[SQUEAKING]

[RUSTLING]

[CLICKING]

MATTHEW VANDER HEIDEN: OK. So welcome back, everybody, to 705. I know this has been a really challenging semester for all of us. I want to particularly acknowledge for the seniors out there the tragedy of missing the end of your senior year. Hopefully us, MIT can somehow make it up to you.

I can say for my part, we will do our best to teach you biochemistry remotely. And just as a couple logistics, a reminder, I'm Matt Vander Heiden. I'm going to be covering the remaining part of 705.

For the rest of the semester, we get to focus on biochemistry of metabolism. We're going to learn what metabolism is, why it's important. And basically, this can be quite a polarizing topic.

In my experience, people tend to really love or really hate metabolism. It's interesting, because there are some people out there who pretend to hate it, but actually deep down really love it. Hopefully I will inspire you over the rest of the semester to at least have some appreciation for why metabolism is important.

And regardless, for those of you who want to go to med school, this is a favorite topic of MCAT Exams. And also you will see that it will come up in many different areas of biology, regardless of whether you go to med school or not. So if you do anything further in biology, hopefully you'll find this material somewhat useful.

OK, so getting into what metabolism is. So I think it's good to start with a textbook definition of metabolism. And so metabolism is the chemical reactions that all cells and organisms use to do two things. One, extract energy from the environment. And two, synthesize the macromolecules that make up all life.

So really, what metabolism is then is the chemistry that makes life possible. And so understanding metabolism is really something that will help you better understand your food. There's lots of material out there in the popular press about what's healthy and what's not. At least this will allow you to form some of your own opinions about those claims, and how they relate to what really goes on in our cells and bodies.

There's lots of implications for medicine, very relevant to agriculture, the energy sector, biofuels come from. And so metabolism really matters to tackle many of the challenges that face us in society.

Now as a topic, this can be very daunting. As shown here on this image, we have hundreds, this is a typical metabolic pathway chart that is hanging in the walls of my office, many other laboratories and academic places around the world. If you look at this chart, it's filled with hundreds of enzymes, complex pathways.

Our goal here is not to memorize this chart. You can always look up details of any pathway or reaction that you want. The goal here really is to see beyond this complexity. I want you to appreciate why metabolism works the way it does, why this chart is organized as it is. Because what we will find is that this chart is really variations on relatively few reactions. It basically is life repurposing similar chemistry to do many different things.

I think there is a beauty in how life can use these reactions to get such a diversity of macromolecules, and enable cells to solve all kinds of different problems. You will see that all of the pathways really follow common principles. These are shared across all species and all forms of life.

And so at the chemical level, all life is really related. And this is why it's so relevant to understanding many of the challenges in medicine, understanding ecology, evolution. And for you MIT students, you'll see it's also very critical for using engineering approaches based on biology to solve various problems in the world.

OK. So now let's start getting into this a bit more. Now, the first topic that we're going to cover over the next several lectures really relates to coming back to sugars and carbohydrates, which I talked about and introduced in my previous lecture. And we're going to do this because sugars and carbohydrates are key energy transduction molecules and cells. And it really facilitates a discussion of the principles for how metabolism works.

Now our goal here isn't to memorize pathways. We could read about the glycolytic pathway in an hour. I could draw it up here in a very short period of time. Many of you have probably already done this in high school. Our goal is instead to understand this pathway at a deeper level, really see why the breakdown of sugars works the way it does, how cells use this to release energy, and how that can be used to support other cell functions.

And so before we get to that, I want to start by introducing just a couple high-level concepts about metabolism. And so really, based on our definition before, metabolism is really about two things. It's about making stuff, and we have a fancy term for that. That is called anabolism.

And it's about breaking stuff down. And the term for that is called catabolism. And anabolism, building stuff-- that is, producing biomass, growing all of the material that's out there, anabolic steroids help you grow. This is anabolism to build stuff.

That is one side of metabolism. The other side is breaking stuff down, catabolism. That is, breaking down our food, eating food. Digesting our food is a way to get energy.

Now of course, to reproduce, life has to build more cells. You need more stuff. You can't create something from nothing, and so that requires energy input. Anabolism typically requires energy input. Whereas that energy has to come from somewhere.

And that's where catabolism comes from. We have to break down food. And ultimately, catabolism is the source for biological energy for many different systems.

Now, some of this is actually somewhat intuitive. We all learned as little kids that we need to eat food if we're going to grow up and be big and strong. You also know that if you're going to run a race, you've got to eat a bunch of food, you need that energy to fuel your activity.

But maybe what's less intuitive to many of you is we also need energy to sustain life, even if we're doing absolutely nothing. OK, adults, sit on the couch all day, hopefully they're not growing. But they'll still starve if they don't constantly eat some food, even if they're inactive.

And so why is that the case? Well, hopefully some of you recall the second law of thermodynamics. So what is the second law of thermodynamics? It's, in effect, the entropy of the universe is increasing. That the universe continually tends towards disorder.

Life is exactly the opposite of this. Life is actually maintaining extreme order in the face of the second law. And so all life must constantly battle entropy. And many have described life at the very highest level as being really the ability to maintain order, to fight entropy, which of course requires constant energy input. And metabolism is the process that all cells use to do this.

Now for humans, I think we all know we need to eat and breathe in order to stay alive. For you future MDs out there, what happens if your heart stops, or you stop breathing? Well, of course, you die in a matter of several minutes. Why is that? Well, that's because every cell in your body has to do constant catabolism in order to derive energy, in order to remain viable.

This means they need constant food, and particularly oxygen delivery. And if you don't get those things, metabolism can no longer work, and the cells die. And so energy is sort of this mystical concept that we like to talk about.

And so before we delve a little bit deeper into what I mean by biological energy, what it is, why we have to constantly do catabolism to maintain it, I want to go back and say just a little bit more about carbohydrates and finish our discussion there, and talk about carbohydrates and sugar polymers, polysaccharides, because this has some additional properties I didn't have time to cover in my prior lecture that is important to understand how it allows these molecules to store energy in a very efficient way. And it will also enable us to talk a little bit about how carbohydrates can also be used as structural molecules for lots of different organisms.

All right, so a little diversion to discuss carbohydrates and polysaccharides. And so if you recall from my prior lecture, sugars that are greater than four to five carbons can exist either in open chain or ring forms, furanose or pyranose rings. I'll just remind you by redrawing up here, glucose.

So this is D glucose, drawn in the open chain form. Remember this can form a ring. OK. So it's a reminder, carbon 1, 2, 3, 4, 5, 6. If we have this hydroxyl group from carbon 5, form a hemiacetal bond with the aldehyde at carbon 1, you get this ring structure.

This would be alpha or beta, depending on whether the hydroxyl group here at carbon 1 is pointing up or pointing down. D-glucopyranose. So this is review of what we already talked about last time.

Now, what I want to talk about is that if we form a disaccharide or a polysaccharide-- that is, begin to make sugar bonds, and if we do that in a way that ties up this hemiacetal or hemiketal bond, this then prevents the ring from opening and gaining access to that carbonyl, that aldehyde carbonyl, as this moves between the open chain and the ring form.

And so a good example of this is the disaccharide sucrose. So sucrose is a disaccharide of glucose plus galactose. And here is what this disaccharide looks like.

OK, so you have here, glucose in the alpha-D-pyranose form. Here's fructose in the beta, because the OH group is pointing out. Fructofuranose form.

And so the formal name for this molecule, sucrose, would be alpha-D-glucopyranosyl 1-2, because we're going from carbon 1 of glucose to carbon 2, there's 1, 2, 3, 4, 5, 6 of fructose. Beta-D-fructofuranose. Sucrose or alpha-D-glucopyranosyl 1-2 beta-D-fructofuranose.

OK, so this disaccharide forms a bond between carbon 1 and carbon 2. That would be the aldehyde in glucose, or the ketone tied up in the hemiacetal or the hemiketal bond of these two molecules. And so there's no way that either of these sugars can access the open chain form, without breaking this O-glycosidic bond. And that's one reason why sucrose is a very good storage molecule for carbohydrates, because without breaking that bond, you prevent access to gaining these reactive aldehyde or ketone bonds, that could react with some other molecule in the cell.

Now, I mention this because whether or not a particular sugar is enabled to get access to this reactive aldehyde or ketone really forms the basis for a classic sugar detection lab test. And this is relevant to discuss this lab test because it explains some nomenclature that is still used, and in fact, we will continue to use a bit as we discuss some aspects of sugar metabolism. So what this test is, is basically, is that if you heat the sugar with copper, which is blue, and that copper can gain access to a free carbonyl--that is, if the sugar can access the open chain form to expose the aldehyde or a ketone, that copper can then become reduced to copper plus. That changes the color from blue to red.

And so if you reduce the copper, you oxidize the bond there. And this test turns positive if there is a reducing sugar, is what it's called. So a sugar that reduces the copper from the blue 2 plus state to the red plus state is a positive test. And so basically, a reducing sugar is any sugar with an ability to access an open chain form. Because that will provide the free aldehyde or ketone to reduce the copper in this test.

So this term, reducing sugar, obviously applies to all monosaccharides, because every single one of them has an aldehyde or a ketone, and can access the open chain form. But it will only apply to some disaccharides or polysaccharides.

So let's come back to sucrose up there. So is sucrose a reducing sugar? No, it's not, because there is no way that you can access a free aldehyde or a ketone in sucrose, because it's tied up in that O-glycosidic bond between carbon 1 of glucose and carbon 2 of fructose. And so you need to break that bond to give them monosaccharide subunits in order to access the open chain form.

However, let's give an example of a disaccharide that is a reducing sugar. And that's the disaccharide maltose. So maltose is basically two glucose molecules. And those two glucose molecules are like this.

So this has a O-glycosidic bond between carbon 1 of this glucose, and carbon 4 of that glucose. And so formally, this would be alpha-D-glucopyranosyl 1-4 alpha or beta. If it's pointing down, it's alpha, pointing up, it's beta. D-glucopyranose.

So maltose or alpha-D-glucopyranosyl 1-4 alpha or beta D-glucopyranose is a reducing sugar, because this sugar could access the open chain form. You could open up this carbonyl and expose the free aldehyde at position 1.

Now, we could also refer to this sugar as having two different ends. We can refer to this end as being the non-reducing end, and this end as being the reducing end. Because of course, this bond, this aldehyde on the first glucose is tied up in this O-glycosidic bond, whereas the one on this one is not. So this end is non-reducing, and this end is reducing.

Now this becomes much more relevant if we start talking about polymers. And so what starch is. So starch, what potatoes are made out of, is basically a polymer of maltose. Polymer of maltose molecules. So basically our glucose with 1-4 linkages.

OK, so I could draw that end, the reducing end of the polymer, in the alpha or beta. But this here, being the non-reducing end. Every other subunit is tied up in this 1-4 O-glycosidic bond. And so starch is really a polymer of glucose molecules with bonds between the 1 and the 4 position of each subunit.

Now we will see later that we build and break down starch polymers from only one end, from the non-reducing end. And so having these terms, reducing and non-reducing, provides the term to specify different ends of a polymer. Reducing and non-reducing ends turns out to also be important for naming conventions for disaccharides or polysaccharides. And this becomes relevant if we consider the disaccharide lactose.

So lactose is also a polymer of glucose plus galactose, except this polymer of glucose plus galactose, unlike sucrose, is different because it has a different linkage between the sucrose is a disaccharide of glucose plus fructose.

Fructose, I wrote the name correctly. But lactose is a disaccharide between glucose and galactose. And so I'll draw it here.

OK, so this is lactose. This is galactose here on the non-reducing end. So the formal name for this would be beta, because there's a beta linkage here between carbon 1 of this galactose and carbon 4 of this glucose.

So be beta-D-galactopyranosyl 1-4. And this is glucose in the alpha or beta. D-glucopyranose. Lactose, or beta-D-galactopyranosyl 1-4, alpha or beta, depending if I drew down, alpha, up, beta. D-glucopyranose.

Now, this also has non-reducing and reducing ends. And by convention, you would name the sugar from the non-reducing end to the reducing end. Hence I put galactopyranosyl 1-4 alpha-D-glucopyranose, named them in that order.

Now you'll note that lactose, unlike starch, unlike maltose, unlike sucrose, links these disaccharides with a beta linkage between this sugar and that sugar, where the other ones had alpha linkages. And structurally, this is very different. And I just want to point that out.

So this here is basically alpha-D-glucopyranose. And so alpha, this is carbon 1. This is the hydroxyl pointing down. That's why it's alpha. If I make an O-glycosidic bond to another sugar, you can see that it points and creates this kinked structure.

Now, if I were to make this beta, this hydroxyl rather than being here would be at this position on carbon 1. You can see that that now creates a very different geometry. Now you have a flat molecule, as opposed to a kinked molecule.

Now, this has consequences, of course, for the enzymes that break down the sugars. Obviously, it's going to be a very different enzyme that breaks a bond in this orientation, versus a bond in that orientation. This has, basically implications for what enzymes we have.

And so lactose, of course, is milk sugar. All mammals make milk, so make lactose, and break it down when we're babies. But most mammals tend to lose expression of the enzyme lactase that's able to break this 1-4 beta linkage as we age. And this is basically what accounts for lactose intolerance.

Now, if you think about it, in the world, much of the world has issues with varying degrees of lactose intolerance in adulthood. The exception to this is often people of European ancestry. Why is this?

Well, Europeans historically drank milk well into adulthood. And so this selected for continuous lactase, the enzyme that wouldn't break that beta 1-4 linkage. The expression as people move into adulthood. And so it's an example of how cultural things, drinking milk into adulthood, really affected evolution, such that that subpopulation of people, people with that genetics to increase lactase expression, don't become lactose intolerant as they age.

Now, this alpha versus beta linkage-- that is, whether or not you have this more kinked versus more flat structure-- also has a major effect on the structure of a polymer as well. And so if we look here at this starch polymer, it's basically this series of glucose molecules stuck together with alpha 1-4 linkages. So that's going to create a kinked structure. This is best shown here on this slide.

So you can see this kinked structure. And so when you build this polymer, you're going to end up more with this helical structure in 3D space. That's a very efficient way to store glucose monomers in much less space than you would otherwise get with beta linkages.

Now, it turns out that nature takes this a step further. And in addition to just having this starch polymer, it turns out sometimes you can add branches to the starch polymer to further increase the efficiency of energy storage. So if you have here a starch polymer, so let me just draw here a couple subunits.

So this here is a starch polymer, glucose polymer with alpha 1-4 linkages that I can put an additional branch on this by adding on a linkage up here on the top.

With an alpha 1-6 linkage. So the 1 position of here, to the 6 position of here, which has the effect of creating branches on this long polymer, such that you have a single reducing end, and lots of non-reducing ends. Each of these non-reducing ends has a polymer of alpha 1-4 linkages that would be stuck to the main chain polymer via this alpha 1-6 linkage. And this has a couple implications for storage.

The first is, by only having one reducing end, it reduces maximally the exposure to a free aldehyde. And so that makes it a good storage sugar. And also as we will see, we're going to break down these sugar polymers biologically from the non-reducing ends, and build from those ends. And so there's lots of hooks or lots of places to either add or remove sugars from, which allows you to access carbohydrates quickly as needed. That's shown nicely here on this slide, better than I can draw it.

So up here, you have your non-reducing end across the polymer. So this here would be starch with the alpha 1-4 linkages on the polymer, or reducing end on one side, non-reducing end on the other. You can then add branch points to that by making an O-glycosidic bond between carbon 1 down to carbon 6, creating a branch.

And so you can see this helical branch structure will then form. That is both very efficient to pack lots of glucose into one place, and give lots of non-reducing ends with which to build and remove sugars from for biology to either store or quickly access sugar molecules as needed.

Now, in plants, of course, potatoes use starch in a straight chain. We know that. However, plants also use this branched structure. This is a molecule called amylopectin.

Amylopectin is better known in the food industry as Sure-Jell. It's the material that allows you to make jelly. It's the jelling agent in jelly. And effectively, this has every 24 to 30 units. There would be a branch with one of these alpha 1-6 linkages to give you this multi-branched structure.

Now, animals don't make starch. Animals don't make amylopectin. But animals make another molecule called glycogen. Glycogen, also a sugar polymer, exactly like amylopectin. Alpha 1-4 linkages with the alpha 1-6 to create branch points.

Just like amylopectin, except glycogen has even more branches with a branch every 8 to 12 glucose units. And so, this is a very complex structure for both plants and animals to quickly store and quickly release glucose molecules when they're needed.

Now, this is in contrast to a glucose polymer that can be done that has a very different 3D structure. So what if we take starch, and rather than have these alpha 1-4 linkages, but instead replace these with beta 1-4 linkages?

OK, so now beta linkages. Looks simple enough, but changing the geometry from the alpha to the beta bond removes the kink. Now it's much more of a flat structure. Turns out this is, shown on an image here.

So here's a beta linkage, and suddenly you have this flat structure. This is the same polymer as starch chemically, but has a different linkage, the beta 1-4 linkage. And that polymer with the beta 1-4 linkage is cellulose. And cellulose is, of course, what wood is made out of.

And so wood and potatoes, cellulose and starch, exactly the same polymer. Same number of calories, if you could access the glucose units. But the alpha and beta bonds make them very different.

Obviously, wood and cellulose is a very good structural polymer for plants. We build houses out of it. We don't build houses out of potatoes, but we eat a lot of potatoes. So equally good molecule for plants to store lots of food for energy.

Turns out wood is a great source of energy too. You just need to have an enzyme that can break the beta 1-4 bond. Termites have symbiotic microbes that allow them to do this, and this is why termites can eat wood, a lot of energy tied up in wood.

Now, we lack the time to go into the details about how this relates to other structural carbohydrates. But in general, structural carbohydrate or carbohydrate-like molecules form polymers of sugar or related molecules, also with these beta linkages. And a good example is chitin, so the material in the insect shells, is basically a sugar-like molecule that's a polymer with a beta linkage in it. Same thing with cartilage in humans and animals. And you can look up the details of what these things look like, of course, if you're interested.

Chitin and cartilage aren't made of true polysaccharide polymers, but they're very related to polysaccharides. And they really illustrate how nature can take carbohydrate chemistry and repurpose it to basically act as an energy source, but also build all kinds of structural molecules that are really useful in biology.

All right, now I want to go back to, here's ways that one can store and use carbohydrates in interesting ways. But now I want to come back to them as energy sources, and assuming you can access the glucose and whatever polymer it's present in, how can you actually metabolize it to provide energy in a way that sustains life? And this means we're going to turn to another topic, a topic that's typically referred to as bioenergetics. Bioenergetics, which is really the discussion of how energy is transduced in biological systems.

I'm going to introduce this topic today, we're going to revisit it throughout the course. But it's really important to ask this question. We really want to consider, what do we mean when we say biological energy? What is biological energy?

Well, someone probably made you memorize in high school, and often you were thinking, oh, it's just ATP. And certainly, ATP, adenosine triphosphate, is a very important molecule for biological energy transduction. But I want to actually explore why ATP is actually useful. And if ATP is energy, why don't we just eat all kinds of ATP?

I can say for a fact that absolutely none of you had ATP today for breakfast. No one sells ATP as an energy booster. And so if ATP is such a great energy source, why don't we just eat it? And to really understand ATP, how it works, what biological energy really is, we really need to revisit some very basic topics in thermodynamics.

Now, thermodynamics strikes fear into the hearts of students everywhere. You can get details of thermodynamics and the theory around these things in other courses. That's not really what we're going to try to accomplish here in 705. Our goal here is really a practical understanding of how thermodynamics applies to biology and metabolism.

And we need to go into this, because it's key to understanding why we store energy, go through all this trouble to store energy as these carbohydrate polymers to begin with. Why we eat potatoes and not ATP, as well as why ATP is actually useful to cells. And so let's take a step back and just think about it. Let's think about wood. I just told you, wood is a polymer of glucose molecules with beta 1-4 linkages.

How can we, forget as organisms, as cells. But how can you release energy from wood, to do other kinds of work? Well, we can burn it.

And so what's the chemistry of burning wood? Well, it's a carbohydrate polymer. So it has this CnH2n formula. If we combine that carbohydrate with oxygen, it releases CO2 and water, plus some light, plus heat.

This light and heat is energy, because we can use it to do work. Boil water, turn a turbine, make electricity. Whatever.

Warm ourselves by the fire. Use the light to do some other things. And so this burning of wood is certainly release of energy.

Now, we lack the enzymes to do this reaction in wood, because we can't break that beta 1-4 linkage. But we certainly can break that alpha 1-4 linkage in starch from potatoes. And we use the exact same chemistry to burn that glucose and release energy.

Except the difference is that if I burn wood, I do this all in one step. But life is a much better engineer than that. It basically, rather than releasing all of that energy in one step, it really releases it stepwise in a manner that's actually useful to cells. But it's exactly the same release of energy.

And so what life is, or extracting biological energy is, is it's really the ability to do stepwise oxidation of glucose or other carbon. I'll write glucose for now. To get energy.

So we burn wood, that's favorable, it releases energy. If we do stepwise oxidation, we also can release energy as well. Same reaction, also releases energy.

How much energy do we get from releasing us burning glucose, as you do and burning it in a fire? Well, it has to be exactly the same amount, because it's exactly the same reaction. So we burn wood, we get light and heat. Cells burn glucose, it releases the exact same amount of energy. It just does so in a way that allows the cells to do biologically useful things.

What are those? Well, it could be heat. All of us maintain temperature. So heat is a conversion of energy that of course, is useful to biology.

But it can do other things, too. It can allow cells to move. It can allow them to do any reaction to fight that entropy that allows life to exist. And so all things, including ourselves, have to follow the same laws of thermodynamics. Life is not special in that regard.

And so burning wood is favorable, because it increases the entropy of the universe. You're breaking up this polymer into a bunch of monomers. That's going to be spontaneous. That is in accordance with the second law of thermodynamics. True if cells burn it as well.

But remember, the second law says that net entropy of the universe must always increase. And so if we're going to do something that's not energetically favorable, like build a glucose polymer, we obviously have to put energy into it. And to do so, the energy that's released must be greater than the energy that we actually store.

And so this is actually a really key point, because anything that we do that requires energy input for a cell to carry out that process requires a source of energy release somewhere else that is equivalent or greater than what is actually put in. And so cells need energy, because they do lots of thermodynamically unfavorable things, fighting that entropy of the universe. And all of those processes must come from the release of energy elsewhere, like the burning of carbohydrate. And so that constant energy input is at the very highest level, why we need to do constant metabolism in order to maintain order and survive as organisms.

Now this, of course, comes from things like burning of carbohydrate. But of course, that carbohydrate has to come from somewhere. And so ultimately, you have to have an external source.

That external source is of course, the sun. Photosynthetic organisms can use light energy from the sun to do exactly the same things. Build those excess polymers, and that's why we as animals or anything that eats other animals as a way to live is ultimately depending on eating photosynthetic organisms, because photosynthetic organisms do this by harvesting energy of the sun.

Now that's all very high level. But I want to come back to the specifics of metabolism, ultimately, and understand those as how they relate to enzymes' reactions and pathways. That is, how do you get that complex series of reactions that make life possible, and ultimately still be guided by these exact same principles? In other words, how do we couple the energy releasing reactions, like burning glucose, in a way that obeys the laws of thermodynamics and operates under biologically acceptable conditions in order to do things that are biologically unfavorable. That's really what bioenergetics is.

And so the next thing we want to consider then, if we want to understand this, is really, let's ask the question. What determines if a specific reaction occurs? That is, what determines if something like burning wood actually spontaneously happens?

If you light wood on fire, it will burn. It does so every single time, everyone has had that experience. But no one has ever seen CO_2 and water spontaneously come together and form a tree. That doesn't happen. So what determines whether you burn wood and that is favorable, versus why it is that CO_2 and water don't come together and spontaneously form a tree?

Do not think that this is determined entirely by enzymes. Enzymes are catalysts. Enzymes are the spark that makes the burning of wood possible. But remember, enzymes cannot change thermodynamics. Enzymes only change the rate at which a reaction happens.

It does not change the equilibrium. That tree wants to, if given the catalyst, form CO_2 and water. That reaction is spontaneous because it is thermodynamically favorable. That tree will not take CO_2 and water and spontaneously reform a tree. And there's no amount of enzyme that will make that happen all by itself.

And so remember that. If you have any reaction between A and B, there is some equilibrium, as defined by the chemical properties of A and B, such that the equilibrium lies far to one side or the other. And that equilibrium is determined by thermodynamics. It is not determined by enzymes. I don't care how much enzyme I add. If the equilibrium lies towards A as I draw it, I can add all the enzyme I want, and I can never create more B.

And so, this is super important, and is actually a point that is gotten wrong by lots of biologists. They think, oh, this enzyme is expressed. Therefore, this pathway must be happening, or this reaction must be happening faster. You need an enzyme to catalyze a reaction.

So a reaction may not occur, your wood may not burn unless you give it a spark, a catalyst to actually make it happen. But you cannot add a catalyst to fight thermodynamics. You cannot take that CO_2 and water and turn it back into a tree. And the same thing is true. You can add all the enzyme in the world you want, and all it will do is help A and B establish the equilibrium that is defined by the thermodynamics of the relationship between A and B.

Now, what determines the equilibrium between any two species? That's a topic for another class, why a species is favored to be one side or another. However, a useful tool to think about this in biochemistry and quantify this for any pair of reactions is something you've probably learned about before. It's something called the Gibbs free energy. Gibbs free energy, or delta G.

Delta G is related to the way we'll talk about the equilibrium constant. And it's basically a term that we can use to specify if a reaction is favorable or not. And so hopefully you learned in an introductory class that if delta G is less than 0, our reaction is spontaneous. If delta G equals 0, our reaction is that equilibrium. If delta G is greater than 0, our reaction is not spontaneous.

All right, if we take our log and ask, what is delta G to turn that log into CO_2 plus water, it is less than 0, because it is very spontaneous. We take a pile of ashes and ask what delta G is to recreate the log. It's greater than 0, because it ain't going to happen. And if it's at some equilibrium, delta G is equal to 0.

Now, delta G depends entirely on conditions, as we will see in a minute. And because of that, this means that there can be conditions where absolutely any reaction can be favorable if the conditions are right. And we'll see that this becomes very relevant to understand how metabolism works. And so let's go back and just now consider some generic reaction, A and B.

So at equilibrium, what happens? So at equilibrium, the concentration of A and the concentration of B are not changing. Delta G equals 0. These two things are at equilibrium, whatever that equilibrium is. A and B are not changing in concentration.

Now if I come and I add more A to this side of the equation, what does that mean? Well, you know from Le Chatelier's principle that that's going to favor production of B. We have shifted things now out of equilibrium. So if I add A, that leads the production of B to re-establish this equilibrium. That means that delta G from A to B is less than 0 until I've reestablished equilibrium, and now delta G is back to 0 again.

Now, what happens if I add B? Well, if I add B, now I'm going to produce some A. So add B. That leads the production of A, until I reestablish that equilibrium.

So delta G, in that case, to go from B back to A. The reverse reaction is less than 0. Or I could also say that delta G, to go to A to B here, because that's not going to happen.

I just added more B. I'm not going to suddenly create more B from A. So here now, delta G is greater than 0.

Now note when I did this, I don't actually have to specify how much A or how much B I've added. The equilibrium depends on the ratio of B to A. Not the absolute concentration of either species. And so having a way to think about this equilibrium, it turns out, is also useful for biological systems to divine some delta G that is helpful to relate to this equilibrium constant. And that is this concept of delta G 0 prime.

A standard free energy, which for biological systems, relates to the equilibrium constant at 25 degrees, or the pH of 7 and 1 atmosphere. Pretty typical biological conditions. And this here is basically related to the equilibrium constant. And we can calculate the actual delta G by the following formula. Delta G equals delta G 0 prime plus RT times the log of the products over the reactant.

So this would be drawn for the reaction A to B. So the reaction A to B is related to delta G 0 prime, which is related to the equilibrium constant. I'll tell you how in the second.

Plus R is the gas constant, T is the temperature in Kelvin times the natural log of the ratio of products, B over reactants, A. This tells you whether or not at specific concentrations of B and A, that is a specific ratio of B and A, whether it is favorable to go from A to B, delta G less than 0, or whether it is favorable to go from B to A, delta G greater than 0. And so this should begin to make it clear that whether or not a specific reaction occurs is affected by the actual conditions present. And you can calculate that based on this relationship.

And so if I specify concentrations of A and B, as well as delta G 0 prime, I can know, at those concentrations, which direction of the reaction is favored. Now of course, my drawing A to B is entirely arbitrary. I could equally draw it B to A. And if I did that, so this is delta G 0 prime, A to B, all I do is flip the signs.

So delta G equals delta G 0 prime. The negative of A to B is equal to the positive of delta G 0 prime from B to A, because the direction is arbitrary. Plus RT log in this case.

Now I have A is my product, and B is my reactant. If I just flip the sign, flip that ratio, it's just going to change the sign of my product and give me exactly the same result with the opposite sign, which makes sense. What's favorable in one direction is not favorable in the other direction, and vice versa.

Now, I can also set delta G equals 0. That's equilibrium. If I do that, then delta G 0 prime equals negative RT. So this would be for A to B. I'll use the top example.

So delta G equals 0. Then I have delta G 0 prime equals RT times log B over A. So if I know two things are at equilibrium, I can calculate delta G 0 prime, and know what the equilibrium constant is. And so it follows from this then that if delta G 0 prime is negative, that means B is favored over A at equilibrium. And if delta G 0 prime is positive, that means A is favored over B at equilibrium.

And so delta G 0 prime really is a convenient way to look at a reaction, and know which direction the equilibrium lies. So A and B, delta G 0 prime is negative, equilibrium lies to B. Between A and B, if delta G 0 prime is positive, the equilibrium lies towards A.

But the key concept is that for any specific reaction, any specific conditions of A and B, whether or not A is converted to B will be defined by the equilibrium constant. But it will also be defined about what delta is under those conditions.

So to be clear, delta G will depend on conditions, because I'll write it again, delta G equals delta G 0 prime plus RT times the log for the reaction, A goes to B. So products over reactants. And so whether or not A is converted to B will be a property of the equilibrium constant, plus the concentration of B and the concentration of A, that ratio of concentrations at the conditions present.

And if in that calculation, delta G is less than 0, that reaction is spontaneous. If delta G is greater than 0, that reaction is not spontaneous. Or to put it another way, if delta G is less than 0 under those conditions, energy is released. Or if delta G is greater than 0, that reaction will not occur without energy input.

If I burn wood, energy is released. But there's no way I can reassemble that log without some kind of energy input. And really getting this concept is central to understanding metabolism, as well as what biological energy means. And hopefully, what is now apparent, based on what I just said, is that what really determines delta G is two things.

Of course it's the equilibrium constant. But in essence, delta G for any reaction is proportional to the ratio of the reactants over the products. And that I can come up, at least in theory, with any relationship, any ratio of reactants to products to make this favorable. At some tiny, tiny concentration, if I have nothing but CO2 and water, and infinite time and infinite catalysis, a tiny bit of wood could spontaneously form. And so life really creates the conditions that allow that, and select for that to happen, and of course, add energy input so it can actually happen more.

And so this should be very clear to you that absolutely no reaction is irreversible. I don't care what your textbook says. Lots of sources will discuss irreversible reactions. I'll talk about irreversible reactions later in the course.

But what we mean when we say the word irreversible reaction is these are under conditions that are found in cells and in nature. And I stress this because if we want to understand the energetics for how pathways work, we have to appreciate that life can create conditions to do unfavorable things, because that's the only way that pathways can work.

All right, what what do I mean by that? Well, suppose again, we'll come back to our reaction. A goes to B. Suppose I need to build a pathway where I need to convert A to B, because B is actually useful for some purpose that I need to do in a cell.

However, what if this is at least by equilibrium, unfavorable? What if the equilibrium lies towards A? That means delta G 0 prime is positive, is greater than 0, for A to B.

What does that mean? Delta G 0 prime greater than 0, for the reaction A to B? That means the equilibrium lies towards A. And so there is no amount of enzyme I can add that will allow me to net convert A to B, because the equilibrium lies far towards A. However, whether or not a cell is able to build a pathway, where it converts A to B depends on delta G, not the equilibrium constant for that specific reaction.

So how can I turn A into B? Well, I could turn A into B if I keep the concentration of B low enough that delta G, not delta G 0 prime, that's the equilibrium constant, but delta G favors A to B conversion? That is, delta G for A to B is less than 0.

How can I do that? Well, remember delta G equals delta G 0 prime plus RT log of B over A. So this term is positive, because I told you, the equilibrium lies towards A. So I need a concentration, a ratio of B to A, such that B is low enough that this term is more negative than that term is positive. And if that's the case, delta G will be less than 0, and I can net move that forward.

So how can I do that? How can I keep the concentration of B low? Well, I can build a pathway that consumes B in a way to keep it low, which favors A to B conversion. In other words, I can build a pathway, A goes to B goes to C.

So even here, where delta G 0 prime is greater than 0, if delta G 0 prime for this reaction, B to A, is much less than 0, such that equilibriums strongly favor C, that means my unfavorable equilibrium here can be overcome by the very favorable equilibrium of B to C. In essence, I can use B to C to pull A to B, such that it actually happens. Produce this intermediate that's useful on the path to making C.

This strategy is used in lots of metabolic pathways. And it's useful to generate lots of chemically useful intermediates. Now note that this only will work if conversion of A to C is favorable.

That is, if delta G 0 prime is less than 0. I can only pull this reaction if delta G 0 prime is less than 0. That is, if equilibrium favors A to C, I can build a pathway this way to do something unfavorable on the way to making C.

This brings up another key point, in that everything that we discuss, relationship between any two metabolites in any single reaction, also must be true for entire pathways. So I have a three-step pathway from A to C. Whether I turn A into C in one step, or turn A into C in multiple steps, the free energy is exactly the same.

If I burn wood by lighting it on fire, it releases the same amount of energy than if a termite has an enzyme that breaks that beta 1-4 linkage in the wood, and can burn that glucose stepwise through metabolism, the exact same amount of energy is released. Glucose on one side, CO_2 and water on the other side, exactly the same amount of energy released, whether I do it in one step, or I do it in multiple steps. The equilibrium constant between those has to be the same, and the delta G has to be the same between all of it.

And so it should become apparent, then, that A to C has to be favorable if I'm going to use this trick of keeping product low in order to pull an otherwise unfavorable reaction forward. In other words, there's no way for me to keep B low and net convert A to B all on its own. That will not work. And it's these unfavorable reactions that ultimately then require energy input. And this is where ATP now starts becoming useful for cells.

OK, so now I want to talk a little bit about how ATP provides energy that allows otherwise unfavorable reactions to occur. And ATP is useful because delta G applies to sets of reactions in the same way it applies to single reactions. That is, it applies to whole systems. All reactions are a series of reactions, whether they happen alone or coupled together. All have to follow the same rules.

So now let's make our reaction more complicated. Let's say A plus B goes to C plus D, where this is really two reactions coupled together. A being turned into C, and B being turned into D.

All right, so will this reaction happen? Can I turn A plus B into C plus D? Well, how do I know? Well, it's going to be, got to calculate delta G. And so it's going to be delta G 0 prime for A going to C, plus delta G 0 prime for B going to D.

So those will relate to what's the equilibrium constant, the equilibrium relationship between A and C and B and D. If it's less than 0, equilibrium will favor to the right. If it's greater than 0, equilibrium will favor to the left of A and C and B and D.

Add them together, that gives me my overall delta G 0 prime for those two reactions. Plus RT times the log of the products. So that's C and D over A and B. Products over the reactants.

So if I take an unfavorable reaction-- say, A to C, if delta G 0 prime is greater than 0, so it would favor A, and I couple it to another reaction, B to D that's very favorable, because I add these two terms together. I can now take something where the equilibrium would be unfavorable, and make it favorable.

However, whether or not this actually happens still depends on this ratio of products to reactants. So it still depends on the ratio of C to A, and D to B in our hypothetical thing here. So to know if a specific reaction will actually occur, we have to take into account both the sum of the delta G primes related to the equilibrium constant, and the actual ratios that are present in cells.

And so the net effect is that I can couple more favorable reactions to make otherwise unfavorable reactions now become favorable. That is, have energy input. But it still depends on conditions.

And so let's think a little bit about the polymer synthesis that we learned from Professor Yaffe. And so in that process, what did we do? We made several polymers, and you learned about the chemistry to do this. We made DNA, we made RNA, and we made protein.

These are polymers of nucleic acids. Protein is a polymer of amino acids. All of these fight entropy, right? We're building polymers, not breaking them down. And all of them were synthesized using reactions that hydrolyzed ATP.

In fact, those reactions actually hydrolyzed ATP in the following way. And every single one of those cases, if you look back, what you will see is that they all had steps where ATP was taken to AMP plus 2 inorganic phosphates. This reaction is very favorable. In fact, it's two reactions. It's really ATP goes to AMP plus pyrophosphate.

And then that pyrophosphate goes to 2 inorganic phosphates. It turns out that delta G 0 prime is less than 0 for both of these reactions. That means the equilibrium lies towards AMP plus pyrophosphate. And here, the equilibrium lies towards 2 inorganic phosphates.

And so there's actually two tricks here by metabolism. One is, it's two very favorable reactions that we're coupled to do something unfavorable, build a polymer. And use the trick of keeping pyrophosphate low by coupling it to a downstream, very favorable reaction, which even further pulls this reaction, and is why ATP hydrolysis here becomes so useful as a way to build these polymers.

Let's just go quickly and add a few numbers to show exactly what I mean by how ATP hydrolysis can be useful in this setting. And so, let's do something a little simpler. Let's go to ATP plus ADP plus to inorganic phosphate.

So let's give this some numbers, delta G 0 prime in this case is equal to negative 7.5 kcals per mole. All right, what does this mean? Delta G 0 prime is related to the equilibrium constant. It's less than 0, which means the equilibrium lies towards ADP plus phosphate.

Now let's see how this actually helps by considering what the first step in glucose metabolism by cells is. So the first step cells do in glucose metabolism is add an inorganic phosphate group to glucose. So it's this reaction, glucose plus inorganic phosphate goes to glucose phosphate.

Just a quick note on shorthand that I will use in this course. So PI, of course, is inorganic phosphate. That is this PO43 minus group. When it forms a phosphodiester bond, like to an alcohol and one of the glucose molecules, I'll draw this in a future lecture.

I'll oftentimes indicate it by putting a circle around the phosphate. And so adding this phosphate to glucose is a step that traps that glucose in cells.

For now, let's just consider this reaction. And so delta G 0 prime of this reaction is positive 3.3 kcals per mole. What does that mean? Delta G 0 prime relates to the equilibrium constant. It says the equilibrium constant lies here to the left, to the glucose plus phosphate side.

So if we want to net trap glucose phosphate in cells, well, it's not going to happen spontaneously. You need some kind of energy input. That can come from ATP.

And so if we couple those two reactions as follows. So we now have glucose plus ATP goes to glucose phosphate plus ADP.

Now let's draw this out. Will this happen? So we can calculate delta G. Delta G equals delta G 0 prime.

So there's two reactions happening here, glucose to glucose phosphate, and ATP to ADP. We know what delta G 0 prime is by adding those together. So it's 3.3 plus negative 7.5. That equals negative 4.2.

What does that tell us? That says, now we've created conditions where the equilibrium constant lies towards the right, towards the glucose phosphate plus ADP side. But how much of this occurs or whether it occurs isn't just the property the equilibrium constant. It's also how much is there. And so that's RT times the log of the products, glucose phosphate.

And ADP over glucose. And ATP. And so, if this term is less than 4.2-- that is, if the ratio of glucose phosphate to glucose and ADP to ATP in this term ends up being less than positive 4.2, this reaction is spontaneous.

It is favored. Lots of conditions where that would be the case, although if this term is 4.2 or greater, now it will no longer occur.

How much ATP is needed? Well, it's hard to answer this question in absolute terms, because if there's a lot of ADP around, you need a lot more ATP for the ADP to be useful. And this is the problem with equating ATP with energy. The energy here is in the ratio of ATP to ADP, or ADP to ATP. And this is something that we we'll come back to in the next lecture, because in the end, this is what will determine if a reaction has happened.

It is not the absolute concentration of ATP. It's the fact that ATP hydrolysis is favorable. But how favorable that is depends on the relevant concentrations of ATP or ADP. And relative concentrations are all that matters in thermodynamics. It is not absolute concentrations.

It is the ATP-ADP ratio that is the correct way to think about energy. And this is true for any reaction with respect to energetics. If an ATP-ADP ratio exists, such that when coupled to a reaction, delta G is less than 0, then it becomes spontaneous.

And this is how ATP provides energy. And we will come back to this more in the next lecture. Thank you.