JOANNE STUBBE: OK, so what I want to do today is hopefully finish up or get pretty close to finishing up module 6, where we've been focused on bacterial uptake of iron into cells. In the last lecture, I briefly introduced you to gram-positive and gram-negative big peptidoglycan, small peptidoglycan, outer-cell membrane. They both have the same goals. They've got to get--

They take up iron the same way from a siderophore, which is what we talked about last time, or by a heme. And we'll talk a little bit about that. And that's what you focused on in your problem set. But they have different apparati to do that, because of the differences between the outer-- because of the cell walls' distinctions between gram-negative and gram-positive.

So we were talking, at the end of the class, about, this was for the siderophores which we talked about. We need to take them up. These are common to all uptake systems. You have some kind of ATPase system and ABC ATPase. We're not going to talk about that in detail, but it uses ATP to bring these molecules and also heme molecules across the plasma membrane. And then, in all cases, you have this issue of how do you get the iron out of whatever the carrier is, be it a siderophore where the carriers can bind very tightly or heme where you also have to do something to get the iron out of the heme so that it can be used.

And so what I want to just say, very briefly-- and this you all should know now. So now we're looking at heme uptake. I'm not going to spend a lot of time drawing the pictures out, but, if you look at the PowerPoint cartoon, what you will see is there is a protein like this, which hopefully you now have been introduced to from your problem set. So this could be IsdB or IsdH. And we'll come back to that, subsequently.

And it sits on the outside of the peptidoglycan. So this is the protein. The key thing that is present in all these Isd proteins is-- let me draw this differently-- is a NEAT domain. OK? And we'll come back to that later on. But this domain--

So you have a big protein, and there's one little domain that's going to suck the heme out. And so what happens is we'll see in Staph. aureus, which is what we're going to be focused on, you

have hemoglobin. And somehow-- and I'm going to indicate heme as a ball of orange, with a little planar thing as the protoporphyrin IX. OK, are you all with me? And then somehow this gets sucked out into the NEAT domain, where--

And again, all of these gram-positive and gram-negative systems are slightly different, but in the Staph. aureus system we'll be talking about today and you had to think about in the problem set you basically have a cascade of proteins which have additional NEAT domains from which, because this is such a large peptidoglycan, you need to transfer the heme to the plasma-membrane transporter. And what's interesting about these systems and is distinct is that they end up, they're covalently bound to the peptidoglycan. And I'm going to indicate peptidoglycan as "PG." And we'll talk about that reaction today-- the enzyme that catalyzes those reactions. And all of these guys end up covalently bound to the peptidoglycan-- which is distinct from all of the experiments you looked at in your problem set. Nobody can figure out how to make the peptidoglycan with these things covalently bound. So what you're looking at is a model for the actual process.

OK, so, also-- so that's the gram-positive. And in the gram-negative, one has two ways of doing this. And again, these parallel the ways with siderophore uptake. So you have an outer membrane--

So this is the outer membrane. And you have a beta barrel, with a little plug in it. And so these beta barrels, they're at, like, 20 or 30 of these things in the outer membranes. And they can take up siderophores, as we talked about last time, but they can also take up hemes. OK? So each one of these is distinct, although the structures are all pretty much the same.

And so what you see in this case is, there are actually two ways that you can take heme up. So you can take up heme directly. And we'll see that what we'll be looking at is hemoglobin, which has four alpha 2 beta 2. So this could be hemoglobin. That's one of the major sources, and it is the major source for Staph. aureus.

And so this can bind directly to the beta barrel-- gets extracted. The heme gets extracted. The protein doesn't get through. And so the heme is transferred through this beta barrel. OK.

So that's one mechanism. And then there's a second mechanism. And the second mechanism involves a hemophore. And the hemophore is going to pick up the heme.

And so every organism is distinct. There are many kinds of hemophores. And I have a

definition of all of these-- the nomenclature involved. And so, after class today, I'll update these notes, because that's not in the original-- the definitions aren't in the original PowerPoint. OK?

So what you have, over here, is the hemophore that somehow extracts the heme out of hemoglobin or haptoglobin. We'll see that's another thing. So this gets extracted and then gets transferred, in that fashion.

And so these hemophores come in all flavors and shapes. They're different-- for example, in Pseudomonas or M. tuberculosis. And we're not going to talk about them further, but the idea is they all use these beta-barrel proteins to be able to somehow transfer the heme across. And what happens, just as in the case-- if you go back and you look at your notes from last time, there's a periplasmic binding protein that takes the heme and shuttles it, again, to these ABC transporters.

OK? So, in this system, again, you have a periplasmic binding protein. And this goes to the ABC transporter, which uses ATP and the energy of hydrolysis of ATP, to transfer this into the cytosol. OK, so this is the same. That remains the same. And the transporters are distinct.

And then, again, once you get inside the cell, what do you have to do? You've got to get the iron out of the heme. So the problems that you're facing are very similar to the siderophores. So, in all cases--

So the last step is, in the cytosol, you need to extract the iron. And you can extract-- usually, this is in a plus-3 oxidation state. So you extract the iron. And this can be done by a heme oxygenase, which degrades the heme. OK.

In some cases, people have reported that you can reduce the iron 3 to iron 2, when the heme can come out, but that still probably is not an easy task because you've got four-- you've got four nitrogens, chelating to the heme, and the exchange, the ligand exchange, rates are probably really slow. So I would say the major way of getting the iron out of the heme is by degradation of the heme. And we're not going to talk about that in detail at all, either. OK.

So that's the introductory part. And here's the nomenclature, which I've already gone through. I've got all these terms defined. And if you don't remember that, or you don't remember it from the reading, you have a page with all the names-- which are confusing.

And so the final thing I wanted to say, before we go on and actually start looking at

peptidoglycans and gram-positive bacteria and heme uptake in Staph. aureus, which is what I was going to focus on in this little module, is to just show you, bacteria desperately need iron. So what do they do? This is what they do.

OK, so, here you can see-- and some bacteria make three or four kinds of siderophores. Others only make one or two kinds of siderophores, but what they've done is they've figured out how to scavenge the genes that are required for these beta barrels. So they can take up a siderophore that some other bacteria makes. OK?

And that's also true of yeast. Yeast don't make siderophores, but most yeast have, in their outer membranes, ways of picking up siderophores and bringing it into the cell, since-- and remember we talked about the fact there were 500 different kinds of siderophores.

But you can see that the strategy is exactly the same. You have a beta barrel. You have-- these are all periplasmic binding proteins. This picture is screwed up, in that they forgot the TonB. Remember, there's a three-component machine, TonB, ExbB and D, which is connected to a proton motive force across a plasma membrane, which is key for getting either the heme or the iron into the periplasm. And you use a periplasmic binding protein, which then goes through these ATPase transp-- ABC-ATPase transporters.

So what I showed you was heme uptake, iron uptake, but in all of these cases, like Staph. aureus we'll be talking about, we can also get iron out of transferrin. We've talked about that. That's the major carrier in humans. The siderophores can actually extract the iron from the transferrin. And remember the KD was 10 to the minus 3, so somehow, again, you've got to get iron transferred under those conditions. And that's how these guys survive.

So they're pretty desperate to get iron. And inside, once they get inside the cell, you have all variations of the theme to get the iron out. But they're all sort of similar. Somehow, you've got to get rid of whatever is tightly binding it. And if you're creative, you can reuse whatever is tightly binding it, to go pick up some more metal. OK.

So that just summarizes what I just said. And so, in two seconds, I'm going to show you, now-- we've spent one whole lecture, a little more than a lecture, talking about iron uptake in humans via DMT1, the iron-2 transporter, and the transferrin transfer receptor. So, in the plus-two and plus-three states, we just started looking at the strategies by bacteria and saw how widespread they are. And then the question is, how do you win?

OK, bacteria need iron. We need iron. And the question is, how do you reach-- and we have a lot of bacteria growing in us, [LAUGH] so we've reached some kind of homeostasis. But with the pathogenic ones, of course, we really want to get rid of them.

And so that's what the issue is. And there have been a bunch of articles. You can read about this in a lot of detail, if you're interested in the more medical aspects of this. But this war between bacteria and humans. And really it's sort of fight for nutrients. And, in this case, the nutrient is iron.

Has received a lot of attention, because we're desperate for new kinds of targets for antibiotics, because of the resistance problems. And so nutrient limitation and iron sequestration from a pathogenic organism might represent a new target. Of course, what are the issues? The issues are, we also need iron. And so, if you lower the amount of iron, then you might be in trouble, as well.

So what we know is, bacteria, viruses-- bacteria have been extensively studied; viruses, less so, also protozoa, such as the malaria system-- all are known to depend on iron for growth. And so, again, if you want to read about this, you can read about some of the strategies these organisms [LAUGH] use to get iron away from the human systems. And it's sort of amazing, when you look at the details of how things have evolved, back and forth, back and forth, [LAUGH] in terms of survival.

And so really what it's all about is homeostasis. OK? And that's what was all about in cholesterol. And we'll see, with reactive oxygen species, that's what it's all about.

So, somehow, using hepcidin-- which is the human master regulator, the peptide hormone-- we need to figure out how to keep ourselves alive while killing off these bacteria, in some way, by sequestering the iron from the bacteria. OK. So this is an important problem that has received a lot of attention. And most of you know that the Nolan Lab is doing beautiful studies in this area. OK.

So what I want to do now, for the rest of the lecture, is focus on Staph. aureus. OK? And Staph. aureus is-- methicillin-resistant Staph. aureus is a major problem, throughout the world. We don't have any ways to kill this guy. And so that's why I decided to pick this target, but there are many other [LAUGH] of these pathogens around that have problems-- have also resistance problems, Staph. aureus being the one that's been most extensively studied in the last decade or so.

But bacteria has come back in vogue. For years, nobody on campus cared anything about [LAUGH] microorganisms or bacteria. The microbiome has brought it back in vogue, because people think they're going to be able to figure that all out. OK.

But anyhow, bacteria have always been extremely important, not only in terms of human health but in terms of how the whole world functions. There are so many of them, and they do so much interesting stuff. And we have to live with them, side by side.

So anyhow, we're going to look at Staph. aureus. That's what we're going to focus on, because of this problem. And I think Staph. aureus, which many people don't realize, is that 30% of all people have Staph. aureus on your skin or in regions that are not breaching into the bloodstream. So we all have Staph. aureus.

So 30% of us have this bacteria. If you get-- if wherever it's localized is breached, and it gets into our bloodstream, then it's all over, because Staph. aureus can colonize almost anywhere. That's different from other organisms. Some organisms can only colonize in the lungs. Some colonize in the heart.

So these can colonize almost all tissues. And what you know is, if you start thinking about physiology-- and again, I'm not an MD-- but different tissues have different environments. OK? And so a lot of organisms find siderophore an environment where they can best live and then take up-- make their home there. But Staph. aureus is one of these guys that can go anywhere.

And so this makes it specifically very insidious. And you can get septicemia, or you can get endocarditis, or you can get all kinds of horrible diseases associated with Staph. aureus, once it breaches the barrier. OK. So what we need to do, as you've already seen from your problem set, to understand how Staph. aureus can get heme into its cytosol to be able to function, to be able to grow effectively, is, we need to look at the outer cell wall or the peptidoglycan.

So what I'm going to do is spend a few minutes talking about the structure of the peptidoglycan. And then we'll go back in and we'll talk about how these proteins that you worked on in the problem set covalently bind to the peptidoglycan and allow you to take up iron to the cell. And why is heme a major target?

Heme is a major target for Staph. aureus. They've evolved. The major source of iron, we all

know, is hemoglobin now, in red blood cells. And so Staph. aureus has developed proteins-- endotoxins, really-- that can go in and-- there's proteins that can insert into red blood cell membranes, make a pore. The blood cells lyse, and now the bacteria are extremely happy because they have huge amounts of heme. And then they want to take that heme into-- to help them survive. So Staph. aureus are amazingly creative, in terms of getting the heme that they need for survival. OK.

So, peptidoglycan. Most of you have probably seen peptidoglycan before. I'm just going to say a few things about peptidoglycan. So let's look at-- let's see. Where do I want to do this? All right. So I'm going to erase this.

We're going to look at the cell wall. OK. And what you can see, here, I'm going to draw just a few things on the board. But what you can see here, in this cartoon, is you have two kinds of sugars-- N-acetylglucosamine and N-acetylmuramic acid. N-acetylglucosamine is a precursor to N-acetylmuramic acid.

And what you see, attached to N-acetylmuramic acid, are little blue balls. And that's the peptide that turns out it starts out with a pentapeptide and goes to a tetrapeptide. And what you see here, in the purple balls-- and this is unique to Staph. aureus-- is, other amino acids, they're all the same, and this is glycine.

So, if you look down here, here are the disaccharides, shown up here. Here is-- yeah, one, two, three, four, five. Here is the pentapeptide.

And what do you notice unusual about the pentapeptide? You have a D glutamine. OK? And I was just reading a whole bunch of papers on somebody's thesis-- tomorrow, actually. And you're trying to make this guy, nobody can study this stuff. Why? Because you have to make a peptidoglycan. And I'll show you. It's complicated.

You have to stick on a pentapeptide. You have to stick on the glycines. And how do you get the substrates for your enzymatic reactions?

So we've known this pathway for decades, but it's taken really good chemists to be able to figure out how to look at these individual steps. And so what's unusual, here, is, if you replace glutamine with a glutamate, it doesn't work very well at all. OK, so it's that subtle. Here you've got this huge macromolecule, and you're replacing an NH2 with an OH, and you alter the resistance to different bacteria.

And again, you have this unusual pentaglycine. And you'll see in the cartoon, in a few minutes, where do you think this glycine, pentaglycine comes from? Well, it actually comes from a tRNA that binds glycine. OK, you've seen that before. But, instead of using the ribosome to make this little peptide, it uses nonribosomal peptide synthetases. And this all happens in the cytosol of the cell.

So, what do we know about the structure? I'm just going to draw N-acetylglucosamine. And what I'm going to do is put some R groups on here. So I'm going to put OX.

And then here we have N-acetyl. So that's an acetate group. Here I'm going to put another OR group.

OK, so the two things I want to focus on, the two things I'm going to focus on, is this X and this R. So is N-acetylglucosamine. And then the second one is N-acetylmuramic acid. And, in both of these cases, X is equal to UDP. So we're going to come back to this in the last module on nucleotides. So nucleotides play a central role in RNA and DNA, but they also play a central role in moving around all sugars inside the cell.

So what you have here, actually, is a pyrophosphate linkage to UDP. OK? And if we look at N-acetylglucosamine, R is equal to H. OK? But if we look at muramic acid, what we're going to see is that nature has put on a lactic acid in this position.

OK, so here's your methyl group, from your lactic acid. And here's the carboxylate. So this is the R group in N-acetylmuramic acid. OK.

Now, what we're going to see is, while most sugars-- and this is true in humans, and it's also true in bacteria-- are carried around and transported within the cell as linked nucleotides, what we'll also see in the cell wall-- which has made them extremely challenging to study, made the whole pathway extremely challenging to study-- in addition to X equal to UDP, X can also be equal to sort of an amazing structure. And the structure is slightly different in different bacteria, but this strategy is also used in humans, where you have a lipid and you have a lipid that acts as-- is made from-- hopefully you now know-- is isopentenyl pyrophosphate. OK?

And there are seven of these, where you have the trans configuration. There are now three of these, which have the cis configuration. Just make sure I get my-- is that right? Yeah, that's right. OK, so you have three of these that have the cis configuration. And then you have a terminal dimethyl L configuration.

And this is C55. So, if you're a synthetic chemist, and you're trying to stick on a couple of these sugars with hydrocarbon on the tail, with C55, you can imagine you would have one heck of a trouble, number one, synthesizing it but, number two, dealing with it. And so this goes to the question which I think is really interesting is, many people think about polymerization reactions. We're going to see this polymer is non-template-dependent, in contrast to polymers of DNA RA, where you have a template. And furthermore, DNA and RNA are pretty soluble. These things become insoluble. So you're making a phase transition from soluble state to an insoluble state, around the bacteria.

And I think it's really sort of a tribute to Strominger, who worked on this many years ago, that he figured out sort of the pathway. But now it's only with recent studies, and really some very hard work synthetically, and also in terms of the microbiology and biochemistry, that it's really allowed us to elucidate this.

So X, in this case, can also be this lipid. So I'm just pointing out what the issues are. And if you look at the cell wall, biosynthetic pathway-- so this is inside-- you're not going to be responsible for the details of this. But this is outside. OK.

So you start out with a couple of sugars. These are the sugars we just talked about. OK. So now what you do is add on these five amino acids. So, over here, we ultimately need to add on five amino acids. And what do we see about the amino acids? They're unusual, because they can have the D-- they are not necessarily L-amino acids. They can be D-amino acids. And these things unfortunately are unique to different organisms. So, if you worked out a synthetic method for one, you're still faced with the problem that every one of them has different pentapeptides stuck on the end of it.

Now, how would you attach-- you've now had a lot of biochemistry, where you've dealt with amino acids, in the first half of this course. How would you attach amino acids-- form and the linkages-- to this lactic acid? Can anybody tell me? What would you do, to make that attachment?

AUDIENCE: You activate the carboxylate.

JOANNE STUBBE: Yeah, so we have to activate the carboxylate. How do you activate the carboxylate?

AUDIENCE: Make an AMP.

JOANNE STUBBE: Yeah. So you make an AMP, just like you've seen with nonribosomal-- the adenlyating enzyme of nonribosomal polypeptide synthases, and you've seen with tRNAs. OK. So you see the same thing, over and over and over again. So you add these things on. The difference is that, again, these things, which are all soluble, down here, these are all soluble with the nucleotides.

Now, because ultimately this needs to go from the inside of the cell to the outside of the cell, what you do, presumably, is take this lipid-- so you have the C55 lipid, with one phosphate on it. And then you attach it to one sugar. So here it's attached to the muramic acid, and that's called "lipid 1."

You add N-acetylglucosamine with a glycosyltransferase. That's lipid 2. And that's the substrate for the polymerization reaction. What is the issue? The issue is, it's in the cytosol and all the chemistry happens on the outside of the cell. But, of course, if you move it from the inside to the outside, you don't want your substrates to float away. You've got to keep them there. OK? And that's especially [LAUGH] true in gram-positives, where we have no outer membrane.

So the question is, how does this species get from this side to this side? OK. In the last couple years, people have proposed-- and so this has taken a long time. People have been looking for these proteins for decades. These are called "flipases."

So you still have this issue-- again, this big, huge thing that needs to be transferred. And I think what's even more amazing, in the case of Staph. aureus, is that you put on the pentaglycine in the cytosol. So, here, what you'll see-- I think this is E. coli. I can't remember one from the other. But, instead of having DAP, which is diaminopimelate, you actually have lysine.

So, here, what you have in Staph. aureus is a lysine, and the lysine has an amino group. And attached to this amino group is the pentaglycine. And this all occurs in the cytosol.

So this is quite remarkable. So then, not only do you have to get the disaccharide with the pentapeptide on it, you need to have, here, the pentaglycine on it, as well. And this becomes really important in thinking about trying to study what's going on in the polymerization reaction, which is the target of natural products that are currently used, clinically. OK.

So this thing's got to flip. And then what you have is a substrate. You have a growing chain.

OK, and then what you need to do is extend this chain, so you have a glycosyltransferase.

So you have two things. You have phosphoglycosyltransferase. And then the other thing you have is a TP, which is a transpeptidase. OK. And so the transpeptidase-- we're going to come back to this in a second, but-- is ultimately responsible for making a cross link. Which is what gives the bacteria cell wall rigidity.

Now, in many organisms, the glycosyltransferase and the transpeptidase are on the same protein. They're two domains. But, in many organisms, they're not. OK, so you have two separate proteins. And furthermore, in Staph. aureus there are now five of these kinds of proteins.

So the question is, what are all five of these glycosyltransferase doing? Which ones are involved in which? Which ones are involved in antibiotic resistance?

And I think, when you start looking at it like this, you know, it's very complex. You realize what a hard problem this actually is. But we now have the tools, I think, because of beautiful studies that have been done in the last few years, to start investigating this.

So this just shows, here, again, we have our lipid 2. We have our growing chain. And here we have our pentaglycine.

So this is Staph. aureus. And we take D-alinine D-alinine, and form a cross-link and kick out D-alinine. And many of you have probably seen this before. I used to teach this in high school. [LAUGH] So that D-alinine D-alinine looks like penicillin. And we understand that this works-- it looks amazing, like a serine protease, which you're all very familiar with. We've seen this hundreds of times, now, in the earlier part-- to form this cross-link. And that cross-link is essential for the viability of the organism, in different ways.

And you can imagine, if a bacteria is dividing, that you might have different peptidoglycal structure at the site, where the two dividing bacteria are going to split apart. So that might be why you want to have multiple glycosyltransferases in this overall process.

OK. So this is just a cartoon that shows you targets. These are all natural products. Here's penicillin. It targets--

It looks-- not in this picture, but you can use your imagination. It looks just like D-alinine D-alinine. Binds in the active site, and covalently modifies a serine involved in that reaction.

Moenomycin. What does this look like? This is sort of amazing. It's got this lipid thing, hanging off the end. That's a natural product.

It binds, also, to the glycosyltransferase. And people are actively investigating this. You can imagine, this is not so easy to make as a new antibiotic.

And then we have vancomycin, and vancomycin is able to bind D-alinine D-alinine. So these are all natural products that target cell wall. And, by far and away, the penicillins are the ones that are used much more prevalently. We have hundreds of variations of the theme. And, again, it's the war between the bacteria and the human, to figure out how to keep themselves growing.

And so we have many variations on the beta-lactams. And you can take this even a step further, if you go-- in addition to the peptidoglycan, you have polymers of teichoic acid-- which I'm not [LAUGH] going to go into. But now people, for the first time, this year, have been able to reconstitute this polymer biosynthetic pathway. And this is a new target for design of the antibacterial. So I think it's exciting times, and we have really smart people working on this problem. And they now, for the first time, can set up the assays, so they can screen for small molecules that hopefully can target cell wall, which is unique to bacteria. OK.

So what I want to do is talk about, in the last few minutes, as we're now moving into Staph. aureus. OK? And we're going to focus in on heme uptake rather than siderophore uptake. But if you look at this, what do we know about Staph. aureus? We know what a bit, because everybody and his brother has been studying it because of the problems with resistance.

So, here, again, Staph. aureus actually has two biosynthetic pathways encoded in its genome. And what these pathways code for are these two siderophores. OK? And if you look at this, what's unusual? Does anybody see anything unusual about the siderophore structure, if you look at it carefully?

I don't want to spend a lot of time on this, but what do you see in the structure? Can you read it? Or, if you brought your handout, you can probably read it. Since I insist on having the windows open, it's harder to read this.

But what do we see, in siderophore, in this siderophore, Staphyloferrin A? See anything you recognize? Yeah.

AUDIENCE: Some citrates?

JOANNE STUBBE: Yeah, citrates. So, again, we're using citrate. We saw polycitrate can bind iron as a siderophore in itself. And, in fact, most gram-negative bacteria have iron-siderophore uptake system.

Here, actually, all of these-- if you look at this carefully, the biosynthetic pathway, you know, is made out of basic metabolites. OK? That you see out of normal, central metabolic pathways. And what happens is, there's an ABC transporter and an ATPase-- FhuC is an ATPase-- all of this is written down in your notes-- that allow the siderophore to bring iron into the cell.

And I think what's interesting here, and I've already pointed this out, in addition to the siderophores that the organism makes it also has a generic transporter that allows siderophores made by other organisms to bring iron into the cell. And so, again, that's a strategy that's used over and over again. So here's a xenosiderophore transport system, desperately trying to get iron. OK.

So the ones we're going to be talking about and focusing on specifically are the heme uptake systems. And these are the ones you've already hopefully thought about, now, from your problem set. We have to extract-- I just told you that red blood cells have most of the iron. So Staph. has been incredibly creative in generating endotoxins that lyse red blood cells, allowing the heme-- hemoglobin, OK?

So we have endotoxins from the organism that lyse red blood cells. And so what you get out, then, is hemoglobin. Which, again, has four hemes and iron. And you want to get-- the key thing is to get the iron out of the heme. So you want to be able to extract the iron out of the heme.

And also-- and I have this down in your nomenclature-- it turns out red blood cells have another protein, called "haptoglobin," that binds to hemoglobin. And that's another place that these organisms have evolved to extract the heme-- to extract the heme. So, in all of these cases, you're extracting the heme out of the protein.

And so, over here, you see the two different ways to do that. And we have different proteins that are able to do this. And then, eventually, the heme that's extracted is passed through this peptidoglycan, eventually to the plasma membrane, where the heme goes into the cytosol. And in this organism, to get it out, you have to break down the heme. You have to cleave it

into pieces by the enzyme called "heme oxygenases." OK.

So I don't want to really say very much about the siderophores, except to say-- let me comment on iron sensing. And you saw-- and this would be Staph. aureus, but it's in true iron-sensing for most bacteria. You saw iron-sensing predominantly at the translational level. Which was unusual. That's why we talked about it, in humans.

Here, iron-sensing is predominantly at the transcriptional level. So this sensing occurs transcriptionally. And so you have a transcription factor, which is called "Fur." And Fur is a transcription factor. That name is used for almost all organisms. And I'm not going to say much about this, but we're going to look at the operon in a minute.

But here's Fur. And if Fur has iron bound, what it does is a repressor. And it shuts down transcription of all the proteins that you might think it would shut down. They can no longer take up iron into the cell, because you have excess iron and you don't need anymore. Again, you want to control iron, because you have problems if you have too much iron with oxidative stress. OK.

So, if you look at the operon-- let's see. So look at the operon, here. So here's the operon. And we're going to see that the key proteins involved in heme uptake are called the "Isd" proteins.

And so, if you look at all of these Isd proteins, this Isd protein and that one, they all have these little Fur boxes. [LAUGH] So we have a Fur box ahead, which regulates whether you're going to make a siderophore or whether you're going to make all this equipment required to take up heme. So all of that makes sense, and people have studied this extensively, in many of these organisms. OK.

So what I want to do now is, I'm going to show you this cartoon overview. And then we'll look at a few experiments that people have done to try to look at what basis in reality this cartoon model has to what actually happens inside the cell. So let's look at--

I can never remember the names of these things. I'm just going to call it the "Isd proteins." And so there are two proteins, we're going to see, that are closest to the surface, that directly interact with hemoglobin-- or haptoglobin and hemoglobin-- the other ones that are going to somehow get the heme out of the proteins. And then these each have little NEAT domains. So N1 is a NEAT domain.

So they have a name for that, which I've also written down. It's, like, 120 amino acids. And each one of these proteins sometimes has two, sometimes has three, sometimes has one, and they're structurally all the same. But it turns out that you can't just pick up one and replace it with another. There's something about the spinach on each side of these NEAT domains that is key, you can imagine, for the directionality of the transfer.

So you want something that the heme is going to get down here. You don't want something where the equilibrium is going to stay up there. So this is not an easy problem. And this is a problem that we discussed in the beginning-- the importance of exchange ligands.

Because somehow we're going to have a heme in a little NEAT domain, but it's going to move into the next domain. It just doesn't hop. It's covalently bound. So how do you transfer one heme to the next heme? And we have a lot of structural information, but I would say we still don't understand how these transfers actually occur. OK.

So there's a couple other things that I want to point out, here. So IsB and IsH extract from heme and hemoglobin. This gives you a feeling, which you also saw from the problem set, that these little domains-- N1 domains, N2 domains-- are all NEAT domains. So we have multiple domains.

And what we're going to see, and this is key to the way these organisms function, is that these Isd proteins are covalently attached to the peptidoglycan. So the issue is, we need to covalently attach the Isd proteins to the peptidoglycans. And the protein--

There are two different proteins that do this. So the Isd proteins have ZIP codes. Where have we seen this? We see this over and over and over again. We have little sequences of peptides that are recognized by another protein. OK?

So we have ZIP codes. And the ZIP codes, I'll just say "see PowerPoint" for the sequence. And it turns out, if you look over here, all of these proteins with a yellow anchor have little ZIP codes in them. OK? [LAUGH] And they're recognized by a protein called "sortase A." OK.

So we'll see that, in addition to the Ist proteins, we have sortases. And we have sortase A and B, and they recognize the ZIP codes, distinct ZIP codes, and are required to attach the Isd proteins covalently to the peptidoglycan. And in the peptidoglycan of any gram-positive, a lot of things are covalently attached to the peptidoglycan.

So, I mean, can you imagine-- how dense do you need these proteins, to be able to do these switches? I mean, this is a cartoon overview that really doesn't tell you anything about the complexity of all that-- what does a peptidoglycan look like? Well, it's got a lot of water and a lot of space in between these N-acetylglucosamine, N-acetylmuramic acids.

So this is involved in the covalent attachment. And it, in fact, involves what you've seen over and over again-- involves covalent catalysis with a cystine in its active site. OK? So what I want to do is briefly look at what these sortases actually do. I'm not going to write it on the board. I'll walk you through it and then, next time-- hopefully, you've already thought about this in some form, but I'll walk you through it and go through it next time. And then what we're going to do is simply look at a few experiments with Isd proteins, to look at this movement of heme across the membrane, similar to the kinds of experiments that you had on the problem set that was due this week.

OK. So, because I don't have much time and I can't write that fast and you can't write that fast, either, [LAUGH] I'm going to walk you through sort of what's going on in this reaction. OK. So, remember, all of these things are anchored to the plasma membrane. OK, so that's the other thing. Sometimes they have single, transmembrane-spanning regions. Sometimes they have lipids that are actually bound.

I wanted to say one other thing, here. So these yellow things are anchored by sortase A. The blue thing is anchored by sortase B. And IsdE is anchored by a lipid, covalently bound. OK, so we have three different strategies, to anchor. OK?

And every organism is distinct. Whoops, I'm going the wrong way. OK, so what happens in this reaction?

So here's our ZIP code. OK, and what we know about this-- and here's sortase A. Sortase A is anchored to the plasma membrane. In a further cartoon, they don't have it anchored, but I tell you it's anchored.

And we know we get cleavage between threonine and glycine. And we know we have a sulfhydryl on the active site. So, this chemistry, we've seen over and over and over and over again, whether it's with serine or with a cystine, you have to have the right equipment to acylate the enzyme.

So what happens here is you acylate the enzyme. And so this is the part of the protein that's

going to get transferred, ultimately, to-- this is lipid 2, with the pentaglycine. And at the end of the pentaglycine you have an amino group. That's-- you're going to attach this protein, IsdA or IsdB to this lyse-- the end-terminal amino group of glycine in the pentaglycine.

So you form. Again, you cleave this peptide bond. And you have this piece left over from your Isd protein. You now have this covalently attached to the sortase.

And again, what you're doing is going to regenerate the sortase, so you can do more of these reactions. And here you're forming your linkage to the Isd protein. OK? Does everybody see what's going on in that reaction?

So another cartoon version of this, and then I'll stop here. This is a more chemical version. Again, this is the sortase. Here is your amino-acid sequence. You go through a tetrahedral intermediate. This is all a figment of our imaginations, [LAUGH] based on what we think-- what we do understand, in the test tube of peptide bond hydrolysis-- not so much in the enzymes. But you generate an acylated attached protein. And then we have our pentaglycine, the terminal amino group that goes through, again, a tetrahedral intermediate to form this linkage.

So what's happening-- I think this is, like, so, again, amazing-- what's happening is, you're transferring-- you've got your lipid 2, and you've transferred it across this membrane into the outside of your bacteria. So you've gotta hang it there. That's why you need these big, huge lipids.

And what you're going to do is attach to this pentaglycine. You're going to attach each of these Isd proteins, covalently. And then what you do--

So you make this guy. Then you attach this whole thing onto the growing polypeptide chain. I mean, this is, like, an amazing machine that they've unraveled, I think, from studies that have been done in the last five years or so.

So, next time, we'll come back and talk a little bit about the Isd proteins, but I think you should be fine, looking. You've looked at-- all you're doing is transferring heme, and we don't understand the detailed mechanism of how that happens. That's something hopefully some of you will figure out.