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PROFESSOR: What I want to do is proceed where we left off. We're in module seven on reactive oxygen species. I'm introducing you to the concept, what was the big picture. And at the end of the last lecture, I gave you a few introductory slides, but what we're going to be focusing on, I told you was oxygen can be one electron reduced to superoxide. Superoxide can pick up another electron. They should have a couple protons here to form hydrogen peroxide.

So these are two of the species we'll be looking at. If you have iron 2 around, so this goes back to the connection to iron homeostasis that we talked about in module 6, this is called Fenton's chemistry. And you produce hydroxide radicals, so that's the third reactive species. And hydrogen peroxide in the presence of chloride with myeloperoxidase can form hypochlorous acid, which then can chlorinate amino acids or sugars or a lot of other things. So you get rampant chlorination inside the cells.

So this cartoon also has reactive nitrogen species. We're only focusing on the reactive oxygen species. So that's what we're going. And so at the beginning of the lecture, I wanted to introduce you-- and I did give you an overview of where we were going. We were going to look at what are the reactive oxygen species, what does reactivity mean, how do you define chemical reactivity-- I think that's a key issue-- and then what are defense mechanisms against these reactive oxygen species, before then focusing on the NADPH oxidases, which those are the enzymes that we're focused on in this whole module on reactive oxygen species.

So identification, and I had put this on the board the last time. We have-- just repeating what's over there, superoxide hydroxide radical, hydrogen peroxide. Because this is together, and hypochlorous acid. And we're going to look at the reactivity of these guys. These are one electron oxidants. These are two electron oxidants. So reactive species don't need to have free radicals. They can do two electron chemistry, or they can do one electron chemistry.

OK, so a key thing to think about, and I don't expect you to remember the detailed reduction potentials, but again, as the inadvertent consequence of our environment, you know, 1.8 to

0.8 billion years ago we moved from an anaerobic to an aerobic world, where we have metals all over the place. The question is how do you control these reactivities.

And so we need to think about the redox chemistry of oxygen. So oxygen, and the details the actual reduction potentials, and these are some of them given here. And this one somehow got lost from here to here, which is 0.94. But it's in a table later on in the PowerPoints, depend on the balanced equation.

So if you're looking at this, and you really want to think about the reduction potentials, you need to count the numbers of electrons and protons and balance the equation that you're looking at, because the reduction potentials vary. But what you need to remember is the more positive the number is, the easier it is to reduce, the more powerful the oxidant.

So we're going to have oxygen. And in the first one, we have one electron reduction. And this is the only one where the reduction potential is negative. So this is uphill. It doesn't want to be oxidized. And this produces superoxide So this is one of the guys we're going to be talking about, and here's the reduction potential.

This can be further reduced with an electron and a couple of protons to form hydrogen peroxide. So I'm not going to write that out. Everybody hopefully knows what hydrogen peroxide is, and this is the number that for some reason got left off that handout, and this is favorable.

And so are all the other numbers. And the numbers are actually large and favorable. That means they're good oxidants. OK, so more positive, better oxidants. These are all biological reduction potentials, relative to the normal hydrogen electrode. So these are the ones-- there's actually a table in there where you see the numbers that people interested in biological systems focus on.

Now, we'll see later on that hydrogen peroxide gives rise to hypochlorous acid in the presence of chloride. But hydrogen peroxide can also get further reduced to-- and this is one where I don't have the balanced equation to hydroxide radical. And so the number I have written down here is 0.38 volts. But again, the numbers aren't so important.

And then this can get further reduced to water, and this number is-- you'll see in a number of the tables I give you is 1.31 volts. So we have different states, and all three states, consequence of moving from the anaerobic to the aerobic world that give you species that are

called reactive oxygen species.

And the one that's hardest to form is superoxide and this is the one that's probably the least reactive. But what I'm going to do is give you a couple sets of criteria for reactivity, because when people say something is chemically reactive, you know, what does that mean? It depends on what it is reacting with. And so this, I think, is the real problem in the field is that people really don't define this very well. And so this is why I think the Winterbourn paper is so important.

So she has a table in there. I'm going to have the table up there. I reorganized the table to focus on what I want to focus in this brief introduction to this topic and show you how they define reactivity. But if you are thinking about something, you need to define what the reaction is that you're interested in with any of these reactive oxygen species.

So these are the guys we care about. And the second question I wanted to address is-- so first we were identifying last time the identification. Second is a chemical reactivity. OK. And I've taken this-- maybe it wasn't from her paper. I can't remember where I've taken this. I should have referenced it. But anyhow, what I'm going to do is give you a simple table that I think is useful to think about reactivity.

So if we look at reactivity. We're going to look at the oxidant. And then another category is going to be the biological defense. And the third category is thermodynamic properties. And you all know that things can be thermodynamically favorable, but not happen at all, like oxygen oxidizing glucose on the table.

So you not only need to think about the thermodynamics, which involves the redox potentials, and you need to define the sorts of conditions that you're looking under, but then you need to also think about the kinetics. OK, so we have thermodynamics. So we have biological defense, thermodynamics, and then the kinetic properties.

And the kinetic properties are often given in terms of reactivity with a molecule called glutathione. I'm not going to draw out the structure. But it's a tripeptide with gamma glutamyl cystine glycine. OK, so it's a tripeptide with an unusual linkage to the next amino acid. What do you know about glutathione? Have you guys ever seen that before?

So it's a major redox buffer inside human cells. Now, if you're in a microorganism, we don't use the same major redox, so you need to look at that. But all organisms have redox buffers.

And as you can imagine, and this is why we focused with the mass spec stuff, sulfenylation, one of the major targets of out of control reactive oxygen species is oxidation of cystines.

There are other amino acids that get oxidized, but the focus is on the cystine [INAUDIBLE] and all the changes that can be made and all the signaling, most of the signaling, through reactive oxygen species all go through cystine oxidation. So this is a reasonable choice, gamma glutamate.

So let me just write down, it's a tripeptide of glutamate cystine and glycine, with an unusual linkage here, with an iso peptide linkage. So the first two are one electron oxidants, and the first one to talk about is hydroxide radical. Hydroxide radical you'll see by far and away is one of the most reactive, is dying to be reduced, as you can tell, by this reduction potential, no matter what the variation on the theme is.

And there is no defense. So you don't want to get to hydroxide radical. So there's no actual defense. And that's not completely true, because in reality, if you have glutathione around, the glutathione will reduce this by hydrogen atom transfer. So an important component is the redox buffer.

So glutathione-- let me just write that down again, because we're not going to have time to talk about this, but redox buffers play a central role in reactive oxygen species. And so the thermodynamics of this, it's dying to be reduced. If I have the numbers right, I think-- I don't remember what the numbers were. OK, so the numbers here are 0.31. I have different numbers in different places.

But anyhow, it doesn't matter. It's dying to be reduced. It's a hot oxidant. And then the rate constant for reaction with glutathione. OK, so it would be h dot transfer. And this is a bimolecular. Rate constant is 1×10^{10} per molar per second.

So this is really fast. And so if you've got glutathione around, your hydroxide radical is gone. We're going to look at another way of trying to define reactivity, but this is the way that people, who were trying to think about the kinetics of all this, are starting to do this.

OK, so this is one. The second species, which is also a one electron reductant, is superoxide. And this is the one seen described most frequently as a reactive oxygen species. In reality, it's not very reactive at all. It is reactive, but not anywhere near as reactive as some of the others. And do we have a defense mechanism? We'll come back to this a little, and I'll write a

balanced equation.

I'm just going to list things. We have enzymes, called SODs, and so this is superoxide dismutase. And I'll come back and write a balanced equation in a minute. So we have proteins that are devoted to this, but in reality metals, like manganese inside the cell can actually function as a superoxide dismutase at reasonable rates. Protons cause rapid dismutation to form hydrogen peroxide and oxygen.

This guy is also dying to be reduced, so thermodynamically, this is a good oxidant. But the key is thinking about the kinetics. And it obviously depends on the reaction you're looking at, the kinetics are going to be different with every small molecule or large molecule it interacts with. But, again, we're using glutathione as an example. And the numbers that people report for superoxide compared to 1 times tend to the 10th are now 10 to 1,000 per molar per second.

OK, so this is chemically much less reactive than hydroxide radical. And even for this one, we have a defense mechanism. This one, again, is problematic. OK, so now what we're going to switch to is two electron oxidants. And the one we're going to focus on today, in this section, what happens in neutrophils to defend against invading organisms, like bacteria or viruses or parasites.

The major way that this becomes neutralized inside the cell is, again, in a mammalian cell is with glutathione. So this is a small molecule. It also is a very strong oxidant, but the mechanism of oxidation is distinct, two electrons, versus one electron. We're going to look at examples of this. And if you look at the rate constant for reaction with glutathione--

And again, you need to really think about a balanced equation in the kinetics of all of these things. If you're ever going to work in this area, that's what you need to do. You need to educate yourself about what the species are with which you're going to interact. But you can see from this number under the sets of conditions, they did everything the same way, so that they could compare the relative reactivity of these molecules, 2 times 10 to the 7th.

So this is much more reactive, for example, in superoxide And then the last one is hydrogen peroxide. So this is also two electron. And two electron, we will see that there are a number of proteins that mount a defense. These are called-- and I'm going to show you this in a minute-- peroxyredoxans. I'll show you what they do.

What did you see-- do you remember the enzyme that was used in the Carroll paper this week

in recitation? To get rid of hydrogen peroxide, what did they use? Anybody remember? We use catalase. I'm going to come back and write the equation, so catalase, and then the other one, which we also talked about, but we didn't talk about the chemistry in the Carroll paper, was peroxyredoxins. And there were like seven or eight different isozymes.

So we have a number of ways of dealing with hydrogen peroxide. Again, it is thermodynamically favorable to be an oxidant. But as we've already talked about before, hydrogen peroxide is really not very chemically reactive at all. And so the numbers that they quote under these sets of conditions are 0.9 per molar per second. So it's much, much slower.

You see numbers that range from 0.9 to 20, but this has really important implications in the paper we talked about in the mass spec analysis, where hydrogen peroxide is functioning as a signaling agent. We're going to come back to this later on. And this for years made more chemical-y type people not believe that hydrogen peroxide was involved in signaling, because the rate constants were just too slow, compared to the biological response of the other side.

So this is sort of a superficial overview of the differences in reactivities, but the real take home message is these molecules have different chemistry and different reactivities. And I guarantee if you're studying stuff inside the cell, you're going to worry about these kinds of things, and you need to educate yourself about what you're worried about in terms of the redox potentials of these systems.

So there's a second way. So those are just the redox potentials. So there's a second way to look at reactivity, and this is also, I think, in the paper you had to read. So the second way is by just looking at diffusion, how far-- this is within a cell-- can you still feel the effects of the oxidant.

And so I'm going to say see PowerPoint for the cartoon. So I think this is a good way to look at this. And again, the numbers are squishy, but here we are inside-- this is the cell, and the question is, do some of these oxidants get out of the cell and go to the next sets of cells. And so if you look at something like hydrogen peroxide.

So hydrogen peroxide is the least reactive from this criteria of kinetically, the least reactive. And it goes way outside the cell. So it diffuses farthest away. So that means it's the least reactive. So the distance is used to define reactivity. And again, this is a squishy number, but I think it's informative.

Now, what you see here-- so hydrogen peroxide, we just went through, is the least reactive. But I also told you that hydrogen peroxide, there are many ways to remove it inside the cell, peroxyredoxins, glutathione, glutathione peroxidases catalases. They all remove it. They all have different rate constants.

Peroxidative redoxins account for, I think, it's 1.5% of mammalian cells, and they're very important in controlling redox balance. And what do you see here? If you are in an environment where you have a peroxyredoxin, what happens? This diffuses a lot less quickly. Why? Because the enzyme rapidly reacts with this molecule.

So we know that the enzyme can react. I haven't given you that number. But we'll see that this number is on-- instead of being a number of 0.9 to 20, is going to be 10 to the 6th per molar per second. So this now-- something about the active site of this-- and it's not SH versus thiolate. We all learn now. Everybody is good at this. Thiolates are the reactive species. It has nothing to do with that. Thiolates are always more reactive.

But there's something else special about these proteins that allow them to control hydrogen peroxide. Now why might you want to do this? If you have a signaling agent, like hydrogen peroxide, you don't want it going all the way over here. You want to control the effective concentration near we want the chemistry to happen. So these peroxyredoxins play an incredibly important role in controlling the effective concentrations.

And so if you look here within the cell, again, we're only focusing on oxygen, but both hydroxide radical and hypochlorous acid are very, very reactive. You can't go very far without having them react with something, and that, again, is consistent with the kinetic analyses that people have done over the years.

So the take home message from all of that is that these reactive oxygen species have different chemistry and different reactivities, and you've got to educate yourself. But some of these things, HOCL and hydroxide radical, are very reactive, no matter what. So the last thing I wanted to focus on was in this section, which is basically the introduction, is the defense mechanisms. OK, so this is the defense.

And I already have listed what the defense mechanisms are, but I wanted to give you a few equations. You've already seen that they can be enzymes or small molecules. And so one example we already looked at is-- we already described, but didn't look at in chemical details is superoxide dismutase. OK, what does superoxide dismutase do?

It takes two molecules of superoxide and they disproportionate in the presence of protons to hydrogen peroxide and oxygen. And these enzymes have a k_{cat} over k_m , a catalytic efficiency on the order of 7×10^9 per molar per second.

So these are incredibly efficient. In fact, metals-- again, manganese in solution, in some organisms, they have a lot of manganese, they can actually do disproportionation. But it's all about the rate constants. So this is incredibly efficient. These enzymes are in all organisms. And obviously, this reaction is very important. You don't want superoxide completely uncontrolled, and there are some of these enzymes-- these are all metal catalyzed reactions.

Some use iron. Some use manganese. Some use copper. Some use-- humans use copper and zinc. And there are others that use nickel. And they all have different properties, and they've all been studied in some fashion. So depending on where the organism lives, they might use different superoxide dismutases to control the levels of this reoxygen species.

The second defense mixing mechanism of the peroxyredoxins. I think I have this one up here. So any of you that are interested in this, there were like a seven or eight isozymes. They keep finding new isozymes everywhere inside the cell. They are at high concentrations. They are clearly very important in controlling the redox balance.

So they do react with hydrogen peroxide, but they also react with other peroxides, and they're important in controlling the redox balance. And so, here and in each one of these isozymes has its own characteristics. You don't need to remember the details, but the chemical mechanisms of sort of the same, even though some are dimers, some are monomers.

It turns out they all have in the monomer two reactive cystines. So one is called Cp. That means that's the species that reacts with the hydrogen peroxide. So we have-- they can be monomers or dimers. This is the protein. And so you could have a Cp which can react to get sulfenylated. And then you have CR, which can react to resolve the sulfenylation process.

So you're going to get rid of the sulfenic acid. So here, if you have a CR I'm being sloppy here. In other words, you probably have-- this is probably protinated. It's all controlled to generate the anionic form of the cysteine, which then can form-- in this case, I'm drawing an intramolecular disulfide. OK, so this forms a disulfide.

And this is intramolecular. So what do we see over here? Over here, we see you can form an

intermolecular disulfide, if the molecule's a dimer. So the chemistry is exactly the same. But sometimes that occurs to the monomer. Sometimes it occurs through the dimer.

And then the question is once you have the disulfide, so now you have-- how do you re-reduce this? And you re-reduce this by some kind of reductant, such as thioredoxin which we will see if we get it as far as talking about ribonucleotide reductase.

So where have you seen these kinds of proteins before? Does anybody remember. So thioredoxin-- this is thioredoxin. There are probably 10 different kinds of thioredoxins inside the cell, these small little proteins, as is peroxiredoxin.

And they're all involved really in redox balance. So we can intercept the hydrogen peroxide. Say we want to get rid of the hydrogen peroxide fast, we've done our signaling, we want to get rid of it, you need to get something in the air that can react with hydrogen peroxide that they are fast to remove it. And then you want to reset your protein, so it can react with another molecule, so you need a reductant.

So these are the key-- for two of the four things I was going to describe in terms of defense. Another one is catalase, this is the one that if you go back and you look at the Carroll paper, which we discussed actually in class, these are heme proteins, and these are distinct from the myeloperoxidase that we'll talk about in this section. But they can take hydrogen peroxide, and they can convert it to oxygen plus water.

So what they've done is removed a putative reactant species. Again, how reactive it is depends on the environment, and turn it back into oxygen and water, which are completely unreactive. And the fourth, which is used quite frequently, are the glutathione peroxidases. And this is the one-- I just told you what the structure of glutathione is, peroxidase is.

You all know from the Carroll paper that you have a single reactive cystine in glutathione peroxidase 3. That's what we used as the model for all of our redox chemistry. Some of the glutathione peroxidases actually use selenium. So there's the 20 second amino acid is selenocysteine This is one of the few enzymes, as our thioredoxin reductase, which are involved in this whole redox balance system, are selenoproteins. We're not going to talk about those.

But the glutathione peroxidases is actually take two molecules of glutathione plus hydrogen peroxide. Again, there are many, many isozymes, and they can oxidize this to the oxidized

form. So this is the reduced form. You have a cystine. And this is the oxidized form. So you have a disulfide. Now, again, where have you seen this thiol disulfide system before?

I mean, bacteria have these things. We're talking now we're focused on human systems. Do you remember what happens in the periplasm bacteria? Did you talk about that this year? So you haven't seen this before in past. So bacteria in the periplasm enzymes that control what thiols you have and the state of the thiol.

So this redox balance by this disulfide interchange, very similar to this kind of chemistry, is everywhere. And the chemistry is pretty simple. But if you go from cystine to a disulfide, you just don't go there. You just don't go there with oxygen. I think this is something that people get confused about all the time.

You're doing an oxidation, something has to be reduced. So hydrogen peroxide, [INAUDIBLE], you go through sulfenic acid. You can now picture that you can have general acid catalyze, general base catalyze, cleavage of disulfide bond formation. So just because you have oxygen around and reduced cystines around doesn't mean you automatically rapidly have disulfides around. Again, you need to think about the chemistry of what's going on.

So now what I want to do is then show you sort of the general model. And then we'll talk about the NADPH oxidases, which is the focus of module 7. The general model is as follows. So you have an oxygen. And we have the N-- sorry. The proteins we're going to be talking about are NO₂ or another NO_x isozyme. OK, I'm not going to write out the name. We talked about that in the last recitation section.

These enzymes use-- and I think this is important because part of the redox switches that I think are under appreciated are the levels of NADPH, NADP. They play incredibly important roles inside the cell. So you have NADPH going to NADP. And we talked about the fact-- and we'll come back to this that this protein has a flavin and two hemes. And it produces superoxide

So this is incredibly unusual. Superoxide is usually an artifact of some uncoupling reaction that happens all the time inside the cell. This enzyme is a professional superoxide generator. That's what its goal is. OK, most other times you see superoxide something has gone astray. So this is a professional superoxide generator.

And so what happens then, when you generate superoxide you could have SOD, or you could

have protons. So if you're in a place where the pH is slightly lower, you generate rapidly, very rapidly hydrogen peroxide. OK, so superoxide doesn't sit around all that long. If you have iron 3 around, it could be bound to something. What happens is the superoxide combines with the iron 3 to reduce it to iron 2 plus oxygen.

So superoxide if you look at the reduction potentials, obviously what does it depend on? It depends on the ligand environment of the iron 3. That affects the redox potential. Hopefully, you all know that and have thought about that at this stage, given the last module. So what happens now is the hydrogen peroxide can react with iron 2. And this is the killer. That does what's called Fenton's chemistry, which generates hydroxide radical.

So these two guys, hydrogen peroxide and iron, now combine by what is called, in the review, Fenton's chemistry. And I'm not going to write out the detailed mechanism of how this works in fact, I think we still really don't completely understand it. But anyhow, you're generating this reactive species, hydroxide radical, which is dying to be reduced.

So this guy is responsible. It hits anything, and it reacts. So it ultimately is responsible for modifying lipids, modifying sugars, modifying amino acids, modifying nucleic acid. This guy-- because it's so reactive-- damages proteins, DNA, RNA, I'm not going to write all this out, lipids. This is the guy. And that's described in the review article you had to read.

And what can this hydrogen peroxide also do? We'll also see that the hydrogen peroxide, which is going to be generated inside the neutrophil, which we're going to be focusing on, the white blood cells that are going to be trying to take care of the bacteria or viruses, and that you have chloride. Now, you form hypochlorous acid.

So these are the kinds of guys, HO dot, HOCl are guys that are going to actually do destructive things when they react with things that help us to defend ourselves against bacterial insults. So that's a picture of the big overview. And so that's that and we're going to be simply focusing on two proteins. The first protein we're going to talk about is the one we went through in recitation, NO_x.

And I'm not going to write down that reaction. Hopefully you all know this by now. I just sort of said that over there. And there are 11 different isozymes, and then myeloperoxidase, which both of these are found in the neutrophils in the phagosome of the neutrophils.

OK, so let me-- so the chemistry that goes on with the NO_x proteins is complicated. So it's not

just the NOx protein. We're going to talk about the NOx protein. But as with everything, there are other factors that play a key role, and I'm going to show you a cartoon with what the other factors are. But we're not going to talk about the details of how those factors help the NOx2 protein make superoxide OK. Make superoxide in a controlled fashion, that's the key thing, in a controlled fashion.

So we have a NOx protein. The only one I do want to look at is the NOx protein itself, because we're going to use it not only in this lecture, but also the lecture of NOx2 proteins in signaling. So the chemistry is the same in destroying the bacteria and in signaling. So you need to know what the protein does. So if you look at NOx2 NADPH oxidases, what do you know?

They exist in a membrane, and we'll see this membrane can be the phagosome, or it can be the plasma membrane, or can be-- I'm going to show you a cartoon of this-- a vesicle membrane. These proteins are located in many places inside the cell. But they all sort of have the same predispositions. So they have one subunit with a domain that has the FAD on it.

So if we're looking at with the epidermal growth factor, or if we're looking at the neutrophils, this would be the cytosol, if we're looking at the neutrophils, and this would be the inside the lumen of the phagosome. And the FAD can be-- what is the function of FAD? We've talked about this. It's a major mediator between two electron chemistry and one electron chemistry.

And you've seen that before. Hopefully that was grilled into you in the respiratory chain. So in the respiratory chain, you have iron sulfur clusters, or you have hemes. Here we're going to have hemes. And so what we have is on this face, we have NADPH going to NADP plus a proton.

This turns out to be important, because that also controls the pH, and there are voltage channels controlled by pH that you need to think about if you looked at the detail biology. And so this protein is gp91. That is it's 91 kilodaltons, and gp means it's a glycoprotein. And then you have a second protein that's also an integral membrane protein, that's also critical. And this is gp22.

And so what you see is you have iron-- you have two hemes, cytochrome b heme, dependent systems. And these are going to change redox state. And the interesting thing is that these two teams are completely coordinated. So where have you seen heme before that reversibly binds oxygen? We need to do something with oxygen. Oxygen is getting reduced.

But what I'm telling you is that oxygen does not get reduced by binding to the heme. It's going to be using this method of electron transfer that we talked about. So they've got to be close enough so you can do electron transfer, perhaps through the heme edge in the protein.

So ultimately, oxygen is getting converted into superoxide not by direct binding to the heme. So this is distinct. And we'll see, this is completely distinct from the myeloperoxidases. This is completely distinct from the P450s we alluded to when we're talking about cholesterol homeostasis. And the key that makes all of this work is that it can form complexes with other proteins.

So let me just tell you what those other proteins are, and that was described in some detail in the reading. So we're going to have a GTPase. RAC2 is a G-protein. OK, so G-proteins can mediate phosphorylations. This one mediates phosphorylations. And it remains in the inhibited state till you need to trigger off your signaling cascade by another protein. The nomenclature is horrible, but there is an inhibitor protein that binds to the G-protein making it inactive, when some sensor comes in, they dissociate, and then the G-protein can function.

And we're going to look at that kind of signaling. We already have looked at that kind of signaling in the Carroll paper. But we'll look at it again in the signaling by NOx. The second group of proteins-- again, they're based on this size. These were identified a long time ago. They are unique to the phagosome. They're called phagosome oxidases. That's going to be the organelle where we're going to kill the bacteria. And so this p47 needs to be phosphorylated and it's phosphorylated by the G-protein. And that's key to have everything come together to allow the chemistry happen.

So this chemistry, in this form, it's inactive. It's only when everything comes together that you actually start doing the chemistry that's going to help us. So here's the model. So here's a resting cell. This is the nucleus. Here, the blue thing is the NOx protein. And the little blue thing is the second subunit. This is the 91. This is the 22. Here we can see that it's located in the plasma membrane. That's one of its locations. That's not the predominant location in the resting state.

The predominant location apparently is in little vesicles within these neutrophils, the white blood cells that are the first defenders against invasion by bacterial systems. And then we have these little complexes. Here's RAC2, a GTPase that's inhibited. And here is the phagosome oxidase.

And then what happens when the bacteria comes in, somehow the bacteria is coming in over here, it gets engulfed, and you form these little phagosomes inside the cell. And now what happens is the NADPH, the NOx proteins are located like this. The NADPH is on the outside. It's been activated by this GTPase. And now it's ready to generate superoxide inside the cell.

So there's a lot of membrane fusion and reorganization. Obviously, the signaling is really complex. We know a lot about the signaling. How do these guys even know there's a bacteria out there? How do they sense all of that? And I don't know if this is going to work. But this is a sort of a cool picture if it does work. Although it worked in my office, but it might not work here. Oh, here it goes.

So here we are. This is a white blood cell. These are red blood cells. The bacteria, these little things, floating around. It's sending off a signal. The white blood cell is chasing the bacteria. So there's some sequences chasing it through all of these cells, and you're going to see that in a minute, it gets it. There it goes. Gets inside. It's now in the phagosome, and puff, everything disappears.

And that is really what's going on in the system. So the question is-- it's a really cool picture. The question is what's the chemistry that's actually going on in these systems. So the chemistry-- whoops, somehow I lost-- I'm already over. That chemistry, it's all over already. What we'll do next time is come back and talk about how this flavin works, and then we'll see in that little phagosome also is a myeloperoxidase. We'll talk about how that works, and those are the two things I want-- how did they degrade this bacteria once they get inside this little organelle?