**Introduction to Complex Biological Systems**
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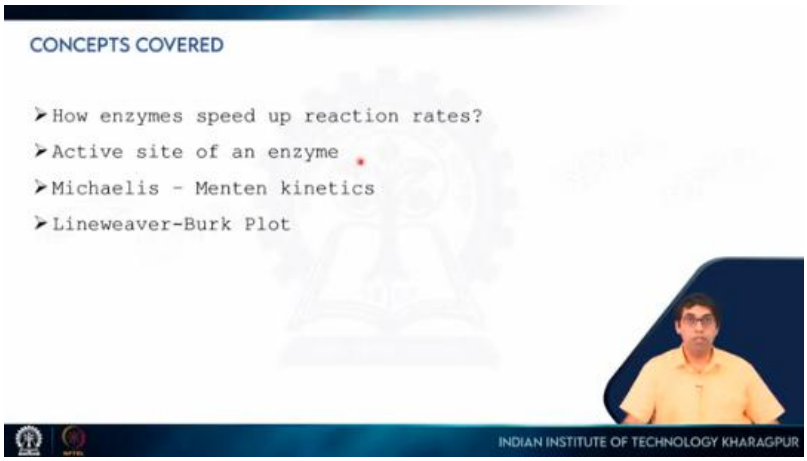
**Lecture 16**
**Introduction to enzymes**

Welcome to week four. This week, we are going to learn about enzymes, which are nature's catalysts. So, in this first lecture, I will introduce enzymes. And I will talk about how enzymes speed up chemical reactions.

I will introduce the concept of the active site of an enzyme. I will talk about Michaelis-Menten kinetics and the Lineweaver-Burk plot, which is the linearization of the Michaelis-Menten plot. So, it turns out that enzymes, or rather proteins, can be classified according to their functions. So, one type of protein is enzymes, which catalyze chemical reactions. There are 1,300 different types of enzymes in the human body.

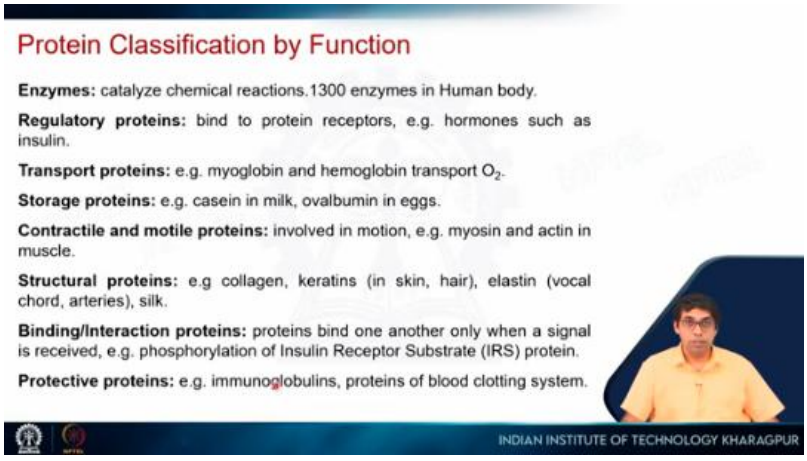



I will talk about many enzymes in week six when I discuss metabolism. Apart from that, there are regulatory proteins that bind to receptors, for example, hormones. In the previous week, we saw examples of these FGF receptors and growth factors. So, they are regulatory proteins. Last week, we also saw the example of hemoglobin, which is a transport protein.

We all drink milk and eat eggs, so these contain casein in the case of milk and ovalbumin in the case of eggs. These are storage proteins. Then, in our body, we have muscles, which are contractile and motile proteins, so they help us to do all sorts of work. There are structural proteins like collagen and keratins in our skin and hair, and elastin, which forms our vocal cords. So, these are all structural proteins. Then there are binding and

interaction proteins, for example, proteins that bind to other proteins and result in signaling. So, phosphorylation of insulin receptor substrate protein. So, you'll see such signaling events a lot when I talk about the cell cycle. And then finally, there are proteins which are protective proteins like immunoglobulins, which play a very important role in our immune system.

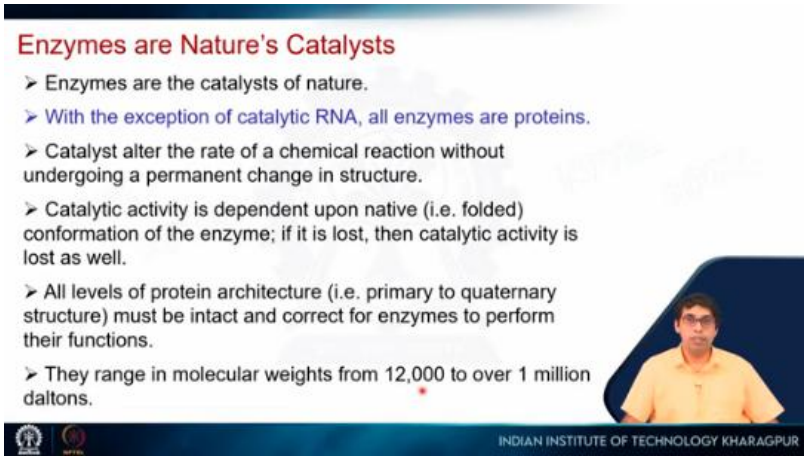
So, we will see proteins of this type in the week 9 lecture on our immune system. So today, I'm going to focus on enzymes. Enzymes are the catalysts of nature. So, just like catalysts that we design in the lab, nature has designed these catalysts so that reactions which do not occur or which are very, very slow under normal conditions of our body temperature and neutral *pH*, which do not occur, they can actually occur within a few seconds because of these enzymes. So, it turns out that most enzymes are proteins with the exception of a few catalytic RNAs.




We have already seen one example, which is the ribosome, where the ribosome is the one which catalyzes the formation of the peptide bond in proteins, and there the active site has RNA. So, it is the ribosomal RNA which catalyzes that reaction. So, what are catalysts? They alter the rate of a chemical reaction without themselves undergoing any permanent change in structure. So, the catalyst or the enzyme is regenerated at the end of the chemical reaction, which means that they can take part in multiple reactions, one after another.

In the case of enzymes, the catalytic activity depends on the folded state of the protein. And if the folded state is lost, then its catalytic activity is also lost. So, just like proteins that we have seen in the last week, enzymes have all the hierarchy of protein structure. That is, they go from primary to tertiary, and in some cases, they will also have quaternary structures. Enzymes are found in various sizes.

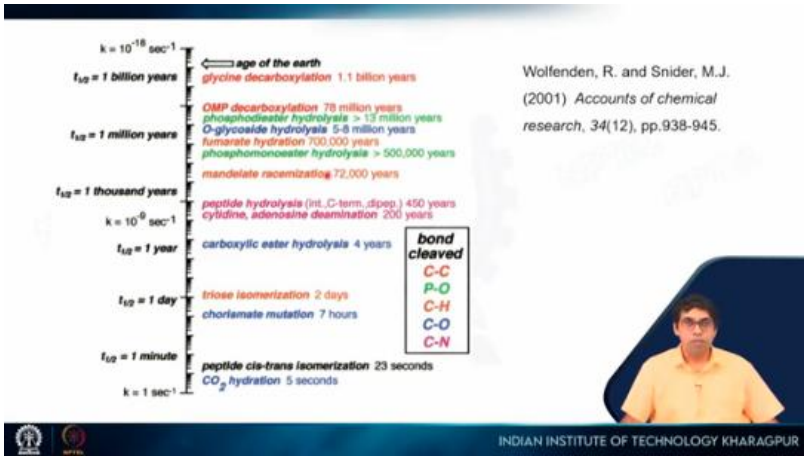
They can range from 12,000 Daltons to over 1 million Daltons in size. So, these are some of the reactions that are very important and occur in our body, and their half-life is listed here. So, what is half-life? It is the time that is needed to reach 50% of the reaction, and that is denoted as T half. So, you can see there are some reactions where the half-life is one year, and then there are reactions where it can go up to a billion years.




So, let's take the example of peptide hydrolysis. So, a peptide, if it hydrolyzes on its own, takes around 400 years. Right. The half-life is around 400 years. Now, for the reaction to reach almost 99 percent completion, you need around five half-lives.

Right. So if it is 400 years or 450 years, then five times that. So 400 times five will be 2000 years. Right. So all these reactions are color-coded according to the bonds that are cleaved.

So you can see that the red ones are the carbon-carbon bonds, and these are the ones which are most difficult to cleave. And we will see some examples in subsequent lectures. So let's take up these macromolecules. Peptide hydrolysis at room temperature has a half-life of 400 years. If we increase the temperature, the reaction will become faster, but still, it will be five and a half weeks.

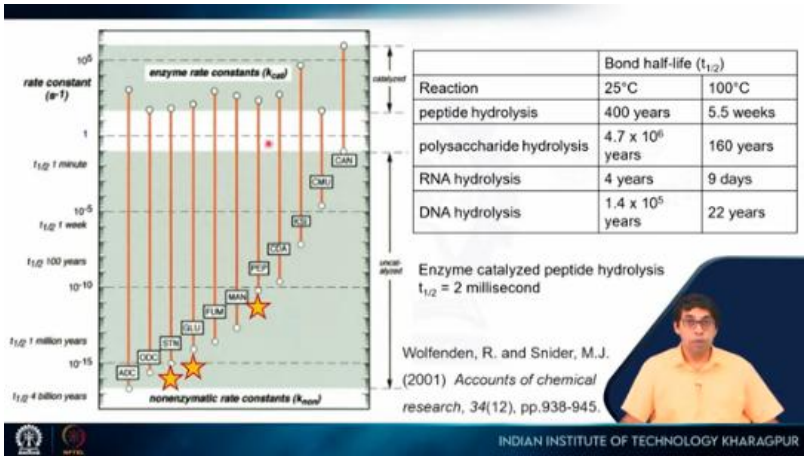



Sugar molecules, polysaccharides, are very stable, so 4.7 million years is the half-life. RNA, we know that among all these macromolecules, is the least stable, so its half-life is also less, around 4 years and DNA hydrolysis takes place in 1,400,000 years. So what happens when there is an enzyme? So these are some of the enzymes that are listed here.

So let's take the example of peptide, DNA, and sugar molecules. You can see that their half-lives are very high. So, the reactions are very slow. But when they're catalyzed by enzymes, these are the enzyme-catalyzed reaction rates, all of them become very fast, and their half-life is in seconds. So, for example, for protein, the half-life is around 500 seconds, right?

So, the rate constant is 500 per second. So, the half-life is 2 milliseconds, which means that the reaction will reach 99% completion in 10 milliseconds, which is very, very fast. So, enzymes have this impressive ability to speed up reactions, which are very slow under normal conditions, to such impressive rates that they can be completed within seconds. Not only that the enzymes also have the ability to catalyze some really complex reactions.

So, we will see one example here. The enzyme oxidosqualene: lanosterol cyclase. This enzyme results in the production of a sterol called lanosterol. This is an intermediate in cholesterol biosynthesis. Lanosterol is produced from this linear molecule. You can see that this is actually a linear molecule that is drawn in this folded manner.

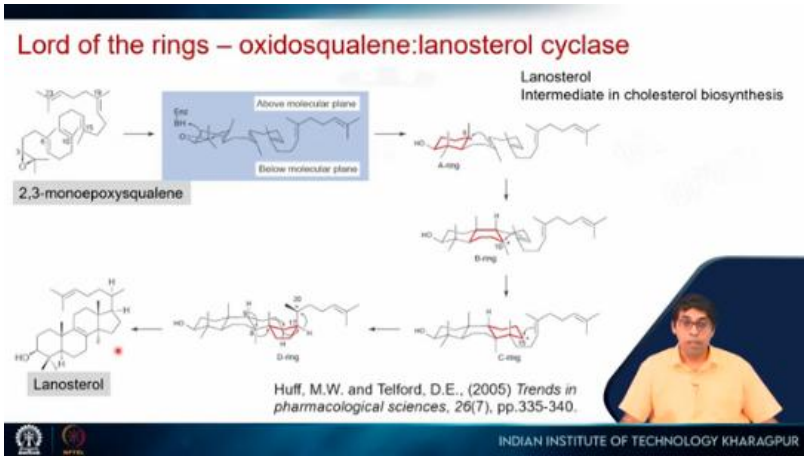



| | Bond half-life ($t_{1/2}$) | |
|---|---|---|
| Reaction | 25°C | 100°C |
| peptide hydrolysis | 400 years | 5.5 weeks |
| polysaccharide hydrolysis | $4.7 \times 10^6$ years | 160 years |
| RNA hydrolysis | 4 years | 9 days |
| DNA hydrolysis | $1.4 \times 10^5$ years | 22 years |

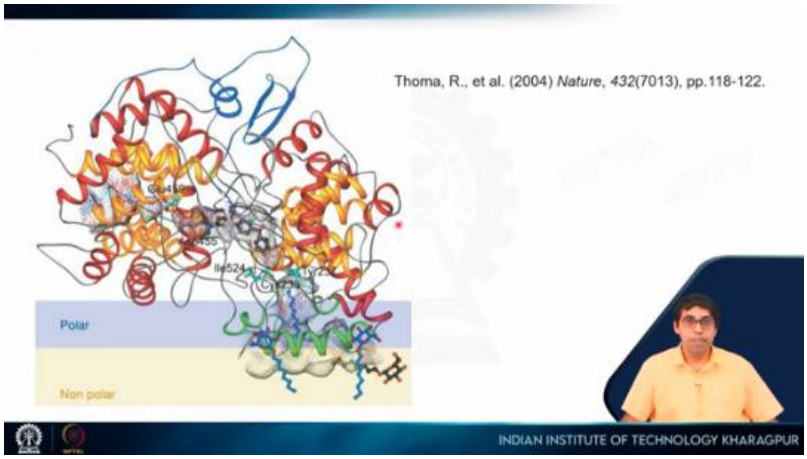
So it has a 24-carbon-long chain. And from that, this molecule is formed, which has four cycles, right? It undergoes this series of reactions. So in the first step, the first ring is formed, then the V-ring, then the C-ring, then the D-ring. These four rings are formed consecutively to give rise to this final molecule.

And all of these steps are carried out by this single enzyme, right? Not only that, this final product, if you count carefully, you will see that there are 1, 2, 3, 4, 5, 6, 7, and 8 chiral centers. So the enzyme not only catalyzes this series of complex reactions but also forms the correct isomer at the end of the reaction with almost 100% accuracy. So that's the fantastic ability of enzymes. They can speed up reactions and also perform really complex reactions.

They do so under very normal conditions with very high accuracy. So this is the structure of the enzyme that I just discussed in the previous slide, and it is a membrane-bound structure. We will talk about the cell membrane in detail. So normally, you have this polar head group and the hydrophobic chain. So that is one leaflet of the membrane, and that is where this membrane



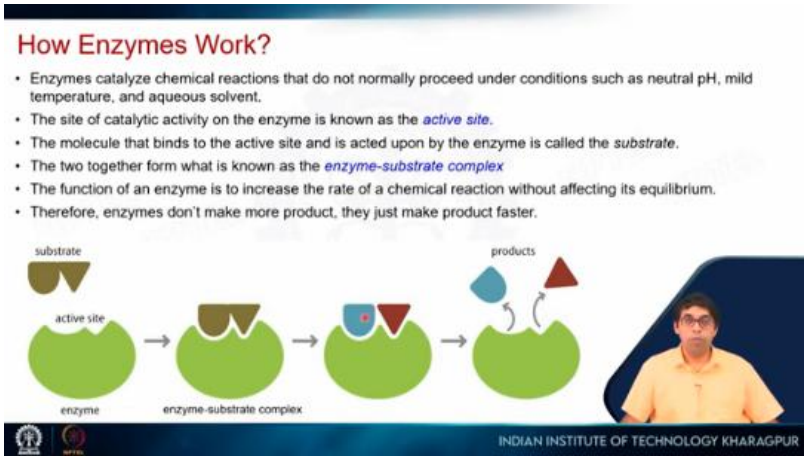

The embedded part of the protein is there, and the active site is somewhere here. And hopefully, you can recognize by now that these spiral structures are the alpha helices, and these arrow-like structures are the beta strands. So, these are the secondary structures of this protein. And this structure was solved by X-ray crystallography in 2004. So, how do enzymes carry out these reactions?




How do they speed up reactions, and how do they carry out such complex reactions resulting in such stereoisomers with high accuracy? The reason for that is that enzymes are molecules, but they have a particular region where the reaction or the chemical reaction is carried out. That is called the active site. In this case, the reactants are called substrates. So, the substrate will bind to the active site in a very precise manner.

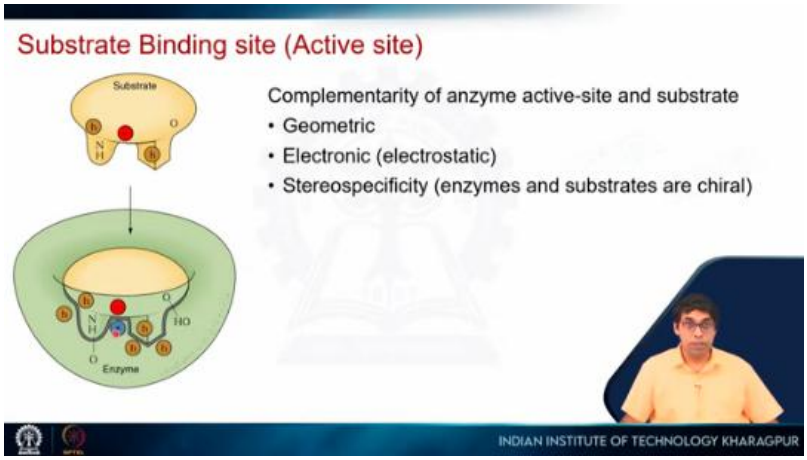
The reaction takes place in this bound state where the reactant gets converted to the product and once the products are formed, they diffuse out. So, how do these substrates bind to the active site? They bind to the active site because there is a huge

complementarity between the substrate and the active site. So, you can see that there is a shape complementarity.

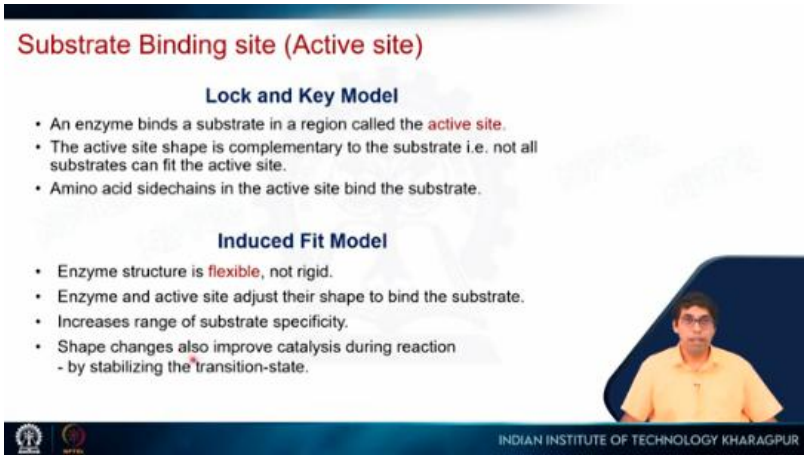



So whatever this shape is, it looks complementary to that so it can fit nicely into the active site. Once it sits there, there are also interactions that are complementary to each other. For example, this *H* denotes a hydrophobic side chain. So it can be a methyl group, it can be an aromatic side chain, which is present in the substrate, and a similar side chain is present in the enzyme. So hydrophobic-hydrophobic interactions take place.

There is a negative charge here and there is a positive charge here on the enzyme. So when the substrate binds, we get this charge-charge interaction, which also makes it favorable. More hydrophobic interactions here, and then also hydrogen bonding interactions. So this is a hydrogen bond acceptor, there is a hydrogen bond donor here, similarly there is a hydrogen bond donor here, and there is a hydrogen bond acceptor on the enzyme. So together they will allow this substrate to bind this enzyme in a particular orientation, which helps in the specificity of the reaction and also they will help in speeding up the reactions. Substrate binding, as we have seen in the previous week, can be of two types, the lock and key and the induced fit. So as we saw in the previous lecture, lock and key can be similar to a lock and key reaction, where the substrate has a perfectly complementary structure to that of the active site.



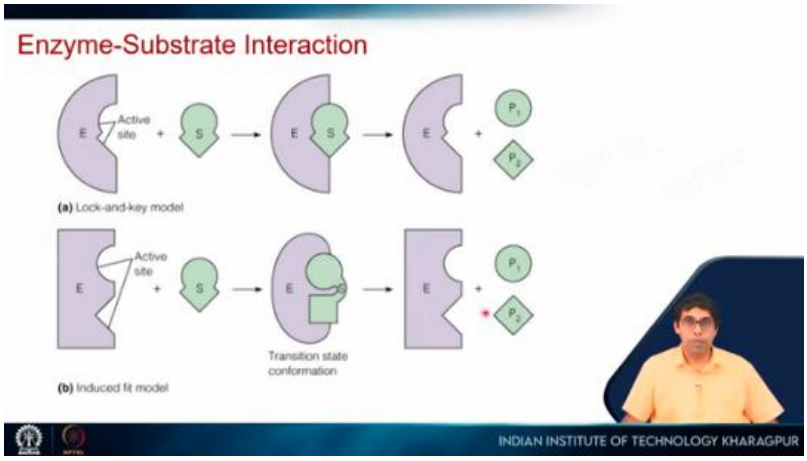

But in the case of induced fit, the enzyme structure is flexible, and once the enzyme and substrate form a complex, there is a readjustment resulting in a slightly changed structure. And this shape change can help in the catalysis by stabilizing the transient state. So I will discuss this in more detail in the next lecture. So here is the pictorial diagram of the lock and key model and the induced fit model.



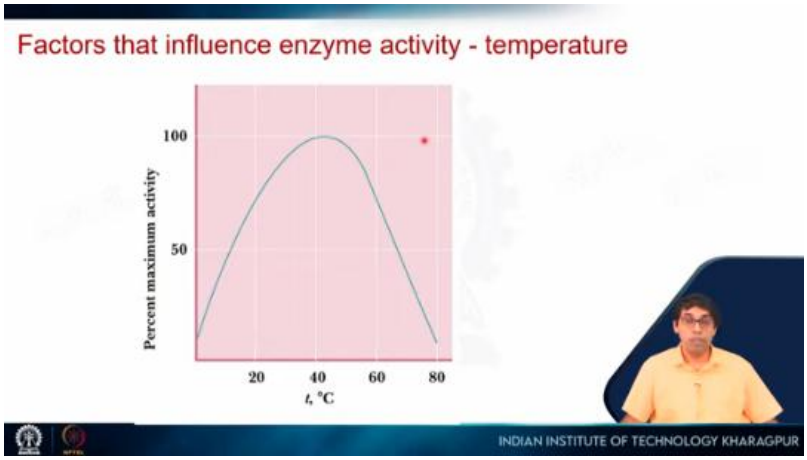

So you can see that the substrate has a perfectly complementary structure to the active site, so that it fits nicely. Whereas, in the induced fit model, this and this are slightly different. So, once the complex is formed, it results in, It induces this structural change. So both the enzyme and the substrate have changed their structure, and then the reaction happens, and we get the enzyme plus product.

So, the enzyme is regenerated at the end, and we get the products. So, what are the factors that influence the reaction? Just like any chemical reaction, temperature is one factor that will influence enzyme activity. So, we know that as the temperature is increased, the reaction rate increases. So, for most enzymes, if we think about enzymes

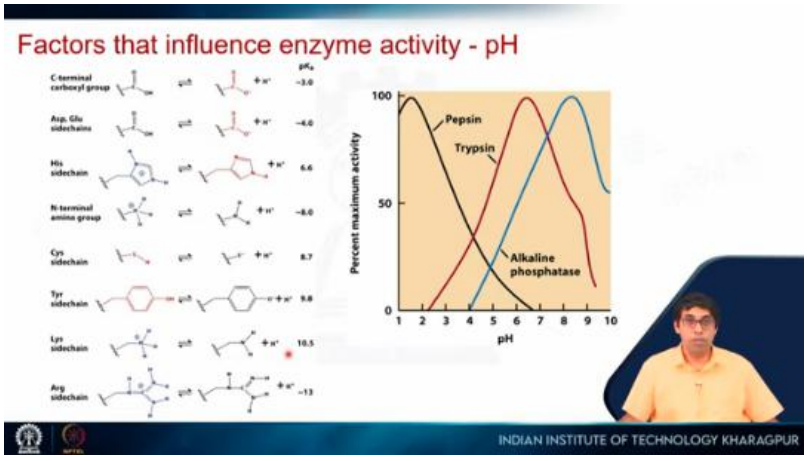
that are present in humans, we will see something like this that with an increase in temperature, the activity increases.




However, after a certain point, the activity decreases. And this is because When you increase the temperature beyond a certain point, the enzyme becomes unstable and can start denaturing or unfolding. And that means that the active site will lose its characteristic, and of course, it will not be able to catalyze the reaction. So, there will be an optimal temperature. For most enzymes in our body, the optimal temperature is around $40^{o}$C because our body temperature is $37^{o}$C. But if we take bacterial enzymes, let us say thermostable bacterial enzymes, in that case, we will see that their optimal temperature will be shifted to much higher temperatures, like $70^{o}$C or $80^{o}$C. That is needed because those bacteria survive at much higher temperatures. Hence the enzymes in those bacteria have evolved to have optimal temperatures at much higher levels that match their environment. Similarly, $pH$ also influences enzymatic activity. We have already seen that all these different side chains have different $pKa$ values. So, depending on the $pH$ at which the enzyme has to work, these different side chains will be found in the active site. So, some examples are shown here.



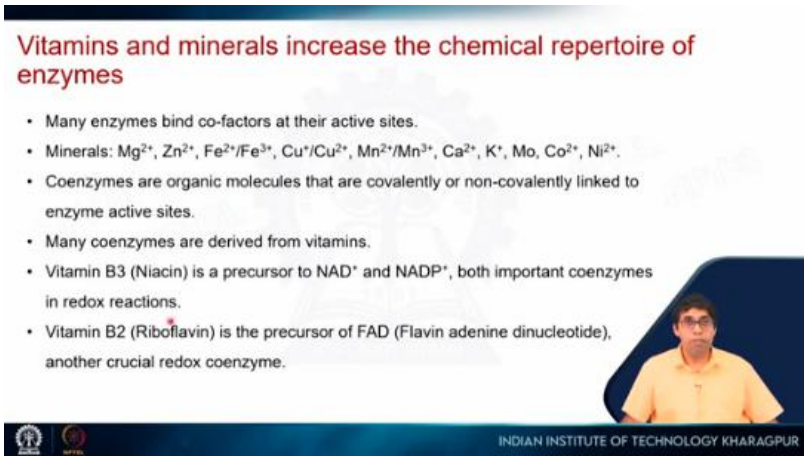

For example, alkaline phosphatase or trypsin works very close to neutral *pH*. Because they are found in environments where the *pH* is neutral, around 7.4, but pepsin is found in our stomach where the *pH* is very low, less than 2. Now, pepsin is optimized such that its optimal activity matches that *pH*, and this is something that is found quite often in different enzymes. But one may wonder that these are the side chains. So, these are the functional groups that are present, but then enzymes tend to catalyze so many different reactions. So, how do they catalyze such reactions using such a limited number of functional groups?




So, it turns out that enzymes recruit other molecules to carry out specialized reactions, and these molecules are called cofactors. So, enzymes bind cofactors at their active site, and cofactors can be classified into two types, the organic and the inorganic. So, most inorganic cofactors are minerals which are found in active sites, and we have already seen an example of magnesium in the case of our polymerases, right? So, apart from minerals, there are also organic molecules which are called coenzymes. They can be

either covalently or non-covalently linked to the enzyme active site and most of these coenzymes are derived from vitamins.

So vitamins that we take as supplements from different foods undergo some changes and are then incorporated into enzymes to carry out their functions. Two examples I am citing here. One is vitamin B3, which is a precursor for NAD+ or NADP+, which are coenzymes used in different oxidation-reduction reactions. Similarly, vitamin B2 or riboflavin is the precursor of FAD, flavin adenine dinucleotide, which is also important in many enzymes that catalyze oxidation-reduction reactions. I will talk about vitamins and these coenzymes in more detail when I discuss metabolism in week 6. So let's look at enzyme kinetics.

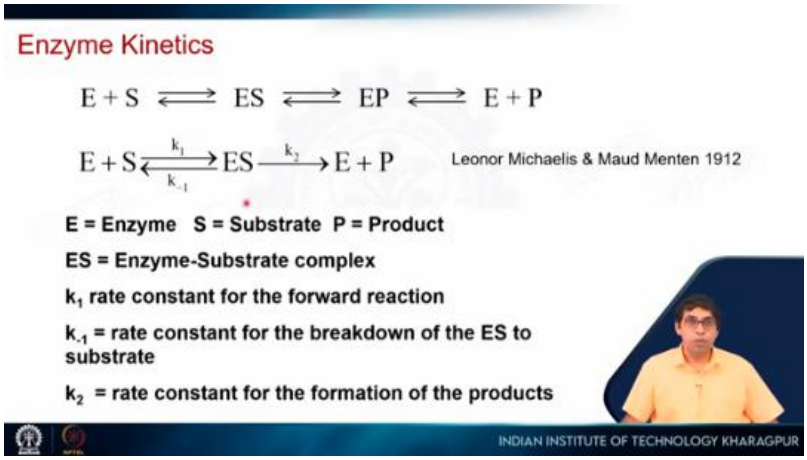



Based on the diagram we saw in one of the previous lectures, or previous slides in this lecture. So we have the enzyme plus the substrate, which forms the enzyme-substrate complex. Once it's in this bound state, we have the enzyme-product complex. So the reaction happens here. This is where the real chemistry happens, and then the product dissociates.

We can consider all of these as reversible steps. So we will have rate constants for all of this, which turns out to be six rate constants. But we can actually simplify this to something like this because this will be a very fast step, and this reaction step is also quite fast. So we can combine these two steps into one irreversible step where the enzyme-substrate dissociates into the enzyme plus the product. This type of approximation was made by Leonor Michaelis and Maud Menten more than 100 years ago, in 1912.

So that is when their first paper came out, and they showed that enzyme kinetics can be explained very nicely by a very simplified kinetic reaction like this. So $K_1$ and $K_{-1}$ are the

$ON$ and $OFF$ rates of the substrate binding, and $K_2$ is the rate at which the enzyme-substrate complex is converted to the product and dissociation. So if we have this equation, we want to know what the rate of product formation is. So the rate of product formation is also called the velocity, which will be given as $d[P]$ by $dt$ or the rate of product formation.

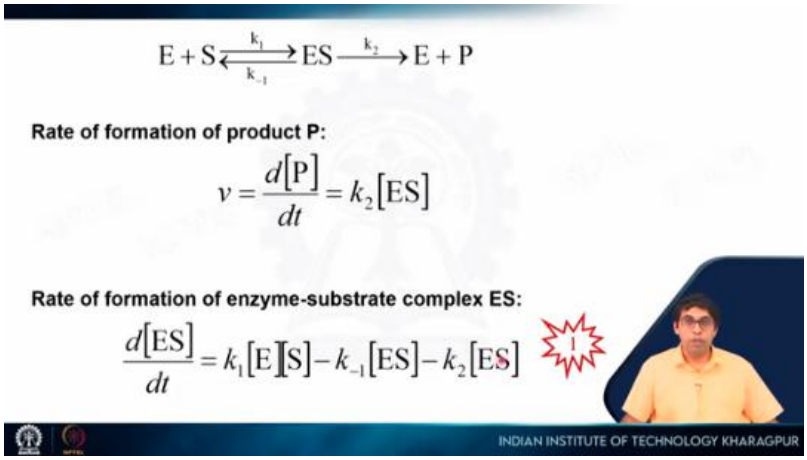



So if we look at this equation, the rate of product formation will be this rate constant multiplied by the concentration of the enzyme-substrate complex. So $K_2$ times $[ES]$. But of course, we do not know what $[ES]$ is, or what the concentration of the enzyme-substrate complex is. And that is what we want to figure out. So if we look at the rate of formation of the enzyme-substrate complex, which is $d[ES]$ by $dt$, from this equation, we can write it as, so the rate of formation is this reaction.

$$v = \frac{d[P]}{dt} = K_2[ES]$$

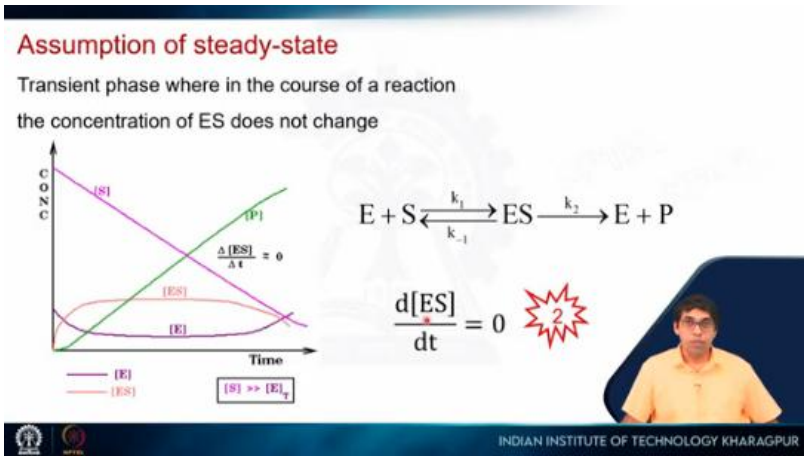
$$\frac{d[ES]}{dt} = K_1[E][S] - K_1[ES] - K_2[ES]$$

So $K_1$ times the free enzyme plus the free substrate, which is this and that is how it is formed. But then it is destroyed by this and this, these two steps. So $K_{-1}$ times$[ES]$, which is this, and $K_2$ times $[ES]$, which is this. Now, since these two steps decrease the concentration of $[ES]$, we have negative signs here.



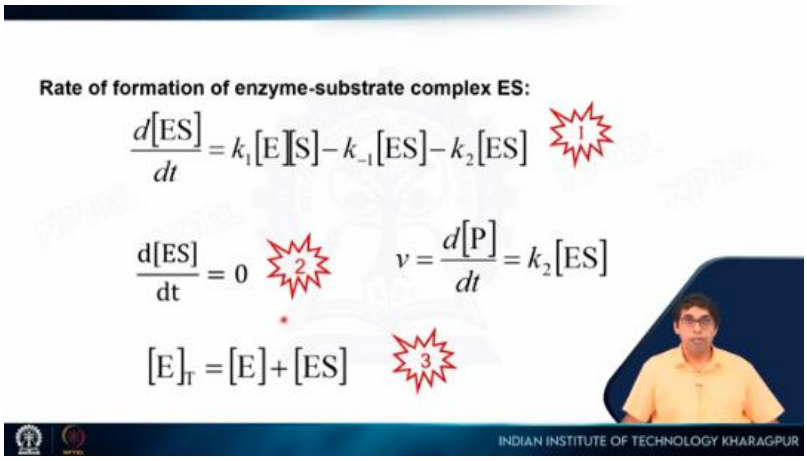

So, this is something that was not known when Michaelis–Menten came up with this equation. But now, using numerical analysis, we can actually plot the concentration of the substrate, the product, and the enzyme-substrate complex. So, of course, the substrate starts with some value. At time zero, it will decrease over time. The product will start from zero and it will increase over time. However, the enzyme-substrate complex shows a very interesting behavior. It will start with zero, quickly increase to a particular value, remain steady or constant at that value, and then finally decrease. So, this period of time is when the enzyme-substrate concentration does not change.

Michaelis-Menten correctly assumed that there would be a steady state where the enzyme-substrate concentration would not change with time, as a function of time. So, $d[ES]\ by\ dt$ will be $0$. So, let us say this is our equation 2. So, this $d[ES]\ by\ dt$ is $0$ under this steady-state condition. What we want to do is find the velocity, which is $d[P] by\ dt$, and that equals $K_2$ times $[ES]$.

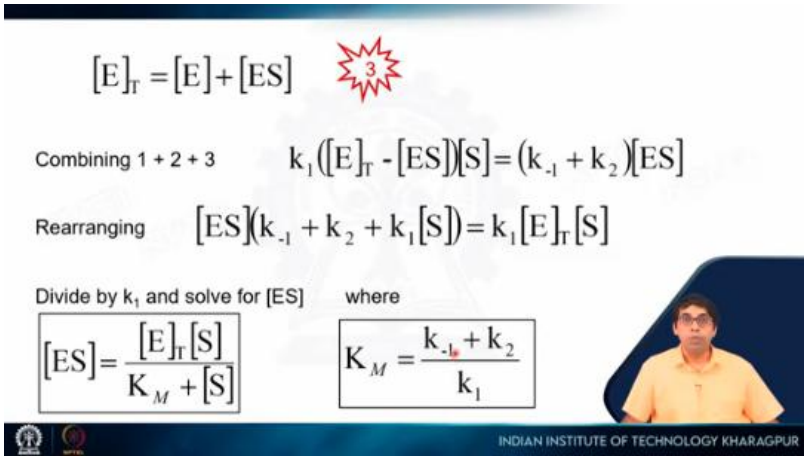


So, what we want to do is solve for $[ES]$ from this equation. But here, you see that we have $[ES]$ and $[ES]$, and these constants are fine. But here, we have free enzyme concentration and free substrate concentration. So, we do not know the concentration of the free enzyme. We can replace that by this because we know the concentration of the total enzyme.

That is something that we have added in the experiment and that will be the summation of the free plus bound enzyme. So $[ES]$ or the free enzyme $E$ will be equal to $E_T$ minus$[ES]$ and we can substitute that here. So that is what is done here, and then if we separate all the variables, we will get the enzyme substrate concentration as $E_T$ times the substrate concentration divided by $K_M$ plus substrate concentration.

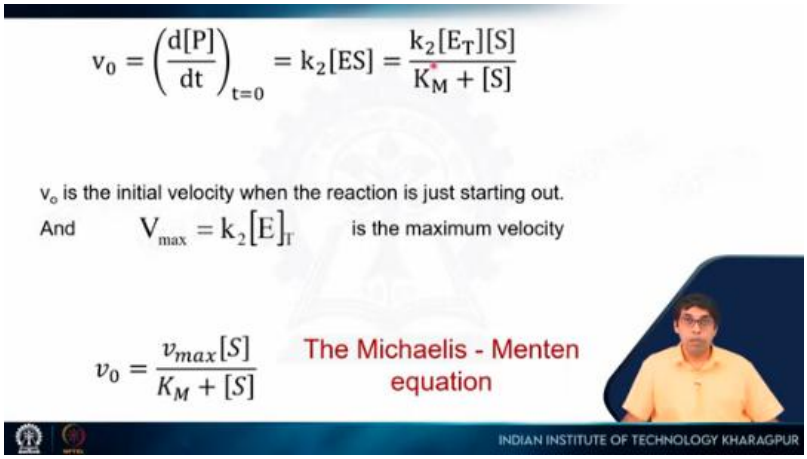



Now, what is this $K_M$ $K_{-1}$ plus $K_2$ by $K_1$. So when we do this separation of variables, we get that. Now, since all three are constants, so instead of writing it like this, we can combine them together into one constant, which we denote as $K_M$, where $M$ stands for Michaelis and Menten. So again, coming back to this $V$, which is $d[P]$ by $dt$ equals to $K_2$ times $[ES]$, we know what is $[ES]$, so we substitute for that.




Now, if you think about this, the velocity equals to $K_2$ times $[ES]$. $K_2$ is a constant, and $[ES]$ is the enzyme substrate concentration. This will have a maximum value of $E_T$ because the maximum that can happen is all substrates are bound to all the enzymes are bound to substrate. So $[ES]$ can have a maximum value of $E_T$.

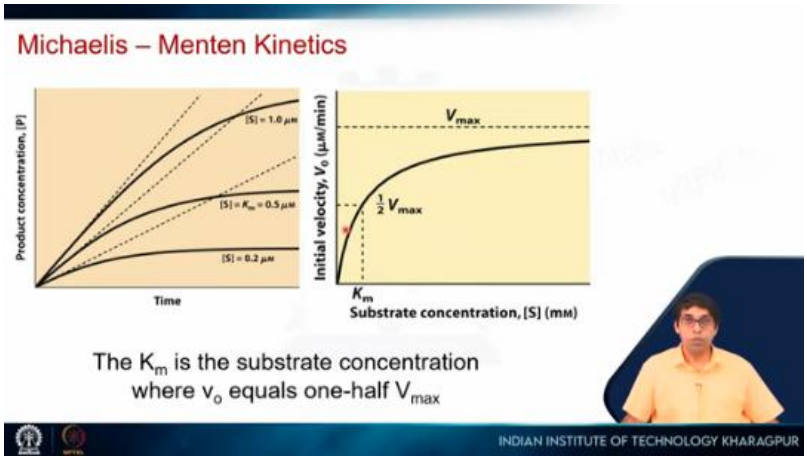
So, $K_2$ times $E_T$ can be denoted as the maximum velocity. So, that will be the maximum rate of product formation. So, we can replace this $K_2$ times $E_T$ with $V_{max}$ and this is the final form of the Michaelis-Menten equation. So, if we plot $V_0$ versus the substrate concentration, it looks something like this. How do we get this plot?




We get this plot by doing a series of experiments. So, for a particular substrate concentration, we will calculate the rate of formation of the product. So, this is the rate of formation of the product. You can see it starts from zero and will saturate. Since we want the initial velocity, we will take the initial slope here.

As we increase the substrate concentration, this slope will increase, which means that the $d[P]$ by $dt$ is increasing, or the velocity is increasing. Remember, in all this series of

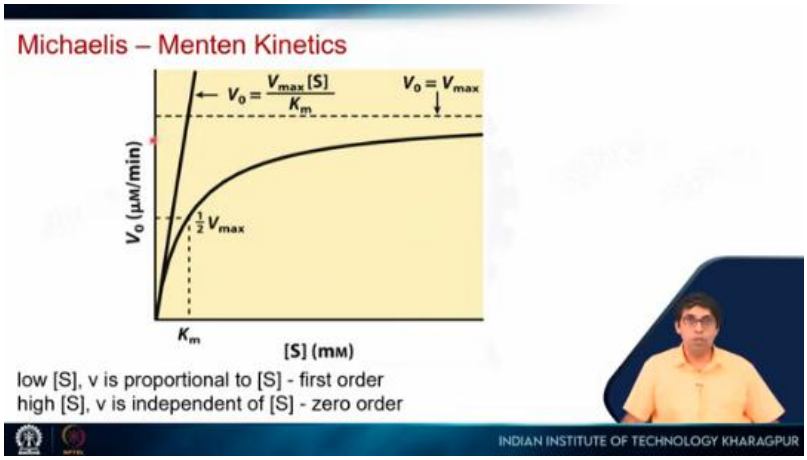
experiments, we have to keep the enzyme concentration exactly the same. We are only increasing the substrate concentration. So, from this series of experiments, different velocities are obtained for different substrate concentrations, and those are plotted here. So, velocity will be plotted here, and substrate will be plotted here, and it gives us a curve like this.




So, if we look at this curve, when the substrate concentration is very high, it will be much more than $K_M$. So, $V_0$ will be equal to $V_{max}$, and that is what we get here. When the substrate concentration is low, then we get this because $S$ is less than $K_M$. So, $V$ will be proportional to the substrate concentration and it will increase linearly.

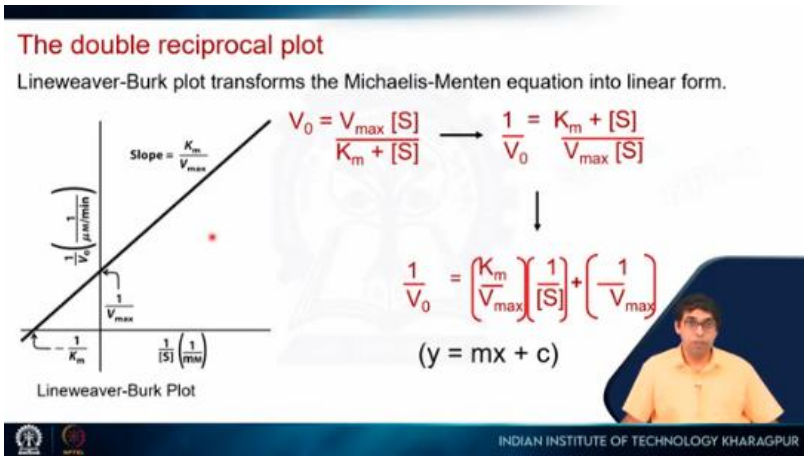
Also, if we go to the Michaelis-Menten equation and we plot $K_M$ equals to $S$, we get that the substrate concentration and $K_M$ will be equal when the velocity equals half $V_{max}$. So, once you get this plot, we have to make sure that we reach this saturation state. From there, we will get $V_{max}$ and then if we go to half $V_{max}$, we can extrapolate to this and we can get the $K_M$. So, we can get $V_{max}$ and $K_M$, which are two very important parameters of an enzyme. But you can see that this is problematic because if we stop here, then we will consider this as $V_{max}$.

So, our $V_{max}$ will be underestimated, and our $K_M$ will also be underestimated. So, to get rid of this problem, Lineweaver and Burk proposed that we can invert this equation like so and make it a linear equation like so. So, $V_0$ becomes *1* by $V_0$ and $S$ becomes *1* by $S$. So, you can see that *1* by $V_0$ equals some constant times *1* by $S$ plus some constant. So, this is very similar to $y$ equals $mx$ plus $c$, which is a straight line equation. So, if we plot *1* by $V_0$ versus *1* by $S$, instead of $V_0$ versus $S$, we will get a straight line plot.

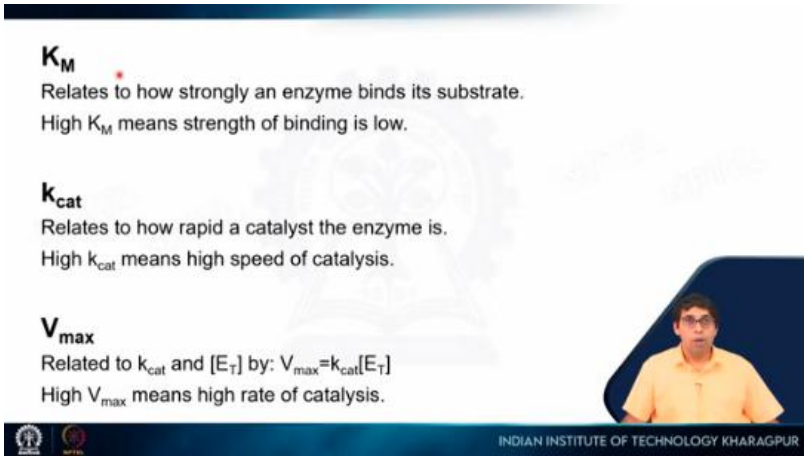



And the straight line will start from somewhere here and go up to here, right? But since it's a straight line, we can extrapolate it and get the *y-intercept* and the *x-intercept*. And if we get these two, we can get $V_{max}$from y-intercept and $K_M$ from the *x-intercept*. So, that is the power of this Lineweaver-Burk plot and I will come back to this in the third lecture this week when I talk about inhibitors.

We will see that this plot becomes very useful in determining which type of inhibitor we have. So $K_M$, $K_{cat}$, $V_{max}$, these are very important parameters. $K_M$ gives us how strongly an enzyme binds its substrate. So this is very similar to $K_D$. So you can actually compare $K_M$ with $K_D$ and see that they both have the unit of concentration, and $K_M$ will be slightly different from $K_D$ because it also has the constant $K_2$ in it.

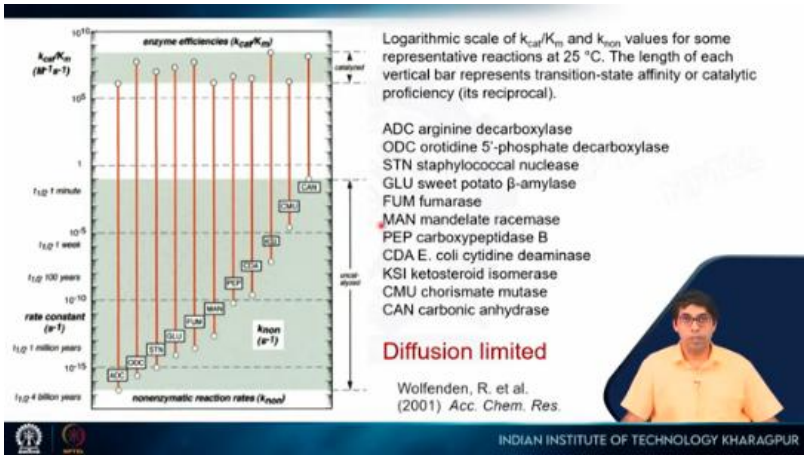



$K_{cat}$ gives us how fast catalysis is done, and $V_{max}$ is the rate of catalysis for a given enzyme concentration. Also, $K_{cat}$ over $K_M$ is a parameter that is often used to indicate the specificity of an enzyme. So that is somewhat shown here. This is very similar to the plot that I showed before.

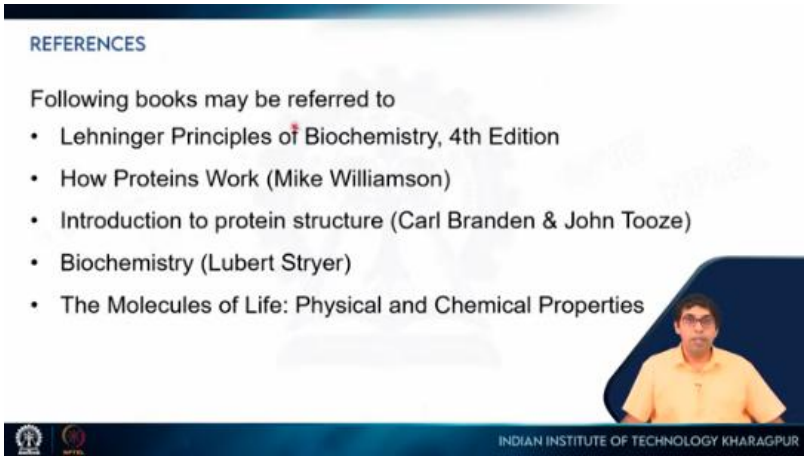



So these are the uncatalyzed reactions and the $K_{cat}$ over $K_M$ is plotted here. So the $K_{cat}$ over $K_M$ will have the unit of per molar per second. So this is a second-order rate constant. And you see that these values are all in the range of $10^6$ to $10^8$ per molar per second. This number is important because it is reminiscent of the rate of diffusion of molecules under normal conditions in water at room temperature. So it means that enzymes, all these enzymes, they speed up this reaction so much that the rate of catalysis is limited by diffusion. So how fast the enzyme and substrate diffuse towards each other and bind to each other.

So that rate determines the rate of the reaction because you cannot speed up the reaction more than that. Only once they bind can you do the catalysis. So evolution has optimized these enzymes perfectly so that their rates are now limited only by the diffusion constant. So these are called diffusion-limited.




So, I think that is all for today. Again, these are the books that you can refer to for this lecture.


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.