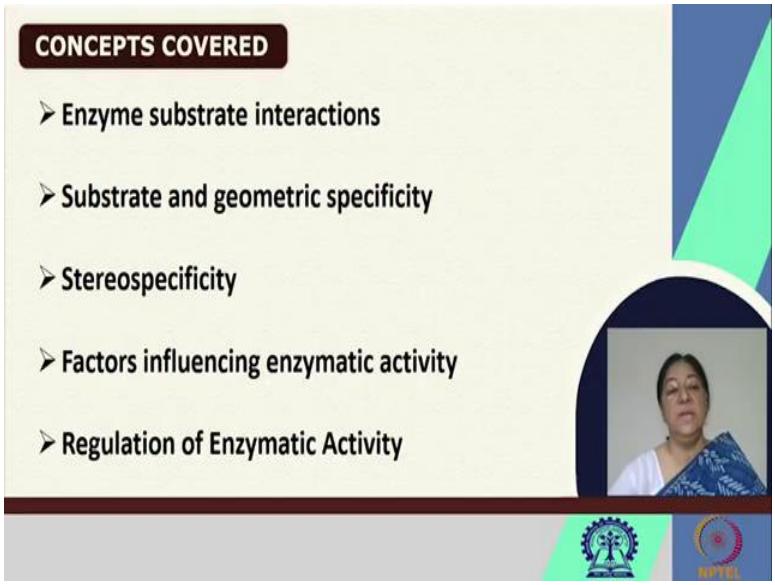
Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 06
Enzymes and Enzyme Mechanisms
Lecture - 27
Enzymes - II

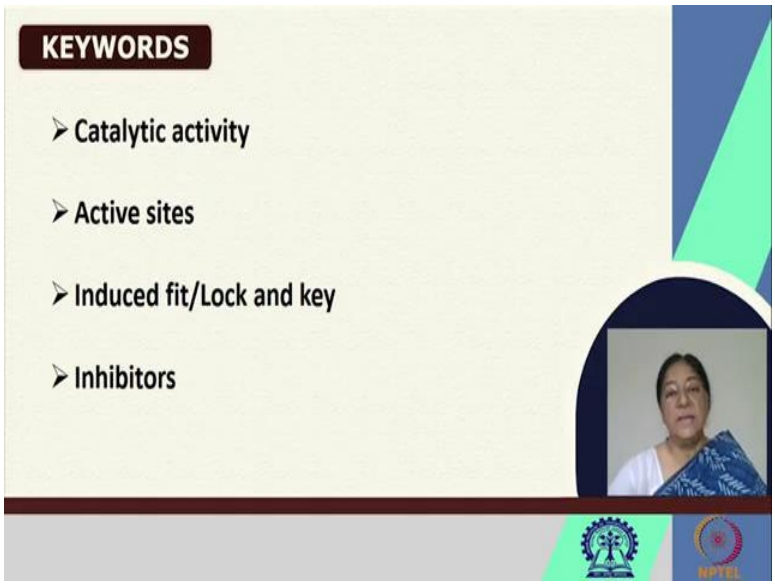
We continue our discussion on enzymes in this particular lecture.

(Refer Slide Time: 00:21)



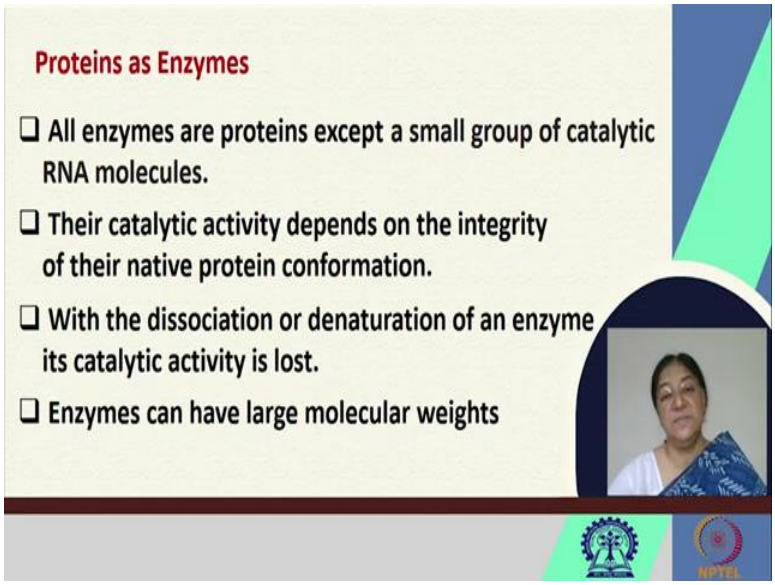
What we are going to talk about is something related to the enzyme substrate interactions. Discussions on how they have their specificity, stereo specificity, and factors that would influence enzymatic activity and how they may be regulated.

(Refer Slide Time: 00:41)



Catalytic activity and active sites of enzymes in terms of the specificity, or what we have in terms of geometric complementarity, is something extremely important for the enzyme substrate complex to form, which is a prerequisite for any enzymatic activity.

(Refer Slide Time: 01:03)



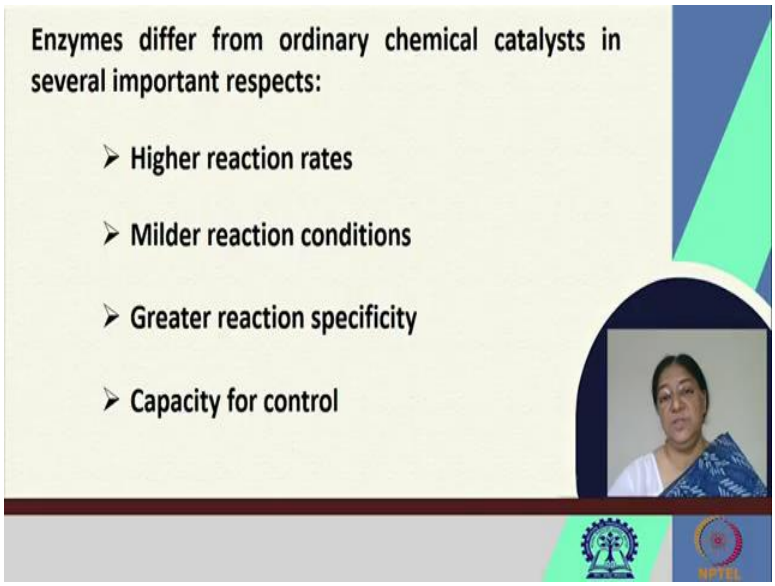
Proteins as enzymes: All enzymes are proteins, except a small group of catalytic RNA molecules known as ribozymes, which we will discuss briefly. And we will look at their mechanism when we look at enzyme mechanisms in general. The catalytic activity of enzymes, depends upon the integrity of the native protein conformation.

And with the dissociation or denaturation of the enzyme, the catalytic activity is lost. We realize this because there is a geometric component to the specific enzyme, that holds the specific

residues that are involved in the mechanism in place. Breakdown of the structure therefore, would result in a loss of the catalytic activity.

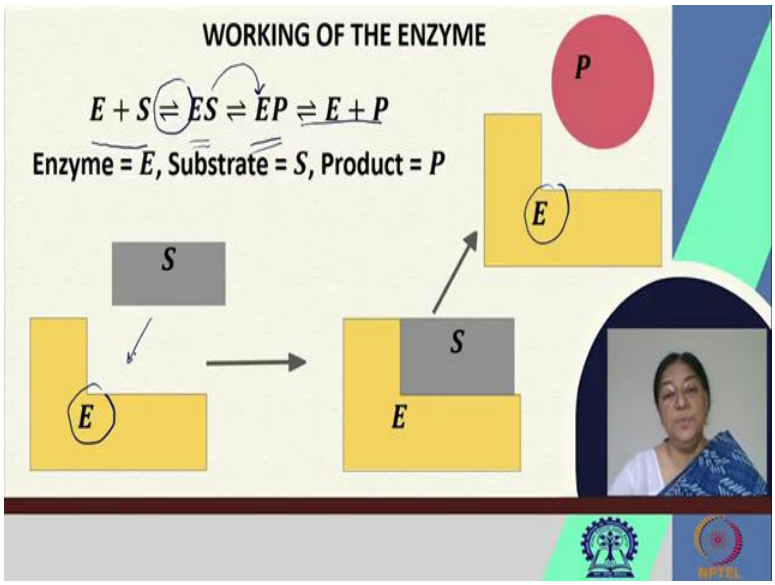
The enzymes like proteins, have large molecular weights, but their specific zone of action is limited to the active site. Which is why there is a lot of research in the area of looking at mimicking enzymes, where only the active site residues are taken into consideration, to bring about a catalytic activity in mimicking the enzymatic function.

(Refer Slide Time: 02:37)



Enzymes differ from ordinary chemical catalysts in several important aspects. They have higher reaction rates, they have milder reaction conditions, they have greater reaction specificity and there is a capacity for control.

(Refer Slide Time: 03:01)

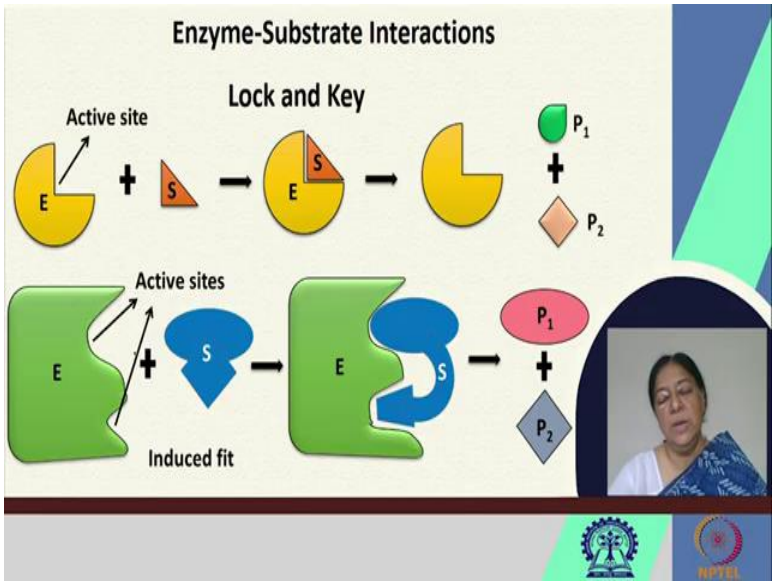


If we look [refer to slide] at the generic working of the enzyme, we see that there is a specific methodology where the enzyme and the substrate together are going to form the enzyme substrate complex. This may form an enzyme product complex where the catalytic mechanism of the enzyme occurs in this step, which then breaks down to the E + P.

This is a pre equilibrium that occurs, but all of these may or may not involve a pre equilibrium step. This therefore, has the structure where we can look at the enzyme, we have the substrate, we have a fit and then we have the breakdown of the enzyme, to release the product.

What is important in the catalytic activity of the enzyme, is the enzyme itself is restored to it's original conformation in terms of the active site residues in their specific geometry, because the enzyme has to be ready to bind another substrate. This is the beauty of enzyme catalysis.

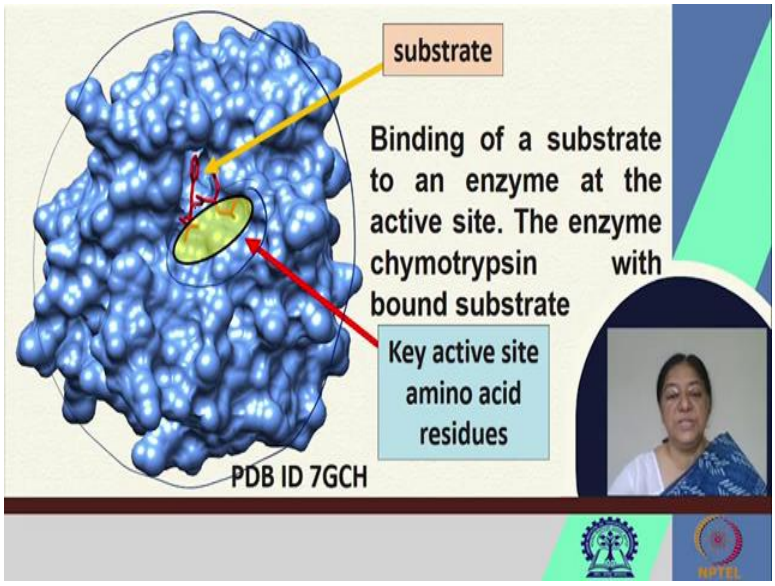
(Refer Slide Time: 04:25)



If we look at enzyme substrate interactions, we have an active site and we know that this active site is going to bind our substrate, subsequently we would have product formation. We could have various types of product formation, we could have 1, we could have 2, depending on the type of mechanism that occurs.

If we look at enzymes that change their conformation due to the presence of the substrate, we form what is called an induced fit mechanism. Where in this case the enzyme accommodates to the substrate molecule and we again have product formation.

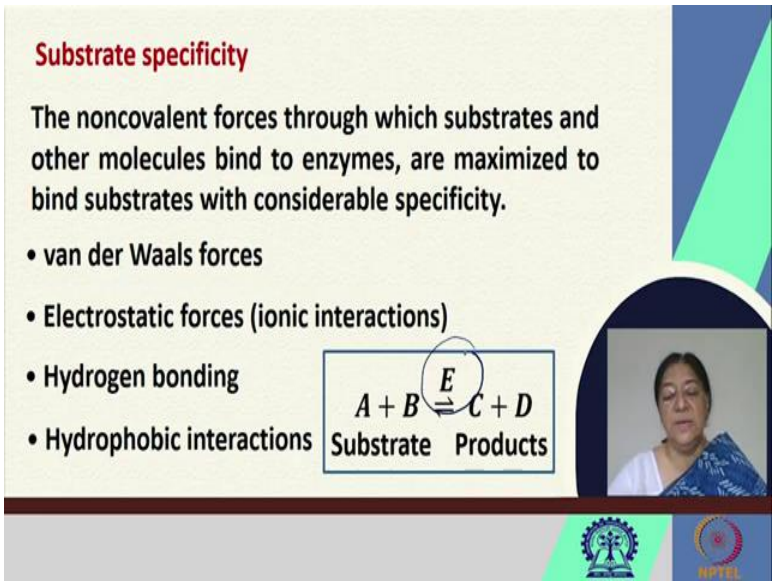
(Refer Slide Time: 05:09)



The binding of a substrate to an enzyme at the active site is therefore, an extremely important component, the molecular recognition as we spoke about in protein ligand interactions. Where we realize that, the binding of the ligand in this case the substrate to the enzyme is an important factor in the recognition and in this case a particular reaction to take place to form the product.

When we look at the substrate bound to the enzyme in this case chymotrypsin, we realize that there is the specific active site residues that are involved, the important residues that are involved in the recognition of the substrate to the enzyme. So we see that even though this protein is very large in it's size, there is a specific region that is the active site that is required for the recognition of the substrate.

(Refer Slide Time: 06:17)



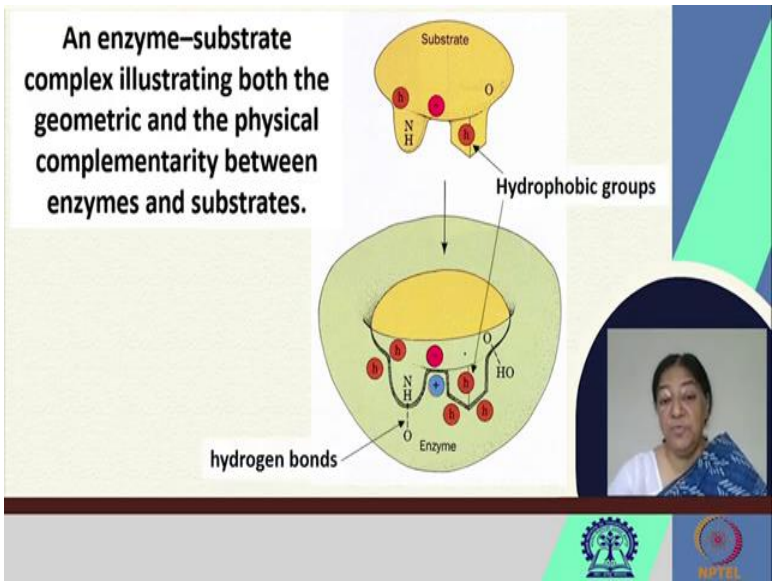
The substrate specificity therefore, is important because there is this important role of recognition. We have the substrate, we have the product form and we have the enzyme as the catalysts that works here. The noncovalent forces through which the substrates and other molecules bind to the enzymes, are maximized to bind the substrates with considerable specificity.

So we have van der Waals forces, we have electrostatic forces, the ionic interactions, and hydrogen bonding. And we realize that since we have the enzyme substrate complex and this dissociates to give the product, we cannot have the ligand binding, in this case the substrate binding, to be too strong to the enzyme.

However, when we are designing an inhibitor we would like the binding to be strong. This is something we will discuss when we look at the specific enzyme kinetics and enzyme mechanisms.

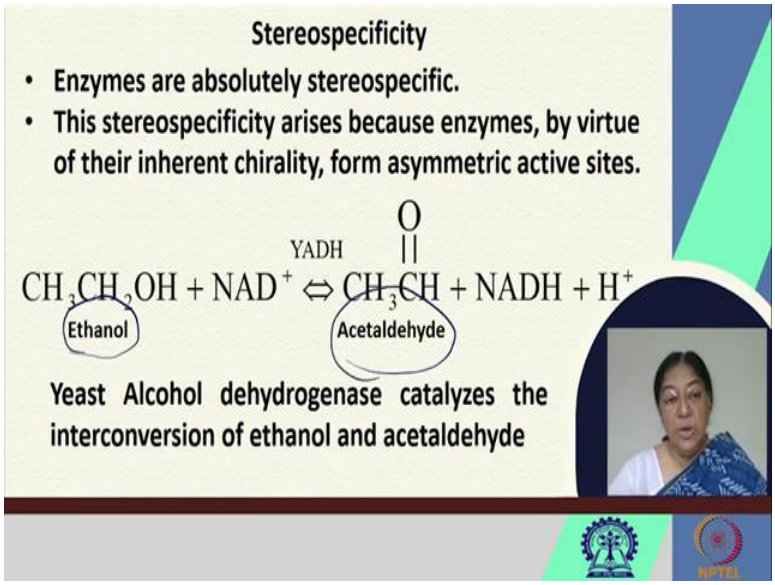
These are the types of interactions possible, the noncovalent forces that would result in specific substrate recognition of the enzyme. There are some enzymes that are involved also in covalent linkages, but during the catalytic mechanism, they revert to their original enzymatic structure.

(Refer Slide Time: 07:53)



So we have our substrate and our enzyme. We realize that it is a complex that illustrates physical complementarity, chemical complementarity, geometric complementarity, in bringing the formation of the enzyme substrate complex.

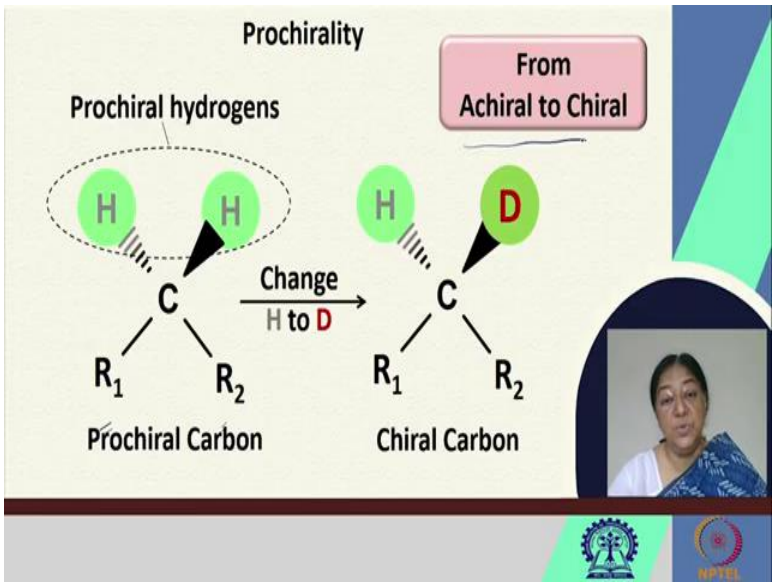
(Refer Slide Time: 08:23)



If we have hydrophobic groups, we can have hydrogen bond formation and we have stereo specificity, a very important factor in the chirality that results in these molecules. Now, enzymes are known to be stereospecific.

This stereospecificity arises because the enzymes by virtue of their inherent chirality, form asymmetric active sites. So what we have is we can have a situation, where we would have the yeast alcohol dehydrogenase that catalyzes the interconversion of ethanol to acetaldehyde.

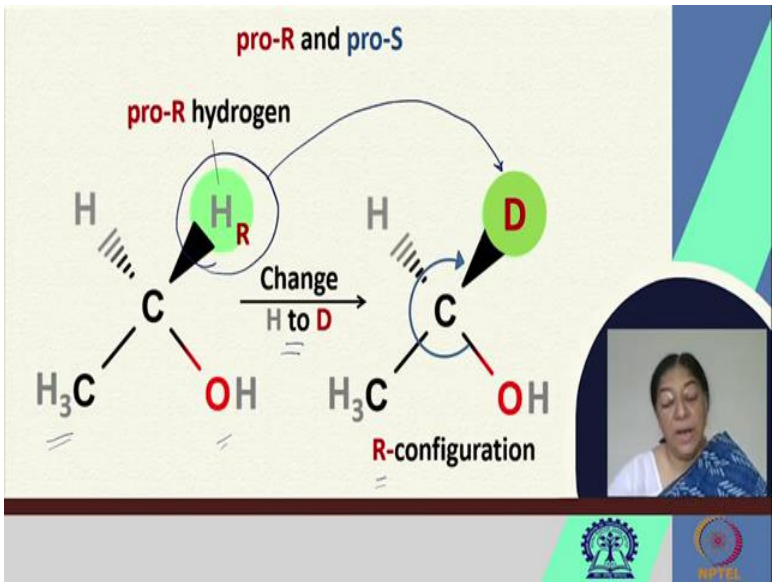
(Refer Slide Time: 09:05)



If we look at this particular interaction, we have to understand what we mean by prochirality. In prochirality we have two substituents R_1 and R_2 to the carbon and we have 2 hydrogens attached. This is called a prochiral carbon, because this replacement of one hydrogen is going to result in a chiral carbon.

If we look at these prochiral hydrogens, we realize that the loss of or the change of one of these hydrogens is going to result in a chiral carbon, because now we have the 4 substituents that are different. This occurrence from achiral to chiral in the substituent changes is important, where we can form the pro-R and the pro-S.

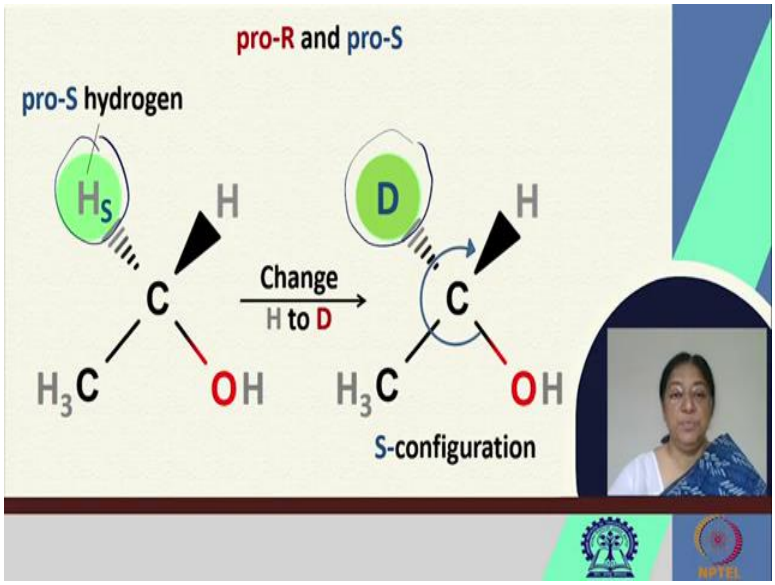
(Refer Slide Time: 10:01)



If we have a representative structure that is CH_3CH_2OH [refer to slide], our ethanol and if we change H to D, then we will have this specific conformation. We now have a chiral carbon, we have an R configuration.

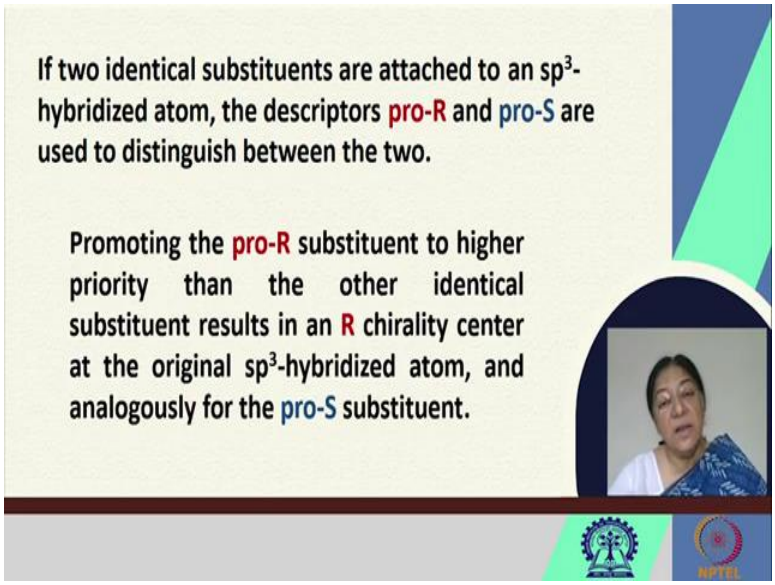
This means, that since we change this particular hydrogen to the deuterium, the change of H to D has resulted in the R configuration. So this is called the pro-R hydrogen.

(Refer Slide Time: 10:55)



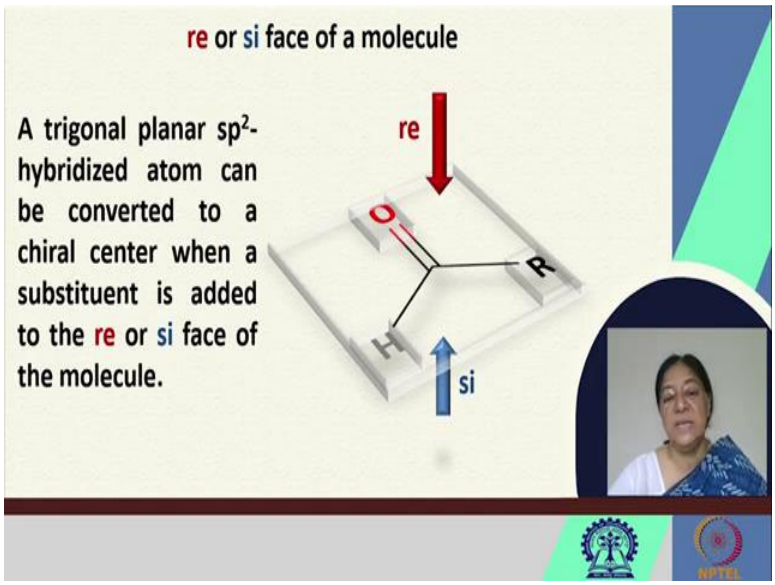
Similarly, if we have the change of the other hydrogen to deuterium, we result in an S-configuration. This S-configuration for the carbon means, that the specific hydrogen is the pro-S hydrogen.

(Refer Slide Time: 11:19)



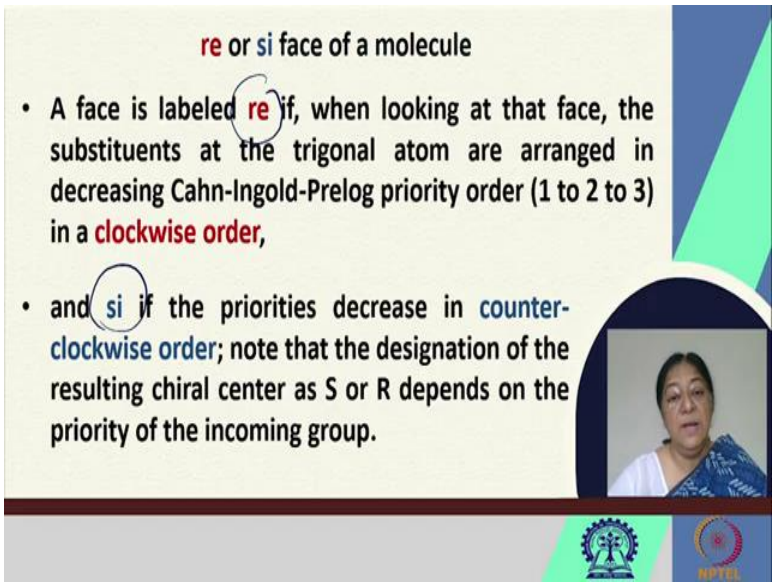
Now, if we have two identical substituents that are attached to an sp^3 hybridized carbon atom, the descriptors pro-R and pro-S are used to distinguish between the two. Promoting the pro-R substituent to higher priority than the other identical substituent, gives us R chirality as we saw, and similarly we can do it for the S one.

(Refer Slide Time: 11:45)



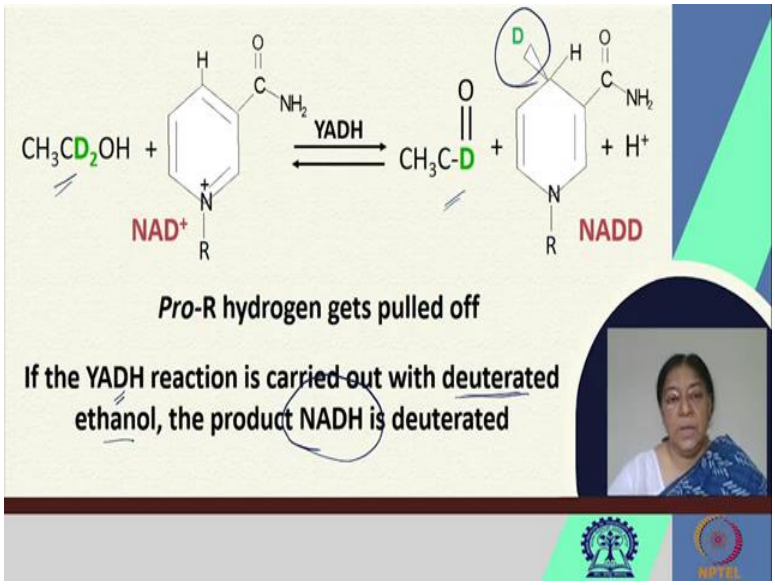
Now we can have two phases of the molecule, we can have the re face or the si face. The trigonal planar SPT hybridized atom, can now be converted to a chiral center when we have the substituents attack from either faces.

(Refer Slide Time: 12:07)



If we look at how they are labelled, when we look at the face the substituents at the trigonal atom are arranged in decreasing priority order, that is 1 to 2 to 3 in a clockwise manner. And if they are in arranged in an anti clockwise manner, then we would have the si face.

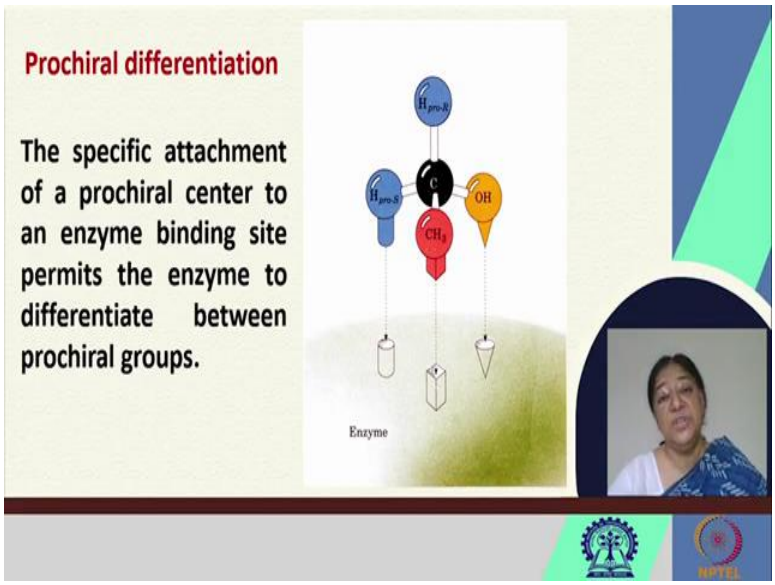
(Refer Slide Time: 12:37)



So if we look at the specific reaction now [refer to slide], where we have the enzymatic activity occur in yeast alcohol dehydrogenase, there is a way in which we can form from the substituted ethanol to the substituent acetaldehyde, giving us our overall reaction.

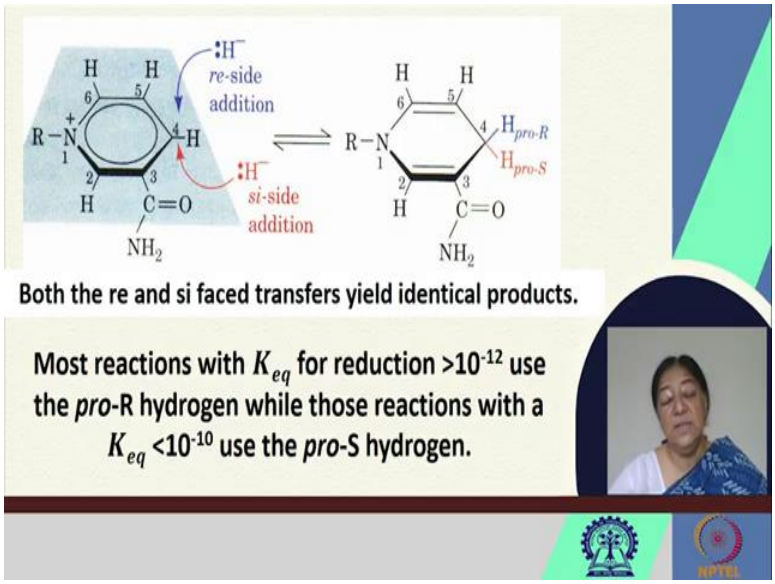
And from this we understand that the pro-R hydrogen is removed. And if this particular enzymatic reaction is carried out with deuterated ethanol, we see that the product NADH is deuterated. This gives us an idea about the enzyme activity, about the enzymatic mechanism.

(Refer Slide Time: 13:29)



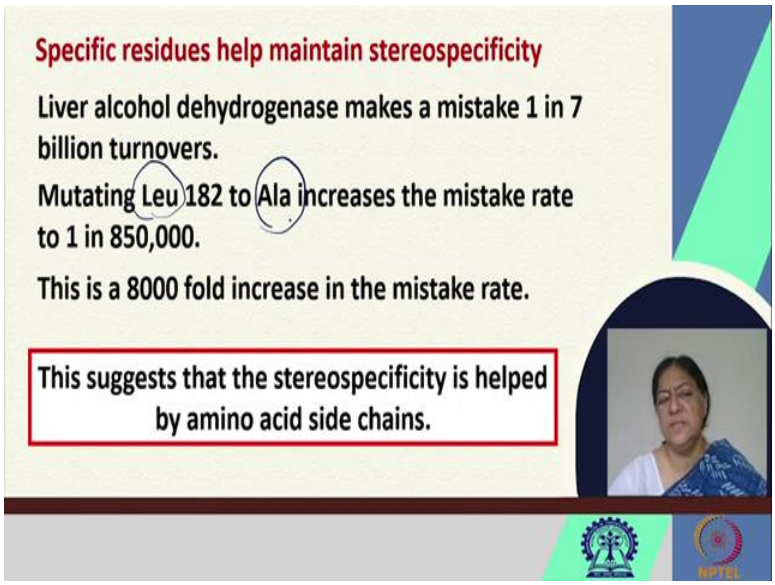
The specific attachment of a prochiral center to the enzyme binding site, permits the enzyme to differentiate between prochiral groups. So we understand the specificity of the enzyme and how important it is to get the specific chirality or the specific configuration of the molecule of interest.

(Refer Slide Time: 13:57)



When we look [refer to slide] at the additions from the two different sides, we get identical products where we have a pro-R and a pro-S. What is known is that most reactions with an equilibrium constant for reduction that is greater than 10^{-12}, use the pro-R hydrogen. And those with reactions with less than 10^{-10}, they use the pro-S hydrogen.

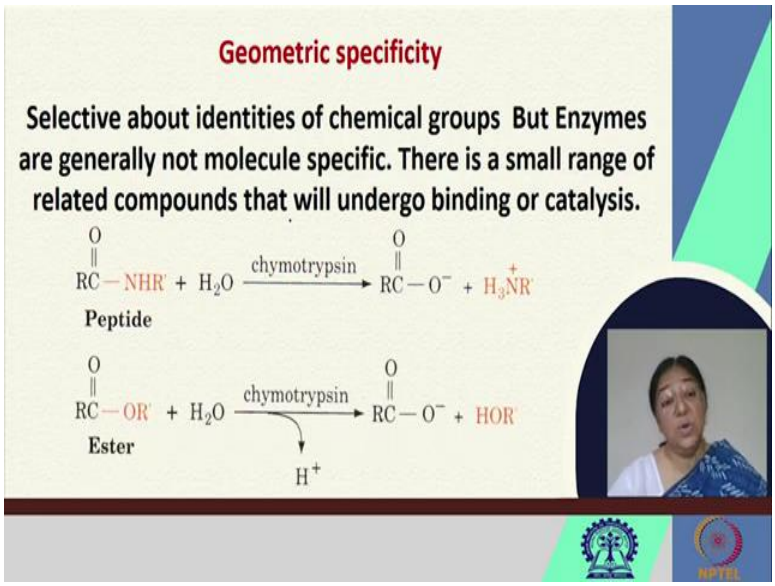
(Refer Slide Time: 14:25)



So the specific residues that we have help maintain the stereospecificity. For example, the liver alcohol dehydrogenase makes a mistake 1 in 7 billion turnovers. So, the efficiency is remarkable.

Now if there is a mutation, which means that 1 amino acid has been changed to another amino acid. In this case a hydrophobic leucine has been replaced by a hydrophobic alanine, it increases the mistake rate. As a result there is an 8000 fold increase in the mistake rate. This suggests that the stereospecificity is helped by the specific amino acid side chains present.

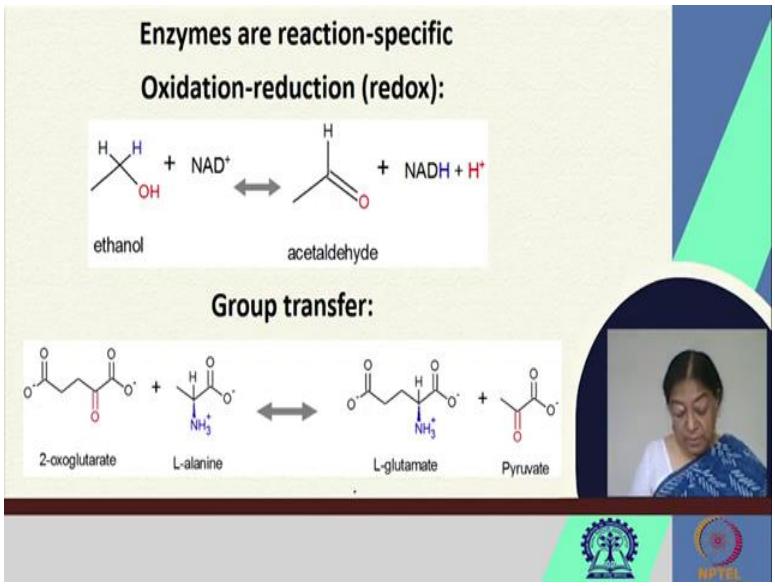
(Refer Slide Time: 15:17)



Apart from the chemical specificity, we understand the importance of the geometric specificity. Here, there is a selectivity about the identities of the chemical groups. But enzymes are not always molecule specifics; there is a small range of related compounds that will undergo binding or catalysis. Say we are looking at chymotrypsin, we have the peptide bond and we have the

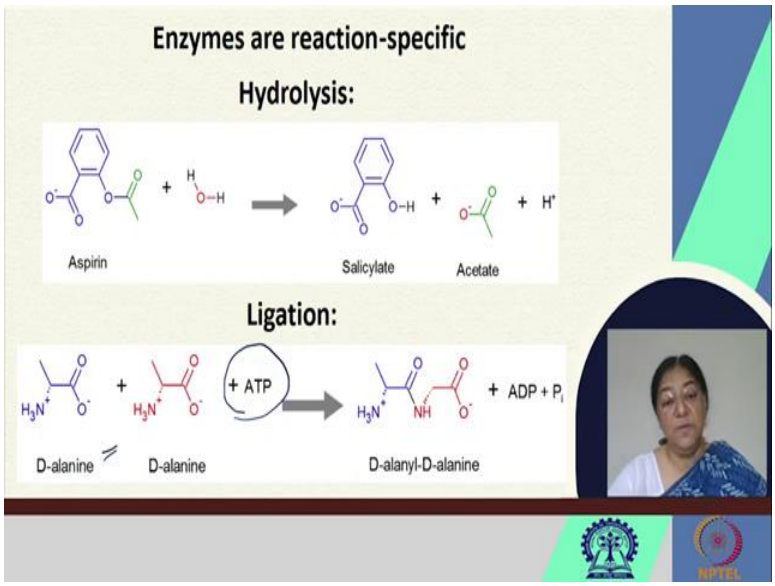
cleavage of the peptide bond. Again we could have the ester and the cleavage of the S, but we have reaction specificity.

(Refer Slide Time: 15:55)



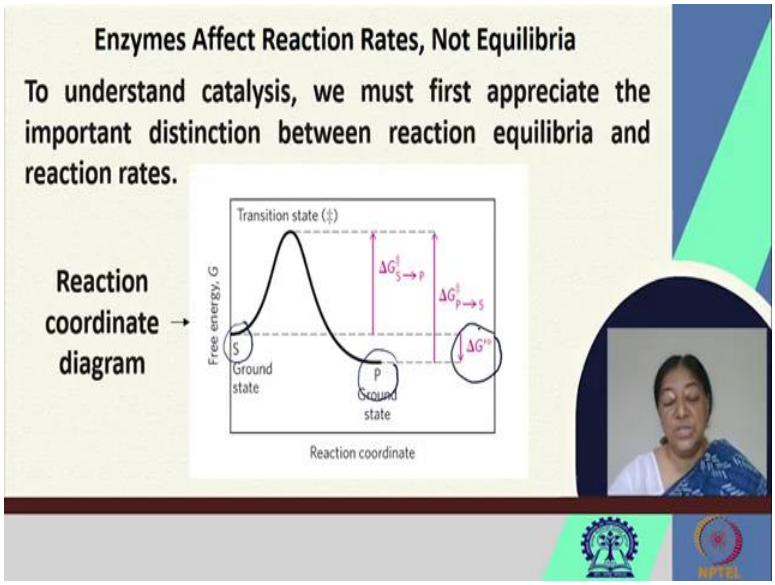
If we are looking at an oxidation reduction redox type, we would have say ethanol go to acetaldehyde. If we are looking at a group transfer type, in this case oxoglutarate and L alanine would form L glutamate and pyruvate. Now all of these reactions as we looked at in the previous lecture, are important enzymatic reactions belonging to the specific classes of enzymes that we spoke about.

(Refer Slide Time: 16:39)



The specific classes have reaction specificity. The third type was hydrolysis, brought about by hydrolases. So this [refer to slide] is a specific hydrolysis reaction. Similarly we can have ligation, we can have isomerization. In this case we remember we used ATP for the specific energy requirement for the formation of this bond. So, the linking is going to result in energy from the cleavage of the ATP to form ADP and Pi.

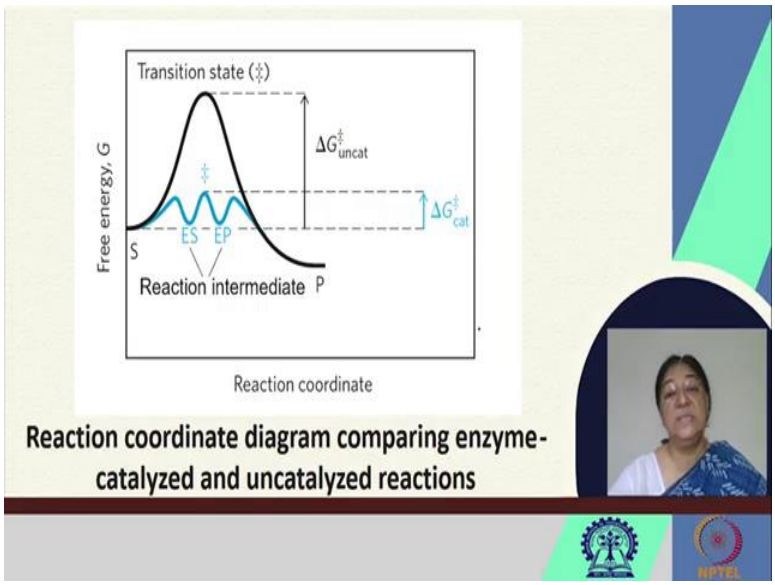
(Refer Slide Time: 17:21)



The most important part is that enzymes affect reaction rates not the equilibria, which is something that we will understand later, when we go to do the kinetics. So to understand catalysis, we have to realize that there is an important distinction between the reaction equilibria and the reaction rates.

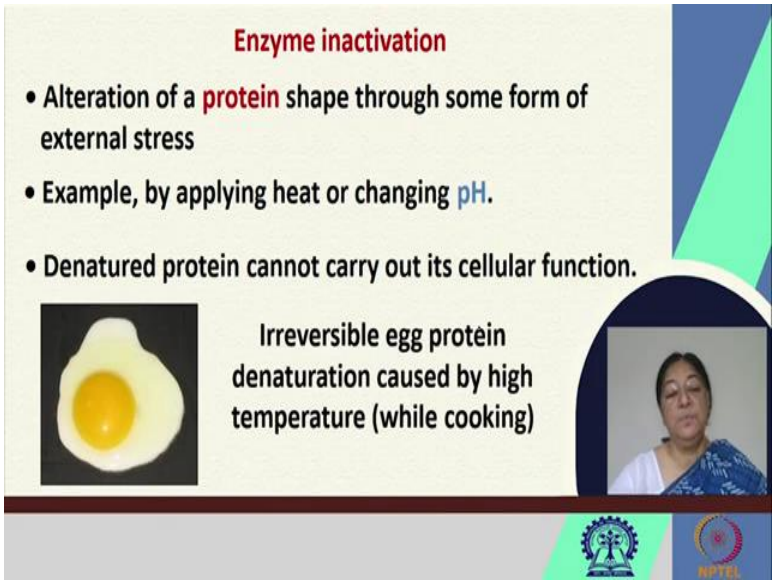
Something that we also discussed in protein ligand binding, where we have a reaction coordinate diagram [refer to slide], that speaks of the free energy change. We have the ground state for the enzyme, we have the ground state for the product that is more stable than the enzyme. Then we have a free energy change associated with the process.

(Refer Slide Time: 18:17)



In the reaction coordinate diagram, we have enzyme catalyzed and enzyme uncatalyzed reactions. We have variations because of the catalysis brought about the enzyme, that makes the reaction easier to occur. We will speak about this in further detail when we study enzyme kinetics. We have in this case the reaction intermediates, the enzyme substrate complex.

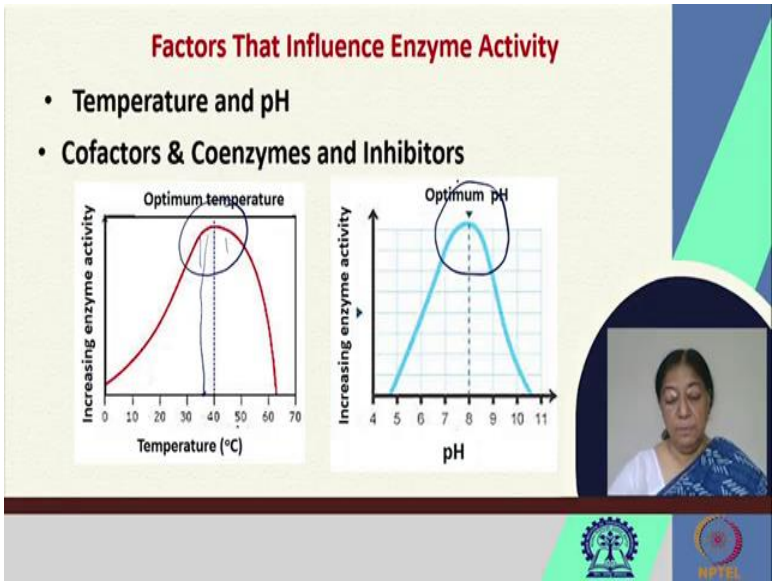
(Refer Slide Time: 18:49)



There is also possibility of enzyme inactivation. This is going to be important in inhibitor design, when we want to have an idea of the active site; we know the substrate binds to the active site. If we want to inactivate our enzyme, we have to design an inhibitor. The inhibitor design can be a substrate analog. But in this case we would like the enzyme inhibitor to be complex and that is occurring because molecular recognition is also a tight complex.

We can result in a protein shape difference, an enzymatic active site geometrical loss, the scaffold being lost, because of the external stress on the protein. For example, by applying heat or changing the pH what will happen is, we will have a denatured protein. And this denatured protein cannot carry out it's cellular function. The most common example is where we have irreversible egg protein denaturation, caused by high temperature.

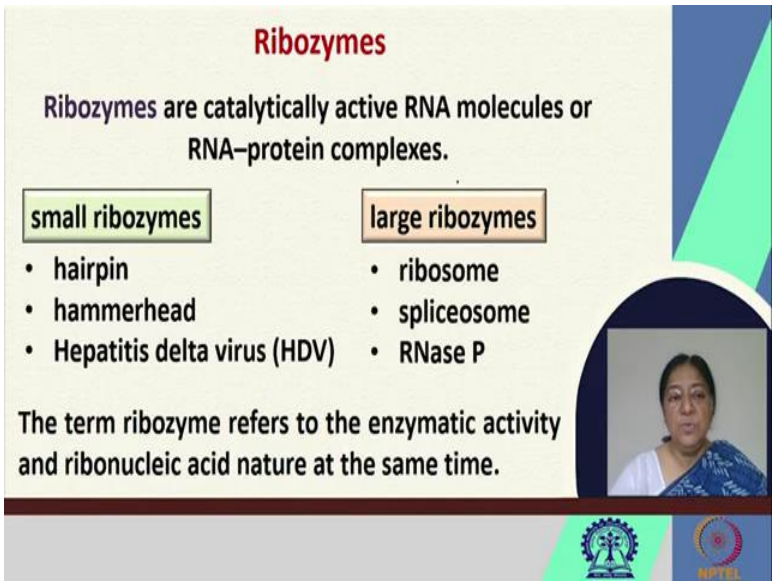
(Refer Slide Time: 20:09)



So factors that influence the enzyme activity are similar to those that would be involved in a protein, that is temperature and pH. Any change would result in a loss of structure, a loss of function, in this case a loss of enzymatic activity and other cofactors, coenzymes and inhibitors. So we would have an optimum temperature for enzyme activity, which we realize functions around our body temperature, around 37^{o}C.

Similarly, we would have an optimum pH for most enzymes around 7 to 8; 7.4 being the physiological pH.

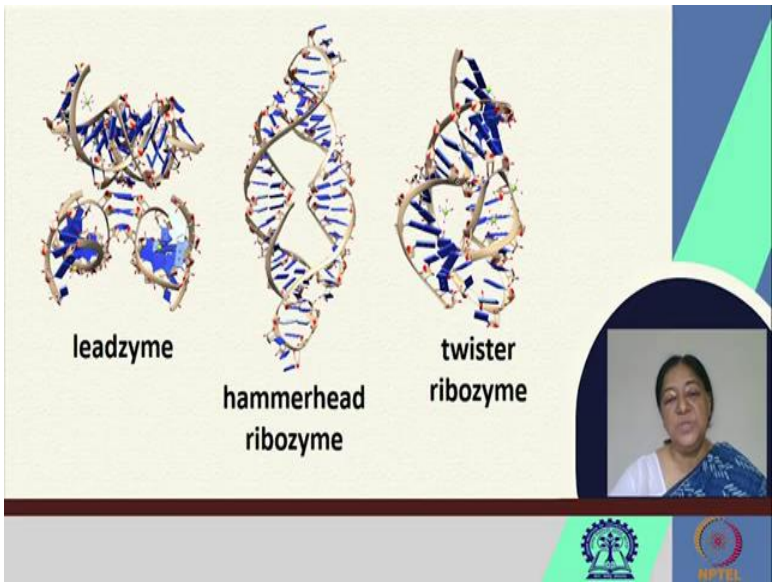
(Refer Slide Time: 20:53)



Another very important enzymatic class that has gained importance over the past years, are ribozymes. These are catalytically active RNA molecules or RNA protein complexes. The term ribozyme refers to the enzymatic activity and ribonucleic acid nature at the same time.

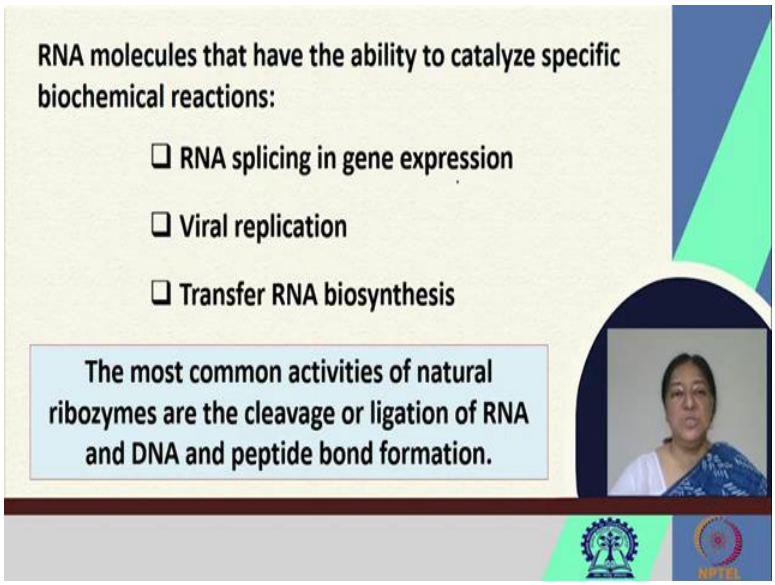
There are small ribozymes that are hairpin ribozymes, hammerhead ribozymes, and the hepatitis delta virus type. There are large ones also, the large ribozymes are the ribosome spliceosome and RNase P.

(Refer Slide Time: 21:43)



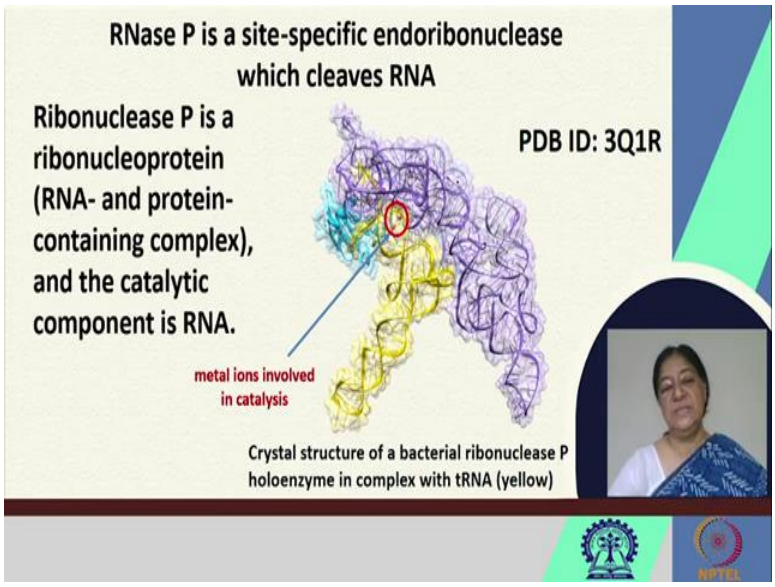
These are also very specific in their activity, in the sense that they look like RNA structures, a leadzyme, a hammerhead ribozyme, and a twister ribozyme.

(Refer Slide Time: 21:53)



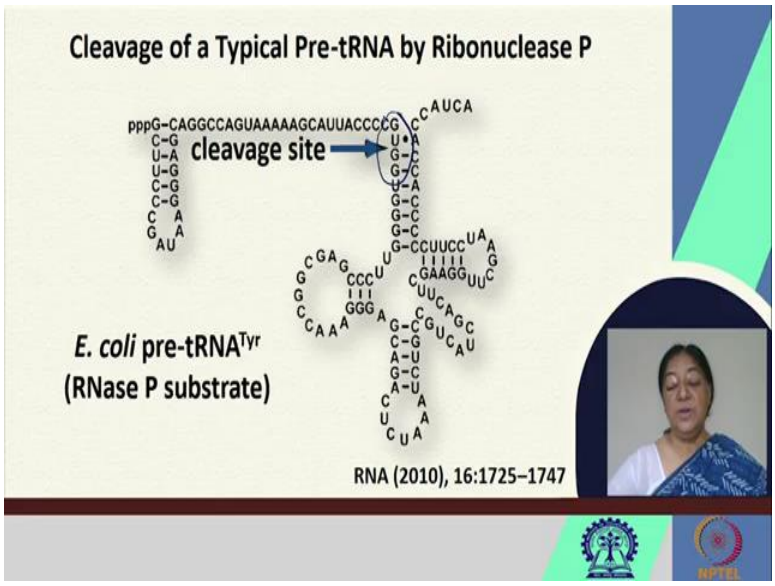
The beauty is that these RNA molecules have the ability to catalyze specific biochemical reactions, just like enzymes that are proteins. We have RNA splicing in gene expression, viral replication and the transfer RNA biosynthesis. The most common activities of natural ribosomes are the cleavage or ligation of RNA and DNA and peptide bond formation. These are all catalytic reactions and in this case the catalysis is brought about by ribosomes.

(Refer Slide Time: 22:33)



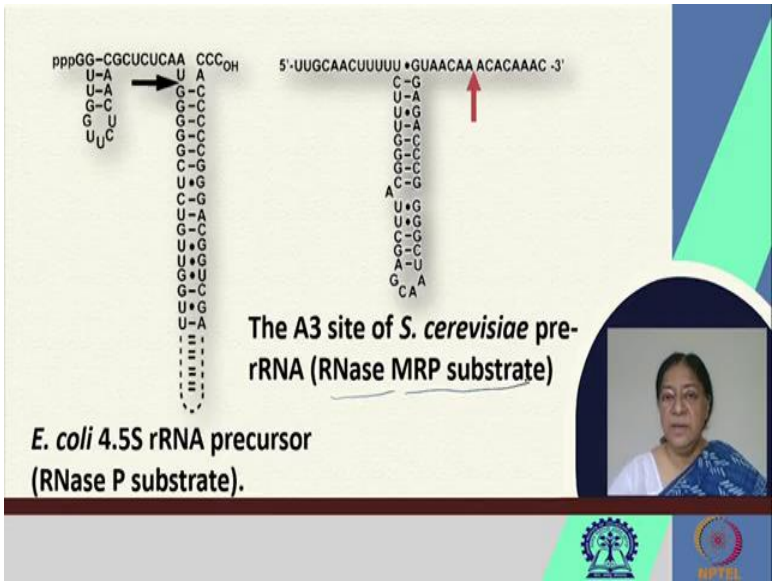
For example if we look at RNase P, it is a site specific endoribonuclease which cleaves RNA. So it has a specific structure, a specific metal ion involved and this ribonucleoprotein (RNA and protein containing complex), the catalytic component in this case is RNA.

(Refer Slide Time: 23:03)



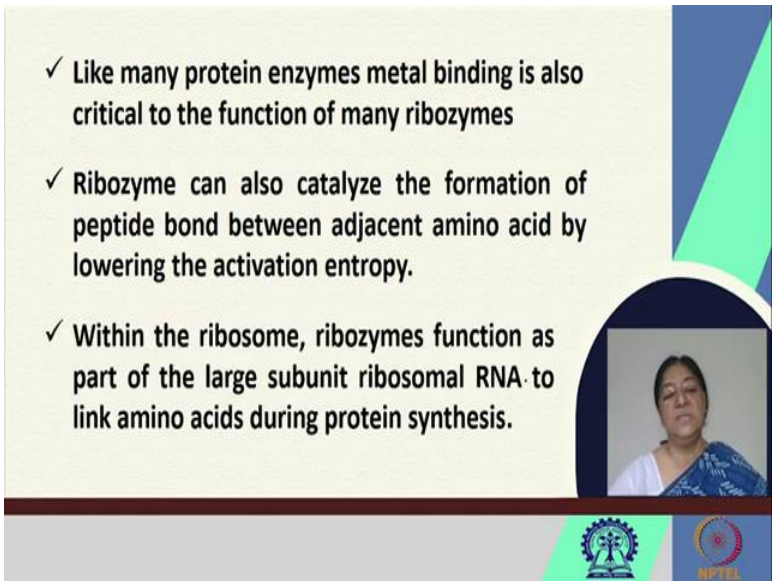
We can have cleavage of typical pre-tRNA by Ribonuclease P and these [refer to slide] are the specific substrates. There is the RNase P substrate and there is the cleavage site that belongs to the recognition here, are the RNA bases.

(Refer Slide Time: 23:25)



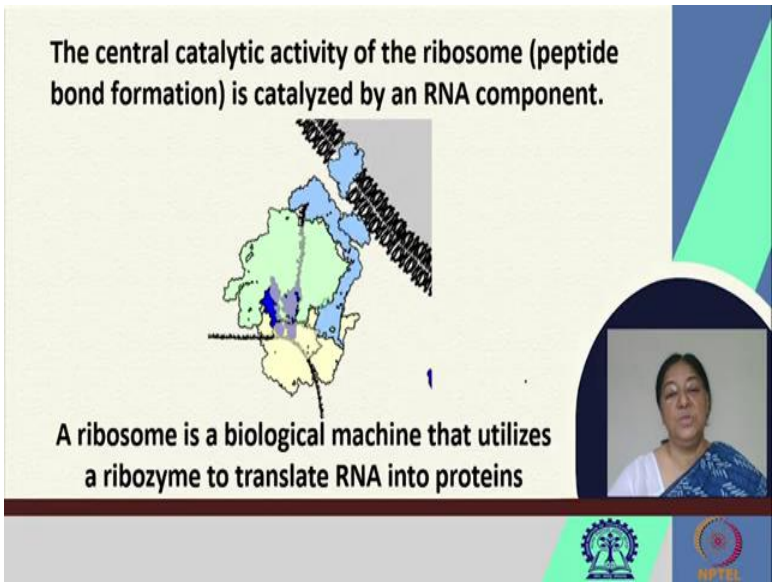
Similarly, there are other [refer to slide] types where we have the RNase P substrate and we have the cleavage site for an RNase MRP substrate. These are specific reactions involving RNA, and the catalysis brought about by these.

(Refer Slide Time: 23:43)



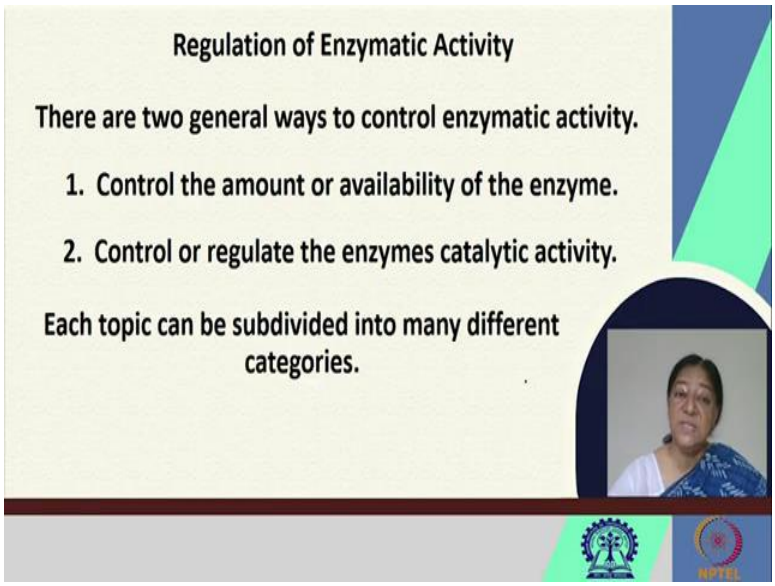
Like many protein enzymes, metal binding is also critical to the function of many of these ribozymes and they can also catalyze the formation of the peptide bond between adjacent amino acids, by lowering the activation entropy, which is what happens in ribosomes. And within the ribosome, ribozymes function as a part of the large subunit ribosomal RNA, to link amino acids during protein synthesis.

(Refer Slide Time: 24:15)



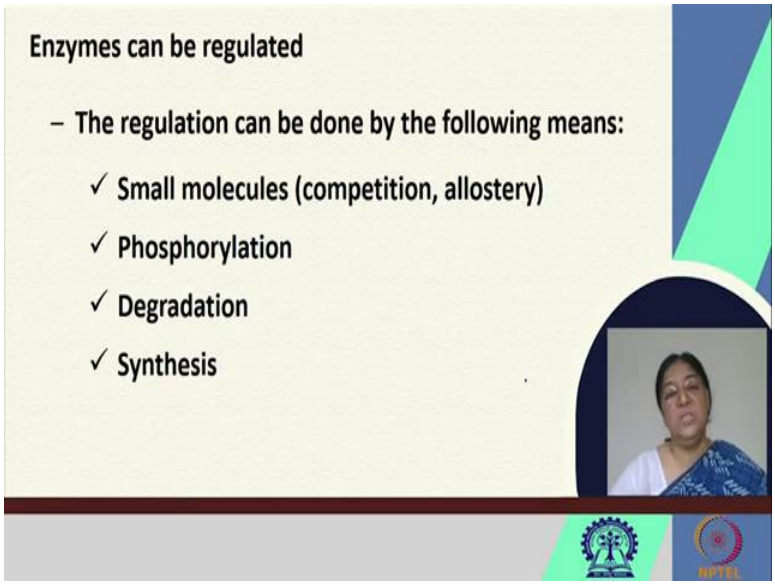
The ribosome is a wonderful biological machine that utilizes a ribozyme to translate RNA into proteins. Here [refer to slide], the central catalytic activity of the ribosome, that is the peptide bond formation is catalyzed by the RNA component. As we see the increase in the linking of the formation to form the peptide, we realize the importance of the ribosome in the formation of our proteins.

(Refer Slide Time: 24:57)



So we have a regulation of enzymatic activity. There are two general ways to control enzymatic activity. One by controlling the amount or the availability of the enzyme and the other by controlling or regulating the enzymes catalytic activity in several ways and each topic can be divided into several subcategories, which we will visit later on.

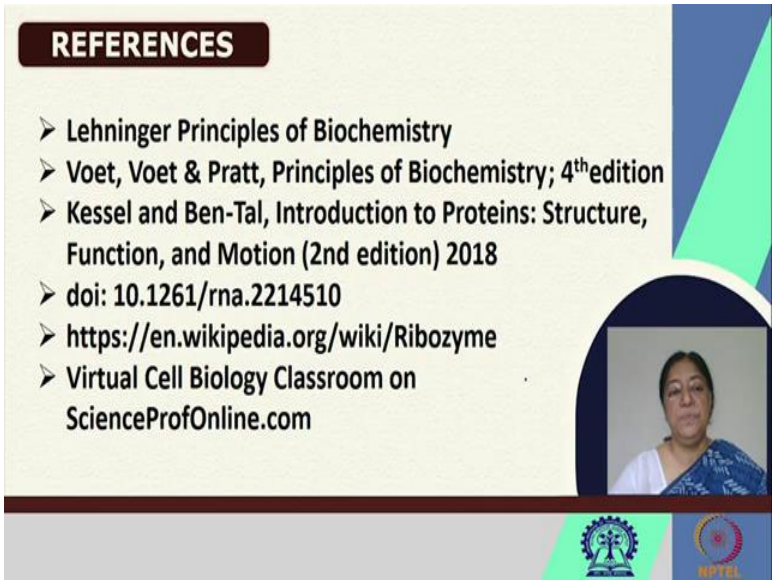
(Refer Slide Time: 25:17)



The regulations can be done by these small molecules that can act as competitors, allosteric molecules that would act at sites away from the active site of the protein, but nevertheless affect the enzyme activity, in terms of enzyme inhibition. Phosphorylation, where we could change the enzyme activity, degradation, denaturation of the enzyme, that will result in a loss of the scaffold, that holds the active site residues in position to recognize the substrate.

The geometric specificity and the chemical specificity are therefore extremely important for an enzyme to recognize a substrate. To form or to undergo it's catalytic action, the enzyme mechanisms which we will be visiting in the subsequent 2 lectures, that will tell us about how specific the substrate is for a particular enzyme.

(Refer Slide Time: 26:33)



These [refer to slide] are the references.

Thank you.