JOANNE STUBBE: At the end of the last lecture, we were talking about some generic properties of metals. And we were talking about the Irving-Williams series that was talked about, and will talk about in recitation this week, and this issue of how do you control metalation inside the cells when inherently copper is going to bind more tightly than zinc. And so if you have same amounts in solution, copper is always going to win, even though it clearly is dependent on the environment, the ligands, et cetera, which we didn't talk about.

So the Irving-Williams series is an overview based on binding. Without that much details, it assumes octahedral environment plus 2 oxidation state, and it gives you an intuitive feeling for what the binding might be. And we focused on one creative solution that is controlling the location of the folding, so that you could pick up the appropriate metal and get correct metalation. In this case, on the cyanobacteria that they looked at in the periplasm, they have two proteins that have the same structures, virtually the same ligand spheres. Yet, they're able to selectively metalate.

So and there are a few more things I want to mention about metals, in general. And the third thing is tuning metals. And the things that are going to tune the metals, we'll see when we look at iron specifically, but this is true of all metals, is the ligands, so the first coordination sphere, the geometry around the metals. So in the cases we were looking at the last time, with the Irving-Williams series, they were looking at an octahedral environment, but most of you know you can have tetrahedral environments, trigonal bipyramidal. You can have square planar. There are all kinds of environments, so you need to think about the geometry.

We'll see later on, with transition metals, we need to look at the spin states. It can control the oxidation and reduction. So we'll look at spin state oxidation and reduction. And we need to also think about-- and this is something that a lot of chemists are now finally trying to build into their molecules-- it's not just the immediate environment around the metals, but the second coordination sphere.

And this is hard to build in, from a chemist's point of view. It's not so hard to tune in from a protein environmental approach because you could have hydrogen bonding to an oxygen, which could then tweak the PKA of the ligand bound to the metal. So why do you have such a big protein? And why can't chemists recapitulate rate accelerations that are actually observed in enzymes? And it's simply because you need the whole protein.

So it really is not just the first coordination sphere and the second. You can make mutations far removed from the active site, and actually affect, in this case, the properties of the metal, or the properties of whatever groups are actually involved in catalysis. And this is an example I took out of their very recent literature which I think is quite amazing. And I also think it's indicative of where chemistry is going in the next five years in the organometallic area.

So this is a paper that was published by Yi Lu, who's at the University of Illinois. And what he was trying to do is he took a small little protein. The protein happens to be azurin that binds copper. It really doesn't matter what the protein is. But it does oxidation and reduction of copper. And what he was able to do is by changing the metal-- so he could either use copper or he could use nickel.

And by changing the first and second coordination sphere around the metal by site-directed mutagenesis-- and at most, he made five mutants-- what he was able to do is tune the redox potential over 2 volts. So that is pretty astonishing, I think. And so he has structures. Here's a thing with two histidines and a methionine. He has structures of all of these species. And we would love to be able to put something-- chemists would love to be able to put things other than copper, or iron, or transition metals into proteins and control the redox potentials.

And I think this is just the tip of the iceberg. I think this is an incredibly exciting result. And it also just allows me to show you that we've talked a little bit about iron sulfur cluster proteins, and we talked about that last time. We can go over 1.2 volts by changing the environment of the iron sulfur cluster. So you saw there were a number of flavors of iron sulfur clusters, and they all look pretty similar. But it's the protein in the environment that's tuning that.

And the question that chemists are asking is, what are the basic principles that govern redox chemistry? Instead of having to select for something to change the redox potential, can we eventually go in and just rationally make a change? Especially now since you all know we can put in a natural amino acid. So we can put in ligands that aren't the normal repertoire for protein. So I think this is an incredibly exciting time because metal-based reactions offer huge

numbers of opportunities.

And I think we aren't going to be limited to the repertoire observed in biology. So and the last thing I wanted to talk about, in terms of generic systems, is that we had a cartoon and you looked a little bit at the diversity of metallocofactors. So we have a huge diversity. Where does the diversity come from? And in general in biology, there are biosynthetic pathways to make the metallocofactors. Also the organic cofactors, as well.

So even something much simpler than the cofactor we looked at that converts nitrogen gas into ammonia-- remember, that had a lot of iron sulfurs and a molybdenum, and a carbide in the middle, and a citrate on one end. Very complex. Even in some of the simpler systems, it's likely there are going to be biosynthetic pathways to control all of this. And we're going to be focusing on, starting in today's lecture-- and the reason that I focused mostly thus far on iron sulfur clusters is we're going to see a major regulator of iron at the translational level are iron sulfur clusters.

So I think looking at an iron sulfur cluster allows you to then maybe think about-- I think people don't think very much about this. What do you need to actually make one of these clusters? So this is a four iron, four sulfur cluster. Let's see. I got to put this up here a little bit more. So we have a cubane structure. And these are attached to proteins through-- irons are attached to the proteins through systanes. And this one is attached-- I'll draw it out here-- through a systane.

And in many of the clusters now where chemistry happens, you're going to have binding through systanes. So each one of these guys is a systane. But you also have an iron that's not coordinated to a systane. So here you have any unique iron, and that is going to be key. This is iron. One of the first systems that people looked at involved-- maybe you remember back to the TCA cycle-- is a cytosolic aconitase.

And people had really thought about iron sulfur clusters as only being involved in oxidation and reduction. And this one was the first example where citrate could bind here. So you had a unique iron allowing you to do chemistry. And now we know there are 100,000 types of enzymatic reactions that use iron sulfur clusters.

So there's a couple of points I want to make because I think it's confusing, and we're going to use this when we think about the regulation. People write the oxidation species as plus 1 or plus 2. That's a typical oxidation of the iron sulfur clusters that we'll be dealing with. And so the

question is, where does that number come from? And so what they do is ignore the cysteines. And so what you have is you have four sulfides, if I've drawn this right. One, two, three, four.

So you have four sulfides, so that gives you a minus 8. And so then what does that tell you? You ignore the cysteines altogether. Then what does that tell you? To get a plus 2 state, what does that tell you about the oxidation state of the irons? So if we need a plus 2, what are the two common oxidation states of iron?

AUDIENCE: Plus 2, plus 3.

JOANNE STUBBE: Plus 2 and plus 3. So if we have plus 3 plus 3 plus 2 plus 2, that gives you plus 10, and that gives you the plus 2 oxidation. People get confused by that is the only reason I'm going through that. So if we want an overall plus 2, then you can have two iron 3s and two iron 2s. Now, it's not as simple as that because in some cases, the electrons can be delocalized, so you have 2 and 1/2 states. They're moving around a lot. And we're not going to talk about anything like that. You need to take bio and organic chemistry if you want to think about that.

So if you look at this complex, you have a cube. It's got irons and it's got sulfides. That's it. What do you need to make something like this? What do you think you need? And this is true of all these cofactors. It's not just this one. This is the one we're going to actually worry about by the end of this lecture or the beginning of next lecture. Where does the iron come from?

So you need something that can deliver the iron. Do you want iron 2 sitting around? We're going to talk about that more in module seven, but no, because iron 2 can undergo redox chemistry. So you need to deliver iron. What about the redox state of the irons? So I just told you they can be plus 2 or plus 3. How do you get that? How would you deliver the iron in the first place, given one of the rules we've already talked about?

So you have iron inside the cell. Would you want it to be delivered in the plus 2 or the plus 3 state to a protein with no metal in it?

AUDIENCE: Plus 3.

JOANNE STUBBE: OK. Everybody agree with that? See, why do you say plus 3?

AUDIENCE: Well, just because it won't undergo redox chemistry with the proteins. But I guess it is also insoluble, so then you have a problem of how do you go about delivering Fe 3 plus?

JOANNE STUBBE: So you have, how do you deliver it. But you also have an additional factor, which you have to pay attention to, is the exchange rates of the ligands. So wherever it starts, it's got ligands on it, whether it's a protein or whether it's something else. And you have to go some kind of mechanism, associative or dissociative, to do exchange into the metals. So in general, we'll see one of the rules is that it's almost always iron 2 that's delivered. And so we need to control the redox state.

And we'll see this big time when we look at humans, and how does iron get into cells. You've got toggle between plus 2 and plus 3 all of the time. And part of the rationale is related to ligand exchange. What else do we need to deliver? We need to deliver sulfide. Where does that come from? So let me just put down there's a paradoxal phosphate enzyme that can deliver sulfide from systane, for example. So where does it come from?

What about these proteins? Can you think of another kind of protein that you've seen before, that if you have an apoprotein-- so the metal's not in there, and the metal doesn't go in during folding. And there is some where the metal goes in during folding, some where it doesn't go in during folding. What else might you have to do to prepare the active site to be able to bind the metal? What kind of a protein might you have to use?

AUDIENCE: Some sort of chaperone [INAUDIBLE].

JOANNE STUBBE: Yeah, so some sort of chaperone. And you've all looked at the heat shock proteins. HSP 70, HSP 40. Almost all of these things have chaperone proteins with HSP 40, HSP 70-like activities. So you require some kind of chaperone. And this could be HSP 70, HSP 40. So I'm showing you this for an iron sulfur cluster because that's who we're going to focus on in the case of iron homeostasis.

But this is true for many, many metal clusters that are generated. And this just shows you the complexity of it. So here are the pathways in bacteria for generic iron sulfur clusters. There are two pathways, one housekeeping, one under stress conditions. And you can see how many gene products you need. And they're involved in different kinds of proteins that do all of this stuff. We have things like scaffold proteins, so if you have to make something really complicated, you make it on a scaffold first and then you transfer and you transfer it.

So ligand exchange becomes extremely important. What about this guy? This is the cluster for that huge formation of that beautiful cofactor you saw in the case of nitrogenase and how to get a carbide in there. So anyhow, I don't want to see say anything more about that. But this is

all controlled, and I think this is something that when people want to study metallocofactors in the chemistry, they always encounter problems of how to assemble the cluster. Because if you heterologously express a protein, it's not a given that the cofactor is assembled correctly.

So that's what I wanted to say about the generic properties of metals. And what I want to say now is just give you a very brief overview of metal homeostasis. So this is in general, and then we'll come back and talk about it specifically with iron. And so I'm going to say-- I'm not going to draw this out. I'm just going to say, see the PowerPoint. But I want to make a couple of points.

And one of the first things that we need to think about that's not shown in this picture is any kind of regulation. How do we control whether you want metal or you don't want a metal? It's the same thing with cholesterol. How do you control whether we want it or we don't want it? So control of metal levels can happen transcriptionally, just like we saw with the SREBP. So in that picture that I have over there, I should have redrawn it.

So this is all in the nucleus, and there is no nucleus in that cartoon, even though it's a eukaryotic cell. Transcription factor is they bind metals in some oxidation state. And they can be either activators or they can be repressors. So there are a lot of people still studying this. So these can be activation or repression. And I think almost all organisms use this in a productive way to control the metals.

We're not going to talk about transcriptional regulation at all, but it's out there. It's a real challenge if you have weakly-bound metals, like you are learning about in recitation this week.

The second thing we have in the cytosol which is also involved in regulation-- which is again not shown, so this happens in the cytosol-- is you have a piece of messenger RNA. And it turns out-- many of you probably know, but messenger RNA has a lot of secondary structures. So this is a secondary structure, which is a stem loop. So this is an RNA. And this is the five-prime end, and this is the three-prime end.

And it turns out that if you're going to convert your messenger RNA into a protein, you want to use the ribosome and you want to have translation. And we're going to see that there are structures-- stem loop structures, in the case of iron-- that can bind proteins. And when they bind proteins, what happens is you can alter. So this could be a protein. Or you can also, at the three-prime end, bind proteins.

And we're going to talk about this. So I'm not going to write all of this down. You're going to see this again. But you can control-- you can stop, for example, the translational process by putting a block over here. And we'll come back to this. Or you can stop messenger RNA degradation by putting a block there. So again, this is just another level of control that you guys haven't thought about. Has anybody seen control of RNA by little structures and small molecules binding? Have you seen that before in some class?

AUDIENCE: Ribozymes?

JOANNE STUBBE: Yes. Riboswitches. So ribozymes is the catalyst. All we're talking about here now is preventing the translational process. And so what you see, which was discovered by Breaker's lab, is that you have riboswitches. They're much more complicated than this. They have a much bigger structure. But they can bind things like adenosylcobalamin. They can bind flavins, all of which you might want to control just like you might want to control metal homeostasis.

So metal homeostasis-- this is the regulation part. So this is regulation. And then if you look at the cartoon over there-- again, I'm not going to write down all the details, but we'll just walk through it. What do we have to be able to do? So we have to be able to take metals up into the cell. Do you think there's one uptake system? What do you think? Yeah. So what you would be surprised is if you want to take iron into an E. coli, they're at least iron 5 transporters.

And the issue is, again, not many people have measured the specificity of all these things and the binding, and it all depends on the environment. So this is, I think, an incredibly important area that needs to have a lot of attention. We'll see in humans. We'll also see in bacteria. Bacteria are desperate for iron. They have an amazing number of ways to take iron into the cell. So once it gets inside, what happens to it? So the metal can come in.

Here's a metal that's sort of free. It can form what people call a labile metal pool. It comes in maybe as aqueous or one of the ligands. They always have ligands. it can then form an interaction with all of your metabolites. So and again, you're thinking about KDs for all of these things. Where does it stay? it depends on the concentrations, and it depends on the proteins you have, and what the binding constants are.

Once it gets in, there-- little things called metallochaperones. So it picks it up. A protein can pick up the metal, and then deliver it in some way to an apoprotein. So you have a protein with no metal on it at all. Can there be another protein that delivers the metal? And the answer is yes. And where this has been studied in detail in the transition metals is with copper. So they

have very well-defined copper. And that's, again, a study in ligand exchange reactions because it binds here, but you don't want it to stay over here. It needs to move over here, and how do you control the transfer so things end up in the right place?

So say you have an excess of metal. What happens? There are different ways of storing the metal. So storing the metal-- we have ways of storing zinc, copper, cadmium, and you'll see we have ways of storing iron. And so we want to be able to control that. And we'd like to be able to get it out of storage when we need it. And in many cases, depending on the metal, it's really important we store it because the metals can be toxic in the reduced state. And so you want to prevent toxicity to the cell.

So the storage also plays a key role in eukaryotic systems. We'll see you also have iron transporters into organelles. It could be into the vacuole. It could be into the mitochondria. It could be into the lysosome. And so that's all controlled. Just like you have the importers, we're going to see you have exporters out of the organelles. And not shown here-- again, this is not a very good cartoon-- you could have exporters. You could control elevated levels-- in some cases, not in humans-- but by exporting the metals out of the cell.

So there are many levels. Doesn't matter what the metal is. You see these kinds of mechanisms in all cases. And we'll focus on the case of iron in both humans and bacteria. So that's the end of the introductory lecture on metal homeostasis.

And now what I want to do, we're going to focus on iron. And as I told you at the very beginning, we're initially going to look at a few pieces of information about iron specifically, rather than metals in general. But all the principles we talked about in general are applicable to iron. And then we're going to look at what happens in humans. And specifically, after you get the big picture-- how much iron do we have? Where do we get it? How much comes from the iron? Is it recycled? Et cetera. Just like we did with cholesterol.

And we're going to be focusing on uptake into the cell, and we will see there are two ways of taking up iron into the cell in the plus 2 state. And there are iron 2 transporters. And we'll also see that there's a protein called transferrin that binds iron in the plus 3 state. So the oxidation state is distinct. You then have an iron 3 protein complex, and that gets taken up into the cell by receptor-mediated endocytosis by mechanisms quite similar to what you've seen with the LDL receptor we have, a receptor that recognizes this a little protein called transferrin. And we'll look at that.

And then we're going to do in the end is talk about, how is iron regulated? We'll see there are a number of mechanisms that regulate everything iron homeostasis, and we're going to focus on one regulation at the translational level, like we were just talking about up here. So that's where we're going in the next couple of lectures.

And so I just want to make a few points about iron. And so the first thing we're looking at is some general issues about iron, the properties of iron that we need to think about. So we're going to look at the properties. And what did we learn in the last lecture? We learned that iron is abundant. We know that 80% of the core of the Earth is iron. But we also learned that the crust of the earth-- the fourth predominant metal is iron. And so it's all over the place.

We also learned it's unavailable because we move from an anaerobic into an oxygen world, and it becomes oxidized, and the solubility goes way down. So iron is abundant. And we know that we have many, many cofactors, but it's unavailable. And if you look, an example of this-- if you take iron 3 aquated at pH 7, what you see is the solubility is 10 to the minus 18th molar. So it's not very soluble.

And again, this poses the problem, not for us, but for bacteria who are desperately trying to get iron, how do you get iron from the environment where it's insoluble? So what is one of nature's solutions that you've already discussed to obtaining iron? You talked about in the first half of the course.

AUDIENCE: Siderophores?

JOANNE STUBBE: Siderophores, yeah. So what do we know? So a solution for the bacteria and fungi is siderophores. And which siderophore-- do you remember which one you talked about in detail?

AUDIENCE: Enterobactin.

JOANNE STUBBE: Enterobactin, yeah. So they estimate that there are greater-- they have all kinds of structures. You saw a structure where you had a cyclical structure with some serines making ester linkages. Does anybody remember what the KD was for iron? This goes into today's recitation section-- today and yesterday's recitation section. How does it bind? What oxidation state does it bind in?

So I'm not going to talk very much about siderophores, but we will see that in the next lecture

after this one. So the iron, in general, binds in the plus 3 oxidation state. And the KD-- I don't remember what it is for enterobactin specifically. For some reason, the number of 10 to the minus 52 sticks in my mind. Is that correct? Or is it 10 to the minus 38?

AUDIENCE: Well, it depends on pH [INAUDIBLE]. It's minus 52 or minus 49 recorded, but then it's in the minus 20s at pH 7.

JOANNE STUBBE: So what's the one that's 10 to the minus 52? All right. How about this? So this is an interesting problem. You're going to be looking at this in class today. How do you measure that? Do you think that's easy? Do you think you're going to have any way of detecting things? So this is where you've got to be creative and think about what you're learning about in recitation. So these things-- the bottom line is, everything is dependent on the ligands and obviously on the pH.

But they bind like a son of a gun. And that's because these bacteria need to get iron to survive. So I think that's pretty important. The second thing, again, I wanted to point out is there exists a diversity of iron cofactors. And you've seen these in the first few lectures. I've blown through a number of structures. But they're found in general ways. What is the one that you're all familiar with? Where do you see iron that you all think about?

AUDIENCE: Heme.

JOANNE STUBBE: Heme, right. Why do you think about it? Why do you know heme and not some of the other? So this is heme. This is my protoporphyrin IX. Actually I can draw the structure of [INAUDIBLE] but I'm not going to draw it. I'm good at drawing structures of organic molecules, but I'm not going to draw it. It's not relevant. But why do you know heme?

AUDIENCE: It's [INAUDIBLE] It's easy to see.

JOANNE STUBBE: It's easy to see. That's it. Why do we know so much about heme? Because it's easy to see. Its extinction coefficient is like over 100,000. So those are the ones that people saw immediately. You prick yourself. It's blue or it's red. Your blood is blue or it's red. So this has a high extinction coefficient. So everybody knows we reversibly bind oxygen, but hemes have an amazing diversity.

Where have we seen heme before? We've seen it, if you remember, in cholesterol biosynthesis in the last 19 steps. We got to get rid of three methyl groups. All of those are heme enzymes which catalyze hydroxylation of unactivated carbon hydrogen bonds. So

hemes can reversibly bind oxygen, but they can also do this really tough chemistry. And how did they do that? They're controlled by the environment around the heme.

So why haven't we seen the other places where-- why don't we think about the other cofactors that involve? So we have non-heme iron, and this can be mono or dinuclear. And why don't we think about those? So no heme. So you have oxygen, nitrogen, histidine ligands, hydrazine ligands, perhaps, sulfur ligands. And you don't see this because they're not colored. In the plus 2 oxidation state, they're really hard to see.

But for every heme-dependent system, there are mono and dinuclear non-heme iron systems that are probably more prevalent that can do the same chemistry. So we don't see them, that doesn't mean they're not there, and it doesn't mean they're not important. It's just they're much harder to study. So these things are very prevalent, and they do the same chemistry as hemes. So I showed you one where you could reversibly bind oxygen. Remember those little worms we saw in the slide that can reversibly bind oxygens, just like hemoglobin?

You can hydroxylate unactivated carbon-hydrogen bonds. And where do you see that? Nowadays, one sees at all over the place because DNA and RNA modification is all mediated by, in many cases, alpha-Ketoglutarate, non-heme iron, dioxygenase. So I don't want to say any more about that, except they're extremely important, and they're hard to study. But we have really good tools to study all of these things.

And then the other one, which we've just been talking about, which is the focus of the section on iron homeostasis, is iron sulfur. And so iron sulfur, for decades, was thought to be oxidation reduction electron transfer, which we talked about. But we now know, again, through these radical SAM proteins, there are just basically hundreds of complex radical reactions that we'd just be scratching the surface in learning.

So this is also on there. Again, greater than 100,000 reactions, and these reactions are chemically interesting. So from a chemical point of view, the frontier, in my opinion, is not in the organic side at all. It's in the metal side. I think we don't have that much. You know, we have a little bit of intuition about what happens, but what we're seeing is things that we didn't expect to happen at all. We're seeing it in proteins, and then people are trying to figure out whether they can make the same things happen in solution and take advantage of all of this.

So we have a diversity of metallocofactors. What about ligands? And almost anything can be a

ligand. So it can be a protein, or it can be a small molecule metabolite. So you can have proteins. What are the ligands you might think you would find on iron? Tell me what the amino-- give me the one letter codes.

AUDIENCE: D, E.

JOANNE STUBBE: D, E. OK. Give me a D. Give me an E. What else? What else? Come on. Let's go.

AUDIENCE: H.

AUDIENCE: C.

JOANNE STUBBE: H. They don't have to be in alphabetical order.

AUDIENCE: C.

AUDIENCE: C.

AUDIENCE: C.

JOANNE STUBBE: C. Try one more. You'll see it in a minute.

AUDIENCE: [INAUDIBLE] for water.

JOANNE STUBBE: Water? Yeah, water is wonderful. I'm not going to write down water. That's not an amino acid. So how about tyrosine? So what's amazing now is we even see things like arginine. That has a PKA of between 10 and 11. And the Drennan Lab has found several proteins where arginine appears to be-- and other people-- a ligand. So we have a diversity of ligands from the amino acid side chains.

If you look at metabolites, we've already talked about citrate. Is that how you spell citrate? Citrate is in the TCE cycle. Alpha-Ketoglutarate-- that's also in the TCA cycle. I'm not going to draw this, but these are major players that mediate chemistry on iron-independent systems. So we have a diversity of these things. What about the geometry of all of these things? The geometry can be octahedral. It can be tetrahedral. It can be trigonal, bipyramidal, et cetera. Almost anything you can imagine, you can find it.

Nature has figured out how to use this. In that paper by Yi Lu, where I told you they were changing the redox potential over 2 volts? One of the things they invoked was figuring out how to strain the metal to enhance the ability to reduce it to change its confirmation, which might be

more favorable. So you have just really a huge number of things that you can deal with in these metals that I think allow the huge diversity of reactions that we're still unraveling, actually.

So the other thing about iron is that, what are the oxidation states of iron? So we have the redox states. I can't remember what number I'm on. So we've just been going over and over again, these are the two common states. Iron 2, iron 3. And in the last lecture, we talked about other oxidation states. And it turns out if you look at the chemistry of what's going on, and you want to hydroxylate an unactivated carbon-hydrogen bond, you frequently see iron 4.

And usually iron 4 is not sitting around in the test tube. It's activated, so it wants to get reduced. And that's what allows it to be able to do the chemistry. So unlike these guys, these are the workhorses you see over and over again. That's what we're going to see in iron homeostasis in general. But one also sees iron 1 or iron 0. And where does one see iron 1 or iron 0? Again, remember those ligands on the hydrogenase I showed you? Iron hydrogenase is what I showed you. There's an iron nickel hydrogenase. There's an iron-only hydrogenase.

People are really interested in this for the energy problem. Hydrogenases are really, really fast. And what kind of ligands? Remember, we discussed this. And the ligands are going to control the chemistry. What kinds of ligands did you see? You saw a CO in cyanide. So that allows very different properties of the metals, in terms of the spin states you'll see, that allows different chemistry to happen. So let's just recall we have CO in cyanide ligands.

So again, this is not the norm. But there are many systems where these have now been formed in unusual bacteria. We don't see these ligands, at least I don't think, in any eukaryotic systems.

So the other thing that you need to think about with metals, if you get into it and start thinking about it-- and this is key to really, how do you know you have an iron 4? How do you know you have an iron 0? How do you study whether it's iron 2, iron 3? And that's different dependent on the spins states, because you have dielectrons associated with both the iron 2 state and the iron 3 state. So if you go back into freshman chemistry, or if you've had 5.03, you need to think about the spin states.

And what we have is high spin and we have low spin states. And this is dependent on the ligands. So this is going to be ligand-dependent. And if you look at iron 2, you have six electrons in the d orbitals. If you look at iron 3, you have five electrons. And so if you look at

the d orbitals in an octahedral field, depending on the ligands, the energetics of the d orbitals are quite distinct.

Again, we're not going to talk about this in any detail. But what you can do, then, is if you want to put in five, depending on what the energy differences are, they might be all unpaired, or they could be paired. So this unpaired is high spin, and the paired is low spin. Do you think they're different spectroscopically? The answer is yes. And we have lots of physical biochemical tools that allow us to look at the differences between all of these things. And so this is, again, an active area of research.

So the last thing I want to talk about in terms of iron properties are going to be key for us thinking about module seven. So there are two kinds of iron properties that you will be introduced to this semester. So we're looking at, now, the chemical diversity. And so one of the things is that, remember, when we're in an anaerobic world, we could use iron 2 because we didn't have to worry about any redox chemistry. Now in humans, we're in an aeorbic world, and we're faced with this issue of reduced metals and oxygen.

So what you're going to see is, in the presence of oxygen-- and we're going to go into this in some detail in module seven. We're not going to spend a lot of time on it. But you learn a little bit about what we call reactive oxygen species. In the presence of oxygen, you can form iron 3, and you can form a molecule that looks like that. That's super oxide. And many people call this a reactive oxygen species. It depends on its environment whether they're reactive.

So again, from a chemical perspective, I think thinking about what's possible is really the key in the kinetics, and what's around-- the concentrations, the kinetics, what's around. That's what has been missing in the reactive oxygen field. And for example, in the presence of protons-- we'll talk about this in detail, I'm not balancing the equations-- we can form another reactive thing that's considered a reactive oxygen species, which is hydrogen peroxide.

And I'll show you that that really, in one or two cases in proteins, that can be very reactive. But in most cases, it's not all that reactive at all. And what we will see is iron 2 can react with hydrogen peroxide. Again, I'm not balancing my equations. We'll come back to this later on. But here's where we do form a reactive oxygen species. And this is hydroxide radical. And hydroxide radical, we'll see, can react with anything it hits. So this is really reactive.

So all I'm pointing out here is you're forming species. They're all reactive. All molecules are

reactive to a certain extent, and you need to put yourself into the context. So this is really a reactive oxygen species. And these guys are the focus of module seven where you'll be introduced to the fact that hydrogen peroxide can, in some way, be used to kill bacteria. We're going to see how that's done.

But hydrogen peroxide is also now thought to be a second messenger in a signaling agent. So again, it's all about homeostasis. So with iron diversity, we've talked about hydroxylation in the cholesterol biosynthetic pathway. We're going to be focused now on this kind of redox chemistry. And so that's all I want to tell you about in terms of introduction to iron. All the properties we talked about-- wrap, exchange, the exchange reactions, et cetera-- you need to think about when you're thinking about iron, as well.

So what I want to do now- so going away from these general ideas about how iron works, and we want to go into an overview in humans. And the first thing, in many of these cases, the pictures are really complicated. So I urge you to pull out your PowerPoint slides and look at them, and then just annotate them a little more. Because I mean otherwise, I won't get through. I'll spend all my time drawing the same pictures on the board.

So one of the things we care about in this section is iron distribution. We cared about that with cholesterol, as well. So this was taken out of some textbook, and I assume it's correct. I don't really know that much about iron distribution in humans. But they say the average adult has 3 or 4 grams of iron. You know, I sympathize with you guys for not being able to read my writing. When I write something now, half the time, I can't read it either. So when I was young, my writing was beautiful, and my board work was beautiful. And it's gone because we don't write that much anymore.

So anyhow, iron distribution. We have 3 to 4 grams. And we'll see that, in contrast with cholesterol, where we take a lot in from the diet and then we have to regulate everything-- the biosynthesis of this, the uptake of all of this-- we don't take that much in from the diet, and almost nothing goes out of us. So it's really the iron is recycled in general. So this is really different from what we saw with cholesterol.

And from this one book, the numbers are all about the same. So I think they're OK. Where would you expect to see the most iron?

AUDIENCE: Hemoglobin.

JOANNE STUBBE: Hemoglobin, yeah. And that's-- so hemoglobin, 2.6 grams. Where else would you expect to see iron? How about myoglobin? Myoglobin takes the oxygen from the hemoglobin and delivers it to the tissue. Remember, we talked a little about metal storage. So these are metal storage proteins. There's a gram there. That's going to be found in the liver. It turns out that only 4% of the iron is found in proteins that catalyze these many reactions.

So next time, we'll come back. We'll have a big overview of iron in humans. And we will also talk about regulation at the translational level.