**JOANNE STUBBE:** In the last lecture, we were focused on the proteasome. And we were focusing on how you targeted the protein of interest for degradation by attachment of ubiquitin. So here's the model that I presented at the very beginning. And right now, we're focused on how do you attach ubiquitins to a protein of interest that's going to be degraded.

OK, and so here's the protein of interest the last time we talked about all the linkages. We're going to be isopeptides where you have lysine on the surface, or a lysine that's accessible, that can then get attached to ubiquitin, which at a C terminal end has glycine. So that's glycine 76-- that's the linkage common to everything.

And then this ubiquitin, and you can see from the structure, has a number of lysines attached depending on what the function is of the ubiquitin. It can be attached almost anywhere. For the proteasome, we're focused on lysine 48. OK, which again, makes an isopeptide linkage here. And you need multiple ubiquitins to be able to get your protein of interest degraded.

OK, so what I want to do now is talk about the equipment that's required to attach this ubiquitin molecule through the glycine to the protein of interest. And so what we're focused on is the three enzymes-- E1, E2, E3. So we want to look at the attachment. And we're going to look at E1, E2, E3, and what their function is in general.

And so E1-- and it turns out in human systems there are only two of these proteins-- by, again, by homology. And this is going to be what they call the activating enzyme.

OK, and so E1 is going to be-- so since we have thousands of proteins that are going to be degraded and only one of these E1s, it's going to play a role in many, many reactions. It's sort of the lynchpin. Over there there's a cartoon of what I'm going to write on the board.

But E1-- and when we look at the chemistry, a key player in the chemistry are cysteines and covalent catalysis by forming thioesters. OK, you've now seen this many times in the polyketide synthases. You've seen it in fatty acids synthases. You've seen it in cysteine

proteases. This is the motif that nature uses over and over again. And what she does is takes ATP and then she takes ubiquitin. And I'm just going to put G 76 at the C terminal end. So this is the C terminal carboxylate. And what she does then is uses ATP to activate the carboxylate so that it can be attached to the cysteine, which then forms the thioester.

OK, and sort of the strategy is the same. I'm not going to write out the details of this strategy. But there are two ways ATP can be used. What does ATP use to activate this into a good dehydrating agent? What does nature do? What are the two options?

**AUDIENCE:** [INAUDIBLE]

**JOANNE STUBBE:** So adenylate or phosphorylate, alpha or gamma, you see this over, and over, and over again. You have seen this used many, many times in the first half of this course. We have talked about the tRNA synthetases, the activating domains of the non-ribosomal peptide synthetases.

And so what you have done in this reaction is activated the carboxylate. And just remember this so I don't have to keep writing this down. The G is there. It's always attached to the C-terminus of ubiquitin.

And now what happens-- so this is activated. And so you have a base. You never do any chemistry with a thiol. And so one then forms that a thioester by nucleophilic attack on the carbonyl.

So what one ends up with then in the first step is ubiquitin attached covalently to E1. OK, so let me put that up. Again, all of this is written down in your hand outs.

And so what we have now is E1, where we have S attached to ubiquitin. OK, so that's the activating step. The second step is-- involves an E2. And that's called the ubiquitin conjugating enzyme. So this is going to react with E2, the ubiquitin conjugating enzyme.

What we see in humans again, that there are about 40 of these proteins. And we still have thousands of proteins that are going to be targeted for degradation. So that would imply that these E2s can be used in multiple processes and during the targeting process for degradation. So we're going to see that E2-- and so there are 40 of these. And we'll see that E2 also has a sulfhydryl group that is going to play a key role in this reaction.

So what we're going to do now is a simple thiotransesterification. So we're going to transfer the ubiquitin to E2 by thiotransesterification. So again, you have base. And you liberate E1.

And now what you have is E2 with the attached ubiquitin.

OK, so there were 40 of these things. And what does that have to tell you-- what does that tell you about E1 and E2? I haven't given you any information about structure, but there are 40 E2's, so they obviously all have different structures. But these things have got to be really flexible.

And so we have a few structures, but I don't think it even comes close to allowing us to understand really sort of the specificity of these processes. And this is a major focus of many people now. They've been discovered for a long time, but people are still messing around. And I think part of the complexity relates to the flexibility, which makes them harder to crystallize.

OK, and so E3 are the ubiquitin ligases. And the latest paper I read-- they keep finding new ones-- but there are greater than 600 of these guys. OK, so even there, since more proteins are targeted for degradation than 600, E3s are going to be used multiple times. And E3s are able to form protein complexes.

And again, I'm giving you sort of a generic overview. But you'll see in the next module, one of the key proteins involved in iron homeostasis gets targeted for degradation by a ubiquitin ligase that exists in a complicated protein complex. So that and then adds to the complexity you need to target all of your proteins specifically for degradation in some fashion.

OK, so what does E3 do? So we have an E2. And we have an E2 with ubiquitin attached. And E2 needs to interact with E3.

And this, again, is a complex. It is not a single, necessarily a single polypeptide. And E3 interacts-- ultimately what we want to do is attach ubiquitin into our protein of interest. So E3 interacts with the protein of interest.

And as I alluded to before, but I'm not going to talk about in any detail, what is the basis of the interaction of a protein? It's going around. It's doing its function. How do you target it for degradation?

In general, you target for degradation by the N-terminal modification or by post-translational modification. And every protein is different. So you might phosphorylate it. You might hydroxylate it.

In fact, I told you and Lizzie told you in the section on ClipX and ClipP, that there was this thing

called the N-end rule. OK, so one of the things you can do is you can attach different amino acids to the N-terminus. And in fact, tRNAs are actually used to do that. And we're not going to talk about the details of that.

But these things, you know, you have so many proteins that are floating around. How you're going to subtly control when you're going to degrade it is not trivial. And that's a focus of many people's attention.

So the model here is that-- remember, everything we're doing is isopeptide linkages. OK so, this needs to be set up. This complex needs to be set up so the lysine to which the ubiquitin is going to be attached needs to be adjacent to the ubiquitin.

So you have-- a lysine needs to be activated for nucleophilic attack. And now you've attached your ubiquitin. So this is a direct transfer. Again, you're forming the isopeptides.

And remember that we said in the very beginning, you don't just have one ubiquitin. You have many ubiquitins. Somebody asked me after class, how do you get the many ubiquitins attached? It turns out that people have started to study this in some detail. And in many cases, the E2s and E3s interact in a processive way to attach multiple ubiquitins.

I gave you a reference if anybody wants to read about this. It was published a couple of years ago about how you control polyubiquitination. And one of the handouts, they had an E4. There could be E4s that also act process to attach ubiquitin. So you can attach more than one to give you, basically, the protein of interest with the ubiquitins actually attached.

OK, so that is the machinery. We know what a bit about this machinery, but that's all I'm going to actually say in this class. But every system-- there are a lot of people studying this. And there are very few of these that are understood in really sort of molecular detail.

So here again is, again, that you have these kinds of isopeptide linkages. Here is that ubiquitin with the carboxylate. And here is the ubiquitin with a lysine 48 which you can attach additional ubiquitins to.

And here is another cartoon of this overall process. So what I didn't tell you in general is that E3s come in flavors. So they have little domains in them.

They have HECT-- is that what it's called? I can't remember the names of these domains. I haven't-- yeah, HECT, H-E-C-T domains, HECT domains, RING domains, U domains, all of

which are distinct and play a role in the details of how this process actually works. So again, this is something I don't expect you to remember the details from, but this is what you can imagine happening.

So in the cartoon over here, this is what I just described, that we have an E2 that has-- this little ball is ubiquitin. Here is our protein of interest, S. And so what happens is, E2 is attaching ubiquitin to the protein of interest. OK, so that's one possibility.

And in this case, the transfer is directly from E2. And that's what the ones that have been studied, the RING-finger-containing domains, do. Alternatively, you can imagine that E2 could transfer ubiquitin to E3. And once it attaches it to E3, E3 could attach it to the protein of interest. So all of those are possible.

And there has been one case where that's been studied, where E3 attaches to ubiquitin. So I think you're going to find actually many variations of this theme. This is an old paper, I think. And I think the more people study it, probably the more complex it will end up getting.

And so that's basically sort of the machinery-- with the major consideration, which I think is actually quite interesting from a biochemical point of view, is the N-end rule, how do you modify the N to target it for degradation. Does it have a half-life of two minutes? Does it have a half-life of two hours? And what governs all of that? And you can imagine that post-translational modification also governs the half-life-- so both of those possible.

And those of you interested in polyubiquitination can look at that reference. And in fact, that paper uses methodologies we've talked about in class and recitation too. They use rapid chemical quench technology to measure the rate constants for putting on multiple ubiquitins. So this rapid chemical quenched technology continues to appear over and over again when you want to look at more details about how these systems actually work.

OK, so that is allowing us to get to the stage where the ubiquitin is attached to the protein of interest. So and that is via the chamber of doom, the 20s proteosome. But now what we also would like to look at a little bit-- and this is a very active area of research-- is the lid. OK, and you saw ClipX in addition to ClipP before.

And so I just want to spend a minute on the 19s lid of the proteosome. And this lid has proteins coming and going. And when you isolate it, you probably lose proteins that are loosely bound. So this is, again, a complex-- you can tell that from this cartoon over here-- a machine of 15 to

20 proteins.

OK, and if you look at this machine, there has been a lot of people-- actually, one of Bob Sauer's students at Berkeley has spent a lot of time studying this human counterpart and has done a lot of really beautiful cryoEM on this. So again, this methodology we've been talking about has been used. Why are they using cryoEM? Because you can imagine, this is really hard to get a picture of because it's moving around a lot.

So if you look over here, what you will see is that you have a species called Rp2-- Rpt. And there are six of these. So they're all slightly different. And this is part of an AAA ATPase system. So you have the Rpt equivalent 1 through 6. And this is an ATPase.

And it sits on top of the proteosome. OK, so its function is exactly like what you guys learned about with ClipX and ClipP. What does it do? It's going to pull to try to unfold the protein. And it's going to use ATP hydrolysis to then try to thread the protein into the chamber of doom.

So the model, which hasn't been anywhere near as well-studied-- the best-studied system is the one that Liz talked about. That's why we chose to look at this. It's sort of doing the same thing. It's just there is orders of level more complexity associated with this. You can imagine how complicated this is in terms of thinking about-- from the Saunders single-molecule talk, you can imagine this is even more complicated.

So what do you have here that's also similar to the ClipX-ClipP system? Here is a hexamer. And remember that we looked at beta and alpha, they were sevenmers. So again, just like with ClipX and ClipP, you have a mismatch. So we have a hexamer-heptamer mismatch just like you did before.

And why nature has chosen to do this, I don't know. But remember, even the beta-- the alpha subunits are inactive. The beta subunits are-- only three out of the seven are active. So it's just really quite complex.

Now, what are the other things that could be involved-- these other proteins could be involved in? Well now, what's distinct from the two you had in the ClipX-P system, you had something that recognized the ssRNA tag. So you had adapter proteins.

So here what we need is something that recognizes the ubiquitins. And these could be-- and in the handouts that I've given you, they tell you which one of these is which. I'm not going to talk about that detail. But you have Rpn proteins that recognize ubiquitins.

OK, and I have another-- people are starting to get cryoEM pictures of all of this. This is a paper in 2012. Here is the protein of interest.

Here is the AAA-type ATPase system that needs to unfold the protein of interest and thread it into the chamber of doom. And you have binding sites for the polyubiquitin tail. OK, so that's one thing you need to do with your lid proteins.

A second thing you need to do is that nature recycles the ubiquitins. So what you have is enzymes that are called deubiquitin enzymes. I think that's whatever they label down here.

R11 in this molecule, Up6 are deubiquitin-- sorry. [INAUDIBLE] DUBs-- OK, yeah, so they are. Both of these guys that are involved in clipping off the ubiquitins and recycling. So you have another set of proteins, deubiquitinating enzymes.

And you have an isopeptide linkage, remember. And what kind of an enzyme might you expect a deubiquitinating enzyme to be? What kind of activity would it have? You want to cut these things off. What are you--

**AUDIENCE:** Protease.

**JOANNE STUBBE:** --going to do? Protease, OK. And it turns out almost all of them, there-- again, we're identifying them continually. They're not so sequence-identifiable by looking at bioinformatics.

The ones that have been looked at or all cysteine proteases. So the ones that have been studied in detail are cysteine proteases. But remember, they're recognizing isopeptide linkages, not peptide linkages. And as in the case of cysteine proteases, what do they involve? They involve covalent catalysis.

So again, here is another example of stuff you learned in the first part of the course that you're going to-- you see over, and over, and over again in nature. And hopefully, this is now becoming second nature to you guys, that these kinds of processes actually happen. OK, so this is the lid. I'm not going to say anymore about that.

You see the equipment. You see how complicated it is. And every system you study in biology, and if you care about the regulation, you're probably kind of have to think about degradation. And you're going to have to individually look at the proteins of interest and figure out what the E2s and the E3s are and what the signals are that control this overall process.

So this was just taken out of some recent review, but it just gives you an idea of where-- you see this is a couple of years old now-- but, where you see this kind of machinery. We're going to see it in the next section. We're going to see a key player in sensing iron is degraded by ubiquitination.

In addition, you can imagine progression through the cell cycle, apoptosis, immune surveillance, they're all regulated by protein-mediated degradation. So this is a fundamental mechanism of regulation. And so having, I think, a cartoon overview that I've given you in class is really important to have in the back of your mind when you're thinking about the system that you might be working on.

And this was a paper that was very recently published. And so we've been focusing on cholesterol homeostasis. And remember, when I introduced this topic, we were talking about Insig and HMG-CoA Reductase. And HMG-CoA Reductase is targeted for degradation by Insig. That's why we made this digression.

And if you go back now and look at what people have pulled out of the literature-- we're going to look today very briefly at gp78 in your problem set due this week. We'll see that gp78, which people thought was the whole story, is not the whole story, that there is another E3. Hopefully you will get that out of the data that I've given you in problem set three.

And there is yet another system involved in cholesterol degradation of HMG-CoA Reductase, but it's not limited to HMG-CoA Reductase. One also has degradation of the transcription factors SREBP. We've talked about those. They use different targeting enzymes.

And furthermore, a lot of people have been studying the enzymes involved in cholesterol efflux. And again, these enzymes here are also targeted for degradation. So the timing of all this and what's recognized is central to think-- people thinking about regulation, not only in systems in general, but cholesterol, specifically. OK, so that's a summary of everything we've said.

And finally, what I want to do now is just come back to where we started in this section to finish up. And where we started was, we were looking at the second mechanism of regulation and the key role of Insig, that you've already seen, plays a key role in SREBP control, keeping it in the endoplasmic reticulation. So now we're coming back and looking at HMG Reductase and the role of Insig in targeting its degradation.

And so we've seen these players now over, and over, and over again. So I'm not going to keep drawing the structures out on the board. But remember, if you have high cholesterol, what do you want to do with HMG-CoA Reductase, if you have high cholesterol? Do what?

**AUDIENCE:** Inhibit it.

**JOANNE STUBBE:** Yeah, you want to inhibit it. And so the way you inhibit it is you target it to remain in the ER. And so the question then is then, how does Insig and HMGR in the presence of cholesterol-- and it turns out, the signal is not cholesterol itself, but the signal is lanosterol.

And we talked about that very briefly a couple of times. Where do you see lenosterol? If you go back to the biosynthetic pathway, it's sort of in the middle. So you have acetyl-CoA. You have lanosterol. And you have another 19 steps before you get to cholesterol.

And somehow, this senses lanosterol. And people are trying to understand the details of that. How do you really know that's true? That's not such an easy thing, as we've talked about in recitation.

So what we want to do then is retain HMG-CoA Reductase in the ER. So these are both ER-bound. And in the presence of lanosterol, we want to target HMG-CoA Reductase for degradation. That's the goal.

The question is, is how did people go about studying that? OK, and so it turns out that they have discovered three proteins, at least in one of these systems. And the protein that I'm going to talk about for a very brief period of time is gp78. That was glycoprotein78, tells you something about its molecular weight.

Again, I don't expect you to remember the details. But gp78 interacts with Insig. OK and if you go back and you look at the little cartoons I've given you, Insig, again, has lots of transmembrane helices and is stuck in the ER.

So what do we know about gp78? And again, you see these cartoons that Liz has used and I've been using, since we really know nothing about the detailed structures of these systems. What we know is, at the N-terminus, we have an Insig binding site.

And so people had to study that. And how did they study that? Probably by mechanisms similar to what you had to-- what you thought about looking at problem set seven. It turns out that gp78 is a ubiquitin ligase, so it's an E3. So this is an E3 ubiquitin ligase. So this is-- gp78 is

an E3 ubiquitin ligase.

It has a RING domain. Remember we said there were little domains that alter the way you stick the ubiquitin on. Again, we don't know the details about this. It has another little domain called Ubc7. We're really into acronym worlds.

But what you need to know is that this is an E2-conjugating enzyme. So what you have now is an E3, they can bind an E2. That's the cartoons we just went through over here, E3 binding to E2. E3 is the gp78. E2 is this little protein domain.

And I think what's really interesting about this protein is it has another little domain called VPC. And this is an ATPase. And if you think about this, if you want to target something for degradation, where is the proteosome located that we've been talking about? Where is it located in the cell? Actually, there are multiple proteosomes, but the ones we've been focused on, where is it located?

**AUDIENCE:** Cytosol.

**JOANNE STUBBE:** Yeah, cytosol, so this is a membrane protein. So how do you get this membrane protein into the proteosome? OK, that's not trivial. And this protein, this VPC domain, uses energy somehow to pull this out of the membrane so it can get degraded in the proteosome.

So the VPC domain, well, pulls HMGR out of membrane. And so it gets degraded in the cytosol by the proteosome, complicated, actually quite interesting-- yeah?

**AUDIENCE:** Is it at all understood how that pulling out happens?

**JOANNE STUBBE:** I don't-- you know, maybe, I don't know how. I haven't found anything, but I haven't looked through the literature of any of this, the details. My guess is the answer is no, but you can go look it up.

And one of the questions you can ask is how frequent does that happen? How often do you want to degrade-- do you have this domain, and how often is that domain used? And what are the characteristics of that domain? Probably a lot more is known. I don't really know off the top of my head.

So this is a cartoon model. And so I'm not going to draw the model out. So I'll say the model, you can just see your PowerPoint. OK, and so this is the same kind of cartoon we've been

using over and over again.

So Insig is the center guy. Insig interrupts with SCAP and cholesterol to keep SREBP in the ER membrane so you don't activate transcription of cholesterol biosynthesis or the LDL receptor. We spent a lot of time on this.

So here, Insig is here again. And it interacts with gp78, which interacts with these other two proteins, the E2 and whatever this protein is that helps extract it from the membrane. A key player in all of this is lanosterol. You have lanosterol in the membranes.

So you could do, potentially, a similar study that we talked about in recitation this past week to look at do you see a switch with lanosterol, what are the lanosterol concentrations? What are the concentrations of lanosterol? And this is a cartoon showing this.

I have no idea about the details of this cartoon, but what you're going to do then is attach the ubiquitin using this E2-E3 machinery onto HMG-CoA Reductase. And remember, that protein-- we've looked at that now a number of times-- has a steroid-- sterol-sensor domain, which is lanosterol.

And it also has a cytoplasmic face. That's the HMG-CoA Reductase. You can cut this off. It's also active. And we've talked about that a number of times.

And so what they have here is just a cartoon of attaching ubiquitin, which then, in the end, magic, you end up with degradation of your membrane-bound system. So this is a major mechanism of regulation involving cholesterol homeostasis. But what you see when you look at the problem set that I've given you is that it's more complicated than that.

So you can knock out genes and you still get it degraded. What is the timescale? How do you do the experiments? And I think that's what people are seeing with all of these things.

And in part, it becomes complicated because, if these proteins need to be modified in some way, it's not so easy to tell whether they've been modified, and what it is that is recognized by the E3 ligase. OK, so I think this is sort of an exciting and interesting area. And we need some new breakthroughs so that we can better understand how these degradation systems are integrated into regulation in general.

So that's just a summary of the role of Insig, in the presence of cholesterol-- or lanosterol, in keeping the levels of cholesterol low. OK, so we finished the section on cholesterol. I think I've

introduced you to a lot of different kinds of concepts. I've told you how important it is in terms of therapeutics. People are continually studying this, as you saw by the news article that Liz had given me last time. We have this PCSK9 that's in clinical trials, in addition to the statins. And I think it's going to be on people's radar screens for some time to come.

So I think cholesterol is cool because of the spectacular discoveries of receptor-mediated endocytosis of transcription factors that are found in the ER as opposed to being found in the nucleus. And we've also introduced you to another generic mechanism of control, that by protein-mediated degradation. So that's the end of Module 5.

And what I'm going to do now-- and we've posted this information. Again, the information will always be posted ahead of class so that you can actually have the PowerPoints out there. Some things, I'm not going to write down. In this section, there is a lot more phenomenology.

What I'll try to do is give you an overview of why I've picked this phenomenology, but I'm not going to write down-- it takes a long time to write down all of the phenomenology on the blackboard. And I'm not going to do that. So integrating your notes of the things I'm going to write down with your PowerPoint, I think, is really important for you to do. And I would suggest that you bring the PowerPoint so you can see what's written down and where you might want to stick in a piece of paper where I expand on something or really tell you something in much more detail than what's written in the PowerPoint.

So Module 6, so as I just told you at the very beginning, these modules are not really linked together except through thinking about homeostasis. Everything in the cell is homeostasis. In the first lecture, we're going to be talking about metals and metal homeostasis in general using the periodic table. OK, but then what I'm going to do is focus on a single metal. And the single metal I'm going to be focused on is iron.

And so the reading is also posted. And there are three things for you to read. One is to think about iron in the geochemical world. You know, why is iron so important?

If you look at the periodic table, why aren't we using aluminum? It's the most abundant in the earth's crust. OK well using iron and not aluminum? Well, as chemists, we ought to be able to think about that. Silicon is the other thing that's one of the most abundant things in the earth's crust. Why aren't we using silica and aluminum as life-- as the basis for life?

And this article, I think it's very interesting from a chemical perspective telling you about how to

think about these kinds of things. Why is it true? And I'll give you a little bit of background on that. And then you can do as much or as little thinking about it as you choose. So the first one, I'm just going to give you an overview of why metals are so darn important and try to convince you that you should all know a lot more about metals than probably most of you have thought about from an introductory course.

Then in Lecture 2, we're going to talk about metal homeostasis in general. And that's going to be-- that could be applied to any of the metals I'm going to show you in the periodic table, but I'm going to focus on iron. And then in the second lecture, we're going to focus on iron homeostasis in humans.

And we're going to look at iron transport from the diet, where we heard this from. How does it get taken into the cell? It can get taken into the cell-- we'll see a number of ways. But receptor-mediated endocytosis, and they told us where have we seen that?

There is a protein that allows iron to be transferred around. Just like with cholesterol, you had to figure out how to keep this insoluble thing soluble with-- we're going to see there is a lot of problems with iron, so we need to figure out how to control iron's chemical reactivity. So we use a protein to do that.

There is a transferrin. It's a little protein called transferrin. There is a transferrin receptor. We'll talk about that.

And then there are many levels at which iron is regulated. Probably the most important regulation is a peptide hormone that I'll briefly mention, but that's not what I'm going to focus on. What I'm going to focus on is a new kind of regulation based on regulation of the translational process and proteins binding to RNA.

And right now, that's a very active area of research here. It doesn't have to be a protein binding to RNA, but small molecules binding to RNA. Riboswitches are being found all over the place. And so I'm going to introduce you to translational control by proteins binding to RNA.

And then the third and fourth lectures are going to be focused on more on bacteria. We know a lot about bacterial systems. Almost all bacteria require iron to survive. And Liz is the expert, so she can correct anything I say incorrectly during this lecture. Where did bacteria get their ion from? Some bacteria get their iron from rocks. How the heck do you get iron out of a rock? OK, well, bacteria have figured that out. We on the other hand are way up here. We can eat

bacteria. We can eat plants. They've already figured out how to get the iron out of the rocks. And so our problem is much easier. But so bacteria are amazingly creative.

And I've just chosen one of the creative ways to look at how iron is obtained. So we're going to talk a little bit about the host-pathogen battle. And I'm going to use specifically *Staphylococcus aureus* as an example because of the resistance problems we currently have in the clinic. You can get an iron in many forms. We're going to focus on getting iron in the form of heme, which is a major source of iron for this organism. OK, so that's where we're going. Will we get finished in four lectures? Probably not.

Anyhow, so what I'm going to do today is the first five or six slides of PowerPoint. And it's more phenomenology. And then we'll get into it, the more details, in the next lecture. So here is the bottom bottle that-- do any of you take Flintstone vitamins? Anyway, I'm not supposed to digress. I can't swallow vitamins, though I like them because they taste good.

**AUDIENCE:** [INAUDIBLE]

**JOANNE STUBBE:** Huh?

**AUDIENCE:** When we were little.

**JOANNE STUBBE:** Do you remember-- does anybody remember who this guy is? No, OK--

**AUDIENCE:** [INAUDIBLE]

**JOANNE STUBBE:** Oh yeah, all right, so [INAUDIBLE] Fred. OK, well you know, I always have this generation-- I'm much older than you. So anyhow, I mean, what you learned about in the introductory course 5.07 is, you learned a lot about the vitamin bottle, really, how the vitamins that you have, vitamin A, vitamin C, vitamin, all the vitamin Bs, et cetera, what they do is greatly expand the repertoire of reactions that enzymes can catalyze in all your metabolic pathways.

What you don't learn about in most introductory courses is the minerals. OK, so they're on the bottle too, but you sort of ignore all of that stuff. And you know, you need iron.

You need copper. You need calcium. You need zinc, et cetera. And so what I want to do is to try to give you very briefly an overview of why these metals are so important. And again, the focus is going to be on iron.

OK so here is our periodic table. And these are the metals that are found inside of us-- yeah, I

guess maybe. We don't have tungsten. Liz, do we have tungsten? We don't have tungsten. I don't think we have tungsten in us. So these are found in bacteria and us.

And so if you look at this, all of these guys over here, where have we seen magnesium before? I've been talking about that over and over again. Magnesium is bound to all nucleotides. We're going to see this again and again. We're going to talk about-- a little bit about the proper use of magnesium which makes it function in that capacity to neutralize the charge on nucleotides. Sodium, and potassium, and iron, conduction-- calcium is involved in signaling.

But what we're going to be focusing on are the transition metals. OK, and specifically within the transition metals, what we're going to be focusing on is iron. And this is-- it's hard to measure the concentrations in their localizations within the cell. But you can measure the total concentration by just taking your cell and then submitting it to some kind of mass spec analysis.

We can see iron versus manganese. And we're going to, again, be focused on iron, which accounts for about 8%. And it's been estimated in this article that approximately 50% of all the proteins have some kind of metal bound.

OK, it might involved in catalysis. It might not. In fact, the metals more likely are not involved in catalysis. And we'll look at that distribution.

So we'll come back to this a couple of times, but we're going to be focusing over here. And what are the properties of metals that make them so special to increase the repertoire of reactions that can be catalyzed inside our bodies? OK, so these guys are unique from a lot of the reactions you've already studied in your introductory biochemistry course. And so what I want to do is sort of just give you a general overview of where you see metals involved in catalysis. And then we're going to focus on iron only.

OK, so where do we see catalysis? We see iron transport. We need to get iron, potassium, and sodium in the right places, or we're in trouble. Signaling-- signal transduction, we use calcium all the time. There is huge numbers of people studying calcium signaling.

Where have you seen oxygen transport? In us-- we're in serious trouble if oxygen can't be carried by our hemoglobin to our tissues. And I'll show you a little bit about that. So oxygen transport is really important.

Of central importance is electron transfer and proton-coupled electron transfer. Where have you seen that? You've seen that in the respiratory chain. If you go back and you look at complex I, complex II, complex III, you see all these metals in there.

What are they doing? They're doing electron transfer reactions. We'll talk a little bit, but not much, about that. So not only is electron transfer involved in respiration. Electron transfer plays a central role in all of the environmental chemistry.

And so while, in many introductory classes, they don't talk about this-- we talk about humans, because most people are more interested in disease-- the coolest chemistry, without question, hands down, is absolutely associated with the bacteria and the Archae. OK, they do, like, amazing things. How do you take nitrogen and do an eight-electron reduction to ammonia? How do we do that as chemists? 200 atmospheres pressure in a 400 degrees.

This is an incredibly important reaction. Where does all the nitrogen from our amino acids come from? What about our nucleic acids? And we skip all this stuff. This is like-- I mean, this, to me, is sort of, like, amazing.

Another thing we skip all the time is where does oxygen, how does oxygen-- how does light take water and make oxygen gas? Without that, we'd be in serious trouble. The bacteria would definitely be taking over the world.

And this, I'll show you, is sort of an amazing reaction-- nucleotide reduction. We may never get there, but the last module is, I'm going to show you, you're making deoxynucleotides. The enzymes can use manganese, iron, cobalt, and iron sulfur. So they use a wide range of metals to make the building blocks required for DNA.

OK, signaling, we've all-- I just talked about calcium as a signaling agent. But now it's becoming clear, because of studies from the lipid group, and studies from Chris Chang who is a former lipid group member, signaling of metals is much more common than we thought. And people are proposing that, not only is zinc a signaling agent, but also copper. And there is a lot of problems in nerve cells with oxidative damage which we're going to come back to.

So thinking about the levels and sensing of these levels I think is going to be a future area that's going to be very exciting to study. You have to regulate these metals. Transcriptional, translational levels, we're going to talk about. And they're involved in many kinds of catalysis.

So let me just close by showing you one last slide, oxygen carriers. You've seen this before. That's hemoglobin. You've all studied, hopefully, hemoglobin and the cooperative binding of oxygen, how it binds, how it's released-- sort of an amazing machine.

That's not the only way that organisms reversibly bind oxygen. This guy, the horseshoe crab, it uses copper. This guy-- these are worms. These are found in-- they're found in the sea, right?

So you go to Woods Hole and they'll extract these worms for you. Anyhow, what do they have? They have a diiron cluster. And the strategies of both-- they all have to reversibly bind oxygen. And they've all adapted to their environments to be able to do this in an efficient way.

So what I'll do next time is come back and-- let me just do one more thing. Anyhow, this is-- think about this. Put it on under pillow. Think about how it works. Look at this. This is the cofactor of nitrogenase. Not only does it have iron and molybdenum, but look at that guy in the center-- carbon, carbon 4 minus. Think about that. We'll come back next time.