JOANNE STUBBE: So that's where we're going in terms of Module Six. We introduced this the last time. And this is the required reading that's also been posted-- and we started this last time.

This introductory lecture is talking about metals, in general-- the chemical properties of metals, and why we have these kinds of metals in our bodies. Why we're using these kinds of metals. And focusing then on metal homeostasis, in general.

And then we will move over into complete focus on iron for the next three-- so, subsequent three lectures. OK.

So here we are-- here we are with the periodic table. And we're going to be focusing on transition-- the transition metals, which are most of the places where you see the chemistry that you're familiar with. And this just sort of tells you the relative abundance of the metals in our bodies. OK?

And we talked about last time-- sort of an introduction to the different kinds of chemistries that we can have. And we talked about iron transport, reversible oxygen binding, and then we were at the place for electron transfer with nitrogen fixation. Again, this is a cursory overview with signaling, where I introduced the fact that you have calcium. And also you can have zinc and copper signaling, which people didn't realize until recently. Zinc is worked on extensively by the Lippard Lab and copper is worked on by other people-- the Chang Lab at Berkeley

We will see we're going to have regulation at the transcriptional and the translational level-- that's true for all metals-- and that many kinds of reactions can happen. I'm only going to focus on the reactions that we're going-- that are related to iron in the course of this module.

We had gone through reversible oxygen binding, and at the end of the last lecture we were focused on the amazing diversity of metallocofactors. These are some of my favorite metallocofactors. Most of you, I think, are not exposed to this. You sort of know there's an interesting cofactor, but I haven't really thought about how these cofactors work. And if you

look at this one-- at the end, we were talking about-- you have-- this is the active cofactor formed-- found in the enzyme nitrogenase, which does an eight-electron reduction of nitrogen to ammonia plus hydrogen.

And there are many enzymatic systems that use multi electrons, OK? And that's an active area from the chemical point of view, as well. How do you control multi electron oxidation and reduction, and what is the multi electrons versus single electrons get you? That's a hot area of chemistry now in the bio-inorganic world. And I think most intriguing is this little carbon in the middle. That's a carbon minus 4. How does it get there? Where does it come from? That should intrigue you and-- actually, where is even the reactive species? Where does nitrogen bind to do the reduction?

Hydrogenase is another active area of research, now-- in energy. People thinking about how do you do-- how do you use catalysts to do oxygen evolution, here? Or hydrogen reduction or oxidation? And people who have taken inspiration from enzymes called hydrogenases, and they come in many flavors. You have nickel iron, iron iron, iron only. And we know quite a bit about the actual chemistry. The rate constants for turnover are amazingly fast, and so people are trying to do that in little devices nowadays, using this as an inspiration to generate these kinds of catalysts.

And what do you see unusual about this iron cluster? I'm digressing, but I think this is a good thing for you to know. I wouldn't expect you to remember the details, but what's unusual about this cluster? Anybody see anything from a chemical perspective that's unusual?

AUDIENCE: You mean, like all of the ligands on--

JOANNE STUBBE: Yeah. Look at the ligands. What's unusual ?

AUDIENCE: Is there a carbon with five bonds?

JOANNE STUBBE: There a what?

AUDIENCE: Carbon with five bonds?

JOANNE STUBBE: I don't see any carbons with five bonds. OK, yeah. So, OK. That's not what I want you-- so again, it depends on what the bonding is.

But what is unusual about the carbon with five bonds?

AUDIENCE: It's attached to both.

JOANNE STUBBE: Yeah. So that's not what I mean, though. What is unusual about the ligand?

AUDIENCE: It's carbon monoxide.

JOANNE STUBBE: Yeah, it's carbon monoxide. What do you know about carbon monoxide? It kills you, right? OK. So how do how the heck do we have organisms that have carbon monoxide ligands, right? And we all have carbon monoxide detectors in our house because it binds to our heme proteins and kills us.

What's the other thing that's unusual about the ligand environment, here? What's the other ligand that's unusual?

AUDIENCE: Cyanide.

JOANNE STUBBE: Cyanide. That also kills you. So automatically as chemists, you ought to be intrigued by where the heck did these things come from, and how do you prevent it from killing the organism? At the same time, is this able to use these ligands to actually do chemistry? And if you think about transition metal chemistry-- we won't talk about this, but we will see-- what are the oxidation states of iron that you're most familiar with?

AUDIENCE: Two, three--

JOANNE STUBBE: Two and three. OK? And then what we'll see is four happens transiently, but we also go to iron zero's. So we have a wide range of redox spanning chemistry by altering the ligands. And that's going to be one of the take home messages from the four lectures. OK?

And this is, I think, totally amazing. We now have an atomic resolution structure without the metals being destroyed, which normally happens when you put a metal into an X-ray beam. The electrons reduce the metal and you don't end up with the cluster you think you're going to be getting.

And what you hear see here is you have four manganeses and a calcium. And again, this is a multi electron process where, in order to go from water to oxygen uphill, you need to have light. And a lot of people are focused on that now, in terms of energy production and chemical catalysts that can mimic these kinds of reactions.

But what we're going to be focusing on now in the case of the iron is the iron cofactors. And

most of you have seen these iron cofactors before. This is just a few of the iron sulfur cofactors. Where have you seen them before? What part of biochemistry in your introductory courses have you seen these before? And then I'll tell you where we're going to see it again.

Nobody has ever seen them before? No?

AUDIENCE: A lot of single-- a lot of single electron transfer--

JOANNE STUBBE: Yeah. So it's single electron transfer. Where? In respiration. Hopefully you all did that as a basic introductory part. You have iron-sulfur clusters all over the place, and these are the clusters that were found in the prebiotic world. So they are incredibly interesting.

What we're going to be focused on are four iron, four sulfur clusters. That's a key-- that's a key component that allows us to sense iron in humans. And so we'll come back to the four iron, four sulfur cluster later on. And all of these-- I just leave you to think about, where do these things come from? You just think you throw in iron and molybdenum and you have a cofactor that looks like that? The answer is no.

The other thing that I just wanted to introduce you to because this is a very active area of research in our department-- the Drennan lab-- and a lot of my former students have been working on enzymes called radical SAM enzymes. What does SAM normally do? S-Adenosyl mithionine? What does that normally do in biology?

AUDIENCE: Does it methylate something?

JOANNE STUBBE: Yeah, it methylates. So, you know-- here is that you have something that's activated for nucleophilic attack-- that's what it normally does. We now know there are 130,000 reactions that involve SAM that doesn't do a methylation. You do reductive cleavage of the carbon methyl bond to form this carbon methyl-- carbon sulfur bond to generate a radical. And you do really complex free radical chemistry. For example, 50% of all the methane gas in the environment comes from a radical SAM enzyme that cleaves the phosphorus carbon bond.

If you look at the antibiotic resistance problem we have now, there's methylation in the active site-- the A site of the ribosome that you guys talk about-- that prevents five different antibiotics from binding. And it doesn't involve methylation in the standard form, it involves complex free radical chemistry. So I'm not going to say any more than that, but radical chemistry is taking off. I mean, there's all kinds of unusual chemistry that peak chemists didn't

think was possible before, and we're-- every time we study another system, we learn something new and exciting from a chemical perspective.

OK. So now what I really want to do is sort of get more into the nitty gritty. And so one of those-- I showed you the periodic table. We have manganese, we have iron, we have copper. Why were those chosen? And in part, those are chosen because it reflects-- the metals reflect earth's history.

And one of the-- so, one of the things in the geochemical-- what we know about the geochemical production of the earth over the eons since its first-- since a big bang, anyhow. Here's the earth's core.

And this is taken from the article by Fry and Reed. And I think it sort of-- this and then the next slide I'm going to be showing you-- sort of, I think, places in perspective why we're using iron and copper and zinc in almost all the enzymes we see inside of ourselves. So the earth's core is here. And then we have-- so we have the inner core, and we have the outer core. These two cores are 80% iron. OK?

We then have the mantle. And then we have the crust. And the crust has-- the fourth most abundant metal is iron. But you also have other things in the crust-- aluminum, calcium, silicon. Why are we using carbon and not silicon, if this is the most-- most abundant-- one of the most abundant elements in the earth's core? And this article sort of goes in and discusses those kinds of issues. Making you think about what you learned in freshman chemistry about the periodic table.

Iron. Iron is everywhere. The most abundant element in terms of mass is iron. OK? And so, iron, you might expect from this description, to be front and center. And in fact, it is front and center.

And so the other thing I think you can think about is solubilities and evolution of-- from the beginning, where we were in a completely anaerobic world. So here's the gaseous environment with oxygen. In the very beginning up to 2.4 billion years ago, it was completely anaerobic.

OK? And so is that important? So if we go to 2.4 billion, it's anaerobic. So now, if you look at-- and this is-- where these data come from and where this model comes from, it-- obviously everything is a model. You can go back and read this in detail if you become interested--

maybe some of you might have had a geology course where you've discussed this before.

But if you look over here, where do you see iron? OK, so iron is going to be the focus. Where is it compared to cobalt, nickel, manganese, all these other-- zinc, copper-- all these other transition metals? It's way up here. So it's most abundant under anaerobic conditions.

What do you think the oxidation state is? So you just told me you had iron that you've commonly encounter is two and three. And that's correct. Everything-- you're going to encounter this over and over again. Hopefully you have encountered this before.

What happens in an anaerobic world? What do you think the oxidation state for iron is?

AUDIENCE: Maybe two?

JOANNE STUBBE: Yeah. It's two. And I think this is incredibly important from a chemical perspective, because many enzymes we're going to see-- metals-- can catalyze reactions by polarizing carbonyls, for example. In an anaerobic world, you likely used iron two all the time.

But what's going to happen when we get over here in an aerobic world? And so that's the key question. And do we see iron two used in that capacity? The answer is no, because in the presence of oxygen some other reaction out competes it. So that's why I'm introducing you to this. It's sort of-- I don't expect you to remember the details, but I think it's an interesting exercise to think about what happened when we transitioned from an anaerobic world-- and this is all in the ocean, and versus the atmosphere-- into an oxygen atmosphere. And this is 0.8 billion years later.

And if you look at this, what happens-- and this is a period where they believe that you had a lot of H_2S around. And remember, we just saw iron clusters with all these sulfides on them. Iron sulfur was in the prebiotic world. They can self assemble. They do all this kind of chemistry that-- until they knew about this radical SAM super family-- they thought was one electron, oxidation, and reduction. And nothing could be farther from the truth. Iron sulfur clusters play a key role, for example, in DNA replication.

OK, so I think-- thinking about this and where these iron sulfur clusters came from, you provided some insight perhaps from looking at the geological record of what people think was occurring. So we went through a period where you had a lot of H_2S. Concentrations of species have changed.

And then we move into the aerobic world, and what happens here? So what happens to the iron? It's dramatically decreased.

So when we go from the anaerobic to the aerobic, why does the iron-- what happens to the oxidation state of the iron? It gets oxidized to iron three. So we're changing in the oxidation state, and so we're going to have to deal with it.

So I'm going to show you, this presents a major issue we face now, both as humans and as bacteria. If you look at this, what happens to copper and zinc-- if you believe this model? That the copper and zinc concentrations increase. And in fact, that becomes really important.

Because if you look at the biological record, and you look at archae and bacteria that are much much, much older, what you see is-- you don't see that many copper catalyzed reactions in zinc, which has a really important role in humans, with zinc fingers. Doesn't play a role like that in bacterial systems. So I think this represents an interesting way to think about metal speciation, oxidation states, what ligands are going to be involved in what's happening-- and also what's happening in bacterial systems.

The key thing that I want you to remember about this is that in the aerobic world-- so we now go from iron two to iron three. And what we'll see is the solubility properties of iron three are dramatically different, and that's something we're going to have to deal with. How do we get-- we talked about this last time-- how do we get iron out of a rock? OK, so that's an issue if you're a bacteria-- you have to figure that out. And bacteria have done some pretty cool things to figure that out.

And so-- OK, so this, I think, also has-- we're going to be focusing on iron here-- important implications in terms of the chemistry. So in terms of being in an anaerobic world, we can use iron as a Lewis acid. OK? And so it can polarize a carbonyl. We'll come back to this in a minute.

Nowadays, we almost never use iron two as a Lewis acid in biological systems. And why is that true? Because when we transitioned to the aerobic world, now we have this problem of-- that the iron three is what? It's insoluble. So that's one problem.

And the second problem is that since it's insoluble, we can't use it. How do you get-- how do you get it to actually use it for chemistry? We're going to-- we're going to talk about that. How do you get it to look at chemistry.

And then we have this issue of oxidation with oxygen, and this is going to lead us into module seven. So while in the very beginning, we used iron to do a lot of chemistry without oxygen around. We then moved into an oxygen-- oxyphilic world, and we have this issue of during this oxidation using oxygen as the oxidant-- what happens? You produce reactive oxygen species.

OK. So, and then you have also the problem of insolubility. So you generated-- by making this transition into an oxyphilic world you're encountering two major problems that we're focused on. How do you deal with the insolubility problem and how do you deal with reactive oxygen species? And that's going to be-- following this module, we're going to talk about what happens with reactive oxygen species as a consequence of moving from an anaerobic to an aerobic world.

I don't want to spend a lot of time on this, but I want to make sure that you understand there are some kinds of reactions that are really distinct from the reactions you meet in the organic world. And a lot of you-- we looked at the vitamin bottle, we learn a lot about flavins, we learn about pyridoxine, we learn about vitamin C-- all the vitamins we learn about. But we sort of ignore the metals on our bottle that's required for life. And so I don't want to spend a lot of time, but there are-- what are the general reactions? So I just want to say a little bit about general reactions.

OK. And one of them is this idea of Lewis acid-- or Bronsted acid. And so what you can have is a carbonyl, and you can have a metal that can activate the carbonyl you for nucleophilic attack. OK? Where have we seen this before? We've seen this before in-- if you go back and you look in the glycolysis pathway, lots of times you use zinc to activate the carbonyl. Sometimes you use shift spaces, maybe. You remember that? In aldehyde dehydrogenase, aldehyde oxidate that converts aldehyde to an acid or reduces aldehyde to an alcohol-- they use zinc. OK?

In the completely anaerobic world, people thought-- most of the time they probably used iron. That was one of the most-- that was much, much more prevalent than zinc. But then things-- so if you go way back and you find bacteria that lived in that period, they still might be using iron in catalysis. But now we almost never use iron two in catalysis, because of the issue of the redox chemistry.

So now they're saying the-- so it's now, you know, your polarize this for a nucleophilic attack. You've seen this over and over again with the Claisen reaction, the Aldol reactions, et cetera.

I'm not going to go through the details.

Another place you see it-- and where have we seen this one? Again we have a metal-- and I'll just leave it in the plus two oxidation state. But what happens to the pKa of the water bound to a metal? And what happens is the pKa is dramatically reduced. You have two positive charges here, depending on the interact-- and that interaction's unfavorable. So the pKa becomes reduced on bonding to a metal.

Where we've seen that before? We saw that in the cholesterol module. We didn't talk about the chemistry-- again, I come from the chemistry side of it so I find the chemistry the most interesting-- but it fits into the biology.

Where have we seen this before? Anybody remember-- in cholesterol? Homeostasis? What happens in the Golgi when you want to go from the Golgi to the nucleus?

AUDIENCE: A zinc--

JOANNE STUBBE: Yeah, we had a zinc protease. So that would be an example. An example of this would be in the cholesterol section. And I'm not going to talk about this in detail. I used to talk about this in a lot more detail, but you can see with different metals-- this is just an example of the first case I'm giving you-- the pKa's of the metal bound are reduced.

Again, it's a play off. Those all with waters, those ligands. Every time you start changing the ligands or you change the oxidation state, these numbers change. OK? So you need to know a lot about the metal you're dealing with.

So that's one place-- you've already seen all of this before. Whoops. So the second thing I want to very briefly talk about is the second kind of reaction-- which maybe many of you haven't seen before-- is electron transfer.

OK. So this is basically oxidation reduction. And so clearly, you've seen oxidation reduction. So if we have some metal m in the n plus state, and we add an electron, it gets reduced. So to get to the reduced state, remember we need two half reactions-- something gets reduced, something else has to get oxidized.

And what's different-- we've looked at redox cofactors-- and most of you have looked at a lot of redox cofactors in primary metabolism, like glycolysis of the pentose phosphate pathway or whatever-- what are the normal redox cofactors you encounter? The organic redox cofactors

you encounter in biology?

AUDIENCE: NAD.

JOANNE STUBBE: Yeah, NAD-- NAD flavins. OK, so this chemistry always involves one electron. So that's distinct. NAD, we've already talked about this, always involves hydride transfer-- two electrons and a proton. So this is one electron. OK. And so one electron.

And if you have other things-- we could have proton coupled electron transfer. So PC is proton coupled electron transfer. And remember, we just saw the example of nitrogen getting reduced to ammonia. OK? You're doing an eight-electron reduction, but you've got to have protons. That involves proton coupled electron transfer.

If you're converting water into oxygen, again, you've got to take care of the electrons and the protons. And if I get that far in the last module, my lab works on ribonucelotide reductases-- that makes a precursor to DNA. You would never think about radicals, at all, but that chemistry involves proton coupled electron transfer. So here is some of the most important reactions in biology, and you really haven't been exposed to what's unique about the chemistry.

So what do we know that's unique about the chemistry? What do what do we know about rate constants for electron transfer? Anybody know anything? Fast, slow.

What's different about electron versus hydride transfer?

AUDIENCE: With the hydride transfer, you have to transfer an entire proton, versus--

JOANNE STUBBE: So you're transferring the proton, which-- what's the difference in mass between an electron and a proton?

AUDIENCE: A lot.

JOANNE STUBBE: Huge. It's 2,000-- 2,000 fold. So you remember, probably from introductory chemistry, when you think about electrons, you think about-- you think about quantum mechanics and quantum tunneling, as well as-- it can be-- electrons can function as both particles and waves. So they can function as waves and particles. And while I'm not going to spend a lot of time talking about this, this is a central reaction in the inorganic part of biochemistry that occurs in humans that you need to take into account.

When things behave as waves, they can function quantum mechanically. And we have an

expression called the Marcus equation, which allows us to calculate the rate constants. So we have a rate constant for electron transfer. And if we have some acceptor her and some donor-- so, all we're doing is redox chemistry.

The question is, what governs the rate constants for electron transfer? Well, it could be the electronic overlap, so that's part of it. This is part of the Marcus equation. What else governs the-- what else governs the redox chemistry if you have a donor and acceptor? The reduction potential of the donor and acceptor. So you need to think about the reduction potentials.

And what other factor governs the chemistry? Does anybody know? Around the metals. So you have to think about, how much energy does it take to go from iron two to iron three, or copper two to copper one? What other factor?

What else happens to the metal during a reduction or an oxidation?

AUDIENCE: A reorganization.

JOANNE STUBBE: Yes, a reorganization. So it can change its geometry. And so the other factor is called lambda, and this is reorganization chemistry.

And furthermore-- and we'll see this is important a little bit with the iron systems-- it doesn't just have to be the immediate coordination sphere of the metals. It can be the second coordination sphere, as well. So the whole protein is important, I think as hopefully most of you know by now.

And we're not going to spend a lot of time on this, but I think this is something you need to think about-- the rate constants for electron transfer. They could be 10 to the eighth, 10 to the 10th per second. How does that compare with the rate constant for chymotrypsin?

What's the turnover number for a protease? Anybody remember? OK. So a turnover number for a typical protease hydrolyzes-- like the cholesterol one hydrolyzes on an amine bond-- might be anywhere from 10 to 50 per second. OK. So how does that compare to this? Slow. Very slow.

So again, the chemistry of electron transfer is quite distinct from most of the chemistry you've encountered, and so you need to know it exists because it's everywhere in biology. We don't spend that much time on it in this class, but it's a unique part of the chemistry associated with metals.

OK, so the third thing I wanted-- the third kind of chemistry I want to very briefly look at is substitution reactions. OK. Now, in organic chemistry, what kind of substitutions reactions do you have? This is something hopefully you all remember from your organic, but what do you-- what do you have? What are the two basic reactions you learn about in the first semester of organic chemistry?

AUDIENCE: SN1 and SN2.

JOANNE STUBBE: Right. SN1, SN2. Associate or dissociate. Same thing in metals, OK? So you need to think about associative-- what does that mean? Dissociative. If you have something with four-- a metal with four ligands around it, you're going to add a ligand to get the reaction to go. That's associative.

If you have something with four ligands around it, one of the ligands could dissociate, and you only have three ligands-- and that's the basis for getting that chemistry to go. And the reason-- the thing that I want to focus on and-- the thing I want to focus on is ligand exchange.

So ligand exchange could occur by associative or dissociative mechanisms. Where have you seen ligand exchange in recitation? I think it was recitation four? You probably didn't think about it. I mean, we were doing something else. But the key to it working is ligand exchange rates.

What about histamine tags? OK, so here you have a metal. What kind of a metal do you have on your column? A nickel. And the nickel is bound. But in order-- so you can hang-- how does your thing hang up? By ligand exchange. How does it come off? By ligand exchange.

So an example of this is histamine tag chemistry. And another example that you've seen is magnesium. OK. What are the rate constants for ligand exchange with magnesium? Where do we see magnesium in biology?

I'm spending too much time on this. But I actually think this is incredibly important. If you take home a few of these basic reactions, this is all you really sort of need to know to deal with metals and biological systems. Where's magnesium? Where do you find it? You find it on--

AUDIENCE: Phosphates.

JOANNE STUBBE: On phosphates, yeah. So you have nucleotides-- like ATP would be an example. Whenever

you have ATP, if you look at the charges of ATP-- we went through this in one of the recitations that I taught-- you never have these negative charges. It's always complex. Just something to neutralize it.

And the major thing-- since magnesium is 10, 15 millimolar inside the cell-- it's always bound. But if you try to isolate magnesium through some kind of a column, what happens? The magnesium-- because of the rate constants for exchange-- falls off. So if you have something else in there that can out compete it-- like protons or something-- it's gone. You never look at-- it depends on the rate constants for exchange-- but you never see the metal bound to those small molecules. So this is rapid exchange.

And we'll see in the case of iron rapid exchange-- and I'm going to show you a table with this-- but rapid exchange is also important. And why is that important? It's important because say you isolate a protein and you're putting it through a column. What-- if the ligands are coming off and on, what happens to the metal by the time you get it out the bottom of the column? There's no metal.

So the issues with iron, which is everywhere, that catalyzes many, many, many kinds of reactions, is it's really hard to tell that there was a metal there inside the cell, because the iron dissociates during-- in the plus two state-- during protein purification.

So what about-- what if I changed the oxidation from iron two to iron three? What do you think would happen to the exchange rate?

AUDIENCE: Slow down a lot.

JOANNE STUBBE: Yeah. So it would slow down a lot. Every metal-- every metal is different. Every set of ligands is different. But you need to think about exchange reactions, because they're all over the place in biology.

Here's is an example that I took out of Lippard's book. I used to give a lot more data than this, but these give you the rate constants for exchange for iron. Here you can see iron two, iron three-- and these are all waters. OK?

That you're never going to sight see inside the cell. You might have a few waters, but you have other ligands around. All of the exchange rates change with different ligands, so you need to think about that. And also magnesium-- 6 times 10 to the fifth per second. So it's exchanging really rapidly.

And that really does govern-- you know, here we're doing protein purification here. We're trying to identify what the metal is. This is it made it really challenging to tell whether you ever had iron two bound to your protein.

Sometimes you isolate zinc bound to your protein. And I'm going to show you-- because of the periodic table, zinc always out competes iron. So when you're purifying something and you have zinc contaminant in your buffers and stuff like that, you'll get the iron replaced with zinc and think you have a zinc protein. And you don't. You really had an iron protein, but because of ligand exchange, you don't know what the real active form of the protein is. This is something that's plagued this area for a long time, and it certainly plagues the area of the iron that we're going to be focused on.

So let me see. I think I want to go up one more. All right. What do I want to say now?

So the other thing I want to talk about is-- that's unique and distinct from what you see in solution-- all of this stuff happens in solution. That's where we learn. Just like with organic cofactors. We sort of study them, we learn how they work, then we take them into biological systems. We use that as a starting point for think about-- thinking about how the enzymes use these cofactors.

And in fact, what you learned over here is exactly what you learn over here, except nature has figured out how to catalyze the reactions by a factor of 10 to the 12th faster. OK? So nature adds her-- adds her two cents worth on top of all the organic and inorganic chemistry we learn. And what is it that at this information? It's the protein environment.

So the last thing that one really needs to think about is how do proteins tune metal properties? OK. So that's the big question. And we're going to spend a little bit of time talking about that.

And to do that, I want to go back to the periodic table. OK, so again we're going to be focused on these metals. And what we see is there is a set of rules that inorganic chemists Irving and Williams-- many of you may have heard of the Irving-Williams series-- it sort of makes a prediction based on what you learned about transition metals in terms of ability to bind. If you compare all of these metals in the same oxidation state, in the same geometric environment.

So one of the questions that we face is binding. And why is that important? Because inside the cell, we will see that copper binds much more tightly than manganese-- no matter what you

do, that's true. And what's the basis of that? It's the atomic number, which changes the atomic radius-- it makes it smaller. It makes the ligands bind more tightly.

So the problem is, when you're inside the cell-- if all these things were floating around inside the cell-- how do you control the metallation state inside the cell? So that's the key issue, and I'm going to give you-- I'm going to show you a little bit about how nature has figured out how to control all of this. It goes awry quite frequently, and that's-- how does it manifest itself? It manifests itself in disease. Just like we saw with cholesterol.

So what we're going to see-- we are going to look at first row transition metals. In general, we'll see that manganese two binds less tightly-- if you look over there, you can see where we are in the periodic table. The atomic numbers increase less than nickel, less than copper. So here are-- here are our transition metals.

And so what we see is the atomic numbers decrease-- increase. And the atomic radius decreases. And therefore what you see is over at this end, you have weak binding-- over at the manganese and iron end, we have weak binding. And over at this end, we have strong binding.

So if you had a protein-- and I'm going to give you an example of this. There is a protein I'm going to show you that combined both copper and manganese. And you had equal amounts? Copper would always win-- by a lot. OK? So you need to study this, but you know-- you'd have to use 10,000 times more manganese to out compete the copper. OK? So this just shows you.

So this is called the Irving-Williams series after the people who described this. And what they compared to get these numbers-- they are looking at all of these things in the plus two oxidation state. OK? And they're looking at it all in an octahedral environment, with six ligands around it.

This is all plus two oxidation state, and all octahedral. Everybody remember octahedral? We have four equatorial ligands, and two axial ligands-- I'm not going to draw that out on the board.

OK. So that's an issue. And the question then is, how do we deal with this issue? So here's our Irving-Williams series that I've given you here. But what do we do-- how do we deal with this inside the cell?

The issue is that-- in vitro, you have an issue. And there's not much you can do about it,

except control the relative concentration of the metals. Inside the cell, do you think it's easy to control the relative concentrations of metals? What do you think?

Concentration is everything in biology, we just don't talk about it that much. Do you think it's easy to-- say I threw in the outside of a cell 15 millimolar copper. Do you think the cell could control that? Do you think it would all get taken in and then all of your enzymes would be loaded with copper?

No. So you have to have a way to actually control all of that. There was a spectacular paper, I think, published a couple of years ago that sort of demonstrates this point. And so I'm going to give you this example, because I think it really-- it was published in 2009.

So in vivo. So this would be, over here, in vitro-- sorry. In vitro.

And we can't get rid of the in vitro part. That's the chemical properties of the molecule, we're stuck with them. So it depends-- in vivo, metallation depends on abundance. Can we control abundance? Absolutely, we can control abundance. You've already seen with cholesterol, you control abundance with transcription factors. That's one way. There are many ways. We're going to see-- that's one of two general ways that iron is controlled.

What about speciation? I've already told you-- and we're going to come back to this with iron later on-- you know, are the metals all bound to waters? But you have ATP inside the cell. Could iron two be bound to ATP? Absolutely. So it's a question of competition and what the binding constants are, which is what are talking about in recitations this week. You know, if it's really weakly bound, then something else will out compete it. But you can purify-- you put ATP in a solution, you'll pick up iron all the time if you do atomic absorption on it. Because iron can easily bind to all the negative charges on ATP.

And the other thing that you need to think about is location. So location is what we're going to be focused on in the example. And what do I mean by location? Even in a bacteria you have location, right? What are the two different compartments? You have a periplasm and you have the cytosol. We're going to be talking about periplasm, and you have cytosol.

In us, we have much more complicated locations in metal homeostasis. We'll see when we get to the second lecture. Has a lot of issues it has to deal with, OK?

Let's look at this example. And I'm not going to spend a lot of time on it, but let me just show

you what you need to think about. And these workers we're interested in a cyanobacteria. And they wanted to find what was the protein that bound the most copper, and what was the protein that bound the most manganese.

So we're looking at two extremes of the Irving-Williams series. So this group identified-- C-- I can never remember the acronym. CucA is the most abundant copper two binder. And they identified MncA. And the way they did it was pretty creative. If you're interested, you can go read the paper. It's the most abundant manganese binder.

Both of these things are made in the cytosol-- both of the proteins are made in the cytosol of the cell. And what they found when they studied this system in more detail is the structures of the proteins and the ligands bound to the metals are exactly the same. So you have a beta barrel in both cases, and you have the same first coordination sphere. The first coordination sphere of the ligands directly bound to the metal.

If you took these two proteins, and you wanted to load MncA-- this is manganese binder-- in the test tube, you would have to add 10,000 times more manganese than copper to get the manganese in there. So that, again, goes back to this-- I mean, it's going to be different for every system-- but it goes back to this question of controlling metallation inside the cell, which is extremely challenging to do. And we don't-- there's a major, in my opinion, on cell problem in biology.

They're going to do this by localization. So let me just walk you through the kinds of experiments they did. So, both these two proteins-- the one that binds copper and the one that binds manganese-- are produced in the cytosol. It turns out that the one that binds manganese folds uniquely in the cytosol. And the cytosol, if you look at metal speciation, how much free-- you're going to learn about free today, or tomorrow in recitation-- how much free copper or zinc do you have-- do you think you have in the cells? Copper two and zinc two in the cell? Do you think you have a lot? A little.

AUDIENCE: For copper, I know it's less than one percent.

JOANNE STUBBE: Yeah, it's less-- yeah. It's tiny. Both copper and zinc bind extremely tightly to-- again, it's all about speciation, so it depends on what the ligands are inside the cell. And in fact, in the cytosol cyanobacteria, they have measured a micromolar of free manganese. And so again, this speaks to this question of is manganese readily oxidized? No. So you don't have to worry about reactive oxygen species with manganese.

What happens is this protein folds in the cytosol of the cell. Comes off the ribosome. It picks up the manganese and folds. But its location is in the periplasm.

How does it get to the periplasm? It gets-- there are two ways you can get proteins from the cytosol to the periplasm. One is through the Tat transporter. And Tat transfers-- it recognizes a couple of arginines. A little zip code-- we've seen zip codes over and over again-- which then takes it in the folded state into the periplasm.

And the manganese, once it's in there, doesn't come out. Doesn't exchange. So there's something about the environment that does not allow exchange. So the manganese is placed into the protein in the cytosol.

Now, what happens to the copper binding protein? In this case, as soon as it comes off the ribosome, it gets grabbed by a second kind of transporter. And this second kind of transporter transfers the unfolded protein through the plasma membrane. And it folds in the periplasm.

And in the periplasm, I don't know what the ratio of copper to manganese is, but remember-- copper, by this model, out competes manganese by a lot. So even if you have manganese and copper in equal amounts, the copper will always win out. And so what happens here is the copper then binds.

And so the copper is loaded in a different location than the manganese. So the way this organism-- this is just one solution-- I think a pretty creative solution-- to how you deal with the Irving-Williams series, which we're faced with all the time with the many, many metallocofactors we actually have inside the cell.

I'm going to come back-- next time I'll talk about two more issues. I want you to be in tune with me when we move on in the iron world. And talk about sort of a big cartoon for metal homeostasis. Doesn't matter what the metal is-- any of these metals. And then we're going to move on and focus on iron.