In beer and wine production, enzymes in yeast aid the conversion of sugar into ethanol.

Enzymes are used in cheese-making to degrade proteins in milk, changing their solubility, and causing the proteins to precipitate. Many industrial processes ranging from fruit juice production to paper production to biofuel production utilize enzymes. In this video you'll see that studying how enzymes catalyze reactions can help us design better therapeutics.

This video is part of the Differential Equations video series. Laws that govern a system's properties can be modeled using differential equations.

Hi. My name is Krystyn Van Vliet and I am a professor in the Materials Science and Engineering and Biological Engineering departments at MIT.

of reactions and after some practice, you will be able to derive a rate law for a general enzyme-catalyzed reaction.

In order to understand the topic of this video, you should be familiar with determining rate laws from experimental data, predicting rate laws from proposed chemical reaction mechanisms, and have some basic understanding of the effects of catalysts on the kinetics of a reaction.

The study of enzyme catalysis has become important for drug development. Many drugs work by inhibiting an enzyme.

For example, enzymes thought to be important for the survival and replication of the parasite that causes malaria are being explored as therapeutic targets. The parasites enter the red blood cells of the host and use proteases to catalyze the degradation of the hemoglobin inside of the red blood cells. Degrading hemoglobin, a protein, yields amino acids, which can then be used by the parasite for its own protein synthesis. Protease inhibitors could help slow the degradation of hemoglobin, which in turn would slow the growth and reproduction of the malaria parasite.

When a potential inhibitor is identified, kinetic data is used to evaluate its efficacy.

In order to understand how this can be done, let's start by describing the kinetics of a simpler case -- that of an enzyme catalyzed reaction without an inhibitor present. Then we'll think about what might happen if we throw an inhibitor into the mix.

This video will help you describe enzyme catalysis mathematically by first reviewing some common biochemical terms, then by describing the general characteristics of enzyme-catalyzed you will encounter throughout your undergraduate experience.

a reactant is called a substrate. This is really just a matter of different fields using different terms, but it's important for you to be aware of this. Next, catalysts found in biological organisms are called enzymes. Textbooks frequently abbreviate "substrate" using the letter "S" and enzyme using the letter "E." How do enzymes work? If we look at a reaction coordinate, recall that the transition state represents a chemical species intermediate between the reactant, or substrate in this case, and the product. The potential energy difference between the transition state and the reactants is the activation energy for the forward reaction. The potential energy difference between the transition state and the products is the activation energy for the reverse reaction. Only reactants with the energy to overcome the activation energy will form products.

Like synthetic catalysts, enzymes accelerate the rates of reactions by stabilizing the transition state, lowering its potential energy, and providing a new pathway by which the reaction can occur. This new reaction pathway has a lower activation energy than the uncatalyzed path making it more likely that a greater number of reactant molecules will have the energy needed to overcome the activation energy and proceed to product.

Enzymes facilitate reactions, but, like other catalysts, are not consumed in the reaction.

Enzymes are typically proteins, but RNA has also been shown to catalyze reactions.

While there are different theories about how enzymes work to catalyze reactions, there are a couple of things that are agreed upon. One is that enzymes have a region called an active site. Substrates bind to this region. The shape and chemistry of the active site determine the selectivity of the enzyme for particular substrates.

The second point is that when a substrate binds to an enzyme, an enzyme-substrate complex is formed. This enzyme-substrate complex is a reaction intermediate, meaning that it is formed and consumed in the reactions, but does not appear in the overall chemical equation.

For many enzyme-catalyzed reactions, if we were to measure the rate of reaction at various substrate concentrations, we would see that the rate of reaction appears to follow first order kinetics at low substrate concentrations and then transitions to behavior that resembles zero-order kinetics at high substrate concentrations. Please pause the video here, turn to the person beside you and discuss what it means for a reaction to be first-order or zero-order.

Remember, in a first-order reaction, the reaction rate is directly proportional to the concentration of substrate. In a zero-order reaction, the reaction rate is constant as the reaction progresses and is unaffected by substrate concentration. So, if we look at this data, we see that, for a given enzyme concentration, the addition of substrate above a certain value has no effect on the rate of reaction.

So let's think about what reaction mechanism might explain this data.

Some scientists hypothesized that after an enzyme and substrate combine to form the enzyme-substrate complex, this complex yields the product and degrades to regenerate free enzyme. Does the rate law derived from this reaction mechanism fit the experimental data?

Let's derive the rate law and see. We can derive this rate law just as we would for other chemical systems.

Remember, each step of a reaction mechanism is assumed to be an elementary reaction. Please pause the video here, turn to the person beside you and discuss how you determine the rate law for an elementary reaction.

Remember, for an elementary reaction, you CAN predict the rate law from the chemical equation.

With this in mind, can you write a differential equation for the rate of product formation with time? Please pause the video, try to write the equation, and continue playing the video to see if you are correct.

From the 2nd step of the reaction mechanism, we can write that the rate of formation of product is equal to the rate constant k2 multiplied by the concentration of our enzyme-substrate complex.

The enzyme-substrate complex is a reaction intermediate and not something that is easily measured in experiments. How can we verify if this expression is correct? It would be nice to restate the rate in terms of quantities that are more easily measured such as the substrate concentration and the initial enzyme concentration.

To get an expression for the concentration of the enzyme-substrate complex, let's write a differential equation for the net rate of change of the enzyme-substrate complex concentration with time. Please pause the video here, try to write the differential equation, and then continue playing the video to see if you are correct.

If we want to write an expression for d[ES]/dt, we see that in the first step of our mechanism, the enzyme-substrate complex is produced in the forward direction and consumed in the reverse direction. In the second step, the enzyme-substrate complex is converted into product.

This equation introduces two additional unknowns: the free enzyme concentration and substrate concentration. So, we need two additional equations in order to solve it.

Can you write an equation for the rate of change of substrate concentration with time?

Please pause the video here, try to write the differential equation, and then continue playing the video to see if you are correct.

If we want to write an expression for d[S]/dt, we see that the substrate will be consumed in the forward direction and produced in the reverse direction of the first step of the reaction mechanism.

We still need one more equation. This equation is a simple relationship that says we know how much enzyme we added to our reaction mixture at time zero, so at any time, we know that the amount of free enzyme plus the amount of enzyme bound in the enzyme-substrate complex should equal the initial amount added. This could also be written in differential form as d[E]/dt plus d[ES]/dt is equal to zero.

So, we have a system of four ordinary differential equations with four variables that all depend on time. Equation 1 and Equation 4 have analytic solutions, but Equations 2 and 3 contain a non-linear term. When you learn about differential equations in your future courses, you'll see why these equations are difficult to solve and that you will need numerical methods to solve them.

Here, we have integrated these equations numerically using the boundary conditions that at time equals zero, the substrate concentration is S0, the product concentration is zero, the enzyme concentration is E0 and the concentration of enzyme bound to substrate is zero.

We see that after a short start-up period, the concentration of enzyme bound to substrate remains approximately constant. In this region, where the substrate concentration is much greater than E0, as enzyme is released from the enzyme-substrate complex, it quickly recombines with available substrate.

As more substrate is converted to product and the substrate concentration approaches E0, the concentration of enzyme bound to substrate is no longer constant.

In the regime where the substrate concentration IS greater than E0, we can make a steady-state approximation and set d[ES]/dt equal to zero. This approximation will allow us to obtain an analytic expression for the concentration of enzyme bound to substrate.

Setting d[ES]/dt equal to zero allows us to solve for the concentration of enzyme-substrate complex, which is equal to the rate constant k1 times the concentration of free enzyme times the concentration of substrate divided by the sum of k-1 and k2.

Let's lump the rate constants into a new term called Km and substitute this into our expression for the concentration of enzyme-substrate complex.

Now we can use this expression... ... to rewrite d[P]/dt.

Let's try to eliminate the concentration of free enzyme from this expression. We can use the enzyme balance that

we wrote earlier, which said that the concentration of free enzyme plus the concentration of enzyme-substrate complex should equal the initial enzyme concentration.

Substituting our expression for the concentration of ES into the enzyme balance and doing a little bit of algebra, we can solve for the concentration of free enzyme.

And finally, we can substitute this into our equation for d[P]/dt and obtain this expression.

Let's check this rate law against our experimental data. At low substrate concentration, the expression reduces to a first-order rate law and at high substrate concentration; the expression reduces to a zero order rate law.

It turns out that the rate law that we just derived is called the Michaelis-Menten equation.

To make our equation look more like the version of the Michaelis-Menten equation that you will see in textbooks, there are a couple of terms that we will use because they are the convention in enzyme kinetics. Instead of saying d[P]/dt is the rate of the reaction, we will call it the velocity of the reaction and use the notation of 'v.' Finally, we'll lump k2[E]o into a term called vmax. At high substrate concentration, the reaction velocity approaches vmax.

Remember that lump parameter Km? We can determine Km from experimental data. If we look at the substrate concentration needed to reach half of vmax, we see that the concentration of substrate required equals Km. Km values for many enzyme-substrate pairs can be found in the literature allowing for comparison.

You may be wondering why we went through all of this. Let's go back to our drug development example.

Let's say that you have designed a drug that you think will inhibit one of the proteases used by the malaria parasite to degrade hemoglobin. How will you test its effectiveness?

First, you might add a fixed amount of protease to solutions of varying hemoglobin concentration in order to measure reaction rates. Let's say that this reaction follows Michaelis-Menten kinetics well and that you are able to estimate vmax and Km.

Next, you repeat the experiment, but this time, you also add a fixed amount of the drug candidate to all of your solutions. You get your rate data and see that the apparent vmax has decreased. But what does this mean? Can you speculate how this inhibitor works?

Is the drug interacting with the enzyme and preventing it from binding substrate?

Is the drug interacting with the enzyme-substrate complex? Is it something else entirely?

The thought process that you used today to derive the Michaelis-Menten equation can be used to derive rate laws

for other reaction mechanisms. Then you can see which mechanism is best supported by the data.

Today we learned a little bit about the importance of enzymes and derived a rate law from a general reaction mechanism using differential equations and a steady-state approximation. This rate law was compared against experimental rate data for an enzyme catalyzing the conversion of a substrate to a product. The equation that we derived is called the Michaelis-Menten equation. While all enzyme-catalyzed reactions may not exhibit Michaelis-Menten kinetics, the same logic that you used to derive the equation can be used to derive rate laws for other proposed reaction mechanisms.