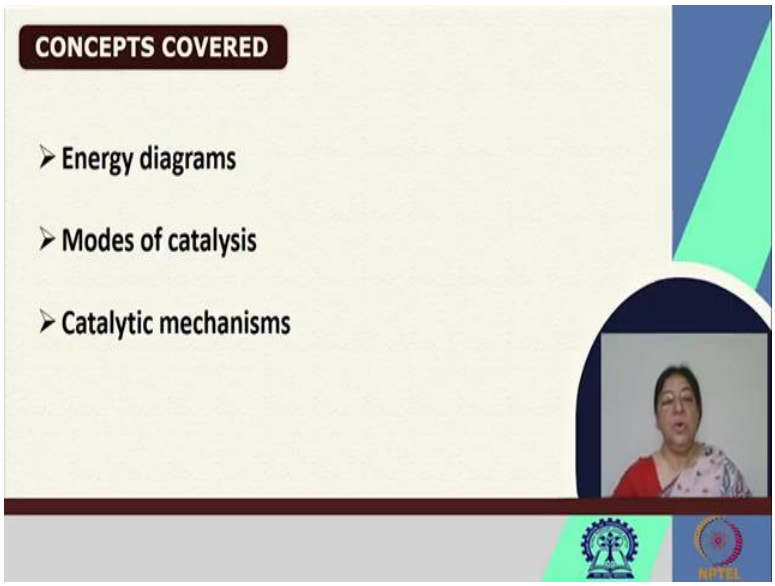
Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 06
Enzymes and Enzyme Mechanisms
Lecture - 28
Enzyme Mechanisms - I

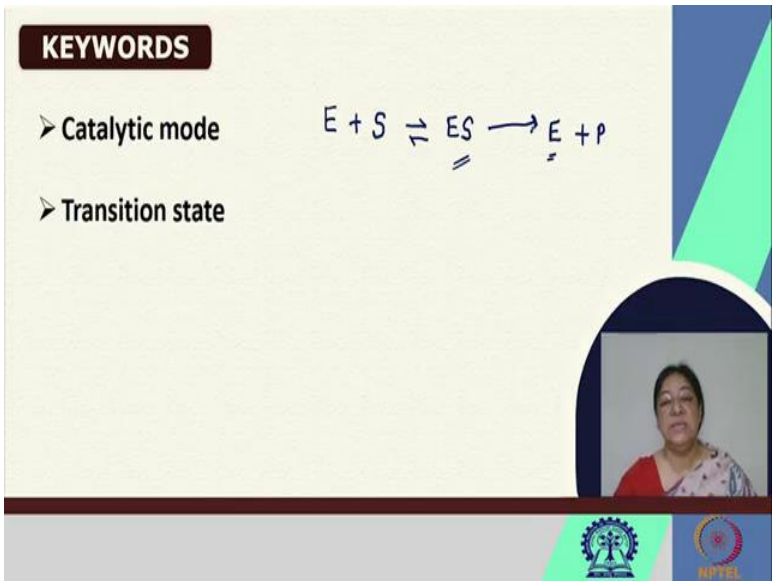
In continuation of our discussion on enzymes, in the previous two lectures we looked at the specific enzyme classes and the geometric and chemical complementarity that is required for an enzyme substrate complex to form. We realize that this is important in the recognition for an enzyme reaction to proceed and the specific catalytic reaction to proceed.

(Refer Slide Time: 00:45)



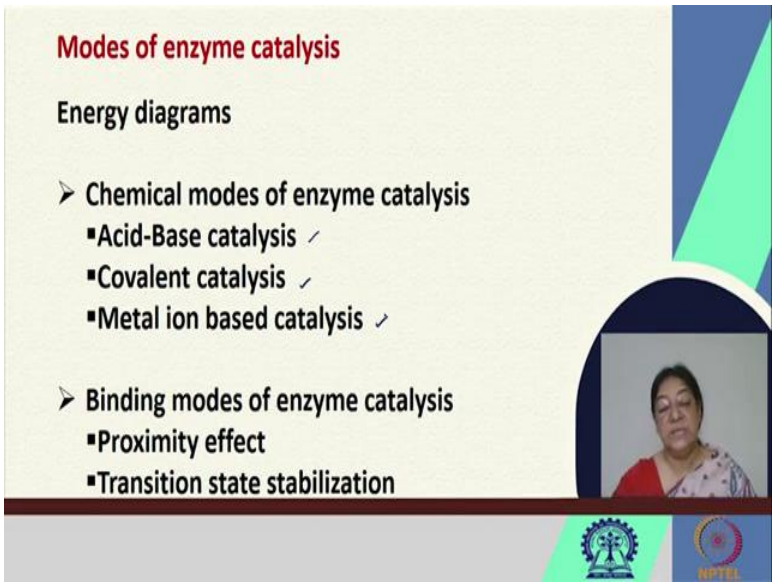
In this lecture we will be looking at energy diagrams, the different modes of catalysis and we will look at specific catalytic mechanisms in the subsequent two lectures.

(Refer Slide Time: 00:59)



In understanding the catalysis, we need to know the formation of the enzyme substrate complex. When we look at the specific enzyme and we understand that we have a substrate that is to bind with it, we know that we have a specific recognition in the formation of this enzyme substrate complex. This enzyme substrate complex is crucial to form our product, where we get back our enzyme in a specific kinetic component, which we will look at in the next module.

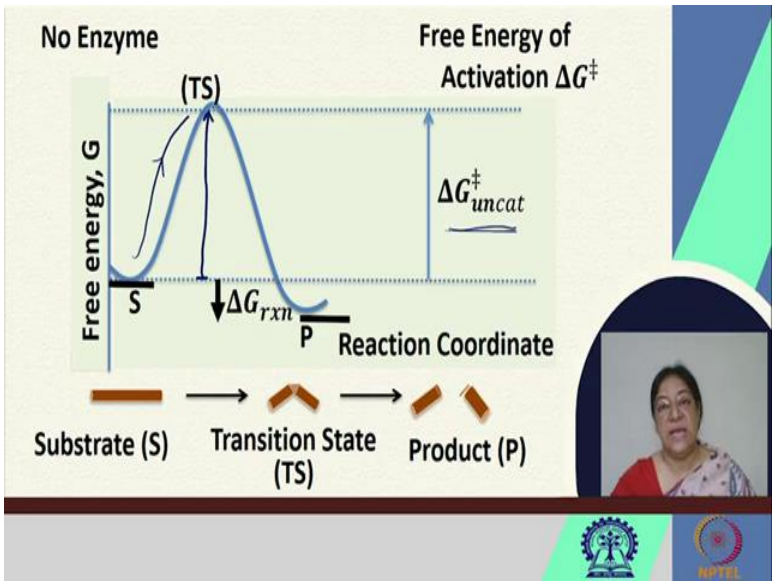
(Refer Slide Time: 01:43)



The different modes of enzyme catalysis involve, the energy diagrams that we need to understand, the specific types of amino acid residues that are involved in the active sites to bring about this crucial recognition of the substrate that is required for the reaction to proceed. So, we will be looking at the chemical modes of enzyme catalysis.

If we go about looking at acid-base catalysis, which are the specific amino acid residues that are involved in covalent catalysis and a bit about metal ion-based catalysis. In this we understand, that it is important for the enzyme and substrate to be in close proximity. We will also be looking at the diffusion controlled understanding of how this is possible.

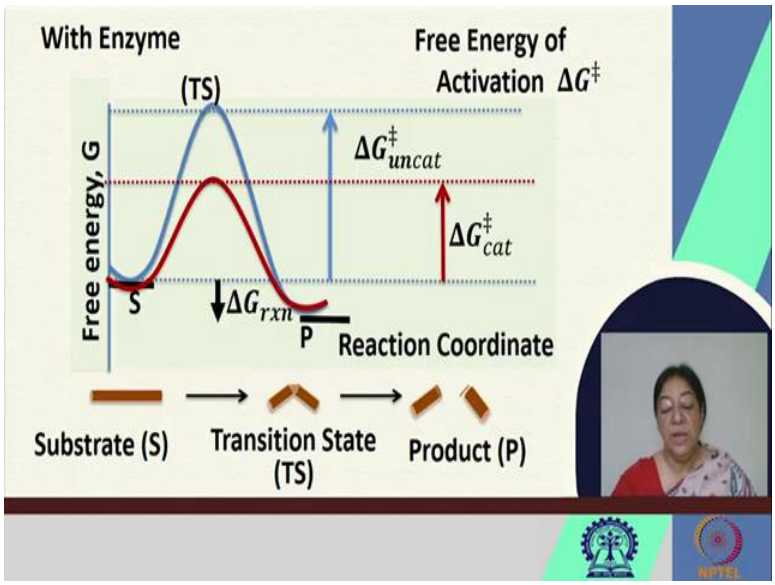
(Refer Slide Time: 02:45)



If we now look at our reaction that has no enzyme, we have the substrate and the product at a lower energy than the substrate, telling us that it is a bit more stable than the substrate in this [refer to slide] particular diagram. And we have the reaction coordinate along the x axis, telling us about the progress of the reaction.

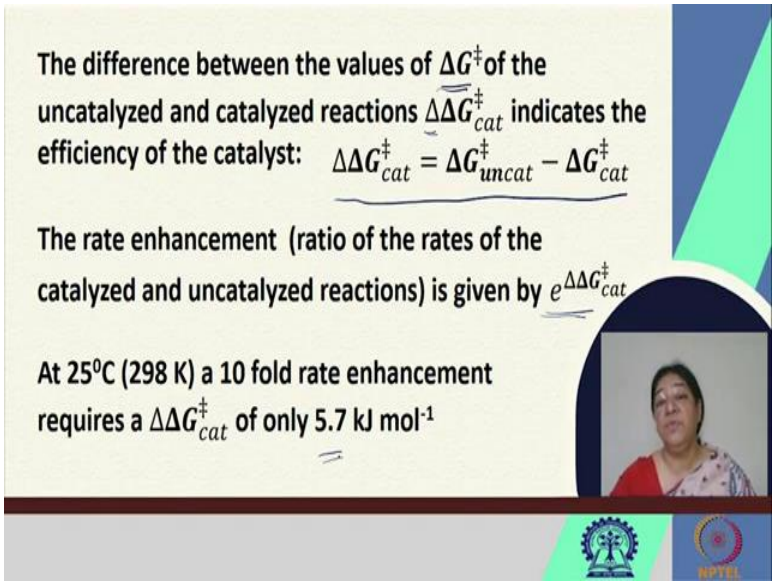
In the formation of the transition state, the substrate has to cross this barrier to reach the product. We have therefore, a ΔG reaction associated with this formation. The uncatalyzed reaction has a free energy of activation that is given by $\Delta G^{\ddagger}_{uncat}$. This uncatalyzed reaction we realize, has a free energy of activation that is given by this energy difference.

(Refer Slide Time: 03:57)



We know from our understanding of catalysis, that if we consider the same reaction with the enzyme we would have the substrate, the product, the transition state, but the ΔG_{rxn} would be the same. We would have the free energy of activation for the uncatalyzed reaction followed by the $\Delta G^{\ddagger}_{cat}$ reaction.

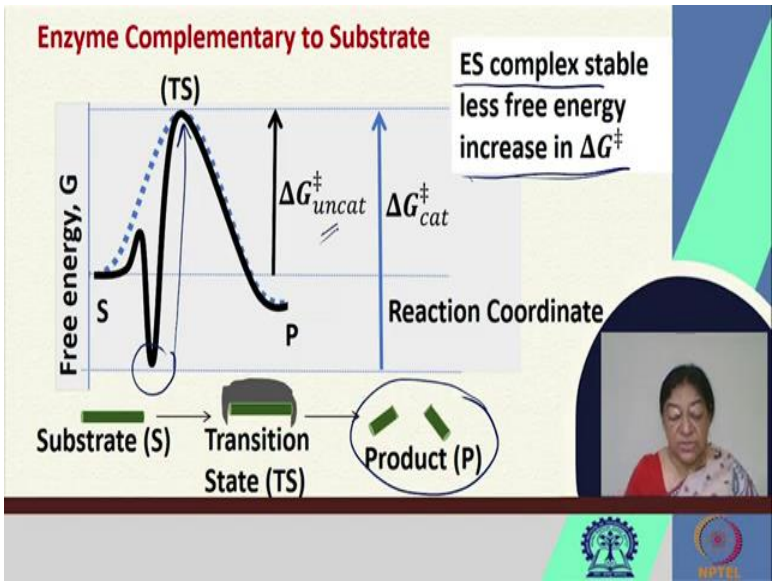
(Refer Slide Time: 04:21)



Now we realize that the value that we get from our $\Delta G^{\ddagger}_{cat}$; the free energy of activation required to reach this particular transition state is lower, indicating that we have facilitated the formation of the product. The difference between the values of the free energies of activation of the uncatalyzed and catalyzed reactions, give us a $\Delta\Delta G^{\ddagger}_{cat}$ that is a measure of how efficient the catalyst is.

The catalysis will proceed in a better manner if we have an efficient catalyst. The rate enhancement, that is the ratio of the rates of the catalyzed and uncatalyzed reaction is given by the $e^{\Delta\Delta G‡cat}$. Just for an example at 25°C, we can get a 10 fold rate enhancement by only 5.7kJ mol^{-1}, which is actually close to that of a hydrogen bond.

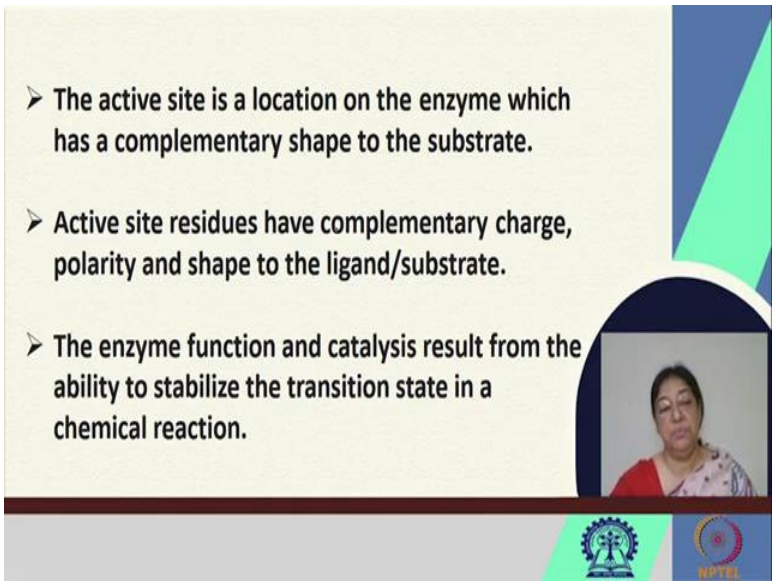
(Refer Slide Time: 05:41)



Now, we have certain conditions. The enzyme may be complementary to the substrate. What do we mean by this? We mean that the enzyme substrate complex formation, may be more stable than the enzyme.

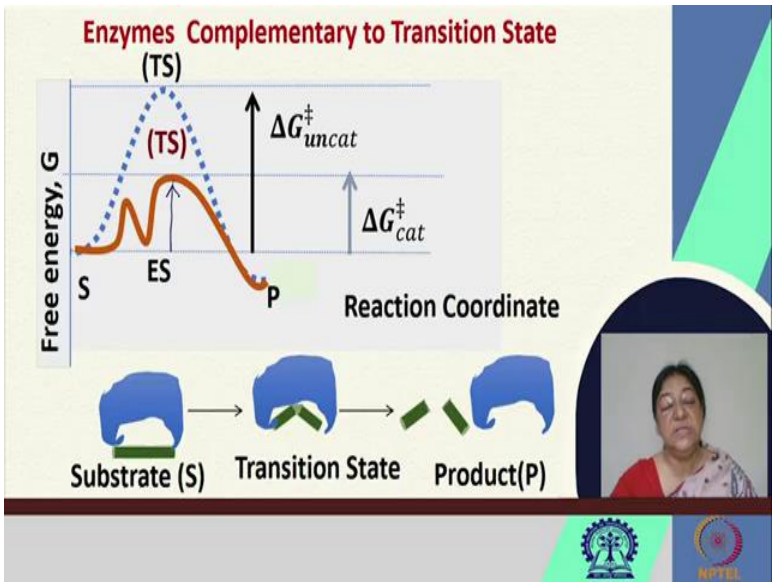
As a result of which what is going to happen, we have a $\Delta G^{‡}_{uncat}$. We have now, a $\Delta G^{‡}_{cat}$ reaction, which happens to be more than that of the uncatalyzed reaction, making this reaction very difficult to go through. So, we have the ES complex that is very stable. There is an increase in the $\Delta G^{‡}$; which means that the formation of the product might be impossible, because the transition state that has to be overcome is higher than the energy of the enzyme substrate complex and higher than the substrate also in the uncatalyzed reaction.

(Refer Slide Time: 06:59)



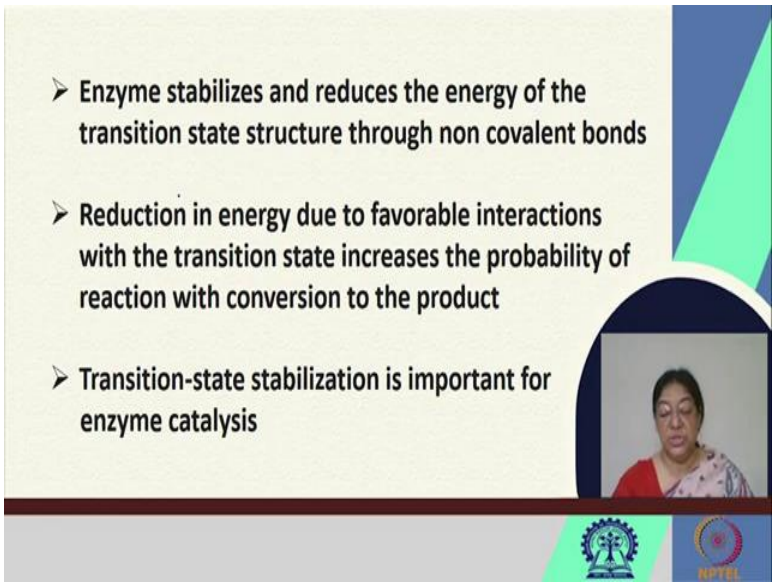
So the active site we know is a location on the enzyme which has a complementary shape to the substrate and active site residues, as we have seen in the terms of recognition, have complementary charge, polarity and shape to the ligand substrate. The enzyme function and catalysis, result from the ability to stabilize the transition state in a chemical reaction. This is important.

(Refer Slide Time: 07:29)



If we have enzymes that are complementary to the transition state, let us see how the reaction can proceed. In this [refer to slide] case, we have the free energy of the uncatalyzed reaction, we have the free energy of the catalyzed reaction. And we realize now, that the transition state barrier that has to be crossed to form the product is much lower when our enzyme is complementary to the transition state, that allows the formation of the product in a much more feasible manner.

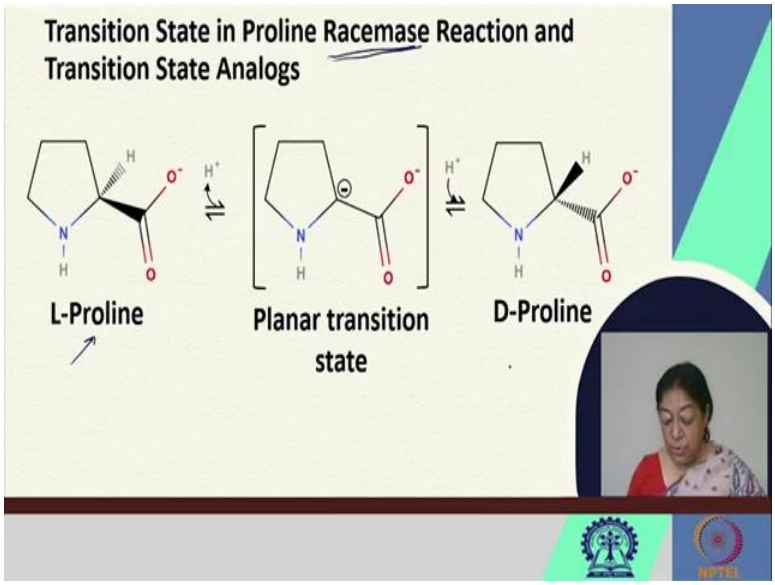
(Refer Slide Time: 08:09)



We know the enzyme stabilizes and reduces the energy of the transition state structure, through non covalent bonds. Then the reduction in energy due to these favorable interactions with the transition state, increase the probability of the reaction conversion to the product.

The transition-state stabilization is more important for enzyme catalysis, because we realize that if the substrate is too strongly bound to the enzyme, just as in the protein ligand interactions, then the enzyme will not release the substrate. If it does not do so, the reaction cannot proceed.

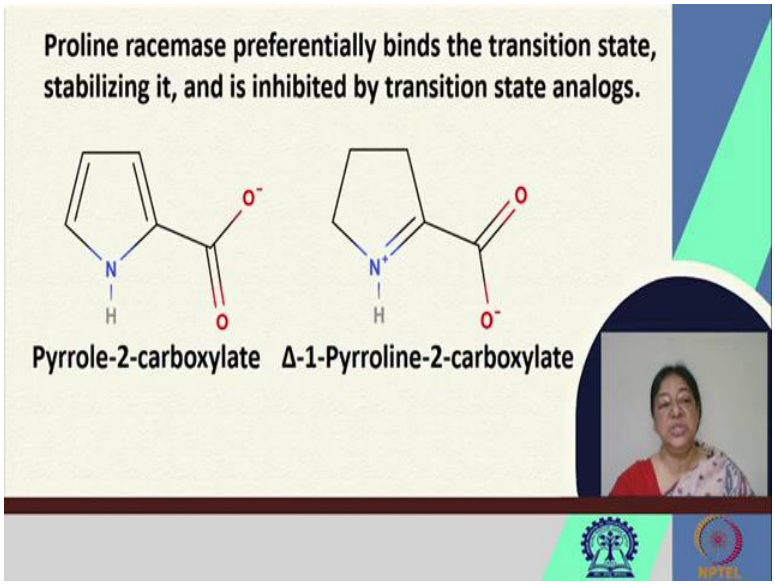
(Refer Slide Time: 09:07)



For example, let us look at a transition state in a specific type of enzyme, the proline racemase reaction and its transition state analogs. So, this [refer to slide] is L-Proline and as we see that we

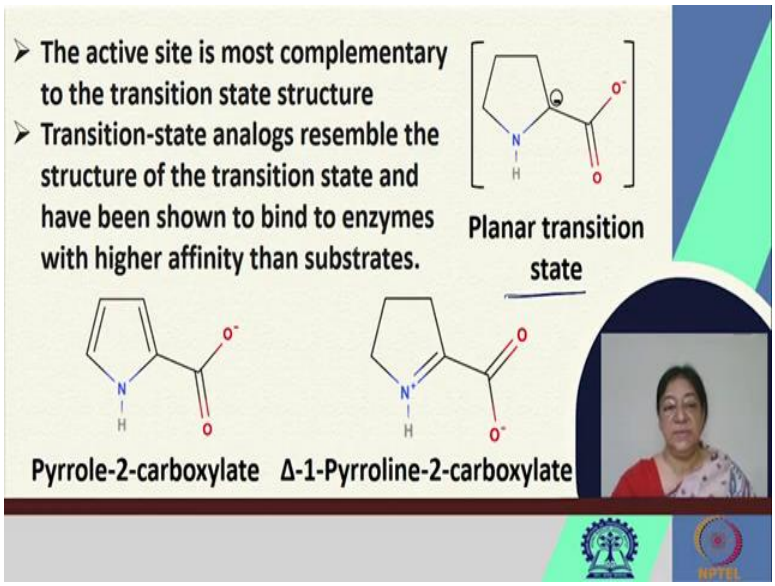
have the reaction or the enzyme as proline racemase. Which means that there would be a specific intermediate transition state involved, that is planar in nature and this would give us D-Proline. Now, we look at the enzyme name and we realize that we are going from L to D proline.

(Refer Slide Time: 09:51)



When we look at the proline racemase, it preferentially binds to the transition state. It stabilizes it and this can be inhibited by transition state analogs. So instead of substrate analogs, we look at transition state analogs to inhibit the enzyme where we could have a tighter binding. These are specific examples of transition state analogs for proline racemase.

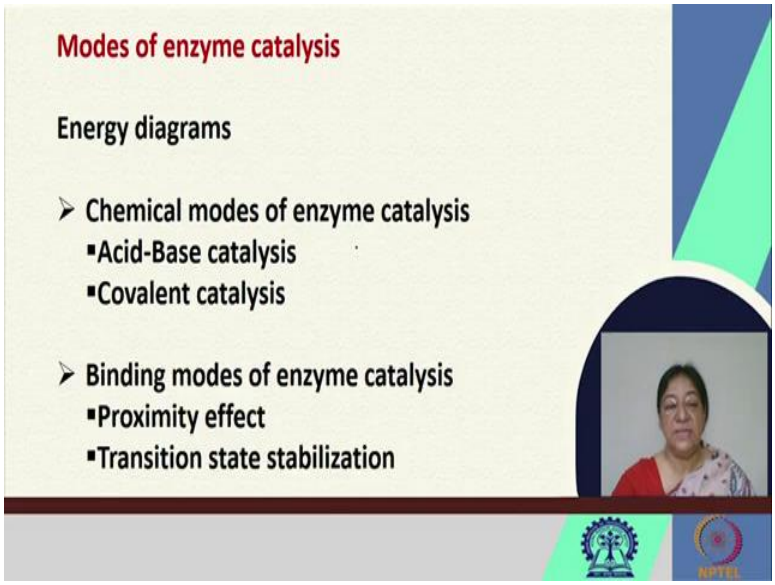
(Refer Slide Time: 10:25)



Let us revisit the structure. If we have the active site that is complementary to the transition state structure, the transition state analogs therefore resemble the structure of the transition state which is this [refer to slide], the planar transition state in the formation from L-proline to D-proline.

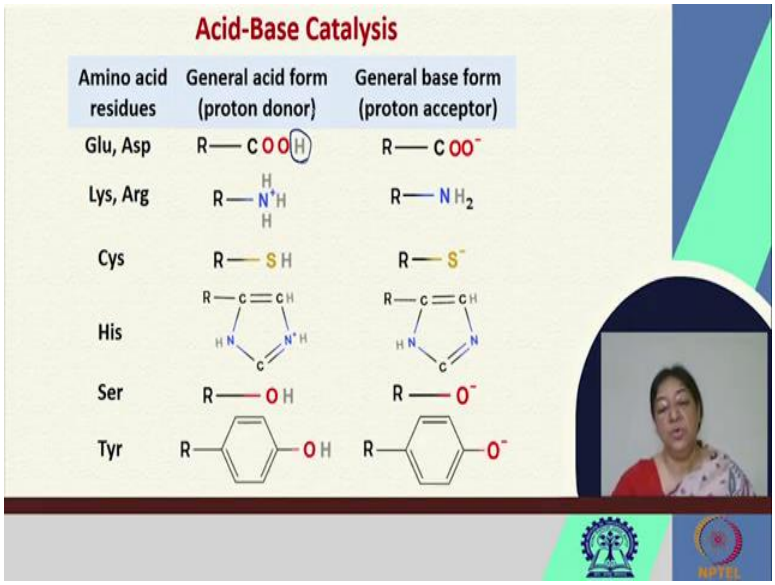
This [refer to slide] resemblance to the two transition state analogs that we showed, indicates that the enzymes will bind with the transition state. So it will have higher affinity than the substrate.

(Refer Slide Time: 11:13)



In energy diagrams, we will now look at specific types of chemical modes of enzyme catalysis.

(Refer Slide Time: 11:21)

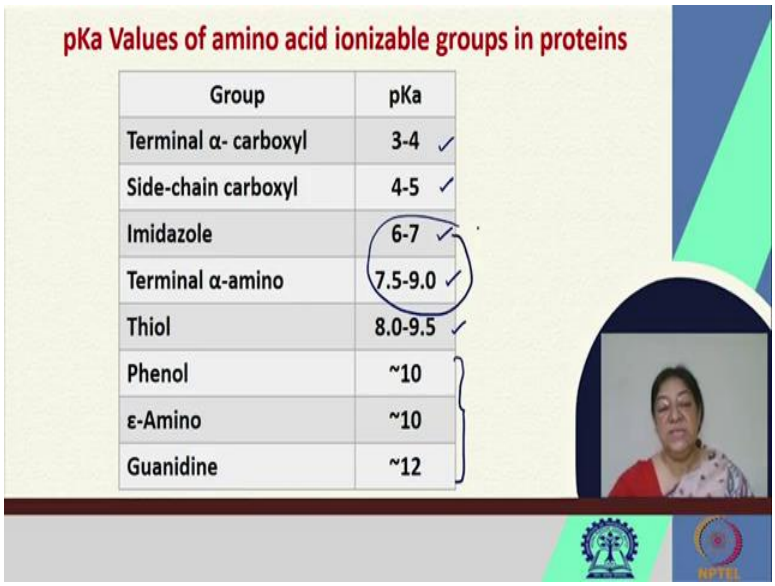


In acid-base catalysis, there are several amino acid residues that act as proton donors in a general acid form and proton acceptors in a general base form. They are glutamic acid and aspartic acid and we realize that the general acid formed, would be the proton donor where it can donate the proton and in effect would become $R\text{-}COO^-$.

We understand this is possible from the type of residue it belongs to. The basic amino acid residues would therefore have a proton donor in the NH_3^+ state and in NH_2, they would be able to accept a proton.

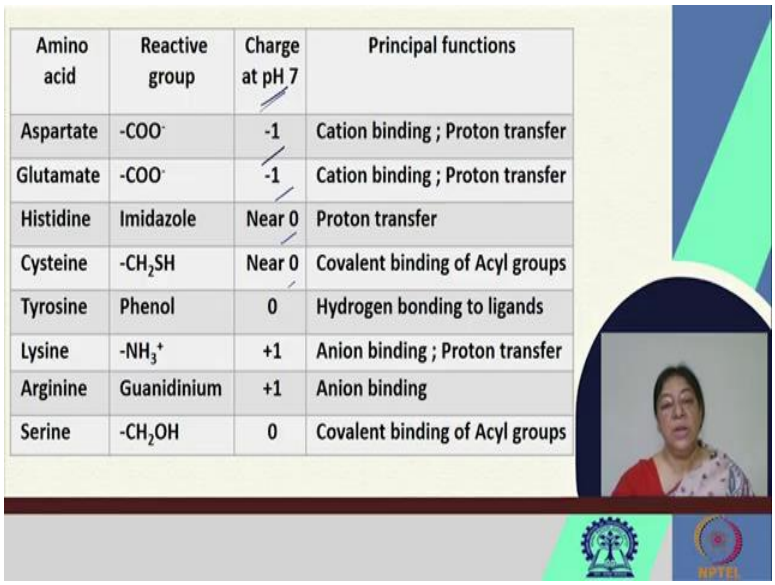
We have the cysteine residue that has the sulfhydryl group. We have the histidine residue, by far the most important one. In the general form, where it can donate a proton and with the general base form, where it can accept a proton. And we can have serine, where we can also have the OH go to O^- and the tyrosine, where we have the OH of the ring go to the tyrosine ring.

(Refer Slide Time: 12:55)



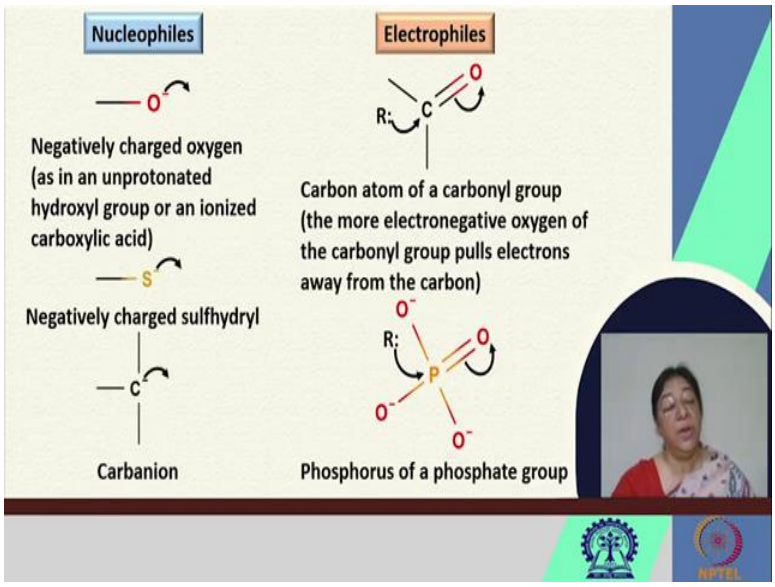
Now, the pKa values of the amino acid ionizable groups in proteins, is important. We see that we have the terminal α-carboxylate group, the side-chain carboxyl group, the imidazole, the terminal α-amino, the thiol. And the phenol, the epsilon-amino and the guanidine group, they are quite high. But the important part is, most reactions occur around the imidazole and terminal α-amino region; particularly imidazole because its range is in the physiological pH range.

(Refer Slide Time: 13:31)



If we look at the net charge at pH 7, we can have a -1 charge associated with the aspartate and the glutamate. We have the imidazole near 0, the cysteine near 0 and these [refer to slide] are the principal functions that these particular residues can participate in, when they form part of the active site. And we will be seeing that in our subsequent discussions on enzyme mechanisms in general.

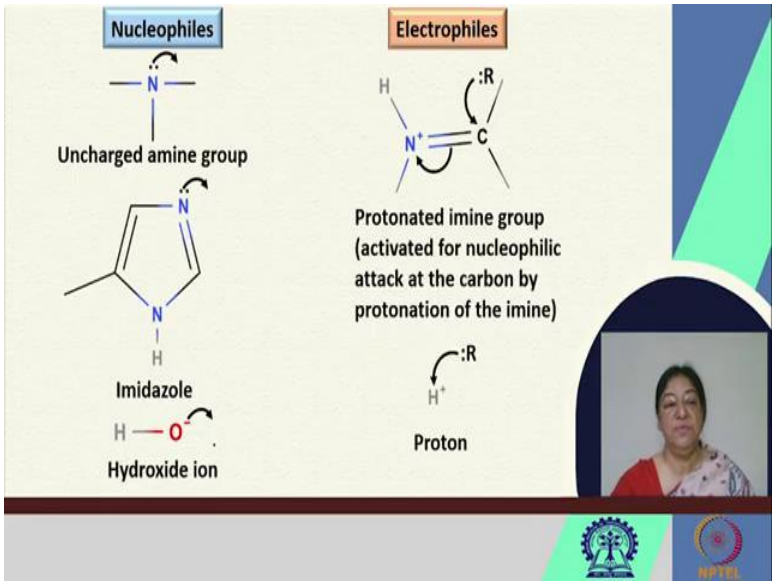
(Refer Slide Time: 14:11)



To look at the specific nucleophiles and the electrophiles that are involved; in nucleophile we have the negatively charged oxygen, that would be the case in an unprotonated hydroxyl group or say an ionized carboxylic acid and in electrophile the carbon atom of a carbonyl group and the more electronegative oxygen in this case, would pull the electrons away from the carbon. We have the negatively charged sulfhydryl group, which would be a nucleophile. We can have this

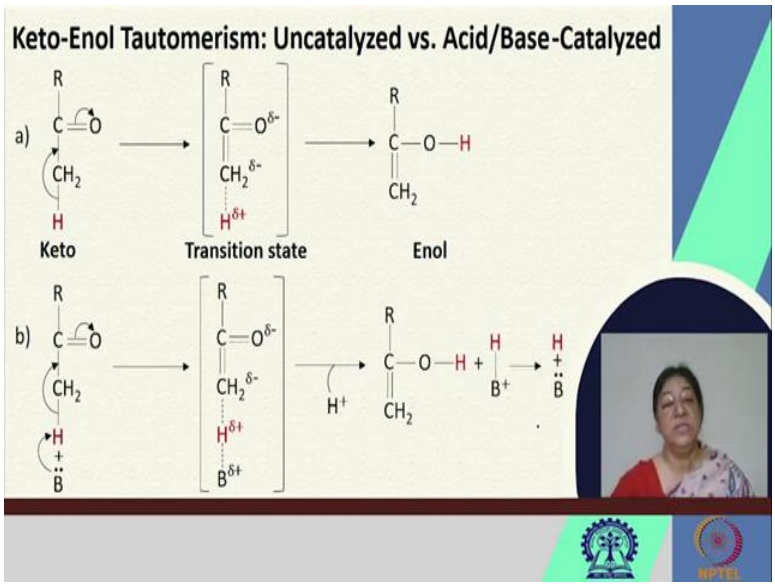
[refer to slide] particular phosphorus of a phosphate group that would be an example of an electrophile and also a carbon ion.

(Refer Slide Time: 15:03)



We can also have the uncharged amine group, which could act as a nucleophile. We could have the electrophilic part where we have a protonated imine group, that would be activated for a nucleophilic attack by protonation of the amine and the imidazole, which would be important in terms of its reaction and a proton in general. Another example would be a hydroxide ion, acting as a nucleophile.

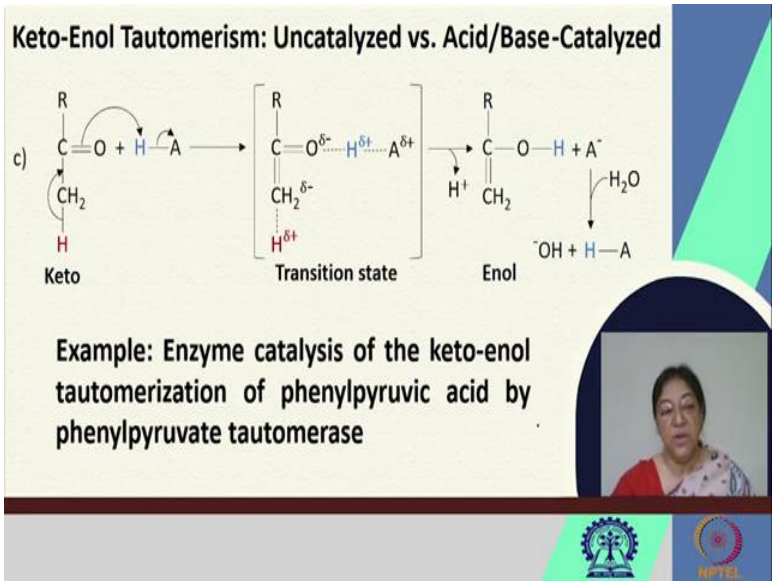
(Refer Slide Time: 15:45)



So when we look at these particular reactions and see an acid-base reaction, we have say a keto. This can now form an enol form in a tautomeric reaction. So, we have the keto and we have the enol form. This reaction can be catalyzed. So, we have the uncatalyzed reaction.

If we now catalyze this reaction by a base, where we have the abstraction of the proton in this [refer to slide] manner facilitating the reaction that would take this to the enol form. Similarly, we can have this reaction and then we get back the base, which is important for a subsequent reaction.

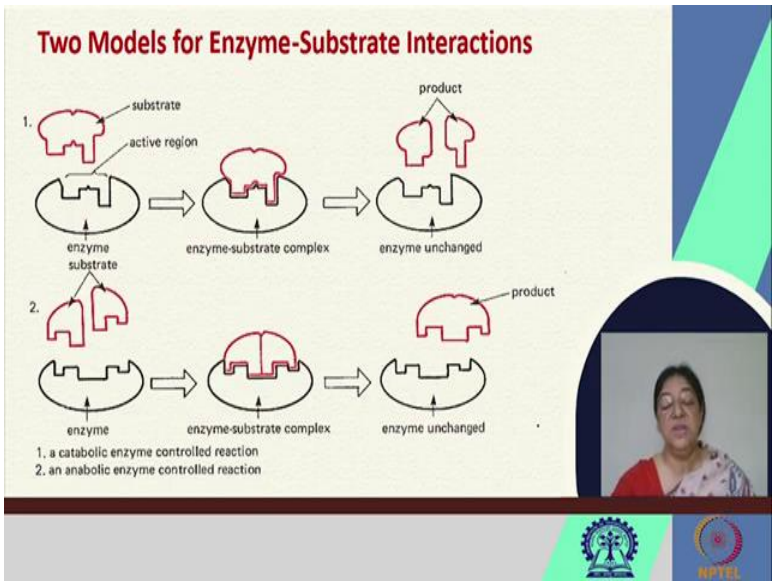
(Refer Slide Time: 16:37)



Similarly, we could have this [refer to slide] facilitated by an acid, where we would again have the keto-enol tautomerism; a transition state which would be different from that in the base catalyzed reaction, giving us our enol.

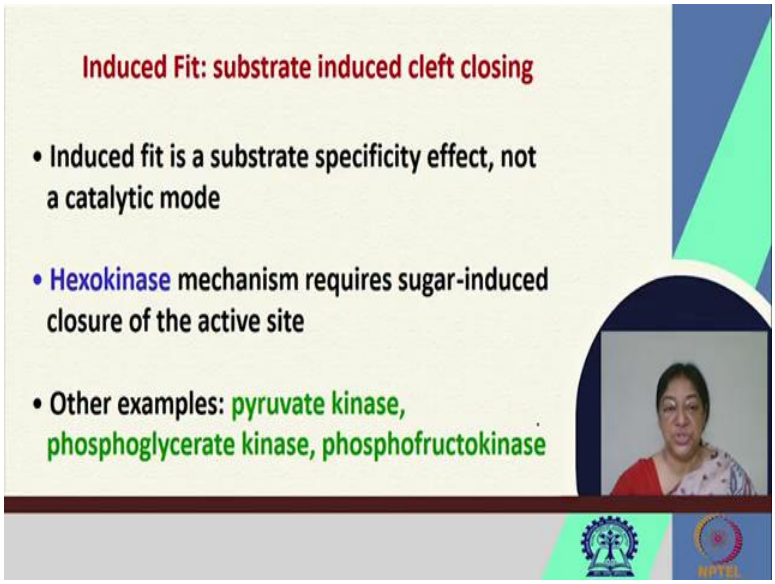
What we have is, we have an example of an enzyme catalysis of the keto-enol tautomerization of phenylpyruvic acid by phenylpyruvate tautomerase. This is one such example that gives a keto-enol tautomerization from phenylpyruvic acid, by the specific tautomerase.

(Refer Slide Time: 17:19)



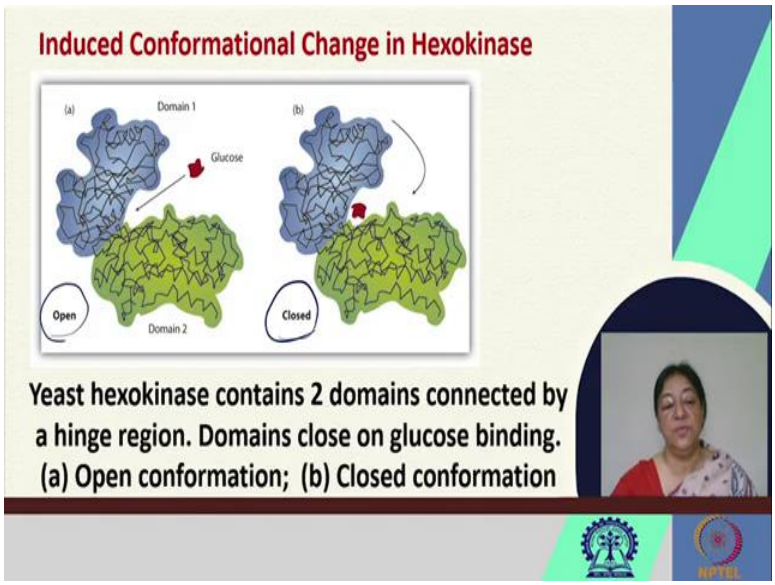
If we look at the two models for enzyme-substrate interactions, we will be revisiting this later on. In trying to understand what kind of product we can have and whether we can have two substrates involved or one substrate involved and how the reaction may proceed in the different types of enzyme-substrate reactions.

(Refer Slide Time: 17:41)



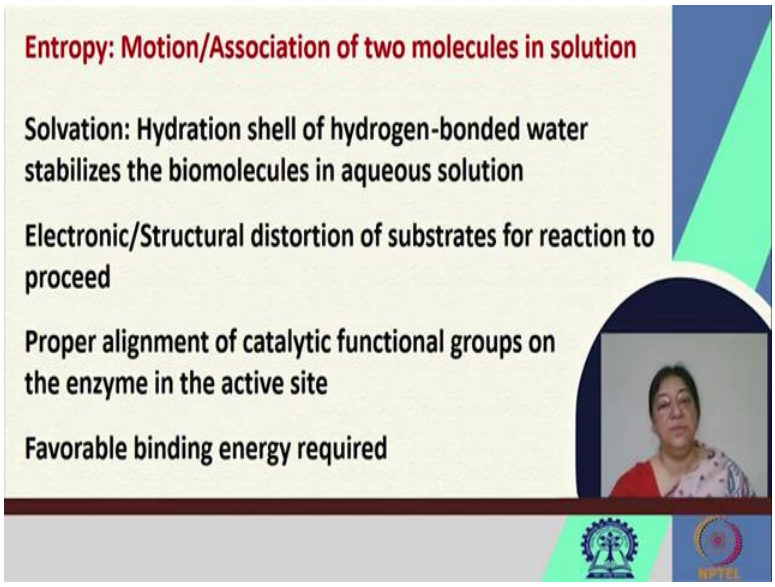
According to this, we have an induced fit, where we have a substrate specificity effect. It is not exactly a catalytic mode and there is an induction of a structural change in the protein, in the enzyme in this case, that results in a closure of the active site that allows the perfect geometry for the reaction to proceed.

(Refer Slide Time: 18:13)



If we look at the example of hexokinase, where we have glucose bound to this [refer to slide] protein, where we have what is called an open state and a closed state. There is an equilibrium between these two states in the binding of the substrate, which in this case is glucose. So, we have two domains that are connected by a hinge region that allow a closure on glucose binding, giving us a closed conformation from the open conformation.

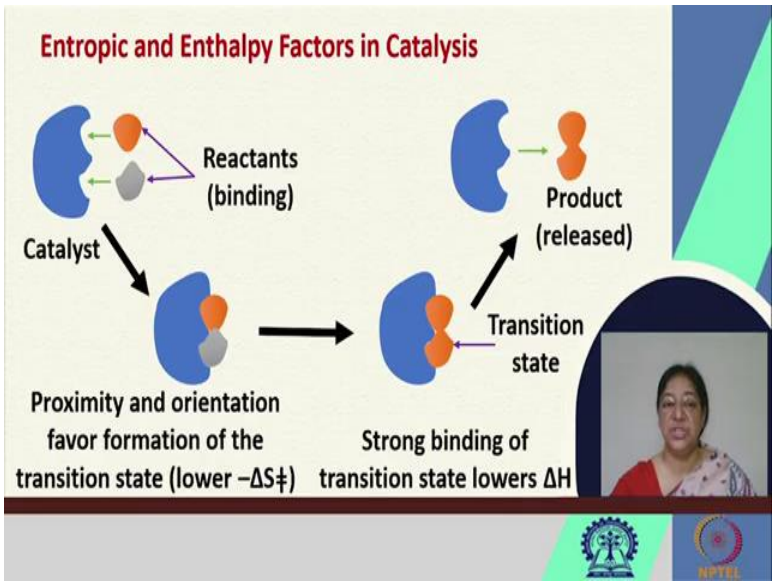
(Refer Slide Time: 18:45)



The importance now of having the protein ligand, in this case our enzyme-substrate come together is a balance of specific effects, there is a solvation hydration shell of hydrogen bonded water that stabilizes the biomolecules in the aqueous solution. So we have our enzyme that is in aqueous solution, we have our substrate in aqueous solution. They have to now come together in a recognition to form our enzyme-substrate complex, that is then going to subsequently give our reaction.

There can be electronic structural distortions of the substrates, that might be necessary for the reaction to proceed and we definitely need proper alignment of the catalytic functional groups on the enzyme in the active site. We need a favorable binding energy that is going to allow the enzyme substrate complex to form and the reaction to proceed.

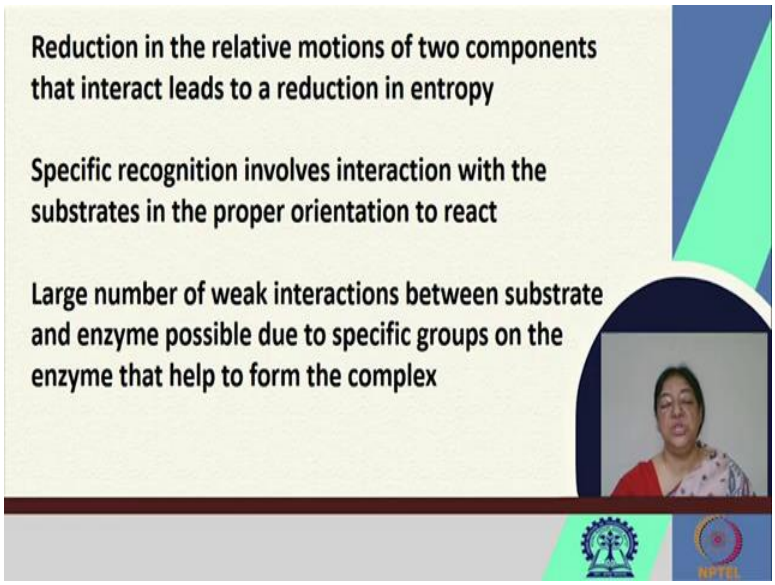
(Refer Slide Time: 19:57)



If we look at the different factors involved, we have our catalyst, our enzyme and we have our reactants binding to the enzyme. As a result of this, we have a specific proximity and orientation effect that will favor the formation of the transition state. However, we realize that there is a loss in entropy, because we have a specific association involved here [refer to slide].

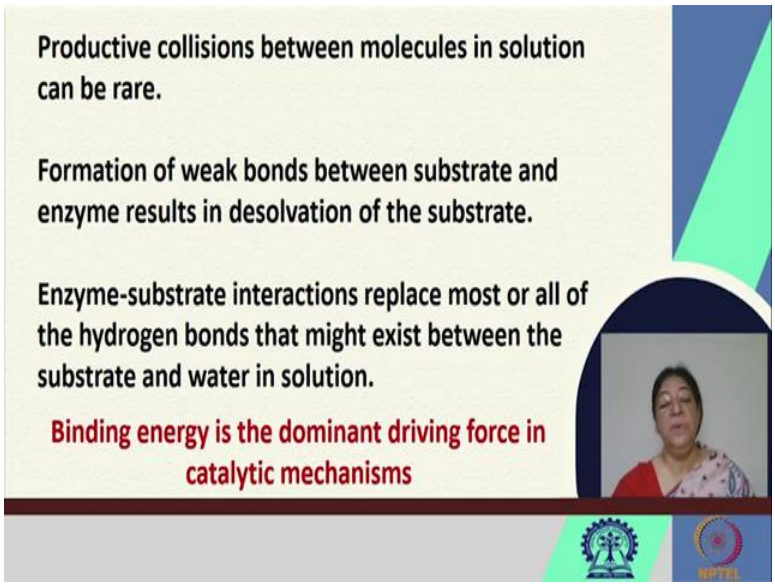
This then gives us a transition state resulting in better binding, that will give us a better enthalphic, because of the enthalphic contributions of the non covalent interactions give us a transition state. Then we would have a release of the product as the reaction proceeds.

(Refer Slide Time: 20:49)



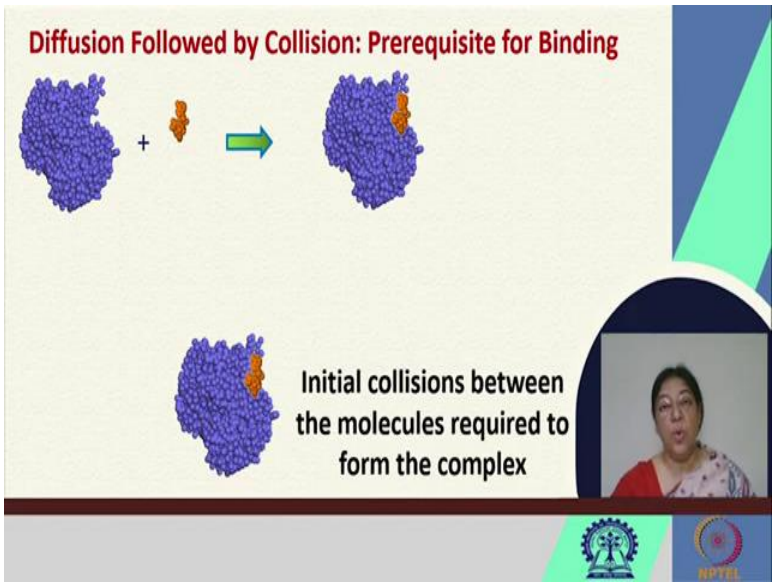
So, there is a reduction in the relative motions of the two components as we saw, that leads to a reduction in entropy, because we are bringing or we are forming a complex. But we require the specific recognition and a proper orientation for the reaction to occur and the large number of weak non covalent interactions between the substrate and the enzyme is possible, due to the specific groups on the enzyme that facilitate the formation of the complex.

(Refer Slide Time: 21:25)



So, if we look at these productive collisions between molecules in solution, they are not very common and we have a formation of these weak bonds, which results in the desolvation of the water molecules that again would result in an increase in the entropy of the water molecules. So it is a balance that occurs in the formation of this enzyme-substrate complex. But we have to realize that the binding energy is the dominant driving force in the catalytic mechanisms.

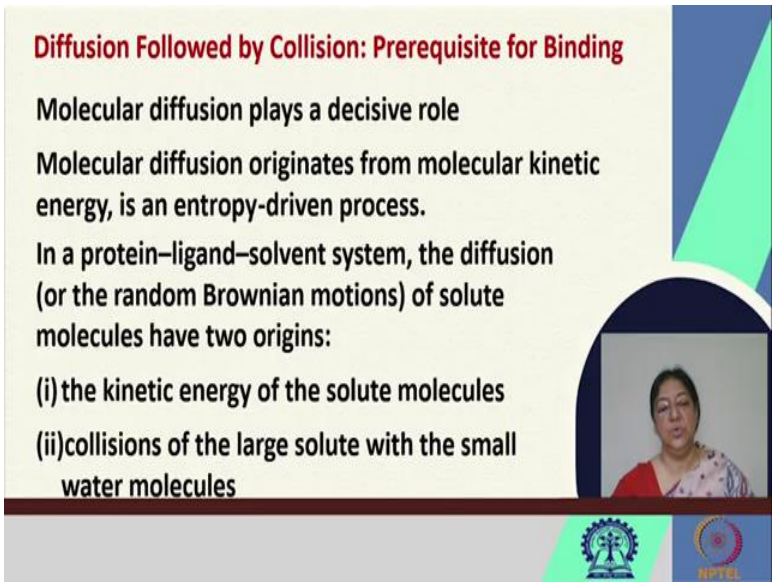
(Refer Slide Time: 22:01)



If we look at a diffusion now, that is going to be followed by a collision, we realize that this is a prerequisite for binding. So if we have our specific enzyme-substrate complex formed, this is true for the protein ligand formation as well, we would have the specific enzyme and substrate come together.

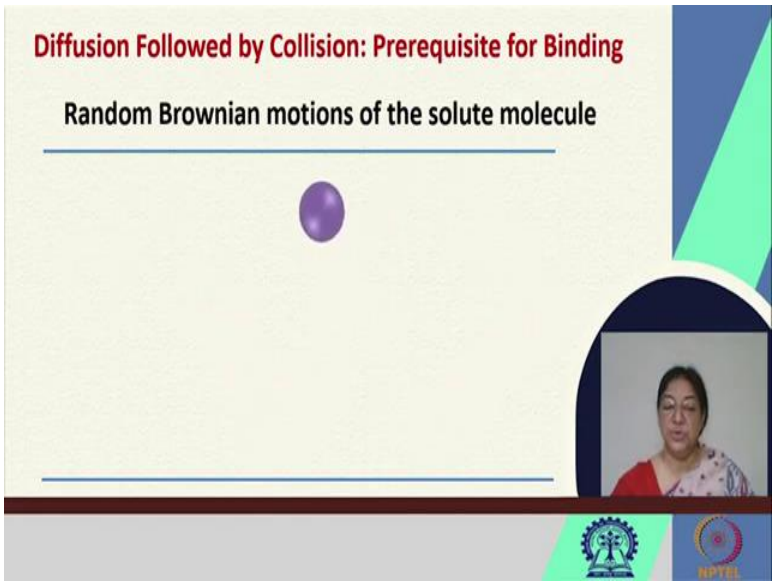
The initial collision in the proper orientation that would be required for the molecules to come together to form the complex and then, have a catalytic reaction and release the product. So, there are many factors involved here, and the specific proximity and the proper orientation is required in the recognition to form the complex.

(Refer Slide Time: 22:47)



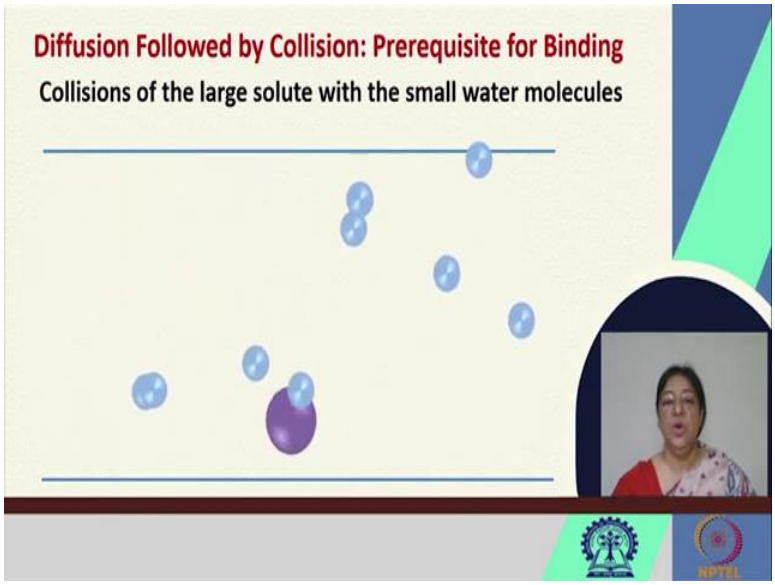
If we look at molecular diffusion in general, we have the kinetic energy that is an entropy driven process. And in protein ligand solvent systems, which applies to enzyme-substrate as well, we have the diffusion or the random Brownian motion of the solute. This could be the kinetic energy and the collisions of the molecules.

(Refer Slide Time: 23:09)



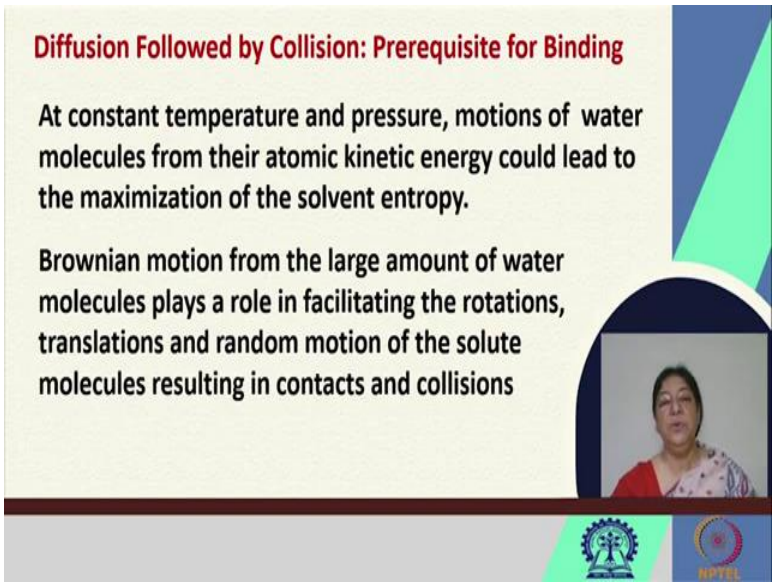
If we look at the random Brownian motions of the solute molecule, they can have random Brownian motions that would take the molecule at different positions around the solution.

(Refer Slide Time: 23:21)



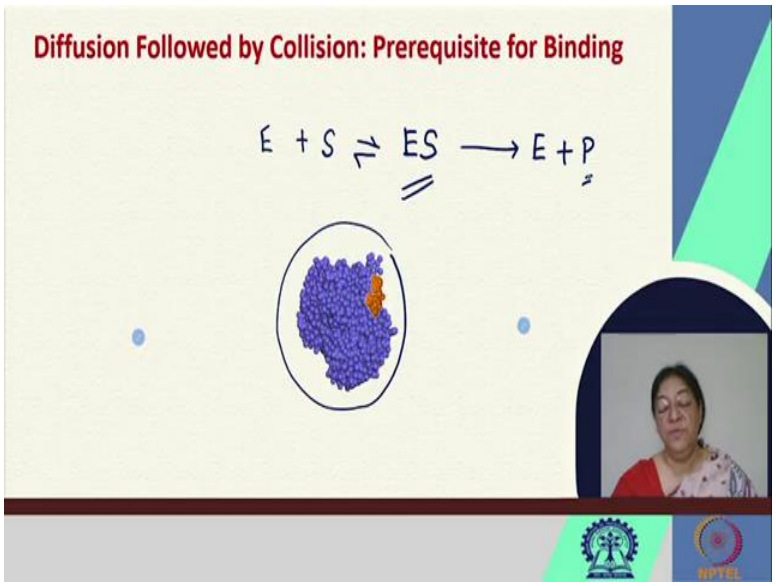
However, in the presence of the solute water molecules, we would have a bombardment with the solute molecules that would interact with our solute in the system.

(Refer Slide Time: 23:37)



At constant temperature and pressure, the motion of the water molecules rise in their kinetic energy and they could lead to a maximization of the solvent entropy. So the Brownian motion from the large amount of water molecules, plays a very important role in facilitating the rotations, translations and the random motion of the solute, that would result in these contacts and collisions.

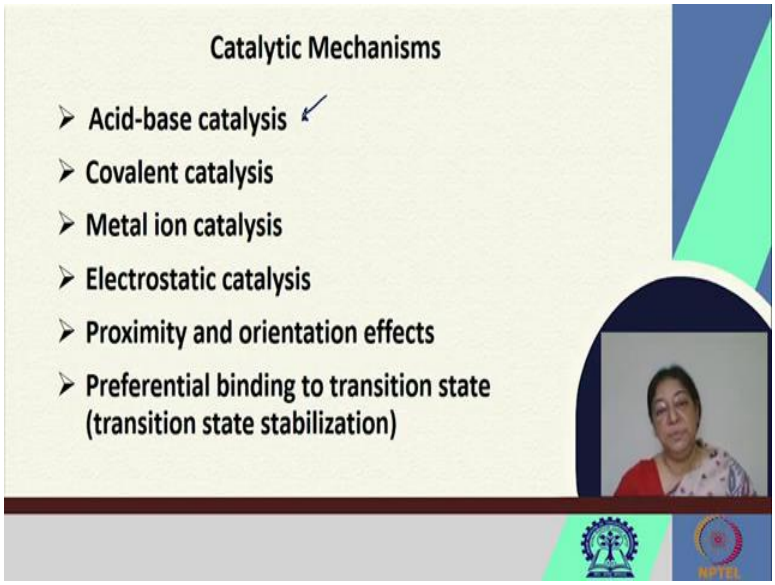
(Refer Slide Time: 24:05)



And we would have a fruitful collision, a fruitful formation of the complex, when we have a look at the specific enzyme-substrate complex bond that would result from our enzyme in solution, our substrate in solution, the proper geometry and the proper association.

We realize from the energetics involved, that we would require the enzyme-substrate to go to the enzyme and the product, which we will see in our discussion on kinetics in the next module of this course.

(Refer Slide Time: 24:55)



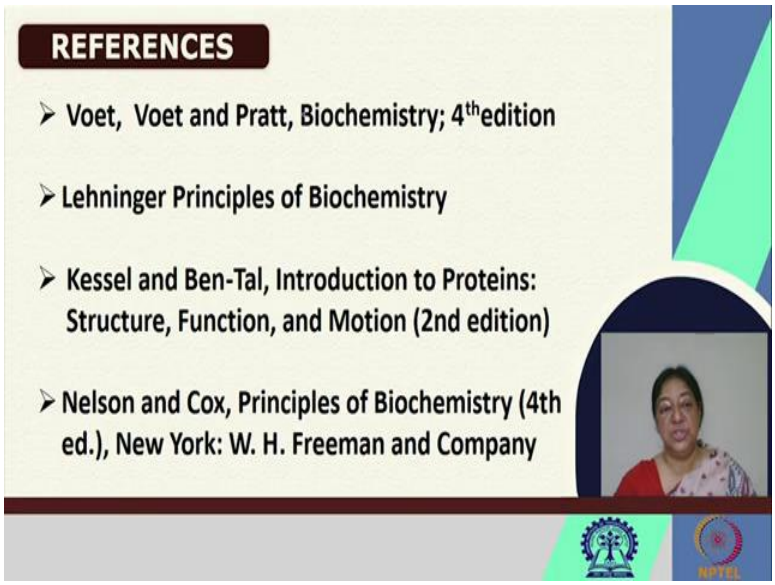
If we look at the catalytic mechanisms, there is acid-base catalysis. And what we did realize, is the importance of specific amino acid residues in this acid-base catalysis. In the understanding that there can be proton donors, they can be proton acceptors. These specific amino acid residues are present in the active site of the enzymes, where they can bring about specific reactions.

We will also look at covalent catalysis, where in specific cases, there are non covalent interactions as we mentioned, in the formation of the enzyme-substrate complex. In some cases, there is covalent catalysis. There is metal ion-based catalysis, which we will look at specific examples to see how the specific reaction can occur in the biochemical process. There is electrostatic catalysis, based on our enzymatic active sites, involving electrostatic interactions.

These are the different types of interactions that can occur between the enzyme active site amino acid residues and the substrate molecules. But the beauty about enzyme-substrate reactions or enzyme catalysis in general, is that the enzyme is not involved in a sense that it participates in the reaction in a manner that it can get back to its original form and be ready for another substrate to be bound to it.

We have proximity and orientation effects, as we saw the importance of the enzyme and substrate to be in close proximity to each other for a fruitful collision. A good orientation for the specific non covalent interactions and the enzyme-substrate complex to form, for subsequent product formation. And the preferential binding to the substrate or the transition state, which we saw in a transition state stabilization, which would lead to a possible reaction.

(Refer Slide Time: 27:27)



These [refer to slide] are the reference books.

Thank you.