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JOANNE STUBBE: --iron homeostasis in module 6. And then, we're going to move on to module 7. And the readings have been posted. And the PowerPoint has been posted for today's lecture as well.

OK. So we were talking about last time peptidoglycans in gram-positive bacteria. And they're actually quite thick, depending on the organism. And so, what is the strategy nature uses to be able to pick up iron where you have a bacteria surrounded by this huge peptidoglycan? And the strategy in Staph aureus that has evolved is they attach everything, almost everything, covalently to the peptidoglycan itself.

So at the end of the last lecture, we were looking at-- we introduced you to the operon and Staph aureus. And there were proteins called sortases, which are transpeptidases, really, basically. And they have different sequence specificities. And I'll just go back one.

So where we were last time, we have this operon. And all of the proteins that are going to be involved in uptake of heme into the cell are called the Isd proteins.

And so, here is the operon. And you can see that each part of this operon is activated by a Fur box. A Fur box is the transcriptional factor that turns off or on regulation at the transcriptional level by the presence or absence of iron, which we're not going to talk about further.

And what I wanted to point out here before we move on is that all of the yellow anchors are proteins that are attached covalently by Sortase A, which has a defined zip code. The blue in IsdC has its own sortase, Sortase B. And IsdE is not attached covalently to the peptidoglycan, but actually has a membrane that's attached covalently to a lipid, which is then inserted into the membrane, which is the other strategy that's used in these systems.

And let me just point out-- you saw this. I'm not going to spend much time on this today because you've already had a problem in your last problem set on this. But you have these little N domains, or the NEAT domains, which are 120 amino acid domains that allow you to pick up heme, and are involved in transfer of heme down a bucket brigade.

So what we're trying to do is attach these Isd proteins to the peptidoglycan. How it does that, how they end up being organized, how many there are, is all an active area of research. We don't really know that. Somebody might know it, but it's not in the published literature.

And so, the idea is, again, you can see all of these proteins in some way are tethered via usually a single transmembrane spanning a region in the protein. And here is one Isd protein here with a little zip code. And it involves the Sortase A, which you've now seen over and over again, happens to have a cysteine in its active site to go through a covalent bond formation.

And you're going to be cleaving one peptide bond and forming another peptide bond. Where have you seen that before? That's how you did cross-linking, the transpeptidation reaction we talked about last time that allows you to form the bag. And the bag of peptidoglycans becomes cross-linked. And that's the key to survival of the organism.

And so, you cleave this bond between T and G. The specificity is understood. And people in the Pentelutes Lab use sortase quite a bit. But it has been engineered by the Liu lab to be more efficient as a catalyst.

So this is a natural catalyst. You do a transpeptidation reaction. And then you have-- this is to me, what's amazing. You have this lipid II, which is that C55 isoprene system with the pentaglycine hanging off the pentapeptide, all of which is generated on the inside of the cell, and gets transferred to the outside of the cell.

One, then, is set up to do peptide bond formation, just like we saw with the transpeptidase reaction. And so, you end up with then this lipid, lipid II attached to the Isd protein.

And then, that's taken-- so this is the detailed chemistry that we just discussed. And you can go through it again if you don't remember it. But it's very similar to things we've now seen many, many times in this course.

So once you attach this, what happens is it gets transferred. The whole thing now gets transferred by the mechanism similar to forming the polymer. But now you have the pentaglycine with an Isd protein attached as well.

And so you can see, then, this is the growing peptidoglycan with alternating units of N-Acetylglucosamine and N-Acetylmuramic acid we talked about last time. And now we have the Isd protein covalently attached.

So this is a pretty amazing strategy. And you can imagine trying to study this under natural systems. With everything attached, it's quite challenging.

And so the problem you had in the problem set, what did they do? They cut everything off. And all you're looking at is a little piece of the protein, not the whole protein anchored in this way with the right topology in the active site.

So, to me, even thinking about how you make a peptidoglycan that's 50-50 nanometers thick-- so you add things. How does it construct itself, a sacculus around this system and build up? I think nobody knows that.

And recent studies have been able to the first time to, in Staph aureus, reconstitute transpeptidase and a glycosyltransferase reaction. So maybe that, with a sort of an imaging technology we have, maybe in the next five or six years somebody will figure out how this amazing thing is organized.

So anyhow, we were able to attach these things covalently. And so, here we are. We've got all of these things anchored. And then, in your problem set-- and in the last part of this, I just wanted to very briefly go through how are people trying to study how the heme that's up here gets transferred.

And so, this is a cartoon model. And so, we have hemoglobin. We've been able to get-- we've been able to lyse red blood cells, get hemoglobin to come out, or haptoglobin hemoglobin. And now somehow you have to extract the heme. And that's done by two proteins, ISB and H.

And then we somehow have to get it to the plasma membrane. And so, the model is that this is transferred from IsdB through a NEAT domain. So all of these transfers up to here are through these little domains that can bind heme. And we have a structure of it. I'll briefly show you that.

And then the question, is there an order? If you get it out of IsdB versus IsdH, is it transferred? Here we have A closer to this than B. But is C closer than A? What is the organization? How many of these guys are there? Can they be transferred back and forth between the IsdAs?

I mean, there's 100 questions you can ask. And I think we don't know the answer to any of them. And the methodology, the problems that you looked at, again, they were looking at something that wasn't the intact system. And the key to all of this, of course, is the kinetics.

And I think when you start taking apart the pieces-- so you have this piece and this piece, and

half the spinach is missing, then you have this issue of how do you design the experiment so you have the right concentrations, so they meet in an efficient fashion?

And so the kinetics of what you were looking at in your problem set are not realistic. Because things aren't covalently attached. And so the question is, does it really give you a good representation of what's going on in the intact system.

So the model has been from studies like the ones that you saw in your problem set, that there is a pathway. That this protein can extract-- the B and the H proteins can extract the heme. It's transferred to the A protein. And so, here we have, can it be transferred directly to the C protein? Or does it need to go through the A protein?

So then you can start doing experiments like that. And you can take this protein and look for transfer to this protein. Or you can add in this protein and see if the rate, if you set the experiment up correctly, increases.

You can also ask the question, are these reactions stoichiometric or catalytic, which was also asked in the problems that you were looking at. If there is an order, how fast is the transfer from here to here versus here to here? And if it's really fast, what can happen is you don't need stoichiometric amounts of this. You can use small amounts of this to get the transfer to work.

And those are the kinds of questions that people are actually focused on. So this is one model that came out of the types of studies that you guys had on your problem set. And eventually, you need to get down here to transfer this to the ATPase, which allows the heme to be transferred into the cytosol.

So these proteins are structurally distinct from this protein, which is structurally distinct from that protein. We have structures of all these things. And they all do heme transfer. That's what their function actually is.

And so the details, the molecular details of how the heme is transferred is a major focus of a lot of energy right now. And so, if you actually look at this, I want to just say a few things about how this happens.

And so, one of the things that you need to think about, so what are the methods to examine Isd-heme transfer? And this is through these NEAT domains, which I defined before.

And so, one of the first things that people did was number one, you need to clone, express, purify, the Isd proteins. And most of these studies have been done in pieces versus full length.

And when you do-- so you could try to do the whole thing. Some of these things have three NEAT domains. Some have one NEAT domain. If you look in those cartoons I gave you, the number of NEAT domains are defined by sequences.

So you can study the full-length thing. Or the other one-- and, in fact, I think in your problem set, I can't remember, but in the problem set I think you actually did an expe-- was an experiment that you had one NEAT domain versus two. And what was the differences in the transfer?

So you can make these things. But when you make them, heme is quite often-- because there is biosynthetic machinery like there is to insert iron clusters, biosynthetic iron clusters, there's also biosynthetic machinery to insert hemes. So it comes out-- these come out to be apo.

So then, what you need to do is you need to add apo plus protoporphyrin IX to get presumably holo-- holo, whatever Isd is. And then, you need to purify that so you can get rid of any heme. You don't have a bunch of heme floating around. So, it's, again, yet another purification to look at all of this stuff that may or may not be easy.

And so, then after you do that, what you want to do is you need to characterize the spectra of the Isd proteins loaded with heme. And so this is the key to the solution of the problem, which is the same thing you saw in your problem set.

But if you look at hemes-- and I'll show you one set of data, a different one from what you had in your problem set. But hemes, you all know this from looking at hemoglobin, when you prick yourself and you bleed, if it's red or it's blue, depending on the oxygenation state, they have a very strong band called the Soret band at 400 nanometers-- about 400.

And that's a key thing. Why do they like the Soret band? Because it has extremely high extinction coefficient. I don't know what the number is. But its extinction coefficient at 400 is approximately 10 to the fifth. So it's easy to see that's why people have studied hemes over the years. Because heme is so much easier to see than any non-heme iron system.

And what you can see here, which also turn out to be quite useful, is these much weaker bands between 500 and 650. And those bands can also be used if you have enough sample

to look at this. And they're more distinct. They're indicative of the coordination environment in heme, whether it's hexacoordinate or whether it's pentacoordinate.

So all of this, I'm not going to talk about the spectroscopy. The spectroscopy of hemes has been extremely well-studied and is extremely rich. And so, you need to do that. You need to have your little proteins characterized and loaded with heme.

You need to quantitate the amount of heme balance. They want to make sure you don't have any free heme around which can interfere with all of your experiments.

And then you can ask the question-- you can start asking your questions once you've got that information. If you start out with IsdA loaded with heme, does it get transferred to IsdC in the apo form? And so, how do you monitor this reaction? Anybody got any suggestions?

One way would be-- so you want to-- the question that they're focused on here is, does IsdA with a heme plus apo-IsdC, does it transfer the heme to give you apo-IsdA plus heme-IsdC? And the question is, what's the mechanism of this transfer?

So I just told you we have spectroscopy. So I'm going to show you you could potentially do that. That's not so easy to do. But is there another method you can think of so we could monitor the reaction?

You need an assay. And that's what you saw in your problem set. So we can take advantage of the region of the Soret band, or perhaps in the longer wavelength.

What other method might you be able to use to monitor this reaction that we've learned about recently in recitations, which hasn't been used in the papers that you've read, but actually has been used to study these systems?

AUDIENCE: Mass spec?

JoANNE STUBBE: Yeah. Mass spec. And so, what people have done-- so you have to carry-- what you will see is that if you look at the sizes of these things, some have two NEAT domains, some have one. The apo also is distinct in size from the non-apo.

So theoretically, if you do all of your homework, you can use mass spec quantitatively to measure these reactions. And a lot of people have recently gone to that method, because this method is challenging.

Now, again, the caveat is, so you always need to remember this, is that all the Isd proteins are not covalently attached to the peptidoglycan. So to me, this immediately raises this issue of how do you decide how to do your experiments? So how much-- do you use micromolar? Do you use millimolar? Is it widely different, depending on whether it's attached or not attached?

And all of that is going to affect the kinetics of this transfer. So you can see transfer. But what you really want is the rate constants for transfer.

So you have two questions, is do you see transfer? So that's the first question. And so, one could tell that by either of these two assays. So you could use one and two.

And what you really want are the rate constants for transfer. And the rate constants for transfer are dependent on the concentrations. So how you set this up is something you've got to do a lot of messing around with.

And so, if you look at this model, you can ask the question, how is this transfer occurred? Do you go through a ternary complex? So does this form a complex with this? And then the transfer occurs through the complex?

Or you can ask a question, does the heme dissociate, and then the heme get picked up? So you can ask the question is, what is the order of addition? So you can look at the mechanism of transfer, and specifically, the order of addition. Do you need the second protein there to see transfer?

So they've done a whole bunch of experiments like this. I'll show you using the Soret band what they actually monitor in this particular reaction. But they did an experiment where they just took IsdA, IsdA loaded with heme, and asked the question, does heme go into solution?

So that's not a trivial experiment either because it can rebind. It depends on the on-rate and the off-rate. So you have to have a way of making sure that if it comes off, you pull it to the right to be able to measure the rate constant.

So this whole problem is really associated with thinking about detailed kinetic models, which I'm not going to go into. But if you look at the data, that's what you need to think about to believe the data, whether the data has been interpreted correctly, that I'm very briefly going to show you.

You need to derive the equations, and look at what your expectations are. Are they consistent

with the kinetic analysis of what's going on?

So here, they see a rate constant. So here, they're just looking simply at this reaction. Does this go to IsdA apo plus heme? And if they look at that rate constant, it's .0007 per second. So it's really slow.

So then the question is, is this transfer-- does anybody remember with the rate constants were for transfer in the problem you worked at? Was it seconds? Was it minutes? Did you think about it? I don't remember what the numbers are off the top of my head.

AUDIENCE: Like, tenths per second.

JoANNE STUBBE: Yeah. So that's much faster than this. And so, this is a really low number. But the question is, how low-- remember, we're missing part of a whole system. And so it could be really low, just because we don't have the system set up to mimic what we see in the native organism. So that's the problem you always face.

And so, if you look at the data, here's what they're monitoring. So here's the Soret band at 400. And so you need to get a good spectrum and convince yourself you're looking at the stoichiometric loading. So you need to know how much heme. Because that's going to affect your absorption spectrum. So you need to know how much is loaded.

And then you can do the experiment outlined over there. And what they're monitoring is these small changes. And so, when they do that, they come up with, if you look at the analysis, and I'll show you a few pieces of data, they come up with-- they favor the model associated with the kinetic analysis of all of their data, that you form a tertiary complex-- sorry, a binary complex of the two proteins, and that the heme is transferred from one to the other. And then the apo IsdA dissociates.

So this is analogous to what you've seen. And here's some data. So they've done it with every single pair in this particular paper. And what you can do-- and they've done all the experiments the same way, been able to see differences in Soret bands. These were all done with changes in the spectra, the visible spectra.

But if you look at this, for example, from the transfer of the heme from methemoglobin. So that's the first step up here. And you look at all the rate constants they measured. The fastest number is 0.31 per second.

So that's slow. But you might expect it. It might not be so easy to extract the heme out of hemoglobin. So you might expect this to be slow.

But, again, if you look here, and this is a thing that I think hopefully some of you thought about, if you look at the rate constants, these reactions are all bimolecular. But what do they have up there? They have first order processes.

So that has got to be telling you something about the interaction-- let's go down-- whether this interaction is rapid and reversible. And in the paper describing this work in detail, and I give you the reference in the PowerPoint for those of you want to look at it, you need to think about the kinetic analysis.

So when you're looking at rate constants, you need to think about whether it's first or second order. Somehow they have to get together. If this is doing half and rapid and reversible, you still have a term for that equilibrium step. But then you're looking at a first-order process.

So, if you look-- I'm not going to go through the whole thing. But if you look at the transfer from holo IsdB, so that's the second step. It got the B, got the heme out of the hemoglobin. And then you can look at the transfer to all the other proteins. You see that in this case one is 114 per second. So that's a fast transfer.

So you can look down. And they've done every single one of these steps. And they've also then also asked the question, can these proteins act catalytically? So then they put it in a small amount of one, and look at the rate constants in the presence or the absence of one. And they conclude that these proteins can act catalytically as well.

So these are the kinds-- I don't want to spend a lot of time discussing this detailed setup. Because I think you still have to worry about being covalently attached to the peptidoglycan. But these rate constants are pretty darn fast.

And so then the other thing that's interesting is, why does it have an order? Does it have an order? So if you were going to take B, can I transfer it to C? And what are the differences in the rate constants? And here, it's 114 versus 15.

So now the question is, did they set up the experiment correctly? They probably used all the same concentrations in the experiment. But one might have a higher affinity than the other. And so, you need to think about all that stuff.

And if they thought about that correctly, they really have learned something about the order of addition and the ability of these proteins to act catalytically. So this is state of the art right now, the way people are studying this. And this kinetic data has allowed us to come up with that model. I just showed you that there is an ordered way to transfer these systems.

And then I just want to say very briefly-- I just want to show you very briefly the structures of these, and just show you again where the state of the art is in this area. All of these NEAT domains-- so NEAT domains are the heme-binding domains, 120 amino acids. They are found in A, B, C, and H. They are found in four different proteins.

This is super position of all the NEAT domains. You can see they all look alike. Furthermore, if you go down here, and you look at binding, they all have a pentacoordinate heme with one axial ligand being a tyrosine. The other one, the top face, is apo in this version of it.

So they also have a structure of two of these things bound together. Again, these are little domains. And so the question, then, you have to ask yourself, which is this question of rates of exchange of ligands. How is this transferred?

Do you have-- how does this interface help this guy move from this protein to that protein? And that's what people are focusing their energies on, trying to think about the detailed structures to come up with a model for how this transfer occurs.

And I'll just show you, this is a-- if you go all the way down, you're going to go from C to E. E has a different structure. So the mechanism of heme transfer is different. People have a model for that. You need to think about the details.

And now, furthermore, you can go from E to F, all the way through the plasma membrane to the ATPase, which then helps you get the heme, provides the driving force getting heme into the cytosol.

So we have a lot of structural data. But what's disappointing, I think, from reading those papers, which I have read, is we still really don't have a good model for how these transfers actually work. So this is an active area of research, and the people interested in the bioinorganic chemistry and how you get heme into cells.

So that is the end of module 6. I think we've learned a lot in the last few years about these proteins. But, as you can see, we still have a long way to go in terms of molecular understanding.

And so the next module, module 7, is going to be the shortest module. And I'll give you an outline of what I'm going to be talking about. And then, today's-- the first lecture is much longer than the second lecture. And we'll see the second lecture is going to be focused a lot on what we're doing in recitation this week.

So if you notice, maybe you haven't, but we posted the readings. And one of the papers for the course, this part of the course, the lecture part of the course, is a Carroll paper you're supposed to read for recitation. So there's a lot of overlap. And so, the second lecture will be much shorter because we're going to draw on what we're doing in recitation, actually today.

So let me give you the outline. So module 7 is the shortest. And this is the required reading. So we've posted a review article by Winterbourn, who, in my opinion-- this area of reactive oxygen species and how [INAUDIBLE] to how you control reactive oxygen species. I'm going to show you they can be good. They can be bad. Just like we saw with iron, it's all a question of homeostasis.

The most thoughtful discussions have been described by Winterbourn, who is in New Zealand, who really thinks about the kinetics of what's going on. And I would argue, you can't do anything in this field without thinking about kinetics, which most people, most MDs in this field, don't think about at all.

So the literature is a mess. But I think the last few years it's become-- it's starting to get unmuddy. And I think it's an incredibly important area. I guarantee you that that's true.

So unmuddying an incredibly important area is going to be up to you guys. But I think it's going to happen in the next five years or something. I think we've already learned a lot in the last couple of years.

So I'm going to have an outline. So we have that paper. And then we have the Carroll paper that you guys hopefully have already looked at in some form. All right. So here, let me just switch.

So where are we going? And so, we're going to have a couple of lectures. First of all, what is ROS? So ROS-- a reactive oxygen species. So automatically, there are a bunch of molecules that are reactive. And so, what you need to think about is what does reactive mean?

So the first thing is we're going to identify them. The second thing is we're going to look at the

chemical reactivity. And, again, the question of chemical reactivity can be quite complex. But I'm going to give you my view of the chemical reactivity and what that view is based on.

And then we'll very briefly look over-- since we move from an anaerobic world, whatever, a billion years ago into an aerobic world, like we learned from the last module, the question is, how do we defend ourself against the presence of oxygen with reduced metals? And that's the issue we raised last time.

So what are our defense mechanisms? Because we saw we had copper. We had iron. And now we have oxygen. And we'll see that that can be a recipe for disaster unless you can figure out how to control all of that. So, again, it's all homeostasis.

So that's the second. That will be the first part of today's lecture. Then we're going to move into the question of the battle between bacteria or viruses or parasites in humans.

And what I'm going to talk about specifically is destruction of bacteria by neutrophils. And we'll see that neutrophils are white blood cells. And we will see that they are the first responders. So if you have a bacteria in our bloodstream, the first guys there are the neutrophils. And that's what we're going to focus on.

So let me see. All right. I'll go over here. So neutrophils are the first responders. Now, we know quite a bit about this. And really, what we're going to be focusing on in both today's lecture and in the next lecture is the group of isozymes called Nox proteins. N-O-X-- NADPH oxidases. And we're going to talk about that particular protein.

And we're going to be specifically focused on Nox2. And we'll see that Nox2's professional job, we'll talk about that, is to generate a reactive oxygen species, superoxide, which is then going to be used in some way to kill bacteria.

So we're going to be talking about neutrophils. We're going to be talking about the Nox protein. Also, if you've read the recitation paper for today, what are we talking about in signaling that's oxygen-dependent? The Nox proteins.

So here we have bad. We're killing the bacteria. Here we have good. We're using the Nox proteins for signaling. So that's sort of the take-home message is homeostasis. How do you control it for bad versus for good? We've already seen that in the case of the iron system.

So the other protein we're going to talk about today, or probably won't get that far today-- how

bad am I? So we have another protein called myeloperoxidase. And we're going to see that this-- so this guy is going to be involved with superoxide. This guy is involved in the neutrophils with generating hypochlorous acid.

So these are the proteins. And these proteins together-- I'll give you the model, the current model. But the current model I'm going to give you is much simpler than reality. But those are the proteins we're going to focus on. And those are two of the reactive species we're going to be focusing on.

And then the second lecture goes back to the Nox2 proteins, and the question now of not killing, but signaling. And as we already saw in the last recitation, how were we signaling? We were signaling by a reaction of sulfide groups with hydrogen peroxide, which can be generated from superoxide. I'll show you how that happens.

So, signaling. And we're focusing on signaling by sulfenylation which, again, is the topic of today's recitation of the epidermal growth factor receptor. So that's where we're going.

And I'm going to give you that-- we're going to follow this outline. And I think you'll get a pretty good feeling for it, an overview of reactive oxygen species, even though we're not going through it in a lot of details. It's really complicated.

So what I want to do before I get to this slide is give you the big picture. So this is the take-home message. So we have a big picture. And so here we have cell. And in this cell we have reactive oxygen species.

And there were also things called reactive nitrogen species. And you'll see that in some of the slides in the PowerPoint presentation. We're not going to talk about that chemistry.

It's interesting. If I had an extra three or four lectures I would also be talking about that. It's central. It's as important as reactive oxygen species. But I've decided to focus on-- that's what I've decided to focus on.

So we have hydroxide radical. We have hydrogen peroxide. We have superoxide. And we have hypochlorous acid. So these are the four species that we're going to be focused on. And you can already see that some of them are radical. And some of them are not.

So reactive oxygen species doesn't mean they have a free radical. They can do one-electron or two-electron chemistry. And we'll talk briefly about that. And so, the question is, where do

these come from?

So remember, we made a transition from an anaerobic to an aerobic world a billion years ago. And during that process, we have a respiratory chain. In humans, the respiratory chain is found in the mitochondria. Otherwise, it's found in the plasma membrane.

So we have complexes I, II, and III. And these guys, to chemistry, their ultimate goal, if you're in an oxygen-dependent world, is to reduce oxygen to water. So this is a goal, is oxygen to water. Although, you all know in bacteria if there's no oxygen, you have to have some other terminal electron accepter.

And so what you get from these complexes is uncoupling. So 100% of the time it doesn't do what you want it to do. And so, you have to-- you get side reactions that you have to deal with. All right. Now, what did I do with my chalk? Anyhow. So you get uncoupling. So that's one way they're generated.

A second way they're generated we've already been through. We went from an anaerobic world to an aerobic world. What do we have under those conditions? We have iron, and we have copper. And, again, we have oxygen. And so, we can generate reactive oxygen species.

We've already talked about the fact that iron just isn't freely floating around inside the cell. But if something happens and you have an imbalance in iron homeostasis, it leads to imbalance in oxygen homeostasis. And hopefully, you remember that one of the proteins regulated by the iron-responsive element, iron-responsive binding proteins, was the oxygen transcription, oxygen-sensing transcription factor.

Another way that we get these things are from xenobiotics or environmental pollutants. If you smoke, which I guess people don't do. But when I was in graduate school everybody smoked. And they smoked in the lab. Anyhow-- you get-- so pollutants can generate reactive oxygen species.

So that's how we get them. What do we do with them? So there's some important things that we can do with them. Here it is. This is what I want. So what are we going to do with them?

So one of the things we can do is we have white blood cells and neutrophils. And we kill bacteria or viruses or parasites. So that's one of the good things we can do with them.

A second thing we're going to do with them, which is what we're focusing on, is signaling. And

we'll see that while we're looking at signaling of growth factors or hormones or cytokines, which is good signaling, we'll see that you can have all kinds of signaling.

So it's not limited to the one system we're going to be studying. So it's very broadly defined. This is a huge area of research right now, people looking at this.

And the third thing that happens when this is completely out of control is you modify, you damage all the macromolecules, the small molecules inside the cell. So you have extensive damage. You can have extensive damage of DNA-- this thing is not writing very well-- proteins.

But it's not limited to that. You have lipids. Lipids are modified by hydrogen peroxide. And so, that's the big picture. That's where we're going.

We're focusing on these guys. And we're focusing on good things. And we're focusing on bad things. And how do we control all of that.

So in the next-- what I want to do is show you why this is-- why I decided to talk about this. I've always found this area fascinating. And, I must say, I've been going to meetings off and on for decades. And I sort of quit going because I lost information every time I went to a meeting because it was so confusing.

Because everybody used different cell types. And they have different kinds of assays. And they didn't pay attention to what the assays were really telling them. But now, I think we're at the time when people really need-- people are doing good experiments. I think we've turned a corner.

And so, one of the things that has been in the front pages of all the newspapers since 2007, there was-- we actually-- there was Jim Collins, who was at Boston University, received a huge amount of press on a paper he published in 2007. And since that time it's been extremely controversial. And so, I think it brings up a lot of issues about, again, how you do controlled experiments.

So his observation and his conclusions were, from the experiments he published, were very interesting. So we just talked about antibiotics. What do they target?

They target cell walls. We have penicillin, vancomycin. They can target the ribosome. You saw that in the first part of the course. You have aminoglycosides. They can target DNA replication. So those are the three sort of major targets. You have the quinolones that do that.

His conclusion from the paper is that all of these things, the mechanism of cell kill is not involved with the primary targets at all. But it's involved with a downstream target, that somehow these guys undo oxygen homeostasis, resulting in bad radicals, reactive oxygen species, that end up damaging macromolecules, damaging the cell, and resulting in bacterial cell death.

So that's the model. This has been quite controversial. I've given you some papers to read. The latest paper was just published online saying reactive oxygen species play an important role in bactericidal activity of the quinolones that targets topoisomerase in DNA replication.

But we've had two articles published in *Science* saying killing by bacterial antibiotics does not depend on reactive oxygen species. So we've had quite inflammatory responses to this paper, which I at one stage didn't believe anything that he did. Because I think if you look at a lot of the original experiments, they use reagents that were completely nonspecific for what they thought they were.

But I think I am now-- I think his observations, in fact, were correct. But not-- some of his observations were correct. But the reasoning behind the observations has changed.

And I think there is something about this. You do signaling up here. And then where do you see something? Way down here, because you trigger off a signaling cascade. And that's the way the world works inside the cell.

So a second example of this which also received a lot of press, this guy-- everybody know who this guy is? The DNA guy. The famous-- he's a male chauvinist pig, but the DNA guy.

Anyhow, what he-- there's been a big fight. Are reactive oxygen species good or bad in fighting cancer? So some people say, again, it's the reactive oxygen species that eventually kill the cancer cells.

Again, you do something up here. You trigger off a set of events. You generate a pathway that generates reactive oxygen species that helps kill the tumor cells. Or do they stimulate growth-- reactive oxygen species stimulate growth? Or do they trigger apoptosis?

So people are still debating that. And there's probably some truth in both of these statements, depending on how you look at it. Anyhow, that's just to get you thinking about the fact that this is an important area that a lot of people are actually focusing energy on.

So what I want to do now is we've given a big overview. What I want to do now is look at what are the identification of the species. And the species we're going to be focusing on, again, are superoxide-- I'll write this down later-- hydrogen peroxide, H202, hypochlorous acid, and hydroxide radical.

So this is a scheme that was taken from a Winterbourn article. So it's in all of her papers. I don't know if it's the exact same scheme in the reading assignment, but you'll see something similar.

And we're not-- I told you we're not going to talk about reactive oxygen species. We're only focusing on reactive-- sorry-- reactive nitrogen species. We're really focusing on reactive oxygen species. But this gives you the big picture with both.

So where are we going to be focusing? Oxygen picks up an electron and goes to superoxide. Superoxide, in the presence of another electron and protons, goes to hydrogen peroxide.

So we're going to be focusing here. Hydrogen peroxide in the presence of Iron(II)-- if we've somehow screwed up our iron and where in the reduced state generates hydroxide radical.

Hydrogen peroxide with myeloperoxidase in the neutrophils forms hypochlorous acid. And so-- at which eventually can chlorinate everything. They chlorinate amino acids. It chlorinates lipids, and can result in extensive damage to whatever hypochlorous acid is adjacent to.

So this is where we're going to be focusing, this part of the scheme. Those of you who have seen reactive nitrogen in the species can look at how that gets integrated into this big picture.

So I just want to write down one of these things that I think it's important to think about. Ultimately, we're doing chemistry. This-- whoops. I mean, this is like, it's terrible. It's all of a sudden, I look up. It's a good thing I looked up. Because I would have kept going.

Anyhow. Sorry. The time is over. But next time we will come back, and we will talk about what I outlined on the board. And I didn't even digress today. I'm just-- anyhow. All right.