Molecular Biosciences 303: Lecture 7 - Protein

[Ron Brosemer] Welcome back to our section on protein. We began our discussion of enzymes last time. That is biological catalyst. And just to review the last two slides that we saw, we considered the fact that enzymes decrease the activation energy for reaction that is how much energy does it take to convert a substrate to a reaction intermediate but then can converge spontaneously that is flow down hill in terms of energy to a product, and this energy barrier, the activation energy determines the kinetics that is the rate or the velocity of a reaction and what enzymes do is decrease the activation energy compared to the uncatalyzed reaction.

All enzymes bind substrate, that is no biological reaction that enzyme catalyze can proceed unless the specific substrate binds to the specific enzyme at a site that is termed the binding site. After binding amino acid residues of the proteins are involved in catalysis. This is the so called active site although many of the amino acids are involved in binding are the same of those involved in the catalysis itself so I have pointed out before binding site and active site are terms that tend to be used interchangeably although strictly speaking they have somewhat different meanings.

To give a specific example of an enzyme one in fact that we have already seen, ribonuclease, to give us an idea of the substrate, in this case it is part of ribonucleic acid, we don’t have to worry at this point what the structure of the substrate is, binds in a cleft and as I have indicated to you all known active sites are clefts are depressions in the protein so that the non covalent and possibly in some cases covalent interaction between the substrate and the enzyme are maximized. This gives us an idea of a relative size of a typical substrate with a typical protein.

We see the ribbon model of the same enzyme substrate complex as this is called that is the binding of the substrate to the enzyme is the enzyme substrate complex and in red is the substrate actually only part of it, part of ribonuclease, for those of you who know what RNA is this happens to be a ribos group here and one of the bonds there will be cleaved or hydrolyzed.

There is a general classification of enzymes that we will not be using in any detail, but I wanted to point out that this classification fact does exist and to also give us a feeling of the general types of reactions that are catalyzed by enzymes. Don’t worry about at this point the classifications, the six classes we are going to be looking at, this is one of those gut feeling excises where I just want you to get this feeling of enzymes and type of reactions that can be catalyzed. I want to remind ourselves that in all cases any of the reactions I show here there is a single enzyme, a single protein catalyst that is catalyzing any particular reaction. Again that isn’t quite correct. One reaction may be catalyzed by more than one enzyme so called isozymes, but for all intents and purposes first approximation one reaction, one specific enzyme.

When we get to metabolism, we are going to see lots of oxidation reduction reactions, that is redox reactions. Remember that a redox reaction is a transfer of electrons from an electron rich compound, substrate, the reducing agent to an electron deficient substrate oxidizing agent. And we get to metabolism we will be looking more specifically and reminding ourselves of what a reducing agent, and oxidizing agent are, but there is an actual transfer of electrons and the example I give here at this point may not make all that much sense since we have not introduced cytochrome c although in fact cytochrome c does bind heem, the compound that we know in great deal, the same compound that is bound to the protein of hemoglobin or myoglobin, but there is
an iron two and it has one extra electron and that is reaction, and it can transfer the electron, actually one at a time, but for cytochrome c transfer electrons to oxygen to reduce the oxygen to water, and to oxidize the iron two to iron three. Arguably this is the most important enzyme in aerobic organisms. And we will see this reaction later. The important point is there are many redox reactions in biological systems.

A second major group of enzymes is one that catalyzes transfer of groups. Now we have not seen the structure of ATP yet although again when we get to metabolism we will, but if you don’t know anything about the structure of ATP simple way to look at this the phosphoreal group here which I have highlighted, the phosphorus with the three oxygen's, notice that it can be transferred from this oxygen to this oxygen on glycerol to form then what is then glycerol phosphate and again ADP and again we will see what the structure of ADP and ATP are but there is a transfer of a functional group from one substrate to another. very very common in metabolism.

Hydrolysis reactions are crucial. In fact in your intestine right, especially if you have had a meal recently, you are breaking down your proteins in the diet, your carbohydrates - starch for example, your lipids mainly by hydrolysis reactions in which the elements of water are added across a bond I happen to have here as an example a peptide bond that we add elements of water across that and get out of the constituent carboxilic acid and alpha amino group and if we hydrolyze enough the peptide bond and the intestine say and the protein that was in the bacon you had can be degraded completely to amino acids which then are absorbed.

The elimination of a group to produce a double bond, or the addition, the opposite direction now, the addition of a group to a double bond is very common in metabolism and there is a whole group of enzymes that catalyze this reaction and we will in fact be seeing the reaction I have on the slide when we get to glycolosis in metabolism.

Another type of reaction is an Isomerization and notice that in this case an alpha hydroxy keytone is isomerized to an alpha hydroxy aldehyde and again just emphasizing, and I realize I am repeating myself but I’m doing it for emphasis, for the reaction to occur, and we will see this reaction when we get to metabolism this substrate, dihydroxyacetone phosphate, must bind to the active site of the enzyme which happens to be a isomerate, reaction occurs, product is formed, and the product is released, and the active site then can receive the next substrate molecule, reaction occurs, product goes on and then repeats.

Lastly the six main groups in the classification of enzymes is the utililization of a pyrophosphate bonds for the sythesis of compounds generally new carbon, carbon, carbon nitrogen, carbon oxygen bonds that are formed. At this point, this won’t make much sense. But when we get to metabolism, we will see that these types of reactions that are driven by the energy that is pumped in by the conversion of ATP to another compound are very common. In this case a carbon carbon bond is made, this new bond between the methalcarbon here, pyruvate acid, and this and this carbon here a bicarbonate.

Let’s now take a look, using a series of graphs at some of the properties of enzymes. What we are going to do is beginning right now we will be taking a look at a prootypic reaction in which substrate is converted to product. I want to give an example here of with and without enzyme. Remember that in the absence of enzyme the reaction does proceed and it will proceed to equilibrium but it does go very very slowly. So here we have the decrease in substrate
concentration, so the slope is negative, that is it goes down with in this case time. Product appears in the presence of enzyme the appearance of product or the disappearance of substrate is much much faster than in the absence of enzyme. That is the enzyme catalizes a reaction, speeds up the rate, but does not affect the equilibrium, the final value which I told the computer was 1, that is the concentration of substrate equals the concentration of product. I just chose that arbitrarily.

Let’s now take a look at the effect of enzyme concentration on rates of reaction. We are doing the same kind of plot as the previous slide. That is at zero time we have no product. We start the reaction by adding substrate. As time progresses, whether this is milliseconds, seconds, minutes, hours whatever, product begins to appear the substrate concentration decreases because it is being converted to product. And arbitrarily let us say that the concentration of the enzyme is 1 unit whatever that unit may be 1 milimoler, 1 micromoler, 10 molecules whatever that unit may be and I have arbitrarily set the equilibrium constant again at 1. So that when the reaction reaches equilibrium way way out here there will be equal concentrations of substrate and product. That is there will be 50% or .5 fraction of substrate and of product. What happens if we have the same reaction, same equilibrium constant? Remember enzymes do not alter the equilibrium constant they only alter rate. Let’s now increase the concentration of enzyme. Let’s double it. I have told the computer to do that. So these are computer generated curves so in fact they are accurate under these conditions. Note that the rate of the reaction is faster. So, let me just return. This is where we have two unit of enzyme (returns to previous slide) here we have one unit of enzyme (returns to a previous slide). Notice that the equilibrium constant has not been altered but the rate at which the reaction approaches equilibrium has increased with increasing enzyme concentration. Now instead of 1 or 2 units of enzyme let’s look at ½ a unit in other words less enzyme than in the first slide. We have again reaching the same equilibrium concentration in this case equal substrate and product eventually because equilibrium constant is one, but note that the rate, the is the slope, and at anytime and especially as we are going to be interested in zero time the slope that is how the change in the y axis divided by the change in x axis, this ratio is the slope. So, this is with low enzyme (returns to a previous slide), very high enzyme (returns to a previous slide), and intermediate concentrations of enzyme (returns to a previous slide). Effect of enzyme concentration on rate.

Now let’s take a look at the effect of the equilibrium constant. We in fact will have three different reactions here. Substrate to product, but there will be three different substrates being converted to three different products, so there will be three different enzymes. But let’s say that every thing else is the same. We have already seen this slide. Equilibrium constant is one. That is at equilibrium the concentration of substrate is equal to the concentration of product. What if we have another reaction in which the equilibrium reaction is two? That is the concentration of the product at equilibrium is twice that of the substrate, so eventually when this reaches equilibrium way out here we will have twice as much product as substrate, but if everything else is the same the rate of the reaction will be the same especially at zero time. And similarly if instead of an equilibrium constant of 2 we have an equilibrium constant of ½ that is at equilibrium the concentration of product is one half that of the substrate that is remaining at equilibrium. We see the same thing; in fact the rates of the reactions, what I told the computer, are exactly the same, but notice that out at equilibrium we will have different concentrations of substrate and product simply because equilibrium constant is different. The reason I have gone through this exercise these two pairs of three graphs each is to show that enzyme concentration effects rate but not
equilibrium. And that the equilibrium constant for a reaction varies with whatever the reaction may be. It has to be experimentally determined.

Let us now lay the groundwork for some of our continuing discussion of enzyme kinetics. That is how do reactions speed it up by binding of a substrate to an enzyme and the first thing we are going to be doing is looking at some quantitative aspects and then later in another lecture we will take a look at a particular mechanism of how the enzyme actually speeds up the reaction, but let’s lay the groundwork by first of all making sure that we realize what a velocity or rate, thus again interchangeable rates, what a reaction is. We will be using a small \( v \) as the abbreviation for velocity or rate. For those of you who know what a differential is, the rate is the rate of change of the concentration of product with time. And remembering that substrate disappears we can also define the rate as the rate of change of substrate concentration with time, but since substrate concentration decreases if we have substrate going to product that is one substrate molecule going to one product molecule then the rate of formation of product is a positive value, the rate of formation of substrate is the same value but negative, same absolute value but a negative sign. This is disappearing. For those of you who don’t know what a differential is, consider that the slope of the line we have been looking at the change in product concentration over a particular time interval let’s say from time zero to time 1 minute maybe product start off at zero and after one minute it is 3.7 milimoler that would be the rate as defined here, or similarly a substrate disappears. If we have one substrate molecule going to one product molecule, then the rate of substrate disappearance has the same absolute value but a negative value.

We define the initial velocity or the initial rate of a reaction often we will see it as \( v_0 \) as the reaction velocity as time zero which is defined as the time when there is no product yet. We start the reaction with substrate. So add substrate and before any product is formed what is the velocity. Obviously this is an infinitely small time scale and for practical reasons we have to allow product to be formed so you can actually make the measurement, but that is no problem there are ways around that experimentally. But mathematically the initial velocity is the velocity at time zero when we started the reaction with substrate and there is no product. In most systems as products begin to form, it will start forming substrate in the back reaction and the reaction actually slows down, that was actually shown by all of the slides that we have seen up till now. With time the reaction slows down, as we will be seeing with in slide coming up pretty soon what we are doing is looking at time zero, no product theoretically, and that is \( v_0 \). The units of a reaction velocity generally in terms of concentration but there is without going into the details biochemists generally using an amount of substrate that is disappearing per unit time which is usually seconds or minutes. If we measure the enzyme activity, and when we use the term enzyme activity we mean the catalytic activity, how fast is the reaction when that particular concentration of enzyme is present, we measure how many micromoles of substrate disappear or how many micromoles of product appear. And if it is one substrate to one product then that’s a one to one ratio so we will often see the units of reaction velocity as micromoles of substrate disappearing or product appearing per minute. Sometimes we will see it per second.

This is an example of how initial velocities are in fact measured. Again I told the computer to do the calculations for me to set up this graph. Arbitrarily set the equilibrium concentration to the product at five we won’t worry about what the equilibrium constant is, but if we knew what the substrate concentration at equilibrium was then we could readily calculate it; here it doesn’t make any difference. Suffice it to say, that the equilibrium concentration of the product under
these conditions is five maybe milioler, or micromoler whatever the units are. We start the reaction by adding substrate. And we have two enzyme concentrations here arbitrarily set this enzyme concentration is 50 units, whatever that might be: 50 molecules, 50 million molecules, 50 micromoler of enzyme and an enzyme concentration that is one half of that, 25 units. If this is the reaction velocity in the presence of 50 units of enzyme and what do we mean by the reaction velocity here, what we do is at zero time we start the reaction by adding substrate or enzyme either one, let’s say at one minute we make a measurement of how much product is formed we get this value at two minutes, at three minutes we do the same measurement, at four minutes we do the same measurement, etc. And eventually after a few hours or so, a couple of hours, this will approach very very closely to the equilibrium value of 5. If we set up a separate incubation tube that has the same substrate concentration at zero time but we have only one half of the enzyme concentration present, the reaction velocity is one half. It will eventually get up to the concentration of product, the equilibrium concentration of 5, but it will take longer and the slope at zero time is one half of the slope when we had the higher enzyme concentration, and in fact again I will let the computer to do this. But this is the slope of the reaction in the presence of fifty units of enzyme. This is the slope at zero time that is this is v0 initial rate, initial velocity in the presence of 25 units. And I will let you take a look at this. And you will see that this slope which in fact is five that is from 0-5, so it is five over five, it just so happens they are the same numbers, the time is 0 to 5 so the slope is one. The slope here is the y axis once again is 5 so that change delta concentration of p is five but notice that the time is 10 and so the slop is five over 10 or one half and that is exactly what we were saying. The slope at zero time, initial velocity in the presence of the 25 units of enzyme is one half that in the presence of that in 50 units of the enzyme.

It is time now to take a look at the mathematical model of measure the relationship between substrate concentration and velocity of the catalyzed reaction. What we are going to do is vary substrate concentration but maintain the concentrations of everything else constantly that includes the enzyme the buffer we will keep the temperature constant so all of the variables except substrate concentration will be altered. And then we will measure quantitatively the relationship between substrate concentration and the rate of the reaction and this will illustrate how enzymes work. This is the so called Michaelis - Menten Model. This model works for many enzymes in cells. We are going to make several assumptions here as we are going to be looking at all of these assumptions are valid for all enzymes in cells. Even though this is an imperfect model it is an understandable one. It illustrates the principles of enzyme catalysis and it is in fact, it gives us for most enzymes in living systems a fairly good idea of how the rate of a reaction is affected by a substrate concentration. Let us take a look at the assumptions in this model.

The first one is the mechanism of the reaction and in fact this is true of any enzyme catalyzed reaction whether it fits this Michaelis-Menten model or not. That is here we are looking at a single substrate molecule being converted to a single product molecule that is one of the simplifications. We will see reactions in which there are two substrate molecules giving rise to three product molecules etc. But there are ways around that in this model. But we are staring off with the simplest model, one substrate molecule being converted to one product molecule. The mechanism of reaction in the Michaelis - Menten model is that the enzyme binds the substrate to form and the enzyme substrate complex and we have already seen a specific example there of RNA the substrate binding to ribonucleic the enzyme. And this is a reversible binding and in an irreversible step the so called rate limiting step the substrate is converted to product, and it is not
show here in the model but then the product is released from the active site. In fact in the model what happens is at the active site substrate is converted to product at the rate limiting step for the overall reaction and then the product comes off very rapidly so that we can neglect the rate of that reaction. So we have this equilibrium between substrate and enzyme for binding and then once the substrate is bound, not always sometimes the substrate will dissociate giving back enzyme and substrate but every once in a while in fact for most enzymes usually once the substrate is bound it will undergo reaction to form product.

We are going to be looking at initial velocity that is the velocity when there is no product form that is why we have a unique directional arrow here. We do not have products going back to substrate which for many enzyme catalyzed reactions does occur when concentration does get high enough. This just repeats what I was saying this just assumes that there is no enzyme product complex although in fact in all cases there is we are just saying that the rate of that reaction is so fast, the rate of release of the product from the active site is so fast, that we can neglect them.

The second assumption is that the concentration of enzyme is much less than that of substrate we won’t go into why this is important. It turns out that in cells there are many reactions in which the concentration of enzyme is not all that different from the concentration of substrate. In fact sometimes that plays a very important physiological roll but the Michaelis-Menten model we are assuming that the number of substrate molecules is much much greater than the number of enzyme molecules.

The third assumption is that the rate of the initial velocity, by the way I want to point out here that just for simplicity I’m not using the subscript zero when consider the Michaelis-Menten model. Some textbooks do, some textbooks don’t but we have to make sure that in this model we understand that we are talking about initial velocity when there are no products present. The initial velocity no matter what the substrate concentration might be is proportional to the total amount of enzyme that is present. We double the amount of enzyme the rate doubles. If we increase the enzyme concentration by a hundred fold the rate of the reaction increases by hundred fold. And again while not all enzymes conform to the Michaelis – Menten model this is generally true and in fact this is one of the ways that living systems control rates of reactions. They actually change the concentration of enzyme.

If there is a very large concentration of substrate all of the active sites can find substrate. And at that point, we say that the enzyme is saturated. And if we add more substrate, there is no more enzyme substrate complex that is formed. And we term this concentration of substrate a saturating concentration of substrate that is all of the active sites are already filled with substrate. Let’s just take a look at an illustration here.

Here we arbitrarily have three enzyme molecules and these are the binding sites, the active sites for substrate, and these rectangle square substrate molecules. In the first box, we have relatively low substrate concentration and it is such a concentration that on the average that one out of three of these active sites is bound. There is an affinity of the substrate for the enzyme and under these concentrations there is in fact an equilibrium constant that can be determined experimentally. And I am just arbitrarily saying that under this substrate concentration that one third of the active site of the enzyme is binding substrate. Notice that at this point in time we are just freezing this time right now this is the only one of the three enzyme molecules that can
catalyze a reaction. Product can be formed here and only here, can’t be formed here because substrate isn’t binding and can’t be formed here because substrate isn’t binding. Let’s now increase the substrate concentration such that on the average about two thirds of the active sites on these enzyme molecules are filled. In fact, we now know the rate of the reaction here is going to be twice as fast as the reaction over here because we are going to have twice as much of the enzyme substrate concentration. We already know that. Now we begin to increase the concentration of substrate and let’s say that we increase it very very high such that at any one time all of the active sites of the enzyme are filled with substrate this then is a saturating concentration of substrate. Now in fact, what happens is the substrate binds, converted to product, and the product comes off and there is a fraction of a second that the site is unoccupied but immediately there is a substrate molecule there ready to come on, to bind, form product, come off, and immediately the substrate comes on because of the very high substrate concentration. Up here with the low substrate concentration the substrate molecule bines reacts to form product the product comes off, now because there are relatively few substrate molecules that active site has to wait a relatively long period of time before another substrate molecule finds its way to that site. And this in fact qualitatively illustrates the effect of reaction velocity upon substrate concentration and this is what we are going to quantify using the Michaelis – Menten model.

The last assumption, this isn’t actually an assumption as such but a description of the Michaelis – Menten model is the introduction of the term Michaelis Constant and in fact this is a ratio of rate constants in the Michaelis – Menten model. I’m not going to show that. And we will be seeing what it fact a Michaelis Constant is in minute or two I just want to point out that the units of the mc are those of concentration. In fact we will see that the Michaelis Constant will defines a particular substrate concentration so we are not surprised that the units are those of molarity. I’m not going to derive the Michaelis - Menten equation. Suffice it to say with all of the assumptions that we have just seen, any textbook will show this derivation it is algebra we come up with the Michaelis – Menten equation and let’s make sure we understand what this equation is doing.

It is relating the reaction velocity at zero time with substrate concentration for a particular enzyme. So, substrate molecule will bind to the active site, reaction will proceed, product will be removed from the active site, it will diffuse off, new substrate molecule will come in, react, product will come off. how fast does this occur? And what is the relationship between substrate concentration that is how many substrate molecules are present with how fast the reaction goes for a given enzyme concentration. Enzyme concentration in this model remains constant. We know if we double the amount of enzyme we will double the rate whatever it may be, half the amount of enzyme we half the rate. So we are keeping the enzyme concentration constant, the temperature, the buffer, etc. The relationship in the Michaelis – Menten model is the following. The initial velocity at a given substrate concentration divided by what is termed the maximal velocity. The maximal velocity is the rate, the velocity at zero time the initial velocity when the substrate concentration is saturated. For that amount of enzyme the reaction simply can’t go any faster. The substrate concentration cannot bind anymore to the active site of the enzyme because all of the active sites are already occupied with substrate. This ratio, the initial velocity with the substrate concentration that we are looking at divided by the maximal initial velocity is the substrate concentration giving this velocity divided by that substrate concentration plus this Michaelis Constant. By the way I point out, that another way of expressing this is that this ratio of velocities how fast the reaction goes in fact, what that substrate concentration divided by how
fast that reaction would go if all of the enzyme were binding substrate is the ratio of the concentration of the enzyme substrate complex in other words how many enzymes are actually binding substrate divided by the total amount of enzyme. Some of the enzyme is free it is not binding substrate, some of it is binding substrate, whatever fraction of the enzyme is binding substrate which then can go on to give product whatever fraction of the total enzyme is binding substrate is the ratio the fraction of the velocity divided by the maximal velocity. Now what is this KM? What is the practical application of the Michaelis Constant? Let’s ask this question and we will see why we ask it in just a second.

What if arbitrarily we set the substrate concentration equal to the Michaelis Constant. Remember that the units of mc are molarity and in fact it is a measure of concentration so it is perfectly valid. So, if we make the mc equal to the substrate concentration what do we have? We then have S over 2S that is the velocity when the substrate concentration equals the km divided by the maximal velocity is 1/2. That is when the substrate concentration equals the Michaelis Constant the velocity the initial rate of the reaction under those conditions is one half of the initial velocity.

Let us take a look at a Michaelis - Menten plot in which the affect of initial velocity on substrate concentration is graphed. Here we have the ratio that we saw in the previous slide of the velocity at a given substrate concentration divided by the maximal velocity. Now these values vary from zero to one. We cannot get a reaction velocity then V max, so that is the maximum velocity but if we don’t have any substrate we won’t have any rate, so V will be zero. So this will vary between 0 and 1. Here we vary the substrate concentration and what the substrate concentration actually is depends upon what the enzyme is this has to be experimentally determined and again I simply told the computer what the km is and this is what it plotted so this is in fact accurate.

What we see is the fact that as we increase substrate concentration the initial velocity of the reaction increases. That makes sense what we have been talking about especially with that semi quantitative figure we had a couple of slides ago where it shows you what saturated concentration was. As we increase the concentration substrate the substrate molecules there is more chance that the substrate molecule will find an empty active site or empty binding site and will get on. The more substrate molecules there are the more the binding sites will be full of substrate will actually be binding substrate the faster the reaction. The product cannot be formed unless the substrate is binding here. The more we have substrates binding the more products we have being formed. The faster it goes.

The next slide is actually the same as this slide except the abscissa has been expanded this maybe isn’t quite clear that this is approaching a value of 1. But notice that the substrate concentration goes up to 10 here and the next slide I plotted this again as exactly the same plot, the plot that we had before would have only gone this far here. But I have expanded the substrate concentration to 100 and you will notice that it approaches closer and closer to a ratio of v to v max of 1. That is as the substrate concentration becomes very high starts to become saturating it starts to get close to this point where the reaction velocity is going as fast as it can. Again those of you who have a mathematical stance understand that it is going to take an infinite concentration of substrate before you actually get up here. But for all practical purposes and biological systems if the rate of a reaction is let’s say about 90% of the maximal velocity biochemists will say hey that reaction is going at its’ maximal range. And the concentration of substrate is saturated.
What if we have enzymes that have different kms? In other words, what is the affect of km on reaction velocity? So we are assuming here that we have the same reaction S to P but we have three different enzymes that have different kms and by this way this is known biological system and we will have more than one protein catalyzing the same reaction and usually the kinetic parameters are slightly in fact significantly different Here we have three enzymes kind of like the same reactions. This is the enzyme in which the km, again I told the computer, is 2, that is the value we saw in the previous two slides. What if the Km value were 1? The velocity reaction of any substrate concentration the initial velocity reaction is faster. And similarly if the KM is greater for any substrate concentration the concentration at a given substrate concentration the velocity is slower. Now let’s make sure that the definition of the mc that we derived that is the mc is the concentration of substrate at which the reaction velocity is one half of the maximal velocity. Let’s make sure in fact that is correct. Let’s take a look at this curve.

Now all of these approach eventually (it isn’t necessarily clear here) but they all approach a value of 1 that is saturating substrate concentration the ratio of v to v max would be 1. And we would be a v max. And so if we are interested in the km the substrate concentration gives one half maximal velocity. What we have to do is look along this y value here of .5 that is the velocity is one half of the maximal velocity. But this curve I told the curve that the km is 2. Let’s take a look at the substrate concentration where the initial velocity is one half the maximal velocity. We go along the curve here. The substrate concentration is now on the x axis there it is, what’s the substrate concentration? 2 milimolers.

What about this reaction? What is the substrate concentration that gives one half maximal velocity? Well here is one half maximal velocity. Let’s go along. What’s the substrate concentration that gives that? Here it is. Let’s read it down. And the value is, and I can assure you that is in fact correct, 1, but what is the km value? 1. Last one. One half maximal velocity, go over, what substrate concentration gives one half maximal velocity, a value of 4, and there it is. I assure you there are no shenanigans here. Just fit the Michaelis - Menten equation into the computer and this is what it spewed out.

What are the value of km and vmax? What do they really mean? First of all both of them depend upon the structure of the enzyme and the substrate. That is the reaction of the substrate binding to the enzyme, reaction occurring, product coming off. These have to be experimentally determined. There is no way of predicting what the km and/or vmax for a given reaction in an enzyme will be. Km is independent of the enzyme concentration assuming that the substrate concentration is much higher than the enzyme. So KM, the concentration of the substrate that will give one half maximal velocity is independent of how much enzyme is present. Now what that velocity actually is does depend upon the amount of enzyme. Remember that if we double the amount of enzyme we double the rate of the reaction. So if the substrate concentration is 2 milimoler and the km is 2 milimoler if we have a given concentration of enzyme there let’s say the reaction is going at 20 units. If we double the amount of enzyme the reaction will be going at 40 units. In both cases the velocity is one half of what the maximal velocity would be under those conditions.

KM measure efficiency and I want to point out I haven’t found a textbook that uses the term efficiency and that is why I have it in parentheses. I like to view it this way. KM measures how much substrate is required to get a given velocity. If the km is low, it takes less substrate to get a given velocity. If the km is high, it takes more substrate.
The max measures the maximal output. How fast can that reaction go with that number of enzyme molecules that happen to be present? The $v_{\text{max}}$ is the rate of the reaction when the pedal is to the metal. And in fact I like to use an automobile analogy for km and $v_{\text{max}}$. Let us compare my 1973 VW bug with a new model Porsche. I own the former I can assure you I do not own the latter; in fact I don’t even lust after it but there are certainly many people who do. My VW bug and a porches will get me from point a to point b. The Porsche can do it much faster (we forget policemen we forget speed limit). The porches can do it much faster. But in order to buy the porches in the first place, and any time anything goes wrong what do you do compared to my VW bug? You lay out big bucks. In other words, it takes lots of dollars to get you that great speed which in fact in most cases you can never use. You are using the porches you can show off, to show others how much money you have or maybe how in debt you may be. My VW bug I don’t like to go over 60 miles an hour but I will tell you when I go in for major operation I generally come out with a 100 dollar maybe a 150 dollar bill. The KM is low the $v_{\text{max}}$ happens to be low in this case. The porches very high in $v_{\text{max}}$ but the efficiency the amount of dollars the amount of substrate molecules you have to put in is very high. And one last comment on the Michaelis - Menten model is just reminding us that at any point the velocity is the concentration of enzyme substrate complex.

This just illustrates what we have been talking about. We have taken three substrate concentrations for prototypic enzyme. If the substrate concentration is saturating let’s say above 95% of the maximal velocity all of the enzyme is bound to substrate so all the enzyme substrate complex and the ratio of velocity to the maximal velocity is one. That is the definition of saturating. At the KM one half of the sites on the enzyme are binding substrate and the reaction velocity is one half of the maximal velocity. And if you don’t believe me, you can plug this into the Michaelis - Menten equation that if we have 10 times the mc, substrate concentration equals 10 times whatever the mc may be then 10/11 of the enzyme is tied up with substrate that is enzyme substrate complex and similarly the ratio of the velocity to the maximal velocity is 10 to 11 or .91.

We introduce the term turnover number which in fact is a measure of a maximal velocity and the definition is simply the number of substrate molecules that can be converted into product by one enzyme molecule in one second. Sometimes turnover numbers are defined in terms of minutes which simply mean the maximal velocity divided by the enzyme concentration. So how fast is the maximal rate per enzyme molecule? We are going to see some examples on the next slide.

And that is enough for now and so let’s say till next time, ciao.