Medicinal Chemistry
Professor Doctor Harinath Chakrapani
Department of Chemistry
Indian Institute of Science Education and Research Pune
Lecture No 04
Protein Structure and Function

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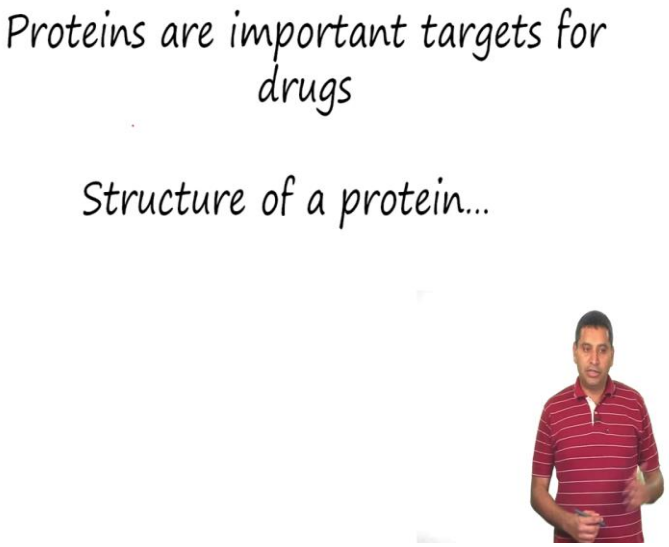

As we discussed earlier, proteins are very important targets for drugs and a large number of drugs interact with proteins. We also looked at what is the structure of a protein and in some detail we discussed about what are the various aspects of a structure of a protein, right.

Now

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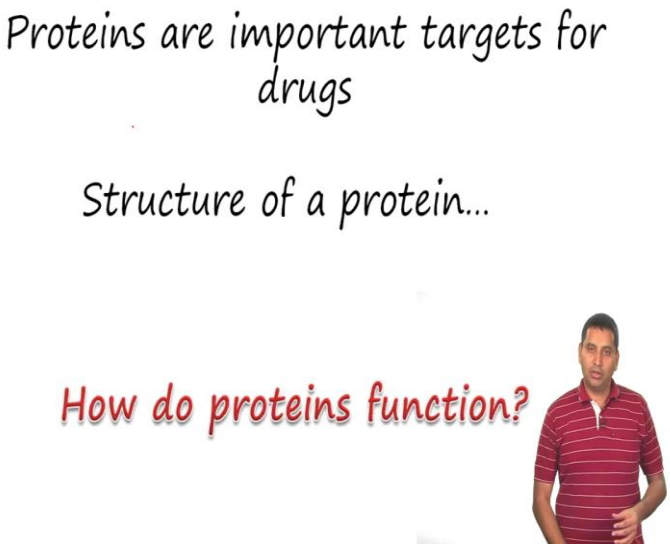

we look at how proteins function. So

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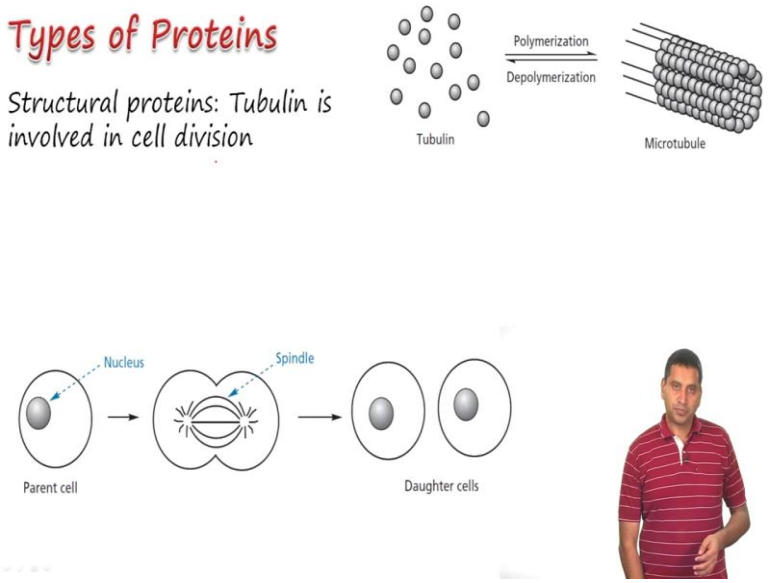

before we go there, let us look at what are the different types of proteins and how they are classified. So the first class of protein that we shall look at are structural proteins. And these are proteins which help, as the name suggests, help with maintaining the structure of the cell.

So an example here is tubulin. So tubulin is a very important

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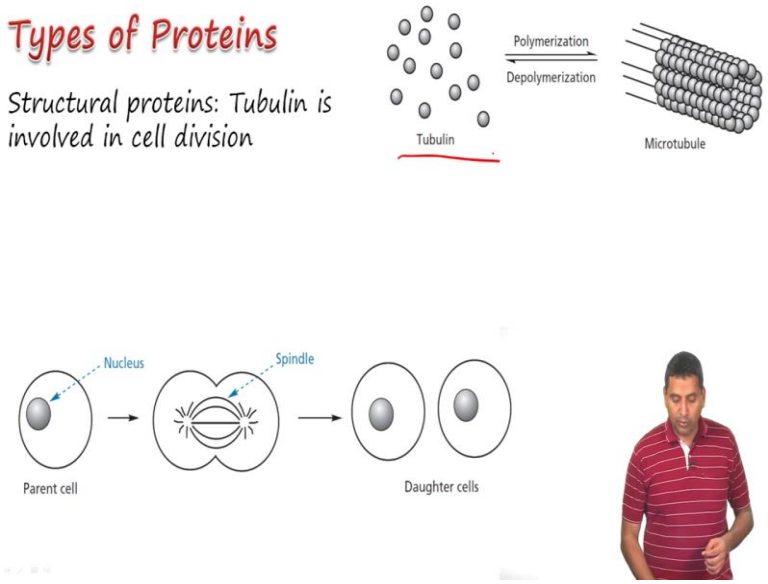

protein because it can undergo polymerization and depolymerization, Ok. So it provides a very important structure of the spindle during

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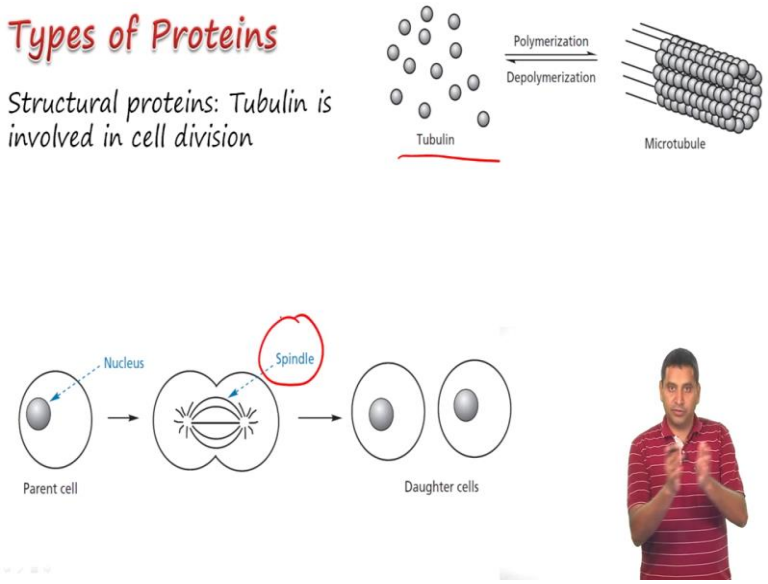

cell division, Ok.

So here in this case, a parent cell

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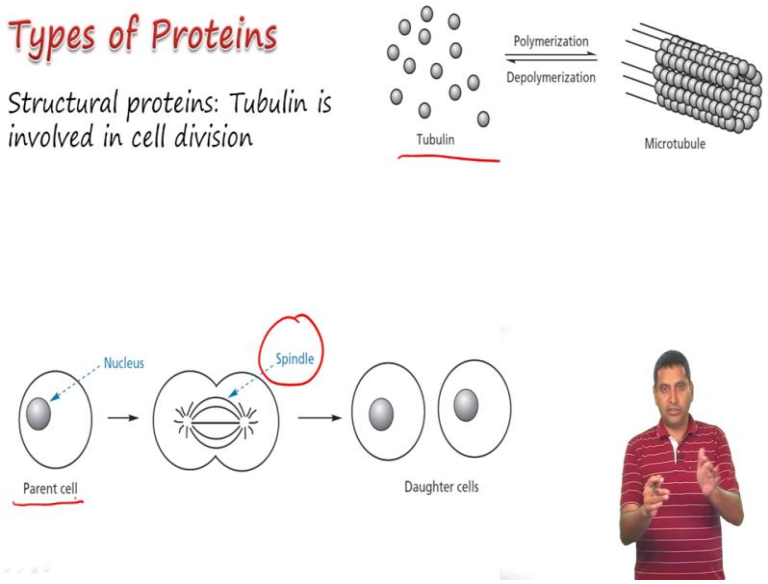

divides into two daughter cells and the spindle formation is a really important part of

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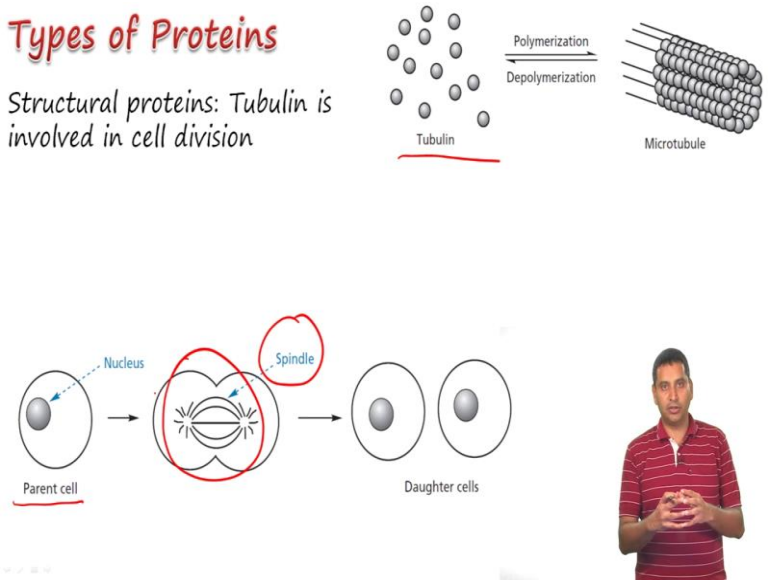

cell division. So during cell division tubulin undergoes polymerization. And

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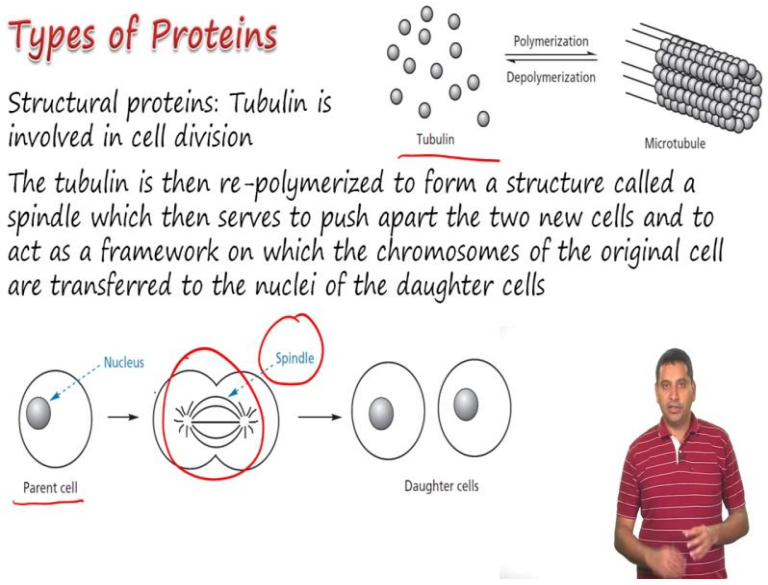

then therefore it forms a structure called spindle and these actually form a framework on which the chromosomes of the original cell are transferred to the nuclei of the daughter cells,

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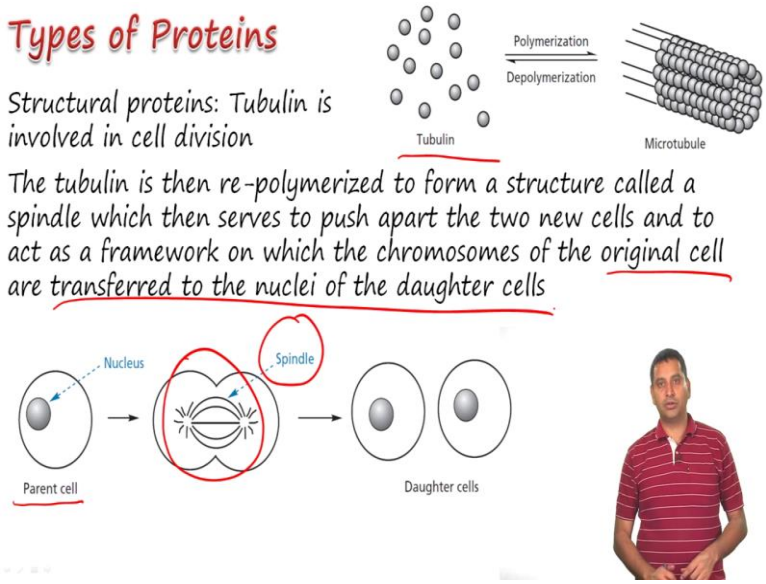

Ok.

So the disease such as cancer, the cells are rapidly dividing and so therefore tubulin polymerization must occur at a very high rate and targeting these

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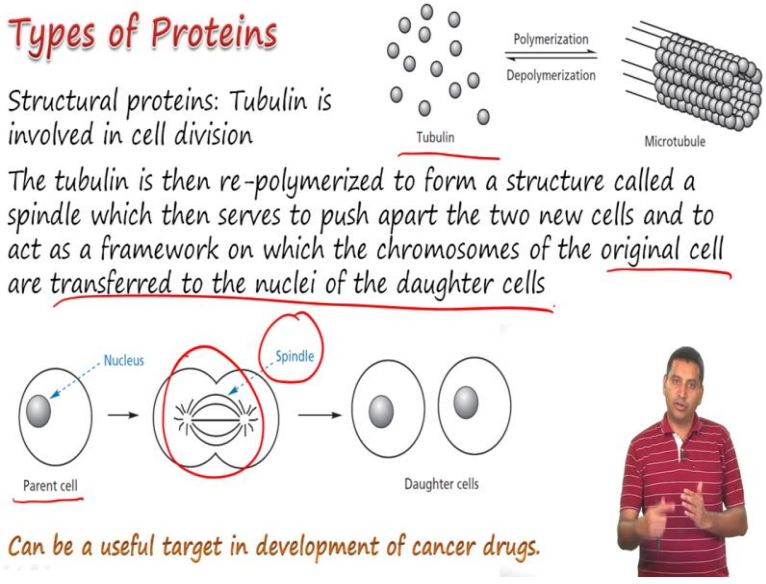

structural proteins can be useful in the development of cancer drugs.

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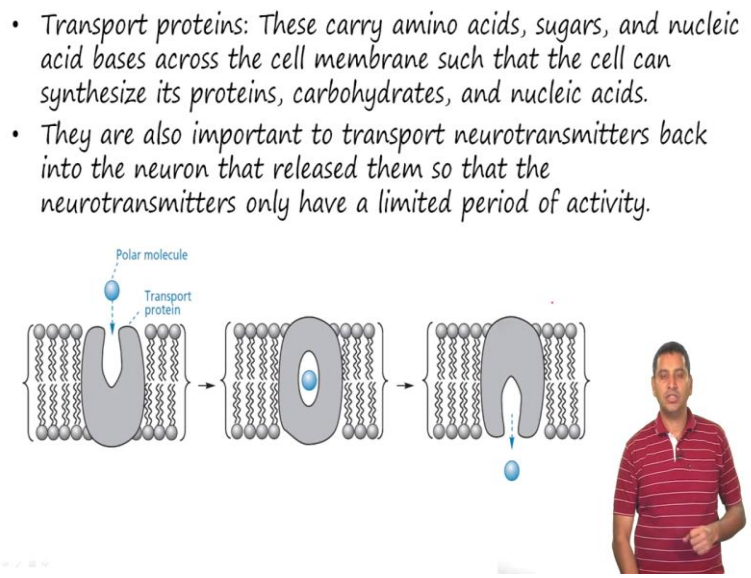

The next class of proteins that are important are transport proteins, Ok. Transport proteins occur on the surface of cells and they help

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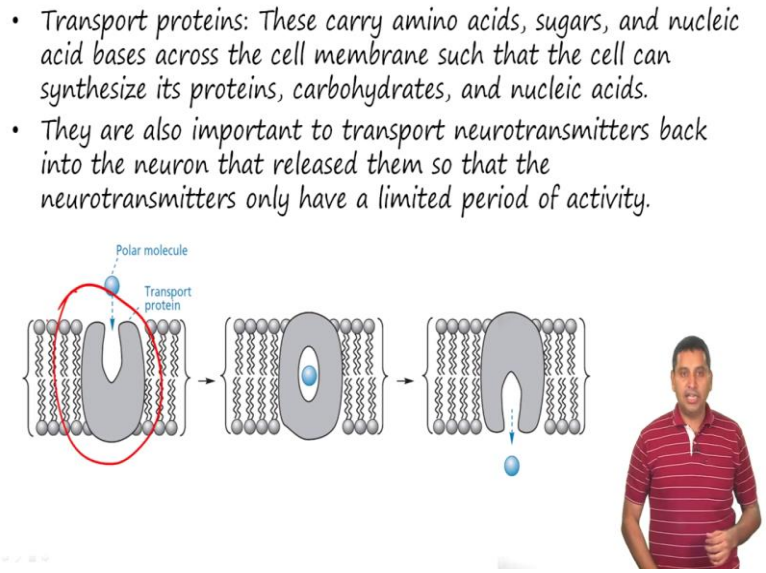

in carrying polar molecules such as small amino acids, sugars or nucleic bases across the cell membrane.

And now the cell can use this for the synthesis of proteins, carbohydrates and nucleic acids. And they are also very important in transporting neurotransmitters back into the neuron, Ok after the signal has been transmitted, Ok.

We look in shortly why this is important.

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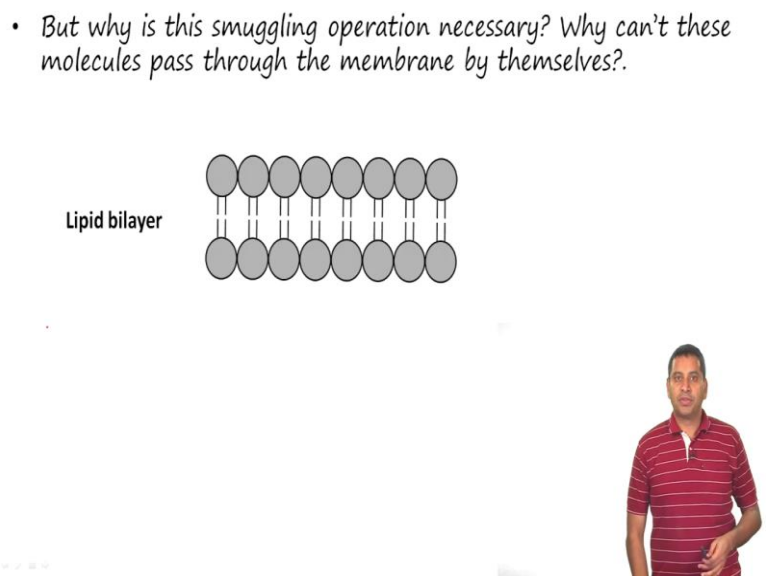

So why is this smuggling operation necessary? Ok. The question is why can't they go through on their own across the

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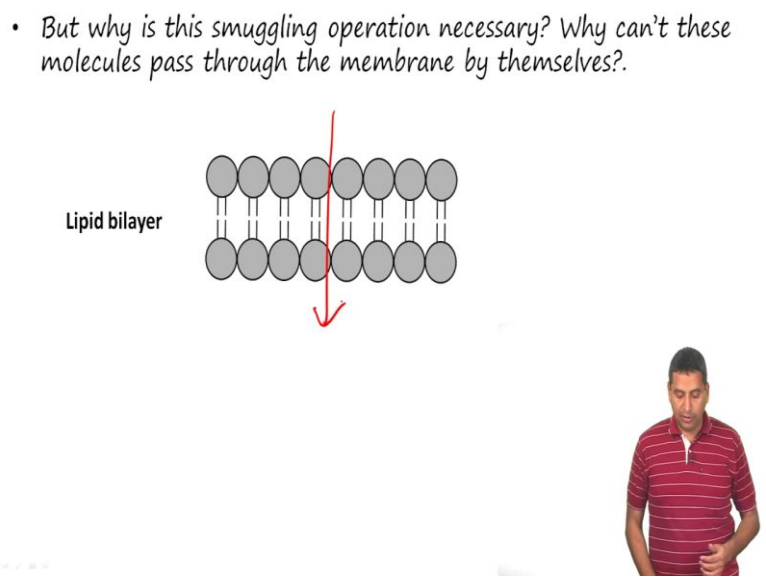

cell membrane, right. As we looked at earlier, the structure of a cell has a lipid bilayer as a, as a barrier or as a membrane.

The lipid bilayer forms an important structure that acts as a barrier in a cell. And these lipids are hydrophobic in nature. So therefore having a

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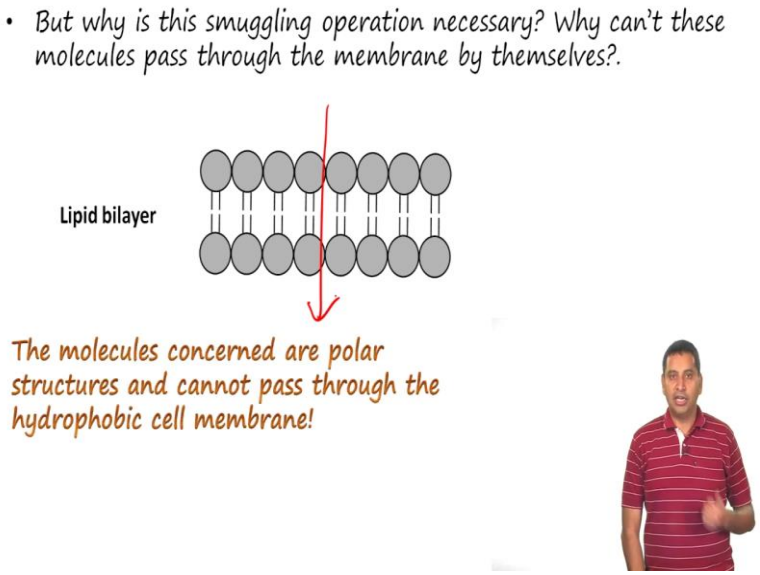

hydrophilic molecule with polar surfaces, polar structures

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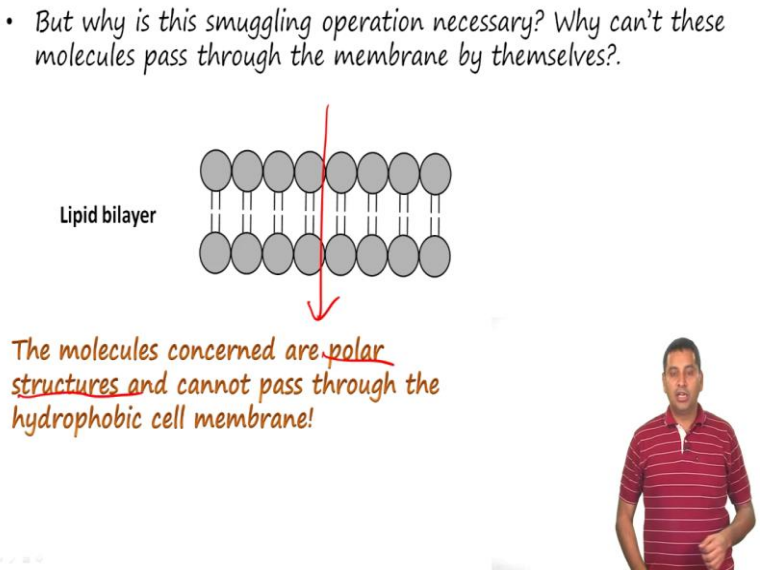

it is very difficult for these to cross the hydrophobic cell membrane.

And if we don't have a mechanism by which these polar structures are taken in, then it becomes impossible for us to synthesize proteins, nucleic acids or carbohydrates which are all important cellular components,

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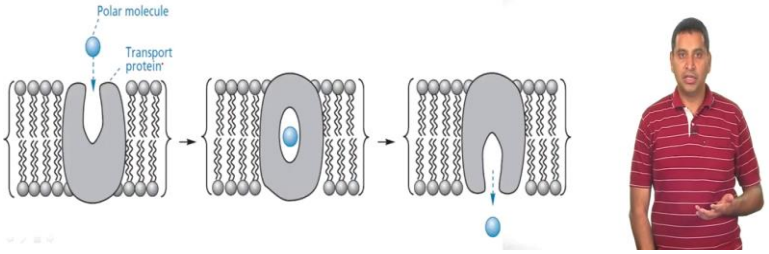

Ok. So let us at how this transport protein typically functions.

So the transport protein has a area, or the surface which

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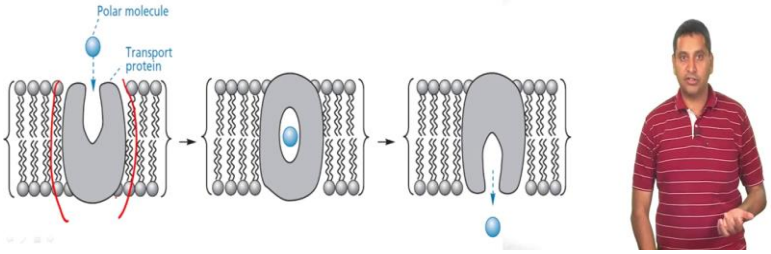

is actually hydrophobic, contrary to how typical proteins are, which we, we just learnt how typical proteins fold. These actually have hydrophobic surfaces

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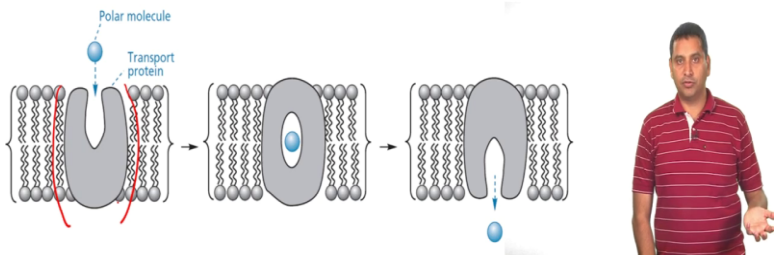
- The transport proteins float freely within the cell membrane because they have hydrophobic residues on their outer surface which interact favourably with the hydrophobic centre of the cell membrane.



and their internal residues are actually hydrophilic,

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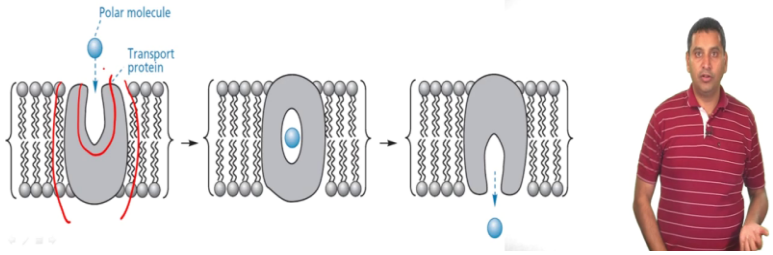

Ok.

And these hydrophilic residues interact with the polar molecule and they can bind it, right?

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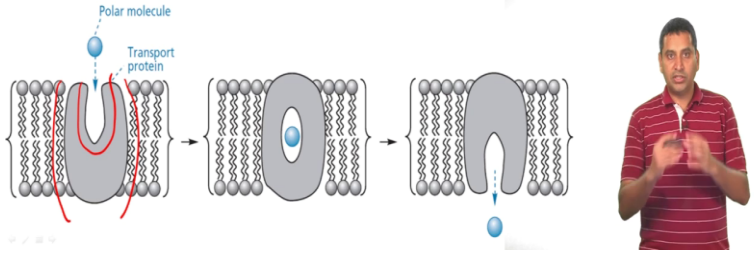
- The transport proteins float freely within the cell membrane because they have hydrophobic residues on their outer surface which interact favourably with the hydrophobic centre of the cell membrane.
- The portion of the transport protein that is exposed on the outer surface of the cell membrane contains a binding site that can bind a polar molecule, such as an amino acid,



Once they bind, it induces a conformational change and it gets entrapped

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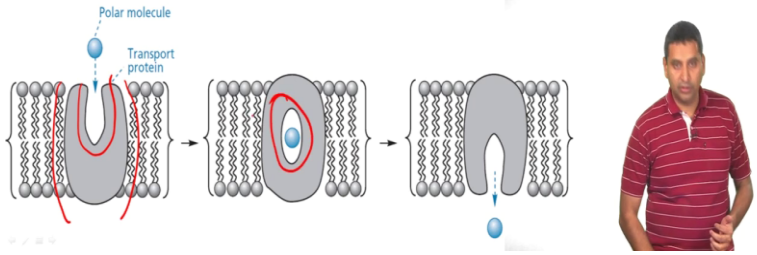

in the protein and now, again another series of conformational changes can occur through which the protein

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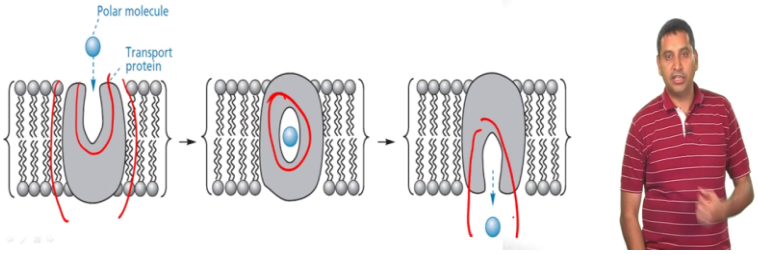

is actually kicking out the polar molecule.

Ok.

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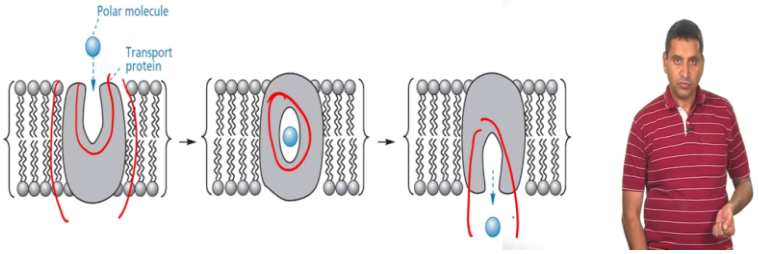
- The transport proteins float freely within the cell membrane because they have hydrophobic residues on their outer surface which interact favourably with the hydrophobic centre of the cell membrane.
- The portion of the transport protein that is exposed on the outer surface of the cell membrane contains a binding site that can bind a polar molecule, such as an amino acid,
- Stow it away in a hydrophilic pocket, and ferry it across the membrane to release it on the other side



So it is almost like how you can stow away a small molecule in a pocket and ferry it across the membrane to release it to the other side. So this is how typically a transport protein works.

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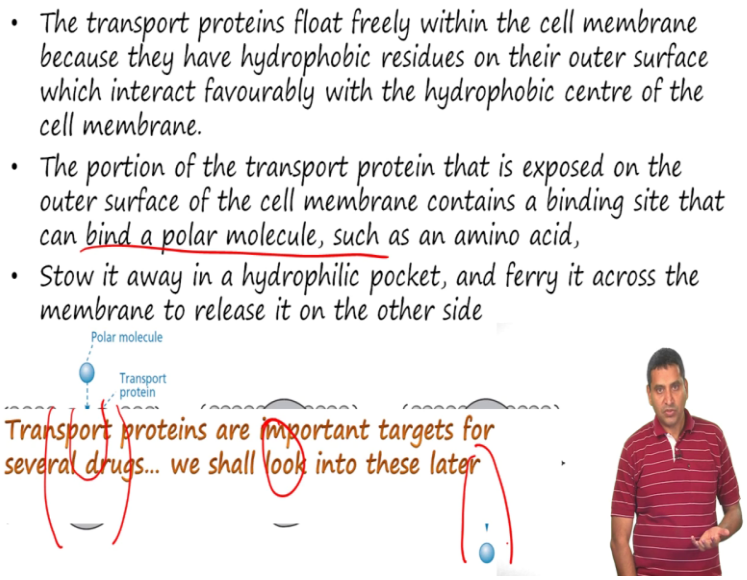

Now transport proteins are important targets for several drugs and we shall look into these in detail later, Ok.

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Now the most important proteins other than structural proteins and transport proteins are enzymes and receptors,

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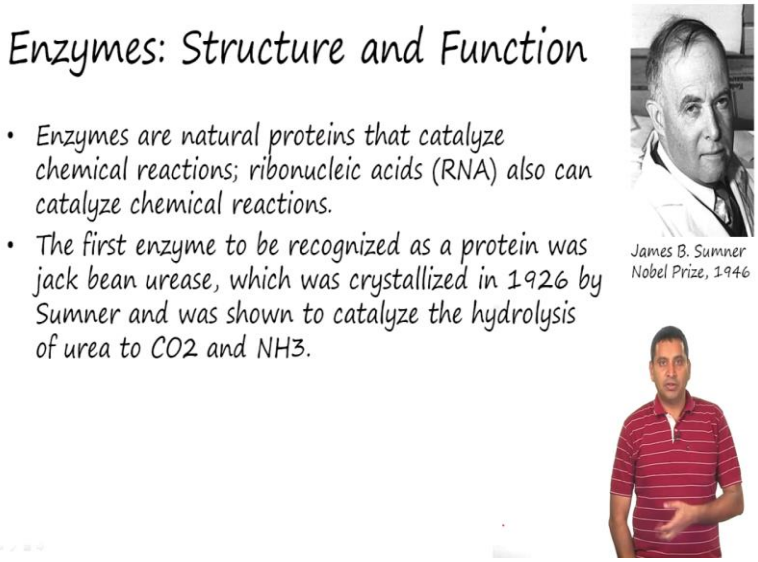

Ok. So now let us spend a little bit of time and look at what enzymes are.

Enzymes are natural proteins that can catalyze chemical reactions, Ok. Of course ribonucleic acid which is also called as RNA can also catalyze reactions but a vast majority of the catalytic processes are mediated by proteins which are called as enzymes.

The first enzyme to be recognized as a protein was urease which was isolated from

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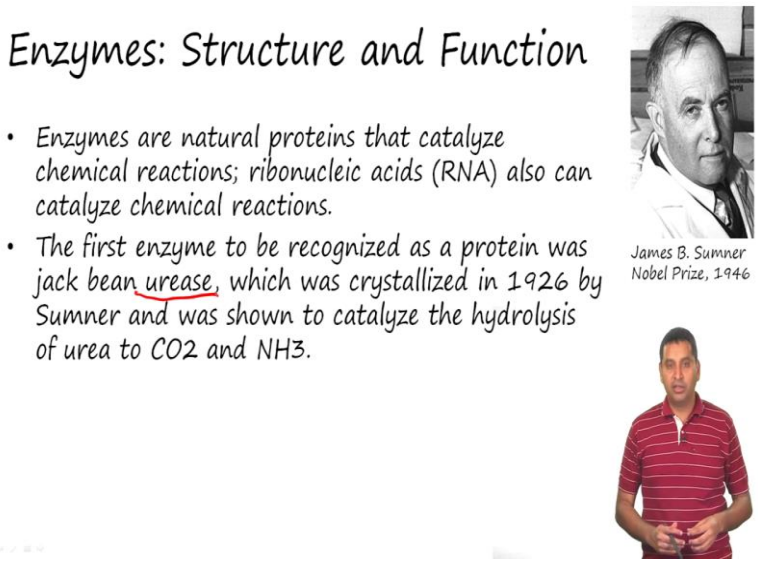

jack bean and it was crystallized in 1926 by Sumner, Ok. James Sumner subsequently won the Nobel Prize in 1946 for this discovery

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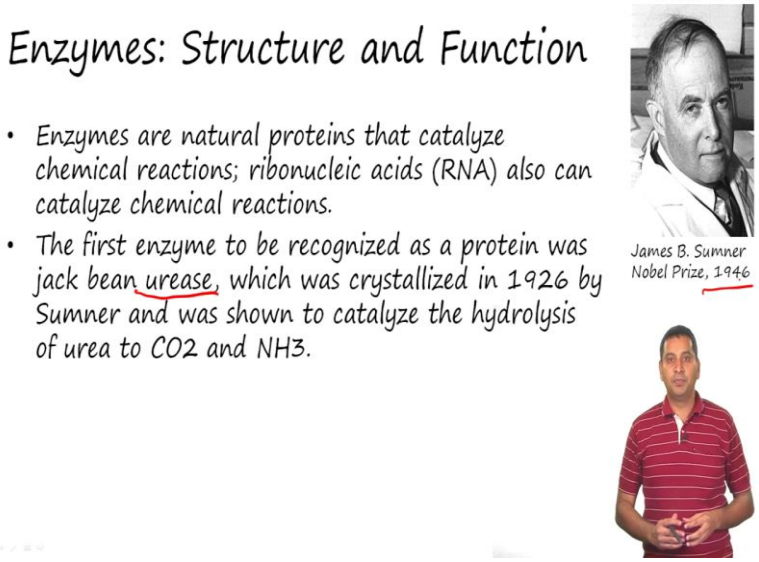

and this enzyme catalyzes the reaction of urea to carbon dioxide and ammonia.

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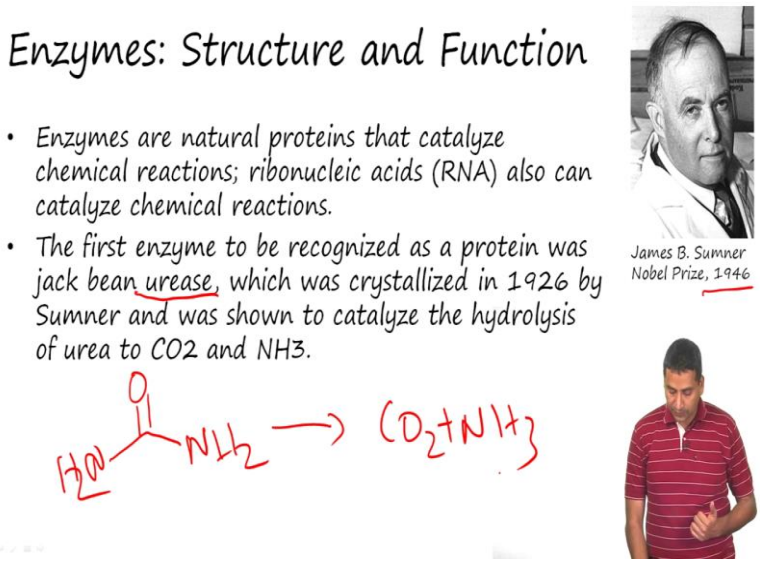

By the 1950s,

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- By the 1950s, hundreds of enzymes had been discovered, and many were purified to homogeneity and crystallized.

there were hundreds of enzymes that had been discovered and many were purified and also crystallized, Ok. In 1960,

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- By the 1950s, hundreds of enzymes had been discovered, and many were purified to homogeneity and crystallized.
- In 1960, Hirs, Moore, and Stein were the first to sequence an enzyme, namely, ribonuclease A, having only 124 amino acids (molecular mass: 13,680 Da).
- This was an elegant piece of work, and William H. Stein and Stanford Moore shared the Nobel Prize in chemistry in 1972 for the methodology of protein sequencing, which was developed to determine the ribonuclease A sequence.

the first sequence of an enzyme was discovered by these three scientists and

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this was of ribonucleus A which had only 124 amino acids, Ok.

And the molecular weight was around

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kiloDalton, 14000 Dalton and this is really elegant piece of work and Stein and Moore shared a Nobel Prize in 1972 for discovering the methodology of protein sequencing, Ok.

And so therefore, by the 1950s and 60s, people had not only isolated a protein but were also able to sequence it and identify what sequence was, right.

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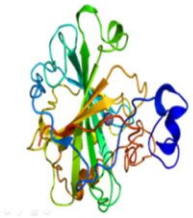
- Enzymes can have molecular masses of several thousand to several million daltons, yet catalyze transformations on molecules as small as carbon dioxide or nitrogen.



Subsequently enzymes which have masses of several thousands to millions to Daltons are, have been discovered. But they can also carry out transformations of extremely small molecules such as carbon dioxide or even nitrogen,

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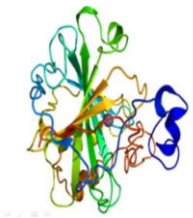
- Enzymes can have molecular masses of several thousand to several million daltons, yet catalyze transformations on molecules as small as carbon dioxide or nitrogen.



Ok.

So here is an example of

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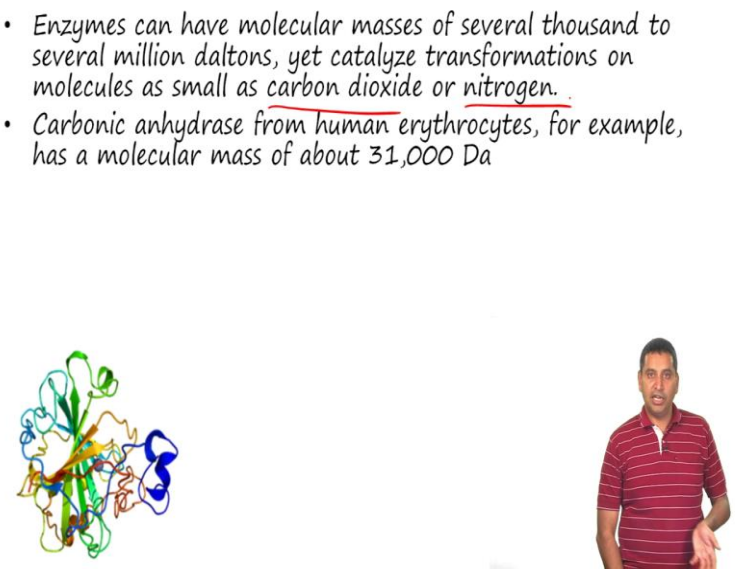

carbonic anhydrase which has a molecular weight of 31000 Dalton but catalyzes the reaction of

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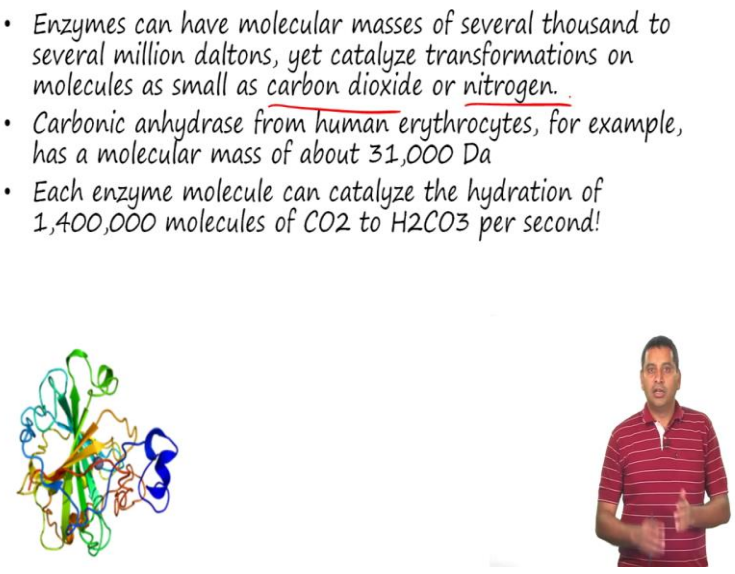

breaking of, hydrolysis of carbon dioxide to bicarbonate or carbonic acid, Ok. Each enzyme molecule can catalyze the hydration of 1 point 4 million molecules of CO2, Ok. So it is a huge number and the efficiency is extremely high.

But keep in mind that the molecular weight of this protein is 31000, Ok so a catalyzed reaction

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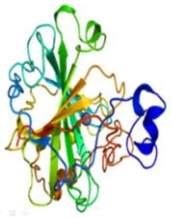
- Enzymes can have molecular masses of several thousand to several million daltons, yet catalyze transformations on molecules as small as carbon dioxide or nitrogen.
- Carbonic anhydrase from human erythrocytes, for example, has a molecular mass of about 31,000 Da
- Each enzyme molecule can catalyze the hydration of 1,400,000 molecules of CO2 to H2CO3 per second!
- This is almost 10^8 times faster than the uncatalyzed reaction, which is actually on the low side of rate enhancements for an enzyme.



is about 10 power 8 times faster than the uncatalyzed reaction, Ok and this rate enhancement is actually on the lower side for a typical enzyme catalyzed process.

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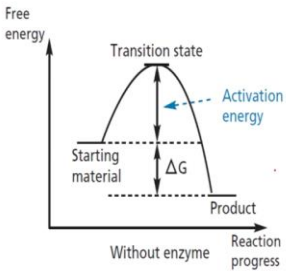

- In general, enzymes function by lowering transition state energies
- A high-energy transition state that must be formed before the starting material (the substrate) can be converted to the product.

In order to understand how this catalysis occurs, let us look at a typical free energy diagram. So here is, on the y axis of free energy and here is the reaction coordinate

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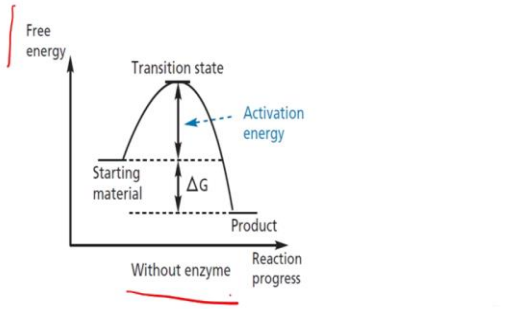

- In general, enzymes function by lowering transition state energies
- A high-energy transition state that must be formed before the starting material (the substrate) can be converted to the product.

and this is the starting material which is represented here.

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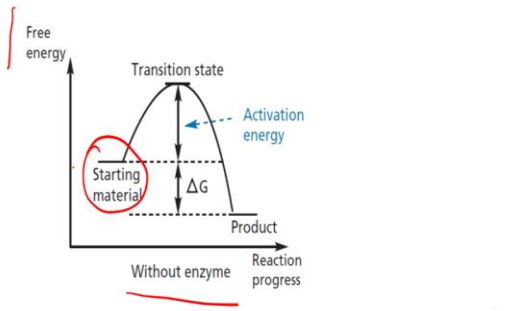

- In general, enzymes function by lowering transition state energies
- A high-energy transition state that must be formed before the starting material (the substrate) can be converted to the product.

And here is the product.

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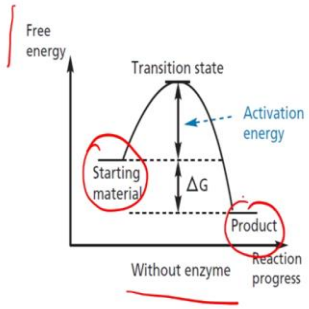

- In general, enzymes function by lowering transition state energies
- A high-energy transition state that must be formed before the starting material (the substrate) can be converted to the product.

This difference in energy is delta G for the reaction

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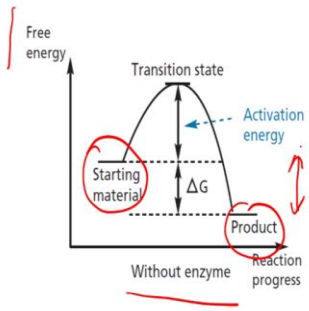

- In general, enzymes function by lowering transition state energies
- A high-energy transition state that must be formed before the starting material (the substrate) can be converted to the product.

and here is what we call as activation energy or

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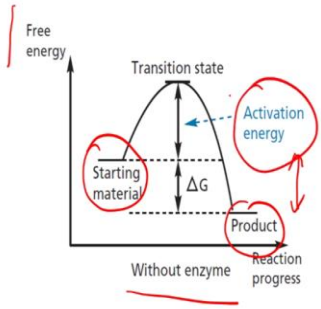

- In general, enzymes function by lowering transition state energies
- A high-energy transition state that must be formed before the starting material (the substrate) can be converted to the product.

the barrier required for the reaction to occur, Ok.

The general understanding of how enzymes function is that they lower the barrier

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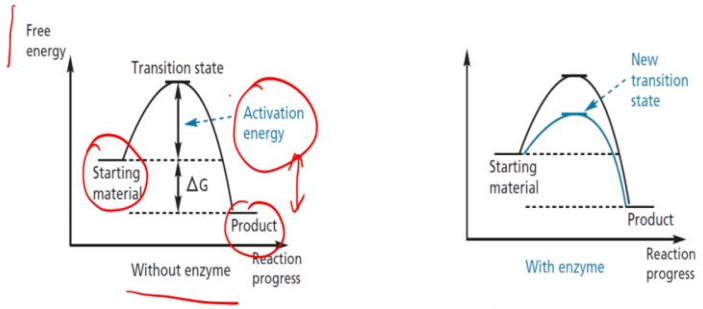

- In general, enzymes function by lowering transition state energies
- A high-energy transition state that must be formed before the starting material (the substrate) can be converted to the product.

or reduce the energy required for the starting material to go to the product. And so therefore

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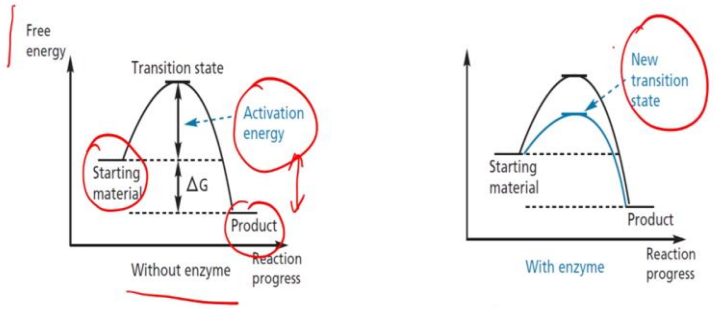

- In general, enzymes function by lowering transition state energies
- A high-energy transition state that must be formed before the starting material (the substrate) can be converted to the product.

enzymes create new transition state which is lower in energy compared to the original transition state for conversion of a starting material to a product, Ok.

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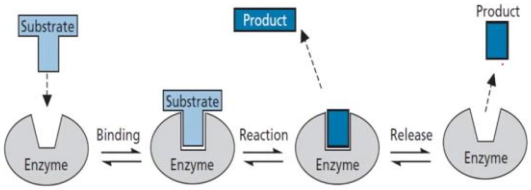

- There is an **active site** in an enzyme
- However, the site could be a groove, hollow, or gully allowing the substrate to sink into the enzyme.
- Normally, the active site is more hydrophobic in character than the surface of the enzyme
- A suitable environment for many reactions which are difficult in an aqueous environment.

Now as we, we can imagine that an enzyme must have a site at which the substrate must bind, Ok. This is called the active site. Now this site can be a groove, a hollow, a gully or any of these small structures where the substrate can actually get in, right.

And once the substrate gets in

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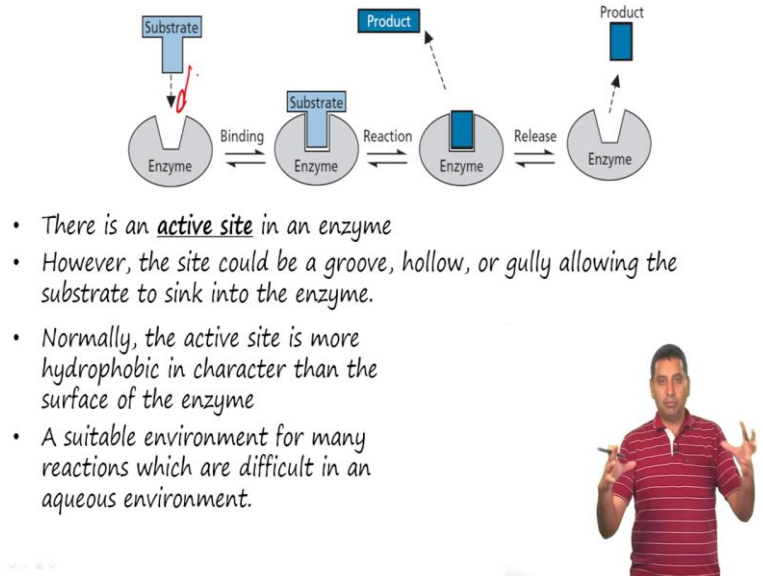

there is a binding that occurs and this binding results in a change in the conformation and of course the enzyme can now do whatever it is supposed to do and then finally the product

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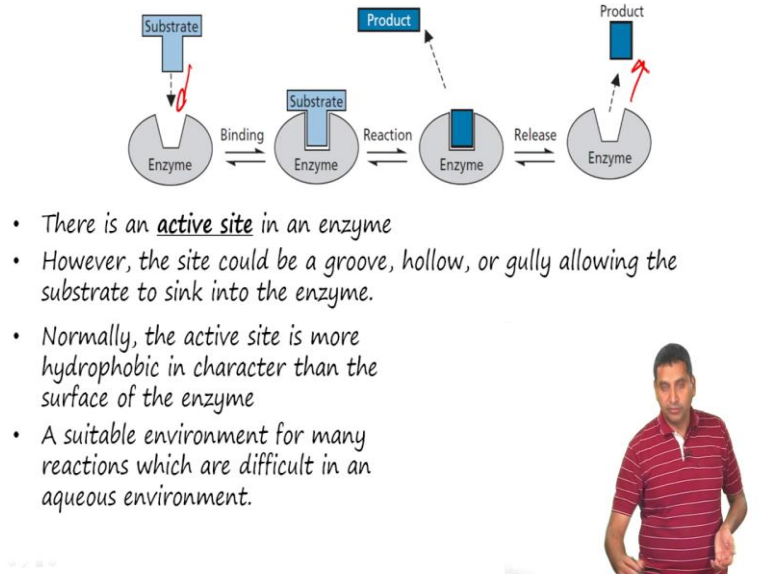

is released.

Now this bind site or the active site is provides a suitable environment for many difficult-to-carry-out reactions. And not just that some of these reactions are not even possible in aqueous environment and as we discussed earlier the hydrophobic environment that is produced in the active site is responsible for binding.

And subsequently we look at some of the residues that can help with catalysis but binding is a important part of enzyme catalysis.

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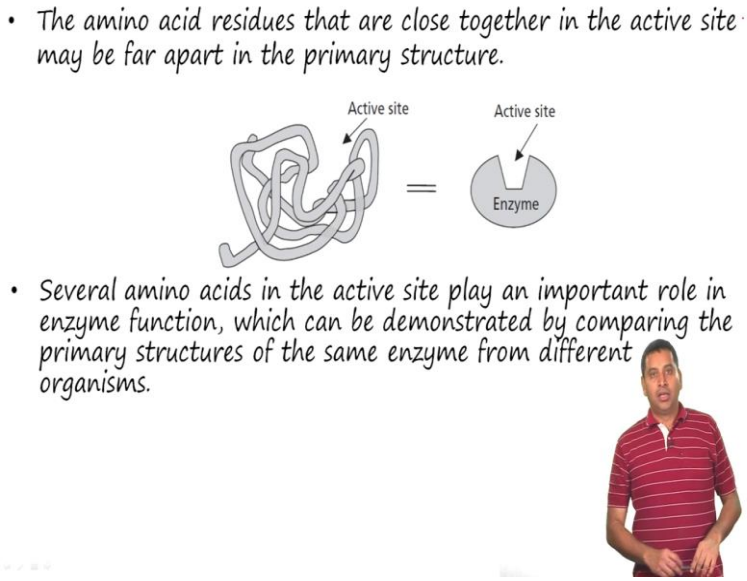

So the active site contains an, several amino acids which play an important role in catalysis, Ok.

Now one can derive some information about how well the active site is conserved across various organisms evolutionary by comparing the sequence or the primary structure of enzymes from various organisms, Ok.

Now it is

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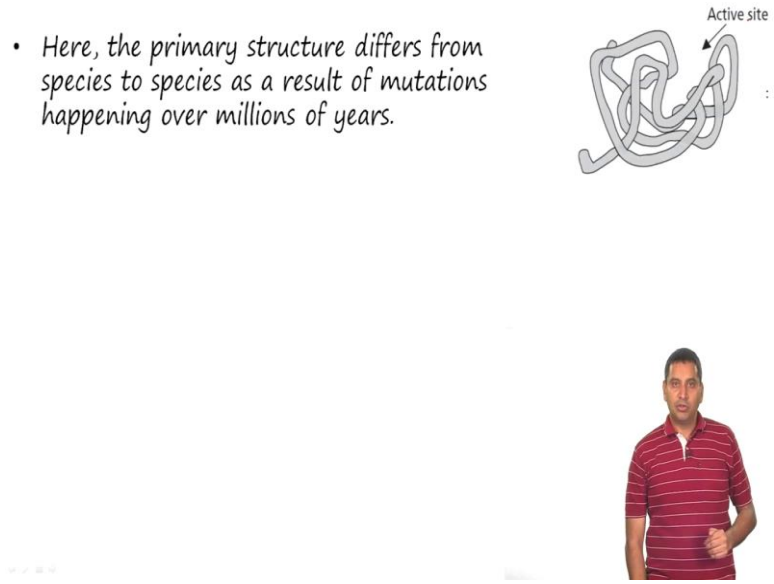

possible that the primary structure differs from species to species because this primary structure has gone through a number of iterations or mutations happening over millions of years,

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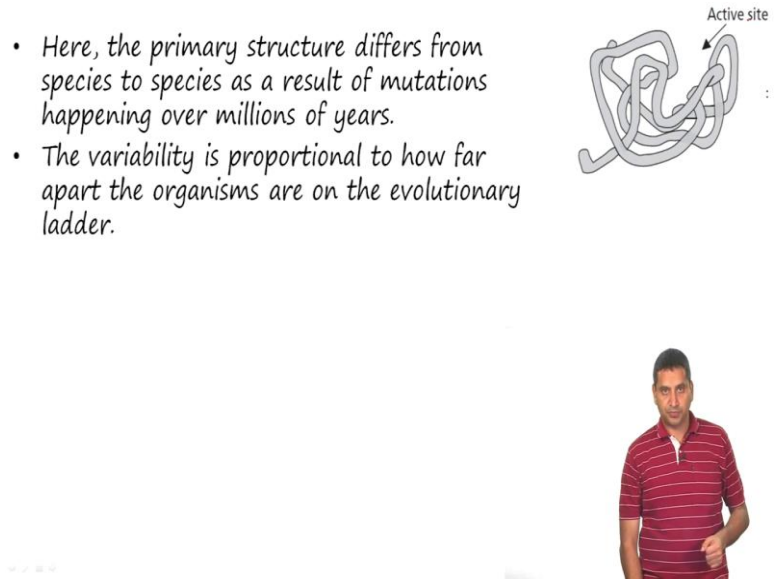

Ok. The variability in this is somewhat proportional to how far apart these organisms are on the evolutionary ladder.

So for example, something that is a evolutionarily in a early phase can have very different primary structure from some enzyme which is in humans,

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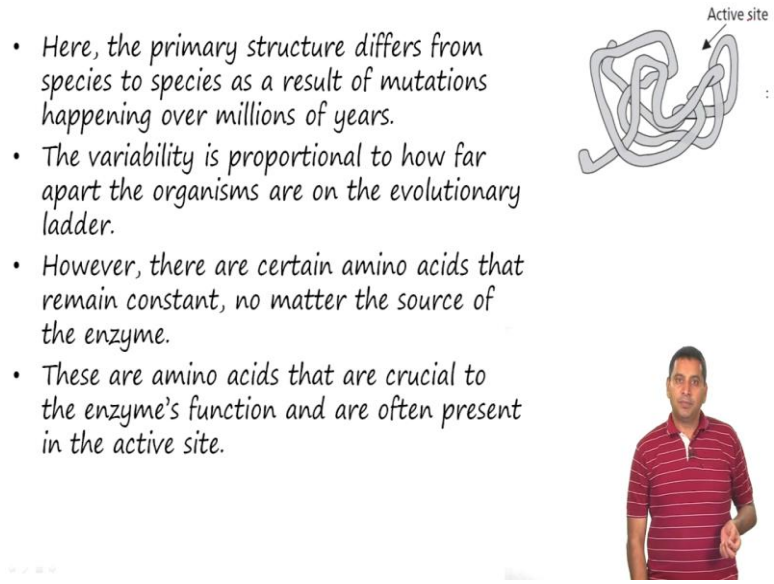

right. But there are amino acids which remain constant through evolution and these can somewhat, can be crucial to the enzyme's function, Ok. And these amino acids can often present themselves in the active site,

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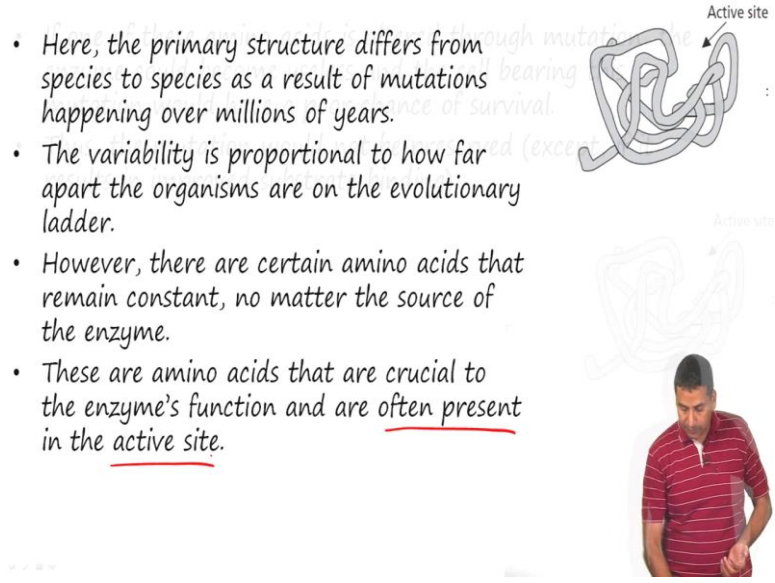

right.

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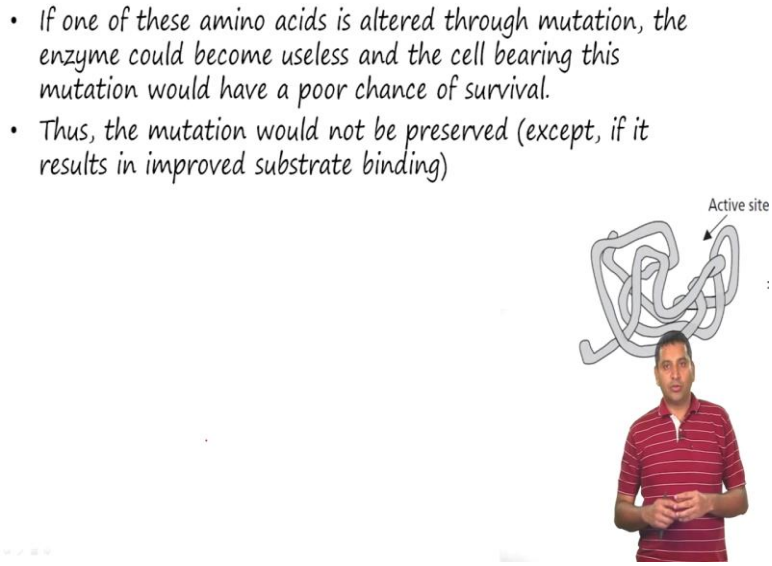

Now it is also possible that once the active site has evolved, it is also possible that it can be altered through mutation. Now if the mutation is not useful then the enzyme can lose its function and the cells which lose the function can, have a poor chance of survival.

And, so therefore there are enzymes which are very crucial to the functioning of a cell wherein even a small change in a particular amino acid can have dramatic impact on survival. Now it is also possible that this mutation would therefore not be preserved unless it, of course improves the substrate binding.

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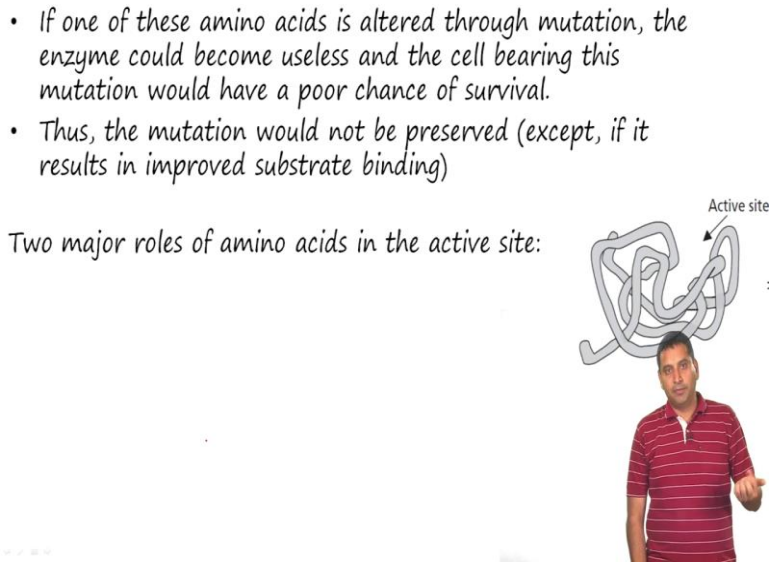

So to sum it up, there are two major roles of amino acid in the

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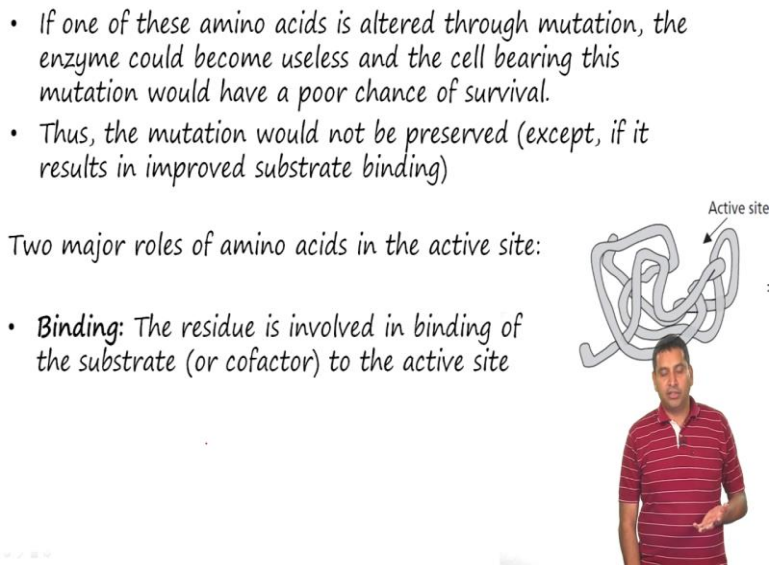

active site. The first one is to bind the substrate or in, we will look at shortly, it has to bind a cofactor and the second part is in

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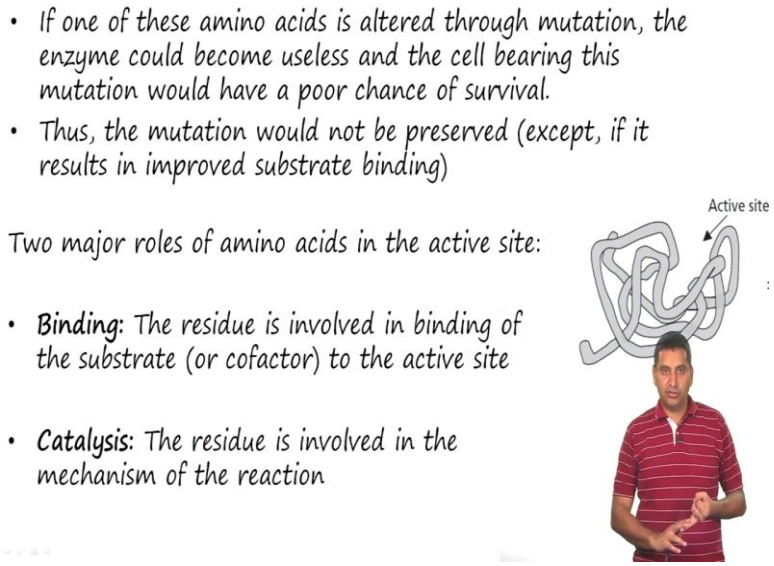

catalysis where the transitional state energy is lower, right. And so both these processes are very crucial for an enzyme to function.

The third part which is basically disassociation is also important because if the product binds very strongly to the enzyme then the catalysis, the turnover is not very good and the catalyst is not going to function very well,

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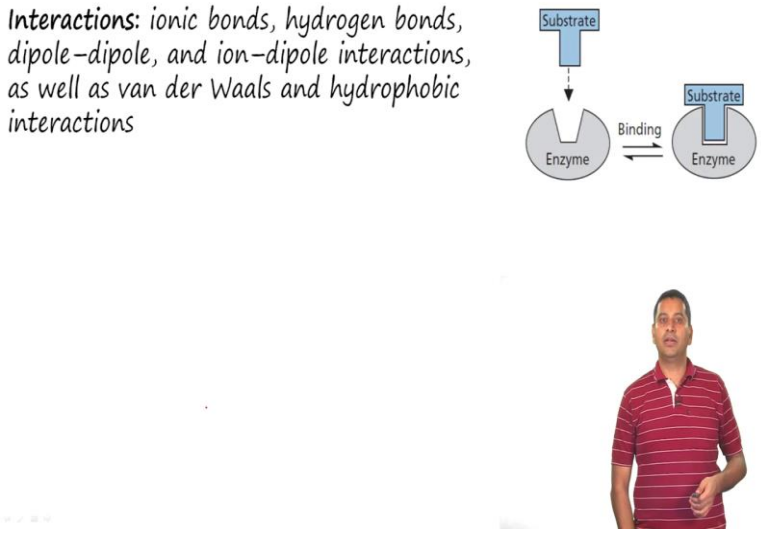

Ok.

Now as we looked at earlier, the binding interactions between the substrate and the enzyme are largely non-covalent in nature. And these non-covalent interactions we have already classified them as ionic bonds, hydrogen bonds, dipole-dipole or ion-dipole interactions and hydrophobic interactions and vander Waal's interactions, Ok.

So

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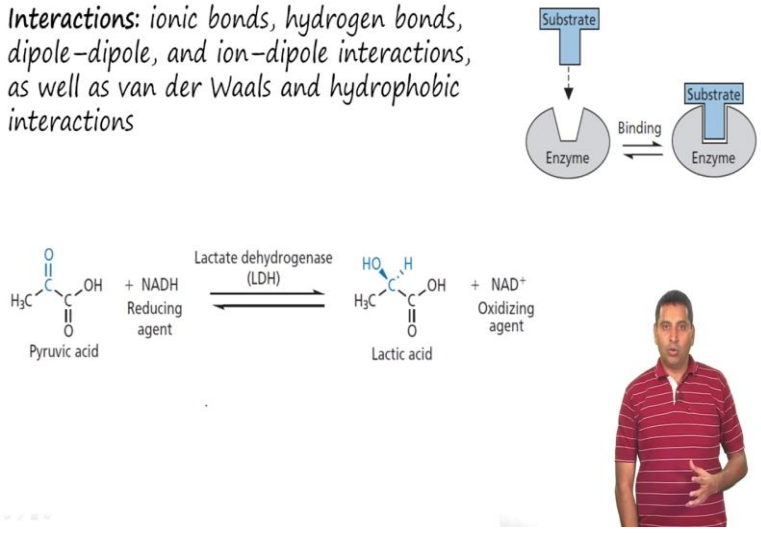

one of the examples we can look at is the conversion of pyruvic acid to lactic acid by this enzyme called as lactate dehydrogenase. So here it uses NADH as a

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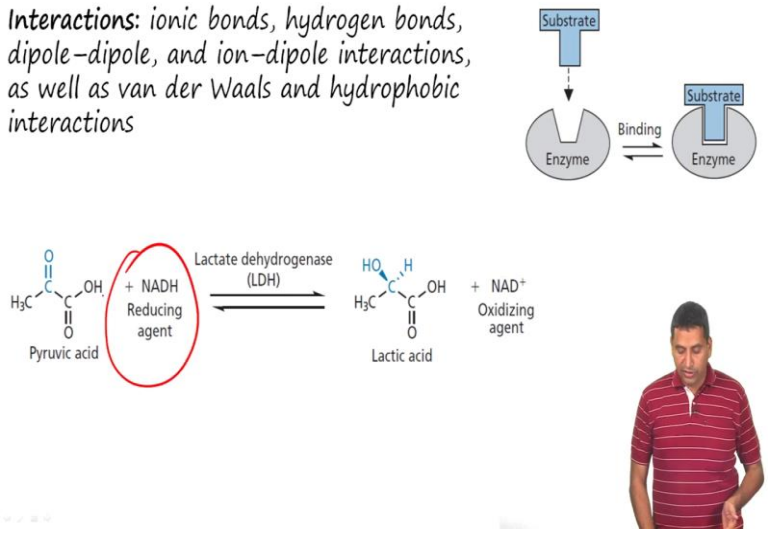

reducing agent and this ketone

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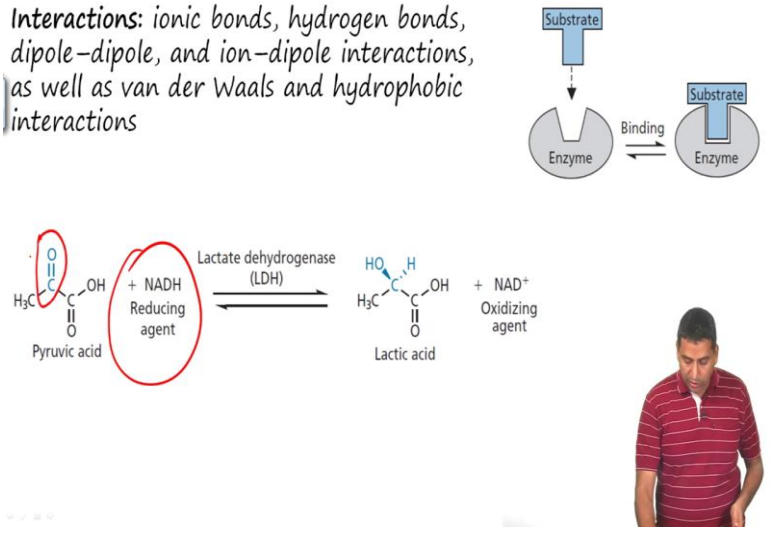

is converted to an alcohol.

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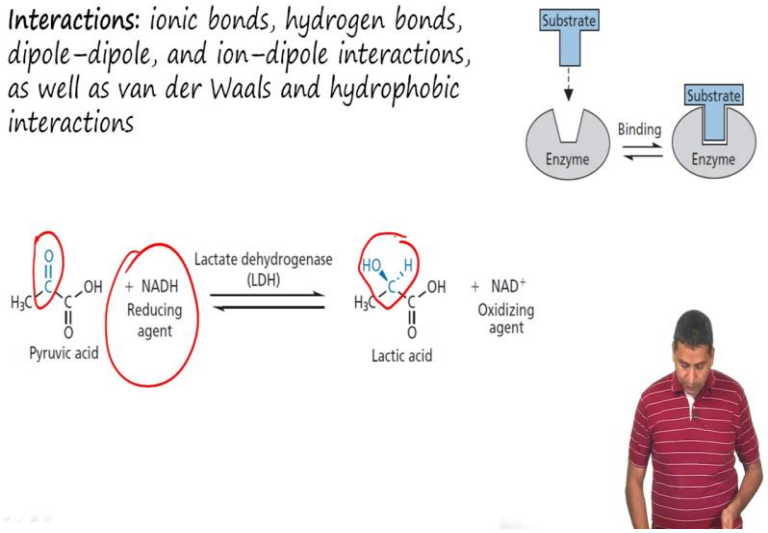

If we look

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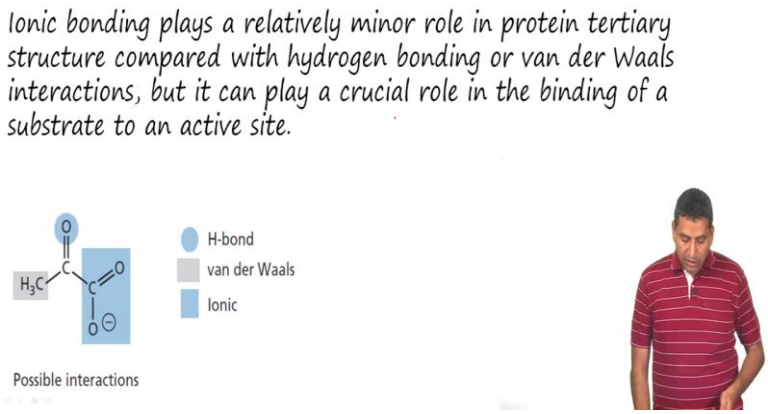

at the kind of interactions that the substrate would have with the protein then you would see that there is a metal group which can involve

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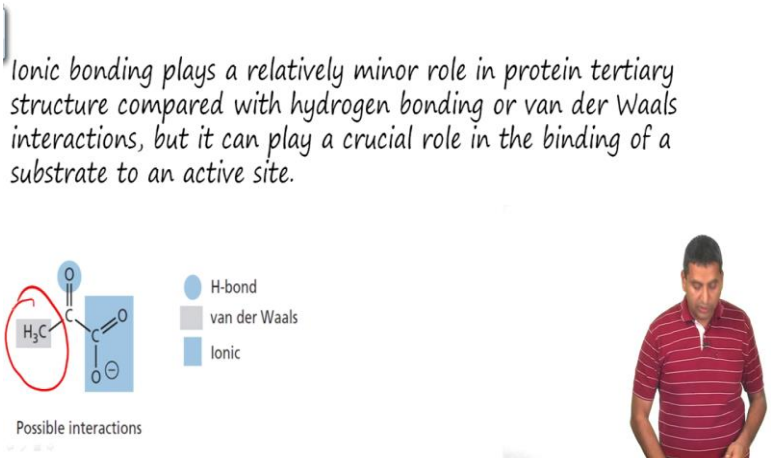

itself in hydrophobic interactions or vanderWaal's interactions and there is a

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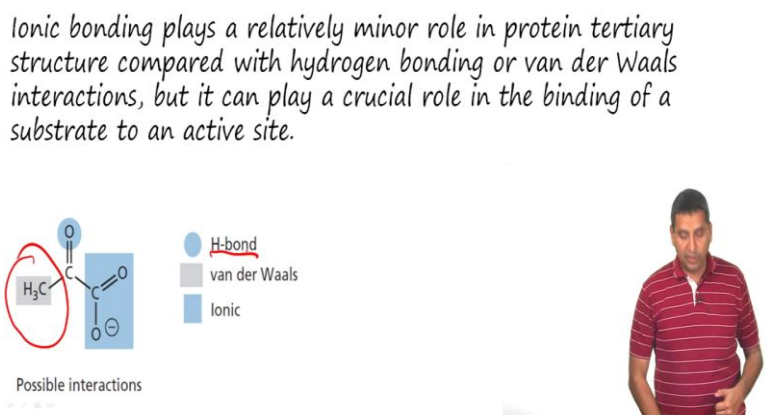

hydrogen bonding capability, all these carbons and the O minus can involve themselves in hydrogen bonding.

And of course there is an ionic interaction

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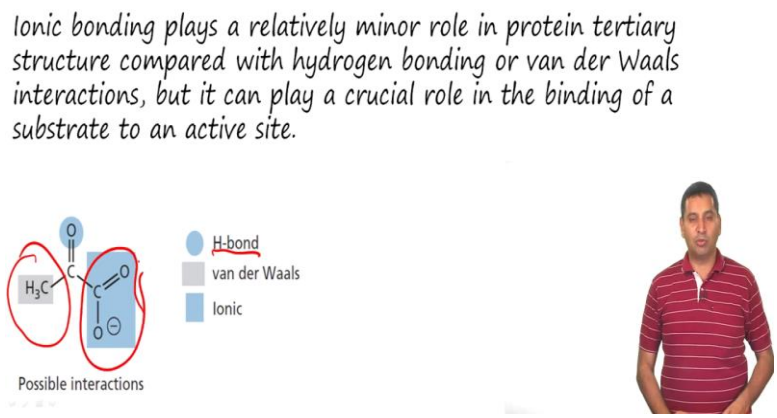

here in the carboxylate, right.

So if we have to divide these, this molecule into various modes of binding, then all these 3 interactions must play or could play an important role in order for

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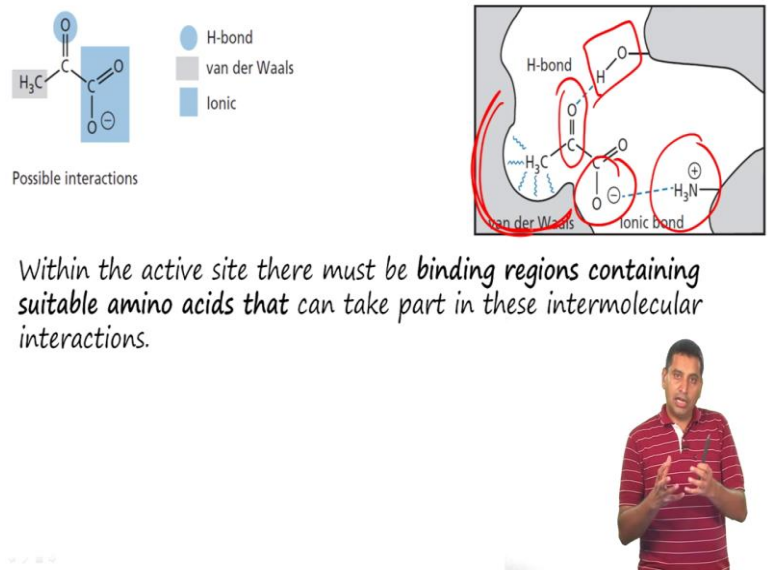

effective binding to take place, Ok.

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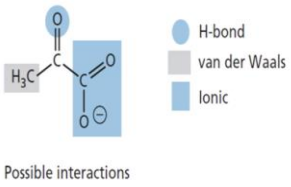

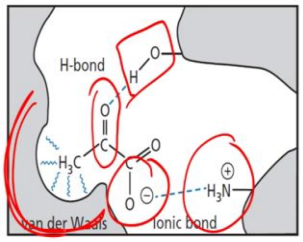

Within the active site there must be **binding regions containing suitable amino acids that** can take part in these intermolecular interactions.

A knowledge of how a substrate binds to its active site is invaluable in designing drugs that will target specific enzymes

Now, therefore the knowledge of how a substrate binds to the active site is really important because if we want to inhibit that enzyme or if we want to design a drug that can inhibit that particular target enzyme.

So

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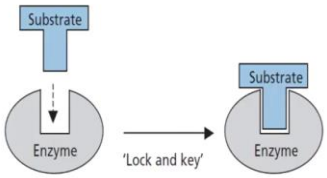
Lock and Key hypothesis: The enzyme has a rigid structure and a specific structure that the substrate (key) will fit into this...



However, this scenario does not explain how some enzymes can catalyse a reaction on a range of different substrates.

let us understand how this, the enzyme catalyst reactions occur. The first major hypothesis that was presented was the Lock and Key Hypothesis. Now the Lock and Key Hypothesis is a very simple mechanism.

So you have a lock which has a particular groove in it which is very selective to a key which is a substrate. Now once the key presents itself to the lock, it binds and forms a very rigid binding. And therefore then you can have the catalysis occurring and the dissociation of the product.

Although this is a very interesting and important hypothesis it does not really explain how some enzymes can catalyze a range of reactions. So if you have a lock that is very specific to a particular key then how is it that we are able to open the lock with number of different types of keys? So

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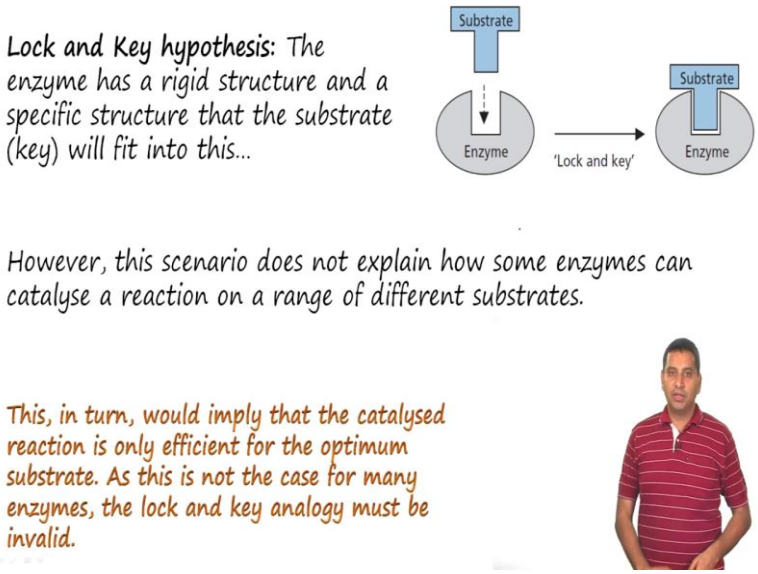

therefore the lock and key analogy is not exactly something that is very optimum for us to understand enzyme catalysis.

The next set of

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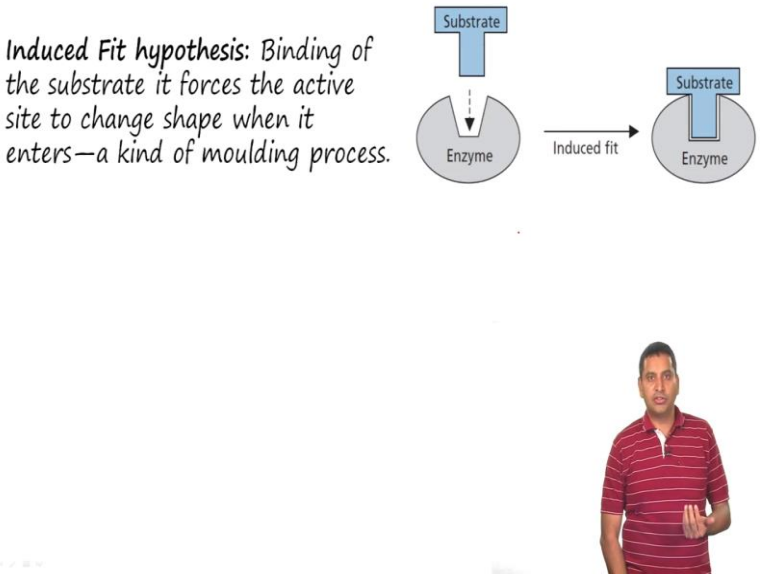

hypothesis that came up was the Induced Fit Hypothesis, Ok. So here the enzyme binds to the substrate but during the binding the shape of the enzyme changes and this is some sort of a moulding process which then results

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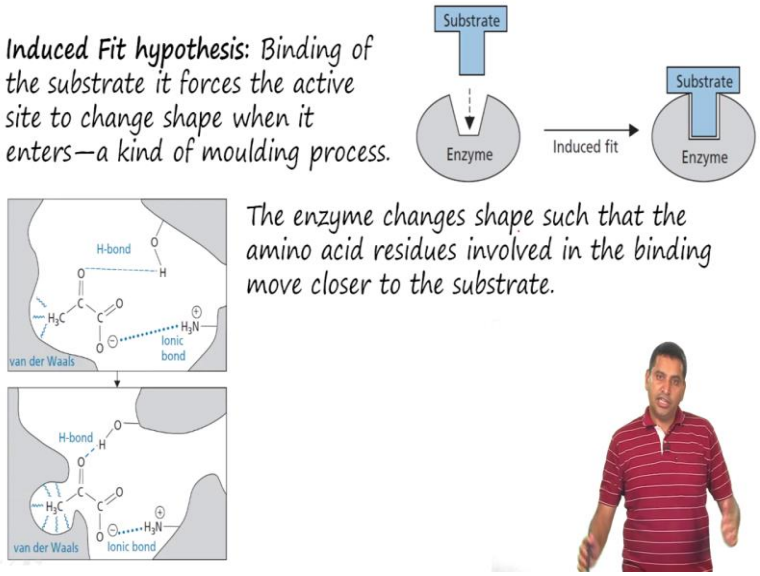

in effective binding.

For example you have pyruvate here which is sitting over here and

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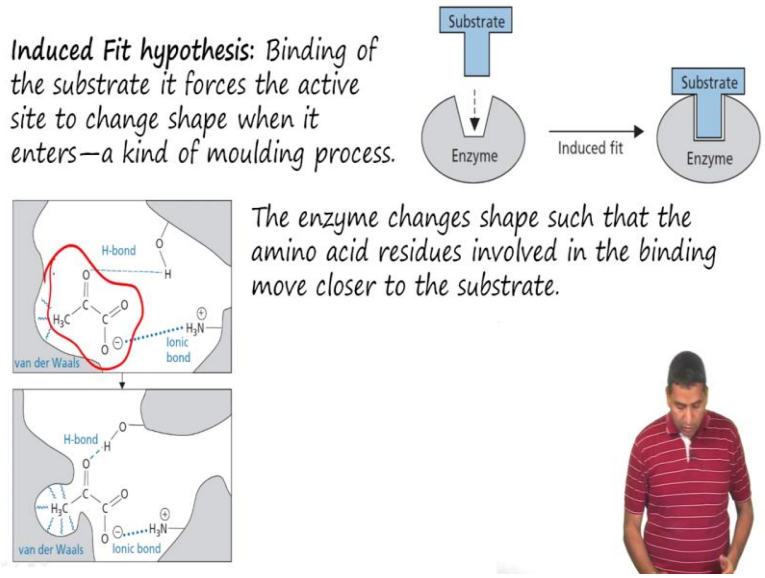

now once pyruvate binds to or starts to bind to the active site, there are conformational changes which can occur which help with improved binding

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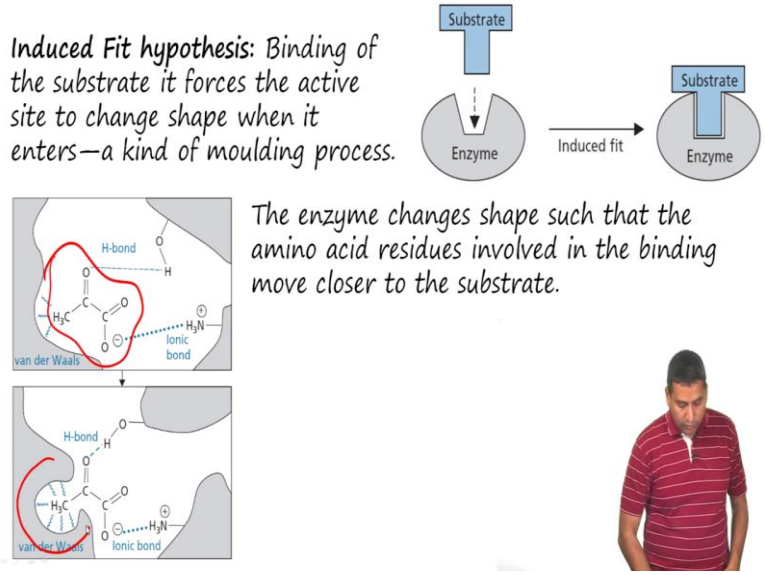

of certain areas of the substrate with certain areas of the active site.

So this helps us understand why an enzyme can catalyze reactions with a number of substrates which do not necessarily have the same structural features.

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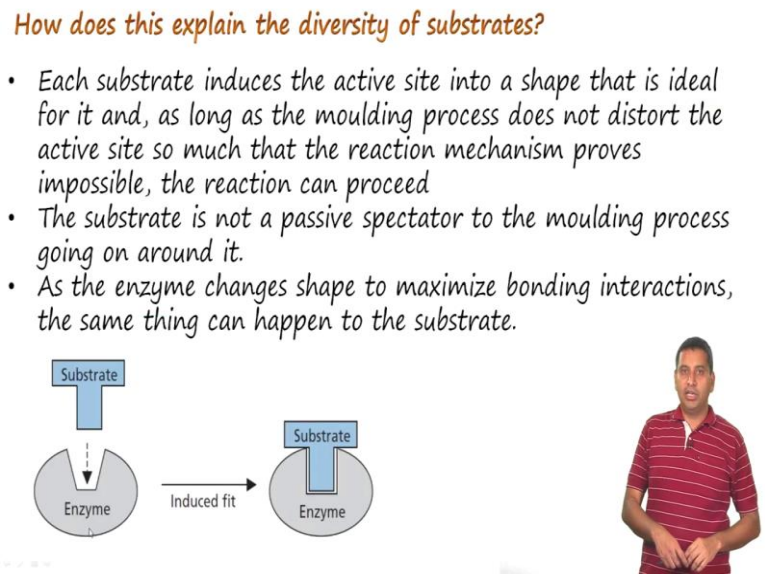

Now each substrate changes the active site into a shape that is ideal for it, Ok. So as long as the moulding process does not distort the active site such that the mechanism does not occur, the substrate would be turned over, Ok.

Now it is obvious that the substrate itself is not a passive spectator and it also undergoes certain rotations and conformational changes which help with improving binding interactions, Ok, right. So therefore the enzyme changes shape to maximize the binding interactions and the same thing can also happen with the substrate.

So this hypothesis which is the induced fit hypothesis helps us understand how an enzyme functions and how an enzyme can actually catalyze a range of reactions with varied substrates.

And from the substrate standpoint when you have a diversity of substrates this, since the, there is an induced fit that occurs, the binding can help weaken certain bonds. It can stretch certain bonds. And it can strengthen other bonds and therefore this interaction can be useful in accelerating the transformation that can occur.

So the enzyme undergoes a conformational change. It starts to change its shape. The substrate can also undergo a conformational change. And this conformational change results in stretching or weakening of bonds that need to be broken. One can explain how the transition state goes down in energy.

It is also possible that maximizing these bonding interactions may force the substrate into the ideal conformation where the reaction can occur in a very facile manner, Ok. Once it is bound the substrate is, has to undergo a reaction.

It is almost like how you fix the victim which cannot, who cannot evade the attack and once you have weakened the defenses, which is by weakening bonds or stretching bonds, the reaction is easier because the activation energy is low.

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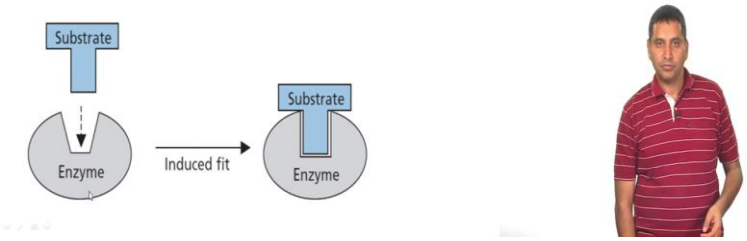
Binding of the Substrate...



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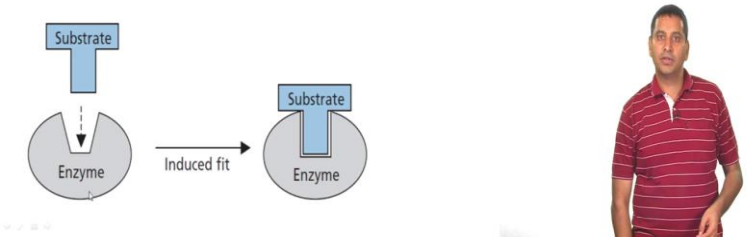
Binding of the Substrate...

- The binding interactions with the active site must be suffi ciently strong to hold the substrate for the subsequent reaction, but they cannot be too strong.
- If they were, the product might also be bound strongly and fail to depart the active site.
- This would block the active site of the enzyme and prevent it from catalysing another reaction... Therefore, a balance must be struck.



As I mentioned earlier, these binding interactions with active sites have to be strong. But they cannot be so strong that they do not let go after the reaction is done. If the product is stuck, it does not dissociate it would basically block the active site from catalyzing another reaction.

Therefore it is important that a balance be struck here and you would want adequate binding and adequate dissociation for a reaction to be effective,

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Transition State

Ok.

Now let us look at what a

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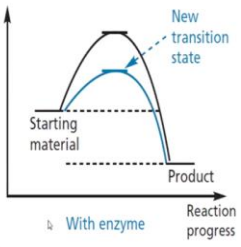
Transition State

- The transition state involved in the enzyme-catalysed reaction. Indeed, the binding interactions involved are stronger than those binding the substrate, which means that the transition state is stabilized relatively more than the substrate. This results in a lower activation energy compared with the non-catalysed reaction.



transition state is. A transition state, as we looked at earlier is a high energy situation between the starting material and product. And now since we have suggested that

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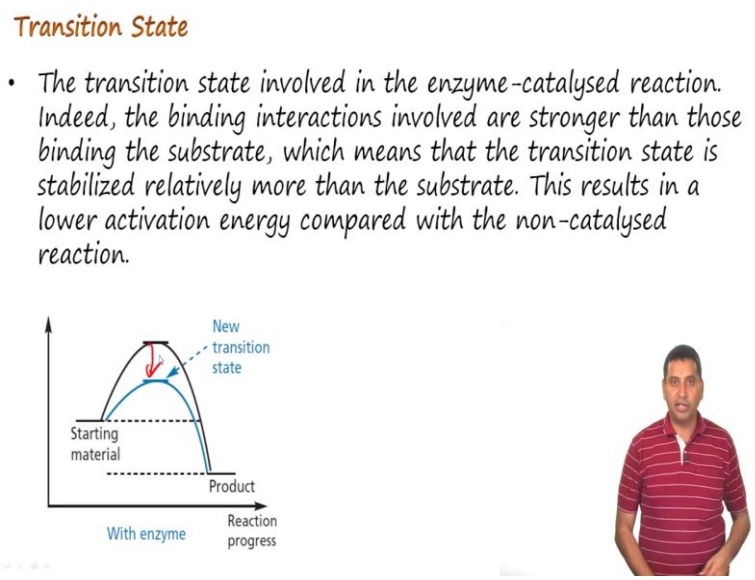

the enzyme reduces the transition state energy, it is, the reaction goes faster when compared to the non-catalyst reaction,

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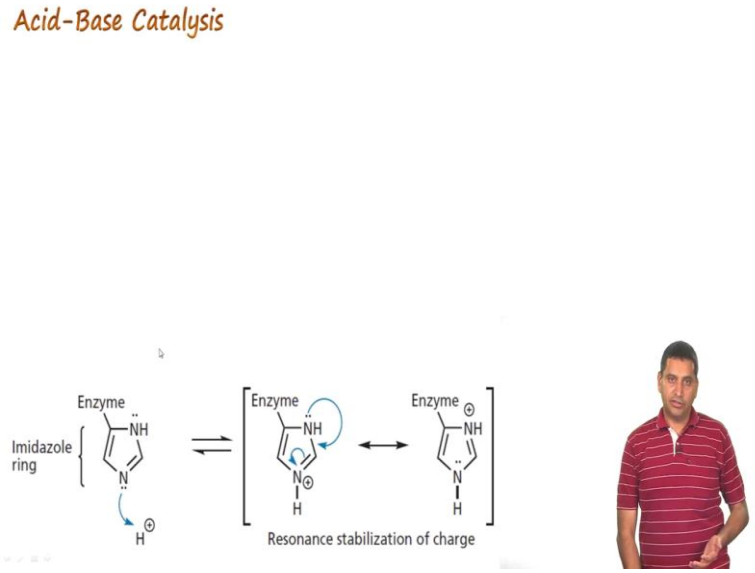

Ok.

One of the ways in which, so histidine is important because histidine is a source of proton and since water molecules are not normally present inside the protein, this can, histidine can act as a source of acid or base and therefore an important way to carry out acid base catalysis,

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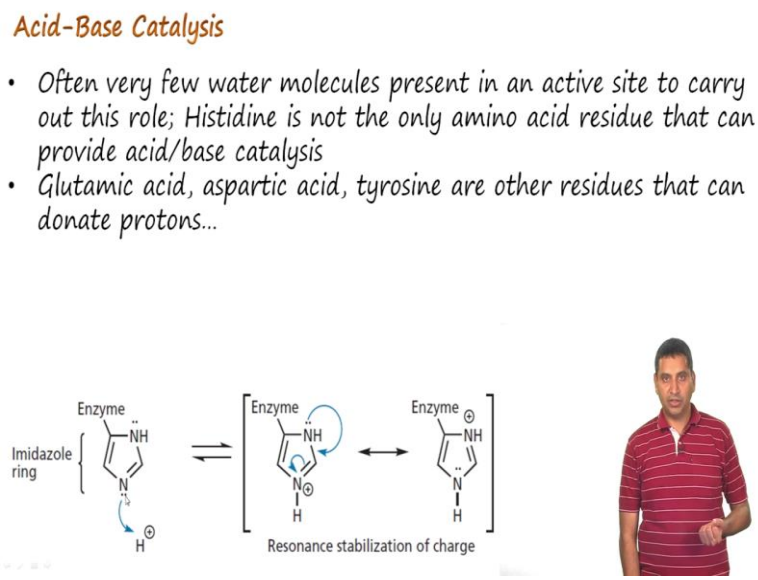

Ok.

There are other amino acids such as glutamic acid, aspartic acid or even tyrosine which can donate protons but this is one way. Therefore it can donate protons and therefore it can act as an acid.

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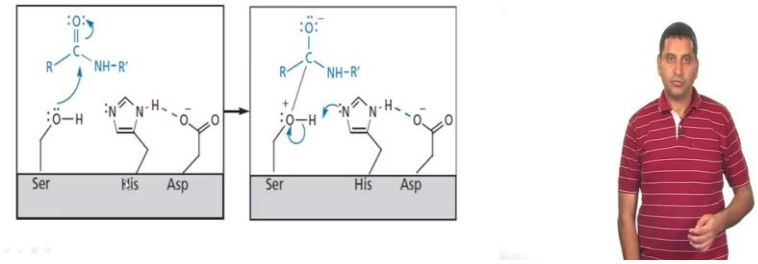

The other important way in which catalysis occurs is through the use of nucleophilic groups, Ok.

One of the best nucleophiles

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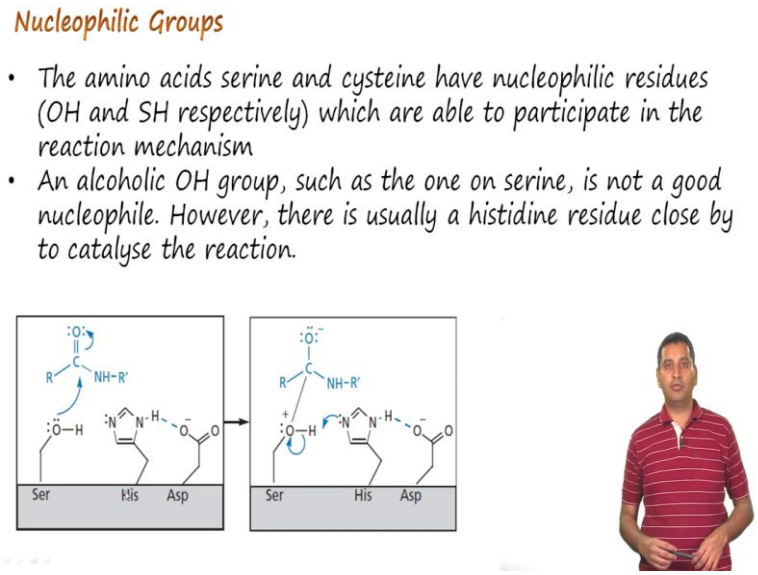

inside the cell contains the thiol which is the cysteine residue. And the pKa of thiols are typically in the range of 7 to 9 but they can go down depending on the local environment. Thiolates are excellent nucleophiles and therefore they can act as a certain nucleophile in certain reactions.

Serine, on the other hand, contains an alcohol and alcohols as you know are not very good nucleophiles. However, when a histidine residue is present right next to serine

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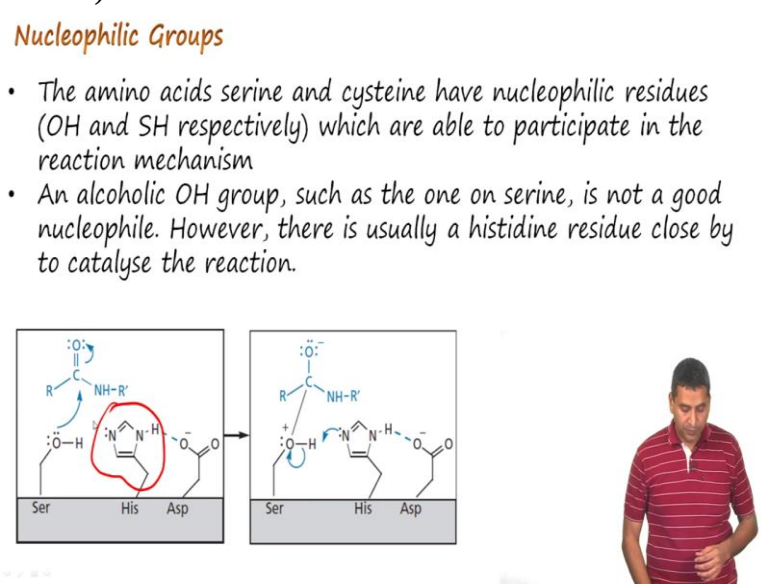

it is possible that the histidine can pick up the proton from serine during a nucleophilic attack. So here is an example of an amide being hydrolyzed by a serine residue, Ok.

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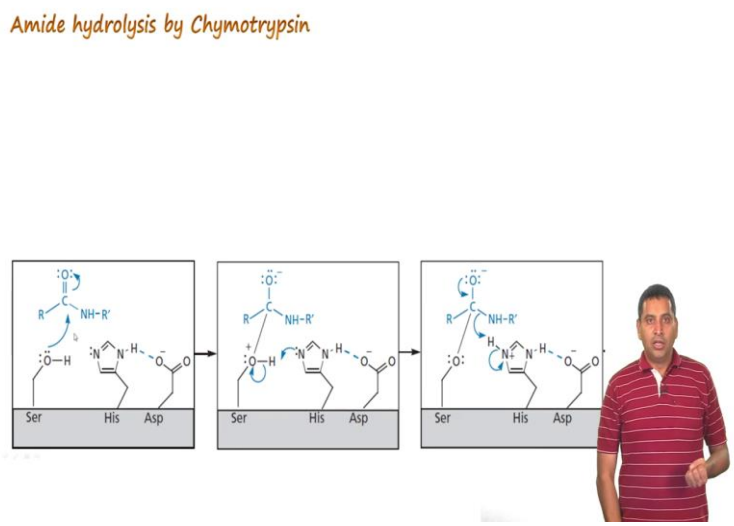

So if you look at this example of chymotrypsin so here a serine residue can donate a pair of electrons.

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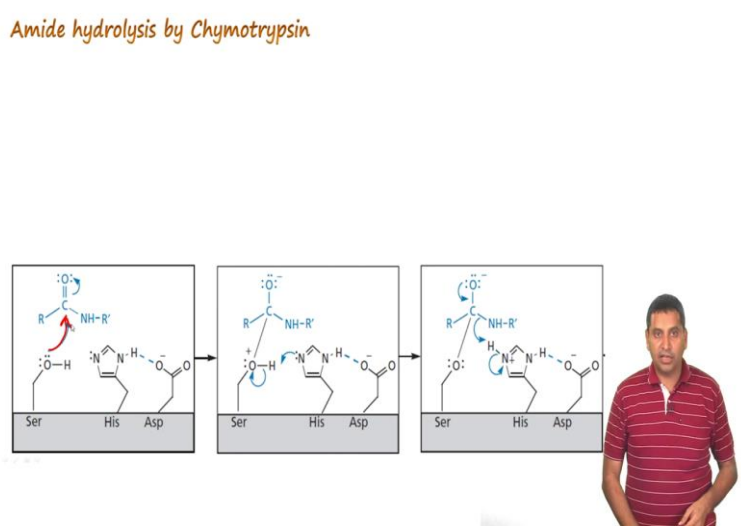

Or it can attack the carbon and subsequently once it forms the protonated

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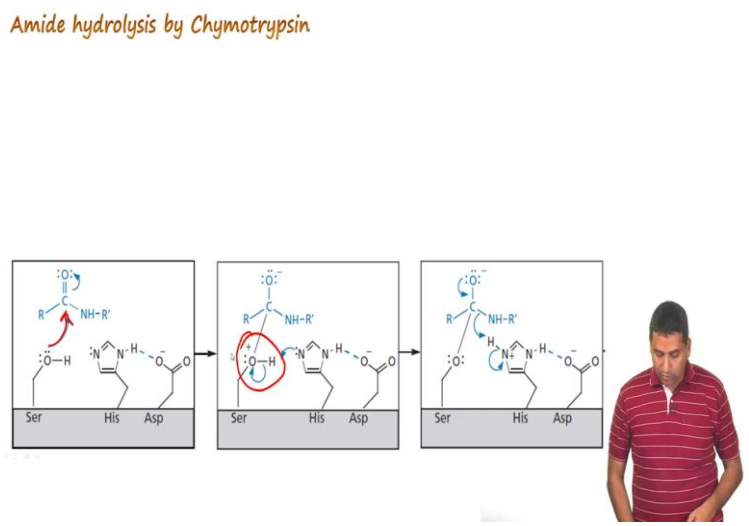

oxonium species, the histidine is present right next to it to pull out the proton and quickly remove the proton from the oxonium species.

And subsequently tetrahedral intermediate which has

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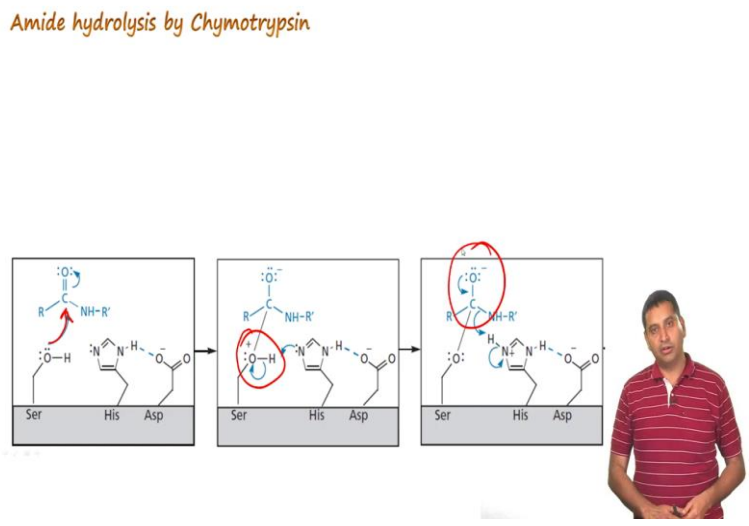

an O minus can pick up a proton from histidine and therefore histidine can act as a acid base catalyst in this case, Ok.

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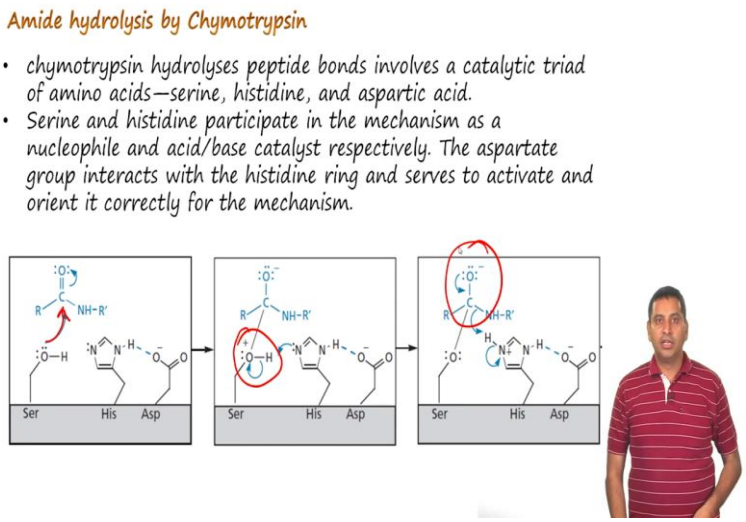

So this is an example of how these three amino acids, serine, histidine and aspartate act as a catalytic triad.