HAZEL SIVE: Let's move on with Biochemistry III. And we have, as you recall, being exploring the fascinating and complicated set of macromolecules inside a cell-- inside the cell that we're using, in a way, analogous to a factory that is a production line for synthesis of various components that are required for function of the cell, for its replication, and for perpetuation of the organism. Today I want to talk to you about some parameters that surround the chemical reactions of biochemistry.

We'll talk briefly about thermodynamics and energy considerations. And then I want to introduce you to a huge and pivotal class of proteins, the enzymes. And then we'll talk very briefly about the currency of energy in the cell.

Let's start with thermodynamics, which we will define as the rules underlying energy usage in chemical reactions. Rules underlying energy use. And really, it's the first law of thermodynamics that we're interested in. We're interested in the fact that energy is neither created nor destroyed, that it is converted from one form to another and that there is conservation of energy. And the other laws are embedded in what we'll talk, but these are the ones that are really important for biochemistry.

Let's think about a basic chemical reaction, some kind of substrate. That's a biological term which we'll use. It's equivalent to the term "reactant." And I could use R, but I'm going to use S, because that's what we'll use in biochemistry, in life. And that gives rise to a product.

And chemical reactions can go in both directions. One reaction may predominate over the other. But the reaction is reversible.

And the questions that we're going to ask is, does a chemical reaction require or release energy? How fast does the reaction go-- which is referred to as the rate of the reaction. And how far, which refers to the equilibrium point. Equilibrium, where equilibrium is defined as the place where the forward rate and the back rate are equal.

And at equilibrium, even though the forward and the back rate are equal, it's not

that you have equal amounts of substrate and product, you have a particular ratio of substrate to product that is characteristic for the chemical reaction. So you get a specific substrate or reactant to product ratio.

Let's make this S a little larger, to match the P. There we go. Good.

The thing that you need to know is called Gibbs free energy and the change in Gibbs free energy with a reaction. So in any chemical reaction, there are a couple of kinds of energy considerations. The one important for us is G, the Gibbs free energy, which is the usable or, the convention is, free energy.

The other term you need is H, which is the total energy, or enthalpy. Entropy, S, the unusable energy. And you also need T, which is the temperature in absolute degrees Kelvin.

And the reaction that you may be familiar with or the equation you may be familiar with is delta G equals delta H minus T delta S. And the thing that's important here is what this means. Delta G is the change in free energy with a chemical reaction. And every chemical reaction has a characteristic change in free energy. And depending on what that is, one can say certain things about how likely the reaction is to proceed.

If delta G is negative for a particular chemical reaction, then as the reaction proceeds, energy is released, the reaction is termed exergonic, and it is sometimes "spontaneous." I'm going to put that in quotes. You'll see why in a moment.

The flip is true if delta G is positive. This means that the reaction requires energy in order to proceed, and it is referred to as endergonic. And if delta G is 0, the reaction is at equilibrium.

The notion that's quite useful to think about this is balls rolling up and down hills. For a reaction that is exergonic, a ball rolling down a hill will decrease its delta G. It will release energy as it rolls down the hill. And you can actually plot this.

And you'll see lots of plots like this where, if you plot energy over the course of the

reaction, you will see that the reactants start off at a higher energy than the products. And as they are rolling down the hill, there is energy released. And the flip is true for an endergonic reaction where delta G is positive. You have to put in energy to get the product out.

Now-- and this is where it gets interesting. And this is where we get back to "spontaneous" in quotes-- chemical reactions can be exergonic, but they may not proceed spontaneously. If you imagine a ball sitting at the top of a hill, if the ball is sitting right on the slope of a hill, and you let the ball go, it'll go zipping down the hill, right?

But if the ball is sitting on the top of the hill, but it's in a little hollow, it's not going to go anywhere unless you give a push. That's very important. And that kind of notion is embodied in chemical principles.

So let's write this out. And then we'll formalize this. Delta G can be negative, but a reaction does not proceed. And that's because you've got to get the reactants out of that little hollow at the top of the hill. And in order to get the reactants out of the little hollow at the top of the hill, you need something called activation energy.

Activation energy is required. I'm going to abbreviate that AC. You can abbreviate it whatever you like. And the thing about chemical reactions that makes them proceed in chemistry and makes them proceed in biochemistry, in life, is that you need something to overcome that activation energy.

Back to the ball analogy-- I think of this because I have a dog. His name is Archer. He's a black Labrador-- in case you're interested-- and he's very fond of balls. And they sit at the top of the hill in my yard. And there's a whole line of them .

And they will sit there forever unless Archer either intelligently or by mistake pushes one. And then it goes rolling down the hill, and he can get it. So you have to do something to start the reaction. You have to give energy.

Where does that energy come from? Well, it comes from somewhere. And you can decrease that activation energy and really get a reaction to proceed quickly by the

use of chemicals called catalysts. So catalysts decrease activation energy and allow a reaction to proceed. Catalysts do not change delta G of the whole reaction or the equilibrium point.

So you're not going to get more. You're not going to really change the reaction. You're just going to get it to go. Catalysts do increase the rate of reaction. That's the point.

And they do this, as we'll explore, by promoting something called a transition state, which is a high-energy form of the reactants. If we go back to our ball analogy, if you kick the ball so that it comes up out of the hollow, that kick that you give the ball, the energy you put in there, is going to get the ball rolling down the hill, and the reaction will proceed. So catalysts promote a transition state, which is a high-energy form of the reactants.

Let's look at a couple of slides that I drew for you. So here is a blank chart. And I've got delta G on the y-axis and the reaction course on the x-axis. And here's a reaction curve.

The reaction overall is going to have a delta G that's negative because you see that the products are at the bottom of the hill, relative to the reactants. But there's also this hump that they have to get over. And that is what requires activation energy.

In an uncatalyzed reaction or a catalyzed reaction, you have to go through this high-energy state. So here is a catalyzed reaction that I put on as blue, the uncatalyzed as red. And you can see what happens to the little hill that has to be climbed before the reaction can proceed.

The amount of energy you need to move that chemical reaction decreases relative to the uncatalyzed reaction. So you decrease the little hollow in which the ball is sitting, in order to get the reaction to proceed. But at the end, you'll end up with exactly the same reaction as you would have if it were not catalyzed.

All right. What does this have to do with biology? Well, it has everything. Because there's a class of proteins that we'll talk about for the rest of the lecture that are

biological catalysts. And without them, there would be no life.

So number two, we'll talk about enzymes. And enzymes are biological catalysts. They are usually protein. And all of the ones that we'll talk about are proteins. But they can also be RNA.

And a Nobel Prize was given some years ago for the discovery of RNA enzymes that are catalysts. And Dr. Sinha has posted on the website a video of the Nobel lecture of Professor Sidney Altman, who got the Nobel Prize for the discovery of RNAs as enzymes. But we're going to focus on proteins.

We're going to rewrite the chemical reaction that I have on that board in a slightly different way, which is the conventional way for thinking about enzymes, where we're going to have an enzyme, abbreviated E, plus the substrate that is going to form an enzyme-substrate complex. And this is the transition state, so called transition-state complex. And that, as I'll draw in a moment, is going to give rise to the enzyme plus the product.

So let me just draw this up there. And then we'll talk about what these mean. E is enzyme, S is substrate, as previously. This enzyme substrate, ES, is the transition-state complex. And what it is in biological terms is some kind of high-energy state of the substrate that is linked to the enzyme. So it's a high-energy substrate linked to the enzyme.

This transition-state complex then resolves to release the enzyme, which can be used again. As in the case of all catalysts, they can be reused to give rise to the enzyme and the product.

So why is this interesting? It's fascinating because -- Actually, let me before I tell you why it's so fascinating, let me show you something. So I drew this for you.

Here is a representation of an enzyme and something I'll explore on the board in a moment called the active site, where the substrate fits into the enzyme. Here is an enzyme-substrate complex, a high-energy transition-state complex, that undergoes

reaction, catalysis, to give rise to the product. The product is released, and you start the whole thing again.

The thing about biological catalysts that are really different from chemical catalysts is their specificity. Now, you may have heard of platinum as a catalyst. Platinum is an incredibly powerful catalyst that promotes almost any chemical reaction that involves hydrogen-- but any chemical reaction. The difference between platinum and enzymes is that enzymes are incredibly specific for a particular chemical reaction.

Let's write that. Enzymes are highly specific, exquisitely specific, for the particular reaction. And they maintain this specificity by fitting the substrate into what I've got on the screen there, the active site, which is a particular part of the enzyme, a particular part of the protein.

And the deal is that the substrate fits into a part of the protein called the active site. The active site is a very small part of the protein. And it's this interaction with the protein and the active site that promotes the transition state.

How does it promote the transition state? Well, you can think physically it might change the shape of the substrate to make it higher energy. You could imagine it adds charge. You could imagine it adds particular orientation between two substrate molecules that happen to be in the active site. Or it might align the substrate in the correct way.

So we can actually say that the substrate is promoted to undergo catalysis by controlling-- let's just list these-- orientation of the substrate in the access site, adding strain to the substrate, or possibly adding a particular charge to the substrate. Let's look through a couple of things here. Okay, this is from your book. Let's not dwell on that. Let's go to this one, which is not quite-- Ah, it's on that screen. Good.

This is an example of enzyme specificity that you see every day, probably. On Diet Coke, it says, "warning, fennel phenylketonurics, contains phenylalanine." What's

the deal? There is a disorder called phenylketonuria where people are unable to digest the amino acid phenylalanine. This is an essential amino acid, but too much of it is bad.

Normally when you eat phenylalanine, you use some of it to build proteins. That's good. But then you convert a bunch of it, using this enzyme called phenylalanine hydroxylase, into tyrosine, which you may remember is another amino acid. And then you use tyrosine. And tyrosine goes on to do a bunch of other things.

If this enzyme is absent, then you get a side product whereby the excess phenylalanine makes this stuff called phenylpyruvic acid. And that is a neurotoxin. And it poisons the nervous system, and people have very severe symptoms.

People with phenylketonuria generally have a single amino acid change in an enzyme that is more than 1,000 amino acids, where there's an arginine that has being changed to a tryptophan. And the single amino acid change lies in the active site. It stops the phenylalanine from binding the active site. And it leads to this really debilitating disorder. And so that's an everyday example of a single tiny change that reflects the specificity of the particular enzyme.

But here's a puzzle. This is a space-filling model of an enzyme, taken from your book. Here's a substrate in red. And you can see that this enzyme has 3D shape. Each of these bubbles is part of one of the amino acids. That's what a space-filling model is.

And the substrate fits into this active site. I think this is called hexokinase. And you can see as the substrate enters the active site, actually the protein changes shape. And it kind of closes up and contains the substrate in the active site. And catalysis takes place.

But the active site is tiny. And there's this huge rest of the protein. And you can't get rid of the rest of the protein. The enzyme doesn't function if you do. So there's a fundamental question that we need to ask, which is, what do the rest of proteins do, the part outside the active site?

So let's ask that question. What does the rest of the protein, which is large, do? And by the rest of the protein, I mean "not the active site," which is the place where the transition state complex is promoted.

What it does is to regulate the activity of the enzyme. So the answer we can give is that it regulates, or controls, activity of the enzyme. What do I mean? Well, if you go back to this notion of the cell as a factory and enzymes as the wheels and the machines that get things done so that you get particular components synthesized, then you really want to make sure that you have got your production line working at the right pace.

If you need more product, you need to speed up. If you need less product, you need to slow down. The enzymes are a place where you control how much product is being made, how much reactant is being used, how much substrate is being used. So the rest of the protein controls the activity of the enzyme.

And there are a couple of ways that this activity is controlled. The end result is that all of these regulations affect the active site, even if they happen far, far away. And so you can imagine that if you are all lined up in a line, and the active site was right at the front of the line of you that was the enzyme prototype, and I pushed you, and you all fell down, the active site, the very front of the line, would eventually be affected although I'd pushed the back of the line. It's a silly example, but it gives you a sense of how you can change one thing and it has an effect far away.

And the way that this is done is through the use of inhibitors. I'll go through some slides in a moment. And there are two kinds of inhibitors. There are reversible inhibitors, and there are irreversible inhibitors.

These inhibitors may be competitive inhibitors. If they are competitive, they actually will bind to the active site as opposed to the rest of the protein. But if they are all of a class that is non-competitive, they will bind to the protein elsewhere. I'll show you a slide in a minute. But let's finish doing some board work here before I do that.

There is also a very important and interesting class of enzyme regulators called

allosteric regulators. There are both allosteric activators of enzyme function or inhibitors, things that will increase or decrease the rate of the reaction. And these allosteric activators or inhibitors will bind to a particular site that's not the active site. It's called an allosteric site-- not the active site, but another particular place on the enzyme.

Other things that can change enzyme function-- pH, the enzymes that work in your stomach to digest your food work at a pH of 2. That's the pH in your stomach. It's very acid. Those enzymes will not work in your small intestine, which is at a pH of about 6 or 7.

So pH, physiologically, can affect how enzymes work. Temperature affects how enzymes work. And all of these things are somehow changing the structure of the protein. And changing the structure of the protein is changing its function. We'll explore this in more specifics as the semester goes by, but this is what you need to understand now.

There are also classes of molecules called cofactors, coenzymes, and prosthetic groups. I'll go through one of these in a moment. But all of these things have the outcome-- all change the structure of the protein so they'll change enzyme structure. And with that, they will change enzyme function.

So all of these things-- I'm going to put it in a box-- change the structure of the protein. They will change the structure of the enzyme, of the active site. And they will change its function. And they're very complicated to think about.

Let's go through some slides that will help us with this. Here's the notion of a competitive inhibitor. Here's the substrate and an active site. And here's an inhibitor. And you can see I've drawn it so that it kind of looks like a substrate, but it's got some extra stuff.

And so when it binds to the active site, it binds specifically. But it's not actually a substrate, because it's got this extra chemical moiety which does not make it available for catalysis. And that stops the enzyme. That competitive inhibitor may or

may not be able to come off the enzyme and allow it to work again. It depends on the inhibitor.

Here's another one, a noncompetitive inhibitor that doesn't look like a substrate and binds somewhere else on the enzyme and, in doing so, changes the shape of the active site. So the active site now can't bind the substrate anymore because its shape has being changed.

Here's the notion of an allosteric activator. Here's the enzyme, and here's the enzyme with its substrate. And here's its allosteric site. And it can't work until an allosteric activator, which is not the substrate-- it's a different molecule-- binds to the allosteric site and then makes the active site the correct shape to fit the substrate so that the enzyme can work. And you can get a cycle here again, so that you get catalysis, and the whole thing can take place again.

I want to show you, to give you a sense of the kind of complexity of what this all looks like, a particular example of an enzyme reaction. And I'll show you an animation that goes with this, to give you a sense of how these things work. The enzyme I'm going to focus on is called dihydrofolate reductase. That's an essential enzyme. It's required for nucleotide synthesis, DNA modification. And the reason you take folate, or folic acid, is to allow dihydrofolate to actually work -- dihydrofolate reductase to work.

The substrate is dihydrofolate. It's a complicated molecule. But I've circled the important part. You can see there's a double bond here which is going to be reduced when the enzyme acts. And here is the reduced form or tetrahydrofolate of dihydrofolate reductase.

You don't need to take notes on this. Just look and listen.

Here is a structure of the protein. You can see the alpha helices in brown and the beta sheets in blue and this kind of unstructured loopy stuff in between. And here is the substrate dihydrofolate binding to its active site. Dihydrofolate reductase requires a coenzyme, something called NADPH, which is a very important

coenzyme. It's also part of the energy production cycle. And dihydrofolate reductase is not only essential for life but it's a target of the anticancer drug methotrexate and of trimethoprim, which is an antibiotic.

This is an animation which will show you how dihydrofolate binds to dihydrofolate reductase, how NADPH comes in, and how NADPH donates the hydrogens that will allow dihydrofolate to be reduced. So let me start the animation, and I'll point things out as you're watching it. And you'll see it's quick.

Here is the coenzyme coming in. And here is the substrate dihydrofolate. And if you look at it, you'll see that the coenzyme is near the active site. And as it binds first, donates its hydrogens to dihydrofolate, which then becomes tetrahydrofolate, is released from the enzyme.

There are a number of things you can see here. You can see the enzyme cycle. You can see this goes over and over again.

You can see that the enzyme, the protein structure, is changing. It's moving as the coenzyme is binding, as the substrate is binding. You can see that there's a chemical reaction going on here as the NADPH is giving its hydrogens to this dihydrofolate. And you can get a sense of the kind of animation that is enzymes.

This is an animation. No one's actually seen an enzyme work like this because it's impossible to take real movies like this. But based on the structure of the enzyme and the binding of NADPH and the substrate to the enzyme, these authors have reconstructed this really beautiful movie which gives you a sense how enzymes work in action.

Okay. I'll post this on your website. And you can watch it.

The last two things I want to tell you are that, firstly, enzymes don't work as one-off deals. They work as part of a production line. And the production line is called a pathway. And like any good production line, as I've mentioned already, there are checks and balances to make sure that the production line is working at optimal speed and making what it needs to make.

So we can talk about pathways and feedback. And let's draw a simple pathway on the board to give you a sense of what I mean. Let's start off with a substrate that's converted through the action of enzyme one to product one. And then there is also a competing pathway where this--

Actually, lets make it product one. Or we can call it substrate two. But let's call it product one, and then through the action of enzyme two, to product one.

And here's a competing pathway where the substrate, through the action of enzyme three makes product three, and then through enzyme four action, makes product four. Okay. This is a pathway. It's a bifurcating pathway. It may be that's if you've got this branch, the E3-E4 branch of the pathway active, you actually want to shut off production of the other pathway.

And it may be that if you get product four made, you actually want to shut off production of product two. In that case, you could draw a line like this-- you could make it up, whatever you like. But for the example I'm giving you, I'm telling you that when you've got lots of product four, it's going to shut off the other branch of the pathway.

And this nomenclature is as follows. An arrow means activate. And what's called a T-bar means inhibit. And these are very standard nomenclatures in biology that you should know. So this T-bar here is inhibit. It's also called negative feedback.

At the same time, you might get positive feedback. It may be that if you have some of product three, you actually want more of product one. And in that case, product three might activate enzyme one so that you actually activate the other branch. This is a complicated pathway. There's a lot of competition going on here.

But in that case, that activation would be positive. And so for this example, my star would be positive feedback. And my T-bar, which I'll make a pound sign, would be a negative feedback.

This is a really pivotal concept in biology. It's not just true of enzyme pathways, it's

true of multiple chemical reactions. And you really need to understand this aspect of biology.

The last thing that I want to tell you has to do, very briefly, with where the energy comes from that cells use for chemical reactions. And what I'm going to tell you very briefly, in about two seconds, is that in the cell, there are very controlled amounts of energy that are contained in a nucleotide called ATP.

ATP is a nucleotide triphosphate. We talked about nucleotides before. The triphosphate is very important here. And this nucleotide triphosphate is kind of like the dollars and cents of energy currency in the cell. If a chemical reaction needs energy, the cell uses ATP to get it.

ATP is hydrolyzed to give rise to ADP, adenosine diphosphate, and PPI, inorganic phosphate, plus an increment of energy. This is an exergonic reaction. This energy is then used for chemical reactions, to build or to break down various molecules.

For this reaction, delta G is negative. That is why there's energy released. And this energy is used for two types of reactions-- those that are catabolic, that break down molecules, and those that are anabolic, which build up molecules.

We're not going to talk about generation of ATP. That is a separate topic. And those of you who are go on to do biochemistry will talk about it in great detail.

But I do have some slides on here that I will refer to. Here is -- This is the last thing I'll show you. Please wait for it. Here is adenosine triphosphate. Here is the high-energy bond that is broken to release energy.

And we'll stop there.