CATHERINE DRENNAN: And now I just want to revisit-- when we started the unit we listed a bunch of things that affected the rates of reaction, and we see where we are. We've talked about mechanism. We've talked about the nature of the material, The Arrhenius constant depends on the nature of the material, the activation energy barrier depends on the nature of the reactants of the material. Concentrations and pressure-- partial pressures, temperatures. The only thing we haven't talked about are catalysts.

So that's what we're going to focus on today. We're going to learn about catalysts and how they affect kinetics. So a catalyst, this is something that's used by non-scientists, a lot of laymen talk about catalyzing something. There's many companies with a name catalyst in it. So a catalyst is a substance-- this is its technical definition-- that will speed up a reaction, but it is not, itself, consumed by the reaction. It doesn't undergo any permanent change. So it can be thought of a sort of a helping hand, a lift up. It makes the reaction go faster.

But it does not appear in the overall balanced equation, because it's not being transformed, it's not being converted to something else. It's just speeding up the reaction. OK so now let's go back to our reaction coordinate diagrams and think about what a catalyst is doing. So back again, potential energy vs. the reaction coordinate. We have the potential energy of our reactants up here, potential energy of our products here, delta E, the difference between them.

But again, before the reactants can go onto products, they must overcome an activation energy barrier. So EA for the forward direction. And if they're going from products to reactants, they don't have to just do that, they have to go way up here first. And this is the activation energy barrier without a catalyst, the transition state, the activated complex. That's the height to which the molecules need to get in potential energy to be able to react and go on.

So we can draw now our curve. So from reactants we go up, overcome that barrier, reach this state, and then can go onto products. So they come together, and you end up at a lower potential energy in this case. But before you get there, you have to overcome this activation

energy barrier. OK. So this is the diagram we've seen before. What is the catalyst doing? So what the catalyst does is it decreases this barrier. So now we have a dashed line for the barrier with a catalyst, and we can draw a new curve over here so you don't have to get as high, you don't have as large a barrier anymore.

And we can put in the new barriers. So in dashed lines now we have the activation energy barrier for the forward direction, and the activation energy barrier for the reverse direction. Both of them are less. This line is below. The catalyst has lowered the activation energy barrier. They typically act by reducing the barrier, both for the forward direction and the reverse direction. So we can also say-- and people do-- that catalysts stabilize or lower the energy of this transition state, also known as the activated complex. So they bring this down, they lower the energy needed. It's easier to get over the hump when you have a catalyst and therefore it speeds up the reaction.

So catalysts have no effect on the thermodynamics of the system. They're affecting the kinetics of the system, and this is because free energy, our friend delta G, is a state function. It's independent of path. OK with these things in mind, tell me with a clicker question what happens to the equilibrium constant if you have a catalyst present? All right. 10 more seconds.

And that is true. It is not changed. So the equilibrium constant is not changed by the presence of a catalyst because this is a thermodynamic property. So delta G is not changed, and the equilibrium constant is not changed. So it doesn't depend on path, and this is a path difference. The catalyst changes the path, but it doesn't change the beginning state or the end state. And so just to help you remember this, I'll give you a little trick to remember this.

So again, catalysts effect the kinetics, not the thermodynamics. And one way to remember this is that the Chinese symbol for catalysts and marriage broker are the same. So a marriage broker increases the rate at which a couple comes together, usually introduces the two people to each other. Of course online services can do this as well, but none of these things can make a couple stable if the couple is not stable.

So online dating services, marriage brokers, increase the kinetics, increase the rate at which people meet, increase the encounter, but do not change the thermodynamics of the relationship. A couple is stable or unstable regardless of the marriage broker, regardless of the catalyst. So affects kinetics, not thermodynamics. All right, so there are two major types of catalysts, homogeneous catalysts-- and that is just when the catalyst and the reactants are in

the same phase.

An example that has actually been in the news a bit this fall is the ozone layer, depletion of the ozone by chlorofluorocarbons, all in the gas phase. So this would be the catalyst for the depletion is in the same phase as the thing it's depleting. And here's a little picture of the increase in the ozone hole over time. But there was some good news this fall that it seemed like the rate of increase was not as dramatic, that it was staying a little bit more level, which is exciting to know that if we stop bad behavior, that we can and we can have a positive effect.

So we shouldn't just say, oh, we polluted so much already, what's a little more? We've already done all the damage. There's no point. No, no there is a point. If we stop doing damage, good thing-- better things will happen. So that was some good news that people were hearing about this fall. OK along the pollution lines of destroying our planet, there's also heterogeneous catalyst, so a different phase. And a common example of this is the catalytic converter in a car.

And so catalytic converters use of metals, solid phase to help catalyze reactions of gases, so solid gas, different phase. And they try to convert these gases to last less toxic pollutants. And so I'll just give you a little example of how this could work. I'm not a catalytic converter, but just in general with this little movie-- so we have this metal surface here. And the metal surface will absorb H2 gas and help to dissociate H2. So here's a hydrogen, here's a hydrogen absorbed onto this metal surface.

And then you can flow something over the metal surface, and that will be reduced by the hydrogens. So let me get this to go. So here we see, now the hydrogens are popping off and going to reduce ethene over here. So this is an example of a heterogeneous catalyst, where we have metal in a solid phase that's catalyzing a reaction that involves materials in a different phase. OK so two types.

But there is also my very, very favorite type, which is enzymes. So we're going to talk just briefly about enzyme catalysis, which will give you a leg up when you go on to courses that have something to do with biochemistry. And these days biochemistry is sort of everywhere on campus. You might think I can escape biology to go into chemical engineering. No, no you can't. There are a lot of chemical engineers that are working only with enzymes.

So a little bit about enzymes as catalysts. So enzymes, large protein molecules, 20,000 grams per mole or more. That's a tiny protein. And they're made up of amino acids, and you've seen a lot of amino acids in this class. We've been using those to think about PKA's. And so here we

have an amino acid with a sidechain, it's abbreviated R. There's 20 different R's, 20 different amino acids. And amino acids come together forming peptide bonds, where we have a connection between this carbonyl and the nitrogen of the next amino acid.

So amino acid one amino acid two and here's the peptide bond. These then form long chains, and will fold up into a compact structure. So here is an example of a structure of an enzyme. And I'll just tell you a little bit about this enzyme. So here there are four polypeptide chains, so one in green, one in red, one in yellow, and one in blue. And they form this compact structure. And this picture shows these ribbons. Here is alpha helices, there's squiggly, the arrows, or beta strands.

And so they draw ribbons through the alpha carbon position, so you don't see all the atoms here you just kind of see how the chains wrap around each other to form this overall protein structure. So this particular enzyme catalyzes the last step in the biosynthesis of an antibiotic fosfomycin. So it converts this reactant or substrate molecule into the fosfomycin antibiotic. Fosfomycin is used in combination therapy to treat MRSA infections.

So I have always tried to give you examples of important areas of science that smart people should go into and solve these problems, and I'll just mention antibiotic resistance with this figure. This was published in the journal *Nature*. They called it a perfect storm, because here we see the increase in antibiotic resistant strains. This is MRSA here. Up to 60% over here. And these are-- this data is old. And then that go up, and here in blue going down are the new antibiotics that have been approved for use.

So we have much more resistance and many fewer antibiotics being approved. And so this what has been called the perfect storm, and this situation the y data here 2010. It's not any better. In fact, it's worse now, so one important area is to come up with new antibiotics. All right so many of the targets of antibiotics are enzymes. Many of the ways to make antibiotics is using enzymes, and so enzyme catalysis is very important for medicine, and also for engineering and biofuels. And people are using enzymes for pretty much everything these days.

All right. So a couple of terminology-- reactants. We've been talking about reactants. If it's an enzyme, it's called a substrate. These terms can really be used interchangeably. The substrate will bind to what's known as the active site on an enzyme, and so as someone who determines three dimensional structures of enzyme, it pains me to draw a picture of an

enzyme structure like this, but I did it anyway.

So here's my enzyme. That's its active site. Here is the substrate molecule, which is about the same size as the enzyme-- usually that's not the case, but anyway-- binding. And so then when you have enzyme plus substrate bind together, we have our ES complex, ES for enzyme substrate. And then the enzyme will undo catalysis, forming product. And product will be released, and we'll have free enzyme again. So this is a very simple mechanism or steps of enzyme catalysis, but that's OK. It sometimes can be written in a very simple way.

And so now, using what you have already learned how to do in our reaction mechanism, we can derive rate laws and rate expressions for enzymes. So you know biochemistry is really not much different than anything else we've been doing. All the things you've been learning in chemistry apply to biochemistry. Biochemistry or life is really just a series of chemical reactions that obey all of the same laws and principles as everything else.

So here we're going to write an expression for the enzyme coming together with its substrate, forming an intermediate enzyme substrate complex, and going on to form free enzyme and product. In step one, enzyme and substrate will come together. And in the forward direction, we have k1 to form the enzyme complex, and it's also a reversible step. In step two, the complex goes on to form enzyme and product. So we can now write these laws, or I should say you can figure out how to write these expressions. These are, again, elementary steps, elementary reactions. 10 more seconds.

OK let's just take a look at that. So over here, again the rate of the forward direction, we have the forward rate constant K1 times enzyme times substrate. And for the reverse direction, it's k minus 1 times our intermediate ES. So now that you remember how those are done, you can tell me-- you can just yell it out-- what am I going to put for this rate? K2 times ES. All right. So we can always, from an elementary step or elementary reaction, write the rate law just based on the stoichiometry, using our rate constants and our reactants here. Or for the reverse direction here.

And then we can write the overall rate at which product is being formed. So the rate of product formation, we can write it from the slow step or from our last step if we don't know anything about slow steps. So the rate at which product is formed can be expressed as DPDT, the change in product over time. And you can also write it by the rate law for this second step, K2 times ES. But we're not done, because ES is an intermediate. It's formed in the first step, and

consumed in the second step. And so it's an intermediate, so we now have to solve for ES in terms of rate constants, products, and reactants.

So how are we going to do this? And why don't you tell me how we do this? And again, this is the change in ES over time. So asking for the rate of change of the intermediate over time. All right 10 more seconds. OK. Yep. So if you remember back to the mechanism lecture, you can solve for ES by looking at the rate at which ES is formed, the rate at which it's decomposed, and the rate at which it's consumed. So the rate of formation is the forward direction of the first step minus the rate at which it decomposes, which is the back direction of the first step. So we have plus K1 E times S minus k minus 1 times the intermediate concentration, and then minus the consumption minus K2 times ES.

So we put all those steps together, and then we need to use the steady state approximation to take this to solve for ES. So again, the steady state approximation says that the rate at which an intermediate forms equals the rate at which the intermediate goes away or the net rate is 0. So that is again the steady state approximation, and they use that in enzyme kinetics as well as pretty much every problem you have in reaction mechanisms-- that's going to be on the final. All right so using that steady state approximation, we can just take this expression that we just talked about and set it equal to 0, and that will allow for us to solve for our intermediate, which is in this case ES. So we set that whole thing into 0.

All right. So this so far is exactly the same as you would do any problem in reaction mechanisms. But now, because it's enzymes, there is a slight difference. So a slight difference. And that is that instead of solving for ES in terms of E or free enzyme, we want to solve for ES in terms of the total concentration of enzyme, e to the 0 or O over here. So total enzyme equals free enzyme plus bound enzyme because your enzyme is either free or bound. It only has two options, and so that's your total enzyme.

And the reason why we want to do this is practical. We don't necessarily know if we're studying its reaction how much of our enzyme is free and how much is bound. But if we're good scientists, we know how much enzyme we put into our experiment. So total enzyme, if we can solve for things and do things in terms of total enzyme, that makes our life much easier. So it's a very practical reason. So what we can do now is replace E, which is our free enzyme, with our total enzyme minus our bound enzyme. So that's what we're going to do.

I'm going to put back those expressions we just had. So this was the clicker question, then we

set it equal to zero. And now we have this E term here. We want to get rid of that because we don't know how much of our enzyme is free, but we do know how much we put in our total. So we're going to place this E with total enzyme minus bound enzyme. So we have a k1, now instead of times E we have times E0 times our substrate. And then we have this ES term, so minus k1 again, ES times substrate. And then you had these two terms. We just put those down here.

OK so now we're back. Now we want to solve for the intermediate ES, and we'll solve for that intermediate ES in terms now of total enzyme. All right. So now we need to do some rearrangement, just putting that expression up here that we just saw. And now we're going to rearrange our ES terms. Everything with an ES to one side of the equation, and then solve for it. So we have all of our ES terms on one side, you remove that one, you move this one, we move that one. And on the other side, we just have this term with k1, total enzyme and substrate.

Now we can pull out the ES terms, so ES is here. We have k1 times substrate, k minus 1, k2, and then this term over here, k1 total enzyme substrate. Now we divide, and we get this term over here. So now we've solved for ES. One more change. There is a constant that's easy to measure called the Michaelis-Menten Constant. And we want to now introduce this term, big Km, and this term is equal sub k minus 1 plus k2 over k1. Now we want to get this new constant, Michaelis-Menten Constant, into this expression because it's easy to measure. Again, practical. So let's do that.

So here's this Km term again, and we want this Km term to appear in this solution to our intermediate. We have k minus 1 here, k2, like that, but there is no k1 underneath it. So let's put one there. So what we're going to do is we're going to divide by k1. So we'll divide the top term by k1. We'll divide and divide this k1 S term by k1 and we'll divide k minus 1 plus k2 by k1, and we do that because then we can get our big Km in here. All right. So with all of these dividing by k1s, we can simplify this expression. And so we'll do that. So we'll cancel those k1s. We can cancel these k1s. And then we can get this. Our total enzyme concentration times substrate over substrate concentration plus Km, because that term equals Km.

And now we're happy because we can measure a Km, and we know how much total enzyme we put in. So we just solved for our intermediate in terms of things we can actually measure. But we're not done. This is just the expression for intermediate. Now we have to put it back into our rate law. So let's do that. Almost done. Here is our expression for our intermediate.

The rate of product formation, the change in product over time, equals K2 times ES, this intermediate. Now we can plug that in to that term and do that. And we get this expression, which is known as the Michaelis-Menten Equation, k2 times total enzyme times substrate over substrate times-- plus Km.

And let me just show you some pictures of Maude Menten and Michaelis. So Michaelis was a professor in Germany, and he was Jewish. And he had a bit of a rocky career, and was encouraged to do things that, perhaps, no one cared about that much, like study enzymes or something. And he worked with Maude Menten, who was Canadian. So Maude Menten couldn't find a faculty position in Canada at the time, so she got a position in the US at University of Pittsburgh. Michaelis decided Germany was not a good place to be, and ended up in New York City at Rockefeller University.

Maude Menten, although she published an enormous number of papers, and really Michaelis-Menten Kinetics is one of the most famous things-- if you ask a biochemist one thing that everyone will know about it's Michaelis-Menten kinetics. She was not promoted to full professor until she was 70 years old, despite the fact that she had accomplished more than most anybody else. And then she retired at 71 years of age. So she was a full professor for one year before she retired. But Michaelis has had a rough time, too, and didn't get his position that was worthy of his accomplishments until he was in his 50s because of being a German Jew.

So both of them had a pretty rocky career, but they were two of the most prominent figures really in biochemistry, setting-- we still use Michaelis-Menten kinetics all the time in biochemistry. So this is really pioneering work. OK so let me now show you how to apply the Michaelis-Menten equation to different conditions. So here we have a plot, a change in product, so the rate at which product is being formed vs. our substrate concentration. So as we-- at low substrate concentrations down here, there's a very fast change in the amount of product that's being produced per time.

So at low substrate concentration, when you add more substrate it increases the rate significantly. And this is because there's a lot of free enzyme. So there's enzyme waiting around to catalyze a reaction. You give it more substrate, you get more product quickly. But this levels off up here, and at high substrate concentration, adding more substrate doesn't really help the rate any. It's leveling off, and that's because all the active sites are already filled, so adding more substrate doesn't make it any faster. You need to form product to

release it for substrate to bind. And so all the active site are filled, the rate levels off. So this is the behavior that you observe when typical Michaelis-Menten kinetics are in play.

So now let's think about those two conditions again, or two conditions, one at high substrate concentration and one in this range here. So going back to our equation, when substrate concentration is much greater than Km-- and I'm going to define Km for you other than the rate constant in a minute. So when that is true, we can look at the Michaelis-Menten equation and just think about what happens if this substrate concentration is way, way bigger than Km. So Km, then is much, much smaller than substrate, and it kind of doesn't matter. So it's very small and we can ignore it. And if we cancel out Km, then we can simplify this equation even further, and cancel out our substrates and we're left with this, that the rate of product formation is just k2 times our total enzyme.

And this has a special name. This is called Vmax, the maximum velocity of the enzyme. So maximum velocity or maximum rate equals k2 times your total enzyme concentration. So this is one equation that you'll find on your equation sheet for the final exam. And if we go back for a second and look at our plot up here, we can now write Vmax, the maximum velocity for that particular enzyme concentration, k2 times total enzyme. This is the maximum rate we're going to get. So now let's think about this down here at low substrate concentrations again, and particularly at a concentration where substrate equals Km, substrate concentration equals Km.

So if substrate concentration and Km are the same thing, we can just put an extra substrate in there, Km equals substrate. So on the bottom we have two substrate concentrations now, and that allows us to cancel out our substrates. And we're left with this equation 1/2 k2 times the total concentration of enzyme, which is the half maximal rate. That's half of Vmax. Vmax was k2 times total enzyme. This is half of that, and this is the definition of Km. Km is the concentration of substrate for which the rate is half maximal.

So if we go back now to our plot, this is the maximum rate at half of that maximal rate. The substrate concentration equals Km. So experimentally, you can plot your data for the formation of product at various substrate concentrations, calculate Vmax, and figure out what was the concentration of substrate when the rate was half that maximal rate, and you can determine Km. That's why you want Km in your equation, because it's something that's not that hard to measure. OK. So let's apply this now.

Let's think about an enzyme, and we're told this is enzyme carbonic anhydrase. It catalyzed

CO2 to bicarbonate, which is the buffering system that happens in your blood, the Michaelis-Menten constant for this enzyme, we have Km 8 times 10 to the minus 5 molar, and a k2 value of 6 times 5 to the fifth, I think-- or sixth. I don't know. I don't have my glasses, [? for ?]. Hopefully you can see better than me. All right. So now with these values, calculate the maximal reaction rate if the enzyme concentration is this, which I think is 5 times 10 to the something molar. They should make bigger fonts. Oh it's easier to read on that. OK, clicker question. All right. 10 more seconds. Yep.

So here you just had to say OK. If you had your glasses-- and I have my now. Enzyme concentration times k2 is going to give you the maximum rate, so Vmax equals K2 times enzyme concentration. All right. So we can put that down Vmax k2 enzyme concentration. Multiply those out together, and you get the rate, and it's in molar per second. Now what about the concentration of substrate for the rate to be 1.5 molar per second? Another clicker question. All right. 10 more seconds.

Wow. OK. So what is this number compared to the number we had a before? It's the half maximal rate. So what is the enzyme-- the substrate concentration at the half maximal rate? Km. Yeah. So all you had to do-- that's the definition of Km is look for the Km value and say that is the substrate concentration when the rate is half maximal. And so this is the kind of problem that you'll have that the extra problems have in them. And this can be the complete answer. You don't have to show any work for these. So a lot of these problems are just looking for you to identify or know what Km means. So again, that's all you have to do for this.

So check out the extra problems on kinetics. They're not really extra, they're on enzymes and things, reaction mechanisms that haven't been on a problem set. They will be on the final exam. OK and then we'll talk briefly about inhibitors, clicker competition Wednesday, and we're going to review a lot of the topics that we've covered on Wednesday. So it's going to be a lot of fun. And our last video. All right. So quickly, we've got to get to our clicker competition. So let's finish lecture 34 notes. So the last thing we've been talking about is one of the most exciting things in chemistry, which is catalysis, how do you speed up reactions?

But we have to end on a slow note, which is that inhibitors are the opposite of catalysts, and they slow down reactions. And if we're talking about enzymes, which we were doing on Monday, then inhibitors will often bind to the enzyme E, forming an EI complex, an enzyme inhibitor complex. And they often bind in the active site, and so therefore substrate, which we have here, little substrate S, cannot bind. It's a very simple idea. If you want to design an

inhibitor to stop an enzyme from doing what it's doing-- and in fact the pharmaceutical industry is largely about this, designing inhibitors to bind to key enzymes to stop processes.

So often what you do in designing this, you want something that kind of looks like a substrate. But most ideally you want something that actually resembles the transition state-- a transition state analog. So you remember catalysts work by lowering the transition state or activation energy barriers, or activated complex. So they lower that energy. And so something that resembles the transition state, where bonds are partly broken, partly formed, it's not reactants, it's not products, it's in the middle. Those molecules, those analogs of transition states will bond very tightly and will block the enzyme from doing its job.

And this is really-- I told you about it-- we had World AIDS Day I mentioned that one of the reasons why AIDS is not such a huge problem in the US anymore, it's not a death sentence anymore, is because the pharmaceutical industry designed transition state analogs targeting HIV protease. And they really worked very well. So for in the developing world, there's a good regime of pharmaceuticals that we can take that keep the viral load low. Now it's different in other parts of the world, but a lot of the pharmaceutical industry is about designing inhibitors for enzymes.

OK so we've been talking about catalysis. We talked about catalytic mechanisms, catalysts, we talked about effect of temperature, all sorts of things. And so we're going to end our catalysis unit with our final video in the series *In Their Own Words*. And this is a former graduate student. She actually just defended I'm talking about her work in Tony Synskey's laboratory, and why kinetics are important to our research and development of biofuels.

[VIDEO PLAYBACK]

- My name is Jingnan. I'm from Chongqing China, and I'm a third year graduate student working in the chemistry and biology department. My research she's on converting carbon dioxide, an environmental pollutant, into a useful biofuel. Right now, currently, we burn ethanol in our gasoline. And ethanol is not an ideal fuel, because it's a shorter chain link, and the combustion of heat is not as high as longer chain length alcohols. Also, ethanol traps a lot of water, so it can cool off the engine. When they first found the Ralstonia eutropha they realized that this organism can store a lot of its carbon as a polymer chain of esters. And these polyesters, when isolated, can be used as biodegradable plastics.

for my research, I'm trying to turn this carbon storage organism into storing the carbon as

biofuels, so an alcohol that can burn in our engines. To change these organisms from making biodegradable plastics to make biofuels, first I have to get rid of the gene that actually makes the biodegradable plastics, and then fine tune another pathway to shun the excess carbon into making biofuels. I really have to think about kinetics, because I wanted my pathway to go as fast as it can to produce as much product as possible within a period of time.

So there are certain parameters for kinetics that I can change, and certain parameters that I cannot change. For the parameters that I cannot change, is the temperature because my organism would not survive under higher temperature. And for parameters that I can change, for example, I can change that enzyme by putting in a stronger and more efficient enzyme from another organism to catalyze the same exact reaction. I can also increase the substrate concentration by eliminating another competing pathway for the same substrate by deleting the gene, so hence making more of the substrate out going into my product. Eventually we'll be able to take the carbon dioxide that's released as a pollutant, and trap it and use it as a carbon source to make useful molecules.

[END PLAYBACK]