PROFESSOR: So today we're going to take a little bit of a lightning tour through some basic topics on chemical enzyme kinetics, before thinking a little bit about some, what you might call, simple input-output relationships, in terms of gene expression. I've got separation of time scale, and also this basic notion that for a stable protein there's a natural time scale over which the concentration will go up or down, and that's dictated by the cell generation time. At the end, we'll then talk about different ways you get this thing of ultrasensitivity.

So, how is it that you can make it so that a small change in the input concentration, say the concentration of a transcription factor, might be able to lead to a large change in the output, or the gene expression of its target? We'll talk about how you can have, for example, cooperative binding at the promoter, or you can have multimerization, kind of leads to similar things here. But also, we're going to talk about this idea of molecular titration. So if you have another protein that acts as kind of a sponge, then this connect can lead to a similar effect. And this is indeed observed in various natural contexts.

So this is motivated by a work by Nick Buchler, B-U-C-H-L-E-R. Turns out I was down at Princeton, no sorry, I was out at Duke yesterday, and so I got to hang out with Nick and talk about this work. I had previously told him that in my first lecture in the systems biology class, we like to discuss this molecular titration effect. He was instrumental in elucidating how it worked.

All right so let's go ahead and get started. So hopefully you all have these cards. We're going to start out with some simple questions, just to make sure that you know how to use the complicated devices that are sitting in front of you. OK?

So what we want to do is to start by thinking about a situation where you have two molecules. We're going to call them E and S, and of course, you can imagine what these might possibly stand for in one context or another. There are two rates here that are describing the rate that these molecules, E and S, find each other, Kf. And Kr is defining the rate at which this complex is going to fall apart.

So there's this forward rate that is sum Kf, times concentration of E, times the concentration of S. Now I'd say for the first part of this lecture, we will indeed use the chemistry convention of concentrations here, although we will quickly get tired of these brackets and we'll just start writing the letters. And hopefully it is self evident in the context that I'm referring to a concentration rather than something else, but if you're ever confused, please ask.

So this is forward rate, and then the reverse rate is something similar. So we have this Kr. Now this is just the concentration of the complex ES. Many of you have spent a lot of time thinking about how we often define things. This Kd, dissociation constant, is defined as the ratio Kr over Kf. Now, just so we can practice using our cards, what we're going to ask first is what the units of this Kd thing is.

Now, in general, when I ask such a question, I will give you some A, B, C, D options. You can start thinking even before I write down the options. Yes? I'll encourage you to think before I start writing down options.

So it's either dimensionless, units of concentration, 1 over concentration, 1 over time, and I will often include at the bottom something that simply is, don't know. And that is if you're really confused about what I'm talking about, then feel free to just flash me that, and that at least tells me that I'm gibbering nonsense.

So there's going to be a very strict set of rules for how we do these flash cards, all right? You don't get to vote before I tell you to vote. You have to keep on thinking. If you think you know the right answer, check limits, just do whatever it is to keep on thinking.

And then we vote simultaneously. That way, it builds up the tension, everyone gets

excited, and then you vote. It also provides me an opportunity to make sure that I can see that everybody's participating. So if you don't vote, then you have the opportunity to tell the group what you think the answer should be and why. And the cards they're both colored, and they have letters on there, so the letters correspond to the answer. We're all on top of this? Have you had a chance to think? Or has my talking bothered you? Both. OK.

So what we do is I'm going to ask, do you need more time? If you need more time, just nod or something like that, and if more than a few people nod, I'll give you more time. But you guys are totally all right. OK let's see how we are. So then I'll say, OK, we're ready. And then we're going to go three, two, one, and then I want a vote by your chest.

You don't need to display it to the group. It's just here, and then tell me what you think. All right, ready. Three, two, one. Broadly, people know how to use the cards. And there's a clear majority of the group, although it's not 100%, that are saying that this thing is a concentration. I'd say that if the group is maybe between 25, 75% correct on these sorts of things, then I will often have you pair off in, well, in pairs. And the goal there would be to try to convince your neighbor that you're right. In this case we're a bit above 75%, so I've already indicated what the answer is. Can somebody just quickly say, why is this a concentration? Maybe in the back.

AUDIENCE: So, we know that both rates need to have the same dimensions--

PROFESSOR: Yeah, and what are the dimensions of these rates?

AUDIENCE: [INAUDIBLE].

PROFESSOR: OK. So, there are actually different conventions, in principle, but we will often be working in numbers in most of this class, in which case it would actually just be a 1 over time. But depending on whether you're doing chemistry-- So the numerators may be ambiguous, depending, but the important thing is that they're definitely the same. These are definitely going to be the same.

But it is true that in an awful lot of this class, we're going to be thinking about

3

numbers rather than the concentrations. Because, for a lot of the class we'll be thinking about finite number fluctuations of stochastic dynamics, in which case, concentration, who knows what's going to happen?

But these have to have the same units, right? And the important thing here is if you look at the right, you see this guy has an extra concentration up here. So, I think this is, on the one hand, a trivial point, but it's just really easy to forget about as you move forward. Because these things, they look awfully similar, right? There's a K, little subscript something, right? So just be careful about this kind of thing. Are there any questions about what I've said so far?

So in these cases. I think that it's really very useful to try to get some intuition for what's going on. These are all just definitions, but you want to ask, well, what happens if concentrations of various things move around? What we want to think about is just the fraction. And the reason we call this E is because, for now, we might be calling this an enzyme and, over here, a substrate, something that the enzyme is acting on.

But we'll see that, in many cases, we might be thinking about one of these as being, let's say, the piece of DNA, and then this is not even the substrate. Then maybe this is the RNA polymerase that will lead to transcription. So in various contexts, we'll think about these things having different molecular identities. But for now, E and S, possibly enzyme substrate.

So the question is, if for now we just think, these are just two molecules of whatever sort, at some concentration, and we just want to make sure that we are on top of what's going to happen if the concentrations of each of these molecular components goes either to 0 or to infinity. I think that before you do any math in life, it's good to just think about these sorts of limits, because it helps to make sure that your intuition is correct.

In many, many cases, if you think about the problem before you do any math, then when you go do the math, you'll get some solution. You can check to see whether your solution is consistent with what your intuition said. And if they disagree it

means you have to update either your intuition, or the solution, or maybe both. It's possible. But at least one of them has to be updated, and that's a way of both getting better scores on your exams, but also improving your scientific intuition.

So, in particular, we just want to do some limits. We'll think in the context, for example, if the total concentration-- your adding of the small S-- if it goes to zero, what we're going to try to get intuition about is this fraction of E-bound. It might be the enzyme. So the fraction of this thing bound, it's defined by the concentration of the complex, divided by the concentration of the enzyme, plus the concentration of the enzyme in the complex, assuming that this is the only two places that the enzyme can be located.

Now, these three arrows, that, in general, means a definition. So the question is, if we come here, what happens to the fraction of this enzyme that's bound? And, again, can't be determined-- which is different from don't know. And we're going to just do a few different limits so we want to maybe go through these quickly.

I'll give you 10 seconds to prepare your card. All right, ready? Three, two, one. So we're pretty good. So I'd say a majority, at least, of the group is saying that in this case, the fraction bound should go to 0. Intuitively, why should that be?

AUDIENCE: [INAUDIBLE]

PROFESSOR: A little louder.

AUDIENCE: You have nothing to bind.

PROFESSOR: Yeah, right. So if there's no S around at all, then you shouldn't have much of this complex, right? But you still have some enzymes. This thing should go to 0, and that kind of makes sense. And, similarly, if you add a lot, a lot of this substrate? I'll give you eight seconds.

AUDIENCE: So, you're moving the substrate [INAUDIBLE].

PROFESSOR: Yes, so S total, this is the total. This is if you have a test tube, and this is the amount

of this sugar that you add in there. So S total, then, is the sum of that and S.

Do you need more time? Ready. Three, two, one.

So we have maybe an island of people that disagree. At least a majority are saying, in this case, it should have to go to 1. Of course, this is a limit and a limit. It's always going to be between 0 and 1, but in the limit, it does go to 1. So if you just add so much of the substrate then you should be able to saturate that binding and drive all of that enzyme into the bound state.

So this one is actually, maybe, a little bit more subtle. So what if we take this limit? I'm going to give you 20 seconds to think about it, just because it's roughly three times as hard as the last one. Do you need more time? Or do you think that you have something you believe in, that you're willing to turn to your neighbor and-- Let's see where we are. Ready. Three, two, one.

All right, so this is good. So we have a fair distribution, and this is one that reasonable people might be able to argue about. It's worth having the argument. Give yourself 30 seconds, turn your neighbor, preferably a neighbor that disagrees with you, and tell them why you said what you said. If in a pair, you've already convinced each other of something, then go ahead and look around to see if there's another pair that has maybe settled on a different answer.

I think that you guys are still kind of passionately arguing, but maybe we'll go ahead and convene, and try to get a sense of-- I think, from the sound of it at least, there's some disagreement about the way to think about this. In general, if you want to volunteer an opinion or an explanation, what I like to do is, I like to tell the group what your neighbor thought. So go ahead, anybody, it could be a neighbor in quotes. Anybody want to volunteer one possible explanation of how to think about this?

AUDIENCE: Well, what my neighbors thought was if E total is defined as E plus ES, then as E total goes to 0, then this goes to 0 over 0 at some vague, unclear--

PROFESSOR: Yeah, although, right. So you're saying, maybe it's all going to 0, and then this is just

philosophy. Not that I'm putting words in your neighbor's mouth. So there's a sense in which this is true, but mathematically, and actually also physically, there are well-defined ways of taking such a limit, right?

So if you get 0 over 0, then you can use L'Hopital's Rule, which we'll have the opportunity to pull out sometime within the class. Of course, the most difficult part of L'Hopital's Rule is knowing how to spell it. So that's one answer, the mathematical answer, that you should be able to just take this limit and so forth.

But I think there is another physical answer, which is that this could happen, right? And something is going to occur, right? You could, in principle, measure. And even if you just had a single enzyme there, this F_b would be the fraction of time that that enzyme is bound. So this is a well-defined experimental question and the answer should arise from these interactions.

But then how do we edit it-- what does it all mean? How do we figure out the answer? What's another possible view on this? Maybe in the back.

AUDIENCE: My neighbor thought that, if there's a non-zero concentration of S, and the concentration of E goes to 0, then all E will be bound [? at some point ?].

PROFESSOR: This is interesting, right? So, if there's a finite concentration of S, if E goes to 0, then you say well there's plenty of S to go around, so I should always get bound. Is that what the neighbor-- that's another option. So, so far, we've had an argument for can't be determined, it's philosophy. We've had an argument for 1. Other possibilities? This is an interesting question, because depending on how you think about it, you can convince yourself that it's anything, right? Other possible answers? No.

AUDIENCE: So, I don't hear the good answer. I said E because, when there isn't very much E, then it's true that a lot of people go into yes, but yeah. I don't know what to say.

PROFESSOR: Now, that's OK. Another take on that answer, or a different one?

AUDIENCE: I don't know. If I had to complete that answer, maybe something like, but there still

might not be a total large concentration of either, so there might still be a decent chance for E to be around for a while without encountering some of S.

AUDIENCE 2: So the forward reaction rate will also go to 0. Even though S is very large, the forward reaction rate will also go to 0, as the concentration [INAUDIBLE].

PROFESSOR: By the forward rate, it's not necessarily a variable. it's just that it depends on the substrate concentration in this case. Other takes on it? This is interesting.

So I'm going to argue that the most reasonable way to view this would give you-- as long as there is some finite concentration of that substrate around, and I think that there's a very well-defined sense in which it's going to go to some finite fraction. And I think that when you're thinking about this in the context of molecular kinetics, the chemistry of you, it still is all well-defined, but the way that I think that I get the most clear intuition is just to imagine myself as being that one and only enzyme in the test tube. Now there's going to be some rate that I bind to the substrates, right? And what's going to determine that rate?

AUDIENCE: The concentration of the substrate?

PROFESSOR: The concentration of substrate, right. So if I double the substrate concentration, what should that do to the rate of me binding? It should double it, yeah.

And then of course there's always this Kf somewhere in there, and some units, right? But there's going to be some rate that I bind. And then when I bind again? Now I'm just an enzyme substrate. Now, instead thinking about this in the context of chemical kinetics, I can just think about this from the standpoint of an individual molecule, where I'm a complex, S-bound, and there's some rate that I fall apart. And it's just the balance of those two rates of finding a substrate and falling part that's going to lead to this fraction bound.

AUDIENCE: Taking what you just explained, couldn't you think about it as E always either 0 or 1, because when you're--

PROFESSOR: Yeah

AUDIENCE: --in the case of having one [? and the same-- ?]

PROFESSOR: Yes. So, if you'd like, we could put an average sign here, to say an average over time. Because in many, many cases, what we're really interested in is the fraction of time that, say, the promoter is bound, or the enzyme is bound, or whatnot. And if you have many, many, many molecules, then at any moment in time, the average is the time average. But once you're down to a single molecule, then you really want to take a time average.

We'll revisit this in just a moment using the math, but what's interesting is the math also can mislead you. Other questions? If somebody wants to argue forcefully what their neighbor said was right, then I'm happy to-- we will come back to this in a little bit.

If I want to do the fourth possibility, which is the total concentration of the enzyme, it's going to go to infinity. I'll give you, again, eight seconds to think and then get your card ready. Are you ready? Three, two, one. OK.

So we actually have a fair amount of disagreement, between As and Bs it seems. Go ahead and, again, turn to your neighbor, but maybe find somebody that disagrees with you. Sometimes there are pockets of people that agree one way or the other. So try to find each other.

Yeah, I know. I understand. You can try to figure out the expression for the fraction bound, and how that behaves. And that's actually kind of weird as well, frankly. Why don't we go ahead and reconvene, just so I can see. And maybe, let's go ahead and re-vote, so I can see if anybody convinced anybody of anything else. Ready. Three, two, one.

So it seems like now there's pretty good agreement. The answer to this is going to be A. There were a fair number of people that said B before. So here comes our curvy lines.

And so the idea here is that in the limit of the enzyme concentration going to infinity,

in that limit the fraction bound has to go to 0, because you just don't have any substrate to bind. And even if all the substrate's bound-- and indeed in this limit, what fraction of the substrate ends up being bound?

AUDIENCE: All of it.

PROFESSOR: All of it, right. So then, indeed, the fraction bound is just going to be the concentration of the substrate divided by the concentration of the enzyme. In the limit of the concentration of the enzyme going to infinity, then the fraction of the enzyme bound is going to go to 0. You're unhappy.

AUDIENCE: I think having made an extremely eloquent argument, number 3 for B, I actually think that that answer is wrong.

PROFESSOR: OK. So this one, right? Yes, I mean, you had convinced me.

AUDIENCE: So if you take E total to 0 at fixed S--

PROFESSOR: Fixed S, yes.

AUDIENCE: --then the reaction rate is--

PROFESSOR: You have to take the limits carefully, I think.

AUDIENCE: --is a ratio of the forward propensity and the backwards propensity.

PROFESSOR: Yeah. I think that the clearest way to think about it is as just that single enzyme. Because, it's certainly going to have some rate of binding, and then once it's bound it's going to have some rate of falling apart. And that ratio is not a function of whether there's one enzyme-- I mean, that ratio is well-defined. The time average of the probability of that enzyme being bound is, indeed, a well-defined quantity.

AUDIENCE: So can Ethan say D and it depends on the Kd?

PROFESSOR: Well, people always like to argue Ds. We can write down what the expression is, actually, now. And then you can decide whether you think D is justified.

We can also just try to figure out, what's the equilibrium of this thing? So the change in the complex concentration as a function of time is just going to be the rate of creation minus the rate of destruction. So there's just going to be this K forward, ES-- oh sorry, these brackets are awful-- Kr, ES. And we just want to set this equal to 0, if we want to figure out what the equilibrium there is. And in one line, you can find that the fraction bound can be written as a concentration of the substrate here, divided by Kd, plus S. Is this correct from standpoint of units?

So there's something that you might find troubling about this expression, though. Is anybody troubled by it?

AUDIENCE: But it equals 0.

PROFESSOR: You could say, what if E total is 0? I'd say that if E total is actually zero, now I think that's an ill-defined quantity. So, at some point, I'll side with the philosophers here. But if there is an enzyme to talk about fraction bound, then-- So it's related to this, but it's--

AUDIENCE: But whenever you have [INAUDIBLE] E [INAUDIBLE].

PROFESSOR: Right. So we've already discussed from our intuition that as E total goes to infinity, then the fraction bound is supposed to go what? To 0, we decided, right? And does this expression do that?

AUDIENCE: Well this is S, not S total.

PROFESSOR: Yes! So this is S, not S total. And once again, really easy to screw this up. Because, in many contexts, S and S total are the same thing. Especially, in context of enzyme kinetics, it's often the case that the concentration enzyme is really rather low, and then the substrate concentration is huge, so then S and S total we can really treat as being interchangeable. But here, in the general context, we can't. And this concentration of S, this thing is a function of the concentration of the enzyme.

So this guy here, it's a function of the total amount of substrate you have and also E total, and for that matter, Kd. So really this thing is a true statement, but it's very

misleading if you're not keeping track of what these things mean. Because, this thing is very simple, except for that it's really actually complicated because S section depends on everything right?

Now there's one context in which you can be safe in assuming that S and S total are the same, and that's in the limit of, for example, E total going to 0. So if you just have 1 enzyme, then this expression is, essentially, always valid. Every now and then, you might be using up one of your substrate molecules, occasionally. Right? But it's pretty safe to say that in the limit of E total going 0, when it's just in the limit of one enzyme, then it's really just described by a curve that looks like this.

And this curve is something that you see over, and over, and over again. And this is the fundamental reason that Michaelis Menten kinetics looks the way it does. But this is going to be very useful for us because, in many contexts that we're interested in, we want to think about, for example, the rate expression of some gene.

And what we want to know about is the fraction of time that it's going to be bound by, say, a transcription factor. So then, in the simplest case, we get an input-output relationship that is just given by this. Because, there's just one, or few copies, of that DNA, so it doesn't really sequester the transcription factor that's going to be binding it.

Do you guys understand why this is weird? A thing you have to be careful of? And more generally, I strongly recommend that, in all of these sorts of problems, it's good to just plot some things.

For example, the fraction bound is a function of if you vary the substrate concentration. Because, often you think that you know what's going on, and then when you just go and sit down to draw some curve, just get your intuition, you realize you don't know where it starts, you don't where it ends, you don't know what it does in between. It's embarrassing, but it's only when you sit down and try to do something like that that you realize that it's not obvious.

So just, for example, it's useful to imagine a situation, just between a similar E and

S, where we, for the sake of argument, say that S total is around this Kd. Now what we want to do is ask, what's the fraction bound as a function of E total? So we're going to fix total substrate, vary the enzyme concentration.

So we already know what the limit here should be. We go to infinity, what should this go to? 0. We know that eventually it should go to 0, and we already figured that out for any finite substrate concentration. Incidentally, on many of the exams, I will ask for plots of curves like this. So basically you want to indicate where it goes on one end, where it starts-- and where is it going to start in the limit of E total going to 0? Half. Then it's actually accurately described by that.

So we start here. This is 1. So we start at 1/2. And it's going to have to go in between those two, right? So what's the characteristic concentration here where something-- where it's changing a lot?

AUDIENCE: Kd?

PROFESSOR: Yeah, Kd. Kd's actually the only concentration in the problem. So that means that's what sets scale. And I don't know, Kd, exactly where it should be, but something in there. So in these sorts of situations, you want to get the limits and what is it that sets the scale? If there's a peak, is where is it? I encourage for a few toy examples, just draw some of these things. It's a fun way to spend a Saturday afternoon.

Are there any questions about what we've said so far? Yes.

AUDIENCE: What's the Fb when E total equals Kd?

PROFESSOR: So the question is, what is the fraction of the enzyme that's bound when E total is equal to Kd? I think we could figure it out, but it might actually be a little bit of math. Let me see. I would have to think about it, but it's going to be around a third or a fifth, somewhere in there. If somebody gets bored with what I'm saying, they can do the calculation and report to us at the end of class.

So what we've just done, it feels like a lot of time to spend on two molecules binding to each other, but I think that it's good to just make sure that you're comfortable with

the simplest kind of process before you start thinking about things that are super complicated, for example, Michaelis Menten kinetics.

So it's not super complicated. E plus S. So now it's the same thing here, where we have K forward, K reverse, to this complex. But here, at some rate, Kcat, enzyme does something, turns it into a product. Now this is a model of how an enzyme works. It is not a perfect description of reality in any given case, or in general. What's the most obvious possible point of concern?

AUDIENCE: There's no way to go back

PROFESSOR: No way to go back, well that's OK. What's that matter with that?

AUDIENCE: Well, sometimes there is.

PROFESSOR: Well sometimes there is. OK. I'd say the problem is, in some ways, more fundamental than just, sometimes there is. Right? It's true that sometimes-- but sometimes lots of things happen. Sometimes the enzyme binds 2 substrates. On any specific case, there are many ways that this thing can fail, but there's a more fundamental sense which is a problem.

AUDIENCE: The rate at which it produces the-- P doesn't depend on any other small molecules. It depends on other concentrations--

PROFESSOR: OK, right. So what Sam is saying is, well this Kcat is not a function of other things.

AUDIENCE: Yeah.

PROFESSOR: It's true, and in many cases in might, but there's a real sense in which this thing is failing fundamentally for any enzyme. And I just want to make sure that we're all--

AUDIENCE: Dissociation of P.

PROFESSOR: Dissociation of P, and what--

AUDIENCE: From the enzyme.

PROFESSOR: So, you don't like the dissociation?

AUDIENCE: We don't have an association there. You're assuming that the Kf of disocciation--

PROFESSOR: Oh, right. Although, I could argue, Kcat is some kind of bulk parameter that tells you about the rate of breaking some bond and dissociating. And it's just a simple model. We don't want to ask too much of it.

AUDIENCE: K reverse is huge.

PROFESSOR: K reverse is huge? Well, I haven't told you what K reverse is. So, it's not huge. I mean, it could be. So far we haven't said anything about what it is.

What are the fundamental properties of an enzyme? Or a catalyst, for that matter? When you go home for Thanksgiving, your grandmother asks you, honey, tell me, what's a catalyst?

AUDIENCE: It's doesn't get used during the reaction.

PROFESSOR: What's that?

AUDIENCE: It doesn't get used up during the reaction.

PROFESSOR: It doesn't get used up in the reaction. OK, perfect, not used up. And does this model violate that?

AUDIENCE: No.

PROFESSOR: So all right. Grandma's happy.

AUDIENCE: You can deactivate or activate these catalysts? I don't know.

PROFESSOR: Right, so it's true, there's some enzymes you can activate, deactivate. How you deactivate a protein enzyme, if you wanted to?

AUDIENCE: Denature.

PROFESSOR: You could denature it from heat or salt. But that's maybe not one of the most

fundamental.

AUDIENCE: You should have a rate from S to P without the enzyme.

PROFESSOR: Right so maybe there should be a rate, S to P, without the enzyme. Although, I'm just trying to tell you about the rate of what the enzyme is doing, so you could write a difference equation. If I just let this model go to infinity, then what happens?

AUDIENCE: You get P.

PROFESSOR: You get P. And how much P is it? A lot of P? A little bit of P?

AUDIENCE: As much as it can make.

PROFESSOR: It's all pee. Right? And how much substrate? None, right? So if I just let this go, you have 0 substrate, all product. Is that OK? I mean is that, in general-- I like product.

AUDIENCE: Time.

PROFESSOR: Time?

AUDIENCE: How much time it would make too.

PROFESSOR: Well, we can calculate what the V is in this model, and then we could figure out what the time. But there's something wrong with that. And normally I wouldn't want to belabor the point, but it's worth belaboring maybe.

AUDIENCE: Yeah. If we had a back reaction, then that wouldn't happen.

PROFESSOR: Right, OK, so this gets back to your back reaction, right? And I like the back rate. It's just there was something a little more fundamental than the way you phrased it, was my concern. Because what you said is, there might be some back rate. Right? And I guess what I would say is that there's kind of always some back rate, or that the equilibrium-- this is fundamental-- the equilibrium ratio between S and P, how does the enzyme change it?

AUDIENCE: Not at all.

PROFESSOR: It doesn't change it, right? So if you take the enzyme, invertase, and you put it in a test tube with sucrose, it's going to break down almost all that sucrose, really fast. It's going to speed things up by a factor of 10 to the 5, or I don't know, by a lot. But if you leave the test tube for a year, it comes to an equilibrium with the enzyme.

If you left it in the test you without the enzyme for a million years, you would get to the same outcome. You come to some equilibrium between the substrate and the product. And that's a function of the kinetics. There's a delta G and so forth, but the important point is that the enzyme does not change that equilibrium.

AUDIENCE: I just have a question. This molecule is effectively [INAUDIBLE]. If you leave it in a test tube for a million years, then the ATP will all be consumed.

PROFESSOR: Yes.

AUDIENCE: But if you keep providing ATP, then you should--

PROFESSOR: Well, first of all, not all enzymes actually are coupled to ATP. So ATP is a way of putting out a big delta G, right? So that you can really push things far. And ATP could, in principle, be included as a co-factor, and then you take the overall delta G of that, and then calculate it.

But if you want to keep on adding, then it complicates things. But I think, for many enzymes, it's more straightforward just to think about enzymes that don't require any extra input of energy. So they're just lowering the energy barrier and they're just speeding up the rate of reaction. But the important point there is that they're speeding up both rates.

So the equilibrium between those two is not going to change. And that's why, this thing, it's a great model, but like all models you have to make sure you keep track of what the assumptions are going into it. Because this is going to violate the laws of physics if you take this model too seriously.

AUDIENCE: I don't understand what the fundamental principle that's being violated is. Because why is it not that if you have it stable, everything is product. You never see as in

nature. I mean, how is that not a physical situation?

AUDIENCE 2: So if you can get a really small test tube with one G and one S, isn't it just like--

PROFESSOR: But the same statement that we talked about for single, then you would want-- if you had just a single substrate going to product-- then you want to look probably at the time average. Because, the thing is that the equilibrium is determined by the delta G of the reaction. And that's going to determine the equilibrium, whether you have the enzyme there or not.

So if the delta G is such that it's at equilibrium-- sort of 90% product, 10% substrate-- then what you can do is go, well if you start out with all substrate, this model may work wonderfully. But then as you're getting closer to that equilibrium, then this model's going to be breaking down because this model is not accounting for the back reaction, as you were saying. But I just want to stress that it's not just a detailed model, or it's not just a failure for some enzymes, this is the way that enzymes work.

Are there other questions about this? Or different ways of thinking about it?

So, it's not used up. It speeds up reaction in both directions.

AUDIENCE: I mean, but that's not necessarily true. You can have an enzyme that is only really capable of going in one direction.

PROFESSOR: Really? We should meet after class and you can give me your--

AUDIENCE: It basically binds in a particular direction.

PROFESSOR: It's just not allowed. So it's true that enzymes can be-- and this is getting to the other fundamental point of an enzyme, which is that they, especially enzymes in biology, can be exquisitely specific. What you're saying is that it's really only catalyzing this one, weird reaction, going from some funny substrate to some funny product, right? But that enzyme also speeds up that back reaction, going from the funny product to the funny substrate. And that's just like the nature of the beast. I'm try to think of what I can--

18

AUDIENCE: That's where you have one enzyme going one way, and another going the other way in biology.

PROFESSOR: So it does happen there, but then what they are often doing is they're coupling things to ATP hydrolysis or something, in order to actually make that reaction go in the single way. Just as kind of like a general statement-- because the way these things work is that there's some over here and it's over here somehow, and these enzymes, they just lower this energy barrier.

AUDIENCE: So the thing that confused me at first is that I was just thinking of rates, and I think the thing that's important is to just realize again that the enzyme doesn't change the thermodynamics, it only changes that variable to change where they are. So the key thing is that it doesn't change the ratio of the product through the substrate, the rates are realatively--

PROFESSOR: Right, because from a thermodynamic standpoint, it's not used up, which means there's an enzyme here and an enzyme here. So these final states, you can think about only in terms of the substrate and the product, because the enzyme was there in both beginning and ending. So from a thermodynamic standpoint, it's just you're not allowed to change one rate without the other.

Now in the reading, you saw the Michaelis Menten kinetics, where you found that once you reach this equilibrium between the enzyme substrate complex, the velocity can be described by something that is rather simple. There's some Km plus S, and then there's some Vmax. And if the substrate concentration, the total concentration is very large, then you can just think about this is the S total. Now in this case, this, once again, can be thought of in this limit of if the enzyme concentration is really small, then this is really just the fraction of the enzyme that's bound.

So we've already spent a lot of time thinking about how to get at the fraction bound, and the question is, what should this Km be here? Now that I've told you that it's the fraction bound, is it just going to be the same thing that we had before? Is the Km the same thing is the Kd? So remember, before, we found that Kd was just Kr over

Kf. But you should, in principle, be able ti just look at that and say what fraction bound should be.

AUDIENCE: Is it Kr over Kf plus-- other way around, Kr plus Kcat over Kf

PROFESSOR: Yes, because now, from the standpoint of the enzyme, there's some rate at which you form the complex. And now the lifetime of that complex has been reduced, because now there are two ways for the complex to fall apart, right? One, is could just go back where it came from, but the other is that you can catalyze the reaction.

So, from the standpoint of the enzyme and the fraction bound, then we can just-- the entire discussion that we had before-- we can just replace Kd with this new Michaelis constant, Km. Where now, we say now it's the Kr up in the numerator still, but now, instead of just being Kf at the bottom, we have to add Kcat, because there are just two ways that that enzyme substrate complex can fall apart.

Oh I'm sorry, I've already messed up. Kf over-- So Kcat has to be with Kr. So it just kind of speeds up the effective rate of dissociation. And of course, depending whether Kcat is large or small as compared to Kr, this can be either a large or small effect. But these rates, they just add. And we'll spend a lot of time thinking about how rates add and so forth in a few weeks.

AUDIENCE: For this expression to be valid, don't you need Kcat to be much longer than the other rates?

PROFESSOR: Right, yes. So you want Kcat to be-- So there's various kinds of limits in which you can talk about this thing. So in general, what you want is Kcat to be small, and you also want the initial transient to have gone away. Because when you first add the substrate, you don't yet have any enzyme substrate complex. So you have to wait until you've gotten to this so-called steady state, where the Michaelis Menten formula applies. And then you also can't have let it go too far, because then of course you're going to start running out of substrate.

In the homework, you're going to get a chance to play with Michaelis Menten kinetics a little bit, and think about the dynamics when you have different kinds of

inhibitors. So you can imagine having inhibitors that inhibit multiple different ways. You could have an inhibitor the binds the enzyme, and prevents the enzyme from providing the substrate. Now should this effect the Vmax?

We'll think about it for 10 seconds and we'll vote because it's so much fun. We have these cards. Vmax change-- and this is with an inhibitor that binds here-- and forming an EI complex, reversibly. The question is, does Vmax change? A is yes and B is no. I'll give you 10 seconds to think about this.

So Vmax is, again, defined as this rate of product formation at saturation, when you have a lot of the substrate. Do you need time? Or will time help? Well who wants more time? Just nod if you want more time. OK, well let's see how we feel. Let's go ahead and vote.

If I add this competitive inhibitor, the question is, will be Vmax change? Ready. Three, two, one. So we have a majority of Bs, but some As. Can somebody give the intuition for why the Vmax should not change? Yes.

AUDIENCE: Vmax is when substrate is far excess to the enzyme and, at that time, all of the enzymes bond to the substrate not to the inhibitor.

PROFESSOR: Right, right. So Vmax occurs when you have lots and lots of substrate. And, of course, the condition you have to be a little bit careful, because it's not just having more substrate than the enzyme, but it's when the substrate is saturating. So if you have lots and lots of substrate, then the important point there is that it's when you've pushed this reaction all the way over here, all the enzyme is bound, and that's when you get this maximal rate of product formation.

And that's true, you might need more substrate in order to get all that enzyme bound, because you have to pull the enzyme away from this side reaction. And, indeed, this kind of inhibitor alters the Km, the effect of Km of the reaction. But it does not affect this Vmax, whereas other inhibitors can bind this complex and prevent it from catalyzing the reaction. And that will instead affect Vmax, but won't affect Km.

So this was a powerful way that enzymologists have used to try to get at mechanism of inhibitors. So if you have some small molecule you know somehow inhibits some enzymatic reaction and you want to know, how is it doing that? One thing you can do is you can titrate in that inhibitor and then measure the Michaelis Menten curve to get out the Vmax and Km to try to get a sense mechanism.

And I always say we should be drawing these things. So V is a function of-- and this is in the [? lit ?] for a lot of substrate relative to the enzyme-- then we can indeed say it's going to plateau in Vmax at concentration Km. It's at 1/2. And then it plateaus. This is a very, very, very common curve. Lots of things in biology and life start at 0 and plateau, and there are almost only two ways you can do that. OK, there are more than two ways, but there are a very small number of ways you can do that. This is one of them.

Any questions on these Michaelis and Menten kinetics inhibitors? You're going to spend a couple hours over the next few days thinking about this.

So what I want to do for the last 20 minutes is switch gears a little bit and to think about the simple dynamics of gene expression. The ideas that we've just been talking about end up being just very relevant for the simple models here.

So what we want to think about is a situation where we have some transcription factor, X, that is activating expression of gene Y. So we have X activating Y. Now, the way we can think about this, for example, is that we may have X, which together with some signal S of X, turns into some X star It's X star that can bind to the promoter and lead to expression of Y.

Now in Uri's book, he talks about this idea of a separation of time scales that is often useful to invoke when thinking about gene expression. In this context, what was the fast event?

AUDIENCE: Activation of X?

PROFESSOR: Activation of X. So in many cases, if this is a sugar or a small molecule that is going

to be, in this case, activating X, that can occur really quite quickly. Often maybe less than a second. The rate-limiting step would then, in many cases, be getting the signal into the cell, so depending on how that works.

So this occurs very rapidly. What that means is if we look at a signal Sx, as a function of time, where it starts out being absent and then, all of a sudden, sugar appears in the environment, we can think about the concentration of X and X star So X starts out high and then quickly goes down, right? Whereas X star will do the reverse here, quickly comes up. And this should be flat.

Now, what is it that Y will do as a function of time? So if X is an activator that means that before X star became available, there was no expression of Y. So it should be low. So X is quickly activated, turns into X star. So we start expressing Y. So, roughly, what does this curve look like? Somebody please help me.

AUDIENCE: It's S-shaped.

PROFESSOR: OK, so it could be S-shaped. The thing that's very fast is activation of X, and then what's really still rather fast is equilibration of X star on this promoter. So that might still be very rapid because these things were nearly instantaneous, But. Coming to equilibrium here still might happen over time scales of seconds.

So that means that you actually, sort of quickly, start getting expression, at least on time scales are relevant in terms of hours kind of time scales. Of course, it still does take time to express. So it takes minutes for the RNA polymerase to transcribe, and then of course the ribosome's going to have to do something. What do I want to ask?

Let's write down the equation that Uri invokes because there's a very real sense in which it does, maybe, look a little bit more S-like. But at least in terms of Uri's kind of formalism, he often would say, the change in the concentration of this protein, it's going to be some function of, in this case X star. And then there's another term here, which was the minus alpha Y. What was the minus alpha Y due to?

AUDIENCE: Degradation.

PROFESSOR: Right, so there are two terms. So there's alpha, and it's going to be the sum of two things. There's alpha due to degradation. So if the protein is degraded actively in some way. If the protein is not degraded, then does that mean that alpha is equal to 0? No. So what is this other term?

AUDIENCE: Cell growth.

PROFESSOR: Right, so it's alpha due to some growth. And cell growth leads to some dilution effect. So if you have the same number of proteins in the cell that the cell is growing, that means the concentration is shrinking. Right?

Now the reality of this process is that it's complicated, because cell growth is not uniform. But if you kind of average over things, then a reasonable description is just to say, just a first order effective dilution rate. If you want to, you can write down a more detailed formula, or a model where, you say if cell growth does this, then-- It's going to kind of wiggle a little bit over the course of the cell cycle, but this is a reasonable description.

Now what this is saying is that even if there is no active degradation, then there still is an effective term due to this dilution. And this means that if we immediately activate, and if F of X star-- at time T equal to 0 here-- if it just goes to some beta, then what is the long time solution of this equation?

AUDIENCE: Beta/alpha.

PROFESSOR: Beta/alpha, right? So we know it should eventually come to beta/alpha. What's the characteristic time scale for it to get there?

AUDIENCE: Cell alpha's rate, it's 1/alpha.

PROFESSOR: Right. So characteristic time is 1/alpha. The solution to this differential equation is just an exponential where, if extend this line here, this is 1/alpha. So that's time. And then, of course, the T 1/2, the time it takes to get to 1/2, is indeed different by log 2, and that's the cell division time. This point here-- this is at T 1/2-- is cell division.

This is for a stable protein, assuming that alpha degradation is equal to 0.

So the thing to remember is that this basic differential equation of Y dot is equal to a minus alpha Y, is an exponential by going to 0. Whereas if you have a constant term here, then it's an exponential going to some nonzero value. So, indeed, if the signal here goes away, then we quickly come back here. So this comes here. This comes here, and then this-- does it go back down to 0? Is it more or less rapid returning to 0 than it took to come up?

All right. OK. So, how can I phrase this? OK, faster decay, question mark. A is yes, and B is no, and you can always do C or something if you don't know what I'm asking.

The question is, we've turned off the signal, is it going to go away faster, or slower, or the same? This is faster. B can even be slower maybe. C is same. Do you understand the options now? So we stopped expressing Y, so concentration of X is going to decrease, right? Question is, it is going to go away faster, slower, or the same as the rate that it came up?

Do you need more time? Ready. Three, two, one. OK so we have a fair agreement that, this thing, it's going to be the same. So there's a characteristic time for it to come and it's the same characteristic time for it to degrade away. So this, I would say, is not a priori obvious, but it's really just the nature of when you have these sorts of situations. This sets the time scale for if you want to change the concentration-- doesn't matter whether you're going to 0, a finite number, or if you go from high to low, but not 0. Again, it's going to be exponential in the same time scale.

So if you want that to be faster, if you want to be able to respond more rapidly, then one solution would be to actively degrade the protein. Right? Now it's obvious that degrading the protein actively will allow it to go way more rapidly. What's perhaps less obvious is that there's a real sense in which degrading the protein allows this response to be more rapid as well. But of course, did we keep everything constant? If I say, oh I want the curve to look like this, can I just increase the degradation rate?

AUDIENCE: [INAUDIBLE].

PROFESSOR: Well let's say that X star is already maximal saturating. So we're already-- well, OK. So I understand. OK, now I understand what you're saying.

You need to increase beta, and that could either be by increasing X star or it could be just by increasing the strength of that promoter. So the idea is that if you want a more rapid on or off, you can also increase the degradation rate. But there's a cost to that, which is that you have to make more protein. And, indeed, many transcription factors are actively degraded.

And that may be because if you are using these transcription factors to turn things on and off, then you want to get rapid responses. And, also, transcription factors are often not expressed at the same high levels as structural proteins. So that means that the cost of degrading them is not going to be as severe. If you're actively degrading cell wall type of things, that's going to be really costly.

Are there any questions of what I mean by this discussion of active degradation, why it might help, costs? Because, over the next week or two, we're going to see multiple possible solutions to this problem. If you want to increase the rate that you respond to some environmental change, one way you can do it is by actively degrading some of the signaling proteins. But there are other solutions we're going to come up with, such as auto regulation.

In the last few minutes here, I wanted to say something about this question of ultrasensitivity. So there are many cases where you would like to get very sensitive responses, i.e. you'd like to be able to make a modest change in the concentration of some, for example, transcription factor, and get a significant change in-- I don't know why I erased that but-- and you want to be able to get a significant change in the output. And one way that you can do this is by having some sort of cooperative binding. If you have dimerization of a transcription factor before binding then you, for example, can get a more sensitive response.

So one way to think that this is if you have an X activating Y right in this simple case,

then the rate of Y expression as a function of-- and here we're going to write X for now and we'll just assume that all Xs are indeed active. OK? Now if it just is a single X binding Y, then this should behave just like this Michaelis Menten formula, where there's some maximal rate of expression here. There's going to be some Kd, which is bound 1/2 the time, and then some curve that looks like this. So this would be an example of something that is not ultrasensitive and that you don't get a significant change in the rate of Y expression-- or the equilibrium Y value, if you'd like-- as a function of changing X.

As you start having more and more X, you would need get more and more Y, but that ratio, if you double X, you always get less than a doubling of Y. So the question is, what can you do to make things somehow more sensitive? You'd like something that looks a little bit more-- well the ultimate would be 0 and then beta. And indeed this would be this logic kind of limit. So this is Y expression as a function of X. If you didn't get any until some Kd and then all of a sudden you had beta, that would be as sensitive as you could possibly-- this is ultra-, ultra- sensitive.

So there's one solution that was talked about in the book to get something that's a little bit more like this.

AUDIENCE: Cooperative binding.

PROFESSOR: Yeah, cooperative binding. So we often describe these functions-- this is the rate of expression as a function of X-- as via some hill equation. So I'm just going to write Xs here. So it could be there's X, K plus X here. Now if you have cooperative binding either at the side of the promoter or dimerization, trimerzation, something before binding, you can get some effective hill coverage in here, where this is going to be up X to the n, X to the n, K to then. We put K to n here just so that all the units are still reasonable.

And as n increases, this thing becomes more and more sensitive or ultrasensitive. So this is with n just equal to 1, just a monomer binding in a simple way. Kd is still the 1/2 mark. So things always cross here, but if it's 2 then it might look like this, now three, four. So it gets steeper and steeper as that hill coefficient increases.

As Uri mentions, for many input in many genes, if you go in and you measure these things, you often get something that's reasonably well-defined by the S with a n somewhere between 1 and 4. So things are often moderately cooperative.

Maybe I'll just tell you about this other mechanism for ultrasensitivity, this idea of molecular titration. I'm going to leave you with the basic model, or the basic idea, and then at the beginning of class on Thursday, we will try to figure out what are the requirements for the various binding affinities in order for that model to work. So what's neat about this is it's a situation where you can get something that is ultrasensitive without any cooperativity. The idea is that you have some X that is indeed binding to the promoter to activate expression of Y, but, in addition, you have some other protein, say W, that can bind to X and turn it into this complex XW.

So we can always describe things as there's some Kw here, some Kd for X to bind to the promoter, and for some relations of Kw, Kd, and W total, you can get ultrasensitivity. What happens is that if you look at this rate of expression-- so this is Y expression-- as a function of X, if you don't have any W here, then indeed it just looks like our standard thing here. Whereas in the this is when you add W, when you add this molecular titration phenomenon, you can make this curve slide over so you don't get significant expression until X-- or this is X total if you'd like-- is larger than W total and this whole curve just slides over.

You can see this is ultrasensitive. So nothing happens until all of a sudden you start getting expression. So what we're going to do is on the beginning of class on Thursday, we'll try to figure out what is the relationship between these different binding parameters in order to get something that looks like this. Are there any questions about anything that we've said so far today? With that, why don't we go ahead and quit.