SPEAKER:

JOANNE STUBBE: OK, so welcome to class today. Today, I'm going to be talking about one of my favorite topics-- enzymes and catalysis. And what I would like to do is give you an outline of where we're going today. First, we're going to define what a catalyst is. And we're going to focus on enzymes as catalysts. Then what we're going to do is describe the theory of catalysis.

And we'll show how the theory can account for all experimental observations or most experimental observations. We'll then talk about the mechanisms of catalysis, and we'll see that there are three basic mechanisms. I won't write them out, but we'll come to them. And then if time allows, we'll talk about another property of enzymes. These are all focused with amazing rates of reaction.

And the second property of enzymes, besides the fact that they can accelerate reactions by a million to a billion fold, is their specificity. So that's where we're going. And what I'd like to do in the very beginning is show you why-- spend a little time to show you why enzymes are important.

Why do you care about enzymes? That's why you care about enzymes. Look at this mess. That's what's going on inside your body. There are thousands of reactions going on inside your body. Without enzymes, no reaction.

So you must care about enzymes. So what we're going to see over the course of the semester is that we can break down this mess into a few basic reactions. OK, so here is Waldo. And over the course of this semester, as you've seen many times, we walk through central metabolism and all of the reactions.

Now, a second thing about enzymes that I think will be what you guys do for a living if you decide to become biochemists and enzymologists is can we take our understanding of how these amazing catalysts work and design our own protein catalysts to do any reactions we want not involved in the 10 or 12 basic reactions we have going on inside our body?

And we can't do that now, but I would argue that understanding catalysis is a key requirement for getting to the point where we can actually do catalytic design. And the third thing that I think is really important is that 40% to 50% of all the drugs we presently use in treatment of antibacterial infections, anti-viral infections, anti-cancer infections are all inhibitors of enzyme-based reactions. And understanding catalysis helps us to design better inhibitors.

So understanding catalysis is central to many things that are important to all of us in society. So let me just tell you how I got excited about enzymes. So I went to graduate school. Never had a biochemistry course. They didn't do anything about biochemistry at the molecular level.

When I went to graduate school, I went to a lecture in the first year of graduate school that was given by a faculty member at Stanford. And he talked about an enzyme called lenosterol cyclase that converts a linear molecule. So here's the linear molecule, but I have it folded up into four rings. And these four rings provide the basis for all steroids like estrogen and testosterone and cholesterol.

And look at what this reaction does. One enzyme in a single step converts this linear molecule through a series of cascade transformations in hydride and methyl shifts into this molecule, putting in six asymmetric centers in a single step in 100% yield at 37 degrees in a pH 7. I said, my god, why do I want to be a chemist?

You sweat. There are no blocking [INAUDIBLE]. You to sweat to put in any kind of an asymmetric center, and here, this little protein has figured out how to do all of this under really mild conditions. And so this was a transformative experience for me. I remember the lecture clearly because I thought it was so amazing and I'd never seen that enzymes could catalyze reactions like this. So enzymes really are amazing.

So what you want to do now is start by defining what a catalyst is. And a catalyst, it can be for those of you who have had more chemistry, it can be an inorganic ion for example. It can be a small organic molecule. But for us, we're going to be focused on large macromolecules. And the macromolecules, we'll see that we're getting focused on, could be proteins or RNA.

But most of the reactions found in our body are catalyzed by protein catalysts. And these catalysts increase the rate of the reaction without themselves being changed during the reaction. And furthermore, while they can increase the rate of the reaction, they don't affect the overall equilibrium of the reaction. They just increase the rate of approach to equilibrium.

So they have no effect on equilibrium insolution, but increase the rate of approach to equilibrium. So what I want to do now is define some of the basic properties of the catalysts that we'll be talking about for the next 15 minutes or so.

So the first thing is that the catalysts we're going to be focused on are enzymes. Remember, we've spent the last few lectures talking about proteins. Enzymes are simply proteins, but then we see that they have special regions in the protein structure which allow them to accelerate rates of defined reactions.

I also will mention that we have inside of us a machine called the ribosome. And the ribozome is the machine that makes proteins, makes polypeptide bonds. We're not going to talk about that in 507, but we talk about it in 508. And the amazing observation was made really initially by the seminal experiments by Harry Knoller at UC Santa Cruz that you don't need any proteins to make peptide bonds.

That was heresy at the time. In 2001, Steitz won the Nobel Prize for the structure of the ribosome and Harry didn't get the Nobel Prize. Bad. He's the one that made the seminal discovery, although the structure of a ribosome, which is 2.3 megadaltons, is really sort of spectacular. I still get goosebumps when I think about that structure that was published in 2001. But Harry didn't get it.

Anyhow, so that was a digression. So that took a few minutes off my 50 minutes. Anyhow, we're going to be focused on enzymes as catalysts. So why are enzymes important. They're important because as I already told you, they accelerate the rates of reaction 10 to the 6-- a million-- to 10 to the 15-fold. Whoa. Can you imagine that?

Essigmann always used to say to me, give them an example. That's a lot. It's faster than a speeding bullet. Do you know where that came from? Faster than a speeding bullet? See, if this is when I have a disconnection with my audience. It's a bird, it's a plane, able to leap tall buildings in a single bound. Superman, course five. That's where our course five logo came from.

So let me just give you an example of this. And so this is taken from an article by Wolfenden, and this is the expanse of reactions that it rates that enzymes can catalyze that also can occur in solution. So if you take down at this end a half life of adding water to CO2 is five seconds. That's pretty fast. Why do you need water to hydrate CO2? Anybody got any ideas? Where have you seen that in the last few lectures? Hemoglobin.

Why do you need that? Because in your tissues, all of the fatty acids of the glucose gets breakdown to CO2. The CO2, where does it come out? You exhale it. Somehow it has to be carried around and into your lungs from your tissues. And there's a key enzyme called carbonic anhydrase that accelerates even this very fast reaction by a million fold.

Let's look at another one that might be familiar to you. Let's think about peptide bond hydrolysis. We just told you the ribosome makes peptides. What about peptide bond hydrolysis, which plays a major role in cell media death and blood coagulation and controlling the levels of proteins inside the cell? If you look at the half life of peptide bond hydrolysis, 450 years.

That means if we needed this reaction in our lifetime, it wouldn't ever happen. So if you actually look at the rate acceleration, proteases, which hydrolyze peptide bonds, have rate constants of about 50 per second. This rate constant is about 10 to the minus 9 per second. That's the rate acceleration of 10 to the 12. So without these kinds of enzymes and many other kinds of enzymes, we would not be alive and we would not be able to function.

So the description of rate accelerations is given by a term we're going to derive in the next lecture-- kcat over KM. A kcat is a turnover number. It tells you how good your catalyst is in terms of per second. KM has a concentration dependence. So this is a second order rate constant-- concentration inverse, time inverse.

And this is what we use to think about how efficient enzymes are, as we'll see in the next lecture. So what I want to show you here is another graph that was made by Wolfenden, who we were talking about data in the previous slide. And what I want to do is show you his comparison of enzyme catalyzed reactions and non-enzyme catalyzed reactions.

And we just heard with peptide bond hydrolysis, 450-fold rate acceleration. That's a lot. What do you notice immediately about enzyme catalyzed reactions? The kcat over KM is on the order of 10 to the 6 to 10 to the 8 per molar per second. Does that ring a bell with anybody? Where have you seen a number like 10 to the 6 to 10 to the 8 per molar per second?

What that is is a diffusion constant of any two molecules finding each other in solution. So what is that telling us? That's telling us that inside the cell, enzymes have evolved to be so efficient that the rate-limiting steps are going to be finding each other in solution. It's physical. It has nothing to do with the chemistry.

So you've had billions of years to figure out all this chemistry, and what limits everything-- and we'll come back to this in a minute-- is the enzyme and the small molecule finding themselves. And so that's where this number-- of 10 to the 6 or 10 to the 8 per molar per second comes from.

If you look at the non-enzymatic reactions, we just talked about hydration of CO2 versus enzyme catalyzed reaction, what you see is that they're all over the place. So the staggering rate accelerations of 10 to the 6 to 10 to the 15 that you see are really based on the rates of the non-enzymatic reactions. And the enzymes have evolved-- most of them have evolved over billions of years to be incredibly effective at what they do.

So the other thing that I wanted to say about enzymes at this stage is that enzymes are usually in addition to being great catalysts, they're also-- you learn, I think, if you've seen enzymes before that they are very specific for the substrates, which I'll call S and we'll come back to this in a minute. So they only react-- you have hundreds of metabolites inside of our body. That only will pick up and react with one of those metabolites.

But in reality, I think what we found over the last 15 years or so is enzymes are not all that specific. They are specific for what they encounter inside us. So if you take them out as a biochemist and start messing around with them, they aren't anywhere near as specific. They don't have to be that specific because they never encounter these molecules inside the cell.

So they are very specific for substrates in vivo. And in fact, many of them are promiscuous in vitro. And I think that's something that's been under-appreciated. So this is number three. I wanted to talk about specificity.

Number four, enzymes in general, if you look at that metabolic chart, almost all those reactions can be subdivided into 10 to 12 reactions. And those 10 to 12 reactions, even though it looks like a jungle and a mess, are found in the lexicon that you have been given in the first lecture. So that lexicon provides a framework to think about all of primary metabolism.

Now, in reality, there are many other kinds of reactions. But the ones that you're going to see in 507 can be limited to 10 to 12 reactions. So enzymes have a limited repertoire of reactions in primary metabolism. And so in this case, let me give a plug for the chemists. Chemists have the whole periodic table. Do we have a periodic table here? No. We're in the wrong department. We're in the wrong building.

Anyhow, we have hundreds-- not hundreds-- we have 50 elements where we can use to catalyze reactions. We can do all kinds of reactions catalytically, and we can do it with something small, like a proton, or something small, like a metal with a little organic spinach hanging off of it.

But what are we doing with enzymes? We have these big huge molecules. So there's a playoff. Enzymes have a very limited repertoire of reactions they catalyze, while chemists actually are limited by their imagination to catalyze these reactions. However, as the world becomes more and more green, chemists are no longer allowed to use metals. For example, they can be toxic to people.

And so people are rethinking and refocusing on developing green catalysts. So the question that you can ask yourself, is there any way that enzymes can enhance their repertoire of reactions that they can catalyze? And they can. They do that by using the vitamins on the vitamin bottle. So enzymes have a limited repertoire, but they increase this repertoire using vitamins.

This is what we eat out of our vitamin bottle that are converted into co-factors. So the vitamins we eat have to be subtly modified and then get incorporated into the protein catalysts and greatly expand the repertoire. So many of you probably-- how many of you take vitamins? Everybody should be taking vitamins. Why don't you take vitamins? Anyhow, so you can see vitamin B6, vitamin B2, vitamin B1.

And over the next three weeks or so, we talk about the chemistry of how these vitamins interact with the protein catalyst to increase the repertoire of reactions to 10 that actual enzymes can catalyze.

But in addition to the vitamins, I want to make mention of another type of catalyst. So most of the vitamins are organic molecules. One also needs to think about inorganic molecules. Inorganic molecules-- copper, zinc, iron, all those if you look at your vitamin bottle are at the bottom and they're labeled inorganic. And they almost always in introductory biochemistry courses get swept under the table.

And in fact, many biologists don't think about metals at all. But 30% to 35% of all the enzymes have metals incorporated. And these metals are essential for the repertoire of reactions that enzymes can catalyze. So without going into any details, I just want to whet your appetite.

Look at this guy. Well, what are we looking at here? These yellow things are sulfurs. The purple thing is molybdenum, and the green things are iron.

And in the middle of all these irons is this silver thing, which is a carbon bonded to four irons. Most of you probably aren't sophisticated enough yet to think that's amazing, but it was only two years ago that the x-ray crystallography where we can look at things at atomic resolution was good enough so we can see that guy.

So what does this guy do? What's its function? Pretty damn important. It converts nitrogen into ammonia. So it turns out to be an eight electron reduction because not only do you produce ammonia-- two molecules of ammonia-- but you also have to produce a molecule of hydrogen during that reaction.

So this is the basic way we control nitrogen-- one of the basic ways we control nitrogen in the environment. So chemists would love to understand how this spectacular inorganic molecule can mediate what turns out to be a six electron reduction. Another molecule-- co-factor molecule that's all metal-based that I think is equally amazing is this one.

We recently got an atomic resolution structure down to 1.5 angstroms. It has four manganese and a calcium. Anybody have any idea what this does? This is the co-factor that takes water in the presence of light-- sunlight-- and converts it to oxygen gas. Why is that important? Because we need oxygen gas to breathe.

So anyhow, on this one co-factor mediates that transformation. Pretty amazing. And that's a major focus of people who want to think about how these catalysts actually work, but we won't be discussing that further. We won't be discussing that further in 507.

So I just wanted to point out here that, again, enzymes have a limited repertoire. Their repertoire is much less than what chemists can do, but they're amazingly efficient at what they do. So I would argue if we really could understand the basis of catalysis and how these things evolve to be able to do these amazing transformations, we might, if I was able to come back 50 years from now, see that we had designer proteins all over the place that could catalyze the specific reactions that we're interested in, not the ones that are found in our bodies.

OK, so the next thing I want to briefly mention is that enzymes, so if you look at an enzyme, it's a big macro molecule. We've looked at these in the last few lectures. The region where the chemistry or catalysis occurs is called the active site. And we've seen this before in the TIN

barrel superfamily of proteins. And so there's a region of about 10 angstroms.

We have your amino acid side chains that I asked you to try to remember and think about. We'll see those are key to making these rate accelerations so fantastic. This is where the chemistry happens.

But I think it's now clear from studies that have been done in the last 15 years or so this is not true. One can make changes out here or here. One can change the amino acids and totally turn off the enzyme or turn on the enzyme. So chemists use these small little molecules, biology uses big huge molecules. Everybody initially focused on this one little region where you can see the chemical transformation occurring. But what about the rest of the molecule? The rest of the molecule is also important.

You cannot remove, in general, all of this spinach and come up with a catalyst that has these amazing rate accelerations. So the active site is very important. But so are specific amino acids outside of the active site. And people have studied this because of technology of sight directed mutagenesis, which many of you have probably done in either 702 or in 335.

So what implications does that have? And I just want to mention one more thing. I don't want to spend a lot of time on this, but our thinking about catalysis is changing dramatically and has changed and continues to change. I continue to study this, even to teach 507. Because it turns out, how does change out here govern what's going on in this region where you think the chemistry happens?

And it governs that chemistry because of conformational changes and movements. So another thing about enzymes that we need to do more thinking about-- and this is a major focus of what people are thinking about now-- is dynamics in enzyme catalyzed reactions.

And so if you look at the time scale-- and I made you think about size scale in the first few lectures. Like how long is a hydrogen bond? How long is a carbon nitrogen bond? A carbon oxygen bond? You also need to think about time scales.

And this is particularly true in the case of catalysis. What happens on the fantasecond time scale? That's pretty fast. That's a vibration of the bond. But what are you doing during an enzyme catalyzed reaction? You're breaking the bond and you're making the bond. So we'll see that the transition state of the reaction happens on the fantasecond time scale.

Yet, if you look at the criteria kcat, which is a turnover number, the enzyme, which is given in time inverse, they're usually on 10 per second to 1,000 per second. So they're on the millisecond to second time scale. So catalysis is happening way up here.

Now, I've just told you that mutations outside the active site can affect catalysis, and so one also needs to think about the time scales in between these two extremes. I've also told you that finding an enzyme, finding its substrate in solution, can often be the slow step. So here you have nanosecond, microsecond time scales, and I'm not going to spend any time on this, but you come back and look at this and think about you've got all these side chains of your amino acids.

You might have loops that are moving in and out and covering the active site. All of this dynamic interaction plays a key role in catalysis, making the enzyme as a whole important in the overall catalytic process. So that's my introduction to you for what an enzyme catalyst is.

And so now what I want to do is look at the second bullet we were going to talk about, which I've already lost. How do we describe catalysis? How do we try to conceptualize in a theoretical framework all of the experimental observations that have been made for decades?

And there are many things that are wrong with this theory, but this theory has stood the test of time, not only for biochemists, but for also chemists. And I think it helps us to think about how enzymes are able with just the amino acid side chains for protein to give us these amazing rate accelerations and specificity that we actually observe.

So what we want is a theory to conceptualize catalysis. And this is transition state theory. And this is-- many of you have seen this in some form before, either in freshman chemistry or maybe if you've had 560. People go through and derive all of the rate equations. What I'm going to do is just show you a picture of how this theory helps us think about these catalytic transformations and then how this picture helps us think more specifically about these amazing rate accelerations that we actually observe.

OK, so I can't remember what's on the next slide, but this is a picture you often see when you're thinking about catalysis. So this is chemical catalysis, but again, chemical catalysis, biological catalysis, really the same basic principles hold that we have some substrates A and B going to products and what's required.

So I think all of this is intuitive, but if you have two things coming together, they have to come

together in exactly the right way to be able to make a bond. They have to remove all the solvent from outside them. They have to come together with enough force to be able to get over the barrier, whatever it is, to break one bond and to form a new bond. So that's true of all reactions and everybody faces the same issues in terms of conversion of substrate into products.

And the highest point along the reaction coordinate-- so this is what we call a reaction coordinate diagram. And this is energy. So the highest point along the reaction coordinate diagram is called the transition state-- TS. This is transition state theory. OK, TS theory. And this is where-- this is the point where we can ever isolate it because this is a point where all the chemistry is happening. The bonds are being made and broken.

And the lifetime I just showed you on the previous slide is fast-- fentaseconds. So you can never isolate a transition state. Everything needs to be aligned. That doesn't come free of charge. You have to do a lot of work to get to the stage where you can get this chemistry to happen. That's what our catalysts are doing. And then bang, the reaction is over at that time.

So this is another way of describing the transition state of the reaction. And in reality, this is the cartoon you see in most introductory textbooks that are describing rates of reaction. But the reaction coordinate is much, much more complicated. And that's true in enzymatic reactions as well. So it's true of chemical reactions, it's true enzymatic reactions.

So you might have a plus b, and they might form two or three intermediates along the reaction pathway where you have many transitions-- you have many transition states along the reaction pathway. And each of these transitions states would be non-isolable. But what about these little valleys? These little valleys are where you might have a chance to see an intermediate during the conversion of a plus b into p plus q.

So an intermediate-- and if you're interested in studying catalysis and the chemistry of the reaction and you need to define what these intermediates are, they can be high or that could be lower in energy. They may be easy to isolate, not easy to isolate. But they have all covalent bonds intact.

So in contrast to the transition state where the bonds are being made and broken, you can never isolate this. You have a chance to be able-- if you're clever and creative, which people that study mechanisms are, you can actually look at the intermediates along the reaction coordinate.

So that's a reaction coordinate diagram. We're going to come back to these because I think they really help us to conceptualize how enzymes can go about achieving these fantastic rate accelerations.

So from transition state theory, one assumes the following-- I'm not going to go through the details of this at all. But the key point that one needs to think about in transition state theory is that-- and this was first put forth by Linus Pauling. Who's Linus Pauling? He's my hero. OK, Linus Pauling, he's the vitamin C guy. He lost it when he got old, but in the early days, he's the one that could take a polypeptide chain-- just a string of amino acids-- and he sat there and he played with it.

And lo and behold, he says, we're going to have alpha helices in proteins. How amazing is that? You've heard me talk about him before. He was the one that I think conceptualized-- first conceptualized-- how an enzyme might catalyze a reaction.

What do you want to do to catalyze a reaction? You want to lower this barrier. So how do you lower the barrier? You don't want the enzyme to bind the substrates tightly, and I'll come back to this in a minute. You want to bind the transition state tightly. So he put forth in the 1940s that the way enzymes might be able to catalyze their reactions is by tightly binding-- uniquely and tightly binding the transition state of the reaction.

And I think that turns out to be a really good way to conceptualize most enzymatic reactions. Now, transition state theory tells us, which again is not so appealing to me but it works to describe most experimental data, that the ground state-- so this would be the ground state-- is in equilibrium with the transition state. So you might ask yourself, how the heck can you ever be in equilibrium with something with such a short half life?

That's a good question to ask. But in fact, this framework-- transition state theory-- allows us to able to explain almost all the experimental observations that we make as both chemists and biochemists. So this goes through and derives that equation, which I'm not going to do today. In the old days, I used to spend a lot of time deriving equations. Nowadays, I don't derive equations anymore.

But the key equation that you need to think about is shown here. And the consequences of this equation are quite simple. It tells you that the rate constant for the reaction-- so from transition state theory, the rate constant for the reaction. And where is this rate constant? Where does

this rate constant come from?

A is going to some product p. You can measure it experimentally. So k observed is an experimentally measurable parameter is equal to a bunch of constants called the transmission coefficient. This should be a cappa. Boltzmann's constant, temperature in degrees Kelvin, Planck's constant times e to the minus delta g dagger over rg.

So this is the equation. This is a constant. This is Planck's constant, Boltzmann's constant. This you can measure experimentally. Cappa is telling you basically-- the transmission coefficient is telling you the frequency that this transition state breaks down to form products versus going back to starting materials and in general, is on the order of one in most reactions.

And so the key thing to remember from this equation, which explains the data and helps us to think about catalysis, is that as you increase the rate of the reaction, it's inversely related to the activation barrier. So what you want to do, this equation tells you, is you lower this barrier. The rate of your reaction becomes faster and faster.

So the whole goal is, then, to figure out how to lower the barrier. If you can lower the barrier, this theory predicts that the rate of your reaction will be faster. So that's what we want to be able to do. The rate constant is inversely related to the activation barrier.

And so now let's look at an enzyme system specifically. So I'm going to draw the same kind of reaction coordinate that we've drawn over there for a chemical reaction. And I'm going to use a simple equation. E is the enzyme, s is the substrate forms an enzyme substrate complex. The substrate binds in the region that we call the active site over here.

Somehow, the enzyme is able to convert itself into product. Now, most reactions are much more complicated than this. You have many substrates. You have many products. But it doesn't affect anything in terms of thinking about the problem. And then in the end, the product dissociates. So that's a simple reaction. You get something in there, a catalyst works on it, it gets converted to the desired product, and the product is released.

So what I've told you now a couple of times is that enzymes have evolved to such an extent that often the physical steps and not the chemistry is rate limiting. So what are the physical steps? Here are the physical steps. Enzyme finding substrate and solution, that's a physical step. What is limited by? It's limited by diffusion control. How fast can they find each other in

solution? That's the number 10 to the 8 per molar per second that limits most enzyme-based reactions that I showed you several slides ago.

What about this? We have product dissociation. What about product dissociation? That's a physical step too. You made the product sitting around, but in order for the enzyme to turn over, again, to free up the active site, the product has to come off so it can bind another substrate. And here is the chemistry. Ah, that's what I care about.

But what happens, now, is that if these steps are rate limiting, then you can't see the chemistry. So it's really challenging, often really challenging, to study the chemistry of a reaction because the rate limiting steps have nothing to do with the chemistry. So let me just draw a diagram. So you can draw a reaction coordinate diagram.

And so what you have is some enzyme plus substrate and it can form an enzyme substrate complex. You have a transition state of your reaction. The enzyme product complex can then dissociate to form enzyme plus product. So what you need to think about if you're thinking about how to accelerate the reaction is what is the bottleneck in the overall reaction?

You don't want to start mucking around with something that doesn't control the rate of the reaction. So you need to know what the rate limiting step is in the reaction. And the rate limiting step is the highest barrier along the reaction coordinate.

OK, now I've already told you that this is a simple case. We have one substrate getting converted into product. Most enzymatic reactions are going to have many barriers. And so in order to affect the overall rate of the reaction, you need to figure out what's rate limiting, and somehow the enzyme has figured out how to lower the barrier to make this reaction easier to occur.

Remember, I just told you that the rate constant is inversely related to this activation barrier. So if we can lower this barrier somehow, what we're going to see, if we can lower this barrier, now we have a lower overall rate of the reaction. So this theory allows us to think about what we need to do to make these catalysts actually work with rate constants of 10 to 6 to 10 to 15 times faster than non-catalyzed reactions.

And I want to say one other thing before you move on. As with everything, I think it's good that we're in a field that's rapidly changing. Remember, I told you have to think about dynamics. We no longer think about a single reaction barrier. That's in most of the textbooks now. Really

what we think about is we bring dynamics into this. I told you things outside the active site can modulate what's going on inside the active site.

What we think about is a reaction landscape. And so one has many barriers that one has to get over. Almost all reactions involve multiple barriers. So you've got to figure out which one is rate limiting and lower that activation barrier. And enzymes, if you think about this, they're huge. Do they all fold exactly the same way? No. So we always think we have a homogeneous enzyme. No.

If any of you work in UROPs, you'll find that out pretty fast. You use recombinant technology to fold things inside the cell. They don't all fold right. So you have all mixtures of things. And so you get a reaction landscape.

And so this axis is bringing in the dynamics that I told you about earlier on that you need to think about-- the conformational changes that occur every step along the reaction pathway. The enzyme is moving at all kinds of steps, reorienting everything to get the chemistry exactly right.

So what I want to do now is-- so that gives you a way to conceptualize rate accelerations. Now what I want to do is tell you what the major mechanisms are that the enzyme uses to enhance the rates of these reactions. How do we lower these energy barriers? So let me see. I need to start erasing somewhere.

OK, so we're on the third bullet over here. Mechanisms of catalysis. And what we're going to be talking about is multiple mechanisms of catalysis. We're going to be talking about binding energy, which is the one people have most trouble thinking about. We're going to be talking about general acid, general base catalysis. And we're going to be talking about covalent catalysis.

And we will see that over the course of the rest of the semester, when we start talking about metabolic pathways, all of these mechanisms are used in almost all enzyme catalyzed reactions to give us these tremendous rate accelerations. What I want to do-- that's the first time I did that. That wasn't too bad.

OK, so what are the mechanisms of catalysis? How do we get 10 to the 6 to 10 to the 15 accelerations? And so the first thing, and I think the one that really is unique to enzyme catalysts compared to small inorganic or organic molecules, is the use of binding energy in

catalysis. So this is the one-- and this is also the one that's thought to contribute the greatest amount to these factors of up to 10 to 15.

So binding energy in catalysis, and what does that mean? What do we need to think about? So the enzyme binds to a substrate. If we take this simple case, we need it to bind. We need it to bind specifically. So that's a key part of the enzyme that we haven't gotten to yet-- specificity.

But what if it bound its substrate really tightly? Do you think that would be good? No. So it's not good because what does it do? If it took all of the spinach changing off of your substrate and made hydrogen bonds and Van Der Waals interactions, all the weak non-covalent interactions we spent a half a lecture talking about four or five lectures ago, what would happen is you would have type binding. You would have lower energy.

But what does that then do to the activation barrier? It increases the activation barrier. So the binding energy is the free energy released when enzyme combines with substrate. But the key is that this bind energy is not used to bind completely. It's used for catalysis. So this energy is used both to bind substrate and-- and this is the key thing-- for catalysis.

So what do we want to be able to do and how does it do this? So if we look at this, if we look at our reaction coordinate diagram over here, we don't want to bind substrate tightly because this is the biggest barrier-- the rate limiting step along the reaction pathway. What we want to do is lower this barrier. So how can we lower the barrier?

We can lower the barrier by stabilizing the transition state. That now makes this barrier-- probably can't read anything now, but that makes this barrier lower. How's another way you could lower the barrier? You could lower the barrier by straining the substrate to look more like the transition state. So you could strain the substrate in this form, and now, again, you would have a lower barrier compared to that barrier.

So you're going to use this binding energy to stabilize a transition state. So we want to use binding energy to stabilize the transition state to de-stabilize-- any of these or all of these could be true-- de-stabilize the ground state-- G-S. Or what else do you need to do to get a reaction to work if you have one or two substrates or even one substrate? Your molecules in solution are all solvated.

What you need to be able to do is get rid of the solvent. If you have two substrates, you have

to bring them together. You have to bring them together at the right orientation. That doesn't come free of charge. You have to get the energy from somewhere, and the energy is proposed to come from this binding energy. So the binding energy is not used to completely bind the substrate, but to do all of these things to get your substrates ready to form product. So you can dissolvate and bring reactants together. And you can freeze out rotational, translational entropy.

So you're getting everything ready for the reaction to happen. So in this case, then, let me just erase this and make this so that this is clearer. What you could have, now, is you can-- so in the beginning, this is the barrier. If you stabilize the transition state, this becomes a barrier. If you de-stabilize the ground state, then this becomes the barrier.

So what we're trying to do is lower this barrier to get the reaction to work. And so the major way that we do this is by using the interactions between the enzymes of the weak non-covalent interactions between the enzyme and substrate to help us do these things to enhance catalysis.

So that's one of the major mechanisms of catalysis. A second-- and this type of catalysis is unique to proteins. So the two types of catalysis are used widely in organic or inorganic chemistry when you're designing your catalyst. I mean, when you're designing a catalyst substrate binding, a small molecule, a big product release is still an issue. If you go and read the organometallic literature, people have trouble with product release all the time.

So the issues in catalysis are exactly the same in biochemistry as they are in organic and inorganic. But now we have to deal with this big protein, which has these unique properties, one of which is that the whole protein is playing a key role in catalysis and allowing everything to align within tenths of angstroms to make these reactions work really efficiently, which chemists can't do yet. And I don't think we'll ever be able to design it, but we can evolve catalysts to become better and better so that they can do the same thing.

That's the beauty of proteins is you can evolve them to become better and better catalysts. So the second mechanism-- so the first mechanism is binding energy. The second mechanism-- I can't remember whether they're using I's or 2's. The second mechanism is general acid, general base catalysis.

Now, as a chemist, what do you learn about catalyzing reactions? Well, one way you could do it is with a big fat proton. Protons are pretty good at helping you catalyze reactions if you go

back and think about chemical transformations or hydroxide ions. What are the concentration of protons and hydroxide ions in aqueous solution of pH 7? 10 to the minus 7 molar.

So you don't have much protons and hydroxide ions in the active site. So even though these are very good catalysts that organic chemists and inorganic chemists use all the time, they're using them in organic solvents, you can argue the active site of the enzyme is more like an organic solvent. But anyhow, this type of catalysis is called specific. So when you see specific acid or based catalysis-- where does the general acid and base catalysis come from? It comes from the side chain of your amino acids.

So remember, the second or third lecture I said, oh, here are all the amino acids. Here are all the side chains. You really shouldn't know all of your amino acids. It's a basic vocabulary of all of biochemistry, and the pKas of all the side chains. Why? That's why. Because you can't understand anything about catalysis without knowing what these side chains of the enzymes are actually doing.

So the general acid and base catalysis come from the side chains of your amino acids. So what side chains do you have? You can have carboxylates. Anybody know what the pKa of a carboxylate is? Hey, Boggin, what is it?

STUDENT: Four to five.

JOANNE STUBBE: Good, four to five. See, he remembers. You could have imidazole. This has a pKa at neutral pH. Anyhow, you need to go back and look at what the groups are that can be involved in catalysis. And chemists, for decades, have studied how you can use general acid and base catalysis to give you rate enhancements.

Now, what I haven't told you is the amount of rate enhancement And so people over the years have measured that with binding energy, you can get factors of 10 to the 8. If you look at general acid base catalysis from all the organic and inorganic reactions people have studied for decades, you get factors of 100 to 1,000 fold.

Now, we need to get to a factor of 10 to the 15 in some cases. We've already gotten to the factor of 10 to 6. So obviously, you're going to have to use multiple combinations of these mechanisms to give you these tremendous rate accelerations. So you will see over the course of the rest of the semester many active sites of enzymes with many amino acid side chains that are playing roles in general acid and base catalysis.

And the last type of catalysis is covalent catalysis. And again, covalent catalysis means that you form-- and where have you already seen covalent catalysis? You've already seen this when we talked about, how do you study the structure of the primary structure of a protein? We use proteases with tripsin or kimotripsan that can break down the big protein into small pieces.

We went through the mechanism of that reaction. In the active site of that enzyme, there is a serine that forms a covalent bond. So over the course of the semester, you're going to see many examples. And I'll just put in parentheses for those of you who don't remember, go back and look at serine proteases. This is a classic example that's in every textbook.

And how do we know how much rate acceleration you get from covalent catalysis versus not having covalent catalysis. We know this, again, because of organic chemists studying the detailed chemical mechanisms of these reactions, and we find out that in this case, we get rate accelerations of 100 to 1,000 fold.

So what you see is the enzymes. And these are the three general mechanisms by which all enzymes catalyze their reactions in some variation. Now, attributing out of this 10 to 15, 10 to the 8 is associated with this, and 10 squared is associated with that is extremely challenging. And there are a lot of people still trying to dissect reaction mechanisms in detail.

And I would argue that understanding how these different methods work and synergize to give you these accelerations is a key to eventually designing new catalysts that can do what you want them to do that's distinct from biological transformations. And I think I'm probably over. I just want to say one more thing. I just want to give you a feeling for what you have to do.

If you're thinking about this reaction coordinate, what you need to do is think about how would you stabilize the transition state relative to the ground state? So what we're talking about is stabilization that's unique to the transition state and not the ground state. If you stabilized them both, what would happen? If you stabilized them both-- if you stabilize this guy and you stabilize this guy, the barrier would be exactly the same.

So what you need is some way to uniquely stabilize the transition state over the ground state. So the question is, how much do you think? How much rate acceleration do you think you can get from a hydrogen bond? Does anybody have any idea? One hydrogen bond.

So here, you have a protein with 1,500 hydrogen bonds. But if you can get one hydrogen bond

that's here in the transition state of the reaction, that's not over here, how much rate acceleration do you think you can get? Anybody got any idea?

You can get almost 1,000 fold. I mean, and you can do a very simple calculation. I can't remember whether I have this on the-- OK, so that's it. So we can do a very simple calculation, and I'll use this to show you the calculation. Here, we have our rate. This should be Delta G. The dagger should be up in the air. So this is the enzymatic reaction. This is the [INAUDIBLE] equation.

One has the same equation for non-enzymatic reactions. So here's a non-enzymatic reaction. In general, the non-enzymatic reaction can happen by some mechanism. To the enzymatic reaction is just much, much slower.

So if we assume, for example, that the rate difference between enzymatic and a non-enzymatic reaction is a factor of 10, how much do you get assuming that all of these terms are the same in the enzymatic and the non-enzymatic reaction? You can calculate a Delta Delta G dagger of 1.38 kilocalories per mole.

For those of you who are modern, this is 5.8 kilojoules per mole. Sorry, I'm really old, so I still think in kilocalories per mole. But a hydrogen bond, one hydrogen bond is worth 2 to 7-- compared to no hydrogen bond, is worth 2 to 7 kilocalories per mole. So a factor of 10 is 1.4 kilocalories per mole. So that shows you, then, that if you had 2 to 7 with one hydrogen bond, it can give you these factors of 1,000.

So I think that's an observation that's something you need to keep in the back of your mind. Because you think about it over and over again. It really doesn't take much to align everything in exactly the right way. And when I say hydrogen bond, these hydrogen bond strengths are really dependent on how everything is aligned. If they're exactly aligned, then you get much stronger bonds.

They can even approach-- in the gas phase, they could approach 30 kilocalories per mole. So having everything aligned, that's the job of this whole big protein, to actually give you catalysis.

And I think I'm at the end of my lecture now. I won't have time to talk about-- I went over already about the question of specificity. But let me just say, I think enzymes are really quite amazing. There's nothing like them. Faster than a speeding bullet. They can catalyze the rates a million to 10 to the 12, 10 to the 15-fold. And they use really the simple concepts that

chemists have developed over the years.

But the key to the enzyme is this big huge molecule, and the dynamics within this molecule that gets everything to align exactly right to be able to lower these barriers so that you can convert your substrate into your product. OK guys, see you next time. The end.