**Medicinal Chemistry**
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**Module 02**
**Lecture 09**
**Tutorial-03**
**Binding Forces, Protein Structure and Function**

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So welcome to the tutorials session.

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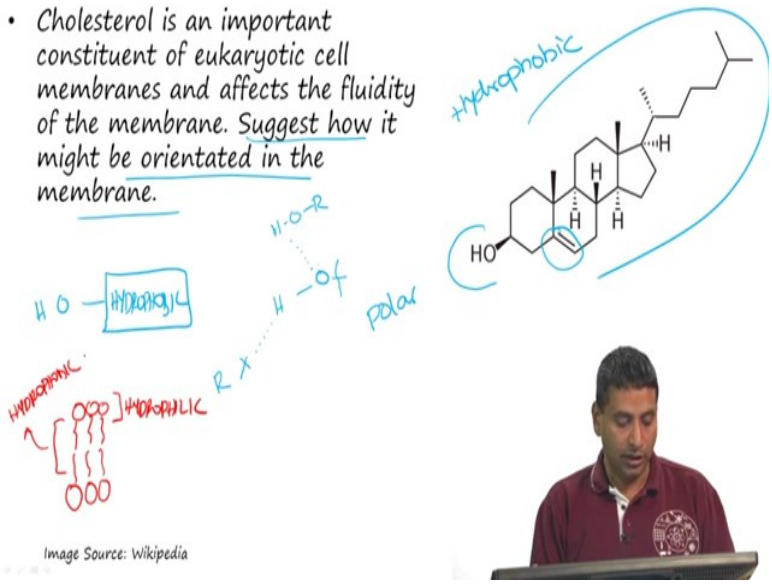

So the first question is about cholesterol. Cholesterol is an important constituent of eukaryotic cell membranes and affects the fluidity of the membrane. So suggest how it might be oriented in the membrane. So the question is how cholesterol is going to be oriented in the cell membrane? So in order to address this question (we) let us first look at the molecule, so if you look at cholesterol you can see here that there is a polar hydroxyl group because of the electronegative atom oxygen and this polar hydroxyl group can also involve itself in hydrogen bonding so you can think about electronegative atom being involved in hydrogen bonding, also the oxygen of this can also be involved in hydrogen bonding. So these are all possible interactions with this hydroxyl group.

If you look at the rest of the molecule, the rest of the molecule is essentially devoid of any polar groups which mean that it would be quite hydrophobic. So if you look at what this end of the molecule would interact with this end of the molecule would probably interact very well with hydrophobic molecules and clearly it will repel groups which are highly polar, so even though there is perhaps a little bit of possibility of some pi stacking with this olefin. I think one can imagine that it is going to be well buried in the molecule that it may not be very important.

So if I have to break down this molecule I would break this down into a large rectangle which is hydrophobic in nature and a small hydroxyl group which is going to be polar. So let us keep this in mind when we are looking at how it is going to interact with the lipid membrane. The membrane as we know consist of a polar head group and a non-polar or hydrophobic tail. So you can approximate the lipid membrane to be something like this.

So if cholesterol were to interact with this group what we could predict is that the hydrophobic regions are going to come closer to one another and the hydrophilic groups are going to be coming close to one another. So here let’s mark out the hydrophobic and hydrophilic regions, so this is the hydrophilic region and the rest of the molecule over here is actually hydrophobic.

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So with this in mind if we were predict how the lipid membrane would interact with cholesterol then one could draw out a figure like this. So you have the lipid membrane over here and as we looked at earlier cholesterol is basically the structure like this and so it would interact in the following manner, perhaps there is another molecule of cholesterol over here with the OH pointing out here. So what this would do is that the hydrophilic interactions would be favoured over here and the hydrophobic interactions would be favourable over here, okay so that takes care of this question.

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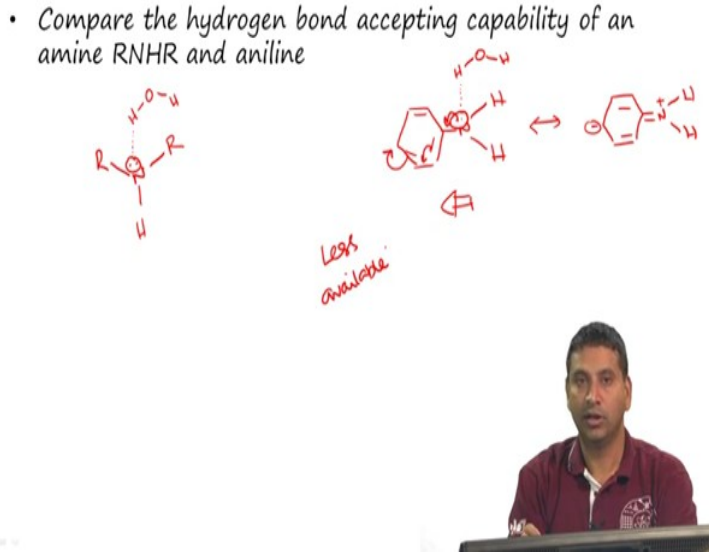


So the next question is compare the hydrogen bond accepting capability of an amine RNHR and aniline. Let us first draw the structure of the amine, so here is the structure of the amine,

next let's draw the structure of aniline this is the structure of aniline and here is the lone pair that we are interested in and the question is about how well it accepts a hydrogen bond. So now therefore this lone pair becomes important for us to understand and this is the lone pair that is going to interact with a hydrogen bond let's say with water, okay.

So if I have to compare these two then there are certain characteristics that we need to understand over here. The first thing is that in aniline being since the nitrogen is attached to the aromatic ring it is quite possible that it is going to interact with the aromatic ring. So the way this might happen is that you may have some sort of a resonance form that one could draw, where you have a negative charge on inside the aromatic ring, okay. So you have a positive charge over here and the negative charge over here.

So because of this what might happen is that you will have a pull (of this electron pair) of this lone pair on nitrogen into the benzene ring, this perhaps makes the lone pair less available for hydrogen bond. So therefore based on this one would argue that aniline would have a less hydrogen bond accepting capability compared to RNHR.

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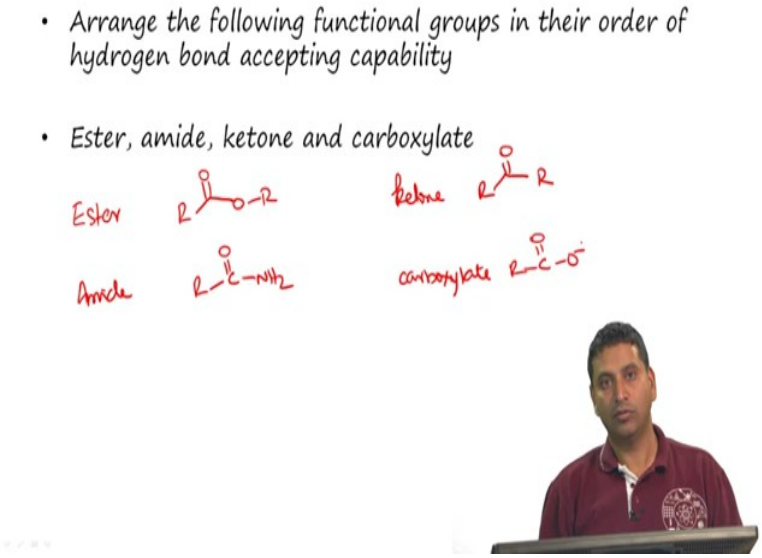


Next question is arrange the following functional groups in their order of hydrogen bond accepting capability which is ester, amide, ketone and carboxylate. So first let's draw the structures of these ester is R C double bond O O R, amide is R C double bond O $NH_2$, ketone is R C double bond O R and carboxylate is R C double bond O O minus, okay. So based on this we can now arrange it in the order of hydrogen bond accepting capability.

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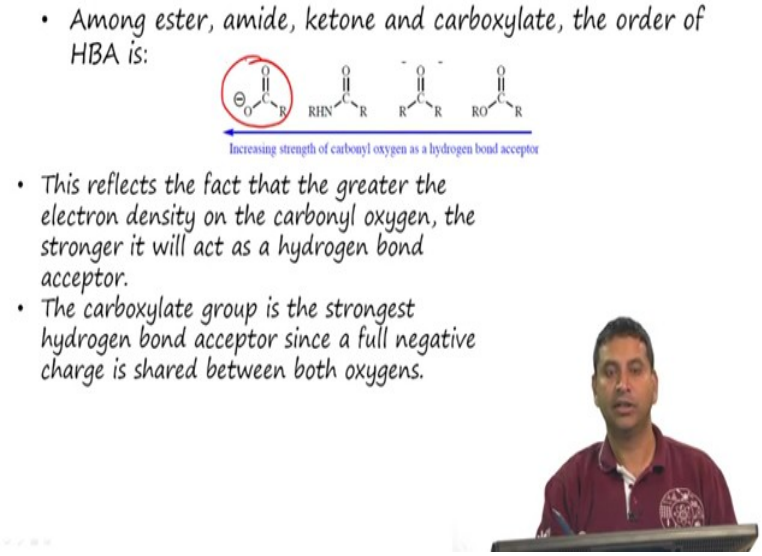


So the order is that since carboxylate has a full negative charge on it, it is more capable of accepting a hydrogen bond when compared with a ketone or an acetone, okay so these are easy to compare, right. So now let us come to the amide shortly but the fact that the greater the electron density on the carbonyl oxygen the stronger it will act as a hydrogen bond acceptor. So because of this the carboxylate is clearly the best hydrogen bond acceptor in the series.

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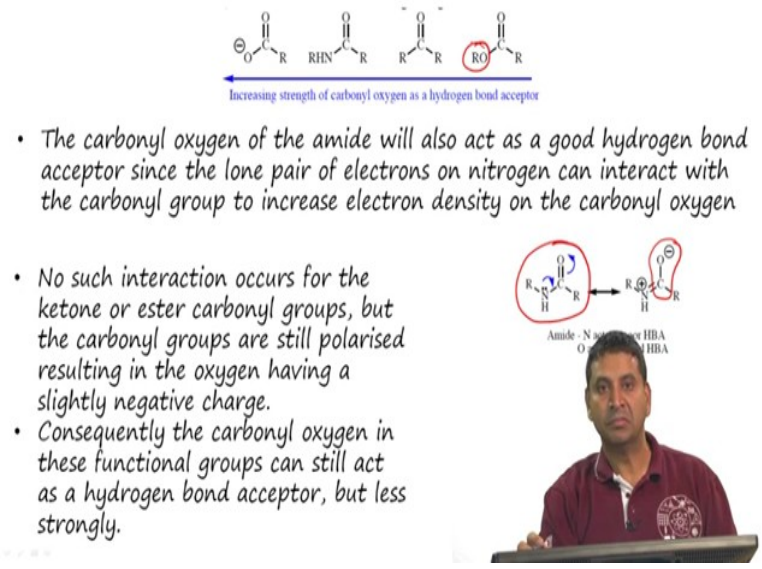


Now if you think about the amide, the amide is very interesting because the carbonyl oxygen of the amide will act as a good hydrogen bond acceptor, since the lone pair of electrons or the nitrogen can interact with the carbonyl an increase electron density of the carbonyl oxygen.

So what ends up happening in the amide is a situation like this where this lone pair is going to go inside and get delocalized and actually it is going to form a CO minus with this CO minus is going to have a slight negative charge on the oxygen.

So consequently, the carbonyl oxygen in this functional group is a much better electron hydrogen bond acceptor compared to a ketone and an ester. So in the case of a ketone and ester the electronegative O R is going to pull electrons from the carbonyl and therefore makes the carbonyl a poorer hydrogen bond acceptor.

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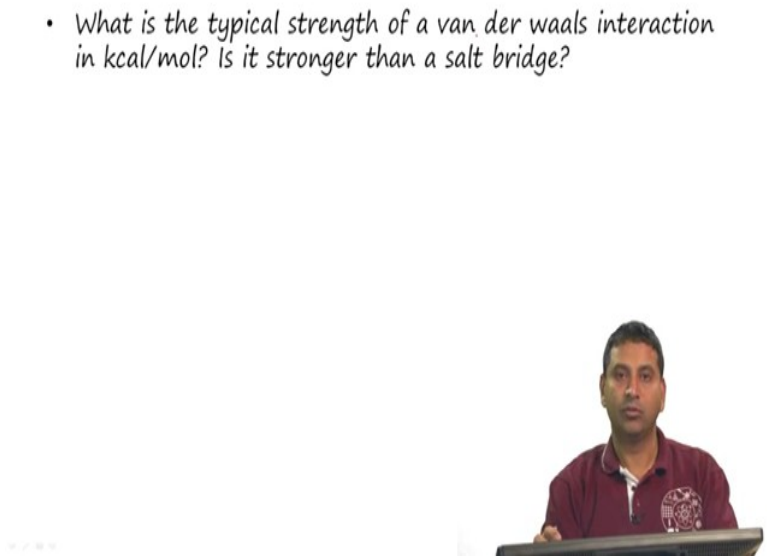


The next question is what is the typical strength of a vander waals interaction in kilocalories per mole? Is it stronger than a salt bridge?

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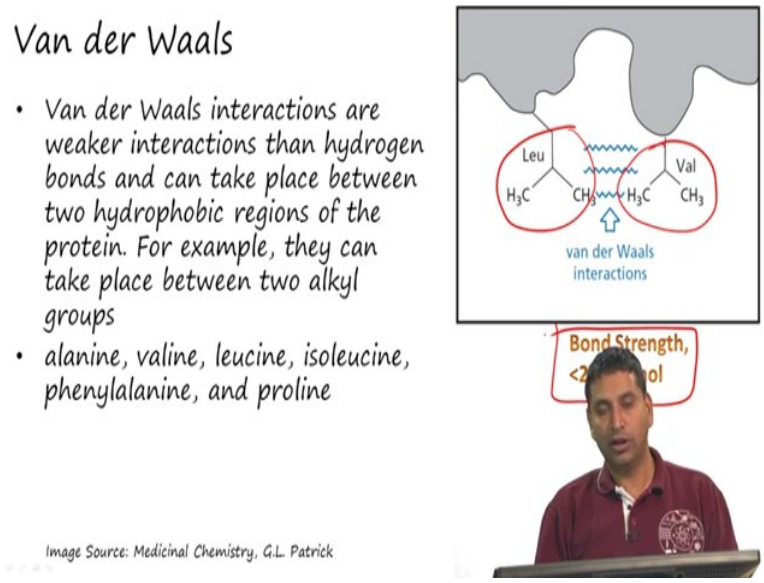


So in order to answer this question let us recap a little bit about what vander waals interactions are? So vander waals interactions are nothing but very weak interactions which are weaker than hydrogen bonds that can take place between two hydrophobic regions of a protein. So here the example that we looked at is a leucine residue interacting with a valine residue and so this type of interactions where you have two hydrophobic groups coming close to each other is called as a (vander waals reactions) vander waals interactions. The bond strength of vander waals interaction is typically less than 2 kilocalories per mole.

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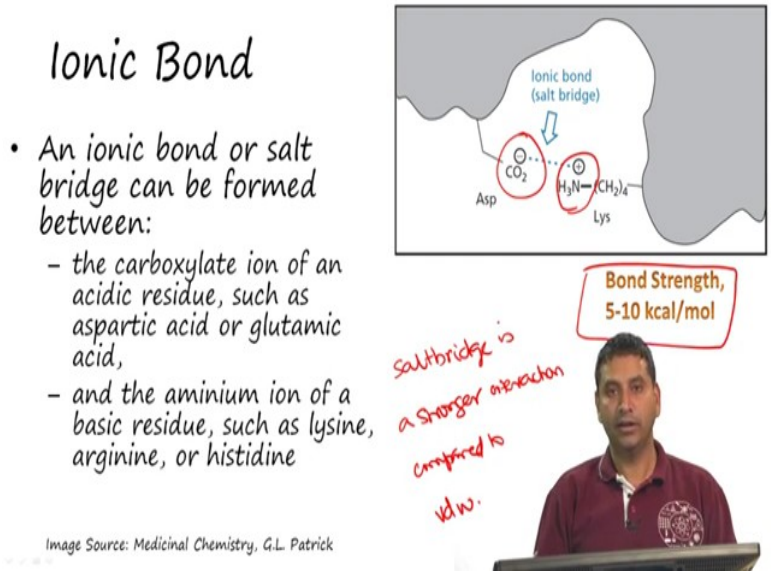
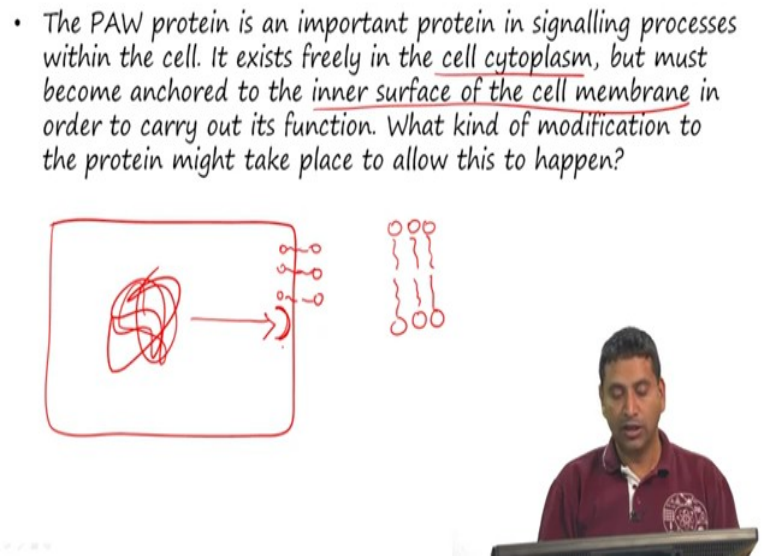



So now if you look at the salt ionic bond or salt bridge an ionic bond is formed between typically a carboxylate ion of an acidic residue, such as the one shown here an aspartic acid and an amine from a neighbouring residue such as lysine or histidine. So here in this

example the protonated lysine has a full positive charge on it and so this kind of interaction is called as a salt bridge. The bond strength here is somewhere between estimated to be 5 to 10 kilocalories per mole. So the answer to the question is the salt bridge is a stronger interaction compared to vander waals, okay.(Refer Slide Time: 10:40)




Now let us look at the next question the PAW protein is an important protein in signalling processes within the cell. It exists freely in the cell cytoplasm, but must become anchored to the inner surface of the cell membrane in order to carry out its function. So question is what kind of modification to the protein might take place to allow this to happen? So let us look at this question a little bit of detail.

So here is this PAW protein which is freely floating around in the cytoplasm. Now for it to be activated, it needs to go and bind to the surface of the membrane. So in order for this to happen we have already seen that the lipid bilayer is the major component of the cell membrane and so you can imagine that the lipid bilayer is going to be like this, right and if it has to associate itself with the lipid bilayer then it would need some hydrophobic residue to actually associate itself with, right.

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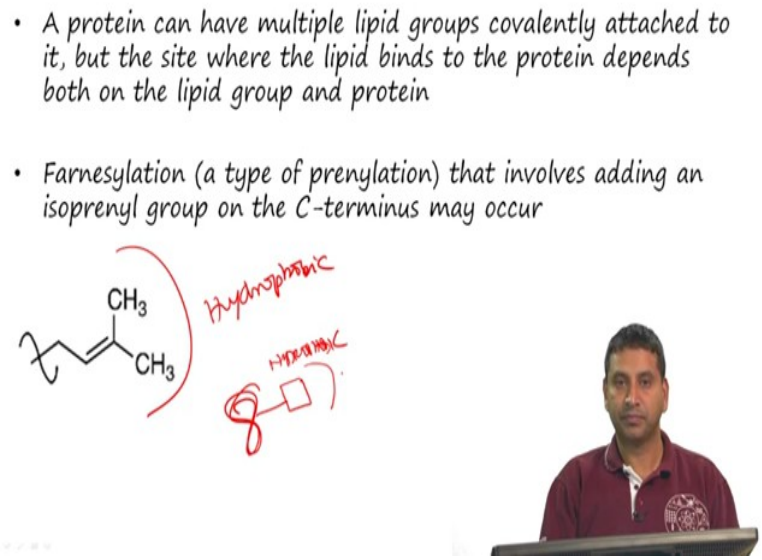


So, therefore the protein can have multiple lipid groups covalently attached to it, but where the lipid binds to the protein depends on the lipid group and the protein, okay. So when this lipid group is attached to the protein one of the examples of this is farnesylation which is a type of prenylation and involves adding an isoprenyl group to the C-terminus (of the enzyme) of the protein. So once this is added it creates a hydrophobic surface for it to interact with the membrane, so it is likely that the protein PAW will have to probably undergo some sort of a modification with a hydrophobic group for it to go and interact with the lipid membrane.

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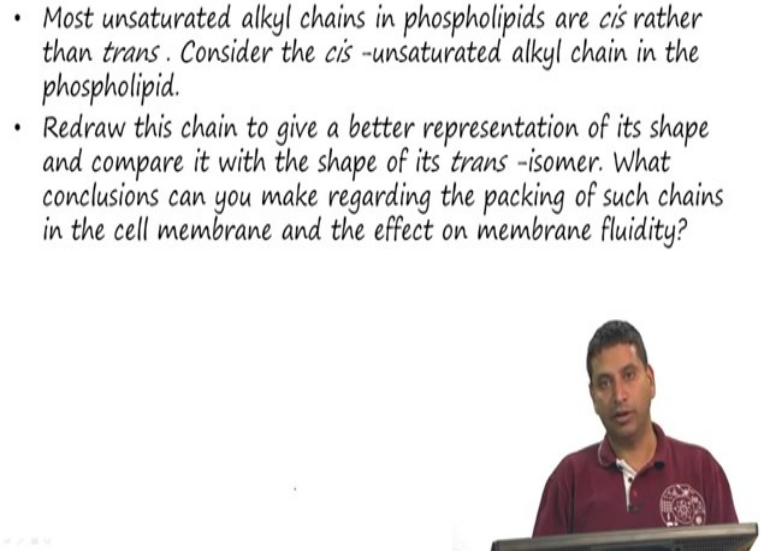


The nest question is most unsaturated alkyl chains in phospholipids are cis rather than trans. Consider the cis unsaturated alkyl chain in the phospholipid, then let's redraw it to give a

better representation of its shape and compare the shape with the trans isomer. What are the conclusions that you can make regarding the packing of such chains in the cell membrane?

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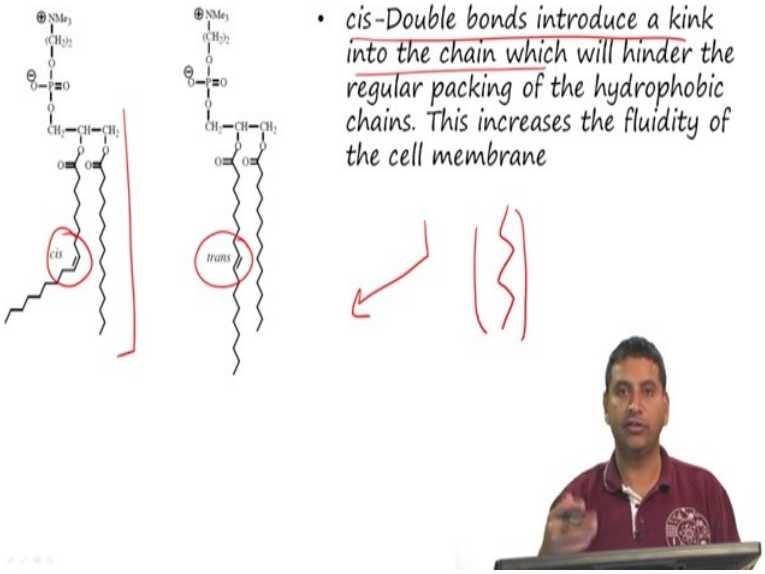


So if we were to draw a cis unsaturated lipid it would be in the following manner where you have a cis double bond over here. Similarly if you want to draw this in the trans (conformation) configuration you would get the structure like this. So as you can see the cis configuration actually undergoes a change in the direction ad this is going to increase the or it is going to make the regular packing of this more difficult, whereas in the trans you have an orientation like this which can improve the packing. So therefore cis double bond introduces a kink in the cellular chain which will hinder the regular packing of the hydrophobic chain and increase the fluidity of the cell membrane.

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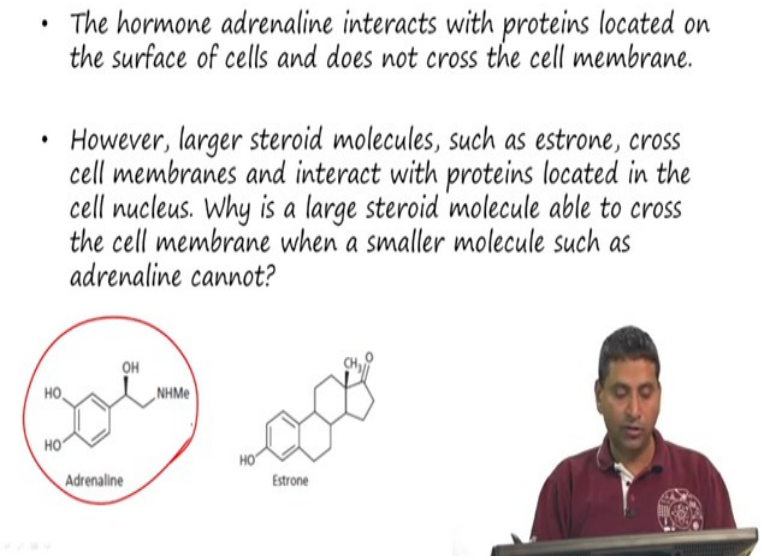


The hormone adrenaline interacts with proteins located on the surface of the cell and does not cross the membrane, so adrenaline structure is shown here. However, larger steroid molecules, such as estrone, cross the cell membrane and interact with the proteins located inside the cell nucleus. Why is a large steroid molecule able to cross the cell membrane when a smaller molecule such as adrenaline cannot?

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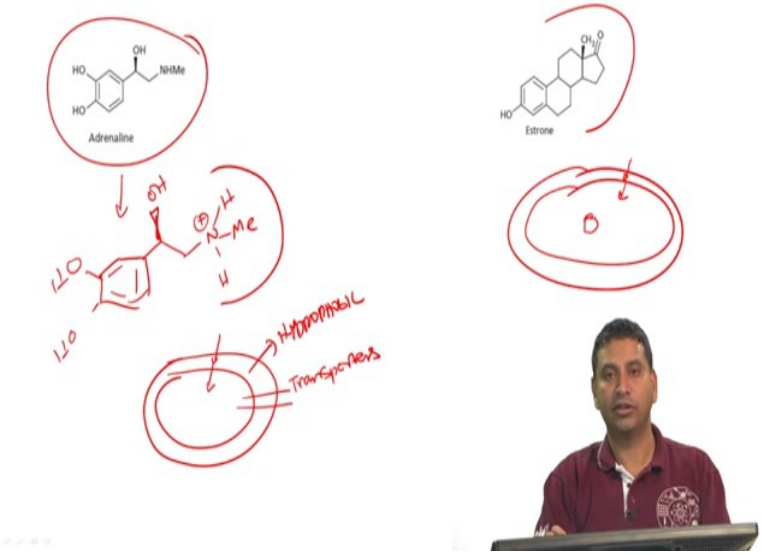


So in order to address this question let us look at the structures of these molecules once again, so adrenaline as shown here is more likely to be protonated and it would have a structure like this where you have two hydroxyl groups and you have this aliphatic hydroxyl group and you

have NHMe perhaps this is going to be protonated and you will have a positively charge species.

So as we looked at earlier in a cell, the lipid bilayer is present and this is going to be hydrophobic, okay. So in order for the compound to get in, it has to get in to the hydrophobic region and then get out of this into the cell. So considering the positive charge that is present over here one would predict or one can understand why adrenaline does not into the cell. So we have already looked at this previously, one of the ways in which nature solves this is to create small areas wherein such (small molecules can get in) small polar molecules can get in.

So these are transporters which help with taking in this highly polar small molecules, whereas estrone having a (hydrophobic) highly hydrophobic surface can actually better interact with the membrane can get in and then it can also get across the membrane because it dissolves or (it can interact it can) there can be a favourable interaction with the lipid membrane which can allow it to permeate so which is why estrone can get in and then go all the way to the nucleus and exert its effect.

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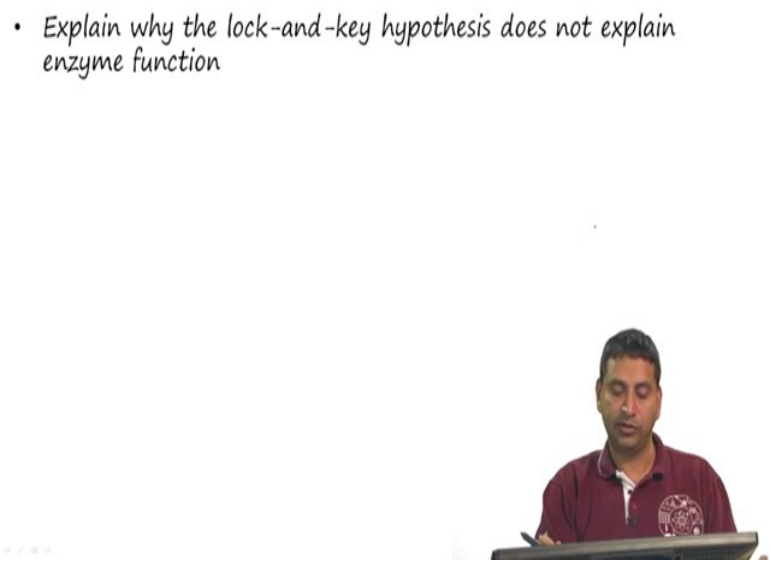


Next question is explain why the lock-and-key hypothesis does not explain enzyme function.

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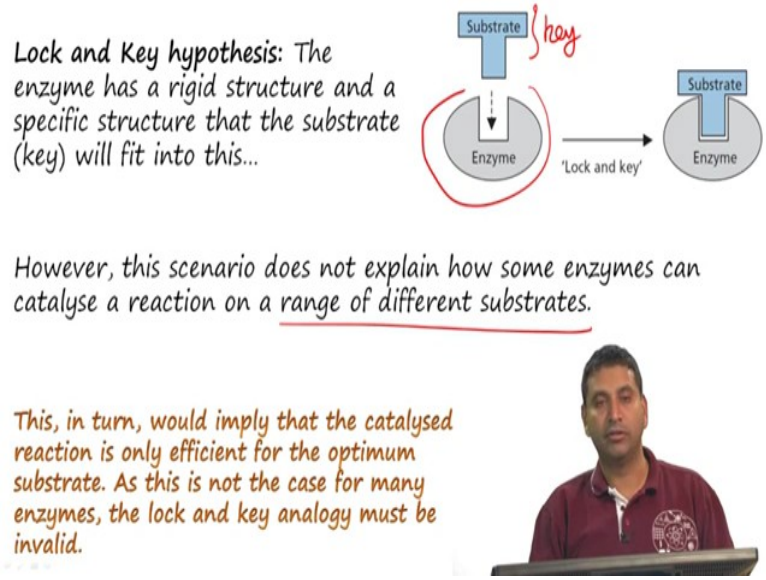


So in order to understand this let us look at what lock and key hypothesis is. Lock and key hypothesis is nothing but the enzyme has a surface or has an area where the substrate which is the key will come and bind to it and since the lock and the key have exactly very well paired shapes, the key can go in and interact with the lock and may be there it binds and then it is going to turnover.

However, this scenario does not explain how some enzymes can catalyse a reaction on a range of different substrates. So the lock and key hypothesis would necessarily or would tell us that the substrate shape and size has to be very highly conserved, but that is not the case in certain enzymes some enzymes bind and react with number of substrates and so this lock and key hypothesis while it is useful does not explain all the functions of an enzyme.

So because this in turn, would imply that the catalysed reaction is only efficient for the optimum substrate. As this is not the case for many enzymes the lock and key hypothesis must be invalid.

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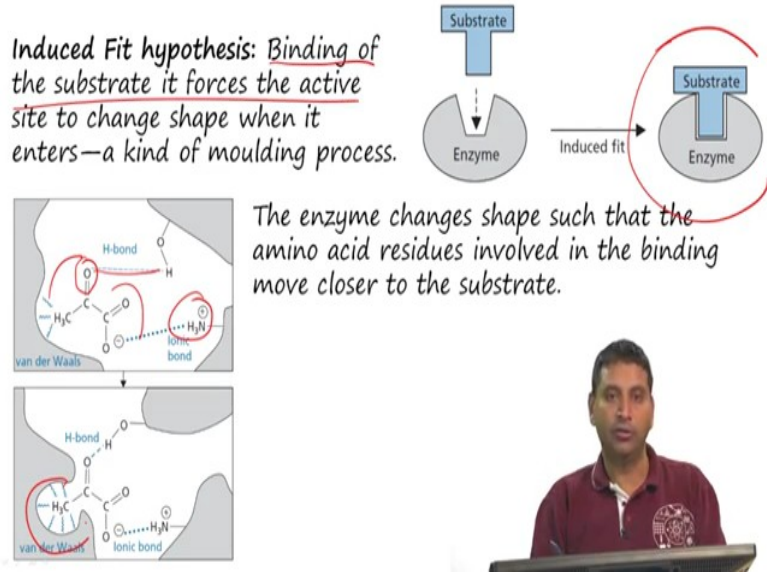


So instead the hypothesis that explains us a number of the observations better is the induced fit hypothesis. So here the binding of the substrate forces the active site to change shape when it enters it is like some sort of a moulding process that happens and once that happens you can have the substrate fit into the pocket much better, right. So enzyme changes shape such that the substrate can fit, the substrate can also change its shape a little bit to accommodate itself into the enzymes.

So here is the example that we looked at previously where you have a carboxylate ion interacting with an ammonium through ionic bridge and this carbonyl interacting with through hydrogen bond with the substrate. Now after it binds it is possible that this vander waals interaction which was previously quite weak can now come and wrap itself around and so that is the conformation change that is required for the induced fit to occur.

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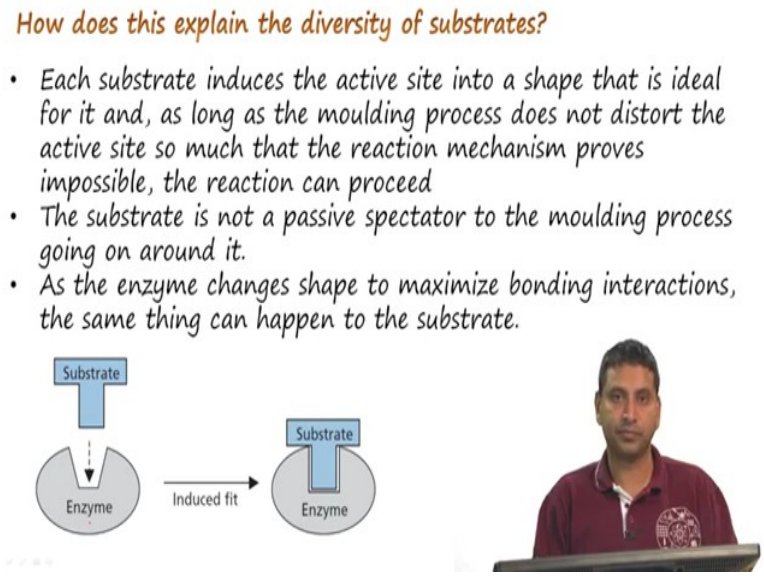


So how does this explain the diversity of substrates? So each substrate induces the active site into a shape that is ideal for it, and so even with a different substrate one can have the same effect or the same binding or similar binding because this is the moulding process and this moulding process does not distort the active site, so the reaction mechanism happens quite consistently even if the substrate is different.

Also the substrate is not a passive spectator as we have looked at previously and it also changes shape into accommodate itself into the active site. So as the enzyme changes shape to maximize bonding interactions the same thing can also happen to the substrate. So therefore, this model allows us to explain why there is a large number of substrates that can be turned over by a particular enzyme even though the substrate may look very different.

It is quite likely that the interactions of the active site with the substrate is quite similar and therefore it can recognize substrates which are similar in not just in shape but also in binding interactions.

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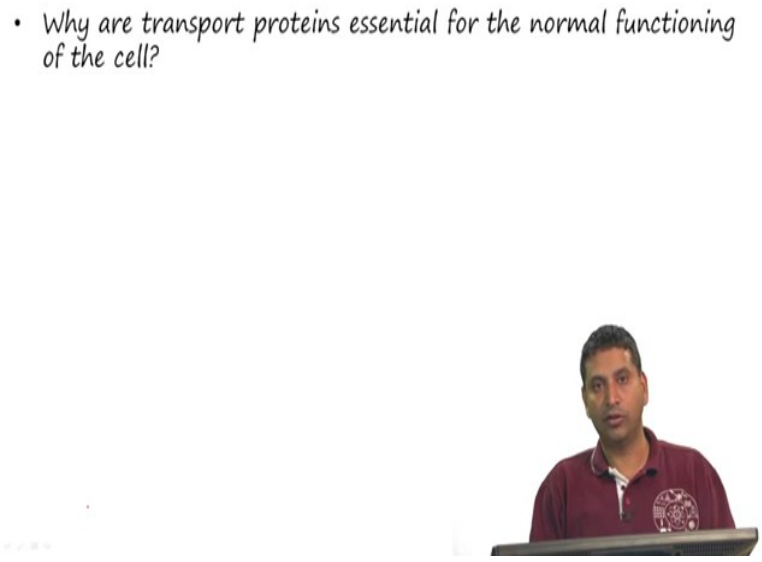


Next question is why are transport proteins essential for the normal functioning of the cell?

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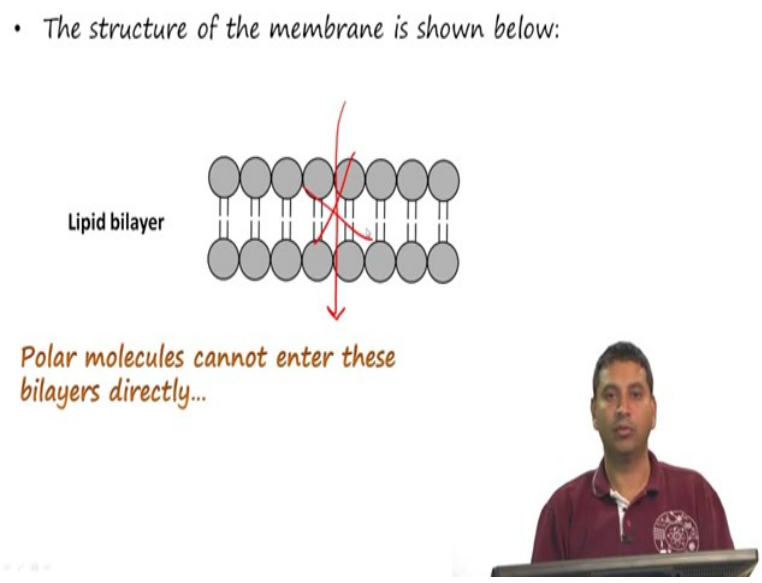


So we have already seen this the structure of the lipid bilayer is shown below and now polar molecules as we discussed earlier cannot enter because they do not get into the hydrophobic surface.

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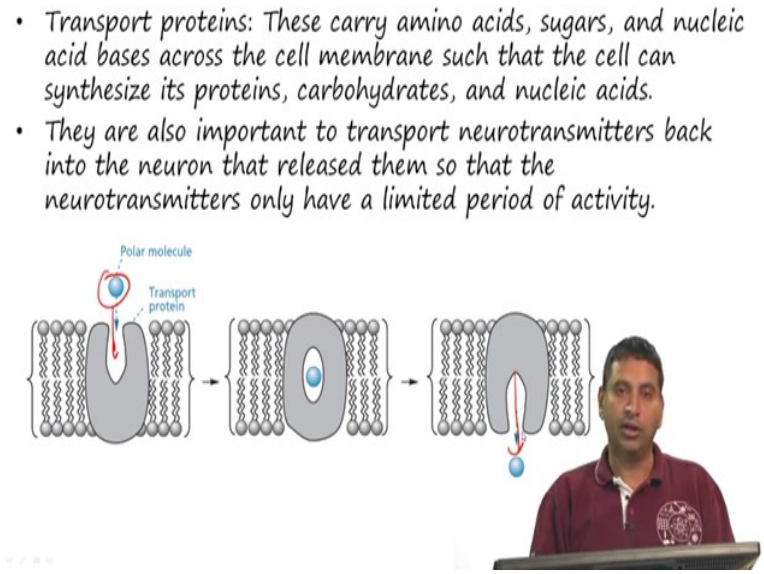


So in order for this to happen we have looked at in the previous case of adrenaline the transport proteins can carry can act as carriers. So the way in which we understand this transport proteins is that when there is a polar charged molecule it goes and binds itself to the (protein surface) the transport protein surface and then the protein acts as if it is a carrier it wraps itself around the polar molecule and then through a process of diffusion the molecule can get out, okay. So this is very important when neurotransmitters have to be transported back into the neuron so that they have a limited period of activity.

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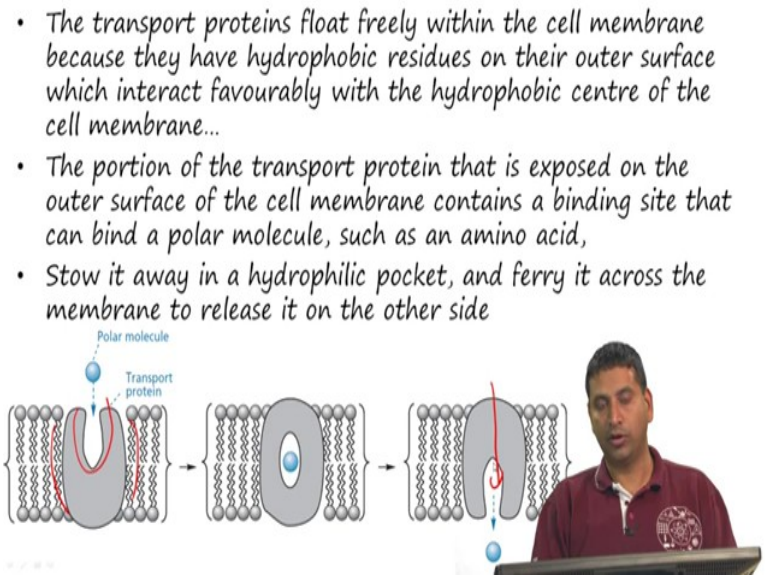


So the transport proteins float freely within the cell membrane because they have hydrophobic residues on their outer surface which interact favourably with the centre of the

cell membrane. The portion of the transport protein that is exposed to the surface contains a binding site that can bind the polar molecule. So therefore, transport proteins are very important for the functioning of the cell.

So they act as something like they stow it away hydrophilic pocket, ferry it across the membrane and release it on the other side, okay. Therefore transport proteins are very important for the normal functioning of a cell.

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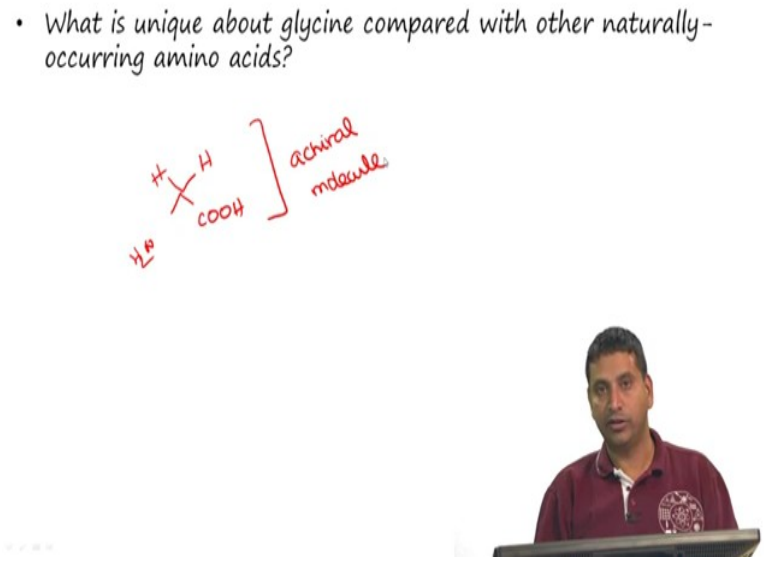


Next question is what is unique about glycine when compared with other naturally occurring amino acids? So let us draw out should structure of glycine, glycine is $NH_2CH_2COOH$. So one thing that really stands out here that this is an achiral molecule, so this is one thing that is very different about unique about glycine when compared with other naturally occurring amino acids. If you go and look at the structure of all the other amino acids they will have a chiral centre in it.

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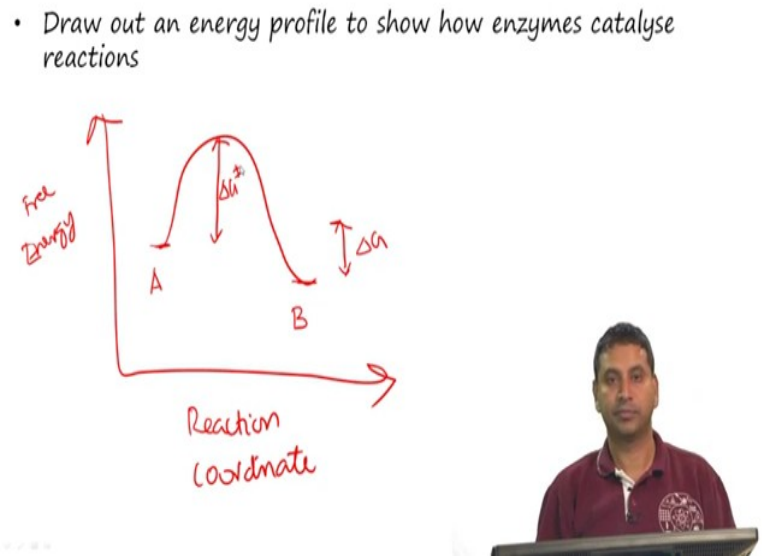


Next question is draw out an energy profile to show how enzymes catalyse reactions, okay. So in order to this task let us draw out the coordinates which we have looked at several times. So here is your reaction coordinate and here is energy it can be free energy or delta G for example and so on. So let's draw hypothetical reaction A going to B let us assume that it is a exothermic reaction or exergonic reaction this would be our delta G. Now what we understand from kinetics is that there is going to be a barrier to this reaction and this barrier is shown here which is delta G double dagger.

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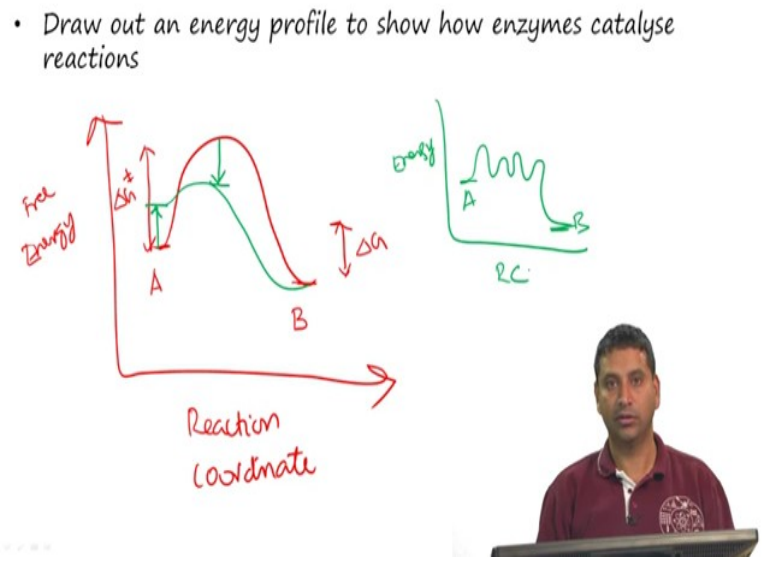


Now in order for us to understand how an enzyme catalyses a reaction we have already looked at what delta G double dagger is which I am just redrawing here for convenience. So

if the reaction goes faster we know that there is going to be either a change in the ground state energy or a change in the activation energy. So that means that the ground state energy is going to go up which will result in the substrate being destabilized so that it can cross the barrier let us assume that it is a similar barrier, the other option is that you can have the transition state coming down in energy and what may end up happening is if you have a combination of these two the enzyme is going to go much faster and it is going to or the reaction is going to go much faster and the turnover is going to be extremely fast.

So in reality it is more likely that an enzyme catalyses may happen in phases such as this. So here is the starting material A, here is the product B, this is the energy and this is the reaction coordinate, okay.

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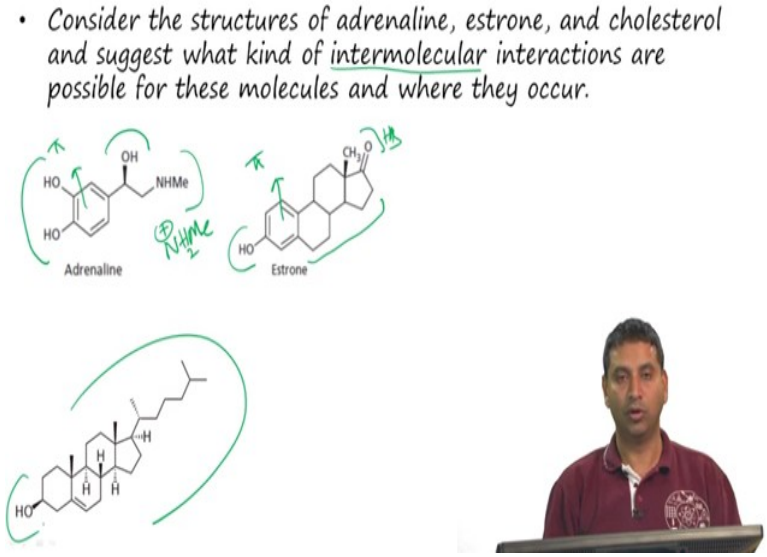


Now next question is consider the structures of adrenaline, estrone and cholesterol and suggest the kind of interactions that are possible in these molecules and the interactions that we are interested in this intermolecular interactions. So we have already looked at adrenaline previously so adrenaline molecules is over here so it can undergo protonation and form $NH_2$ Me plus that will create a possibility of salt bridge type interaction, you also have hydrogen bonding capability, hydrogen bonding capability over here and this will give us a pi interaction over here.

In the case of estrone, there is a hydrogen bonding capability here, pi stacking over here, there is a hydrogen bonding capability here and the rest of the molecule is actually

hydrophobic. Cholesterol we have already looked at previously it is going to have a large hydrophobic interaction under small area where it can do polar interactions.

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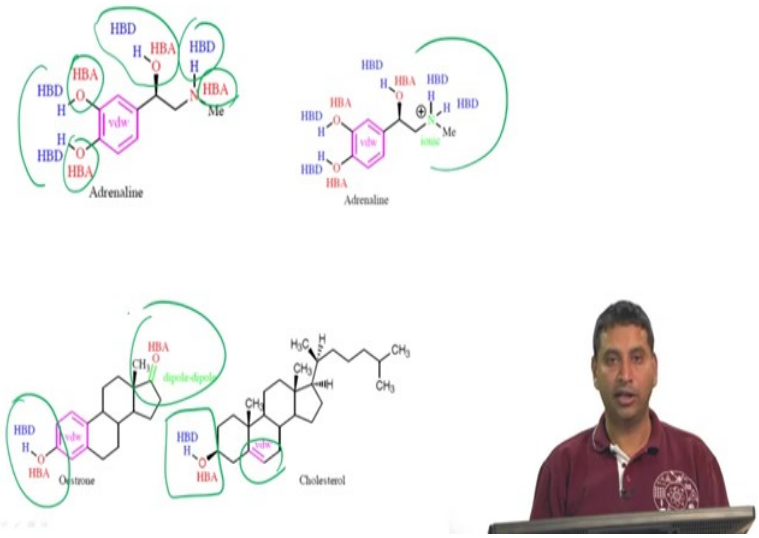


So the answer is drawn out here so you can see here there are hydrogen bond donors, there are hydrogen bond acceptors in the case of adrenaline and you have a hydrogen bond donor over here, hydrogen bond acceptor over here and here is another hydrogen bond acceptor donor pair and as we discussed earlier if it is ionised then if it is protonated then it is going to be ionic over here and the rest of the hydrogen bond capability is similar.

In the case of cholesterol there is some possibility of vander waals interaction and there is a hydrogen bond acceptor donor pair over here. Similarly in the case of estrone is going to be like this and there is also possibility of hydrogen bond acceptance and a dipole-dipole interaction.

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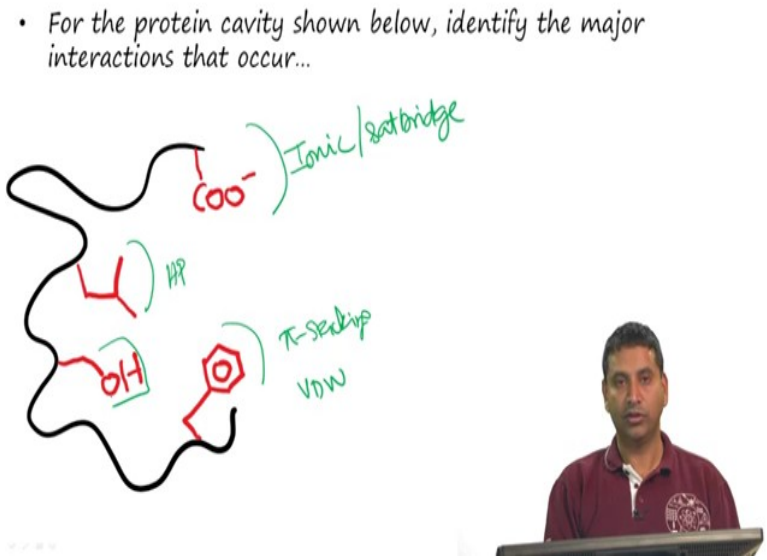


So now let's look at a situation where a protein has the following cavity in it, identify the major interactions that occur. So if you see here the major interactions that might occur here are here would be an ionic interaction or salt bridge interaction, here would be a hydrophobic interaction, here would be a pi stacking vander waals and here is a situation where you would have hydrogen bonding occurring through this hydroxyl group.

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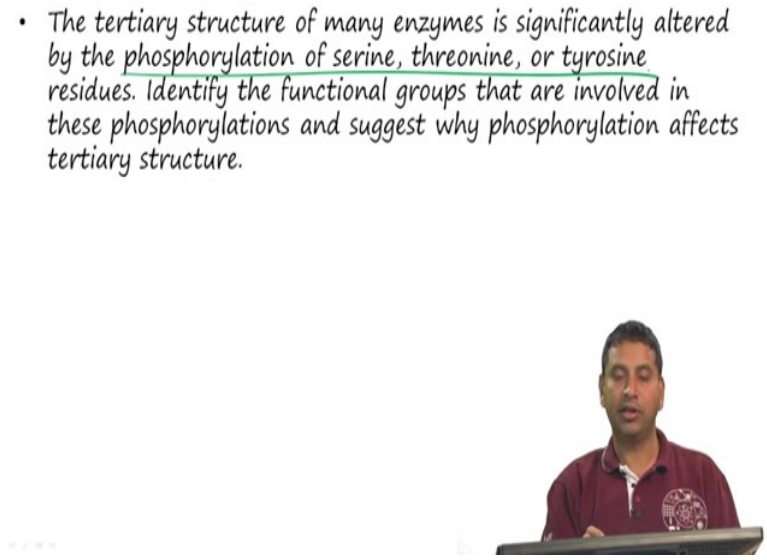


The next question is the tertiary structure of many enzymes is significantly altered by phosphorylation of serine, threonine, or tyrosine. Identify the functional groups that are involved in these phosphorylations and suggest why this phosphorylation might affect the tertiary structure.

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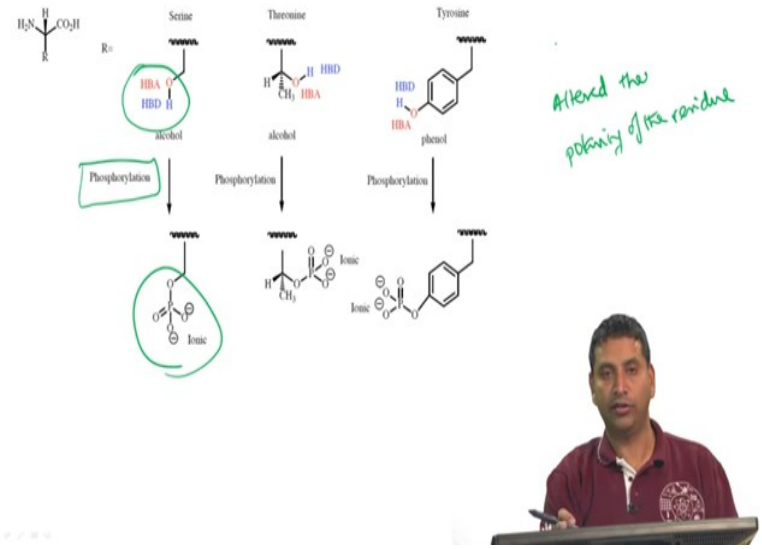


In order to answer this question let's first look at what phosphorylation? Phosphorylation is a situation where you have an alcohol in the form of a serine or threonine or tyrosine and it undergoes a phosphorylation, which involves addition of a phosphate group on it and what was previously a situation where you had only hydrogen bond acceptor and hydrogen bond donor now is converted to a functional group which is ionic in nature.

So what happens in this process is that you have altered the polarity of the residue, okay. So you have converted a simple little relatively polar group into an ionic group. So this is an example of a post translational modification that we have looked at previously.

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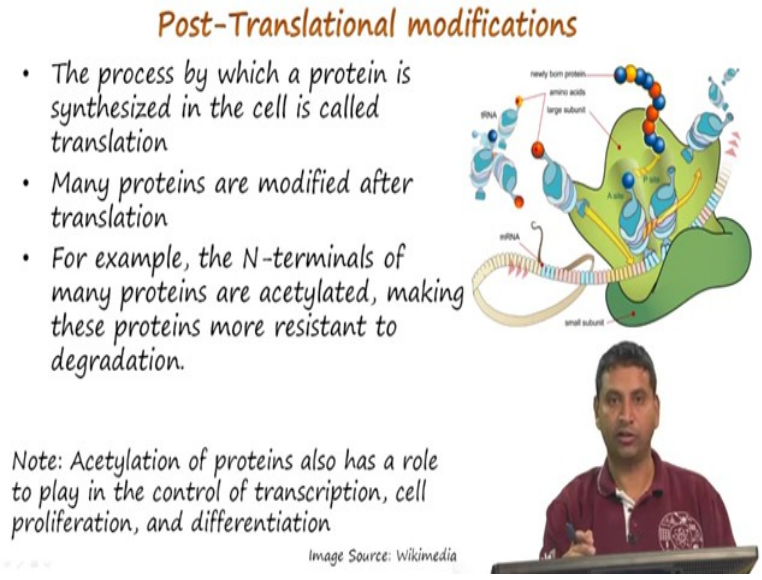

In post-translational modification, translation is the process by which a protein is synthesized in the cell and many proteins are altered after they are translated. So for example N-termini of many proteins are acetylated, making these proteins more resistant to degradation. And we have also looked at later that acetylation of proteins has a major role to play in the control of transcription, okay.

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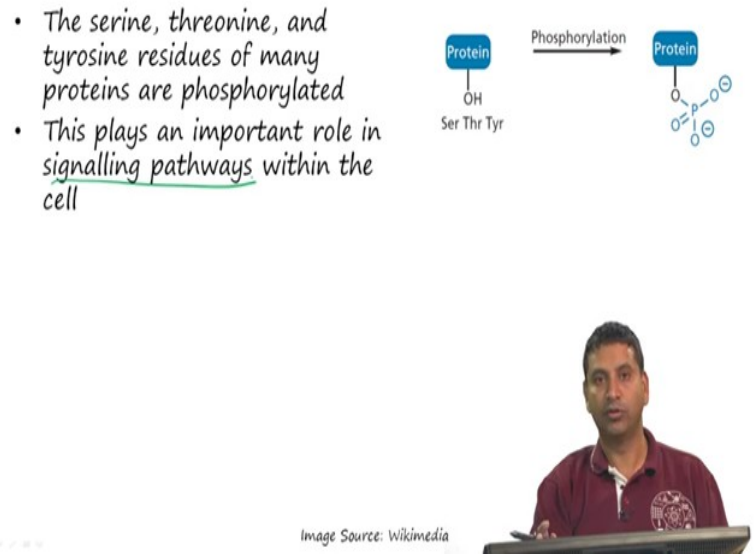


Now once we do phosphorylation, the serine, threonine or tyrosine residues are once they are phosphorylated they can be a part of many signalling pathways. And therefore it is a very important post-translational modification that occurs.

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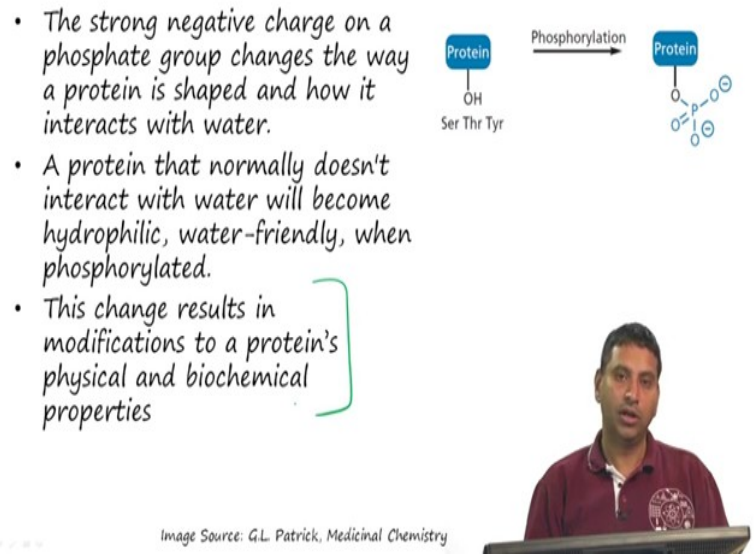

But now because of the strong negative charge on the phosphate group this completely changes the way in which the protein is shaped and how it interacts with water. So a protein that normally does not interact with water will suddenly become hydrophilic and become water friendly when it is phosphorylated. So this change results in the major biochemical properties of the protein and so therefore it has been used phosphorylation has been used to carry out many signalling events inside the cell.

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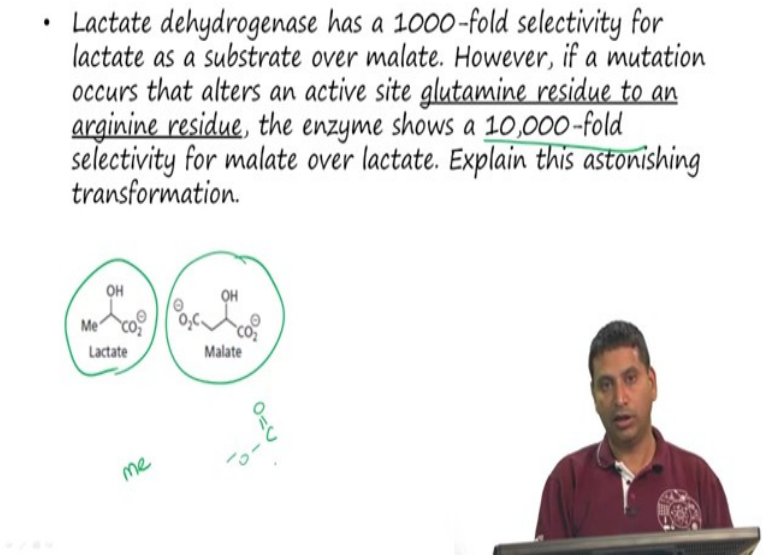


Next question is lactate dehydrogenase has a 1000 fold selectivity for lactate as a substrate over malate. However, if a mutation occurs that alters an active site glutamine residue to an arginine residue, the enzyme shows a 10000 fold increase in selectivity, okay. So explain this astonishing transformation. So here are the structures of lactate and malate, so if you look at these two structures closely, the major difference is here a methyl group which is hydrophobic is converted to a carboxylate which is an ionic residue.

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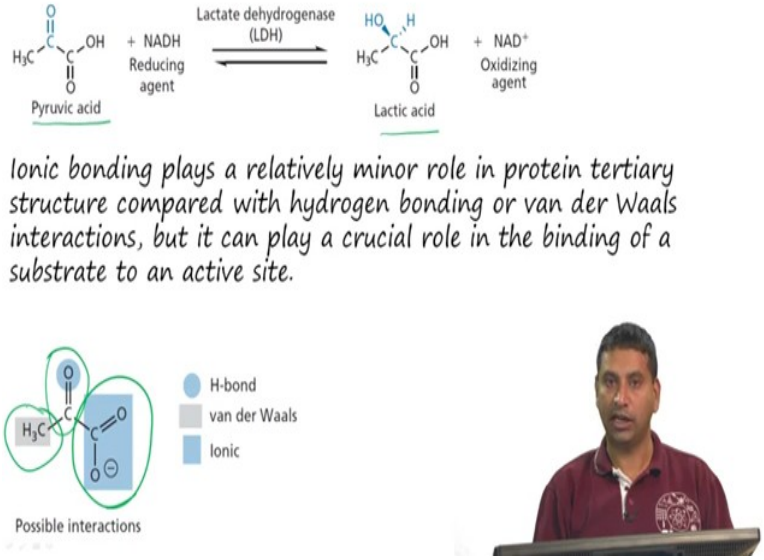


So ionic bonding so first let us look at the reaction here is a molecule pyruvic acid. It interacts with lactate dehydrogenase and gives you lactic acid as the product. So here the possible interactions that we have looked at in the past are the ionic interaction which is shown here, hydrogen bonding interaction and hydrophobic interactions or vander waals interactions. So ionic bonding plays a relatively minor role in the tertiary structure compared with hydrogen bonding or vander waals interaction, but it can play crucial role when it is binding to the substrate of an active site.

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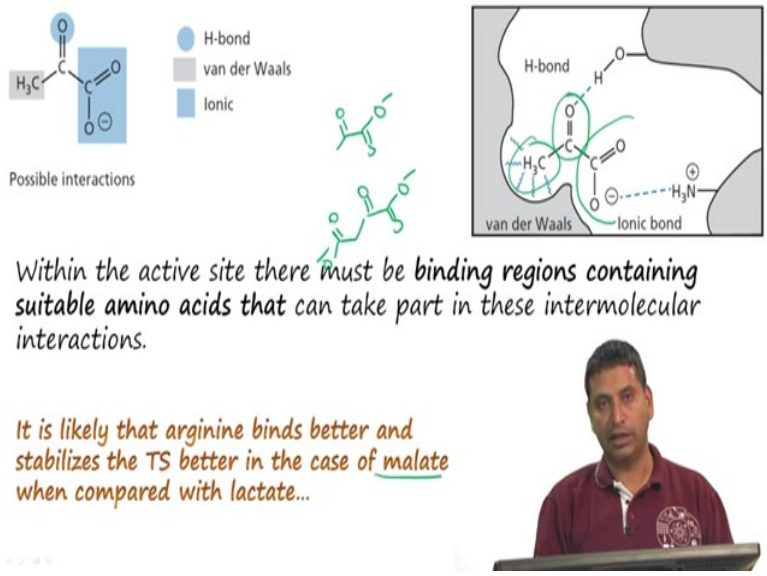


So here it is redrawn and one can imagine that the active site of the protein must contain an area where it binds to this carbonyl group, it must have an ionic bond over here and a

hydrophobic or a vander waals interaction, okay. Our data tells us that the reaction of this is much faster when compared to, okay. So therefore it is possible that the arginine binds better and stabilizes the transition state better in the case of malate when compared to the lactate.

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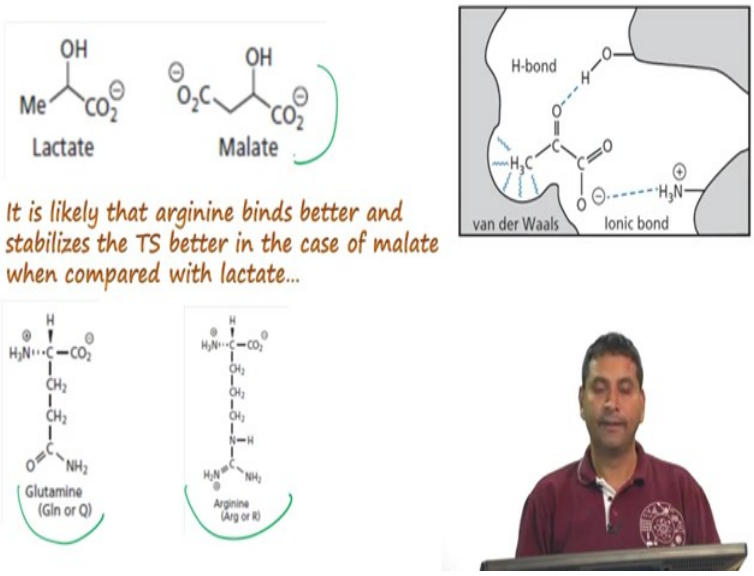


So this is structures of lactate and malate as shown here and when we mutate the glutamine residue to the arginine residue the arginine residue is quite likely to bind to the carboxylate much better when compared to glutamine. So from this example what is clear is that by changing the residue in the protein's active site or it is possible that the substrate specificity can be altered. So here we can the enzyme has become more selective towards malate when compared to lactate, okay.

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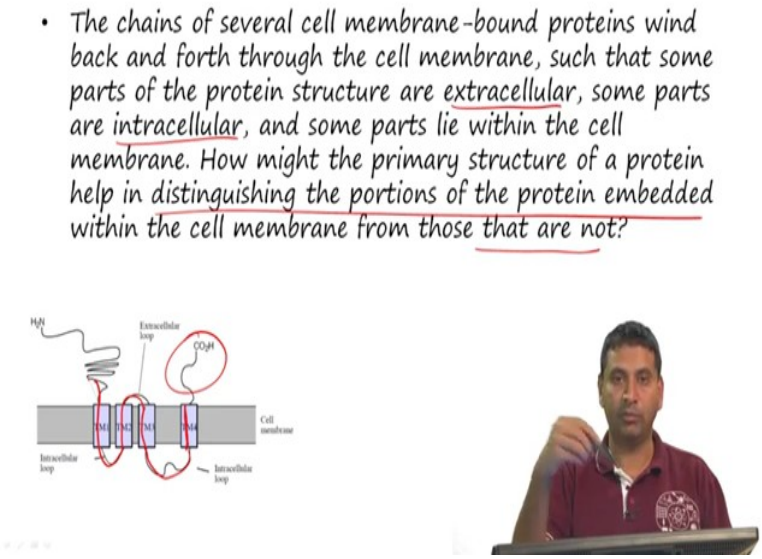

Next question is the chains of several cell membrane bound proteins wind back and forth through the cell membrane, such that some parts of the protein structure are extracellular and some parts of the protein are intracellular, okay. So the question here is how might the primary structure of a protein help in distinguishing the portions of the protein embedded within the cell membrane from those that are not?

So I have drawn here a cartoon which shows a protein which goes back and forth into the membrane and out, so here you have an extracellular domain it is over here and then there is a region where it goes inside or embeds itself into the membrane and then there is another loop and then again it goes through the membrane, then it goes out, comes back in, goes in, comes out and so on. So there are several loops here going back and forth.

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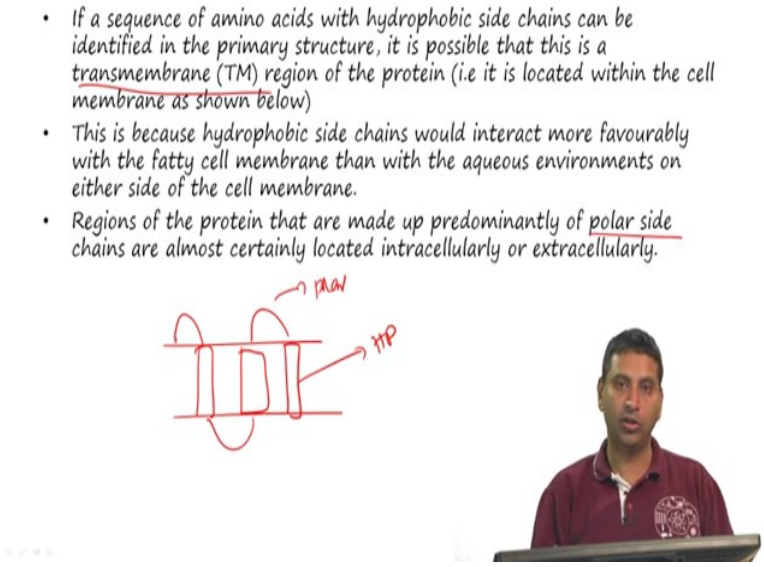


We already know that if a sequence of amino acids with hydrophobic side chains are identified in the primary structure that this hydrophobic side chains will more slightly interact with the lipids in the lipid bilayer. So it is possible that this is the transmembrane region because they are located within the cell membrane as shown. This is because hydrophobic side chains would interact more favourably with the (fatty acid) fatty cell membrane than the aqueous environment on the either side of the cell membrane.

So regions (that are) of the protein that are predominantly made of polar side chains are almost certainly located intracellularly or extracellularly, what we could understand here is that the regions which are over here are likely to be hydrophobic and the regions which are over here are going to be most likely polar, so you have again binding regions and so on,

right. So these are some predictions that one could make based on our understanding of how the cell membrane works and the hydrophobicity and hydrophilicity.

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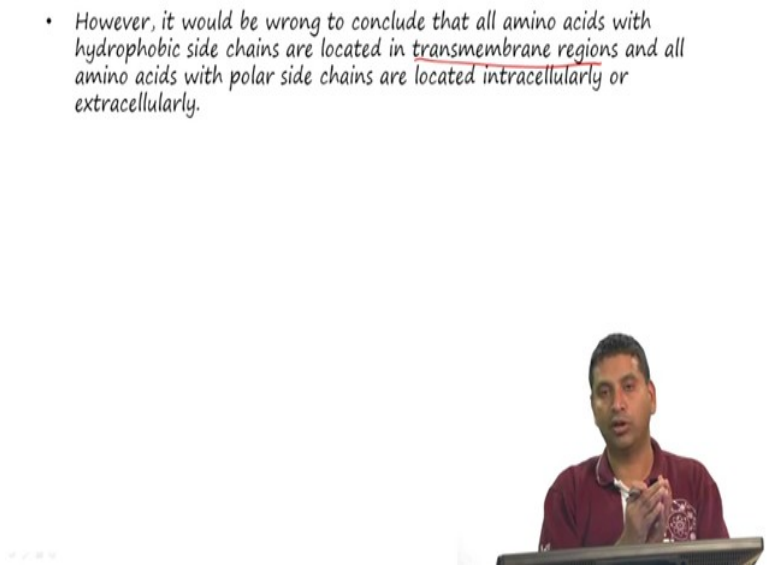


However, it would be wrong to conclude that all amino acids with hydrophobic side chains are located in the transmembrane region and all amino acids with polar side chains are located intracellularly or extracellularly because it is possible that their particular shape wherein the polarity become less important or more important.