Introduction to Complex Biological Systems
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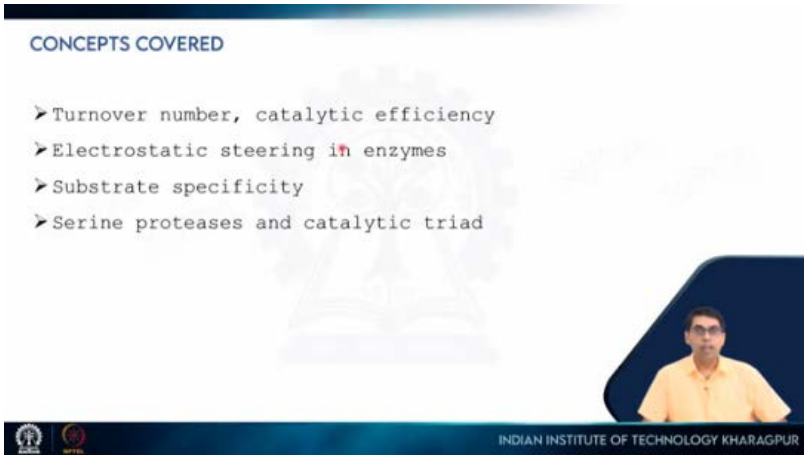
Lecture 18
Specificity of enzymes, e.g. Chymotrypsin

Welcome back. So in this lecture I am going to discuss some more concepts on enzymes and I will also discuss one specific example of chymotrypsin which is a protease. So in this lecture I am going to cover concepts on concepts like turnover number catalytic efficiency I will discuss one very interesting property that happens in some enzymes which is called electrostatic steering. I will talk about substrate specificity which is very important in case of enzymes. We have already seen specificity in the context of protein ligand interaction. So here we will see in the context of enzyme catalysis and finally as I said I will talk about serine proteases and I will take a specific example of chymotrypsin and in that context I will discuss catalytic triad which is found in the active site of serine proteases. So, let us look back at this Michaelis-Menten equation.



So, this equation even though it is called Michaelis-Menten equation, the concept was first introduced by Victor Henry which was further developed by Michaelis and Menten and their paper came out in 1912. So, in all textbooks this is popularly known as Michaelis-Menten kinetics. So, in this kinetics we saw that there is this enzyme and substrate. So, free enzyme and free substrate the first step is binding of this enzyme and substrate which is the ON and OFF rates are given as k_1 and k_{-1}. Then the next steps are simplified.

So conversion of the substrate to the product and the product release are combined into one irreversible step, which is given by this rate constant k_2. So from this we go to enzyme plus product. So in the derivation of the rate constant $DP\ \ DT$ or the velocity of the reaction, we used steady state kinetics. So we assume that the concentration of this enzyme substrate complex remains constant under steady state condition. So, today I am going to discuss a little bit more about what we mean by steady state condition and under what circumstances this condition prevails.

So, steady state condition again means that this $[ES]$ remains constant and we saw that the rate of product formation will be equals to k_2 times the concentration of $[ES]$. So, we have to solve for $[ES]$ the concentration of the enzyme substrate complex. So, this is what we get when we set $D\ [ES]\ DT$ equals to 0.

So now we start from this equation. What I have done in the next step is I have divided this by k_{-1} plus k_2. So this becomes 1.

So this divided by k_{-1} plus K_2. So this is what you get. And then this divided by k_{-1} plus k_2. So this is the second step. Now, what we do is we take k_{-1} plus k_2 divided by k_1 $[S]$. So, we take that and we divide both sides.

$$[ES](k_{-1} + k_2 + k_1[S]) = k_1[E]_T[S] \qquad [ES] = \frac{1}{\left(1+\frac{K_M}{[S]}\right)}[E]_T$$

$$[ES]\left(1 + \frac{k_1[S]}{k_{-1}+k_2}\right) = \frac{k_1[E]_T[S]}{k_{-1}+k_2} \qquad [ES] = f[E]_T$$

$$[ES]\left(\frac{k_{-1}+k_2}{k_1[S]} + 1\right) = [E]_T \qquad f = \frac{1}{\left(1+\frac{K_M}{[S]}\right)}$$

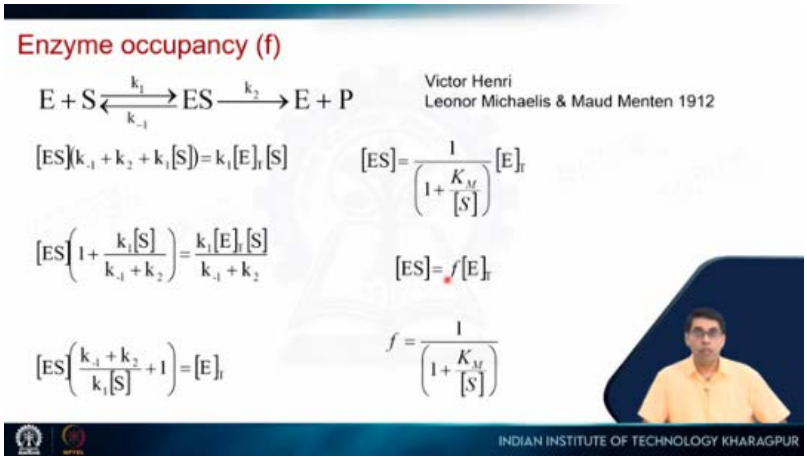
So, if we do that then this $k_{-1}\ k_2$ goes to the numerator and $k_1\ [S]$ goes to the denominator. Here you see $k_{-1}\ k_2$ were multiplying up. So, that will cancel out and $k_1\ [S]$ we are dividing. So, this will cancel out. So, this becomes 1.

And on this side also $k_1[S]$ and k_{-1} plus k_2 that cancels out. So, we are left with $[E]_T$ so enzyme substrate complex multiplied by some constant. So remember that in this case we have taken substrate, which is a constant number at this type. So normally we assume that this substrate, free substrate and the total substrate are almost equal because the substrate concentration is much much higher than the enzyme concentration so very small amount of substrate will be bound.

So this free substrate and total substrate will be almost equal. So this you can assume as a constant. So, all of this is a constant. Now, we can divide this whole thing on this side. So, $[E]_T$ by this.

Now, you will remember that k_{-1} plus k_2 by k_1, this 3 together we have clubbed into one single constant called the Michaelis constant, which is K_M. So, this becomes 1 plus K_M by $[S]$, which is shown here, 1 plus K_M by $[S]$. So, what we have the enzyme substrate complex equals to some constant multiplied by the total enzyme concentration. So, this actually we can put this as f where f equals to this. So, this is what we define as enzyme occupancy. So, when f equals to 0, it means that no substrate is bound to the enzyme.

So, the concentration of enzyme substrate is 0. When f equals to 1, then $[ES]$ equals to $[E]_T$. It means that 100% of the enzyme is bound to the substrate. When f is let us say 0.5, it means that half of the enzyme are bound and half of the enzymes are free. So f will have a value between 0 to 1 and that is given as occupancy.

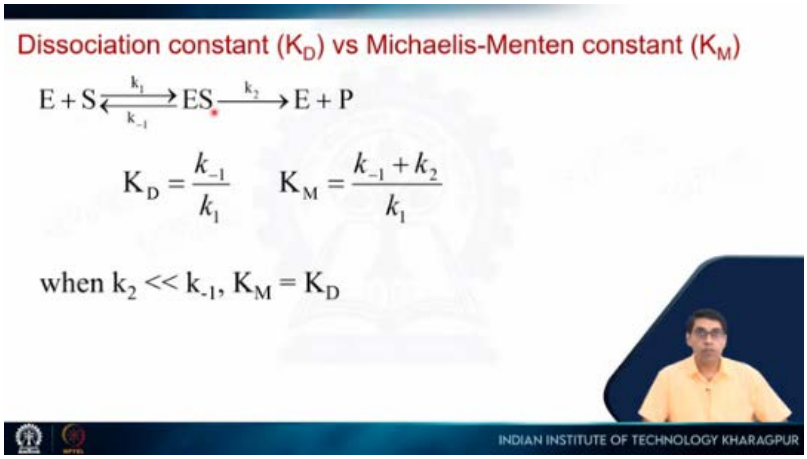


So now let us look at two more numbers. So during protein ligand interaction, I introduced this concept called dissociation constant or K_D which gives us a measure of how tightly a ligand binds to the protein. So, if we just consider the first step, this is where the substrate binds. So, we can define the dissociation constant for the substrate binding to enzyme by this, where K_D will be equal to the ratio of the OFF rate and the ON rate. So, k_{-1} divided by k_1.

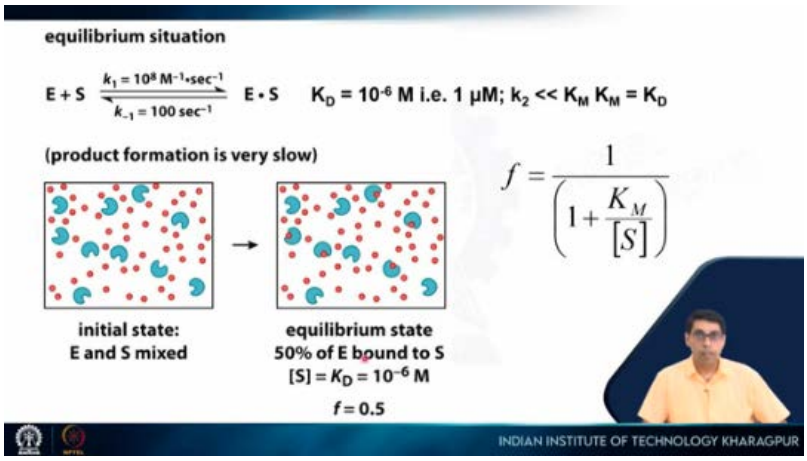
$$K_D = \frac{k_{-1}}{k_1} \qquad K_M = \frac{k_{-1} + k_2}{k_1} \text{ when, } k_2 \ll k_{-1}, K_M = K_D$$

K_M is something that we have defined as this, k_{-1} plus k_2 divided by k_1. So, if you consider this, then in case of K_M, this is the extra term. Now, if for a particular enzyme, K_2

is much less than k_1, so k_2 is much much less than k_{-1}. So, we can ignore this term then K_M will be equals to k_{-1} by k_1 which is K_D so, under this condition where this rate is much less than the rate of dissociation, this rate k_{-1}. So, the reaction will precede more in this direction compared to this direction under that condition K_M equals to K_D and this is what is called the equilibrium condition.



So let us look at that. So that is the equilibrium situation and we can take some actual numbers to understand it better. So let us say k_1 equals to 10^8 per molar per second. So remember that this is the rate of association and the maximum value that this rate of association can become is the rate of dissociation or in other words how fast the two molecules that is the enzyme and the substrate the free enzyme and the free substrate they found each other. They find each other.



So they can find each other at the rate of diffusion. So that is what we have taken. Let us say k_{-1} is 100 per second. So then K_D will be the ratio of these two, so 100 by 10^8. So we get 10^{-6} molar or 1 micromolar and these are very realistic numbers. So enzyme K_D values

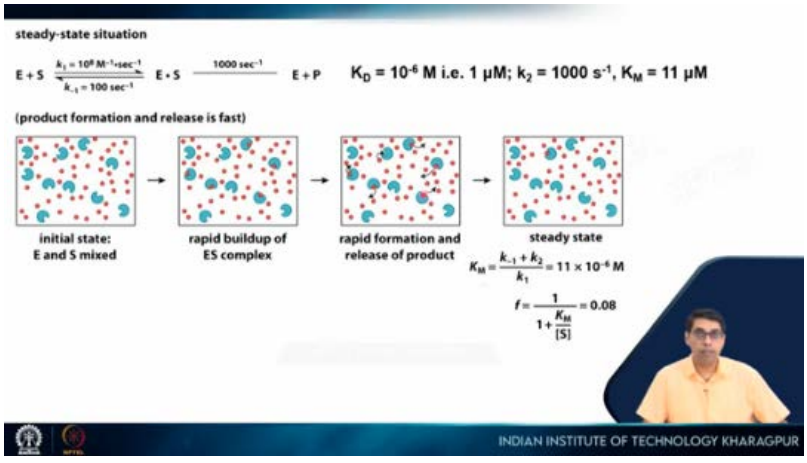
will be somewhere in this range of 1 micromolar. We assume that K_2 is much much less than K_M. So, K_M will be equals to K_D. Now, if we consider a situation where we have taken a substrate concentration of 1 micro molar which is equal to the K_D or the K_M value, then if we plug in this K_M is 1 micromolar because it is equal to the K_D substrate concentration is also 1 micromolar.

So, we have taken substrate also as 1 micromolar. So, these two are exactly the same. So, this is the ratio is 1. So, 1 plus 1 is 2. So, f will be 1 by 2 or 0.5.

So, it means that under this condition a rapid equilibrium will be set up where 50% of the enzyme will be bound to the substrate and 50% of the enzymes will be free. So if I go back here, so the equilibrium is set up in this step. So this is much faster because both these rates are much faster than this. So a rapid equilibrium is set up where 50% of the enzyme is bound to the substrate and 50% is free. And then this reaction happens very slowly from the bound state.

So at any given point of time, we will get 50% saturation. And remember this is only when the concentration equals to the K_D or the K_M value. The other situation is the steady state situation. So, under this condition, so let us assume the same k_1 and k_{-1} values. So, that K_D is 1 micromolar, but now the K_2 , we have assumed as 1000 per second.

So, this is 10 times more than k_{-1}, but again this is 10% of this. So, we cannot exactly ignore k_{-1}. So K_M becomes 100 plus 1000 divided by 10^8. So that turns out to be *11* micromolar. So K_M is *11* micromolar, K_D is *1* micromolar.

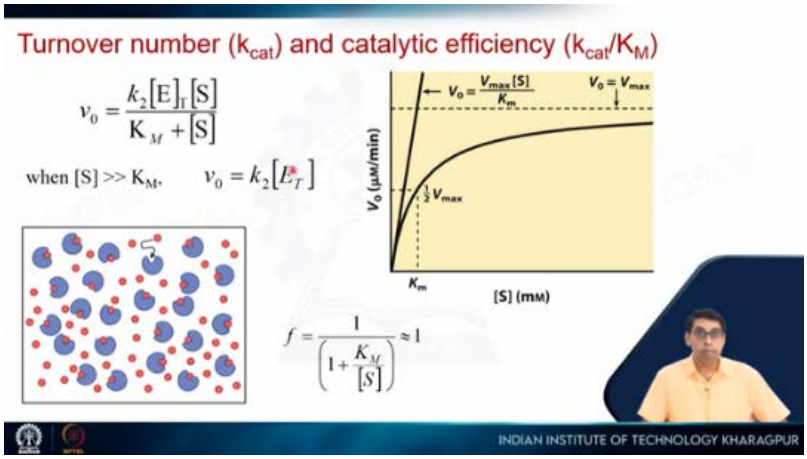


So what happens in this case is that the substrate binds to the enzyme and once it binds, it gets very rapidly converted to the product and the product is released. So, under this

condition, what is our occupancy? Occupancy is this. So, K_M is *11*, substrate concentration is 1. So, that is the ratio is *11*, *11* plus *1* is *12*.

So, *1* by *12*, which is roughly *0.08* or at any given point of time, only 8% of the enzyme will be bound to the substrate. So, there is a steady state of where the enzyme is binding to the substrate and then rapidly getting converted to the product and the concentration of the enzyme substrate complex is around 8%. So, this is our steady state condition.

So, let us look at the Michaelis-Menten equation and there are two terms that are very often used. So, if you are reading research papers or if you characterize an enzyme, you will come across this one is called the turnover number and the other one is called the catalytic efficiency. So, what are these two numbers? So, let us look at the Michaelis-Menten equation here. So, remember that k_2 times $[E]_T$ is V_{max}. So, we can either write it as V_{max} or we can break it down as k_2 times $[E]_T$.

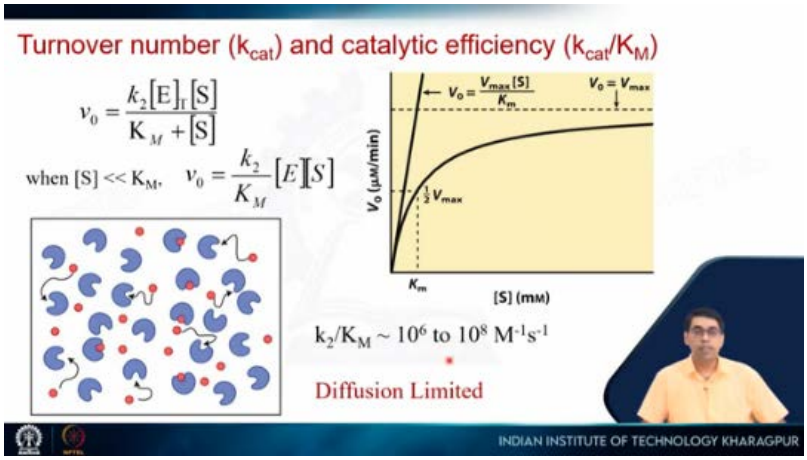


So when the substrate concentration is much much greater than K_M. So this is much much greater than K_M, so we can ignore K_M, so denominator will be $[S]$, so these two cancel out and v_0 will be equals to k_2 times $[E]_T$, which is given here, which means that the rate of velocity is independent of the substrate concentration. So if we increase the substrate concentration, it is not going to increase, which means that we have reached this saturation point. So when substrate concentration which is somewhere here is much, much greater than the K_M value, then our v_0 will reach this V_{max} value or k_2 times $[E]_T$. And we can see that what happens to the occupancy. $[S]$ is much much greater than K_M, so we can ignore this, this ratio will be a very small number. So this is essentially *1*, so *1* by *1* is *1*, so the occupancy is *1*. It means that all the enzymes are bound to the substrate. So if all the enzymes are occupied, then under that condition you cannot increase the rate, so you have reached the maximum rate.

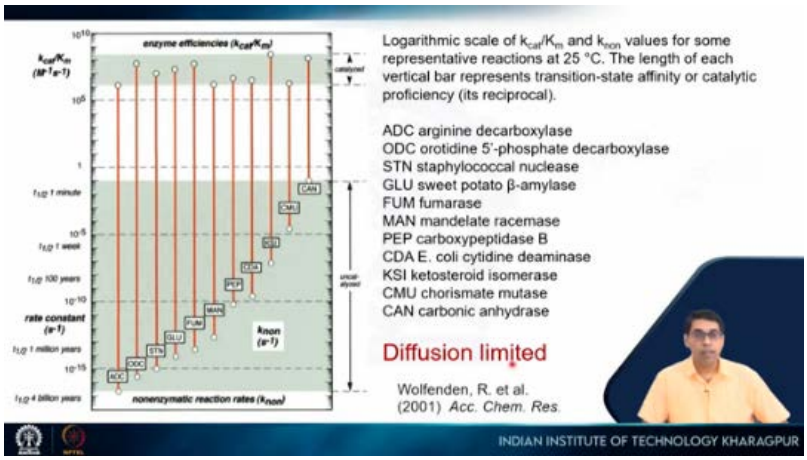
This k_2 is also referred to as k_{cat} and that is the turnover number. So, if you look at this value, so k_2 will be v_0 by $[E]_T$. So, rate of product formation per mole of enzyme. So it means that k_2 gives a number where you get how many reactions happen per second per enzyme. So that is why it is called the turnover number that these many reactions are happening per second per molecule of the enzyme or per mole of the enzyme. Other factor is called the catalytic efficiency and for that we have to look at the condition where our substrate is much less than K_M. So, we are somewhere here.

So, if substrate is much much less than K_M then we can ignore this. So, our equation becomes k_2 $[E]_T$ S by K_M or if we take these two together k_2 by K_M times $[E]_T$ times $[S]$. So, this looks like an equation where these two molecules are finding each other and that is multiplied by some rate constant. So, this is the rate of association of the enzyme and the substrate. So again you can see that the velocity will depend on this $[E]_T$ and we can assume that since the substrate is much much less than K_M, very less amount of enzyme is bound so $[E]_T$ will be almost equal to the free enzyme concentration. So we have replaced $[E]_T$ by $[E]$. So this is the rate of association.

Now, the maximum value that the rate of association can become is 10^6 to 10^8. So, again this is the rate at which two molecules find each other. So, under these conditions the velocity will increase linearly as a function of $[S]$ and the rate constant is given by this k_2 times K_M which will have a maximum value of this which is the rate of diffusion. And as we have seen, I will show again in the next slide that many enzymes through evolution have been optimized in such a way that this k_2 by K_M value is actually in this range which means that cannot make these enzymes any better because they are working as fast as they can and they are limited only by the rate of diffusion so how fast the substrate and the enzyme can find each other. So the substrate goes and binds to the enzyme. The moment it binds the reaction happens and the product is released. So this is finding of the substrate to the active site of the enzyme. This is what limits the rate of the reaction. So this is diffusion limited. And this is something that I have already shown you in a previous lecture.



So, these are the uncatalyzed reactions and then in the presence of enzyme this is the catalyzed reaction and here we are plotting k_{cat} over K_M and this value is in the range of 10^6 to 10^8. So, all these enzymes are as best as they can be because their rate is limited by the rate of diffusion. So, these are diffusion limited. Now, there is something very interesting that happens that in some cases, some enzymes, I will show you two examples where the rate of association or this k_{cat} over K_M is greater than 10^8.



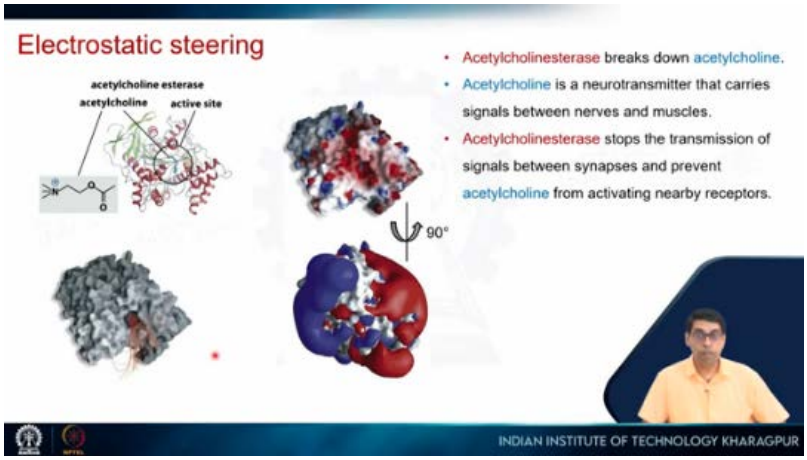
So, it approaches 10^9 or maybe even 10^{10} and this is something called electrostatic steering. So, we have seen this concept in the case of protein DNA interaction and here I am going to talk about it for protein ligand binding or protein substrate binding. This is an enzyme called acetylcholine esterase. So what it does it breaks down acetylcholine, so that's acetylcholine. Acetylcholine is a very important molecule. it's a neurotransmitter that carries signals between nerves and muscles. So whenever we are doing something, this is one of the molecules that results in this signaling event. So what it does is acetylcholine esterase breaks down acetylcholine which means that it stops the transmission of this signal between the synapses.

So the two nerve endings or the joint where two nerves meet and it prevents acetylcholine from activating nearby receptors. So acetylcholinesterase is a very important enzyme. Now because the signal has to be transmitted so fast and then this enzyme also has to work so fast, it uses electrostatic steering to ensure that the substrate binds to the protein or the enzyme very fast and in the right orientation. So, if we look at the protein you can see you can see several alpha helices and beta strands. So, this is the surface representation.

So, again this is the cartoon representation of the protein and this is the surface representation of the protein and in this case the side chains are colored in red and blue. So, oxygen is red, nitrogen is blue. Oxygen means aspartic acid and glutamic acid cytins, they will have negative charges and arginine, lysine, histidine, they will have positive charges. So, red means negative charge, blue means positive charge.

And you can see the active site is here. So, this is the same orientation and this is the active site. So, you can see that the active site is in the middle of these negative charges. And what is the charge of our substrate? It is positively charged.

So these negatively charged residues will drive or attract this molecule towards this active site. And if you see so this molecule is now turned by 90 degree so you can see the front phase is here and the back phase is here. So you can see that the back phase where the active site is not there the active site is on this side so if the molecule comes and binds here that will be futile binding. So you want to discourage that. So we have positively charged residues here, the substrate is also positively charged so it will repel the molecule out of this region. So any futile interactions are prevented and it will drive the molecule towards this side and you can see that the lines of forces are drawn here, the electrostatic lines of forces. So that tells you the path that this molecule will follow to reach the active side. So this is called electrostatic steering. So by decorating the surface of the molecule by positive and negative charges, what this enzyme ensures is that the substrate finds the active site very quickly in the right orientation and that enhances this on rate, which actually goes beyond the diffusion limit. So diffusion limit is set when the two molecules are neutral.

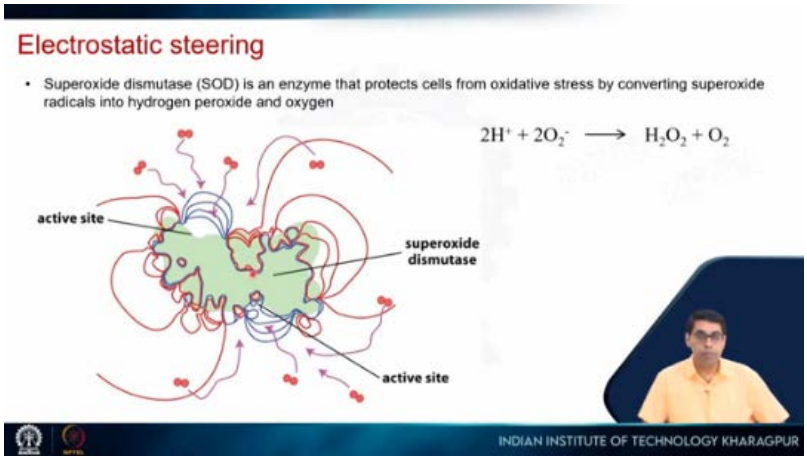


So this is another example. So this enzyme is called superoxide dismutase. So this is superoxide. So it's a very highly reactive species and it is a dangerous molecule because it can oxidize all these different macromolecules like DNA, RNA, protein. So we don't want this.

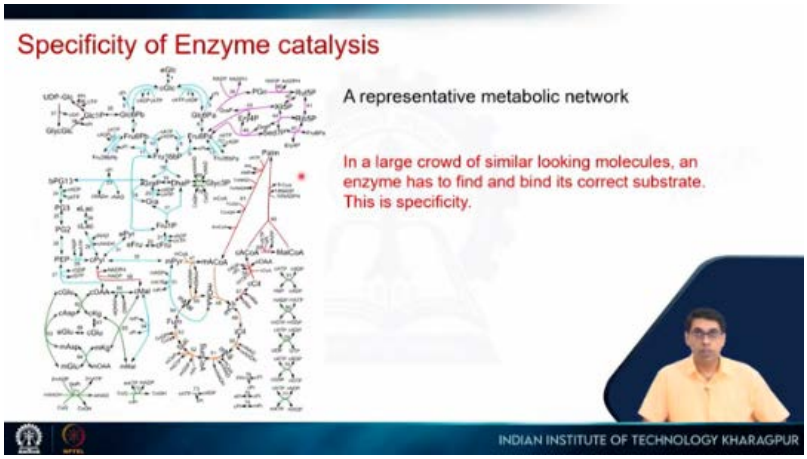
So the enzyme should quickly neutralize superoxide dismutase and convert it into less harmful molecules of hydrogen peroxide and oxygen. So, again if you look at the charge distribution on this enzyme and this enzyme is a dimer. So, there are two active sites. So, most of the enzyme is negatively charged. So, again you see that the substrate is negatively charged, red means negatively charged residue.

So, if the superoxide is here or here it will be quickly repelled out of this region. So, it is not going to bind the enzyme here or here. On the other hand once it drives it out it can get into this region which is positively charged. You see blue lines so positively charged and it will get attracted and get directed into the active side. So this is the active side this is one active side this is the other active side. So this negative electrostatic interaction will repel the molecule from these regions and drive them into this active site.

So it sort of funnels it into the active site so that the substrate is bound very quickly and it is converted into less harmful hydrogen peroxide and oxal. So apart from those enzymes also have to bind their substrate with high specificity. So this is one example and we will see this in more details when I talk about metabolism and bioenergetics later. So, these are different molecules which are converted to, so this will be a substrate, this is a molecule, this will be substrate, this will be the product. So, each of these steps are catalyzed by different enzymes and you can see that some of these molecules look very similar in structure, maybe they are just different stereoisomers.



So, enzymes have to be very specific, so that they bind the right substrate and carry out the reaction. So, this is called specificity and we have already seen specificity in the context of protein ligand interaction when I talked about the FGF receptors. Now I am going to talk about one specific enzyme which is a protease. So what are proteases? Proteases are enzymes which break down peptide bonds.



So you can see there is this peptide bond, so one amino acid is written here. So the side chain is written as $P1$ and the other one is $1'$.

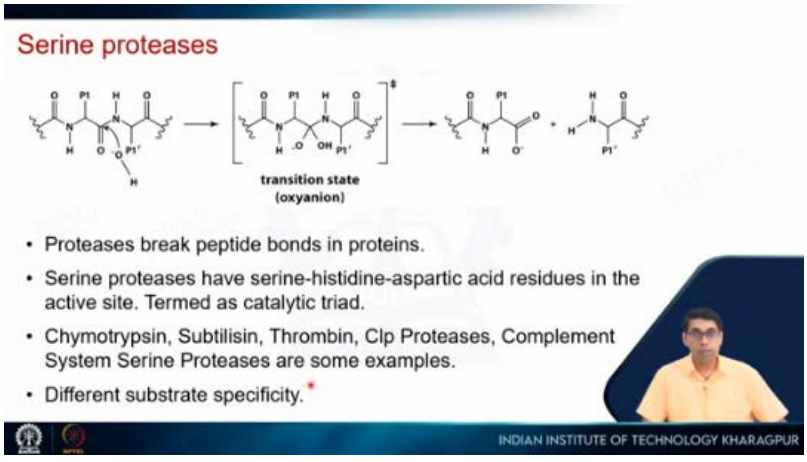
And this particular peptide bond between these two amino acids is going to be cleaved. Now uncatalyzed reaction happens something like this. So you have a hydroxide ion. It will attack the carbonyl group, the negative charge goes here. So this is the intermediate, the tension state will be where a partial bond is formed here and partial bond is formed here and partial charge is developed here. Now this will be a very short lived state and then it will quickly get converted into this. So now here you can see there are two possibilities this negative charge goes back here and this bond breaks. So if this bond breaks then again it

goes back to the substrate but instead of if this carbon-oxygen bond, if this carbon-nitrogen bond breaks, then this will go to the product and this is what you want. So there is always a chance that it can revert back to the actual substrate. So that will be a futile step. But if it goes in this direction, then you will get the substrate and the product, get the two products.

We have already seen that uncatalyzed, so this is the uncatalyzed reaction. So this uncatalyzed reaction is very slow and the half-life of this is almost 400 years. So to get 99% product formation you have to wait for 5 half-lives so almost 2000 years. So, proteases speed up this reaction and there is a class of proteases which are called serine proteases. There are also metal proteases, but I am going to discuss only serine proteases. And serine proteases, they have in their active site a set of three residues, serine, histidine and aspartic acid. And we will see that serine is the one which actually takes part in the reaction.

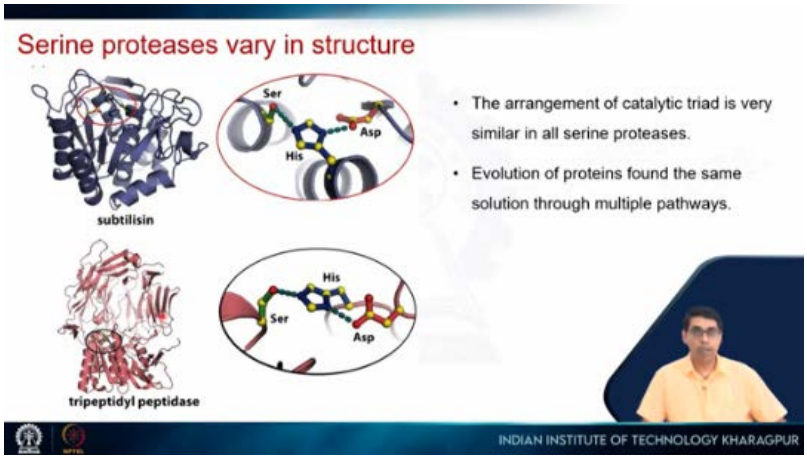
So, these are called serine proteases. But these three are very important and together they are called a catalytic triad. So, there are several examples like chymotrypsin, sirtulicin, thrombin, cleft proteases, and complement system of serine proteases. So, these are some examples. These become very important in our immune system also.

So, these proteases they have all sorts of functions. So, I am not going to discuss those I am going to talk about the enzyme mechanism. Now, these different proteases have different substrate specificity. Some proteases have no specificity. So they do not care what is $P1$ and what is $P2$. Some care what is $P1$ and what is $P1'$, some care what is $P1'$, and some proteases have very high specificity means that not only these two but also the neighboring amino acids will determine whether the enzyme will cleave it or not. So let us look at two serine proteases.



These are two examples, subtilisin and tripeptidyl peptidase. So you can see that the structures are shown here and you can see that these structures are very different. But if we zoom into the active site, so this is the active site of subtilisin and this is the active site of tripeptidyl peptidase. So, if we zoom into this you will see the arrangement of this catalytic triad is exactly the same. So, both have serine followed by histidine followed by aspartic acid and you can see that this serine, histidine, and aspartic acid arrangement is very similar.

So, it means that evolution of proteins has found the same solution through multiple pathways. So, it has come up with all these different enzymes which uses the same mechanism, but you can see that the overall structure looks very different. And I will talk about this in more details when I talk about protein evolution. So this the basic reaction mechanism and we will see take it in the context of chymotrypsin. So, here is your peptide.



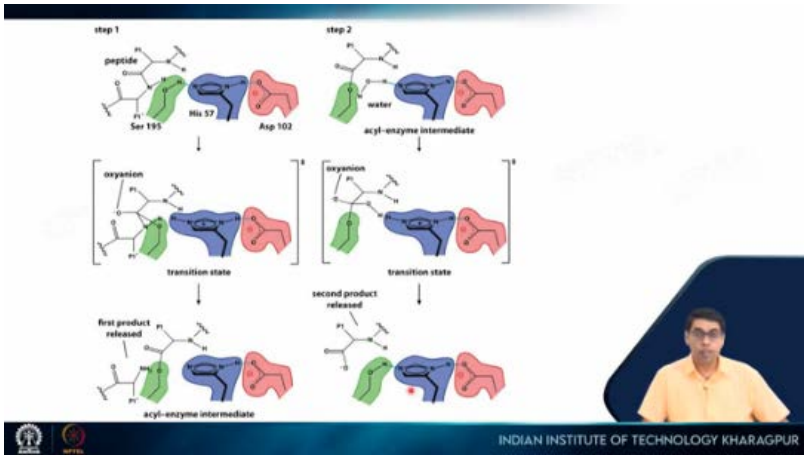
So, the peptide is bound here, this is serine its side chain is there OH, this is histidine, this is aspartic acid. So, histidine forms a hydrogen bond with this and the aspartic acid. In the first step this serine oxygen attacks this carbonyl carbon. So, it will attack this carbonyl carbon for that it has to be O minus. So, it gives up this proton to this histidine.

So, you see the histidine becomes positively charged and this double bond breaks and it becomes O minus. So, there is a negative charge here, there is a positive charge here. So, that is sort of the transition state. And this is very similar to this, where you have this O minus and this OH.

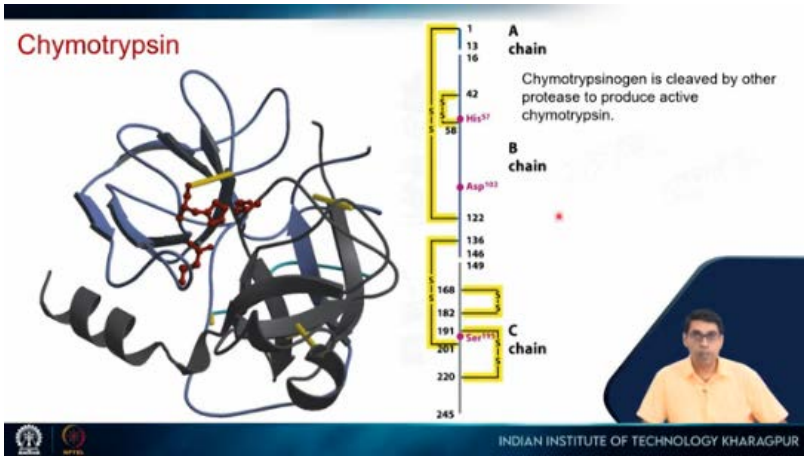
Now, instead of OH, we have the serine. So, instead of the hydroxyl ion, serine is performing this attack. Now, what happens is this bond, this carbon nitrogen bond will be broken. So this O minus will come back here this carbon nitrogen bond will be broken. So

you see that the first peptide or first part of the peptide is released but now the second part of the peptide is actually covalently linked to the serine by this ester linkage. Now the second step happens. So a water molecule comes in which is activated by the histidine. So this O minus will attack here, the proton of the water is handed over to the histidine. So now histidine is again positively charged and we have a negative charge here. So this is the second transition state. This will come back here and now this bond will break so the carbon oxygen where the oxygen is belongs to the serine. So this bond breaks so the second part of the peptide is released and the enzyme is regenerated. So how does the enzyme drive this reaction? Because again you see that this bond is broken here not this bond if this bond breaks it goes back to the original state but the enzyme ensures that it is this carbon nitrogen bond which is broken here. Similarly in this case this carbon oxygen bond is broken and not this one. So the probability of this breaking is very less because if this breaks it goes back to this. But if this breaks then you get the product formation and the regeneration of the enzyme. So, we will see that how the active site and small structural changes of the enzyme ensures that the reaction is driven in the forward direction and this also enhances the rate of reaction.

So this is the structure of chymotrypsin and again you can see these three enzymes in the active site aspartic acid, histidine, serine. Chymotrypsin is synthesized like this big polypeptide containing 245 amino acids and initially it is called chymotrypsinogen which is inactive.

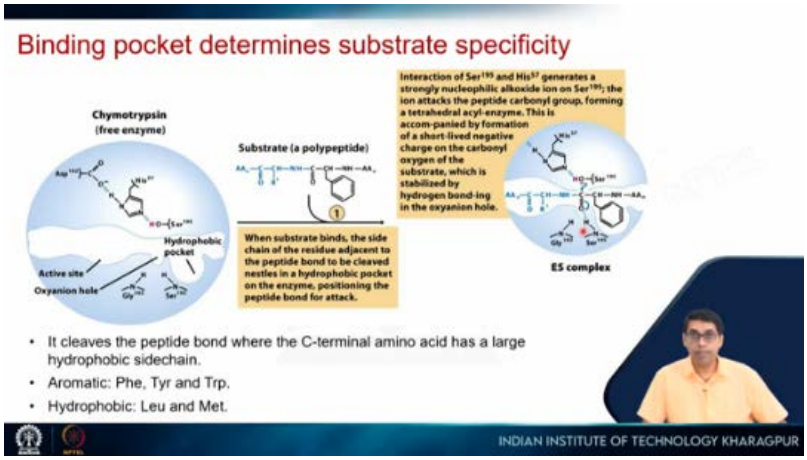


There are two places between this amino acid 13 and 16. So 14, 15 are gone. And here 47, 48, these two amino acids are gone. So this cleavage is performed by another protease and that activates chymotrypsin. So then it becomes activated enzyme, which is called chymotrypsin.

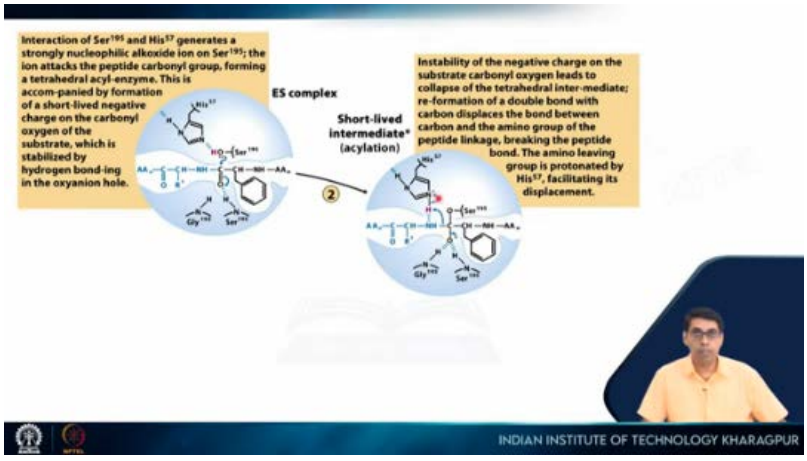


So in a cartoon like diagram chymotrypsin will look something like this and there are certain features. So again you see that the serine is here histidine is here aspartic acid is here. There is a big pocket which is lined by hydrophobic residues which is called the hydrophobic pocket and it binds to hydrophobic residues. So chymotrypsin prefers hydrophobic residues in this part. So if this bond is being broken then this amino acid will have hydrophobic residues so it can be anomatic residues like phenylalanine, tyrosine or tryptophan or it can be large aliphatic residues like leucine and methionine. So they bind, the side chains bind here. We will see that this oxygen will form O^- $minus$ and that fits in this small oxygen ion hole and it is stabilized by these two backbone amides. This is where the peptide bond is and the reaction will happen and this is where the first part of the peptide will fit in.

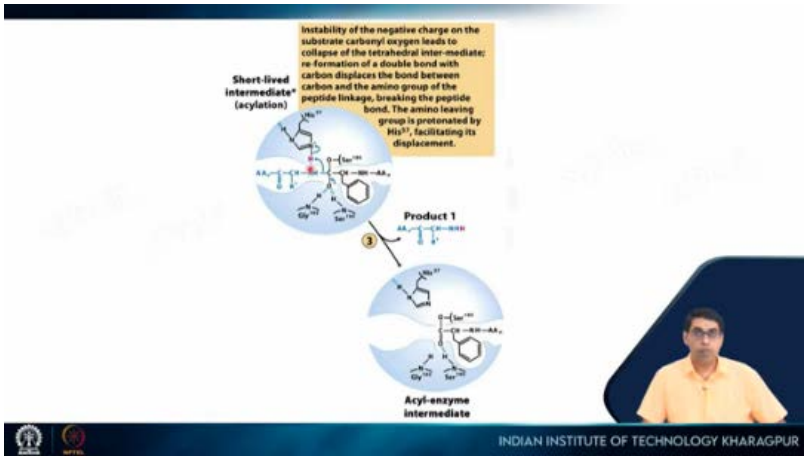
So, this is where the substrate is bound in the first step the serine will attach this the serine OH side chain OH will attach this carbonyl carbon and this oxygen is activated by forming hydrogen bond with these two side chains. So, this is glycine and this is serine amides. This hydrogen bond activates this oxygen and it stabilizes the tension state which looks something like this.



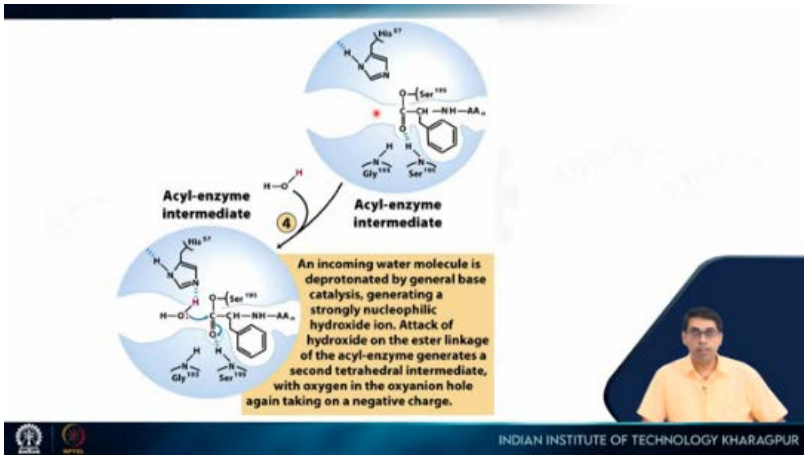
So, the O minus attacks here, the oxygen attacks here this becomes O minus and this hydrogen is handed over to the histidine. So, it becomes positively charged. There is a negative charge here and that is stabilized by this oxygen ion hole.



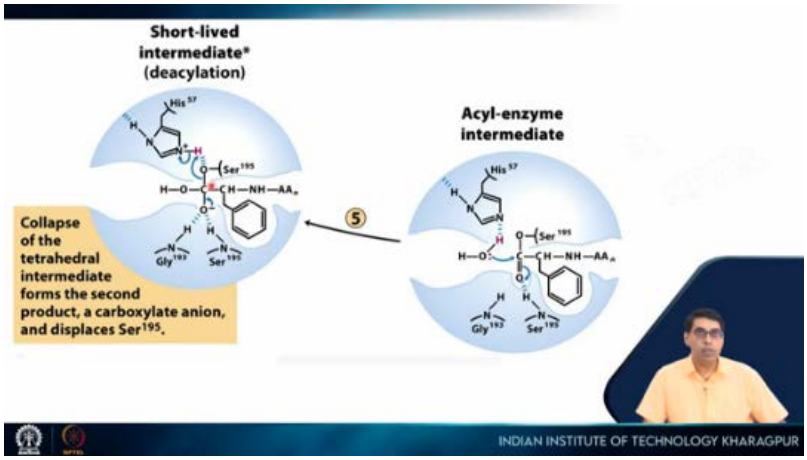
Now two things can happen either this carbon oxygen bond can break or this carbon nitrogen bond can break. You will see that this nitrogen forms a hydrogen bond with the histidine side chain which is positively charged. So this makes this a very good living group which means that it destabilizes this nitrogen carbon bond and ensures that this is the bond which breaks more often compared to this bond this carbon oxygen bond so it drives the reaction forward. So this one breaks so the first amino acid is released and now the second part of the amino acid is actually covalently linked to the serine by this ester linkage. So that's the acyl enzyme intermediate. Now this is the intermediate this peptide is gone so water molecule will diffuse in from this and it will sit here and this water molecule is now activated by this histidine by forming a hydrogen bond.



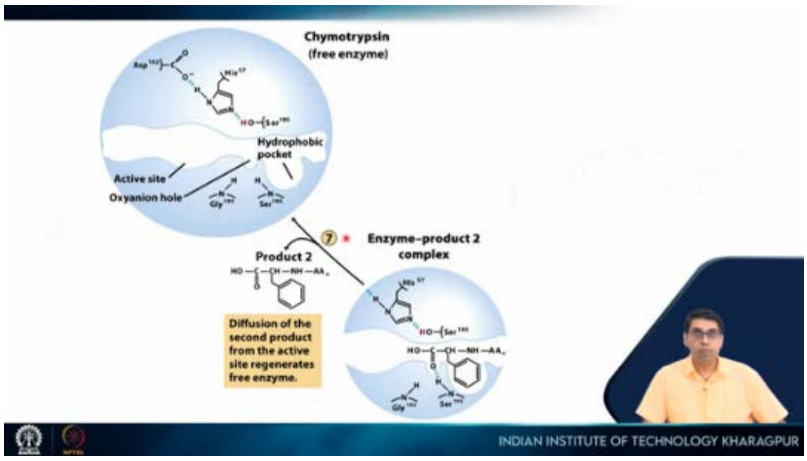
So it will abstract this proton this becomes O minus it will attack here and the negative charge ultimately comes here. So that is the second transition state and that is again stabilized by this oxyanion hole. So, this is the reaction and this is the intermediate that is formed.



Now this carbon oxygen bond can break or this carbon oxygen bond can break. So, now the histidine, just like the previous step it activates this oxygen. So, it forms a hydrogen bond with this which means that it makes this a better living group compared to this OH. So, this carbon oxygen bond will break like so.

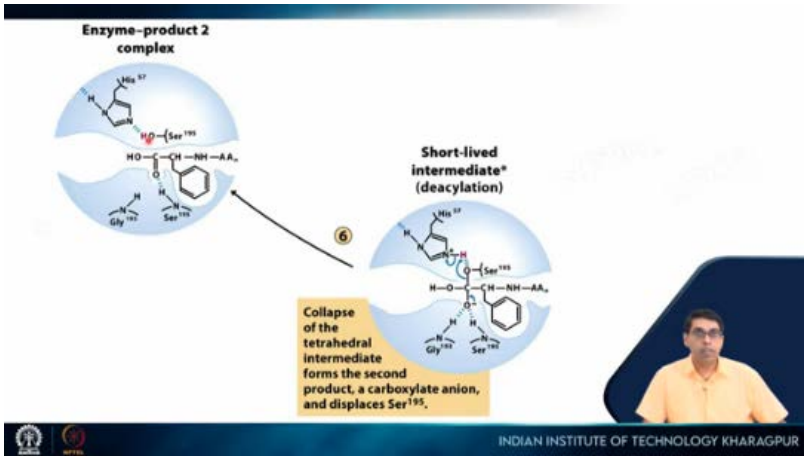


So, you see that this carbon oxygen bonds bond breaks and this is your free peptide. So, the serine is regenerated, the histidine is regenerated, the second part of the peptide is there and it will diffuse out.



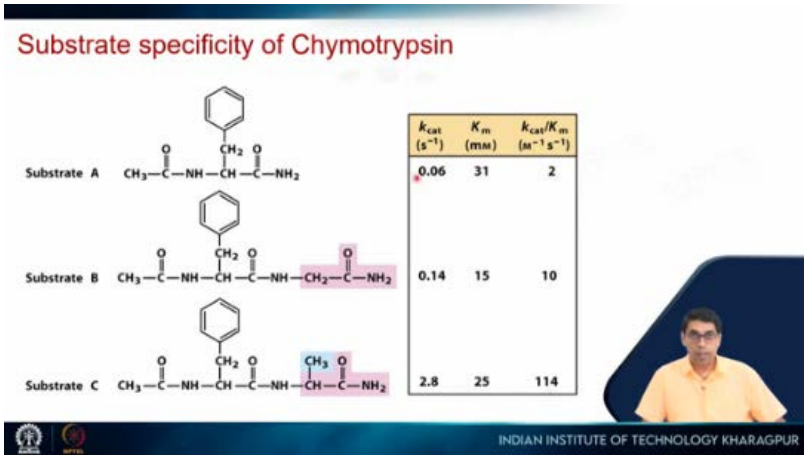
So, the second part of the peptide is formed, it diffuses second part of the peptide diffuses out and the free chymotrypsin or the free enzyme is regenerated.

So this is where we see substrate specificity so I told you that for the second part of the substrate it has to be a bulky group. So we have kept it a bulky group but the first part of the substrate also determines a little bit of the specificity. So, this is the peptide bond in this case instead of an amino acid we have just hydrogen. So, this is a $CONH_2$ group and for this one the k_{cat} values and the K_M values are given.

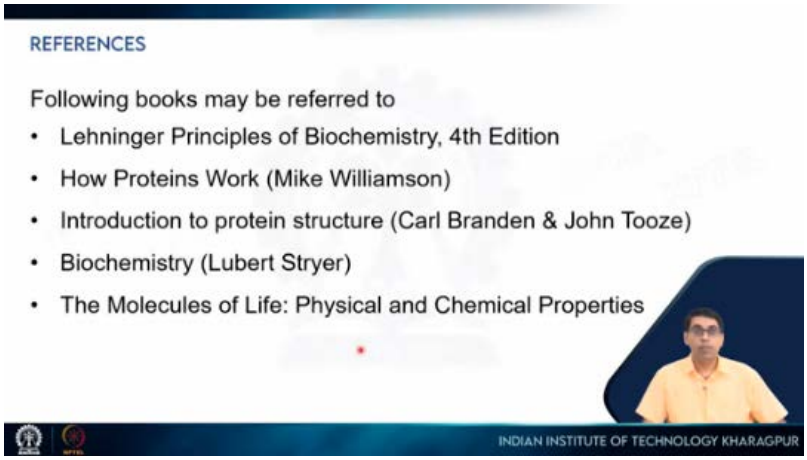


So, k_{cat} over K_M is 2. So, instead of that if we have this alanine and the carbonyl CH_2 group is protected by NH_2, sorry this is glycine, in this case k_{cat} is 0.14, K_M is 15 and the specificity has increased from 2 to 10, the k_{cat} over K_M value. If we put alanine which has a big more bulky group which means that this binds even better now you see that the k_{cat} over K_M has increased to 114. So, this is how we can actually determine substrate specificity.

So, we are not even changing this we are changing this part and we can see that the k_{cat} over K_M value is increasing. So, again if we make it even a bulkier group maybe this will go down. So, by using these different types of substrates we can tell that chymotrypsin has higher specificity for which type of substrate.



So, that is all for now again these are some of the books that I have referred to and you can go through them.


REFERENCES
Following books may be referred to
• Lehninger Principles of Biochemistry, 4th Edition
• How Proteins Work (Mike Williamson)
• Introduction to protein structure (Carl Branden & John Tooze)
• Biochemistry (Lubert Stryer)
• The Molecules of Life: Physical and Chemical Properties
INDIAN INSTITUTE OF TECHNOLOGY KHARAGPUR

Thank you.