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PROFESSOR: So, let me start here with the temperature dependence of k . And this turns out to be extremely important. And it's due to Arrhenius in 1889. And Mr. Arrhenius is famous for many reasons. Not just for his rate law. So I learned recently, turns out that he was also one of the first people to calculate the potential effect of carbon dioxide in the atmosphere. And its potential effect on global warming. This was the Industrial Revolution and people were starting to use fossil fuels at increasing amounts. And back then, already, some people started to get worried. And so he did the calculation. It was a very crude calculation. And he extrapolated, assuming that the rate of fossil fuel use would keep going, he got his calculation pretty much right. What he didn't get right was the amount of fossil fuel that people would be using. And so when he did his calculation, which is again a very crude calculation, he got that we would get in trouble at about 2,000 years from the time of his calculation. So he said, no problem, 2,000 years, we've got a lot of time.

And ever since then people have redone these calculations and more and more sophisticated. But his crude calculation was good enough. And as people redo the calculations, the time that they say we're in trouble to the time of the calculation gets closer and closer together. And the reason for that isn't that their calculations are wrong, it's that the rate at which we put out carbon dioxide just keeps getting faster and faster and faster. So it's interesting to go back to these calculations through the last century and a half and see that. Anyway, so Mr. Arrhenius predicted global warming. And he wrote his rate equation, k is equal to e to the minus E_a over RT . Famous Arrhenius rate equation. If you plot it as $\log k$ is equal to $\log A$ minus E_a over RT , you find that it looks like a straight line. With an intercept at $\log A$, where A is the pre-factor here and E_a is going to be the activation energy. And the slope is $-E_a$ over RT . R is the gas constant. T is the temperature.

And if you put in some typical numbers, activation energies typically are on the order of a few tens to hundreds of kilojoules per mole. Let's say 50 to 300 kilojoules per mole. And typical activation factors, typical pre-exponential factors, are depending if it's a first order or second order. Let's say, first order 10 to the 12 th, 10 to the 15 th, per second, so the A carries the units for the rate here. So if you have A , so this is for first order, if you have a second order, then the units of A will be different. And typically they're going to be on the order of 10 to the 11 th or so, 10 to the 10 th, 10 to the 12 th, one over molar per second, for second order.

And it's interesting to do sort of a back of the envelope example to figure out how important this rate is. And one of the interesting things to look at is, suppose that I'm at some temperature and I want to know how do I change the

temperature. How much do I need to change the temperature to double the rate. And let's say my temperature's at, say, T_1 is on the order of 300 degrees Kelvin, room temperature. Room temperature, and let's take a typical E_a on the order of 100 kilojoules per mole. Room temperature, or body temperature are roughly the same, right? These things are.

And so the question is, what does T_2 need to be to double the rate connected with an activation energy which is fairly typical of 100 kilojoules per mole. So we want to know, what is, if I have k_2 over k_1 is equal to two, what's the new T_2 . Alright so $A e^{-E_a / R T_2}$ divided by $A e^{-E_a / R T_1}$, we want that equal to two, the A's cancel out. So $\log k_2 / k_1$, which is equal to $\log 2$, is equal to E_a / R , $1 / T_1$ minus $1 / T_2$. We know what this is, we know what this is, we solve for T_2 and we find that T_2 is 305 degrees kelvin. It's a pretty small change to double the rate.

So it's pretty important that your body temperature doesn't change very much. If you have a fever and your temperature goes up, rates start going up. In your body, you could cause a lot of problems if the rates of some reactions go up by a factor of two. So, very sensitive to temperature. Rates are very sensitive to temperature. What are these things physically? E_a and the pre-exponent factor. Let's take a look at that.

So physically what's going on is, you have your two, let's say it's a bimolecular reaction, $A + B \rightarrow C$. So mechanistically what we think is happening is that the molecules come together and collide, right? You have A and B getting together and colliding. And the hypothesis is that when they collide they form a complex. They sort of bind together momentarily. And form this complex that has all the kinetic energy, or part of the kinetic energy of this collision as part of it. So A and B are bound together in some highly excited complex. Which then falls apart to give the product by rearranging its atoms around.

And if you plot the energy of this process as a function of the reaction, we call this the reaction coordinate, then we're going to start at some ΔG , or some energy. For the reactants. We're going to end up with some energy for the products. And it could be higher or lower. Let me make them higher here, just because I don't have enough room on the board. Products. So if this is an endothermic reaction, that's the energy for C, this is the energy for A plus B, and then along the way we have this complex that captures some of the energy of the collision. Up here somewhere. And this is the energy, then, at A B star, which we call the activated complex.

So for the reaction to happen, then, we have to go over the barrier and then back to the product. And this distance from the energy of the reactants to the top of the barrier, that's E_a . That's the energy of activation. How much extra energy you have to put in there to go over the barrier. So it's very clear that as you increase the temperature and you increase the amount of energy that the reactants thermally have, or in terms of their kinetic energy, as you raise the temperature you get more and more kinetic energy, you're going up higher and higher in the

Boltzmann distribution. And the number of reactants that can make it over the barrier clearly goes up exponentially. As the temperature goes up.

So this is E_a for the forward reaction. Clearly there's going to be an equivalent activation energy for the backward reaction. The two are related. The difference between the forward and backward reactions, so $E_{\text{backwards}} - E_a$ forward gives you the ΔG for the reaction itself. So, E_a backwards minus E_a forwards gives you ΔE for the reaction. Where E could be enthalpy or it could be free energy.

So that's the physical origin of this E_a , this activation energy. Now, A , the pre-exponential factor, that's the rate of attempt for the molecules to try to go over the barrier. So it has units that make sense for that. And this A is different than this molecule here. Rate of attempt. How many times per unit time, or how many times per second, do these two molecules try to collide? They never collide, they never try, they'll never make it over. So it's one over second, or one over mole second. So this is the rate of attempt. Then $e^{-E_a/RT}$ is the probability of success, of making it over the barrier. So the rate of attempt times the probability of success gives you the rate per unit time of making it over the barrier. Any questions on the dissection of this Arrhenius rate law?

Alright, so here's some examples, a few examples that you know of. Let's look at OH^- plus methyl bromide. Displacement reaction going to this OH^- attacks the carbon right here. To form an activated complex $\text{H}_3\text{C}-\text{OH}^\ddagger$, with the OH^- coming in like this. Let me go like this. OK, there's the intermediate right here. The activated complex in your reaction. Which then falls apart. It can fall apart two ways. The OH^- can be spit out again, forming back to the reactants, or the bromine can be spit out, forming the product. Which in this case here is methanol. $\text{H}_3\text{C}-\text{OH}$, plus Br^- . Typical example.

So, given this picture here, which you're probably already somewhat familiar with, we can then move on to talk about how to affect this barrier. How to change the rate. Oh, I should say a couple more things. One more thing, too. Now, the rate of, so clearly the E_a 's are related. Because the difference of the two E_a 's is the ΔE for the reaction. But the pre-exponential factors, these A 's, for the forward reaction and the backward reactions aren't necessarily related. You can imagine that in one case you have, first of all they don't even have to have the same units. You could have a bimolecular reaction one way. And a unimolecular reaction the other way. So you can't really say anything about the forward versus the backward rate this way. All you know is about the energies.

OK, catalysis. So now we have this barrier we have to overcome. And, so suppose that you have two motivators. Suppose you have an equilibrium case which is slow. And a reaction which is slow in both directions, k_1 , k_{-1} one. And you don't want to wait for this to happen. And this could be in a biological environment. Or it could be an industrial process, like the Haber process. You don't want to wait. And so one of the ways that you can speed it up, you know is by changing the temperature. You change the temperature, the rate goes up. You change it by

five degrees, the rate goes up by a factor of two. You can make a huge change by changing the temperature. But, so if you raise T , speeds up. But, K equilibrium also changes. And we saw that when we did the Haber process. We raised the temperature. The rate speeds up, but the equilibrium switches to the reactants. That's no good. So changing the temperature is not always the best thing to do if you want to change the rate. Instead, what you can do is use a catalyst. if you find one. A molecule that reacts with your reactant to form a product, that's B, spitting back that molecule C again without using it up.

And now with the activation energy, we can understand what the catalyst does. What the catalyst does is speeds up those rates, k_2 and k_{-1} , by changing the activation energy. By making the E smaller. So now I have a catalyst, and I can make this energy smaller. So this would be $A + C$ activated, getting ready to spit out B.

So in my example here where I have $A + B$ goes to products, so I would have A, some combination of A, B, and C together, to give out the products. In this case, the difference in energies doesn't change. All that you're changing is that the hump, in both ways. Equilibrium constant doesn't change. Just the rate changes through the Arrhenius rate law. And this is extremely powerful. Especially in biology. So let me give you some examples here.

This is sort of a typical example of increasing rates. Let's say you have the reaction, hydrogen peroxide, goes to water plus oxygen. If you take a little bit of hydrogen peroxide and you put it on your skin, it starts to bubble. But if you let it sit on the bottle nothing happens to it. Put it on your hair, your hair turns white. Blond. But again, if you just let it sit in the bottle very little happens. Well, it happens but very, very slowly over time.

So if you look at the rate of this reaction here, if the rate, moles per second, with no catalyst at all, the rate is 10^{-8} molar per second. Which is very slow. And the activation energy in kilojoules per mole, in this case here is 71 kilojoules per mole.

Now we can start adding catalysts. We can start adding inorganic catalysts like hydrogen bromide. Increase the rate at 10^{-4} . So this creates a complex with the hydrogen peroxide, which lowers the barrier to 50 kilojoules per mole, a small amount of lowering. But because the energy is in the exponent there, it makes a big change in the rate. Yes.

STUDENT: [INAUDIBLE]

PROFESSOR: The rate is independent of the catalyst concentration.

STUDENT: [INAUDIBLE]

PROFESSOR: The rate would have to change. You're right. That's a good question and I'm not prepared to answer it. I'm going to have to think about this.

Let's pretend now that we are at per unit concentration of the catalyst. And then, and I'll look into it. OK, so this is what happens with an inorganic catalyst. And instead if you use a generic biological catalyst, an enzyme catalase, it's a sort ubiquitous enzyme which is why you have it on your skin, et cetera, then this rate become 10 to the 7th. And the activation energy drops to eight kilojoules per mole.

So your question really has to do with the units of A. Of the pre-factor right there.

OK, and so there are all these examples of reactions. That are very important biologically. Where with an inorganic catalyst, an organic catalyst, you can change the rate by maybe a few orders of magnitude. But as soon as you put in an enzyme, the rate changes by ten orders of magnitude. Or in this case eight plus seven, fifteen orders of magnitude. Humongous change in the rate.

So you've probably done some enzyme catalysis before. But it's probably a good idea to quickly do it again. Because it's just so important. And it ties together our approximations that we've learned about. So enzyme catalysis, so enzymes can be either, could be heterogeneous catalysis, can be homogeneous catalysis. Enzymes are ubiquitous in the biological environment. They serve to regulate the cellular activity in very complicated ways. The cell will up-regulate or down-regulate the concentration of enzymes as it needs to make more or less products. And there's whole cascades of events that happen in this way. And they're in very small concentrations. But they play an extremely important role. Because they're also extremely specific. So you can have an enzyme that will only act on one part of the biological cycle. And affect it by 10 orders of magnitude or 15 orders of magnitude. But not affect any other protein that's around. And that's amazing. And it turns out that a lot of the diseases of old age like, what I'm about to face, or am facing already, you, not yet, but, are the result of some of these enzyme up-regulation, down-regulation, beginning to break down.

So we have all these reactions going on that need to be essentially perfect. When you have DNA replication or protein folding, or things like this. And errors are made. Errors are made all the time in these processes. And errors cause diseases. And so, even as a baby, your biological processes make errors. But you have these processes, these enzymes especially based on enzymes, that can go in there and sort of fix things. Fix things and make sure that you don't end up getting Alzheimer's at age six months. But as we get older, for some reason, these repair processes lose their bearings. Just like we lose our bearings. And and they can't repair things any more. They don't do it very well. And that causes all sorts of diseases. Cancer is probably one of the diseases, due to the lack of being able to repair problems. Alzheimer's. All sorts of dementias. MS. Just name a chronic disease and it's probably due to a problem with up-regulation or down-regulation of some proteins, some enzymes, that are due to a repair process.

So anyway, these enzymes are big proteins. 10 to the 4th, 10 to the 6th molecular weight proteins. On that order

or so. They tend to be fairly large in size. On the order of nanometers. Let's say ten nanometers to 100 nanometers. That could be, that's a little bit on the big side. Probably closer to ten nanometers. Ten nanometers is kind of big, for any sort of biological molecule. And they always end with their name ase. Like catalase, uricase, RNase. Esterase, clips ester bonds. Your liver is full of esterases. Because it likes to break things down into smaller and smaller pieces and lots of ester bonds and things that are not very biologically interesting. And that's one way of the liver breaking things down.

So the way it works is that you have your reactants, which in the biological language are called your substrate, come in, into an enzyme. Gets bound up in a pocket of, there's the substrate, reactant. There's the enzyme here. Forms a complex. And then product gets spit out. And the product floats off. And does its thing. It probably gets bound to another enzyme, which makes another product. Et cetera, and the cascade goes on. The signaling cascade goes on. And this enzyme is in very small concentration. The product goes away, so that's going to stay as a very small concentration as well.

So let's observe experimentally, then. Experimentally, what's seen is that the substrate makes products in the presence of the enzyme. With a rate dP/dt , that's, at t equals zero this is the initial rate is proportional to the concentration of the enzyme. This is initial rate. And in the language of enzymatic kinetics, this is called the velocity. dP/dt is also called the velocity. Velocity, moles per unit time. And this would be called, then, the initial velocity of v_{initial} . v_{initial} 's proportional to the enzyme concentration. And what else is seen? For fixed, if I fix my concentration of enzyme, I look at the velocity over time, let's say minus dS/dt , which is dP/dt , I find that that's proportional to S . For small S . Small concentration of substrate. And then at large concentration, it's a constant.

So it's not a straight line. In fact, I don't want to do it on this board here. I'll do it on this board here. Plot the concentration of substrate on this axis, and I plot the velocity, or the rate, of the reaction on that axis here. What I find is that it's a constant. So it's going to saturate, the rate is going to saturate to some number. And it's going to start linear with substrate at the beginning. So it's going to be a straight line at the beginning. And eventually it will saturate. That sort of slope. And the saturation point, that's the maximum velocity, or maximum rate that it can have. So we call this v_{max} . And this part here, the velocity is proportional to S .

And somehow we have to find, explain this. Using what we know from kinetics. So we have to come up with a mechanism, and then solve the mechanism. And make sure that it reproduces the data. So we're not the first ones to do this, obviously. Michaelis and Menton did this many years ago. For this mechanism. And the idea is, you have the enzyme plus a substrate react, k_1 , k_{-1} , to form a complex. Enzyme substrate complex. But unlike what we've drawn before in terms of this hump, where there's an activated complex which is not stable, in this case here this enzyme substrate combination lasts a long enough time that it's basically a stable complex.

So we're going to write this as a real intermediate that lasts long enough for you to be able to fish it out. And characterize it. And then eventually, that, k_2 , k minus two, goes to product plus enzyme. So the enzyme is a catalyst that forms a long-lived complex. And if you were to draw this, then, in our energy diagram, where we have the reaction coordinate here, you start out with your enzyme plus substrate. Go up, and you form your intermediate up here. ES. And I put a little dimple in there, because it's stable enough that it's not on the top of the hump, but it lives long enough. And it comes back down to form the product. Plus the enzyme. Without the enzyme in there, this hump would be way, way up there. Would be maybe a factor of ten higher. So this really lowers it a lot.

Now we can start to solve this mechanism. And I forgot one arrow. There which is the arrow going back. Which you usually don't see, but we might as well keep it there. For the sake of completeness. So what do we know? We know that this intermediate concentration is very small. The enzyme concentration itself is very small. Intermediate is very small. And it doesn't change very much. Not changing much. So that means that we need to use a steady state approximation.

So let's write down the rate for this intermediate, $d[ES]/dt$. It gets formed through the forward process. I'm going to put my brackets back in because E and ES would look the same otherwise. Gets destroyed. Through the backward ways. And I'm going to use a steady state approximation. So I'm already going to start adding steady state here every time I see an intermediate. Get it destroyed to make products. It gets recreated through the backwards reaction from the products. And I'm going to set that equal to zero for the steady state approximation.

Now, we don't really want to have this E floating around here. Because this is something that is very hard to measure. It's much easier to measure the initial concentration of the enzyme. What you put in there, instead of the amount that's not being bound up. So we're going to solve, instead, in terms of $[E]$ is equal to $[E]_0$ minus $[ES]$, where this is the initial concentration and this is the amount that's binding substrate. And this is the amount of free enzyme here then. And when you do that, and you plug in here, and you plug in here, you get your result. Which is that $[E]$ steady state, $[ES]$ steady state, is this ratio. k_1 times $[S]$ plus k minus two times the product divided by k minus one plus k_2 plus k_1 times $[S]$ plus k minus two times the product, times proportional to the initial concentration of enzyme.

And then you can take your steady state approximation for the intermediate and plug it back into your rate equation. So the velocity, we defined as the rate which is minus $d[S]/dt$, which is k_1 times $[E]$ times $[S]$, this is the destruction of the substrate minus k minus one, times $[ES]$ steady state, the backwards process. So we put in, instead of this $[E]$ we put in $[E]$ is equal to $[E]_0$ minus $[ES]$ steady state. And then we put in for $[ES]$ steady state, we put in what we found here. We turn the crank on the algebra. And we find that the velocity, then, is $k_1 k_2$ times the substrate concentration. Minus k minus one times k minus two times the product concentration. The whole

thing times the initial enzyme concentration. And then $k_{-1} + k_2$ on the bottom. Plus $k_1 [S]$ plus k_{-2} times the product.

So, as I mentioned, this product here usually just floats away. And so locally, where you're doing the, and then it gets used by the next step in the cycle. So this is pretty much zero. We can pretty much ignore this. We can ignore this guy here too. Because it's not, you don't have any steady state or an equilibrium. You have a steady state but not an equilibrium situation. And so in that case here, you can rewrite this then as $k_1 k_2$ times the substrate times $[E]_0$ divided by $k_{-1} + k_2 + k_1$ times the substrate.

And now we can look at these experimental observations and see whether they match our mechanism. If we can understand something. So let's look at the initial rate. Initial rate is supposed to be proportional to the substrate. Initial rate is supposed to be proportional to the substrate. So the initial rate, $k_1 k_2$ plus k_1 , if at early times, plus one. So the initial rate is going to be, somehow I've got the product missing here. No, I got it backwards here. This is not the initial rate. This is the rate where $[S]$ is small.

The initial rate is when you don't have any products made. Or when the product concentration is very low. And because we're making the assumption that the product concentration is basically equal to zero here, this is basically the initial rate here. So by taking the product equal to zero here, we're also saying that this is the same thing as the initial rate. So this is what it looks like here.. The initial rate here. And this is, this, you rewrite as $v_{initial}$ is equal to k_2 times $[S]$ times $[E]_0$. You divide by k_1 up and down. And you have this $k_{-1} + k_2$ over k_1 and then plus $[S]$ sitting down there. And you define this ratio of rates, $k_{-1} + k_2$ over k_1 as the K_M , the Michaelis constant, by definition.

And this is an interesting ratio. This is the rate, k_{-1} is the rate of destroying the enzyme substrate complex by going back to the reactants. k_2 is the destruction of the complex by going to the product. And k_1 's the creation of the complex. So this is the rate of destruction of the complex divided by the rate of creation of the complex. So if the rate of destruction of the complex is much faster than the rate of creation, meaning that this is a large number, then you're not going to pile up any complex. It's going to be destroyed as soon as you create it. So if K_M is large, then the concentration, $[ES]$, is going to be very small. Compared to $[E]_0$. But if the rate of destruction of the complex is small compared to the rate of creation, you create complexes, you create complexes but you don't keep up in terms of destroying them, in terms of making products, or going back to reactants. And so you end up saturating your enzyme. Every enzyme ends up having substrate bound to it. So when K_M is very small, then $[ES]$ goes to saturation. Basically, when K_M is very small, then you're limited by the rate, the second rate in the process, of the enzyme falling apart. To form the product. You have to wait until that happens. Because then the product just floats away. And that becomes your rate limiting step.

Another way that you also will see this written is as this, then, is equal to, we'll define k_{cat} is equal to k_2 . k_{cat} times the enzyme concentration times the substrate, $[E]_0$, divided by K_M plus but the substrate concentration.

So let's look at a few limiting cases then. First limiting case is, suppose, that $[S]$ is large. Let's take $[S]$ to be much larger than K_M . Because you look at the denominator, and you see it's this ratio of rates but there's this concentration that's important here. So in one case K_M is going to dominate, and in the other limiting case the substrate concentration is going to dominate. So let's say that the substrate concentration dominates. Meaning $K_{sub M}$ is small. In which case we already saw. If $K_{sub M}$ is small then you reach saturation. And in the equation, then, the velocity, K_M is small, $[S]$ is large, the $[S]$'s cancel out and the velocity is equal to k_{cat} times the initial substrate concentration. Both of these are constants. The velocity is constant. And the rate is constant, it's that limit up here. That's experimentally seen.

And the rate is depending on the initial substrate concentration. Initial enzyme concentration. All the enzymes have a substrate in there. The more enzymes you have to begin with, the more intermediates you're going to have, the faster you going to make products. And then it's going to depend on this on the rate, k_{cat} , which is just k_2 , which is the rate of formation of products. That becomes the rate limiting step here.

The other special case is if substrate concentration is very small compared to K_M . And in that case here, and the other thing that we're going to do is, we're going to, because we now understand this as this maximum rate up there, we're going to call this v_{max} . k_{cat} times $[E]_0$, we're going to call it v_{max} . And so when $[S]$ is very small, v , then, is equal to v_{max} , which is k_{cat} times $[E]_0$. This is v_{max} here now. So we can rewrite this as v_{max} times substrate divided by K_M plus the substrate concentration. Another way of writing it. Capital K .

v_{max} times the substrate concentration. And we have K_M plus $[S]$. But $[S]$ is very small. So we drop it. And this is then proportional to the substrate concentration. And then that's small substrate concentration compared to K_M , we're sitting right here. Where it's linear, where the rate is linear. And there's a third place on the graph which is interesting. Which is when the substrate concentration is equal to K_M . In that case there, you plug $[S]$ equal to K_M , and you end up with the velocity then is equal to v_{max} divided by two.

So when $[S]$ is equal to K_M , you are halfway up. There's v_{max} over two and there's K_M sitting here. When $[S]$ is equal to K_M , you're at v_{max} over two. And so enzymes, then, are labeled by their K_M 's. Because then it becomes very important to know how strongly they bind the substrate. Sometimes you want the enzyme to bind it very strongly. Sometimes you don't, you want it to be fleeting. Depends on the role that the enzyme plays.

Now there's a way to plot this that extracts out these important numbers. k_{cat} and K_M . And that's the Lineweaver-Burk plot.. Lineweaver-Burk plot.. And I just looked up this morning to see if Mr. Lineweaver was still alive. And as far as I can tell he's still alive. He was 97, in 2003. So as of 2007 he was still alive. He's getting up there. One of

the most cited papers that you have in your notes is the in Jack's, was the paper that showed how to go from this curved line to a straight line by plotting one over v versus $[S]$, instead of v versus $[S]$. So if you take your equation and massage it, $\frac{1}{v} = \frac{K_M}{v_{max}} \times \frac{1}{[S]} + \frac{1}{v_{max}}$, we haven't done anything except rewrite the equation in terms of one of v versus one over $[S]$. So it becomes linear. In one over $[S]$, and there's one over v sitting here. And you get a straight line. With an intercept here that's one over v_{max} . And if you keep going, extrapolate out, you get this point here to be minus 1 over K_M and the slope is K_M over v_{max} . And v_{max} was equal to k_{cat} times $[E]_0$, so you get k_{cat} out of this. So it turned out to be a very useful plot. It's very easy to plot a straight line, especially before computers. In the age of computers. And the referees, there were six referees that got this paper and pretty much turned it down because they didn't think there was any new chemistry in it. Which is true, there's no new chemistry. It's just a way of rewriting the plot. But it was very important nevertheless.

OK, any questions on catalysis? Enzymes? Arrhenius? Alright, next time we'll oscillating reactions and recap the course.