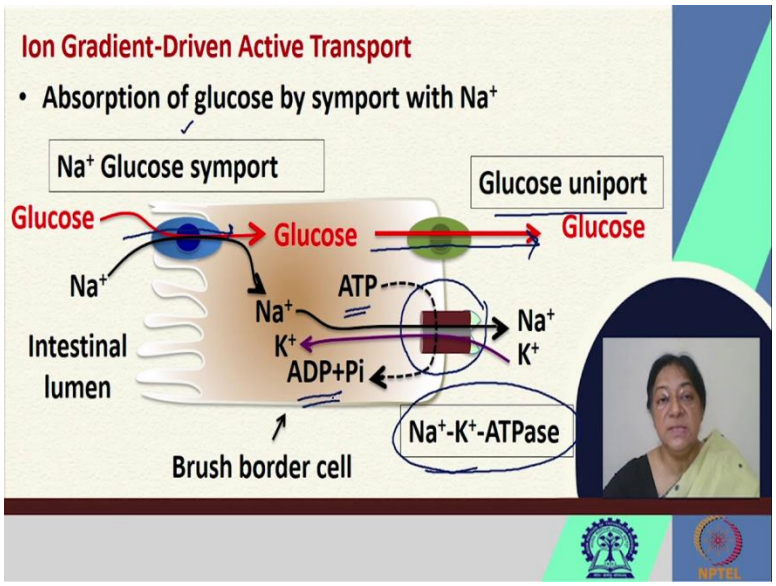

So what happens is, the transport occurs again on ATP binding. If we look at an overall system where we can picture an ion gradient driven active transport, we have an example where we have the sodium glucose symport, where we have the transport of the ion, the transport of the molecule across.

Now, a glucose uniport means that it allows the transport of glucose across this. A facilitated diffusion may then occur with the use of Na^+-K^+ ATPase, that provides the potassium required inside the cell and this pump we realize, is triggered by the ATP hydrolysis to ADP and Pi. The conformational changes that occur in the protein due to the binding of ATP, is important in bringing about the active transport of the ions against a specific concentration gradient. So we have the absorption of glucose by a symport method with Na^+.

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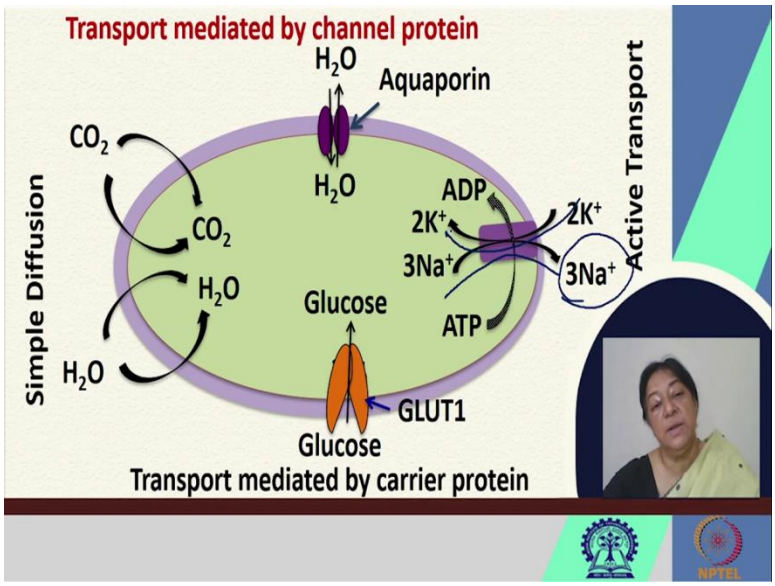

If we look at our overall picture again, we have simple diffusion of the ions, we have aquaporin, we have the transport mediated by a carrier protein, we have an active transport, where we have 3 sodium from inside the cell come outside and 2 potassium go inside of the cell. This occurs with the ATP hydrolysis that brings about the active transport.

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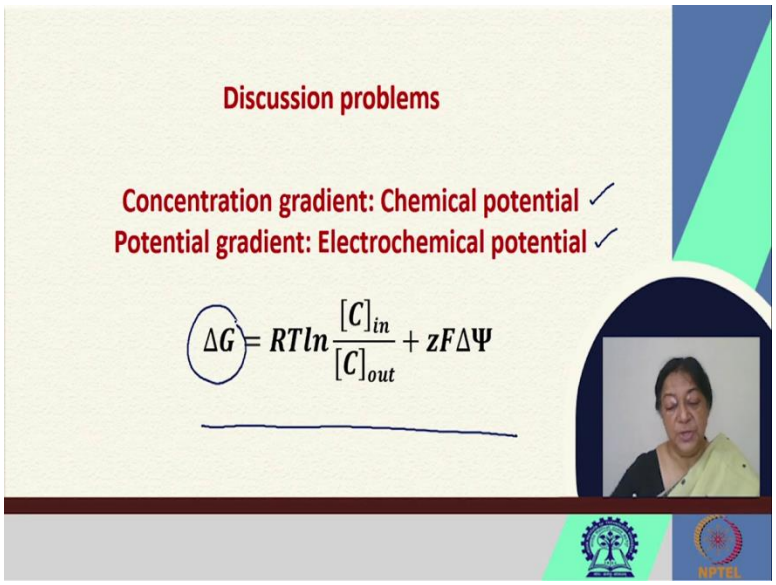

When we looked at our specific idea of how we can address these problems, we realize that we have a concentration gradient that is given by a chemical potential, we have a potential gradient given by an electrochemical potential and the expression is: $\Delta G = RT \ln [C]_{in}/[C]_{out} + zF\Delta\Psi$

We realize that for the diffusion to occur, we have to have a favorable ΔG. This occurs in the following fashion.

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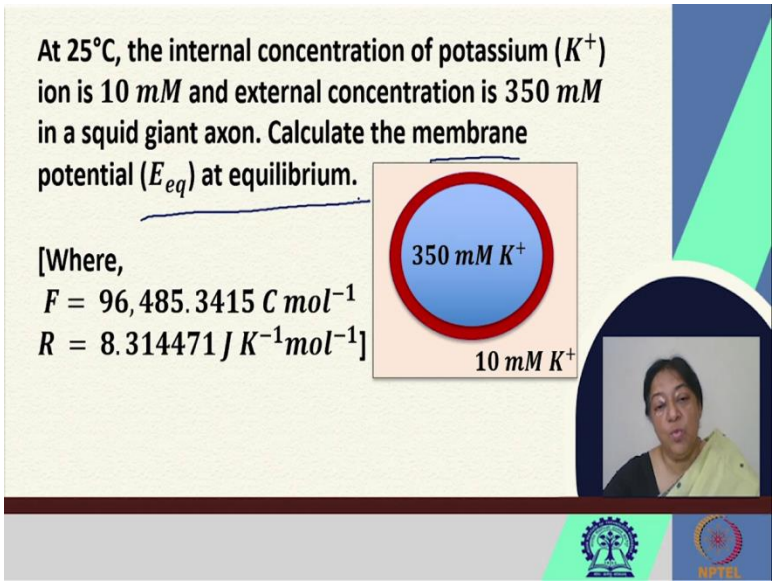

So, if you look at the specific problem that says at 25°C there is an internal concentration of potassium given by 10 mM, and the external concentration is 350 mM in a squid giant axon. Now, the membrane potential has to be calculated at equilibrium.

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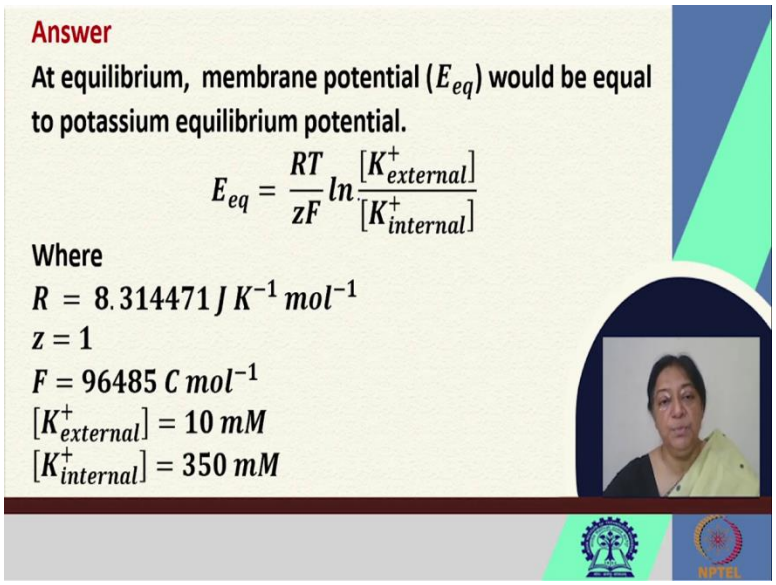

At equilibrium, we realize that membrane potential would be equal to the potassium equilibrium potential. So, we have the overall ΔG that would be 0. We can equate these two specific processes where we have our concentration of the external ion of potassium, the concentration of the internal ions, where we have the value of R, we have z = 1 because we have potassium, we have the Faraday constant the [K^+external] and the [K^+internal], are both values that have been provided in the problem.

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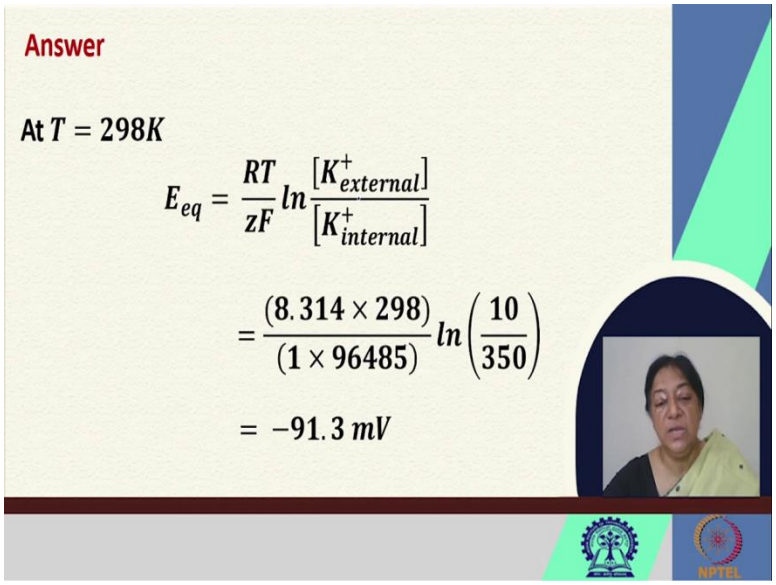

If we were to solve this problem, we have T at 298K and we find out what the E_{eq} is by plugging in the specific values and we get a value of -91.3 mV. So that is the answer to that problem.

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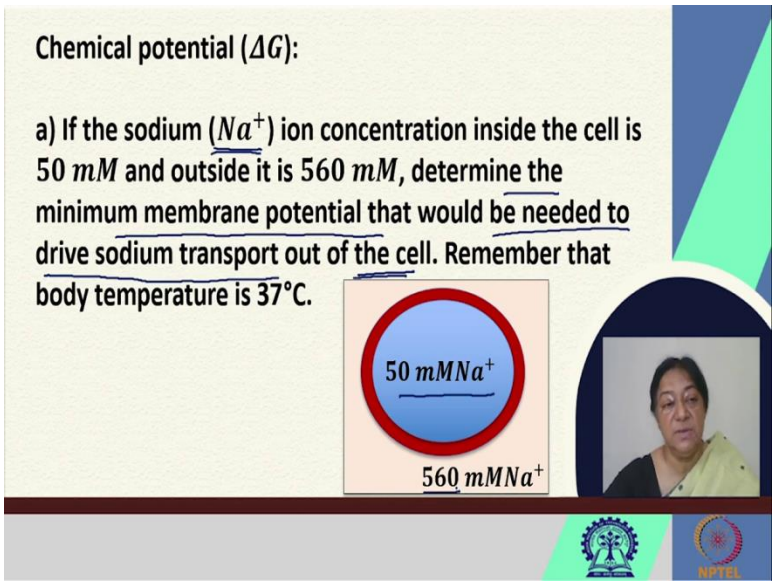

When we look at the chemical potential ΔG, if we now have another situation where the sodium ion concentration inside the cell is 50 mM and outside it is 560 mM, the question is that we have to determine the minimum membrane potential that would be needed to drive sodium transport out of the cell. So, we have a body temperature of 37ºC, which we have to use for our expression in this case.

Inside the cell we have 50 mM sodium ions and outside we have 560 mM sodium ions.

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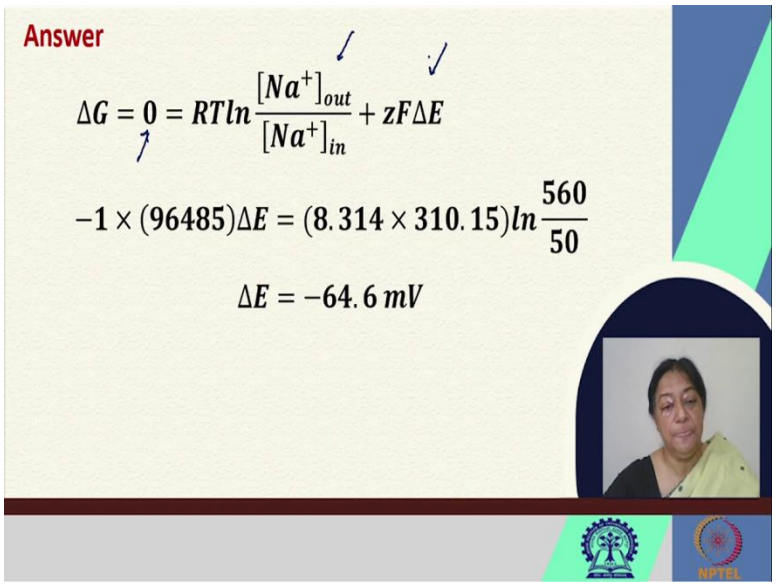

So again we have a ΔG = 0; we consider this to be 0 because we have an equilibrium situation. We have our chemical potential and we have our electrochemical potential. Together we find out from this what the ΔE value is going to be.

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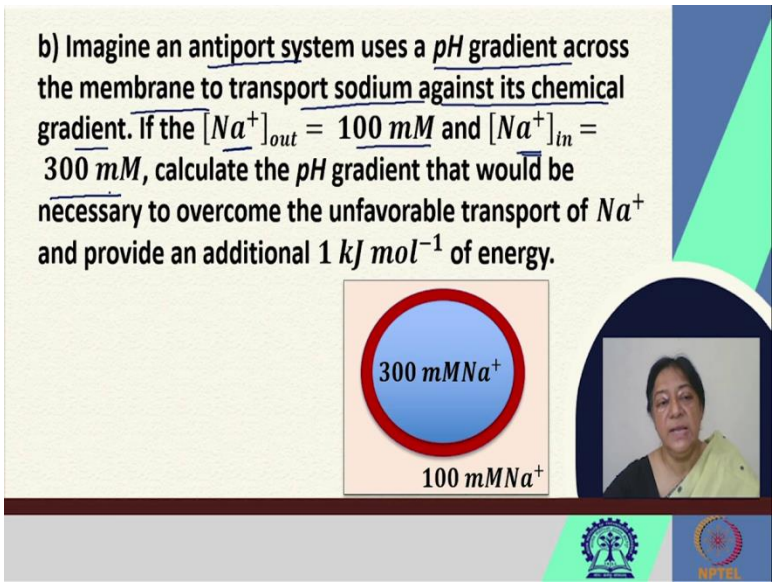

If we now look at an antiport system, which uses a pH gradient across the membrane to transport the sodium against its chemical gradient. In this case, we are looking at a $[Na^+]_{out}$ of 100 mM and a $[Na^+]_{in}$ of 300 mM. We have to calculate the pH gradient that would be necessary to overcome the unfavorable transport of sodium ions and in addition provide 1kJ mol^{-1} of energy. We have this situation here, where we have the concentration outside as 100mM and inside as 300mM.

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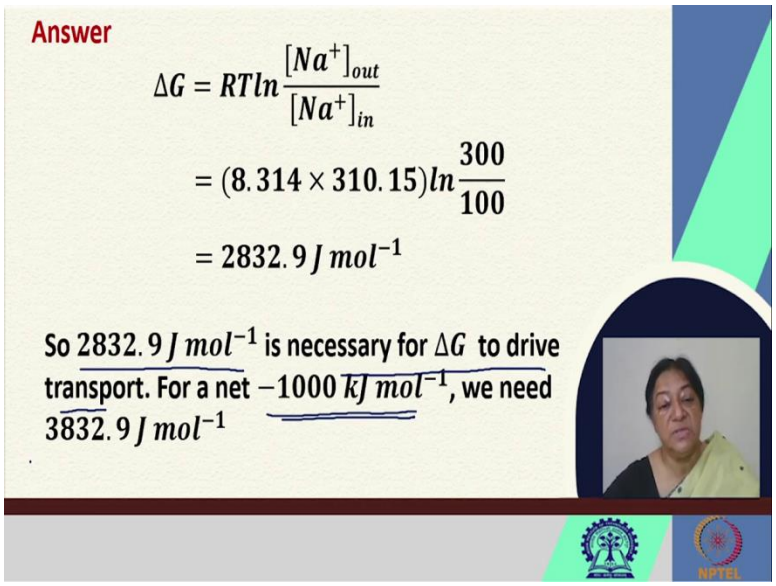

So, in this case we are looking at a $[Na^+]_{out}$/ $[Na^+]_{in}$ concentration ratio, where we get the ΔG associated with this. Now this means that we have to give 2832.9 J mol^{-1} amount of energy for the transport to occur and if we want an additional -1000 kJ mol^{-1} of energy, then we have to give a supply of close to 4000 J mol^{-1}, for the specific transport to occur.

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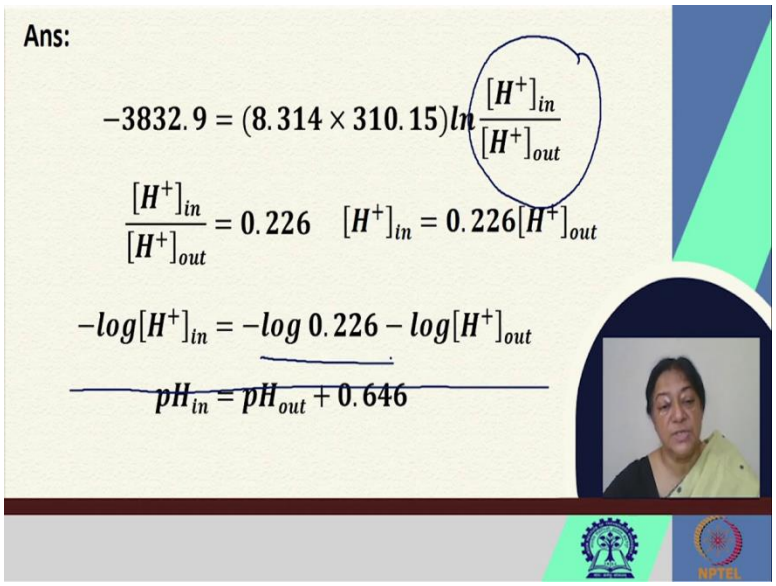

This [refer to slide] is our value and we want to know what pH has to be done. We have a proton transfer associated with this process. The problem that we were looking at is, we have an antiport here and we want to find out how these two can be connected.

In this case, we have a concentration ratio of $[H^+]_{in}$ to $[H^+]_{out}$ is 0.226, which would give us a concentration ratio of $[H^+]_{in}$ to $[H^+]_{out}$ and based on this, we can actually determine what the pH is; a $pH_{in} = pH_{out} + 0.646$, that is the logarithm of 0.226, which is -0.646.

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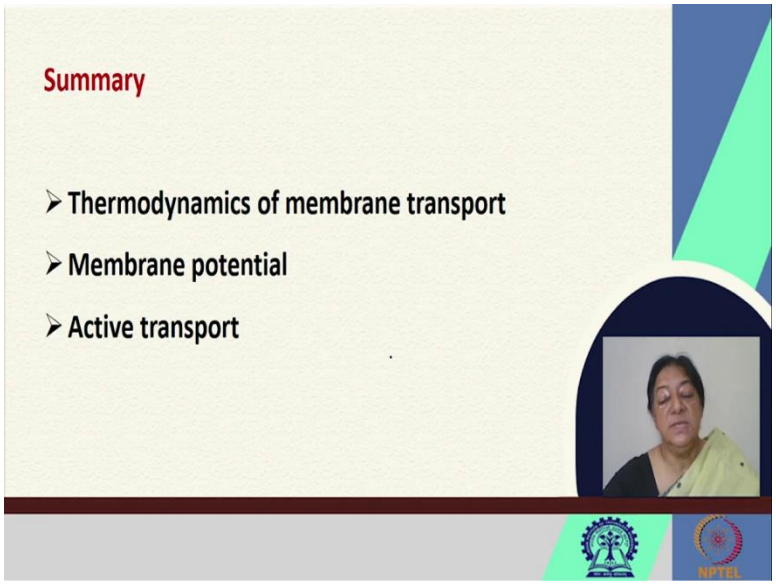

What we have learnt in this lecture is the thermodynamics of membrane transport. At the end we looked at some specific problems associated with this transfer, where we can determine based on

an equilibrium situation, the electrochemical potential, the chemical potential and the concentration of the ions that are going inside and outside the cell.

This is an important aspect of trying to understand how membrane transport occurs. We looked at active transport, where we realized that we need the energy from the ATP hydrolysis, to bring about the transport of ions across the membrane and this can be brought about by specific sites on these membrane proteins, that would be able to bind the ATP and on binding, there would be conformational changes associated that would create an inward to outward conformation, that would allow the substrate to bind, followed by the transport across the membrane.

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These [refer to slide] are the references.

Thank you.