**PROFESSOR:** So what are we going to do today? So, today we're going to continue with amino acids, peptides, and proteins. And I want to talk about a different protein variant that is the causative, the cause of sickle cell anemia. And it's a very interesting structural issue. But let me very briefly recap what we did last time and then talk to you a little bit about a process known as denaturation.

So last time, we discussed how the primary sequence of a polypeptide chain defines its folded structure. The folded structure is put in place with secondary and tertiary interactions, non-covalent interactions. Secondary just amongst backbone and its tertiary sort of everything else, even including backbone amides, but either with water, or a side chain, and so on. And then there are some proteins that dissociate into quaternary structure.

So these monomer subunits, as they would be called-- and I'm going to depict this as a closed circle or an open circle-- may form dimers of some kind. The dimers may be heterodimers. Or they may be homodimers. Or you could form trimers, tetramers, and so on.

And when we talk about hemoglobin, which is the protein that gets, that has a problem-- that is the cause of sickle cell anemia, you'll see that that is a heterotetrameric protein. So in this sort of rendition, you would kind of draw it like this where there are four subunits. Two are of one flavor and two are of the other. And that's the quaternary structure of hemoglobin.

Now proteins fold. There are weak forces that are holding them together. But there's a lot of weak forces. But if you subject a protein to various treatments that may break up those weak forces, the protein will undergo a process of denaturation. So can anyone think of what kinds of things would cause protein DNA denaturation? Yes.

**AUDIENCE:** Some heat.

**PROFESSOR:** Heat is a bad one, is a serious one, obviously. And heat-- yes, I'll write them all down. What's yours?

**AUDIENCE:** pH.

**PROFESSOR:** pH. So pH. Acidity. Basicity. And we'll talk about why those things cause changes. Any other thoughts? Yes?

**AUDIENCE:** [INAUDIBLE]

**PROFESSOR:** Oh. Yeah. So for example, salt. Organic solvents. And a process that a lot of people don't necessarily think about, but as engineers some of you will, is shear forces. So if you're shooting a protein through a very narrow tubing and there's high shear forces, those who will also denature nature proteins.

So with heat, it's very clear. You're going to break those weak bonds. And then they can either reform. Or if you go to too high heat, the unfolded protein starts to form aggregates. And anyone who has ever scrambled an egg knows that that is an irreversible process. You don't get to cram the egg back into the shell. It's not the same anymore.

Because what you're doing when you're scrambling eggs is denaturing proteins through heat treatment. So that's what heat does. It breaks the forces. The proteins stretch out into their denatured state. And instead of refolding to a compact structure, they just start aggregating with each other. And that's pretty much irreversible.

pH is interesting. Why would pH break up at low temperature? Why would pH cause changes? Yeah.

**AUDIENCE:** [INAUDIBLE] amino acids have a certain structure. So they're either protonated or deprotonated, then the pH, that would change.

**PROFESSOR:** OK. So pH, perfect. So pH will change the charge states of many of your sight chains. And once you've changed it, you might have had a lovely electrostatic interaction. But then you go and protonate the carboxylic acid. And it can't form-- in fact, it wants the form, it wants to break apart as opposed to come together. So that is changing charged state, which causes denaturation.

Salts and organics. For example, they may make interactions with parts of the protein. For example, organics, organic molecules may slip into a hydrophobic core and break them up. Just push them apart. They want to be there. And then too much of a high concentration of an organic solvent that is miserable with water. And we would say ethanol, acetonitrile, DMSO. But you don't need to worry about too much of which details.

Well actually, once you get above 10% or so, we'll just start denaturing proteins, sometimes reversibly but often irreversibly. So this is very important to know that proteins are stable, but you've got to treat them nicely. There are some human diseases that are a result of misfolded

or aggregated proteins.

So for example, all the prion diseases are proteins gone bad, pretty much, where they are not in a folded structure anymore, but they are in aggregates that cause problems with cellular processes and toxicity. So Alzheimer's disease. Mad cow disease. A lot of those are neurologic disorders caused by poorly folded or very misfolded proteins, for example.

So these are the things we talked about last time with respect to the flux from primary to secondary, to tertiary to quaternary. And that's a perfect time for me to introduce to you what we'll talk about today. So last time we talked about structural proteins. And I showed you how collagen, just with a simple defect, changing a glycine an alanine in one of its subunits, really alters the quaternary structure of the protein to make very weak collagen that's no longer supportive of bone strength.

But what I'm going to talk to you about today is a defect in a transport protein that carries oxygen around the body. So we're going to talk about hemoglobin. These diseases are what are known as inborn errors of metabolism, or that's kind of a complex term. Or genetically linked diseases, because there is a single defect in a DNA strand that then gets transcribed into an RNA strand.

So one base defect that then becomes an amino acid defect in your protein strand. So these are tiny changes in the protein that cause dramatic changes in the structure and function of the protein. And what you will see with hemoglobin is it causes a real problem with the quaternary structure and causes proteins to aggregate.

So hemoglobin is the dominant protein in red blood cells. Or erythrocytes. And in fact, the differentiation of the red blood cell as it comes from progenitor cells goes through a process where the red blood cell dumps out its nucleus so it can't divide anymore. And basically, the content of the cell is extremely high in hemoglobin. You've packed the hemoglobin into the red blood cell at the cost of losing the nucleus.

So that's terminally differentiated. Can't become a red blood cell. It can't divide anymore. And it has about a half-life, they have about a half-life of 100 days. So they turn over, and then that's it. And when red blood cells turn over, the hemoglobin has to be taken care of in order that it's not toxic.

Red blood cells are red because of a particular molecule that's in the hemoglobin called the

heme molecule, which is bound to iron, which provides the hemoglobin with the capacity to pick up oxygen in your lungs, travel it around the body, and then leave it where it's needed. And then replace the oxygen with CO2 and take the CO2 back to the lungs in order for you to respire it out. OK?

So hemoglobin carries oxygen and CO2, from oxygen from the lungs, CO2 back to the lungs. And the reason why you need the iron is that the iron is coordinated to the oxygen. So the heme molecule-- I won't draw it. If you want to see it, it's a big, complex organic structure. Very interesting structure. But something for another day here.

But I want to just stress to you that the iron heme complex is red. That's why your blood cells are red. Your blood cells don't have a nucleus so they can cram in lots more hemoglobin. So it's kind of a fascinating situation.

So hemoglobin is an example of a homotetrameric protein. And it has four subunits. Two of one flavor and two of another. So we call this an alpha 2 beta 2 protein, differentiating the alpha subunits and the beta ones. Yes.

**AUDIENCE:** Why isn't it homotetrameric?

**PROFESSOR:** Why isn't it homotetrameric?

**AUDIENCE:** [INAUDIBLE]

**PROFESSOR:** You could ask why is it? I don't know. I mean, there will be interactions amongst the subunits that favor that particular packaging. The subunits are kind of similar in shape. They have what's called a globin fold. You can more or less pick out those tubes, remember, alpha helices.

They could form tetramers that are all the same, but the energetically favored form is the two and two. Hemoglobin is a tetrameric protein, because that's really advantageous for picking up oxygen and dropping off oxygen in a very narrow oxygen range. So there are proteins called globins that just one of these that can bind oxygen.

Hemoglobin is tetrameric because it has a cooperative oxygen binding. So in a very narrow range of oxygen, it fills all four sites in the tetrameric protein with an oxygen Molecule. So it's very advantageous from a physics perspective that it responds to very narrow changes in oxygen. Does that make sense to everyone? Yeah.

**AUDIENCE:** [INAUDIBLE]

**PROFESSOR:** OK. It means, anything that's cooperative means that one, let's say I've got a tetramer of hemoglobin. One oxygen binds to one of them. So I'm a binding oxygen here. And then binding to the next, the next, and the next gets easier and easier.

So they sort of want to come in as a team. And that's handy for maximizing oxygen transport around the body in a narrow oxygen range, which we can only deal with what's out there in the atmosphere, so we have to make this work. Does that answer your question? OK. All right. So where was I? OK.

So what we're going to do today. We're going to look at hemoglobin. It's the tetramer. Those discoid structures are the hemes that I just mentioned. I've drawn them as this sort of four-leafed clover here just for simplicity. And there is a single defect in the sequence of the single monomer subunits in hemoglobin. So each of these-- let's go here.

So there are four proteins-- beta globin, two copies of beta globin, and two copies of alpha globin. They are all-- let me see. What's the size? Do, do, do, do. [INAUDIBLE] You know, I can never see things when I'm up at the screen. But they're about 150. 156. OK. So they're about 146 amino acids long in each of them.

And a single defect in the beta globin where you have a change from glutamic acid residue 6 to valine at residues 6-- one change in beta globin, which means two changes in the whole structure, because there are two beta globins-- alters the properties of the hemoglobin and causes what's called sickling of your red blood cells.

So let's take a look at what that would look like at the amino acid level. Glutamic acid is one of your charged amino acids. I'm just going to draw a little bit of it as it were in a peptide. And it's at position 6 in the sequence. So it's six residues from the amino terminus because we always write things in this direction.

And the change takes place to put in place a valine. And there's a pretty big change in identity and personality of those residues. You've gone from polar charged, to neutral, big, fluffy, hydrophobic residue. And it's really amazing. So the beta globin is expressed on chromosome 11. It's 134 million base pairs. One base has changed.

So what you have in the DNA, in the normal DNA that encodes the normal beta globin gene,

there's a particular sequence of nucleic acids. This is what the double strand would look like. We're going to see way more about nucleic acids next week.

When that gets converted to the messenger RNA, you get a particular code that in the genetic code codes for glutamate acids. Everything's normal. A single change, if we change the center nucleic acid within the DNA, it makes a different messenger RNA. And one base pair puts in valine instead of glutamic acid out of 134 million base pairs.

So what happens in the normal hemoglobin, you have normal behavior. You had this tetrameric structure. It cooperatively carries oxygen. It moves around the blood no problem. Excuse me, it sits in the erythrocytes or red blood cells no problem.

The minute you have that mutation, the hemoglobin molecules start to associate into clusters like fibrillar clusters, because each tetramer gets glued to another tetramer, and another one, and another one. So you have hemoglobin not behaving as this beautiful, independent quaternary structure, but rather sticking to, physically sticking to other Molecules

And those tangles get, those molecules get so large that they start to form long and inflexible chains. And it's such a dramatic change that that discoid structure that you're familiar with for red blood cells suddenly becomes a sickle shape. So that would be the normal cell with normal hemoglobin. But sickle cell, they look like this. They're kind of curved, odd, a very odd shape.

And the problem is red blood cells have evolved to move really smoothly through your capillaries. As soon as you get a different shape that's sort of not that discoid structure, they start clogging in the capillaries. And when you have the defect where all of your hemoglobin is messed up with this variation, it's incredibly painful, because think of all your capillaries going out to the farther reaches of your joints. Those very thin blood vessels are blocked up with the sickle red blood cells that are caused by the variation in hemoglobin. So that one little defect takes us all the way to a serious disease. All right?

So what I want to do very briefly is show you the molecular basis for this. All right. And the defect actually appears on the two beta globin chains, but right on the outside of the protein, not in the middle of the protein. Because this is a defect that affects how proteins interact with other proteins, not the function of the protein on its own. Probably still carries oxygen just fine. But it's the mechanical change in the hemoglobin that causes the disease.

OK. So sickle cell anemia, the hemoglobin is now called hemoglobin S with that mutation that I

just described. And when people are heterozygous, it means they have one good copy of the gene that's normal and the copy of the gene that's the variant. And you'll learn much more about this in human genetics when we talk about that later on. So you have a mixture of the OK hemoglobin and the sickle cell hemoglobin.

People who are homozygous for the defect, all of their hemoglobin is disrupted, and those are the people who really end up in hospital with a lot of transfusions, and so on. The heterozygous, actually, you can manage quite well with. And I'm going to show you in a minute that in some parts of the world, being heterozygous-- i.e., having some of your hemoglobin with a defect and some without it-- actually confers an advantage. It's a really cool story.

So what I'm going to do is quickly show you the wire structure. OK, so this is the structure that elucidated the real reason for the interaction. What happens when you have this mutation. And it was a structure that was captured of a dimer of hemoglobin molecules where you could really see what was happening at the interface and the sorts of changes that had been put in place by that variation from the charged to the neutral structure.

So for any of you who want to pop by, I can start to show you how to manipulate PyMOL. We can do that separately from class. But this is a dimer of tetramers. And if I just show you just some of the subunits, I can actually show you how there's two of each subunit in each structure. So if I, go I can pick some out. Every other one.

And then I can color them a different color. You can see where the globins, where the beta globin are and where the alpha globins are. That's still looks like chicken wire. It's very unsatisfactory. So what I can do is I can show you everything as a cartoon and get rid of all those little lines. And then you can see perfectly the structure where you see two beta globins and two alpha globins in each structure. OK? So what we're going to do next is zoom in to see what's happening where we've done this mutation, what's going on with the placement of the valine in that structure. All right?

And wherever I put a four-letter code-- so that one was 2HBS-- that's what's known as the protein data bank code, and it enables you to go fetch the coordinates of that protein. So if any of you for the late project want to do a protein structure and print it, come to me and I'll explain a lot more about that. Or the TAs can also do that.

So let me now move you to looking in closely to the variations. So what I've done here is I've actually colored-- the beta globin is purple, and the alpha globin is cyan colored. You can see

the hemes in each of the subunits. Those are those red wire things.

And now we've zoomed into the place where the mutation is where you have a valine instead of carboxylic acid. And what you can see from this image which should stop is that the valine on one subunit in one homotetramer interacts with a sticky patch on another subunit that's made up of phenylalanine 85 in the adjacent protein and leucine 88 in the adjacent protein.

So this sticky patch on one surface glues onto a sticky patch on the surface of another tetramer. If you had glutamic glutamate there, would that form? No. In fact, it would be quite deterred from forming because you don't want to cram that negatively charged element into those two hydrophobic residues.

So what you've gone from is a situation where this really is fine on the surface. It's hydrated. It's not sticking to anything. To another situation where you have phenylalanine and leucine, which are both hydrophobic, providing a patch on the one tetramer where the valine from the other tetramer combined.

And because the molecule's a tetramer, on each of the subunits, there is also another valine that will go off and do that elsewhere, and another valine. And there's one you can't see that's tucked behind. So that's why the hemoglobin forms these structures, because every hemoglobin molecule has two places to stick to another hemoglobin tetramer, and so on. So think of the repercussions from one nucleic acid change that's really quite remarkable.

So what we've seen here is that that change occurs. And just a couple of moments for you to think about this, you can have variations at that site that won't cause a problem. Which ones of these do you think are least likely to cause a sickle cell type of phenomenon? So tyrosine, serine, aspartic acid, and lysine? So I'm going to change the glutamate to something else. Which one's going to have a perfectly normal hemoglobin? There's one that stands out. Yeah.

**AUDIENCE:** [INAUDIBLE]

**PROFESSOR:** Aspartic. That's fine. No problem. It just switched it for its younger brother. Well, which one of the others? And in many cases here, you could probably argue your way to all of them. But one would be pretty bad. Which one would be pretty bad? Tyrosine, exactly. It's another. Even though it's got that OH group, it's still pretty hydrophobic because of that ring system there.

What about the other two, serine and lysine? What do you think? Which one would probably

be, in fact, the least detrimental of those remaining two? And give me the reason as well. Yes.

**AUDIENCE:** Lysine.

**PROFESSOR:** Lysine. I think it would be lysine because lysine is now positively charged. It's equally unlikely to want to do this goofy interaction because it is also charged, just charged in the other direction. But one could also argue that serine would be OK because it's a little bit more polar, so it wouldn't cause as much problem.

OK. Finally, this issue with sickle cell anemia, there's some fascinating data that shows in parts of the world-- for example, during a drug trial for plasmodium falciparum, one of the causative agents of malaria, they found that 1 out of 15 people with the sickle cell trait was infected with malaria, whereas then the people who were healthy, normal homozygotes for the right hemoglobin, 14 out of 15 were infected with plasmodium falciparum.

Now why do you think that is? How can we relate the infectivity of a parasite with the shape of a cell? We've gone from these juicy-looking red blood cells, nice and round and probably quite open, to a cell that's sort of difficult to shape. So it turns out that the parasite doesn't want to infect the sickle cell red blood cells anywhere near as well. And there are, for example, other bloods tested which shows the same correlation.

And here's a map of Africa where you see a massive overlap of the prevalence of the sickle cell trait and the presence of plasmodium falciparum. So there is an evolutionary advantage to having the heterozygous variant where you have some normal hemoglobin but some of the sickling hemoglobin, because it confers you some resistance to malaria. It's not good to have both of them, the variant that causes sickling, because that's painful and it really causes a lot of health disorders. It's just when you have one of each gene encoding both variants. OK?

All right. Great. OK. So now we're going to talk about enzymes. And these are the proteins that catalyze reactions. Any questions about that? So while a lot of disease states actually might be bred out because someone would be at a disadvantage with a particular disease, in this case, that trait has been maintained because it offers a very different advantage with respect to disease.

OK. Let's talk about enzymes for a moment. Or for the rest of the class, in fact. OK. So enzymes are the heavy lifters of the protein world because they catalyze all the reactions in metabolism, in biosynthesis, all kinds of transformations that make you want you are. Enzyme

is a protein-based catalyst. You all know that. Terrible writing again.

There were a couple of other times I just quickly want to give you. So an enzyme, there is also a term known as an isozyme. And an allozyme. You may see them. You'll see allozyme less commonly, but you'll see isozyme quite commonly. An isozyme of one enzyme is a variation on the enzyme that catalyzes the same reaction, but it's expressed on a different gene.

An allozyme is the same enzyme, but with a variation in it. So it's encoded by an allele of one gene. So it's just a variation of the gene that might have happened through a mutation. Still catalyzes the reaction, but there's a slight change in the sequence. But they're coded by the same gene. Same gene, with a variation. And as I said, you will see the isozyme term more commonly than the allozyme term.

Now why do we need enzymes? Well, the problem is there are physiologic reactions that we need to carry out that are just too hard to carry out at room temperature pH 7 in water. They just don't occur. So you need enzyme catalysis for all of your metabolic reactions. Let me just give you one trivial example. This bond you already know nicely now. Peptide or amide bond.

If I want to hydrolyze that, if I want to break it open, pH 7, physiologic temperature, so 37c, in water, it would take me-- how many years is it? The half-life of that bond would be 600 years. OK? That's pretty untenable for digesting a Big Mac even that even under the best of circumstances. So we need enzymes to speed up breaking down proteins and carrying out reactions because otherwise, we just can't-- we can't do anything. So what I want to describe to you are some of the details of how enzymes work and then how we can control the function of enzymes.

So typical enzymes take a substrate to a product. Some enzymes may take two substrates and make one product. Some enzymes maybe take one substrate and make two products. It just depends on the transformation that you're doing.

Enzymes are classified into a bunch of different families. But the thing that will tell you that something you're reading about is an enzyme is the suffix ASE at the end of the name of the enzyme. So the enzyme that hydrolyzes the peptide bond or hydrolyzes proteins is called, no big surprise, a protease. And you'll see later on ribonuclease, DNAs, oxidoreductases, all kinds of reactions where if you see this term at the end of the name it's telling you quite loud and clear that it's an enzyme. Just a very sort of simple way of remembering that.

Now enzymes promote reactions in order that we can have them carried out at room temperature. But we want to think about how they carry out these changes and transformations. What is it about the structure of the protein that enables these reactions?

But the first thing we have to do is take a look at the thermodynamics and kinetics of a transformation. So before I go anywhere, what I want to do is describe to you how enzymes work by thinking about the physical parameters that we describe the energetics of a transformation. So in thermodynamics, you all know delta G is delta H minus T delta S. And we're really only going to worry about one of these terms. We're going to worry about delta G, and I'll explain why.

So delta G is the Gibbs free energy. H is the enthalpy T is the temperature in Kelvin. And then S is entropy. So these are the two terms when you're looking at an energy diagram, we generally think about reactions where we describe the y-coordinate as the change in delta G, the change in the free energy, and the x-coordinate is your reaction coordinate.

So in going from a substrate to a product, we generally have a situation where we have a substrate at a certain energy, and then maybe a product at a different energy. And we're going to talk about the details of that. So why do we deal with Gibbs free energy, not enthalpy? Does anyone know?

OK. Enthalpy describes the energies of all the bonds in a molecule. But when you're doing an enzyme-catalyzed transformation, you're not busting open all of those bonds. You're not breaking something down to carbon, hydrogen, and oxygen. You're only dealing with parts of the energetics of the molecule. You're only dealing with what's known as the free energy changes.

So looking at the enthalpy changes isn't going to get you very far. It's not going to describe the reaction because the enthalpy changes would be enormous breaking down that molecule. And that's not what you want to achieve. In a chemical transformation, we care about delta G.

Now the next thing to think about is what are the energetics of the reaction, and how does an enzyme-catalyzed reaction manipulate those energetics? So the key thing here is we want to talk about Gibbs free energy. I shouldn't have written quite this much stuff here because I need the Blackboard.

All right. So when you describe a reaction, you want to understand how far that reaction goes

and how fast that reaction goes. So when you go through a reaction, we can describe how far the reaction goes by thinking about the free energy of the substrates and the products. So in this case, the substrate is at a higher energy than the products. So you will go a long way through the reaction to make quite a lot of products in a transformation.

So that describes how far the reaction goes. So that is the difference between the energy of the substrate and the product. How fast the reaction goes is described in a different part of this diagram. Does anyone know what it is? Yes.

**AUDIENCE:** Activation rate.

**PROFESSOR:** Yes, exactly. How fast the reaction goes is literally how high the mountain is that you have to get over to carry out the transformation. And that height is described as the energy of activation. So that tells you how fast, and the difference here tells you how far. The energy of activation is a really important parameter because it's actually what gets manipulated when you're dealing with catalyzed reactions. So the energy of activation-- the higher that mountain is, the slower the reaction will be because it's a much harder transformation to go through.

The reactions in our bodies can be of different flavors depending on the difference in energy of the substrate and the product. So shown there, substrate going to product where the product is at lower energy than the substrate, we would call this an exergonic reaction because we're releasing energy in the transformation. So S higher than P. Exergonic.

And if we have a different reaction-- and I'll sketch this one in here-- where the product is higher energy-- and this is a reaction coordinate-- then that will be an endergonic reaction. Both reactions happen in enzyme-catalyzed systems. And we'll explain why you're able to catalyze even ones that require energy.

So exergonic releases energy. And endergonic requires. OK. What else have I got on here? We also, in the situations where energy is produced, the exergonic reactions, we call these catabolic processes. And if you have trouble remembering catabolic and anabolic, just join me in that because I always forget which is which.

But the ones that produce energy are catabolic. The ones that require energy are anabolic. And when we think about metabolism, the catabolic reactions are when we're breaking molecules down because we need energy. We need to use it to do something. The anabolic reactions are when we want to store things. Store fats, build proteins, because they're going to

be endergonic. They're going to be requiring energy to take place.

I just forgot one thing that I have shamefully done. Remember, this axis is kilocalories per mole most commonly when we're talking about delta G, or kilojoules per mole if you're in a different part of the world. But it's important to have units on these diagrams.

So that tells us a little bit about enzyme-catalyzed reactions. We need the enzyme to do something about this energy of activation. Because if we didn't have a high energy of activation and I brought a Snickers bar to eat during class, I would just burst into flames, right? It needs a high energy of activation to keep it stable under regular conditions, but only break down the bonds at times when you require that breakdown.

All right. So what did the catalyst do? OK. Now I'll show you the simple reaction. The enzymes are a very large structure. It binds to a substrate, chemistry happens, and it releases a product. But at the same time, you can't disobey the principles of thermodynamics. So there are certain criteria we have to think about when we consider an enzyme-catalyzed reaction.

So first of all, do not disobey whichever law of thermodynamics it is. They do not change delta G. Delta G is a property of the two reactants. You're not going to change it with a catalyst. It's going to have a much more, a more important impact on a different parameter. Which parameter do enzymes change and help lower? Over there.

**AUDIENCE:** [INAUDIBLE]

**PROFESSOR:** Right. So catalysts do change and in fact lower energy of activation. And we'll talk about how they do that the end. And then the last rule about a catalyst is you can recover them unchanged after a reaction. It would be a lousy catalyst if it did its chemistry and then you've used up the catalyst.

So enzyme catalysis are the ultimate green reagents. You can keep using them thousands and thousands of times to continuously turnover transformation. So you haven't changed a catalyst. So the things that we want to think about is how-- what are the processes that enzymes can manipulate?

And I should probably just quickly run through these slides so we've talked about these entities. But I put them on the board because they're particularly important. So the energy of activation of a catalyzed reaction is lower than the uncatalyzed. And I'm not going to bore you with these questions because you can work this out quite readily. So delta G is the free energy

that changes. And these are endergonic because the energy of the products is lower.

So this is the slide I want to get to with respect to the enzyme-- to enzyme catalysis. So we always think, well, gosh, the enzyme is really large relative to the size of the product. That's because all the energy within the protein-folded structure is very useful for lowering the energy of activation of a transformation.

So let's say I have a reaction that involves two substrates coming together to make a product. If I'm off the enzyme, these guys, it's going to take them a long time to bump into each other to do chemistry. The way enzymes catalyze those types of reactions is they have binding sites for both of those compounds.

In fact, the enzyme acts as a stage. One substrate binds. The other substrate binds. They're binding close to each other on the enzyme. Chemistry can happen. It favors reactions that involve multiple molecules. What about another situation where you have a bond-- for example, the amide bond-- the proteases break? It's hard to think of how that-- how can we make that more easy?

Well, amides are most stable when they are flat and planar through this arrangement of atoms. But what can happen on the enzyme is that they can twist bonds to make them less stable and then more easy to hydrolyze. So the structure of that enzyme basically holds onto the substrate and twists or distorts the bond that you're trying to do chemistry on to once again lower the energy of activation.

Another way enzymes work is in a reaction where you're breaking this bond, you might make charged intermediates. The enzyme's there to hold those charged intermediates in order to stabilize them. Once again, to lower energy of activation.

So it's funny when you get the question that's well, how do enzymes catalyze reactions? There is no one rule. You want to think about the reactions and then just think about the ways in which an enzyme could contribute to that.

For example, orienting two substrates ready to do chemistry. Causing physical strain in a bond that you want to break. Or comforting electric charges that form during a reaction coordinate. So there are loads and loads of different principles, and it's a really important study that is carried out.

So finally, I think I have a couple-- oh no, I have a couple of minutes. But I want to just describe this to you. It'll also be covered in the sections, because I'm going to rush it a bit because this last bit features a little bit on the P set. So finally, enzymes are very commonly the targets of drugs. We like to think that some drugs are important targets. If we deactivate the enzyme, we might mitigate the symptoms of a disease.

Now you can't go in and heat the enzyme or denature the enzyme if you're trying to treat a person. So we do a lot of work to mitigate disease by inhibiting enzymes with small molecules. So in these slides, I describe to you the types of molecules that may alter the chemistry of a transformation.

So if a substrate binds to an enzyme-active side-- we often do this Pac-Man rendition-- you could design a molecule that binds there instead and basically inhibits the substrate from getting there. This would be called a simple reversible inhibitor that's competitive with the active site.

There are other inhibitors that will bind to the enzyme but do chemistry with it and stay blocked at the enzyme. And that would be called an irreversible competitive inhibitor. You can't get the inhibitor off. And there's differences in the way you can reverse this. Because for example, up here, if I add a lot more substrate and these are equilibria, I can get my reaction to happen any way.

But here, I could add as much substrate as possible but it won't help. It won't reverse the transformation. OK? And there's a question here to restore the reaction. The answer really is, you just have to start with a new enzyme cause you covalently changed the protein structure.

The last type of inhibitors that are important are the ones that bind at different sites on the enzymes. And they are called allosteric. Allo always means different. So if you have a compound that's an allosteric inhibitor, it might bind on another face of the enzyme, but it will alter the active side so it doesn't work. That's an allosteric inhibitor. And the final type of compound is an allosteric activator that may bind somewhere else on the enzyme but make it more active.

So these are the way small molecules work. I'd like to encourage the TAs to just cover this in a little bit more detail because I've rushed It. And I'll also re-mention it at the beginning of the next class. But bear in mind, we should have everything covered now so the problem set 1. And if you have any questions, reach out to us. Covered them in section. And I'll reiterate a

little bit of this in the next class.

And finally, there's a little bit of reading. If you would like to prepare, we'll talk about carbohydrates next time, one of my favorite molecules. And there's also a fabulous set of videos on how enzymes work at the Protein Data Bank site. And you will see this little handout on the version of the slides that's posted.